

THIRD EDITION



Essentials of **MEDICAL MICROBIOLOGY**

As per the Competency Based Medical Education Curriculum (NMC)

Students' Feedback

- This book is the reason why I started liking Microbiology!
- My Holy Grail book, highly recommended!
- All the chapters are well organized, contains all the necessary information
- Must try out this book for the best learning experience.
- I got silver medal in micro only because of this book, just go for it
- Very good presentation, easy to understand and remember
- Extraordinary book, each topic is crystal clear in presentation
- Only textbook written in a PG entrance-oriented way

First Indian Clinical Microbiology Book, in true sense

- Infective syndrome based (rather than traditional organism-based) presentation
- Parasitology—incorporated under the respective infective syndromes
- Hospital infection control, the need of the hour—thoroughly updated
- Overview chapters incorporated, for better understanding
- COVID-19, added as a separate chapter
- AETCOM module in Microbiology added
- Recent advances and latest informations in epidemiology, treatment and laboratory diagnosis incorporated
- Bulleted format in concise, simple and lucid language

Revised Reprint

**Apurba S Sastry
Sandhya Bhat**

Forewords
**Pallab Ray
Sujatha Sistla**



Essentials of Medical Microbiology



Essentials of Medical Microbiology

THIRD EDITION

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Dedicated to

Our Beloved Parents, Family Members

And, above all, the Almighty

"Life is the most difficult exam. Many fail because they tend to copy others, not realizing that everyone has a different question paper."

"Gold medalists are not made up of gold. They are made up of determination and hard work and ready to kill themselves to achieve their goals."

"Success is not the key to happiness. Happiness is the key to success. If you love what you are doing, you will be successful."

"You can succeed only if the fire inside you burns brighter than the fire around."

Golden Rules of Goal Setting

Dear Students

Here are some important tips which will help you in setting your goals in studies:

1. Set Goals That Motivate You: This means making sure that they are important to you, and that there is value in achieving them
2. Set SMART Goals
 - Specific: Your goal must be clear and well defined, not vague or generalized
 - Measurable: Goals must have measurable objectives
 - Attainable : Make sure that your goals are achievable and within your limit
 - Relevant: Will take you to the direction you want your life and career to go
 - Time Bound: You must know when you have the deadline and can celebrate success
3. Set Goals in Writing: Written commitment in presence of your close people (parents, close friends) will always push and remind you whenever you tend to deviate from your goal
4. Make an Action Plan: Do not focus only on the outcome, but make planning of all small steps that collectively take to the outcome. This is especially important if your goal is big and demanding, or long-term
5. Monitor Yourself: Compliance to the action plan should be monitored at least weekly (for one month goal) or monthly (for a yearly goal), depending upon your goal size.

Remember,

"Success is not final; failure is not fatal: It is the courage to continue that counts."

—Winston S Churchill

"There are two types of people who will tell you that you cannot make a difference in this world: those who are afraid to try and those who are afraid you will succeed."

—Ray Goforth

"Success ke piche mat bhago. Kabil bano kabil. Kamyabi toh sali jhak maar ke peeche ayegi."

—Aamir Khan

Foreword

It gives me immense pleasure to know that the third edition of *Essentials of Medical Microbiology* is to be released. The first two editions were very well appreciated among faculty and students across the country. The third edition is fully updated with a simple lucid presentation style like its earlier editions. This updated edition of the book has come at a much-needed time when NMC has implemented competency based medical education (CBME) and there is a strong need for a clinical microbiology textbook with stress on the Indian perspective. There is also an urgent need for clinical microbiologists with patient-centric bedside orientation rather than the age-old microbe-based laboratory limited attitude. This textbook has been written from a syndromic rather than traditional organism-based approach, in accordance with the revised competency based MBBS curriculum.



The book is aptly divided into two parts. The first part comprises of General Microbiology, Immunology and Hospital Infection Control and the second part, Systemic Microbiology (Infectious Diseases), includes several sections, each starting with a clinical infective syndrome followed by chapters covering detailed information about the etiological agents and their specific pathogenetic, epidemiological and clinical characteristics.

Parasitology has been added and therefore it obviates the need for a separate book. General Microbiology section has been meticulously restructured with the inclusion of chapters on general virology, general parasitology and general mycology. General bacteriology is reorganized into a single chapter with several subchapters. To help understand individual organisms better when discussed under systemic microbiology, overview chapters have been included in the general microbiology section.

Hospital Infection Control section has been extensively updated with addition of contemporary topics like major types of HAI, monitoring of antimicrobial stewardship, escalation and de-escalation strategy, donning and doffing of PPE and transmission-based precautions. The chapter on sterilization and disinfection has been completely revised based on hospital use of sterilizers and disinfectants and shifted to hospital infection control section.

Microbiology in 2020 cannot be complete without COVID-19 and a chapter has been rightly added covering details about the most catastrophic and enigmatic disease, the COVID-19 and its agent SARS-CoV-2. Annexures section has been expanded to include several new and relevant topics such as opportunistic infections, transplant infections, national health programmes for communicable diseases, vector-borne diseases, transfusion-transmitted infections and AETCOM (attitude, ethics and communication) in Microbiology. AETCOM module has been added as a new annexure, which covers topics pertaining to confidentiality in disclosing laboratory reports and demonstration of respect for patient samples.

Updates and recent advances have been made in epidemiology, laboratory diagnosis, treatment guidelines and vaccine prophylaxis of infectious diseases including tuberculosis and HIV.

I congratulate Dr Apurba S Sastry and his wife Dr Sandhya Bhat, for this commendable work and I am sure that this book will be widely read and appreciated by both undergraduate and postgraduate students of microbiology.

Pallab Ray MD

President

Indian Association of Medical Microbiologists (IAMM)

Professor

Department of Microbiology, PGIMER, Chandigarh, India

Foreword

I am happy to know that the third edition of *Essentials of Medical Microbiology* is to be released. This is a Clinical Microbiology textbook written in a system-wise approach, rather than traditional organism-based approach, with Indian perspective. JIPMER has implemented system-based Microbiology in MBBS curriculum since four years and we observed that students are finding it difficult to read organism-based book when the syllabus is system-based. Therefore this book has come at a much desired time when system-based Microbiology curriculum has been introduced pan India, by NMC.



- ❖ Book is divided into two parts.
 - The first part comprises of General Microbiology, Immunology and Hospital Infection Control.
 - The second part 'Systemic Microbiology (Infectious Diseases)' comprises of several sections, each comprising a first chapter on clinical infective syndrome followed by several chapters covering detailed information about the etiological agents
- ❖ Parasitology has been incorporated and therefore it obviates the reading of a separate book.
- ❖ Hospital Infection Control section has been thoroughly updated with the inclusion of new topics such as major HAI types, monitoring of antimicrobial stewardship, escalation vs de-escalation strategy, donning/doffing of PPE and transmission-based precautions.
- ❖ General Microbiology section has been meticulously restructured with the inclusion of general virology, general parasitology and general mycology chapters. General bacteriology is reorganized into a single chapter with several subchapters.
- ❖ Overview chapters have been incorporated in the general microbiology section, which will help in better understanding of individual organisms when discussed under systemic microbiology.
- ❖ Sterilization and disinfection chapter has been completely revised based on hospital use of sterilizers and disinfectants; and has been shifted to Hospital Infection Control section.
- ❖ A chapter on COVID-19 has been added, covering the latest pandemic in detail.
- ❖ Annexures section has been expanded to include several new topics such as opportunistic infections, transplant infections, national health programmes for communicable diseases, vector-borne diseases, transfusion-transmitted infections and AETCOM in Microbiology.
- ❖ AETCOM module has been added as a new annexure, which covers topics pertaining to confidentiality in disclosing laboratory reports and demonstration of respect for patient samples.
- ❖ I really appreciate the efforts made by my student and now colleague Dr Apurba S Sastry and his wife Dr Sandhya Bhat, for this praiseworthy work and I am sure that this book will be widely read by both undergraduate and postgraduate students of Microbiology.

A stylized signature in black ink, consisting of the letters 'S' and 'J' intertwined.

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Preface to the Third Edition

It gives us immense pleasure to announce the release of third edition of *Essentials of Medical Microbiology*. The excitement reaches its pinnacle as our sleepless nights of last ten months have come to an end. The first two editions were highly appreciated among students and faculty. Many have described it as 'highly recommended', 'extraordinary book', 'boon for exam', 'well organized', etc.

Clinical Microbiology, in True Sense

The making of this third edition is a revolutionary event in our life, as we went through a journey where we experienced how Microbiology can be changed to suit to the need of an Indian Medical Graduate.

- ❖ This book is prepared according to new MBBS curriculum and the content of the book was modified from the traditional organism-based teaching to system-based teaching. The content has been **updated, concised and reshuffled**—the three major types of changes incorporated in this edition.
- ❖ **Parasitology** has also been incorporated into this book under the respective infective syndromes, thus obviates the reading of a separate parasitology book.
- ❖ The reduction of the content is made by keeping the perspective of an Indian medical graduate in mind. The book is categorized into two parts, which is further divided into eleven sections.

Part I: General Microbiology, Immunology, Hospital Infection Control

Part I comprises of three sections—General Microbiology, Immunology, and Hospital Infection Control

- ❖ **Section 1: General Microbiology section** is meticulously restructured with the inclusion of general virology, general parasitology and general mycology chapters. **General bacteriology** is reorganized into a single chapter with several subchapters. **Overview chapters** have been incorporated in this section, which will help in better understanding of individual organisms when discussed under systemic microbiology.
- ❖ **Section 2: Immunology section** is thoroughly updated. Various topics have been updated such as MAC ELISA, National Immunization Schedule 2020, diagnosis of type I hypersensitivity (atopy), etc. Outdated topics such as precipitation, neutralization, complement fixation, etc. have been concised.
- ❖ **Section 3: Hospital infection control (HIC) section** needs a special mention. We must say that this section underwent a major update. In the era of COVID-19 pandemic, the significance of HIC is being increased to thousand folds. Every healthcare personnel is supposed to be well verse with the finer details of HIC. Therefore, the updated version of this section will be a key in making of a skilled Indian medical graduate.
 - The content has been thoroughly updated with the inclusion of new topics such as Major HAI types, monitoring of antimicrobial stewardship, escalation vs de-escalation strategy, donning/doffing of PPE and transmission-based precautions.
 - The **sterilization and disinfection** chapter is completely revised based on hospital use of sterilizers and disinfectants rather than traditional 'Microbiology use' and aptly shifted from general microbiology section to HIC section.

Part II: Systemic Microbiology (Infectious Diseases)

It comprises of eight sections, each comprises of a chapter on clinical infective syndrome followed by several chapters covering detailed information about the etiological agents. Chapters on clinical infective syndromes—represent the first chapter of every section; covers various infective syndromes pertaining to that system in detail.

- ❖ **Section 4: Bloodstream and cardiovascular system infections section** covers topics such as infective syndromes (infective endocarditis, rheumatic fever, bloodstream infections, etc.), enteric fever, rickettsial infections, brucellosis, leptospirosis, borreliosis, HIV/AIDS, viral hemorrhagic fever, malaria, visceral leishmaniasis and trypanosomiasis, lymphatic filariasis, systemic candidiasis and systemic mycoses.
- ❖ **Section 5: Gastrointestinal (GI) infections section** covers topics such as infective syndromes (diarrhea, dysentery, food poisoning, etc.), GI infections due to Enterobacteriaceae (diarrheagenic *E.coli*, shigellosis, yersiniosis, nontyphoidal



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Sandhya Bhat

salmonellosis), cholera, *Helicobacter*, *Campylobacter* and *Clostridioides difficile* infections, viral gastroenteritis, intestinal protozoan and helminthic infections.

- ❖ **Section 6:** *Hepatobiliary system infections section* covers topics such as infective syndromes (liver abscess, peritonitis, etc.), viral hepatitis, yellow fever, amoebic liver abscess, hydatid disease and Trematode infections of liver.
- ❖ **Section 7:** *Skin, soft tissue and musculoskeletal system infections section* covers topics such as infective syndromes, staphylococcal and streptococcal infections, gas gangrene and infections due to non-sporing anaerobes, leprosy, anthrax, actinomycosis, nocardiosis, non-venereal treponematoses, viral exanthems and other cutaneous viral infections, parasitic and fungal infections of skin, soft tissue and musculoskeletal systems.
- ❖ **Section 8:** *Respiratory tract infections section* covers topics such as infective syndromes, bacterial pharyngitis (streptococcal pharyngitis, and diphtheria), bacterial lobar pneumonia (pneumococcal pneumonia, *Haemophilus influenzae* pneumonia and others), bacterial atypical (interstitial) pneumonia (*Mycoplasma*, *Chlamydia* and *Legionella*), tuberculosis and non-tuberculous mycobacteria infections, pertussis, infections due to non-fermenting gram-negative bacilli, viral infections (myxoviruses—influenza, parainfluenza, mumps and respiratory syncytial virus, coronavirus, rhinovirus, adenovirus and infectious mononucleosis), parasitic infections (e.g. paragonimiasis) and fungal infections (zygomycosis, aspergillosis and pneumocystosis). Coronavirus has been added as a completely new chapter covering in detail about the most catastrophic disease, the COVID-19.
- ❖ **Section 9:** *Central nervous system infections section* covers topics such as infective syndromes, bacterial meningitis (meningococcal, pneumococcal, *Haemophilus influenzae*, *Listeria*, tubercular meningitis, spirochetal meningitis, and others) tetanus, viral meningitis and myelitis (poliomyelitis and others), viral encephalitis and encephalopathy (rabies, HSV and arboviral encephalitis), parasitic infections (neurocysticercosis, free-living amoebae infections, toxoplasmosis and others) and fungal infections (cryptococcal meningitis and others).
- ❖ **Section 10:** *Urogenital tract infections section* covers topics such as infective syndromes (UTI, pyelonephritis, genital ulcers, urethritis, vulvovaginitis, etc.), urinary infections (Enterobacteriaceae, *Enterococcus*, *Schistosoma haematobium* and others), and genital tract infections (syphilis, chancroid, donovanosis, gonorrhoea, genital *Chlamydia trachomatis* infection, trichomoniasis and genital candidiasis).
- ❖ **Section 11:** *Miscellaneous infective syndrome section* covers topics such as ocular and ear infections, congenital infections, organisms with oncogenic potential and zoonotic infections.
- ❖ **Annexures:** It has been expanded to include several new topics such as opportunistic infections, post-transplantation infections, national health programmes for communicable diseases, vector-borne diseases, and transfusion-transmitted infections. AETCOM module has been added as a new annexure, which covers several case scenarios pertaining to confidentiality in disclosing laboratory reports and demonstration of respect for patient samples
- ❖ **Most features of the previous editions have been maintained:** Such as concept of more content-less pages, concise, bulleted format and to-the-point text, simple and lucid language, separate boxes for summary of laboratory diagnosis and treatment for quick review, clinical case-based essay questions at the end of each chapter.

As you know, human errors are inevitable; and no book is immune to it. We would request all the readers to provide any errata found and also valuable suggestions and updates via e-mail.

This is probably the first book in India on 'Clinical Microbiology', in true sense. We are confident and hoping that you all will fall in love with this edition of the book.



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Preface to the First Edition

The association of man and microorganisms, and their fight to survive each other is never-ending. With the increasing complexity of interaction between man and microbes, the relevance of studying medical microbiology has increased manifold. Our book titled *Essentials of Medical Microbiology* attempts to provide smart ways to master the world of microbes relevant to the mankind.

The idea of yet another book on the subject, but with a quite different approach was born after several discouraging and unsatisfying experiences with several existing books regarding many needs of the enthusiastic students of the subject and a strong desire to make medical microbiology more interesting, up-to-date, clinically relevant and yet palatable to mainly undergraduate students of medicine and also the postgraduate aspirants and students. This book was conceptualized and brought to reality to meet the strongly felt diverse needs of the Indian students, such as gaining essential concepts, acquiring contemporary knowledge, approaching university exams with ease and confidence, scoring high in postgraduate entrance examinations, etc.

The book focuses on providing good foundation in clinically important concepts and principles of microbiology. Enough (over 300) tables and flowcharts have been included along with the text. Over 200 schematic diagrams have been drawn to simplify difficult concepts, and they are easy to reproduce where necessary as in examinations. Plenty of clinical photographs (over 400) included in the book will create a real life-like picture in the minds of the reader and also are meant to help solving image-based MCQs in postgraduate entrance examinations. It has more content in fewer pages, making the book handy. The concise bulleted format and to-the-point text used in this book will be helpful in rapid revision before the examinations. Best attempts have been made to keep the language simple yet lucid to help easy comprehension. Summary of laboratory diagnosis and treatment in separate boxes makes quick review possible. Highlighted boxes are incorporated to cover the important concepts. In a nutshell, this book is carefully written targeting to meet the varied needs of undergraduate students with an approach that will orient them to build concepts and to clear undergraduate examinations as well as to equip them for postgraduate entrance examinations in future.

General Microbiology section deals with principles of microscopy, morphology, physiology, culture identification of bacteria, concepts of bacterial genetics, etc. Principles of sterilization, antimicrobial chemotherapy and susceptibility testing are also explained in detail.

It is our humble hope that this book would change the general feeling of the students regarding immunology as being a difficult section into immunology as an interesting and enjoyable topic. In this section, topics such as *immunity*, *antigen*, *antibody*, *complement* and *structure of immune system* are explained in a simple and logical manner. Chapters like *Immune Response* and *Antigen-Antibody Reaction* have been fully updated according to the current need. Appropriate diagrams and flowcharts are incorporated to make critically tough content easy to grasp. Topics such as *autoimmunity*, *immunodeficiency* and *immunization* provide complete and latest information compiled in tabular form at one place.

Systematic Bacteriology section deals with individual bacterial pathogens in detail. Flow of information follows a very logical and clinically relevant course. More stress is given to the knowledge that helps in clinical setting and a careful attempt has been made to reduce the obsolete and not-so-useful core microbiology content. Sections like *laboratory diagnosis*, *treatment* and *prophylaxis* are most updated and referenced from internationally accepted literature and guidelines.

Virology is another section where the readers will find a different approach from the existing books. The updated and succinct information provided in this section with emphasis on *pathogenesis* and *laboratory diagnosis* will be useful to the students.

This book also addresses to the long-time complaint of the undergraduate students about unavailability of a concise and pictorialized *Mycology* section. Written in a clear and concise manner with appropriate and beautiful schematic pictures, images and illustrations, this section will surely make the students enjoy reading.

Applied Microbiology covers important aspects of various clinical infective syndromes with special reference to the approach towards the diagnosis. Useful information regarding hospital-acquired infections and biomedical waste management have been incorporated. The annexure incorporated at the end covers the recent topics, such as *emerging pathogens*, *bioterrorism* and *laboratory-acquired infections*.

Clinical case-based essay questions and MCQs are given at the end of each chapter to orient and prepare students for the examinations. Advanced and newer postgraduate entrance-oriented topics like H1N1, ebola, polio eradication, bacterial drug resistance mechanisms (such as ESBL, VRSA, VRE), automations and molecular methods in microbiology, etc. are incorporated.

We hope that the undergraduates, postgraduate aspirants, and postgraduate students will relish reading this book and find it useful. We also hope that we have made a good start in addressing the varied needs of students and faculty teaching medical microbiology with a single comprehensive book. We will feel glad to receive your valuable feedback, which will enable us to improve further.



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Contents

	Competency Number (NMC Curriculum)	Pages
PART I: General Microbiology, Immunology and Hospital Infection Control		
Section 1: General Microbiology		
1. Introduction and History	MI 1.1	3
2. Microscopy	MI 1.1	7
3. General Bacteriology		
3.1. Bacterial Taxonomy	MI 1.1	12
3.2. Morphology and Physiology of Bacteria	MI 1.1	15
3.3. Laboratory Diagnosis of Bacterial Infections	MI 1.1, 1.2, 1.6, 8.9, 8.10	25
• Specimen Collection		
• Direct Detection (Staining Methods and Others)		
• Culture, Identification and Antimicrobial Susceptibility Test		
• Molecular Diagnosis and Typing Methods		
3.4. Bacterial Genetics	MI 1.1	51
3.5. Antimicrobial Agents and Antimicrobial Resistance	MI 1.6	61
3.6. Pathogenesis of Bacterial Infections	MI 1.1	68
3.7. Overview of Bacterial Infections	MI 1.1	72
4. General Virology and Overview of Viral Infections	MI 1.1, 8.9, 8.10	80
5. General Parasitology and Overview of Parasitic Infections	MI 1.1, 1.2, 8.9, 8.10	102
6. General Mycology and Overview of Fungal Infections	MI 1.1, 1.2, 8.9, 8.10	115
7. Normal Human Microbiota	MI 1.1	121
8. Epidemiology of Infectious Diseases	MI 1.3	125
Section 2: Immunology		
9. Immunity (Innate and Acquired)	MI 1.7	133
10. Antigen	MI 1.8	140
11. Antibody	MI 1.8	144
12. Antigen-antibody Reaction	MI 1.8, 8.13, 8.15	152
13. Complement	MI 1.8	163
14. Components of Immune System: Organs, Cells and Products	MI 1.8	169
15. Immune Responses: Cell-mediated and Antibody-mediated	MI 1.8	184
16. Hypersensitivity	MI 1.10	193
17. Autoimmunity	MI 1.10	203
18. Immunodeficiency Disorders	MI 1.10	208
19. Transplant and Cancer Immunology	MI 1.11	213
20. Immunoprophylaxis	MI 1.9	221

Section 3: Hospital Infection Control

21. Healthcare-associated Infections	MI 8.5, 8.6, 8.7	229
22. Major Healthcare-associated Infection Types	MI 8.5, 8.6	241
23. Sterilization and Disinfection	MI 1.4, 1.5	251
24. Biomedical Waste Management	MI 8.5, 8.6	263
25. Needle Stick Injury	MI 8.5, 8.6	268
26. Antimicrobial Stewardship	MI 1.6	272
27. Environmental Surveillance (Bacteriology of Water, Air and Surface)	MI 8.8	278

PART II: SYSTEMIC MICROBIOLOGY (INFECTIOUS DISEASES)**Section 4: Bloodstream and Cardiovascular System Infections**

28. Cardiovascular System Infections: Infective Endocarditis and Acute Rheumatic Fever	MI 2.1, 2.2, 2.3, 8.13, 8.15	287
29. Bloodstream Infections	MI 1.1, 2.4, 8.15	295
Bacterial Infections		
30. Enteric Fever (<i>Salmonella</i> Typhi and <i>Salmonella</i> Paratyphi)	MI 3.3, 3.4, 8.15	302
31. Rickettsial Infections	MI 1.1, 8.15	310
32. Miscellaneous Bacterial Bloodstream Infections: Brucellosis, Leptospirosis and Borreliosis	MI 1.1, 8.1, 8.15	316
Viral infections		
33. HIV/AIDS	MI 2.7, 8.15, 8.16	325
34. Viral Hemorrhagic Fever (VHF) Arboviral VHF (Dengue, Chikungunya and others), Filoviral VHF (Ebola and Marburg Virus), Hantaviral and Other Agents of VHF	MI 1.1, 8.15	336
Parasitic Infections		
35. Malaria and Babesiosis	MI 2.5, 2.6, 8.16	346
36. Visceral Leishmaniasis and Trypanosomiasis	MI 2.5, 1.1, 8.16	360
37. Lymphatic Filariasis	MI 2.5, 2.6, 8.16	370
Fungal Infections		
38. Systemic Candidiasis and Systemic Mycoses	MI 1.1, 8.15	377
Section 5: Gastrointestinal (GI) Infections		
39. Gastrointestinal Infective Syndromes	MI 3.1	387
Bacterial Infections		
40. Food Poisoning: <i>S. aureus</i> , <i>Bacillus cereus</i> , <i>Clostridium botulinum</i> and Others	MI 3.5	394
41. Gastrointestinal Infections due to Enterobacteriaceae : Diarrheagenic <i>Escherichia coli</i> , Shigellosis, Nontyphoidal Salmonellosis and Yersiniosis	MI 3.1, 3.2	400
42. Cholera, Halophilic <i>Vibrio</i> and <i>Aeromonas</i> Infections	MI 3.1, 3.2	410
43. Miscellaneous Bacterial Infections of Gastrointestinal System: <i>Helicobacter</i> , <i>Campylobacter</i> and <i>Clostridioides difficile</i> Infections	MI 3.1, 3.2, 3.6	419
Viral Infections		
44. Viral Gastroenteritis: Rotaviruses and Others	MI 3.1, 3.2	424
Parasitic Infections		
45. Intestinal Protozoan Infections: Intestinal Amoebiasis, Giardiasis, Coccidian Parasitic Infections, Balantidiasis, Blastocystosis, and Others	MI 3.1, 3.2, 1.2, 8.15	427

46. Intestinal Helminthic Infections	MI 3.1, 3.2, 1.2, 8.15	446
• Intestinal Cestode Infections: <i>Diphyllobothrium</i> , <i>Taenia</i> , <i>Hymenolepis</i> and Others		
• Intestinal Trematode Infections: <i>Fasciolopsis buski</i> , <i>Schistosoma mansoni</i> , <i>S. japonicum</i> and Others		
• Intestinal Nematode Infections: <i>Trichuris</i> , <i>Enterobius</i> , hookworm, <i>Strongyloides</i> , <i>Ascaris</i> and Others		
Section 6: Hepatobiliary System Infections		
47. Infective Syndromes of Hepatobiliary System and Abdomen	MI 3.1	473
Viral Infections		
48. Viruses Causing Hepatitis	MI 3.7, 3.8, 8.13, 8.15	476
Hepatitis Viruses, Yellow Fever and Others		
Parasitic Infections		
49. Parasitic Infections of Hepatobiliary System	MI 3.1, 3.2	491
Amoebic Liver Abscess, Hydatid Disease (<i>Echinococcosis</i>), Trematode Infections (<i>Fasciola hepatica</i> , <i>Clonorchis</i> and <i>Opisthorchis</i>) and Others		
Section 7: Skin, Soft Tissue and Musculoskeletal System Infections		
50. Infective Syndromes of Skin, Soft Tissue and Musculoskeletal Systems	MI 4.1, 4.2, 4.3, 1.2	503
Bacterial Infections		
51. Staphylococcal Infections	MI 4.2, 4.3, 1.2	511
52. Beta-hemolytic Streptococcal Infections	MI 4.3, 1.2	520
53. Gas gangrene (<i>Clostridium perfringens</i>) and Infections due to Nonsporing Anaerobes	MI 4.1, 1.2	527
54. Leprosy (<i>Mycobacterium leprae</i>)	MI 4.3, 8.15, 8.16	534
55. Miscellaneous Bacterial Infections of Skin and Soft Tissues: Anthrax (<i>Bacillus anthracis</i>), Actinomycosis, Nocardiosis, Nonvenereal Treponematoses and Others	MI 4.3, 1.2, 8.13, 8.15	539
Viral Infections		
56. Viral Exanthems and Other Cutaneous Viral Infections	MI 4.3, 8.15	550
Herpesviruses (Herpes Simplex, Varicella-zoster and HHV-6 and 7 Infection), Poxviruses (Smallpox, Molluscum contagiosum), Parvovirus, Measles, Rubella, Coxsackieviruses and Others		
Parasitic Infections		
57. Parasitic Infections of Skin, Soft Tissue and Musculoskeletal System	MI 4.2, 4.3, 8.13, 8.15	565
Cutaneous Leishmaniasis, Cysticercosis, Tissue Nematodes (Filarial Tissue Nematodes, <i>Dracunculus medinensis</i> , <i>Trichinella spiralis</i>) and Larva Migrans		
Fungal Infections		
58. Fungal Infections of Skin, Soft Tissue and Musculoskeletal System	MI 4.2, 4.3, 8.13, 8.15	574
Superficial Fungal Infections, Subcutaneous Fungal Infections, Candidiasis (cutaneous and mucosal) and <i>Penicillium marneffeii</i> Infection		
Section 8: Respiratory Tract Infections		
59. Infective Syndromes of Respiratory Tract	MI 6.1, 6.2, 6.3	587
Bacterial Infections		
60. Bacterial Pharyngitis: <i>Streptococcus pyogenes</i> Pharyngitis, Diphtheria and Others	MI 6.1, 6.2, 8.15	598
61. Bacterial Lobar Pneumonia: Pneumococcal Pneumonia, <i>Haemophilus influenzae</i> Pneumonia and Others	MI 6.1, 6.3, 1.2, 8.15	606

62. Bacterial Atypical (Interstitial) Pneumonia: <i>Mycoplasma</i> , <i>Chlamydia</i> , <i>Legionella</i> and Others	MI 6.1, 6.3	618
63. Tuberculosis and Nontuberculous Mycobacteria Infections	MI 6.1, 6.3, 1.2, 8.16	623
64. Pertussis (<i>Bordetella pertussis</i>)	MI 6.1	638
65. Infections due to Non-fermenting Gram-negative Bacilli: <i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Burkholderia</i> and Others	MI 6.1, 6.3, 4.3	642
Viral Infections		
66. Myxovirus Infections of Respiratory Tract: Influenza, Parainfluenza, Mumps, Respiratory Syncytial Virus and Others	MI 6.1, 6.2, 6.3	648
67. Coronavirus Infections Including COVID-19	MI 6.1, 6.2, 6.3	660
68. Miscellaneous Viral Infections of Respiratory Tract: Rhinovirus, Adenovirus and Infectious Mononucleosis (Epstein-Barr Virus)	MI 6.1, 6.2	672
Parasitic and Fungal Agents		
69. Parasitic and Fungal Infections of Respiratory Tract <ul style="list-style-type: none"> • Parasitic Infections: Paragonimiasis and Others • Fungal Infections: Zygomycosis, Aspergillosis, Pneumocystosis and Others 	MI 6.1, 6.2, 6.3	676
Section 9: Central Nervous System Infections		
70. Infective Syndromes of Central Nervous System	MI 5.1, 5.2, 5.3	687
Bacterial Infections		
71. Bacterial Meningitis <ul style="list-style-type: none"> • Acute Bacterial (Pyogenic) Meningitis: <i>Neisseria meningitidis</i>, <i>Streptococcus pneumoniae</i>, <i>Streptococcus agalactiae</i>, <i>Haemophilus influenzae</i> and <i>Listeria</i> • Chronic Bacterial Meningitis: Tubercular Meningitis, Spirochetal Meningitis, Lyme Disease and Others 	MI 5.1, 5.3, 1.2, 8.15	692
72. Tetanus	MI 4.1	702
Viral Infections		
73. Viral Meningitis and Myelitis: Poliomyelitis, Coxsackievirus Infections, and Others	MI 5.1, 5.3, 8.16	706
74. Viral Encephalitis and Encephalopathy Rabies, HSV Encephalitis, Arboviral Encephalitis (Japanese Encephalitis and West Nile), Nipah and Hendra, Slow Virus and Prion Disease, and Others	MI 5.2, 8.15	715
Parasitic and Fungal Infections		
75. Parasitic and Fungal Infections of Central Nervous System <ul style="list-style-type: none"> • Parasitic Infections: Neurocysticercosis, Free-living Amoeba Infections, Toxoplasmosis and Others • Fungal Infections: Cryptococcal Meningitis and Others 	MI 5.1, 5.2, 5.3, 8.15	727
Section 10: Urogenital Tract Infections		
76. Infective Syndromes of Urinary Tract and Sexually-transmitted Infections <ul style="list-style-type: none"> • Bacterial Infections: Enterobacteriaceae, <i>Enterococcus</i> and Others • Viral (BK Virus), Parasitic (<i>Schistosoma haematobium</i>) and Fungal Infections 	MI 7.1, 7.3	745
77. Infective Syndromes of Genital Tract <ul style="list-style-type: none"> • Ulcerative Genital Disease: Syphilis, Lymphogranuloma Venerum, Granuloma Inguinale, Soft Chancre and Genital Herpes • Gonorrhoea and Non-gonococcal Urethritis (<i>Chlamydia trachomatis</i> and Others) • Vulvovaginitis (Trichomoniasis, Bacterial Vaginosis, Vaginal Candidiasis) • Other Genital Tract Infections of Females and Males 	MI 7.1, 7.2, 8.15	756

Section 11: Miscellaneous Infective Syndromes

78. Ocular and Ear Infections	MI 1.1, 8.15	779
79. Congenital Infections Cytomegalovirus Infections, Congenital Varicella, Neonatal Herpes, Congenital Rubella, Congenital Toxoplasmosis, Congenital Syphilis, Zika Virus Infections and Others	MI 1.1, 8.15	793
80. Organisms with Oncogenic Potential Human Papilloma Virus, Kaposi Sarcoma, HTLV and HIV, Epstein-Barr Virus, Hepatitis B and C, and Others	MI 8.3	800
81. Zoonotic Infections: Plague, Tularaemia and Bite Wound Infections	MI 8.1	807
Annexures		
1. Opportunistic Infections	MI 8.2	817
2. Transplant Infections	MI 1.1, 8.2	818
3. Emerging and Re-emerging Infections	MI 8.4	819
4. Microbial Agents of Bioterrorism	MI 1.1	820
5. Laboratory Acquired Infections	MI 1.1	822
6. National Health Programmes for Communicable Diseases	MI 8.16	823
7. Vector-borne Infections and Ectoparasite Infestations	MI 8.1	824
8. Transfusion-transmitted Infections	MI 1.1	827
9. AETCOM in Microbiology	MI 8.11, 8.12, 8.14	828
10. Pandemic Management	NMC's Pandemic Management Module	834
<i>Index</i>		835

Organism Assignment into Various Systems

This book has followed system-wise approach rather traditional organism-based approach to discuss the etiological agents of infectious diseases.

The assignment of organisms under each system is mainly based on the organs/systems they principally infect and produce manifestations; *not based on their mode of transmission or habitat or source (e.g. animal)*. For examples,

- ❖ HIV is discussed under bloodstream, not genitourinary section. This is because HIV is only transmitted sexually, but does not produce any genital manifestations
- ❖ Anthrax is discussed under skin and soft tissue infections; not under zoonotic infection
- ❖ Enteric fever is discussed under bloodstream, not GIT section. This is because *Salmonella Typhi* is only transmitted by enteric route, but manifestations are largely extraintestinal
- ❖ *Schistosoma mansoni* and *S. haematobium* are discussed under infections of GIT and urinary tract respectively. Although called as blood flukes (because of their habitat); the manifestations are confined to GIT and urinary tract.
- ❖ For the organism infecting more than one system, it is discussed under the major system it principally infects and is briefly discussed under other system(s) by keeping only the relevant points.

The table below is a guide for the reader to find out the chapters in which the organisms are discussed. The chapter numbers in '**bold**' are the systems in which the organisms are discussed in detail.

Table 1: Etiological agents of infectious diseases			Table 1: Etiological agents of infectious diseases		
Bacterial agents	Chapter(s)	Page	Bacterial agents	Chapter(s)	Page
Gram-positive cocci			Gram-negative bacilli		
<i>Staphylococcus aureus</i> , Coagulase negative, <i>Staphylococcus</i> (CoNS)	51, 28, 40, 61	511, 290, 395, 518	<i>Clostridioides difficile</i>	43	422
<i>Staphylococcus saprophyticus</i>	76	752	Non-sporing anaerobes	53	531
<i>Streptococcus</i> species	52	520	<i>Mycobacterium tuberculosis</i>	63, 55, 71, 76, 77	623, 547, 699, 752, 770
Classification	52	520	Non-tuberculous Mycobacteria	63, 55	636, 547
<i>Streptococcus pyogenes</i>	52, 60	520, 598	<i>Mycobacterium leprae</i>	54	534
<i>Streptococcus agalactiae</i>	52, 71, 77	525, 694, 775	<i>Actinomyces</i>	55	542
<i>Enterococcus</i>	76	750	<i>Nocardia</i>	55	544
Viridans streptococci	28	290	<i>Listeria monocytogenes</i>	71	698
<i>Streptococcus pneumoniae</i>	61, 71	606, 695	<i>Erysipelothrix rhusiopathiae</i>	55	547
Gram-negative cocci			<i>Tropheryma whipplei</i>	39	393
<i>Neisseria meningitidis</i>	71, 55	695, 546	Enterobacteriaceae		
<i>Neisseria gonorrhoeae</i>	77, 60	764, 605	Enterobacteriaceae	41	400
<i>Moraxella</i> species	61, 78	617, 780	<i>Escherichia coli</i>	41, 76	400
Gram-positive bacilli			<i>Shigella</i>	41	404
<i>Corynebacterium diphtheriae</i>	60, 55	599, 547	<i>Salmonella</i>	30, 41	302, 406
Diphtheroids	60	604	<i>Klebsiella</i>	61	614
<i>Bacillus anthracis</i>	55, 61	539, 616	<i>Enterobacter</i>	61	615
<i>Bacillus cereus</i>	40	395	<i>Serratia</i>	61	615
Anaerobes	53	527	<i>Citrobacter</i>	76	749
<i>Clostridium perfringens</i>	53	528	<i>Proteus, Providencia, Morganella</i>	76	749
<i>Clostridium tetani</i>	72	702	<i>Yersinia pestis</i>	81	807
<i>Clostridium botulinum</i>	40	397	<i>Y. enterocolitica</i>	41	407
			<i>Y. pseudotuberculosis</i>		407

Bacterial agents	Chapter(s)	Page
<i>Vibrio</i> and <i>Aeromonas</i>	42	410, 418
Non-fermenting gram-negative bacilli— <i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Burkholderia</i> , <i>Stenotrophomonas</i>	65	642
<i>Haemophilus influenzae</i>	61, 71	610, 697
<i>Haemophilus ducreyi</i>	77	762
<i>Haemophilus aegyptius</i>	78	780
HACEK group	28	291
<i>Bordetella</i>	64	638
<i>Brucella</i>	32	316
Other gram-negative bacilli		
<i>Campylobacter</i>	43	419
<i>Helicobacter</i>	43	420
<i>Legionella</i>	62	621
<i>Klebsiella granulomatis</i>	77	763
Agents of rat bite fever (<i>Streptobacillus</i> , <i>Spirillum</i>)	81	813
Miscellaneous bacterial agents		
Spirochetes	32	319
<i>Treponema pallidum</i>	77, 71, 79	756, 700, 799
Non-venereal treponematoses	55	545
<i>Borrelia</i>	32	322
<i>Leptospira</i>	32	319
Rickettsiae and related genera — <i>Rickettsia</i> , <i>Orientia</i> , <i>Bartonella</i> , <i>Ehrlichia</i>	31	310
<i>Chlamydia trachomatis</i>	77, 78, 62	766, 781, 621
<i>Chlamydomydia psittaci</i>	62	620
<i>Chlamydomydia pneumoniae</i>	62	620
<i>Mycoplasma pneumoniae</i>	62	618
Urogenital <i>Mycoplasma</i>	77	770
Viral agents		
	Chapter(s)	Page
DNA viruses		
Herpesviruses	56	550
Herpes simplex virus	56, 73, 74, 77, 78, 79	551, 708, 716, 762, 785, 797
Varicella-zoster virus	56, 78, 79	554, 785, 797
Epstein-Barr virus	68, 80, 56	672, 802, 550
Cytomegalovirus	79, 56	795, 550
Human herpes virus 6 and 7	56	556
Human herpes virus 8	80, 56	803, 556
Other DNA viruses		
Parvoviruses	56, 79	556, 798
Papillomaviruses	80, 56, 77	803, 557, 771
Poxviruses	56	557
Adenoviruses	68, 78, 44	674
Bacteriophages	4	97

Viral agents	Chapter(s)	Page
RNA viruses		
Orthomyxoviruses (Influenza viruses)	66	648
Paramyxoviruses	66	655
Parainfluenza viruses	66	655
Mumps virus	66, 73	656, 709
Measles virus	56, 74	559, 724
Respiratory syncytial virus	66	657
Nipah and Hendra viruses	74	718
Rubella virus	56, 79	562, 794
Picornaviruses	73	707
Polioviruses	73	709
Coxsackie viruses	73	707
Other enteroviruses	73	707
Rhinoviruses	68	675
Arboviruses	34	336
Classification	34	337
Dengue, Chikungunya	34	338, 341
Kyasanur forest disease virus	34	342
Japanese encephalitis virus	74	716, 718
West Nile virus		
Yellow fever	48	489
Zika virus	79	798
Rabies virus	74	718
HIV and other retroviruses	33, 80	325, 801
Hantaviruses and Arenaviruses	34	345
Ebola and Marburg viruses	34	343
Coronaviruses	67	660
Viral gastroenteritis (Rotaviruses, Noroviruses and others)	44	424
Slow viruses and prion disease	74	724
Other group of viruses		
Hepatitis viruses	48, 80	476
Oncogenic viruses	80	800
Parasitic agents		
	Chapter(s)	Page
Protozoan parasites		
Amoeba		
<i>Entamoeba histolytica</i>	45, 49	427, 491
Nonpathogenic Amoebae	45	432
Free-living Amoebae: <i>Naegleria</i> , <i>Acanthamoeba</i> , <i>Balamuthia</i> , <i>Sappinia</i>	75	727, 729, 730
Flagellates		
<i>Giardia lamblia</i>	45	433
<i>Trichomonas vaginalis</i>	77	771
<i>Leishmania</i>	36, 57	360, 565
<i>Trypanosoma cruzi</i>	36, 57, 78, 79	366, 572, 787, 799
<i>Trypanosoma brucei</i> complex	36, 75	368, 735

Parasitic agents	Chapter(s)	Page
Apicomplexa		
Malaria parasites and <i>Babesia</i>	35, 75, 79	346, 734, 799
<i>Toxoplasma</i>	75, 78, 79	731, 787, 793
<i>Cryptosporidium</i> , <i>Cyclospora</i> , <i>Cystoisospora</i>	45	437
<i>Sarcocystis</i>	45, 57	442, 572
Miscellaneous Protozoa		
<i>Balantidium coli</i>	45	441
<i>Blastocystis</i>	45	442
Helminths		
Cestodes		
<i>Diphyllobothrium latum</i>	46	450
<i>Taenia saginata</i>	46	447
<i>Taenia solium</i> (intestinal)	46	447
<i>Taenia solium</i> (cysticercosis)	75, 57, 78	735, 573, 788
<i>Echinococcus granulosus</i>	49	493
<i>Hymenolepis nana</i>	46	450
<i>Dipylidium caninum</i>	46	450
Trematodes		
<i>Schistosoma haematobium</i>	76	753
<i>Schistosoma mansoni</i>	46, 75, 49	454, 738, 498
<i>Schistosoma japonicum</i>	46, 75, 49	455, 738, 498
<i>Fasciolopsis buski</i>	46	456
<i>Fasciola hepatica</i>	49	497
<i>Clonorchis sinensis</i>	49, 80	498, 806
<i>Opisthorchis viverrini</i>	49, 80	498, 806
<i>Paragonimus westermani</i>	69	676
Intestinal Nematodes		
<i>Trichuris trichiura</i>	46	458
<i>Enterobius vermicularis</i>	46	459
<i>Ascaris lumbricoides</i>	46, 69	461, 678
Hookworm: <i>Ancylostoma</i> and <i>Necator</i>	46, 57, 69	463, 572, 678
<i>Strongyloides stercoralis</i>	46, 75	466, 739
Somatic Nematodes		
Lymphatic filarial nematodes <i>Wuchereria bancrofti</i> <i>Brugia malayi</i> , <i>B. timori</i>	37	370
Cutaneous filarial nematodes: <i>Loa loa</i> , <i>Onchocerca</i> , <i>Mansonella</i>	57	567
<i>Dracunculus medinensis</i>	57	569
<i>Trichinella spiralis</i>	57	570

Parasitic agents	Chapter(s)	Page
Nematodes of lower animals		
Visceral larva migrans	49	499
Cutaneous larva migrans	57	572
<i>Toxocara canis</i>	49	498
<i>Angiostrongylus cantonensis</i>	75	739
<i>Diectophyme renale</i>	76	755
<i>Capillaria</i> species	46, 49	469, 499
Ectoparasites		
<i>Sarcoptes scabiei</i>	Annexure-7	824
Fungal agents		
Superficial mycoses		
Tinea versicolor: <i>Malassezia furfur</i>	58	574
Tinea nigra: <i>Hortaea werneckii</i>	58	575
Piedra: <i>Trichosporon beigellii</i> , <i>Piedraia hortae</i>	58	575
Dermatophytosis: <i>Trichophyton</i> , <i>Microsporum</i> , <i>Epidermophyton</i>	58	575
Subcutaneous mycoses		
Mycetoma: <i>Madurella mycetomatis</i> and others	58	578
<i>Sporothrix schenckii</i>	58	580
Chromoblastomycosis	58	581
Phaeohyphomycosis	58	581
<i>Rhinosporidium seeberi</i>	58	582
Systemic mycoses		
<i>Histoplasma</i> , <i>Blastomyces</i> , <i>Coccidioides</i> , <i>Paracoccidioides</i>	38	379, 381, 382
Opportunistic mycoses		
<i>Candida</i> species	38, 58, 77	377, 583, 774
<i>Cryptococcus neoformans</i>	75, 69	739, 684
Zygomycosis: <i>Rhizopus</i> , <i>Mucor</i> , <i>Lichtheimia</i>	69, 78	680, 789
Aspergillosis: <i>Aspergillus flavus</i> , <i>A. fumigatus</i> , <i>A. niger</i>	69	681
<i>Penicillium marneffeii</i>	58	582
Other <i>Penicillium</i> species	69	684
<i>Pneumocystis jirovecii</i>	69	679
<i>Fusarium</i> species	78	789
Others		
Microsporidia species	45, 58, 78	443, 582, 790
Mycotoxicoses	40	398

Further Reading

1. Abbas' Cellular and Molecular Immunology, 8th Edition.
2. Bailey & Scott's Diagnostic Microbiology, 14th Edition.
3. Gillespie's Medical Microbiology and Infection at a Glance, 4th Edition.
4. Harrison's Principles of Internal Medicine, 20th Edition.
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7. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 7th Edition.
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9. Mackie and McCartney's Practical Medical Microbiology, 14th Edition.
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11. Manson's Tropical Diseases, 23rd Edition.
12. National AIDS Control Organisation (NACO), India.
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24. Various national and international journals and other internet sources.
25. World Health Organization (WHO).
26. Centers for Disease Control and Prevention, Atlanta, USA.

PART I

General Microbiology, Immunology and Hospital Infection Control

Section 1: General Microbiology

Section 2: Immunology

Section 3: Hospital Infection Control

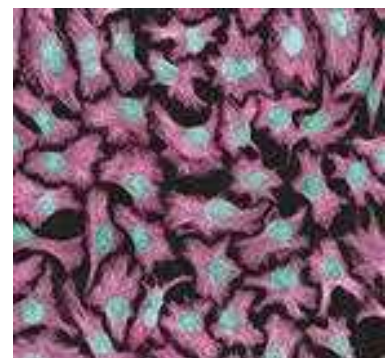
General Microbiology

SECTION

1

SECTION OUTLINE

1. Introduction and History
2. Microscopy
3. General Bacteriology
 - 3.1 Bacterial Taxonomy
 - 3.2 Morphology and Physiology of Bacteria
 - 3.3 Laboratory Diagnosis of Bacterial Infections
 - 3.4 Bacterial Genetics
 - 3.5 Antimicrobial Agents and Antimicrobial Resistance
 - 3.6 Pathogenesis of Bacterial Infections
 - 3.7 Overview of Bacterial Infections
4. General Virology and Overview of Viral Infections
5. General Parasitology and Overview of Parasitic Infections
6. General Mycology and Overview of Fungal Infections
7. Normal Human Microbiota
8. Epidemiology of Infectious Diseases



MALDI-TOF



A Revolution in Diagnostic Microbiology

CHAPTER PREVIEW

- Clinical Microbiology
- Classification of Microorganisms
- History

CLINICAL MICROBIOLOGY

Medical microbiology is a branch of medicine that deals with the study of microorganisms such as bacteria, viruses, parasites or fungi; which produce disease in humans, called as infectious diseases.

Clinical microbiology is a branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases. The various branches of clinical microbiology include:

- ❖ **General microbiology:** It deals with the study of general properties of microorganisms—taxonomy, morphology, pathogenesis, laboratory diagnosis, and treatment for their effective killing
- ❖ **Immunology:** It deals with the study of the immune system, immunological mechanisms of infectious diseases and various immunological methods for diagnosis of infectious diseases
- ❖ **Hospital infection control:** It deals with the study of various control measures to prevent the transmission of healthcare associated infections
- ❖ **Systemic microbiology (infectious diseases):** Microorganisms infect various organ systems of our body. Accordingly, the infectious diseases are classified into various clinical syndromes
 - Bloodstream and cardiovascular system infections
 - Gastrointestinal and hepatobiliary system infections
 - Skin, soft tissue and musculoskeletal system infections
 - Central nervous system infections
 - Respiratory tract infections
 - Urinary tract infections
 - Genital tract infections
 - Infections of eye, ear and others.

CLASSIFICATION OF MICROORGANISMS

Microorganisms are grouped under both prokaryotes and eukaryotes.

Table 1.1: Characteristics of prokaryotes and eukaryotes.

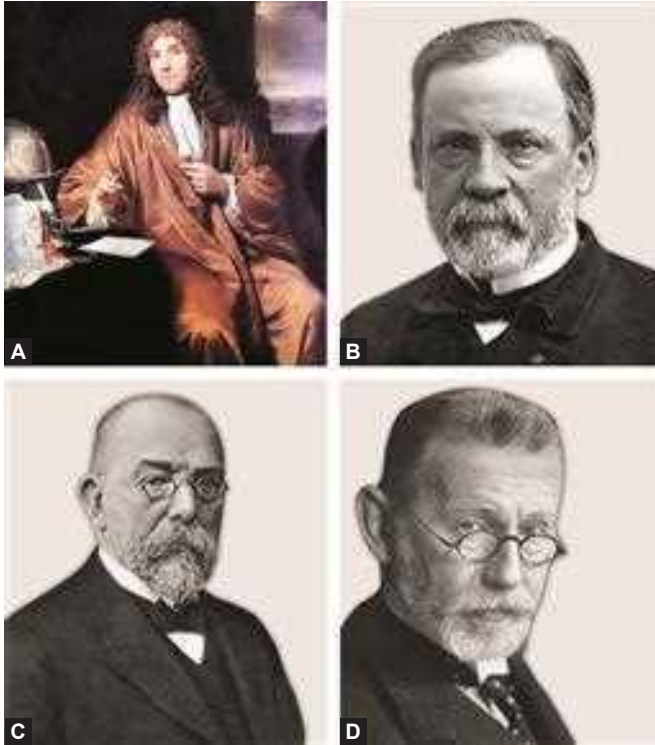
Characteristics	Prokaryotes	Eukaryotes
Major groups	Bacteria, blue green algae	Fungi, parasites, other algae, plants and animals
Nucleus	Diffuse	Well defined
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
Ribonucleoprotein	Absent	Present
Cell division	Binary fission	Mitosis, meiosis
Chromosome	One, circular	Many, linear
Extrachromosomal DNA	Found in plasmid	Found in mitochondria
Cell membrane	Does not contain sterols except in <i>Mycoplasma</i>	Contain sterols
Cellular organelles like mitochondria, etc.	Absent (except ribosome)	Present
Ribosome	70S	80S
Site of respiration	Mesosome	Mitochondria
Pinocytosis	Absent	Present

Abbreviation: S, Svedberg unit.

- ❖ Bacteria and blue green algae are placed under prokaryotes. They have a primitive nucleus, and other properties of a prokaryotic cell (Table 1.1)
- ❖ Whereas other algae, fungi and parasites (protozoa and helminths) belong to eukaryotes; having a well-defined nucleus and various eukaryotic cellular organelles
- ❖ Viruses are neither considered as prokaryotes nor eukaryotes because they lack the characteristics of living things, except the ability to replicate.

HISTORY

The existence of microorganisms was hypothesized for many centuries before their actual discovery. The teaching of Mahavira (Jainism, 6th century BC) and the postulation



Figs 1.1A to D: Eminent microbiologists: **A.** Antonie van Leeuwenhoek; **B.** Louis Pasteur; **C.** Robert Koch; **D.** Paul Ehrlich.

Source: Wikipedia (with permission).

of Varo and Columella (who named the invisible organisms as '*Animalia minuta*') were some of those attempts. The eminent personalities in the field of Microbiology and their important contributions have been described below.

Antonie Philips van Leeuwenhoek (1676)

He was the first scientist who observed bacteria and other microorganisms, using a single-lens microscope constructed by him and he named those small organisms as '*Little animalcules*' (Fig. 1.1A).

Edward Jenner

Edward Jenner 1796, developed the first vaccine of the world, the smallpox vaccine. He used the cowpox virus (*Variolae vaccinae*) to immunize children against smallpox from which the term '**vaccine**' has been derived. The same principles are even used today for developing vaccines.

Louis Pasteur

Microbiology developed as a scientific discipline from the era of Louis Pasteur (1822–1895). He is also known as '**father of microbiology**'. He was a professor of chemistry in France. His studies on fermentation led him to take interest to work in microbiology (Fig. 1.1B). His contributions to microbiology are as follows:

- ❖ He had proposed the **principles of fermentation** for preservation of food
- ❖ He introduced the **sterilization techniques** and developed steam sterilizer, hot air oven and autoclave
- ❖ He described the method of **pasteurization of milk**
- ❖ He had also contributed for the vaccine development against several diseases, such as anthrax, fowl cholera and rabies
- ❖ He disproved the theory of spontaneous generation of disease and postulated the '**germ theory of disease**'. He stated that disease cannot be caused by bad air or vapor, but it is produced by the microorganisms present in air
- ❖ **Liquid media concept:** He used nutrient broth to grow microorganisms
- ❖ He was the founder of the Pasteur Institute, Paris.

Joseph Lister

Joseph Lister (1867) is considered to be the '**father of antiseptic surgery**'. He had observed that postoperative infections were greatly reduced by using disinfectants such as diluted carbolic acid during surgery to sterilize the instruments and to clean the wounds.

Robert Koch

Robert Koch provided remarkable contributions to the field of microbiology. He was a German general practitioner (1843–1910) (Fig. 1.1C). His contributions are as follows:

- ❖ He introduced **solid media** for the culture of bacteria. Eilshemius Hesse, the wife of, one of the Koch's assistants had suggested the use of **agar** as solidifying agents
- ❖ He also introduced methods for isolation of bacteria in **pure culture**
- ❖ He described **hanging drop** method for testing motility
- ❖ He **discovered bacteria** such as the anthrax bacilli, tubercle bacilli and cholera bacilli
- ❖ He introduced staining techniques by using aniline dye
- ❖ **Koch's phenomenon:** Robert Koch observed that guinea pigs already infected with tubercle bacillus developed a hypersensitivity reaction when injected with tubercle bacilli or its protein. Since then, this observation is called as Koch's phenomenon
- ❖ **Koch's postulates:** Robert Koch had postulated that a microorganism can be accepted as the causative agent of an infectious disease only if four criteria are fulfilled. These criteria are as follows:
 1. The microorganism should be constantly associated with the lesions of the disease
 2. It should be possible to isolate the organism in pure culture from the lesions of the disease
 3. The same disease must result when the isolated microorganism is inoculated into a suitable laboratory animal

4. It should be possible to re-isolate the organism in pure culture from the lesions produced in the experimental animals.

An additional **fifth criterion** was introduced subsequently which states that antibody to the causative organism should be demonstrable in the patient's serum.

Exceptions to Koch's postulates: It is observed that it is not always possible to apply these postulates to study all the human infectious diseases. There are some bacteria that do not satisfy one or more of the four criteria of Koch's postulates. Those organisms are:

- ❖ *Mycobacterium leprae* and *Treponema pallidum*: They cannot be grown in vitro; however, they can be maintained in experimental animals
- ❖ *Neisseria gonorrhoeae*: There is no animal model; however, it can be grown in vitro.

Paul Ehrlich

Paul Ehrlich (1854–1915) was a German scientist and is also known as '**father of chemotherapy**' (Fig. 1.1D). His contributions are as follows:

- ❖ He was the first to report the **acid-fast nature** of tubercle bacillus
- ❖ He developed techniques to stain tissues and blood cells
- ❖ He proposed a toxin-antitoxin interaction called **Ehrlich phenomenon** and also introduced methods of standardising toxin and antitoxin
- ❖ He proposed the '**side chain theory** for antibody production'
- ❖ **Chemotherapy:** He discovered **salvarsan**, an arsenical compound (also called as the 'magic bullet') as

the first effective medicinal treatment for syphilis, thereby initiating and also naming the concept of chemotherapy

- ❖ The bacteria '*Ehrlichia*' was named after him
- ❖ In 1908, he received the Nobel prize in Physiology or Medicine for his contributions to immunology
- ❖ He was the founder and first director of what is known now as the Paul Ehrlich Institute, Germany.

Other Important Contributors

- ❖ **Hans Christian Gram (in 1884):** He developed a method of staining bacteria which was named as 'Gram stain' to make them more visible and differentiable under a microscope
- ❖ **Ernst Ruska:** He was the founder of electron microscope (1931)
- ❖ **Alexander Fleming (in 1929):** He discovered the most commonly used antibiotic substance of the last century, i.e. penicillin
- ❖ **Goodpasture:** He described the viral culture technique in chick embryo
- ❖ **Barbara McClintock:** She described the mobile genetic elements in bacteria called transposons
- ❖ **Walter Gilbert and Frederick Sanger** were the first to develop (1977) the method of DNA sequencing
- ❖ **Kary B Mullis:** Discovered polymerase chain reaction (PCR) and was awarded Noble prize in 1993.

Discovery of Microorganisms

Several microorganisms were discovered by the scientists (Table 1.2). The names of some of the bacteria have been coined in the honor of the scientists who discovered them (Table 1.3).

Discoverer	Organism
Ogston	<i>Staphylococcus aureus</i>
Neisser	<i>Neisseria gonorrhoeae</i>
Weichselbaum	<i>Neisseria meningitidis</i>
Loeffler	<i>Corynebacterium diphtheriae</i>
Frenkel	<i>Streptococcus pneumoniae</i>
Bruce	<i>Brucella melitensis</i>
Kitasato	<i>Clostridium tetani</i>
Hansen	<i>Mycobacterium leprae</i>
Yersin and Kitasato	<i>Yersinia pestis</i>
Schaudinn and Hoffman	<i>Treponema pallidum</i>
Daniel Carrion	<i>Bartonella bacilliformis</i>
d'Herelle	Bacteriophages
WH Welch	<i>Clostridium perfringens</i>
Anthony Epstein and Yvonne Barr	Epstein-Barr virus

Nobel Laureates

A number of scientists in medicine or physiology have been awarded Nobel Prizes for their contributions in microbiology (Table 1.4).

Common name	Scientific name
Kleb-Loeffler bacillus	<i>Corynebacterium diphtheriae</i>
Preisz Nocard bacillus	<i>Corynebacterium pseudotuberculosis</i>
Koch Week bacillus	<i>Haemophilus aegyptius</i>
Pfeiffer's bacillus	<i>Haemophilus influenzae</i>
Whitmore's bacillus	<i>Burkholderia pseudomallei</i>
Bathey bacillus	<i>Mycobacterium intracellulare</i>
Johne's bacillus	<i>Mycobacterium paratuberculosis</i>
Eaton's agent	<i>Mycoplasma pneumoniae</i>
Gaffky-Eberth bacillus	<i>Salmonella Typhi</i>

Table 1.4: Nobel laureates in medicine or physiology for their contributions in microbiology.

Nobel laureate	Year	Research done
Emil A von Behring	1901	Development of antitoxin against diphtheria
Sir Ronald Ross	1902	Life cycle of malarial parasite in mosquitoes
Robert Koch	1905	Discovery of the causative agent of tuberculosis
Charles LA Laveran	1907	Discovery of malarial parasite in unstained preparation of blood
Paul Ehrlich and Elie Metchnikoff	1908	Discovered selective theory of antibody formation
Charles Richet	1913	Discovered anaphylaxis
Jules Bordet	1919	Discovered complement and developed complement fixation test
Karl Landsteiner	1930	Described ABO blood group
Sir Alexander Fleming	1945	Discovery of penicillin
F Enders, FC Robbins, TH Weller	1954	Cultivation of polio viruses in tissue culture
J Lederberg and EL Tatum	1958	Discovery of conjugation in bacteria
Sir M Burnet and Sir PB Medawar	1960	Postulated immunological tolerance
Watson and Crick	1962	Discovered double helix structure of DNA
Peyton Rous	1966	Discovered viral oncogenesis
Holley, Khurana and Nirenberg	1968	Discovered genetic code
BS Blumberg	1976	Discovered Australia antigen (HBsAg)
Rosalyn Yallow	1977	Developed radioimmunoassay
B Benacerraf, F Dausset and G Snell	1980	Discovered HLA antigen
Barbara McClintock	1983	Discovered mobile genetic elements (transposon)
Georges Kohler	1984	Developed hybridoma technology for monoclonal antibodies
Niels Jerne		Postulated idiotype network hypothesis (Jerne hypothesis)
S Tonegawa	1987	Elucidated the nature of antibody diversity
Kary B Mullis	1993	Invented polymerase chain reaction
Stanley B Prusiner	1997	Described Prions
J Robin Warren and Barry J Marshal	2005	Discovery of <i>Helicobacter pylori</i> and its role in peptic ulcer disease
Luc Montagnier and F Barre-Sinoussi	2008	Discovery of human immunodeficiency virus (HIV)
Harald zur Hausen		Human papilloma viruses causing cervical cancer
Bruce A Beutler and Jules A Hoffmann	2011	For their discoveries concerning the activation of innate immunity
Ralph M Steinman		For his discovery of dendritic cell and its role in adaptive immunity
Sir John B Gurdon and S Yamanaka	2012	For the discovery that 'mature cells can be reprogrammed to become pluripotent'
William C Campbell	2015	For discovering anti-parasitic effect of ivermectin in filariasis
Youyou Tu	2015	For discovering artemisinin, a novel drug used for malaria

EXPECTED QUESTIONS

I. Write short notes on:

- Contributions of Louis Pasteur to Microbiology.
- Koch's postulates.

II. Multiple Choice Questions (MCQs):

1. Who has described the germ theory of life?

- Antonie van Leeuwenhoek
- Louis Pasteur
- Robert Koch
- Paul Ehrlich

2. Who has introduced the sterilization techniques?

- Louis Pasteur
- Edward Jenner
- Robert Koch
- Paul Ehrlich

3. Who is considered to be the 'father of antiseptic surgery'?

- Edward Jenner
- Robert Koch
- Alexander Fleming
- Joseph Lister

4. Who is known as 'father of chemotherapy'?

- Joseph Lister
- Robert Koch
- Alexander Fleming
- Paul Ehrlich

5. Which of the following organism follows Koch's postulates?

- Mycobacterium leprae*
- Bacillus anthracis*
- Treponema pallidum*
- Neisseria gonorrhoeae*

Answers

1. b 2. a 3. d 4. d 5. b

CHAPTER PREVIEW

- Bright-field or Light Microscope
- Phase Contrast Microscope
- Electron Microscope
- Dark Field Microscope
- Fluorescence Microscope
- Atomic Force Microscopy

INTRODUCTION

Microorganisms are extremely small. The size of the bacteria, fungi and parasites is expressed in micrometers ($1 \mu\text{m} = 10^{-3} \text{mm}$) whereas viruses are measured in nanometers ($1 \text{nm} = 10^{-3} \mu\text{m}$).

- ❖ **Bacteria:** Most of the bacteria of medical importance generally measure 0.2–1.5 μm in diameter and about 3–5 μm in length
- ❖ **Viruses:** The majority of the human pathogenic viruses range 20–300 nm in diameter
- ❖ **Parasites:** They greatly vary in their size; protozoans measure in μm and helminths range from few millimeters to several meters
- ❖ **Fungi:** Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 μm in diameter and up to several centimeters in length. Some fungi grow as yeasts; round to oval unicellular budding cells of 4–8 μm in size.

Some parasites such as adult form of helminths are visible to the naked eye; whereas rest of the microorganisms due to their small size, cannot be seen distinctly with the unaided eye, but need a microscope for their visualization. Most bacteria, parasites and fungi can be observed by light microscope, whereas viruses need an electron microscope. Therefore, it is important to understand the principle and the functioning of each microscope.

Microscopy refers to the use of a specialized instrument—called ‘microscope’ to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye). There are various types of microscopes that are used in diagnostic Microbiology.

- ❖ Bright-field or light microscope
- ❖ Dark field (or dark ground) microscope
- ❖ Phase contrast microscope
- ❖ Fluorescence microscope
- ❖ Electron microscope.

Properties of a Microscope

A good microscope should have at least three properties:

1. **Good resolution:** Resolution power refers to the ability to produce separate images of closely placed objects so that they can be distinguished as two separate entities. The resolution power of:
 - Unaided human eye is about 0.2 mm (200 μm)
 - Light microscope is about 0.2 μm
 - Electron microscope is about 0.5 nm.
 Resolution depends on **refractive index** of the medium. Oil has a higher refractive index than air; hence, use of oil enhances the resolution power of a microscope.
2. **Good contrast:** This can further be improved by staining the specimen. When the stains bind to the cells, the contrast is increased
3. **Good magnification:** This is achieved by use of lenses. There are two types of convex lenses used:
 - Ocular lens with a magnification power of 10x
 - Objective lenses—scanning (4x), low power (10x), high power (40x) and oil immersion (100x).
 The total magnification of a field is the product of the magnification of the objective lens and ocular lens:
 - Scanning field (40x)
 - Low power field (100x)
 - High power field (400x) and
 - Oil immersion field (1000x).

BRIGHT-FIELD OR LIGHT MICROSCOPE

The bright-field or light microscope forms a dark image against a brighter background, hence the name.

Structure

The parts of a bright-field microscope are divided into three groups (Fig. 2.1):

Mechanical Parts

- ❖ **Base:** It holds various parts of the microscope, such as the light source, the fine and coarse adjustment knobs



Fig. 2.1: Bright-field microscope.

Source: Nikon Alphaphot (with permission).

- ❖ **C-shaped arm:** It holds the microscope, and it connects the ocular lens to the objective lens
- ❖ **Mechanical stage:** The arm bears a stage with stage clips to hold the slides and the stage control knobs to move the slide during viewing. It has an aperture at the center that permits light to reach the object from the bottom.

Magnifying Parts

- ❖ **Ocular lens:** The arm contains an eyepiece that bears an ocular lens of 10x magnification power. Microscopes with two eye pieces are called as binocular microscopes
- ❖ **Objective lens:** The arm also contains a revolving nose piece that bears three to four objectives with lenses of differing magnifying power (4x, 10x, 40x and 100x).

Illuminating Parts

- ❖ **Condenser:** It is mounted beneath the stage which focuses a cone of light on the slide
- ❖ **Iris diaphragm:** It controls the light that passes through the condenser
- ❖ **Light source:** It may be a mirror or an electric bulb
- ❖ **Fine and coarse adjustment knobs:** They sharpen the image.

Working Principle

The rays emitted from the light source pass through the iris diaphragm and fall on the specimen. The light rays passing through the specimen are gathered by the objective and a magnified image is formed. This image is further magnified by the ocular lens to produce the final magnified virtual image (Fig. 2.2).

DARK FIELD MICROSCOPE

Principle

In dark field (or dark ground) microscope, the object appears bright against a dark background. This is made possible by use of a special dark field condenser (Fig. 2.2).

- ❖ The dark field condenser has a central opaque area that blocks light from entering the objective lens directly and has a peripheral annular hollow area which allows the light to pass through and focus on the specimen obliquely
- ❖ Only the light which is reflected by the specimen enters the objective lens whereas the unreflected light does not enter the objective. As a result, the specimen is brightly illuminated; but the background appears dark.

Applications

Dark field microscope is used to identify the living, unstained cells and thin bacteria like spirochetes which cannot be visualized by light microscopy.

PHASE CONTRAST MICROSCOPE

As per its name, in phase contrast microscope the contrast is enhanced. This microscope visualizes the unstained living cells by creating difference in contrast between the cells and water. It converts slight differences in refractive index and cell density into easily detectable variations in light intensity. Contrast can also be enhanced by staining the specimen, but as staining kills the microbes, the properties of living cells cannot be studied.

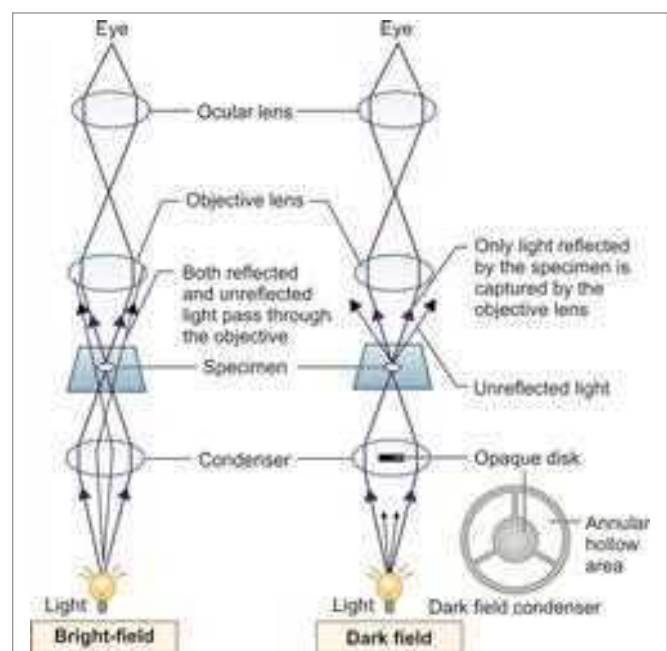


Fig. 2.2: Light pathways of bright-field and dark field microscopes.

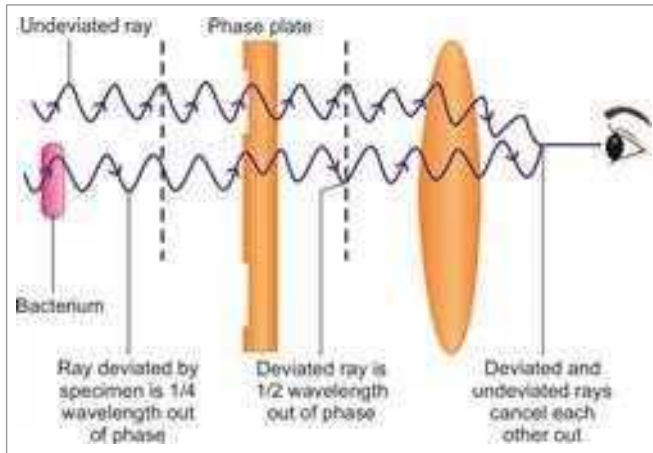


Fig. 2.3: Principle of phase contrast microscope.

Principle

The condenser is similar to that of dark field microscope, consists of an opaque central area with a thin transparent ring, which produces a hollow cone of light.

- ❖ As this cone of light passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about one-fourth of a wavelength (Fig. 2.3)
- ❖ The undeviated light rays strike a **phase ring** in the phase plate, (a special optical disk located in the objective), while the deviated rays miss the ring and pass through the rest of the plate
- ❖ The phase ring is constructed in such a way that the undeviated light passing through it is advanced by one-fourth of a wavelength, the deviated and undeviated waves will be about half wavelength out of the phase and will cancel each other when they come together to form an image (Fig. 2.3)
- ❖ The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined.

The light rays go through → condenser → specimen (e.g. bacterium) → phase ring → objective lens → ocular lens.

Applications

Phase contrast microscopy is especially useful for studying:

- ❖ Microbial motility
- ❖ Determining the shape of living cells, and
- ❖ Detecting microbial internal cellular components, such as the cell membrane, nuclei, mitochondria, spindles, chromosomes, Golgi apparatus, endospores and inclusion bodies; which become clearly visible because they have refractive indices markedly different from that of water.

FLUORESCENCE MICROSCOPE

The “fluorescence microscope” refers to any microscope that uses fluorescence property to generate an image.

Principle

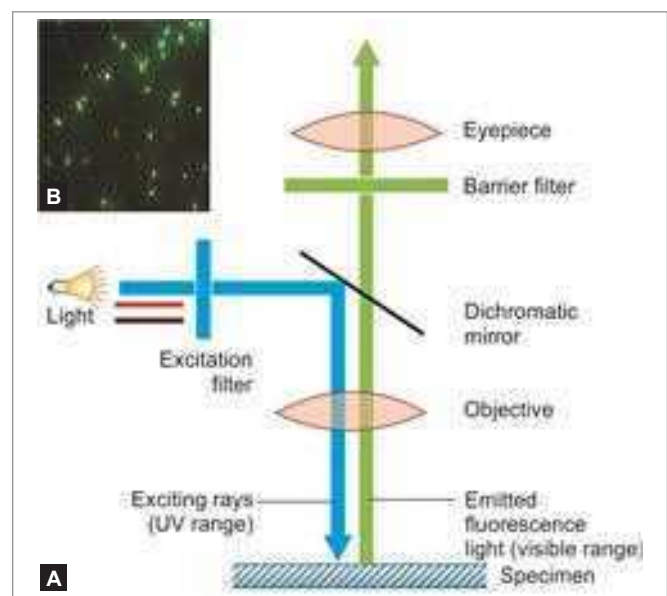
When fluorescent dyes are exposed to ultraviolet (UV) rays, they become excited and are said to fluoresce, i.e. they convert this invisible, short wavelength rays into light of longer wavelengths (i.e. visible light) (Fig. 2.4A).

- ❖ The source of light may be a mercury lamp which emits rays that pass through an excitation filter
- ❖ The excitation filter is so designed that it allows only short wavelength UV light (about 400 nm, called as the exciting wavelength of light) to pass through; blocking all other long wavelength rays
- ❖ The exciting rays then get reflected by a dichromatic mirror in such a way that they fall on the specimen which is previously stained by fluorescent dye and focused under the microscope
- ❖ The fluorescent dye absorbs the exciting rays of short wavelength, gets activated and in turn emits rays of higher wavelength
- ❖ A barrier filter positioned after the objective lenses removes any remaining ultraviolet light, which could otherwise damage the viewer’s eyes, or blue and violet light, which would reduce the image’s contrast.

Applications

Epifluorescence microscope: It is the simplest form of fluorescence microscope, which has the following applications:

- ❖ **Auto fluorescence:** Certain microbes directly fluoresce when placed under UV lamp, e.g. *Cyclospora* (a protozoan parasite)
- ❖ **Microbes coated with fluorescent dye:** Certain microbes fluoresce when they are stained non-specifically by fluorochrome dyes



Figs 2.4A and B: A. Principle of fluorescence microscope;

B. Tubercle bacilli seen under fluorescence microscope.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- Acridine orange dye is used for the detection of parasites such as *Plasmodium* and filarial nematodes by a method called as quantitative buffy coat (QBC) examination
- Auramine phenol is used for the detection of tubercle bacilli (Fig. 2.4B).
- ❖ **Immunofluorescence:** It uses fluorescent dye tagged immunoglobulins to detect cell surface antigens or antibodies bound to cell surface antigens. There are two types—direct and indirect immunofluorescence test (described in detail in Chapter 12).

Confocal microscope: It is an advanced design of fluorescence microscope. It uses point illumination and a pinhole in an optically conjugate plane to eliminate out-of-focus signal and to get a better resolution of the fluorescent image.

ELECTRON MICROSCOPE

An electron microscope (EM) uses accelerated electrons as a source of illumination. Because the wavelength of electrons can be up to 100,000 times shorter than that of visible light photons, the EM has a much better resolving power than a light microscope; hence, it can reveal the details of flagella, fimbriae and intracellular structures of a cell. It was invented by German physicist **Ernst Ruska** in 1931. Differences between light microscope and EM are listed in Table 2.1. Electron microscopes are of two types:

1. Transmission electron microscope (TEM, most common type) (Fig. 2.5)
2. Scanning electron microscope (SEM).

Transmission Electron Microscope

Specimen Preparation

The specimen to be viewed under EM should be able to maintain its structure when it is bombarded with electrons. Hence, only very thin specimens (20–100 nm thickness) are suitable for EM. However, bacterial cells are thicker than this; hence, they need to be sliced into thin layers. To prepare the thin specimen, the following steps are needed:

- ❖ **Fixation:** Cells are fixed by using glutaraldehyde or osmium tetroxide for stabilization
- ❖ **Dehydration:** Specimen is then dehydrated with organic solvents (e.g. acetone or ethanol)

Table 2.1: Differences between light microscope and electron microscope.

Features	Light microscope	Electron microscope
Highest practical magnification	About 1,000–1,500	Over 100,000
Best resolution	0.2 μm	0.5 nm
Radiation source	Visible light	Electron beam
Medium of travel	Air	High vacuum
Specimen mount	Glass slide	Metal grid (usually copper)
Type of lens	Glass	Electromagnet



Fig. 2.5: Transmission electron microscope (parts).

Source: David J Morgan/Wikipedia (with permission).

- ❖ **Embedding:** Specimen is embedded in plastic polymer and then, is hardened to form a solid block. Most plastic polymers are water insoluble; hence, complete dehydration of specimen is must before embedding
- ❖ **Slicing:** Specimen is then cut into thin slices by an ultramicrotome knife. Such thin slices of the specimen are mounted on a metal slide (copper).

Electron Pathway

Electrons are generated by electron gun, which travel in high speed. The medium of travel in EM should be a fully vacuum path because in air path, electrons can get deflected by collisions with air molecules.

- ❖ Electrons pass through a magnetic condenser and then bombard on the thin sliced specimen mounted on the copper slide
- ❖ The specimen scatters electrons passing through it, and then the electron beam is focused by magnetic lenses

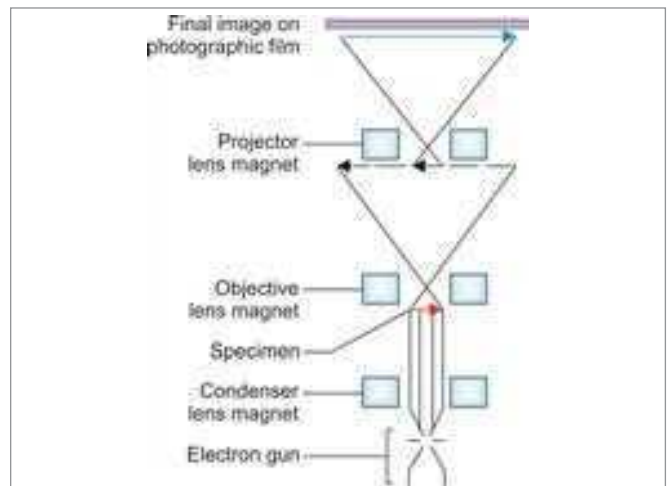


Fig. 2.6: Principle of transmission electron microscope.

(objective lens followed by projector lenses) to form an enlarged, visible image of the specimen on a fluorescent screen (Fig. 2.6)

- ❖ A denser region in the specimen scatters more electrons and therefore appears darker in the image since fewer electrons strike that area of the screen. In contrast, electron-transparent regions are brighter.

Applications and Modifications of EM

- ❖ **Virus detection:** The most important use of EM in diagnostic microbiology is detection of viruses. It can detect viruses either—(i) directly from the clinical specimens (e.g. rotavirus from stool) or (ii) from tissue cultures or (iii) after adding specific antiviral antibody to the specimen (known as immune-EM)
- ❖ The **contrast of EM** can be increased by several methods such as—(i) negative staining with heavy metals

(phosphotungstic acid or uranyl acetate), and (ii) by shadowing

- ❖ **Freeze-etching technique** is an alternative method for specimen preparation, which helps to disclose the shape of organelles within the microorganisms.

Scanning Electron Microscope

Scanning electron microscope (SEM) has been used to examine the surfaces of microorganisms in great detail. It has a resolution of 7 nm or less. The SEM differs from TEM, in producing an image from electrons emitted by an object's surface rather than from transmitted electrons.

ATOMIC FORCE MICROSCOPY

It is an advanced microscope that uses scanning probe technology to study the cellular structure. It has a resolution power in fraction of nanometer.

EXPECTED QUESTIONS

I. Write short notes on:

1. Principle and uses of dark field microscope.
2. Principle and uses of fluorescence microscope.
3. Principle and uses of electron microscope.

II. Multiple Choice Questions (MCQs):

1. Unaided human eye has a resolution power of:

- a. 0.2 mm
- b. 0.2 μ m
- c. 0.2 nm
- d. 0.5 nm

2. Phase contrast microscopy is useful for studying:

- a. Microbial motility
- b. Determining the shape of living cells
- c. Detecting bacterial components, such as endospores and inclusion bodies

d. All of the above

3. Which of the following microscopes, the object appears bright against a dark background?

- a. Simple light microscope
- b. Dark ground microscope
- c. Compound light microscope
- d. Electron microscope

4. Electron microscope differs from light microscope in all, except:

- a. Highest practical magnification
- b. Medium of travel
- c. Resolution
- d. No need for specimen preparation

Answers

1. a 2. d 3. b 4. d

Taxa	Ends with suffix	Taxonomic name
Kingdom	-om	Bacteria
Phylum		Proteobacteria
Class	-ia	Gammaproteobacteria
Subclass	-idae	
Order	-ales	Enterobacteriales
Suborder	-ineae	
Family	-aceae	Enterobacteriaceae
Subfamily	-oideae	
Tribe	-eae	Escherichieae
Subtribe	-inae	
Genus		<i>Escherichia</i>
Species		<i>E. coli</i>

- Biochemical reactions, e.g. coagulase positive *Staphylococcus* and coagulase negative *Staphylococcus*
 - Antigenic structure, e.g. antigenic structure of somatic antigen present in bacterial cell wall.
- ❖ Though, this classification is a **convenient and user friendly** method, it is not a perfect method. Because the weighted characters used may not be valid all the time for a given bacterium. For example, fermentation of lactose though is an important property to classify family Enterobacteriaceae; is not a permanent trait. In due course of the time, bacteria may lose or gain the property to ferment lactose.

Adansonian Classification

To avoid the use of weighted characteristics, Michel Adanson proposed another method (1774) that classifies organisms based on giving equal weight to every character of the organism.

- ❖ This is also called phenetic classification. It has its greatest application in numeric taxonomy

- ❖ Numerical taxonomy: The concept was first developed by Robert R Sokal and Peter HA Sneath in 1963
- ❖ With the advent of computer facilities, the principle of phenetic classification has been extended further so that very large numbers of characters of several organisms can be compared at the same time
- ❖ They have created a taxonomic system by using numeric algorithms like cluster analysis rather than using subjective evaluation of their properties which are arbitrarily given special weightage.

Molecular Classification

It is based on the degree of genetic relatedness of different organisms. Guanine + cytosine (G + C) content of bacteria is estimated after extracting DNA from pure bacterial culture. The nucleotide base composition and the base ratio vary widely among different groups of microorganisms, but for any one particular species, it is constant.

NOMENCLATURE

Nomenclature is the branch of taxonomy, that is concerned with designating scientific names to taxa, based on a particular classification scheme and in accordance with agreed international rules and conventions.

- ❖ Bacterial nomenclature also follows the same rules as proposed by Swedish botanist **Carolus Linnaeus** who invented the modern system of binomial nomenclature
- ❖ Scientific names for taxonomic levels above genus are always capitalized but not italicized; for example, Phylum Proteobacteria
- ❖ In binomial nomenclature system, the scientific name of bacteria comprises of a genus name (starts with a capital letter) and species name. Both genus and species should be written in *italic* or are underlined; e.g. *Staphylococcus aureus* or Staphylococcus aureus

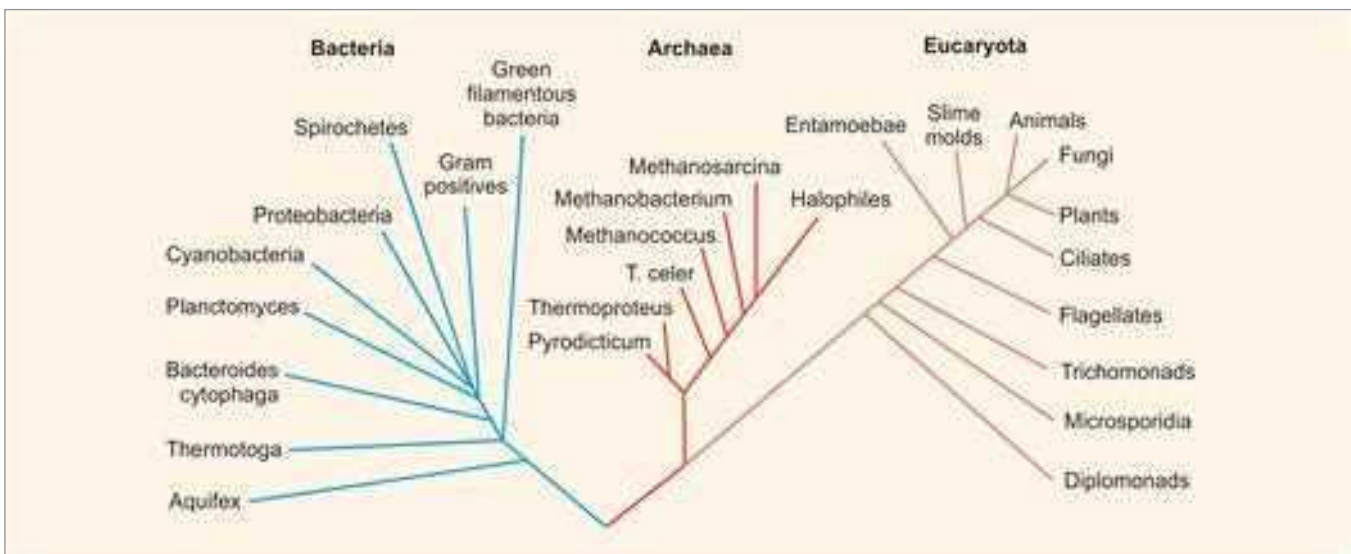


Fig. 3.1.1: Phylogenetic tree of classifying living structures based on weighted characters.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

- ❖ The genus (plural: genera) is usually a Latin noun whereas the species refers to a defined taxon of organisms within a particular genus
- ❖ The genus and species are coined based on some property of the bacteria, e.g.
 - *Staphylococcus aureus* is named after their arrangement in cluster (*Staphyle* means bunch of grapes) and type of pigmentation they produce (*aureus* meaning golden yellow)
 - *Neisseria meningitidis* is named after—the discoverer (U Neisser) and the disease it causes (meningitis)
 - *Brucella suis* and *Brucella melitensis* (named after the discoverer (*Brucella* from David Bruce) and the animal host (*suis* meaning pig) and the place of discovery (*melitensis* from Malta, Europe).
- ❖ The recent changes in the taxonomic name of medically important microorganisms have been depicted in Table 3.1.3
- ❖ **Typing:** The species can also be classified further by various typing methods as described in Chapter 3.3.

Type Cultures

There are many international reference laboratories which are designated as type culture reference centers.

- ❖ They maintain the representative cultures of the established species, which show all the standard characteristics of the original strain
- ❖ The strains isolated in the laboratories are compared using the standard strains supplied by these type culture centers
- ❖ The original cultures of any new species described are deposited in type collection centers

Table 3.1.3: Taxonomic name changes of medically-important microorganisms.

Old name	New name
Bacteria	
<i>Clostridium difficile</i>	<i>Clostridioides difficile</i>
<i>Haemophilus aphrophilus</i>	<i>Aggregatibacter aphrophilus</i>
<i>Haemophilus paraphrophilus</i>	<i>Aggregatibacter paraphrophilus</i>
<i>Enterobacter aerogenes</i>	<i>Klebsiella aerogenes</i>
<i>Propionibacterium acnes</i>	<i>Cutibacterium acnes</i>
Fungi	
<i>Absidia corymbifera</i>	<i>Lichtheimia corymbifera</i>
<i>Madurella grisea</i>	<i>Trematosphaeria grisea</i>
<i>Penicillium marneffeii</i>	<i>Talaromyces marneffeii</i>
<i>Geotrichum candidum</i>	<i>Dipodascus geotrichum</i>
<i>Rhizopus oryzae</i>	<i>Rhizopus arrhizus</i>
Parasites	
<i>Isospora belli</i>	<i>Cystoisospora belli</i>
<i>Balantidium coli</i>	<i>Neobalantidium coli</i>

- ❖ The two most important type collection centers of the world are:
 - **ATCC** (American Type Culture Collection), USA
 - **NCTC** (National Collection of Type Cultures), UK.

BACTERIAL INFECTIONS MEDICALLY IMPORTANT TO MAN

Based on Gram stain, the bacterial infections can be grouped into infections caused by gram-positive cocci, gram-negative cocci, and gram-positive bacilli, gram-negative bacilli and miscellaneous bacteria (that do not take up/poorly take up Gram stain) (Table 3.1.4).

The overview of bacterial infections is given in Chapter 3.7 and individual bacterial infections are discussed in detail under various system-based microbiology sections.

Table 3.1.4: Medically important bacterial pathogens to man.

Gram-positive cocci
<ul style="list-style-type: none"> • <i>Staphylococcus</i> species—<i>S. aureus</i>, coagulase negative staphylococci (CoNS) • <i>Streptococcus</i> species—<i>S. pyogenes</i>, <i>S. agalactiae</i>, <i>Enterococcus</i>, <i>Viridans streptococci</i>, <i>S. pneumoniae</i>
Gram-negative cocci
<ul style="list-style-type: none"> • <i>Neisseria</i>—<i>N. meningitidis</i>, <i>N. gonorrhoeae</i>
Gram-positive bacilli
<ul style="list-style-type: none"> • <i>Corynebacterium</i> species—<i>C. diphtheriae</i> • <i>Bacillus</i> species—<i>B. anthracis</i> • <i>Mycobacterium</i> species—<i>M. tuberculosis</i>, Nontuberculous mycobacteria (NTM), <i>M. leprae</i> • Miscellaneous gram-positive bacilli—<i>Listeria</i>, <i>Actinomyces</i> and <i>Nocardia</i>
Gram-negative bacilli
<ul style="list-style-type: none"> • Enterobacteriaceae—<i>Escherichia coli</i>, <i>Klebsiella</i>, <i>Enterobacter</i>, <i>Citrobacter</i>, <i>Serratia</i>, <i>Proteus</i>, <i>Providencia</i>, <i>Morganella</i>, <i>Shigella</i>, <i>Salmonella</i>, <i>Yersinia</i> • Non-fermenting gram-negative bacilli—<i>Pseudomonas</i>, <i>Acinetobacter</i>, <i>Burkholderia</i>, <i>Stenotrophomonas</i> • <i>Vibrio</i> and related genera—<i>Vibrio</i>, <i>Aeromonas</i> • Fastidious gram-negative bacilli—<i>Haemophilus</i>, <i>Bordetella</i>, <i>Brucella</i> • Miscellaneous gram-negative bacilli—<i>Campylobacter</i>, <i>Helicobacter</i>, <i>Legionella</i>, <i>Klebsiella granulomatis</i>, <i>Gardnerella vaginalis</i>
Anaerobic bacterial infections
<ul style="list-style-type: none"> • Sporing anaerobes—<i>Clostridium</i> species such as <i>C. perfringens</i>, <i>C. tetani</i>, <i>C. botulinum</i>, <i>Clostridioides difficile</i> • Non-sporing anaerobes—<i>Peptostreptococcus</i>, <i>Bacteroides</i>, <i>Prevotella</i>, <i>Porphyromonas</i>
Miscellaneous bacteria
<ul style="list-style-type: none"> • Spirochetes—<i>Treponema</i>, <i>Borrelia</i>, <i>Leptospira</i> • Rickettsiae and related genera—<i>Rickettsia</i>, <i>Orientia</i>, <i>Bartonella</i>, <i>Ehrlichia</i> • Chlamydiae—<i>C. trachomatis</i>, <i>C. psittaci</i>, <i>C. pneumoniae</i> • <i>Mycoplasma</i>—<i>M. pneumoniae</i>

General Bacteriology: Morphology and Physiology of Bacteria

CHAPTER 3.2

CHAPTER PREVIEW

- Morphology of Bacteria
 - Shape of Bacteria
 - Bacterial Cell Wall
 - Cell Membrane
- Cytoplasmic Matrix
- Cell Wall Appendages
- Bacterial Spores
- Physiology of Bacteria
- Bacterial Growth and Nutrition
- Factors Affecting Growth of Bacteria
- Bacterial Metabolism

MORPHOLOGY OF BACTERIA

SHAPE OF BACTERIA

Depending on their shape, bacteria are classified into:

- ❖ Cocci (singular coccus, from; kokkos, meaning berry)- are oval or spherical cells and
- ❖ Bacilli or rods (singular bacillus, meaning rod shaped). Cocci are arranged in groups (clusters), pair or chains. Similarly, bacilli can be arranged in chain, pair, and some bacilli are curved, comma shaped, or cuneiform shaped (Table 3.2.1 and Fig. 3.2.1).

Both cocci and bacilli are further classified based on Gram staining property into (Table 3.2.1 and Fig. 3.2.1):

- ❖ Gram-positive cocci
- ❖ Gram-negative cocci
- ❖ Gram-positive bacilli
- ❖ Gram-negative bacilli.

However, there are some bacteria that are weakly Gram stained and hence need special stains for their demonstration, such as:

- ❖ Spirochetes (*Treponema* and *Leptospira*)—thin spirally coiled bacilli
- ❖ *Mycoplasma* (cell wall deficient free living bacteria)
- ❖ Rickettsiae and chlamydiae are obligate intracellular bacteria.

Bacterial cell anatomy comprises of the following structures (Fig. 3.2.2):

- ❖ The outer layer or the **envelope** of a bacterial cell consists of—(1) a rigid cell wall and (2) underlying plasma membrane
- ❖ The cytoplasm contains cytoplasmic inclusions (mesosomes, ribosomes, inclusion granules, vacuoles) and a diffuse nucleoid containing single circular chromosome
- ❖ Some bacteria may possess additional cell wall appendages such as capsule, flagella and fimbriae.

Table 3.2.1: Classification of bacteria depending on their morphology and Gram staining property.

Bacteria	Example
Gram-positive cocci arranged in	
Cluster	<i>Staphylococcus</i>
Chain	<i>Streptococcus</i>
Pairs, lanceolate shaped	<i>Pneumococcus</i>
Pair or in short chain, spectacle shaped	<i>Enterococcus</i>
Tetrads	<i>Micrococcus</i>
Octate	<i>Sarcina</i>
Gram-negative cocci arranged in	
Pairs, lens shaped	<i>Meningococcus</i>
Pairs, kidney shaped	<i>Gonococcus</i>
Gram-positive bacilli arranged in	
Chain (bamboo stick appearance)	<i>Bacillus anthracis</i>
Chinese letter or cuneiform pattern	<i>Corynebacterium diphtheriae</i>
Palisade pattern	Diphtheroids
Branched and filamentous form	<i>Actinomyces</i> and <i>Nocardia</i>
Gram-negative bacilli arranged in	
Pleomorphic (various shapes)	<i>Haemophilus, Proteus</i>
Thumb print appearance	<i>Bordetella pertussis</i>
Comma shaped (fish in stream appearance)	<i>Vibrio cholerae</i>
Curved	<i>Campylobacter</i> and <i>Helicobacter</i>
Others	
Chain	<i>Streptobacillus</i>
Spirally coiled, flexible	Spirochetes
Rigid spiral forms	<i>Spirillum</i>
Bacteria that lack cell wall	<i>Mycoplasma</i>

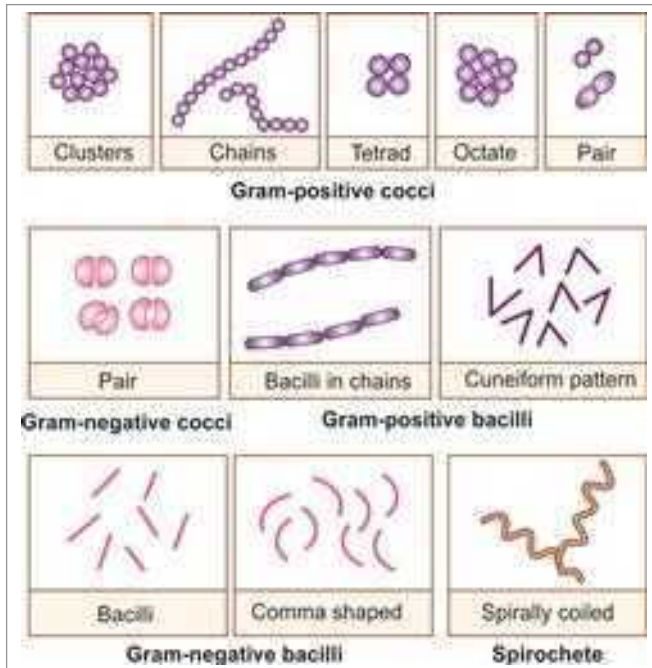


Fig. 3.2.1: Different morphology of bacteria and Gram staining property.

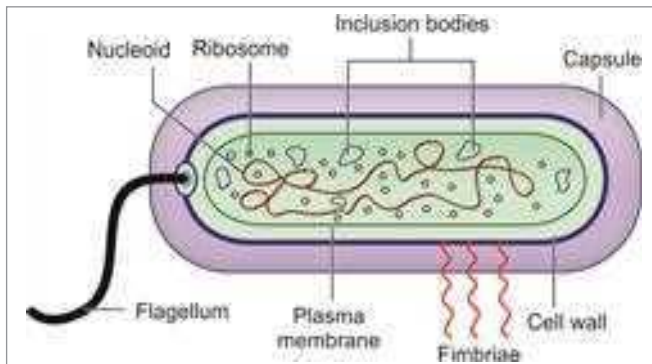


Fig. 3.2.2: Structure of bacterial cell.

BACTERIAL CELL WALL

The cell wall is a tough and rigid structure, surrounding the bacterium. It is 10–25 nm in thickness and weighs about 20–25% of the dry weight of the cell.

The cell wall has following functions:

- ❖ It provides protection to the cell against osmotic lysis
- ❖ It confers rigidity upon bacteria due to presence of peptidoglycan layer in the cell wall
- ❖ The cell wall can protect a cell from toxic substances and is the site of action of several antibiotics
- ❖ Virulence factors: Bacterial cell wall contains certain virulence factors (e.g. endotoxin), which contribute to their pathogenicity
- ❖ Immunity: Antibody raised against specific cell wall antigens (e.g. antibody to LPS) may provide immunity against some bacterial infection.

Gram-positive Cell Wall

Cell wall of gram-positive bacteria is simpler than that of gram-negative bacteria (Table 3.2.2).

Peptidoglycan

In gram-positive bacteria, the peptidoglycan layer is much thicker (50–100 layers thick, 16–80 nm) than gram-negative cell wall (Fig. 3.2.3).

- ❖ Each layer is a mucopeptide (murein) chain, composed of alternate units of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) molecules; cross linked to each other via tetrapeptide side chains and pentaglycine bridges
- ❖ A tetrapeptide side chain ascended from NAM molecule is composed of L-alanine—D-glutamine—L-lysine—D-alanine
- ❖ The L-lysine of one tetrapeptide chain is covalently linked to the terminal D-alanine of the adjacent chain via a pentaglycine bridge (Fig. 3.2.4).

Table 3.2.2: Differences between gram-positive and gram-negative cell wall.

Characters	Gram-positive cell wall	Gram-negative cell wall
Peptidoglycan layer	Thicker (16–80 nm)	Thinner (2 nm)
At third position of tetrapeptide side chain	L-Lysine present	Meso-diaminopimelic acid present
Pentaglycine bridge	Present	Absent
Lipid content	Nil or scanty (2–5%)	Present (15–20%)
Lipopolysaccharide	Absent	Present (endotoxin)
Teichoic acid	Present	Absent
Variety of amino acids	Few	Several
Aromatic amino acids	Absent	Present

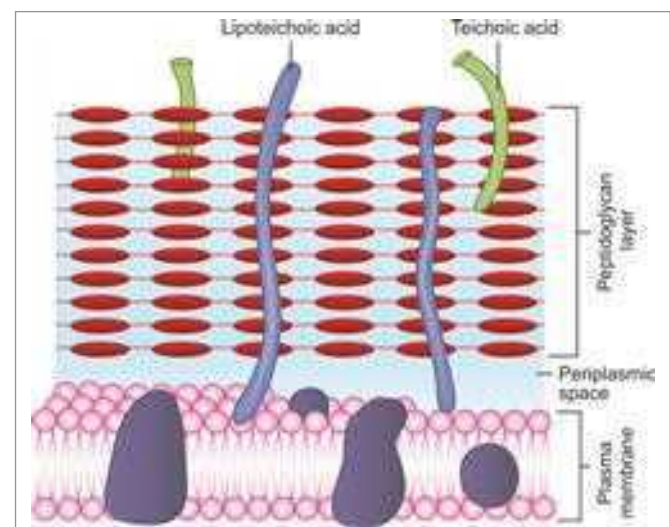


Fig. 3.2.3: Structure of gram-positive cell wall.

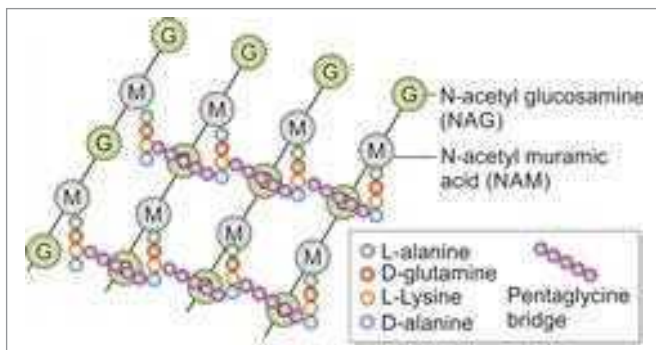


Fig. 3.2.4: Peptidoglycan layer of gram-positive cell wall.

Teichoic Acid

Gram-positive cell wall contains significant amount of teichoic acid which is absent in gram-negative bacteria. They are polymers of glycerol or ribitol joined by phosphate groups. These molecules may maintain the structure of cell wall. They are of two types: (i) Cell wall teichoic acid and (ii) Lipoteichoic acid.

Gram-negative Cell Wall

Gram-negative cell wall is thinner and more complex than the gram-positive cell wall, comprises of the following components (Fig. 3.2.5 and Table 3.2.2).

Peptidoglycan Layer

It is very thin (1-2 layer, 2 nm thick), composed of a mucopeptide chain similar to that of gram-positive cell wall, and consists of alternate NAM and NAG molecules. However, it differs from the latter by (Fig. 3.2.6):

- ❖ Meso-diaminopimelic acid is present at third position of the tetrapeptide side chain ascended from NAM molecule
- ❖ The pentaglycine bridge is absent
- ❖ The tetrapeptide side chains are directly linked to each other by the covalent linkage between D-alanine of one

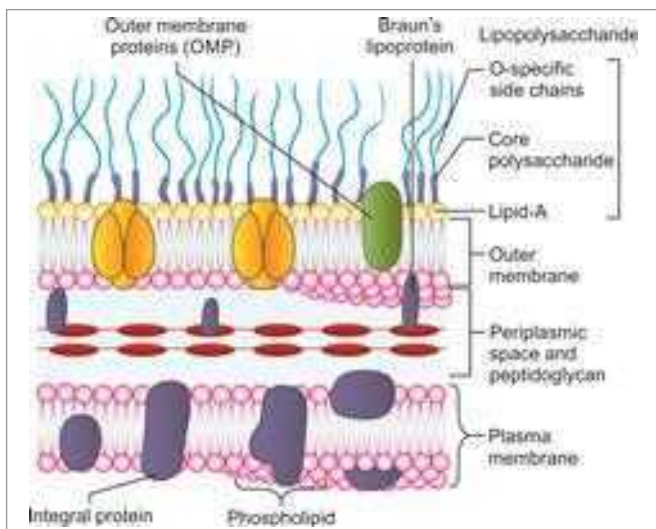


Fig. 3.2.5: Gram-negative cell wall.

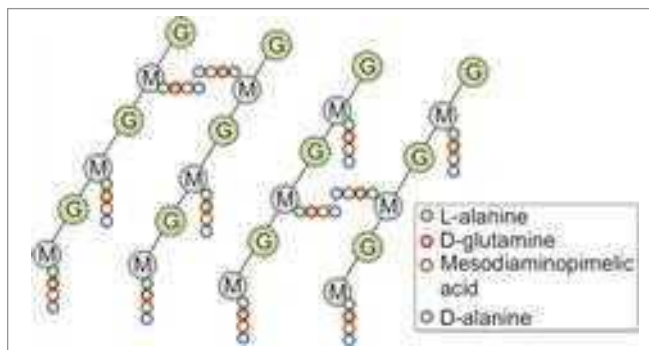


Fig. 3.2.6: Peptidoglycan layer of gram-negative cell wall.

chain with meso-diaminopimelic acid of the adjacent chain.

Outer Membrane

This is a phospholipid layer which lies outside the thin peptidoglycan layer; firmly attached to the latter by a membrane protein called Braun's lipoprotein.

- ❖ It serves as a protective barrier to the cell
- ❖ Outer membrane proteins (OMP) or porin proteins: They are the specialized proteins present in outer membrane; help in transport of smaller molecules.

Lipopolysaccharide (LPS)

This layer is unique to gram-negative bacteria, which is absent in gram-positives. It consists of three parts:

- ❖ **Lipid A or the endotoxin:** It has endotoxic activities, such as pyrogenicity, lethal effect, tissue necrosis, anti-complementary activity, B cell mitogenicity, adjuvant property and antitumor activity
- ❖ **Core polysaccharide:** It is projected from lipid A region. It is composed of 10-12 sugar moieties
- ❖ **O side chain (or O antigen or somatic antigen):** It is a polysaccharide chain extending outwards from the core polysaccharide region.
 - It greatly varies in composition between bacterial strains. Bacterial strains vary in their composition of O antigen; a property which is used for serotyping for bacteria
 - O antigen is also a major surface antigen, induces antibody formation.

Periplasmic Space

It is the space between the inner cell membrane and outer membrane. It encompasses the peptidoglycan layer.

CELL MEMBRANE

The plasma membrane is essential for the survival of the bacteria.

- ❖ **Fluid mosaic model** is the most widely accepted current model to describe the membrane structure
- ❖ It is 5-10 nm thick, composed of bilayered phospholipid in which several proteins are embedded, such as integral proteins and peripheral proteins (Fig. 3.2.7)

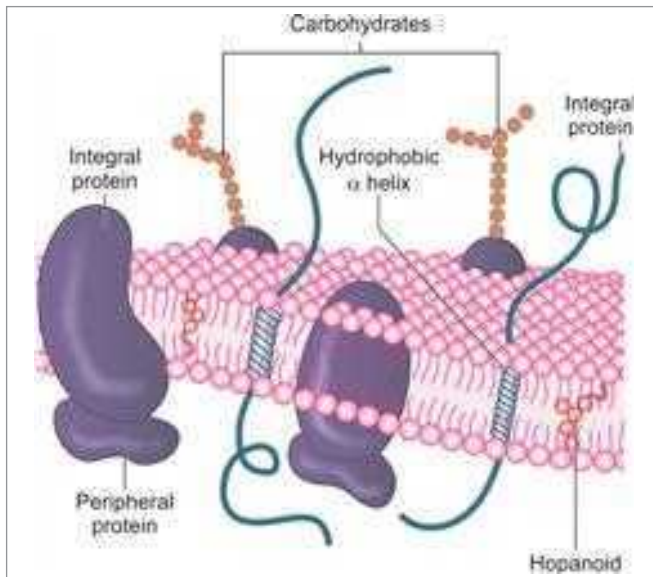


Fig. 3.2.7: Structure of bacterial cell membrane.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

- ❖ It differs from eukaryotic membranes in **lacking sterols**, such as cholesterol (except in *Mycoplasma*). However, many bacterial membranes do contain pentacyclic sterol-like molecules called **hopanoids**
- ❖ **Carbohydrate:** Some carbohydrates are often attached to the outer surface of plasma membrane proteins.

Functions

- ❖ It is a **semipermeable membrane** acting as an osmotic barrier; selectively allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others
- ❖ **Transport system:** Proteins and enzymes present in cell membrane are involved in nutrient uptake, and waste excretion
- ❖ **Site for metabolic processes:** Bacterial cell membrane is the site for a variety of crucial metabolic processes such as: Respiration, synthesis of lipids and cell wall, and probably chromosome segregation
- ❖ Special receptor molecules located in the membrane help the bacteria to detect and respond to chemicals in their surroundings.

CYTOPLASMIC MATRIX

Bacterial cytoplasm, unlike that of eukaryotes, lacks membrane-bound organelles. The cytoplasmic matrix is mainly composed of water (about 70% of bacterial mass is water) and is packed with ribosomes, storage granules called **inclusions** and cell membrane invaginations called **mesosomes**. They lack true cytoskeleton, but do have a cytoskeleton-like system of proteins. The plasma membrane and everything within it is called as **protoplast**.

Ribosomes

Ribosomes are the sites for protein synthesis. These are composed of rRNA and ribosomal proteins.

- ❖ Ribosomes are integrated with the mRNA to form the polysomes
- ❖ At this site, the genetic codons of the mRNA are translated into peptide sequences
- ❖ They are 10–20 nm size, with a sedimentation constant of 70 S (S for Svedberg units)
- ❖ Each 70 S unit consists of a 30 S and a 50 S subunits.

Intracytoplasmic Inclusions

They are the storage sites of nutrients/energy present in some bacteria. They are formed by the bacteria under nutritional deficiency conditions and disappear when the deficient nutrients are supplied. There are two types of inclusions:

1. **Organic inclusion bodies:** Examples include glycogen granules and polyhydroxyl butyrate granules
2. **Inorganic inclusion bodies:** Examples include polymetaphosphate or volutin or metachromatic granules: They are found in certain bacteria, such as *Corynebacterium diphtheriae*.

Mesosomes

Mesosomes are invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae. They are generally more prominent in gram-positive bacteria. They are involved in:

- ❖ **Bacterial respiration:** They possess respiratory enzymes and are analogous to mitochondria of eukaryotes
- ❖ They may be involved in **cell wall formation** and **chromosome replication** during cell division.

Nucleoid

Bacteria do not have a true nucleus, but the genetic material is located in an irregularly shaped region called the nucleoid. There is no nuclear membrane or nucleolus.

- ❖ Bacteria possess a single haploid chromosome, comprising of super coiled circular double stranded DNA of 1 mm length. The bacterial DNA lacks basic proteins
- ❖ However, some bacteria have a linear chromosome and some have two chromosomes (e.g. *Vibrio cholerae*)
- ❖ Bacterial DNA divides by simple binary fission (described later in this chapter)
- ❖ The nucleoid can be seen by electron microscopy or on staining with stain such as the Feulgen stain
- ❖ Bacteria also possess extrachromosomal DNA called **plasmids** (described in detail in Chapter 3.4).

CELL WALL APPENDAGES

Capsule and Slime Layer

Some bacteria possess a layer of amorphous viscid material lying outside the cell wall called **glycocalyx**.

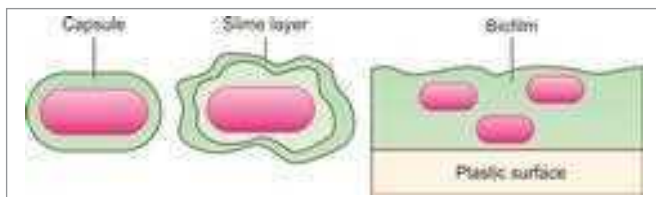


Fig. 3.2.8: Capsule, slime layer and biofilm.

Capsulated bacteria	Composition
Pneumococcus	Polysaccharide
Meningococcus	Polysaccharide
<i>Haemophilus influenzae</i>	Polysaccharide
<i>Klebsiella pneumoniae</i>	Polysaccharide
<i>Pseudomonas aeruginosa</i>	Polysaccharide
<i>Bacteroides fragilis</i>	Polysaccharide
<i>Bacillus anthracis</i>	Polypeptide (glutamate)
<i>Streptococcus pyogenes</i> (some strains)	Hyaluronic acid

- ❖ When the glycocalyx layer is well organized and not easily washed off, it is called **capsule** (Fig. 3.2.8)
- ❖ When the glycocalyx layer is in the form of diffuse, unorganized loose material that can be removed easily, it is called **slime layer** (Fig. 3.2.8). (Some bacteria may possess both capsule and slime layer, as in *Streptococcus salivarius*).

Examples

Most of the bacterial capsules are polysaccharide in nature (Table 3.2.3), except in *Bacillus anthracis* where it is polypeptide in nature.

Function/Uses

The capsule has various functions as follows:

- ❖ **Contributes to bacterial virulence:**
 - Capsule protects the bacterium from phagocytosis
 - It can also prevent complement-mediated bacterial cell lysis
 - Prevent cell from drying out (desiccation)
 - It protects the bacterium from the action of lysozyme and bacteriophages
 - Capsule of certain bacteria (e.g. *Bacteroides fragilis*) may be toxic to the host cells and induces abscess formation
 - Biofilm formation and adhesion (see below):

Biofilm Formation

A biofilm is a living ecosystem made of millions of adherent bacterial cells embedded within a self-produced matrix of extracellular polymeric substance (i.e. the polysaccharide slime layer).

- ❑ Persistent biofilms containing pathogenic bacteria are capable of adherence to damaged tissues and plastic surfaces (e.g. medical devices, such as catheters and pacemakers)

Contd...

- ❑ This is the first step in bacterial colonization and sometimes it leads to disease, e.g. prosthetic valve endocarditis and catheter associated urinary tract infection (Fig. 3.2.8).

- ❖ **Capsules as vaccine:** Capsules of few bacteria are antigenic and anticapsular antibodies are protective in nature. Hence, capsular antigens are used as potential vaccine candidates. Capsular vaccines are available for bacteria, such as pneumococcus, meningococcus and *Haemophilus influenzae* serotype-b.

Demonstration of Capsule

Capsule can be detected by various methods as follows:

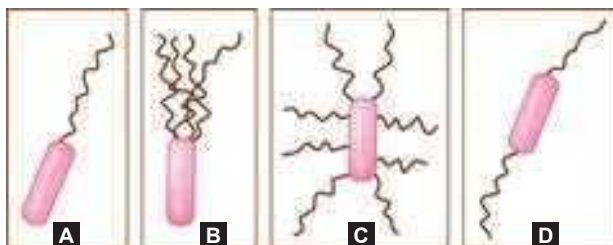
- ❖ **Negative staining** by India ink and nigrosin stain: Capsule appears as a clear refractile halo around the bacteria; where as both the bacteria and the background appear black (Fig. 75.14A)
- ❖ **M'Faydean capsule stain:** It is used for demonstration of capsule of *Bacillus anthracis* by using polychrome methylene blue stain (Fig. 55.3A)
- ❖ **Serological test:** Capsular material is antigenic and can be demonstrated by mixing it with a specific anti-capsular serum
 - Quellung reaction: Capsular serotypes of *Streptococcus pneumoniae* can be detected by adding antisera mixed with methylene blue. Capsule becomes swollen, refractile and delineated
 - Capsular antigen: It can be detected in the sample (e.g. CSF) by latex agglutination test by using specific anticapsular antibodies coated on latex particles. This is available for pneumococcus, *Cryptococcus*, *Haemophilus influenzae* and meningococcus.

Flagella

Flagella are thread-like appendages, protruding from the cell wall, that confer motility to the bacteria (organs of locomotion). They measure 5–20 μm in length and 0.01–0.02 μm in thickness.

Arrangement of Flagella

There are various patterns of arrangement of flagella with respect to the bacterial surface (Figs 3.2.9A to D):



Figs 3.2.9A to D: Types of bacterial flagellar arrangement: A. Monotrichous; B. Lophotrichous; C. Peritrichous; D. Amphitrichous.

Contd...

- ❖ Monotrichous (single polar flagellum), e.g. *Vibrio cholerae*, *Pseudomonas* and *Campylobacter*
- ❖ Lophotrichous (multiple polar flagella), e.g. *Spirillum*
- ❖ Peritrichous (flagella distributed over the entire cell surface), e.g. *Salmonella Typhi*, *Escherichia coli*
- ❖ Amphitrichous (single flagellum at both the ends), e.g. *Alcaligenes faecalis*.

Ultrastructure of Flagella

Electron microscope reveals that the bacterial flagellum is composed of three parts (Fig. 3.2.10).

1. **Filament:** It is the longest portion of the flagellum that extends from the cell surface to the tip. It is a hollow, rigid cylinder, made up of a single protein **flagellin**
2. The **basal body:** This is the portion of flagellum which is embedded in the cell. It is the most complex part of a flagellum, made up of 2–4 rings connected to a central rod
3. **Hook:** It is a short, curved flexible segment that links the filament to its basal body.

Detection of Flagella

Flagella can be demonstrated by:

- ❖ Direct demonstration of flagella
 - Tannic acid staining (Leifson's method)
 - Electron microscopy.
- ❖ Indirect means by demonstrating the motility:
 - Craigie tube method
 - Hanging drop method
 - Semisolid medium, e.g. mannitol motility medium
 - Dark ground or phase contrast microscopy.

Bacterial Motility

Bacteria can produce characteristic type of motility which helps in their identification (Table 3.2.4).

Fimbriae or Pili

Fimbriae or pili are short, fine, hair-like appendages that help in bacterial adhesion; hence called as the organ of

Table 3.2.4: Types of motility shown by different bacteria.

Types of motility	Bacteria
Tumbling motility	<i>Listeria</i>
Gliding motility	<i>Mycoplasma</i>
Stately motility	<i>Clostridium</i>
Darting motility	<i>Vibrio cholerae</i> , <i>Campylobacter</i>
Swarming on agar plate	<i>Proteus</i> , <i>Clostridium tetani</i>
Corkscrew, lashing, flexion extension motility	Spirochete

adhesion. A special type of pilus (called sex pilus) also exists which helps in conjugation.

- ❖ Pili are made up of protein called pilin
- ❖ They are antigenic; however, the antibodies against pilin antigens are not protective
- ❖ They are not related to motility and can be found both in motile as well as in nonmotile organisms.

Type of Pili

According to the functions, pili are of two types.

1. **Common pili or fimbriae:** They help in bacterial adhesion to epithelial surface helping in colonization
 - They are very small, measuring 0.5 μm long and 10 nm in thickness
 - A single bacterium can have as many as 1,000 fimbriae (Fig. 3.2.11A)
 - They are present in gram-negative and some gram-positive bacteria.
2. **Sex pili:** They help in bacterial conjugation by forming conjugation tube through which the bacterial gene transfer takes place
 - They are long thick tubular structures
 - The number of sex pili are less; 1–10 per bacterial cell
 - They are only found in gram-negative bacteria.

Detection of Fimbriae

Fimbriae can be detected either directly by electron microscope, or indirectly through formation of **surface pellicle**.

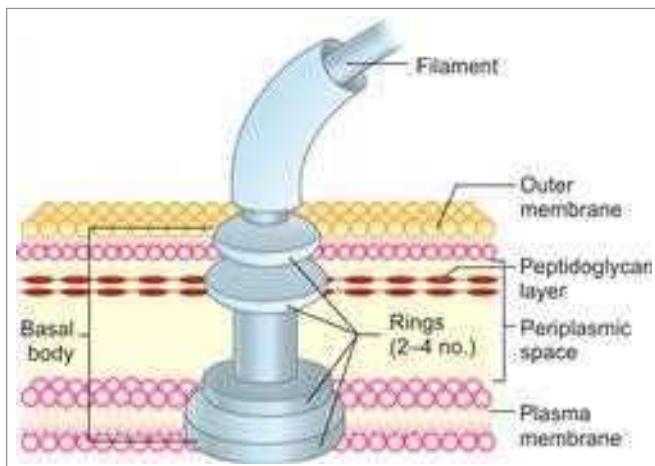
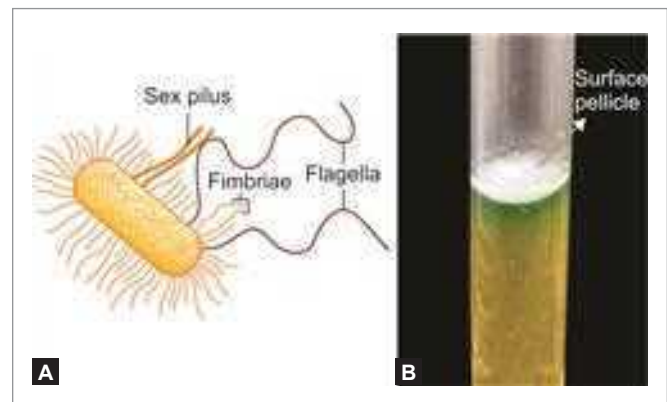


Fig. 3.2.10: Structure of flagella (gram-negative bacterium).



Figs 3.2.11A and B: A. Differentiation between fimbriae, sex pilus and flagella; B. Surface pellicle (arrow showing).

Source: Department of Microbiology, JIPMER (with permission).

It is a thin layer formed at the surface of a liquid culture of strongly aerobic bacteria such as *Pseudomonas* that adhere to each other by their fimbriae (Fig. 3.2.11B).

Atypical Forms of Bacteria

- ❖ **Involution forms:** They are swollen and aberrant forms of bacteria (e.g. gonococci and *Yersinia pestis*) formed in ageing cultures in high salt concentration
- ❖ **Pleomorphic bacteria:** Some bacteria exhibit great variation in the shape and size of individual cells, e.g. *Proteus* and *Haemophilus*. This is known pleomorphism
- ❖ **Cell wall deficient forms:** See below.

Pleomorphism and involution forms are often caused by defective cell wall synthesis. Involution forms may also be formed due to the activity of autolytic enzymes.

L Form (Cell Wall Deficient Forms)

L forms are the cell wall deficient bacteria, discovered by E Klieneberger, while studying *Streptobacillus moniliformis*.

- ❑ She named it as L form after its place of discovery, i.e. Lister Institute, London (1935)
- ❑ When bacteria lose cell wall, they become spherical irrespective of original shape. This may occur spontaneously or after exposure to penicillin or lysozyme
- ❑ L forms play a role in the persistence of pyelonephritis and other chronic infections.

Types of L forms

Two types of L forms are distinguished:

- ❑ **Unstable L forms:** Bacteria lose their cell wall in presence of penicillin, a mechanism of resistance shown by the bacteria against penicillin. Such L forms are maintained only in presence of penicillin and they can revert to the original morphology once penicillin is removed.
 - Protoplasts: They are gram-positive bacteria whose cell wall is entirely removed
 - Spheroplasts: They are derived from gram-negative bacteria whose cell wall is partially removed.
- ❑ **Stable L forms:** As mycoplasmas lack cell wall permanently, it has been suggested that mycoplasmas may represent stable L-forms of bacteria; but genetic, antigenic and biochemical properties do not support this hypothesis.

BACTERIAL SPORES

Spores are highly resistant resting (or dormant) stage of the bacteria formed in unfavorable environmental conditions as a result of the depletion of exogenous nutrients.

Structure

Bacterial spore comprises of several layers. From innermost towards the outermost, the layers are: core → cortex → coat → exosporium (Fig. 3.2.12).

- ❖ The **core** is the inner most part containing the DNA material and is walled off from the cortex by an inner membrane and the germ cell wall
- ❖ Cortex and the coat layers lie external to the core, and are separated from each other by an outer membrane
- ❖ The outermost layer is called as the exosporium.

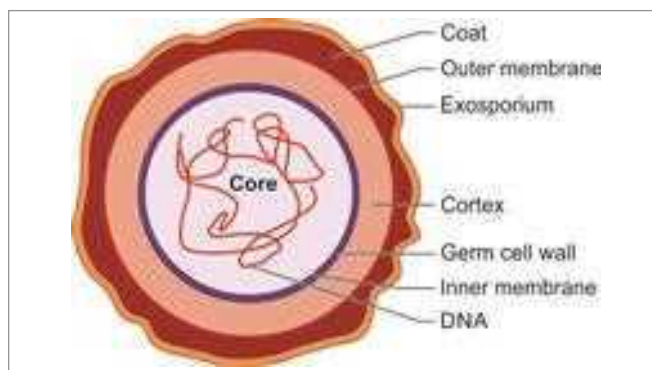


Fig. 3.2.12: Structure of bacterial spore.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

Sporulation

Sporulation (or sporogenesis) refers to the process of formation of spores from the vegetative stage of bacteria. It is not a method of reproduction because the bacteria do not divide during sporulation. Sporulation commences when growth ceases due to lack of nutrients. It is a complex process; takes about 10 hours. The mature spore formed is extremely resistant to heat and disinfectant; which is due to the deposition of calcium and dipicolinic acid into the spore cortex.

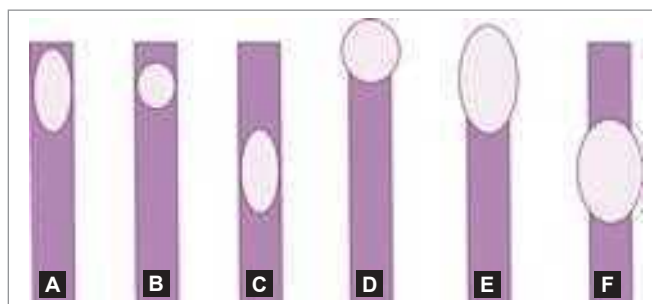
Germination

It is the transformation of dormant spores into active vegetative cells when grown in a nutrient-rich medium.

Shape and Position of Spores

For a given species, the precise position, shape and relative size of the spore are constant.

- ❖ **Position:** Spores may be central, subterminal or terminal (Figs 3.2.13A to F)
- ❖ **Shape:** They may be oval or spherical in shape
- ❖ **Width:** The diameter of spore may be same or less than the width of bacteria (non-bulging spore—e.g. as in *Bacillus*), or may be wider than the bacillary body producing a distension or bulge in the cell (bulging spore, e.g. as in *Clostridium*).



Figs 3.2.13A to F: Position and shape of spores: A. Non-bulging, oval and terminal; B. Non-bulging, round, and subterminal; C. Non-bulging, oval and central; D. Bulging, round and terminal; E. Bulging, oval and terminal; F. Bulging, oval, and central.

Sporicidal Agents

Spores are resistant to most of the routinely used disinfectants. Only limited agents called as sterilants are capable of killing the spores, e.g. autoclave, or ethylene oxide sterilizer, etc. (refer Chapter 23).

Demonstration of Spores

- ❖ **Gram staining:** Spores appear as unstained refractile bodies within the cells
- ❖ **Modified Ziehl-Neelsen staining:** Spores are weakly acid-fast and appear red color when ZN staining is performed using 0.25–0.5% sulfuric acid as decolorizer
- ❖ **Special techniques** for endospore staining include the Schaeffer–Fulton stain and the Moeller stain.

Applications

- ❖ Spores of certain bacteria are employed as indicators of proper sterilization. Absence of the spores (inability to grow) after autoclaving or processing in hot air oven indicates proper sterilization
 - Spores of *Geobacillus stearothermophilus* are used as sterilization control for autoclave and plasma sterilizer
 - Spores of *Bacillus atrophaeus* are used as sterilization control for hot air oven and ethylene oxide sterilizer.
- ❖ Spores have also been used as agents of bioterrorism, e.g. endospores of *Bacillus anthracis* were used in the 2001 anthrax bioterrorism attack.

PHYSIOLOGY OF BACTERIA

BACTERIAL GROWTH AND NUTRITION

Bacterial Growth Requirement

Water constitutes about 80% of total bacterial cell. The minimum nutritional requirements that are essential for growth and multiplication of bacteria include sources of carbon, nitrogen, hydrogen, oxygen and some inorganic salts (such as small amounts of sulfur, phosphorus and other elements like sodium, potassium, magnesium, iron and manganese).

Bacterial Vitamin

Some fastidious bacteria do not grow in the routine culture medium unless certain organic compounds (that are essential to those bacteria) are added to the medium. These are known as growth factors or bacterial vitamins. In most instances, bacterial vitamins are same as the vitamins necessary for mammalian nutrition, particularly those belonging to the vitamin B group—thiamine, nicotinic acid, riboflavin, pyridoxine, folic acid and vitamin B12 (Table 3.2.5).

Bacterial Cell Division

Bacteria divide by a relatively simple form of cell division, i.e. by binary fission. The cell division commences when

Table 3.2.5: Bacterial vitamins.

Vitamins	Bacteria requiring vitamin
Biotin	<i>Leuconostoc</i> species
Cyanocobalamin (B12)	<i>Lactobacillus</i> species
Folic acid	<i>Enterococcus faecalis</i>
Pantothenic acid	<i>Morganella morganii</i>
Pyridoxine (B6)	<i>Lactobacillus</i> species
Niacin (nicotinic acid)	<i>Brucella abortus</i> , <i>Haemophilus influenzae</i>
Riboflavin (B2)	<i>Bacillus anthracis</i>

a bacterial cell reaches a critical mass in its cellular constituents. The nuclear division precedes cytoplasmic division.

- ❖ **Nuclear division:** The two strands of bacterial DNA are separated and then they replicate to form new complementary strands. Thus two identical molecules of ds DNA are formed
- ❖ **Cytoplasmic division:** A transverse septum grows across the cell from the cell membrane, following which the cell wall materials are deposited and then the two daughter cells get separated
- ❖ In few bacteria, the daughter cells may remain partially attached even after cell division; so that the bacterial cells are arranged in pair or in chain (e.g. streptococci) or in clusters (e.g. staphylococci).

Rate of Multiplication in Bacteria

Generation time is the time required for a bacterium to give rise to two daughter cells under optimum condition. The generation time for different bacteria is as follows:

- ❖ *Escherichia coli* and most of the other pathogenic bacteria: 20 minutes
- ❖ *Mycobacterium tuberculosis*: 10–15 hours
- ❖ *Mycobacterium leprae*: 12–13 days.

As bacteria grow so rapidly and by geometric progression, a single bacterium can theoretically give rise to 10^{21} daughter cells in 24 hours. Fortunately, it does not happen in reality, because the bacterial multiplication is arrested after a few cell divisions due to exhaustion of nutrients and accumulation of toxic products.

Bacterial Count

Bacterial count may be expressed in terms of total count and viable count.

- ❖ **Total count:** It indicates total number of bacteria (live or dead) in the specimen. This is done by counting the bacteria under microscope using counting chamber
- ❖ **Viable count:** It measures the number of living (viable) cells in the given specimen. Viable count may be obtained by a technique called as pour plate method.

Bacterial Growth Curve

When a bacterium is inoculated into a suitable liquid culture medium and incubated, its growth follows a definite course. When bacterial count of such culture is

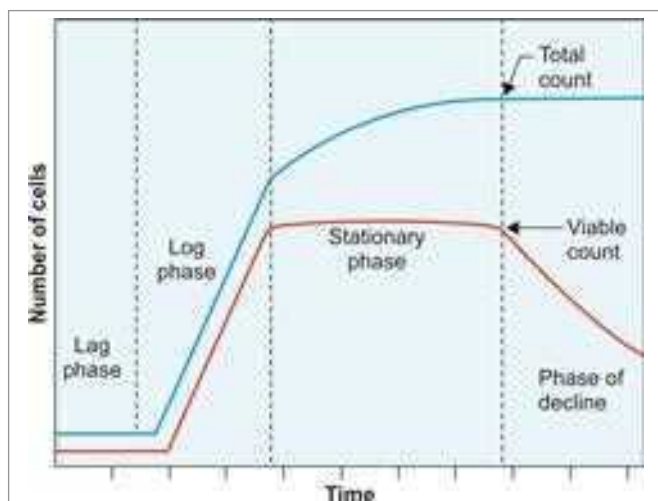


Fig. 3.2.14: Bacterial growth curve.

Table 3.2.6: Various phases of bacterial growth curve.

	Lag	Log	Stationary	Decline
Bacteria divide	No	Yes	Yes	No
Bacterial death	No	No	Yes	Yes
Total count	Flat	Raises	Raises	Flat
Viable count	Flat	Raises	Flat	Falls
Special features	Accumulation of enzymes and metabolites Attains maximum size	Uniformly stained Metabolically active Small size	Gram variable Produce: Granules, spores, exotoxin, antibiotics, bacteriocin	Produce: Involution forms

determined at different intervals and plotted in relation to time, a **bacterial growth curve** is obtained comprising of four phases (Fig. 3.2.14 and Table 3.2.6).

- Lag phase:** It is the period between inoculation and beginning of multiplication of bacteria. After inoculating into a culture medium, bacteria do not start multiplying immediately, but take some time to build-up enzymes and metabolites
 - Bacteria increase in size due to accumulation of enzymes and metabolites
 - Bacteria reach their maximum size at the end of lag phase.
- Log phase:** In this phase bacteria divide exponentially so that the growth curve takes a shape of straight line. At this stage, the bacterium is:
 - Smaller in size
 - Biochemically active: It is the best stage to perform the biochemical reactions
 - Uniformly stained: It is the best time to perform the Gram stain.
- Stationary phase:** After the log phase, the bacterial growth ceases almost completely due to exhaustion of nutrients, accumulation of toxic products and autolytic enzymes

- The number of progeny cells formed is just enough to replace the number of cells that die
- Hence, the number of viable cells remain stationary as there is almost a balance between the dying cells and the newly formed cells. But the total count keeps raising. In this phase:
 - ♦ Bacterium becomes gram-variable
 - ♦ More storage granules are formed
 - ♦ Sporulation occurs in this phase
 - ♦ Bacteria produce exotoxins, antibiotics and bacteriocins.

- Decline phase:** Gradually, the bacteria stop dividing completely; while the cell death continues due to exhaustion of nutrients, and accumulation of toxic products
 - There is decline in viable count and not in total count
 - Involution forms are seen.

FACTORS AFFECTING GROWTH OF BACTERIA

There are several environmental factors that affect the growth of the bacteria.

Oxygen

On the basis of their oxygen requirements bacteria are classified as:

- ❖ **Obligate aerobes:** They can grow only in the presence of oxygen (e.g. *Pseudomonas*, *Mycobacterium tuberculosis*, *Bacillus*, *Brucella* and *Nocardia*)
- ❖ **Facultative anaerobes:** They are aerobes that can also grow anaerobically (e.g. most of the pathogenic bacteria, e.g. *E. coli*, *S. aureus*, etc.)
- ❖ **Facultative aerobes:** They are anaerobes that can also grow aerobically (e.g. *Lactobacillus*)
- ❖ **Microaerophilic bacteria:** They can grow in the presence of low oxygen tension, i.e. 5–10% of oxygen (e.g. *Campylobacter* and *Helicobacter*)
- ❖ **Obligate anaerobes:** These bacteria can grow only in absence of oxygen, as oxygen is lethal to them (e.g. *Clostridium tetani*)
- ❖ **Aerotolerant anaerobe:** They can tolerate oxygen for some time, but do not use it (*Clostridium histolyticum*).

Carbon Dioxide

Organisms that require higher amounts of carbon dioxide (5–10%) for growth are called **capnophilic bacteria**. Examples include *Brucella abortus*, *Streptococcus pneumoniae*, etc.

Temperature

Most of the pathogenic bacteria grow optimally at 37°C (i.e. human body temperature). However, the optimal temperature range varies with different bacterial species. Accordingly bacteria can be grouped into:

- ❖ **Psychrophiles:** These grow best at temperatures below 20°C; example, most of the saprophytes, e.g. *Pseudomonas*

- ❖ **Mesophiles:** These grow within a temperature range 25°C and 40°C; example, most of the pathogenic bacteria
- ❖ **Thermophiles:** These bacteria grow at a high temperature range of 55°C–80°C, e.g. *Geobacillus stearothermophilus*.

pH

Most pathogenic bacteria grow between pH 7.2–pH 7.6. Very few bacteria (e.g. lactobacilli) can grow at acidic pH below pH 4, while bacteria such as *Vibrio cholerae* are capable of growing at alkaline pH (8.2–8.9).

Light

Bacteria (except phototrophs) grow well in darkness. They are sensitive to ultraviolet rays and other radiations in light. Photochromogenic mycobacteria produce pigments only on exposure to light.

Osmotic Effect

Bacteria are able to withstand a wide range of external osmotic variation because of the mechanical strength of the cell wall. However, sudden exposure to hypertonic saline may cause cell shrinkage (**plasmolysis**) and exposure to distilled water may cause cell swelling and rupture (**plasmolysis**).

Mechanical and Sonic Stresses

Though bacteria have tough cell walls, they may be ruptured and disintegrated by vigorous shaking with glass beads and by exposure to ultrasonic vibrations.

Moisture and Desiccation

Moisture is an essential requirement for the growth of bacteria because 80% of the bacterial cell consists of water. However, the drying has varying effects on different organisms.

- ❖ Some organisms like *Treponema pallidum* and *N. gonorrhoeae* die quickly after drying, while *M. tuberculosis* and *S. aureus* may survive drying for several weeks
- ❖ Drying in cold and vacuum (lyophilization) is used for preservation of microorganisms.

BACTERIAL METABOLISM

Bacterial metabolism is the process by which a microbe obtains the energy and nutrients (e.g. carbon) for its survival and reproduction. Bacterial metabolism can be based on three principles:

1. How the bacteria obtain carbon for synthesizing cell mass?
 - **Autotrophs:** These bacteria can synthesize all their organic compounds by using atmospheric CO₂ as their sole source of carbon
 - **Heterotrophs:** They use reduced, preformed organic molecules as carbon sources.
 2. How the bacteria obtain reducing equivalents (electrons) used either in energy conservation or in biosynthetic reactions?
 - **Lithotrophs:** These bacteria obtain reducing equivalents (electrons) from inorganic compounds
 - **Organotrophs:** They obtain reducing equivalents from organic compounds.
 3. How bacteria obtain energy for living and growing?
 - **Chemotrophs:** These bacteria obtain energy from external chemical compounds
 - **Phototrophs:** They obtain energy from light.
- Bacteria usually possess combination of these properties. Most of the pathogenic bacteria fall into **chemoorganoheterotrophs** group. These bacteria obtain energy, carbon, and reducing equivalents for biosynthetic reactions from organic compounds, e.g. *Escherichia coli*.

EXPECTED QUESTIONS

I. Write essay on:

1. Describe in detail the structure and function of the cell wall and cell membrane of a gram-negative rod with the help of a diagram.
2. Discuss the role of a bacterial cell wall structure in diagnosis and antimicrobial treatment of a bacterial infection.

II. Write short notes on:

1. Bacterial capsule.
2. Bacterial growth curve.
3. Bacterial flagella.

III. Multiple Choice Questions (MCQs):

1. **Cuneiform arrangement is characteristic of:**
 - a. *Staphylococcus*
 - b. *Streptococcus*

- c. *C. diphtheriae*
- d. *Bacillus anthracis*

2. Bacterial capsule can be best demonstrated by:

- a. Gram staining
- b. Acid-fast staining
- c. Negative staining
- d. Albert staining

3. Lipopolysaccharide is a component of cell wall of:

- a. Gram-positive bacteria
- b. Gram-negative bacteria
- c. Virus
- d. Fungi

4. Bacterial structure involved in respiration is:

- a. Ribosome
- b. Pili
- c. Mesosome
- d. Flagella

5. Which of the following cocci-arrangement is wrong?

- a. Chain-*Streptococcus*
- b. Pair-*Pneumococcus*
- c. Tetrad-*Gonococcus*
- d. Cluster-*Staphylococcus*

Answers

1. c 2. c 3. b 4. c 5. c

General Bacteriology: Laboratory Diagnosis of Bacterial Infections

CHAPTER 3.3

CHAPTER PREVIEW

- Specimen Collection
- Direct Detection
 - Staining Techniques
 - Other Methods of Direct Detection
- Culture, Identification and AST
 - Culture Media
 - Culture Methods
 - Culture Identification
- Antimicrobial Susceptibility Test
 - Serology
 - Molecular Methods
 - Microbial Typing

INTRODUCTION

Laboratory diagnosis of bacterial infections is useful for the following purposes:

- ❖ **Identification:** To identify the causative bacterial agent responsible for the disease
- ❖ **Treatment:** To provide accurate antimicrobial therapy
- ❖ **Surveillance purpose:** To assess the disease burden in the community by estimating the prevalence and incidence of the infections
- ❖ **For outbreak investigation**, e.g. diphtheria outbreaks in the community, MRSA (methicillin-resistant *S. aureus*) outbreaks in the hospitals
- ❖ **To start PEP** (post-exposure prophylaxis): Useful in infectious diseases such as, anthrax and plague
- ❖ **To initiate appropriate infection control measures:** For example, contact precaution for MRSA infection, droplet precaution for diphtheria and airborne precaution for tuberculosis (Chapter 21).

Laboratory diagnosis of bacterial infections comprises of several steps—specimen collection, direct detection, culture, identification and antimicrobial susceptibility test, serology and molecular methods (refer box).

LABORATORY DIAGNOSIS

Bacterial infections

- 1. Specimen collection**
- 2. Direct detection**
 - Microscopy: Gram stain, acid-fast stain, Albert stain, histopathological staining, dark ground, phase-contrast and fluorescence microscopy
 - Antigen detection from clinical specimen
 - Molecular diagnosis: Detecting bacterial DNA or RNA from clinical specimen
- 3. Culture**
 - Culture media
 - Culture methods
 - Colony morphology, smear and motility testing
- 4. Identification**
 - Biochemical identification

Contd...

Contd...

- Automated identification methods
- 5. Antimicrobial susceptibility testing**
- 6. Serology**
- 7. Molecular methods**
- 8. Typing methods**

SPECIMEN COLLECTION

Specimen collection depends upon the type of underlying infections (Table 3.3.1). The proper collection of specimen is of paramount importance for the isolation of the bacteria in culture.

General Principles

The following general principles should be followed while collecting the specimen:

- ❖ **Standard precautions** should be followed for collecting and handling all specimens (Chapter 21 for details)
- ❖ **Before antibiotics start:** Whenever possible, culture specimens should be collected prior to administration of any antimicrobial agents
- ❖ **Contamination** with indigenous flora should be avoided, especially when collecting urine and blood culture specimens
- ❖ **Swabs** are though convenient but considered inferior to tissue, aspirate and body fluids
- ❖ **Container:** Specimens should be collected in sterile, tightly sealed, leak proof, wide-mouth, screw-capped containers
- ❖ **Labeling:** All specimens must be appropriately labelled with name, age, gender, treating physician, diagnosis, antibiotic history, type of specimen, and desired investigation name
- ❖ **Rejection:** Specimens grossly contaminated or compromised or improperly labelled may be rejected (Annexure 9)
- ❖ If **anaerobic culture** is requested, proper anaerobic collection containers with media should be used

Type of infections	Specimens collected
Bloodstream infection, sepsis, endocarditis	Paired blood culture specimens <ul style="list-style-type: none"> Collected aseptically by two-step disinfection of skin; first with alcohol followed by chlorhexidine 8–10 mL of blood (for adults) collected in blood culture bottles
Infectious diseases requiring serology	<ul style="list-style-type: none"> Blood (2 mL/investigation) Collected by minimal asepsis (one-step skin disinfection with alcohol) Collected in vacutainer
Diarrheal diseases	Stool (mucus flakes), rectal swab
Meningitis	Cerebrospinal fluid (CSF)
Infections of other sterile body area	Sterile body fluids; e.g. pleural fluid, synovial fluid, peritoneal fluid
Skin and soft tissue infections	Pus or exudate, wound swabs, aspirates from abscess and tissue bits
Anaerobic infections	Aspirates, tissue specimens, blood and sterile body fluids, bone marrow (swabs, sputum not satisfactory)
Upper respiratory tract infections	Throat swab with membrane over the tonsil, nasopharyngeal swab, per-nasal swab
Lower respiratory tract infections	Sputum, endotracheal aspirate, bronchoalveolar lavage (BAL), protected specimen brush (PSB) and lung biopsy
Pulmonary tuberculosis	<ul style="list-style-type: none"> Sputum—early morning and spot Collected in well-ventilated area Gastric aspirate for infants
Urinary tract infections	Midstream urine Suprapubic aspirated urine Catheterized patient—collected from the catheter tube, after clamping distally and disinfecting; not from urobag
Genital infections	Urethral swab, cervical swab—for urethritis Exudate from genital ulcers
Eye infections	Conjunctival swabs Corneal scrapings Aqueous or vitreous fluid
Ear infections	Swabs from outer ear Aspirate from inner ear

- ❖ Specimen should not be sent in container containing **formalin** for microbiological analysis.

Specimen Transport

The specimens should reach the laboratory for further processing as soon as possible after the collection. If required appropriate transport media should be used (discussed subsequently in this chapter).

For most of the specimens, transport time should not exceed **two hours**. However, there are some exceptions.

- ❖ Specimens that require an **immediate transport** (<15 minutes)—such as CSF and body fluids, ocular specimens, tissue specimens, suprapubic aspirate and bone specimen

- ❖ **Urine (midstream)** added with preservative (boric acid) is acceptable up to 24 hours, otherwise should be transported within 2 hours
- ❖ **Stool culture:** Stool specimen should be transported within 1 hour, but with transport medium (Cary-Blair medium) up to 24 hours is acceptable
- ❖ **Rectal swabs**—up to 24 hours is acceptable
- ❖ **For anaerobic culture:** Specimens should be put into Robertson's cooked meat broth or any specialized anaerobic transport system and transported immediately to the laboratory.

Specimen Storage before Processing

Most specimens can be stored **at room temperature** immediately after receipt, for **up to 24 hours**. However, there are some exceptions.

- ❖ **Blood cultures**—should be incubated at 37°C immediately upon receipt
- ❖ Sterile body fluids, bone, vitreous fluid, suprapubic aspirate—should be immediately plated upon receipt and incubated at 37°C
- ❖ **Corneal scraping**—should be immediately plated at bed-side on to blood agar and chocolate agar
- ❖ **Stool culture**—can be stored up to 72 hours at 4°C
- ❖ **Urine** (mid-stream and from the catheter), **lower respiratory tract specimen**, **gastric biopsy** (for *Helicobacter pylori*)—can be stored up to 24 hours at 4°C.

DIRECT DETECTION

Direct detection of bacteria in the clinical specimen plays a very important role in early institution of antimicrobial therapy. These methods include microscopic demonstration of bacteria—staining techniques and other methods such as detection of antigen or nucleic acid in the clinical specimen.

STAINING TECHNIQUES

Structural details of bacteria cannot be seen under a light microscope due to lack of contrast. Hence, it is necessary to use staining methods to produce color contrast and thereby increase the visibility. Before staining, the smears are fixed so that they will not be displaced during the staining process. Fixation also protects the internal structures of cells in a fixed position. It is done by two methods.

1. **Heat fixation:** It is done by gently flame heating an air-dried film, used for bacterial smears
2. **Methanol fixation:** Used for blood smears.

Common staining techniques used in diagnostic bacteriology include:

- ❖ **Simple stain:** Basic dyes, such as methylene blue or basic fuchsin are used as simple stains. They provide the color contrast, but impart the same color to all the bacteria in a smear

- ❖ **Negative staining:** A drop of bacterial suspension is mixed with dyes, such as India ink or nigrosin. The background gets stained black whereas unstained bacterial/yeast capsule stand out in contrast. This is very useful in the demonstration of bacterial/yeast capsules which do not take up simple stains
- ❖ **Impregnation methods:** Bacterial cells and structures that are too thin to be seen under the light microscope, are thickened by impregnation of silver salts on their surface to make them visible, e.g. for demonstration of bacterial flagella and spirochetes
- ❖ **Differential stain:** Here, two stains are used which impart different colors to different bacteria or bacterial structures, which help in differentiating bacteria. The most commonly employed differential stains are:
 - **Gram stain:** It differentiates bacteria into gram-positive and gram-negative groups
 - **Acid-fast stain:** It differentiates bacteria into acid-fast and non acid-fast groups
 - **Albert stain:** It differentiates bacteria having meta-chromatic granules from other bacteria that do not have them.

Gram Stain

This staining technique was originally developed by Hans Christian Gram (1884). Even after more than 130 years of its discovery and even in the presence of newer modern diagnostic facilities, still Gram stain remains the most widely used test in diagnostic bacteriology.

Procedure (Fig. 3.3.1)

- ❖ **Fixation:** The smear made on a slide from bacterial culture or specimen is air-dried and then heat-fixed

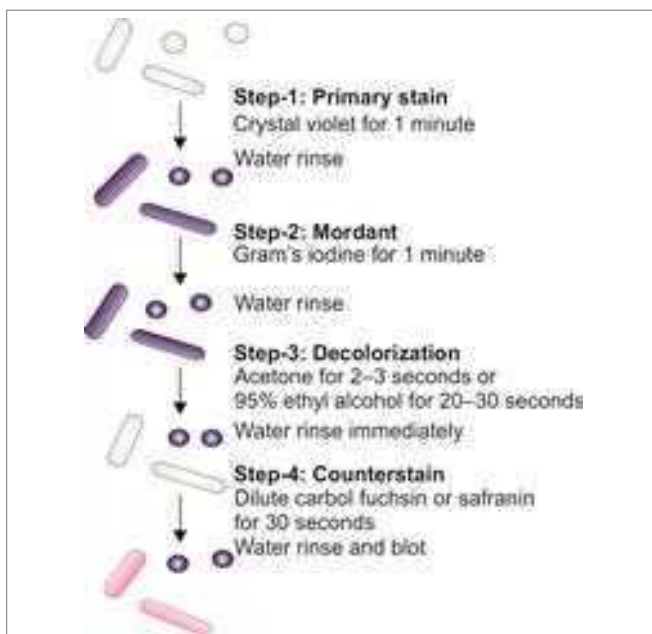


Fig. 3.3.1: Principle and procedure of Gram staining.

- ❖ **Step 1 (Primary stain):** The smear is stained with pararosaniline dyes such as crystal violet (or gentian violet or methyl violet) for one minute. Then the slide is rinsed with water. Crystal violet stains all the bacteria violet in color (irrespective of whether they are gram-positive or gram-negative)
- ❖ **Step 2 (Mordant):** Gram's iodine (dilute solution of iodine) is poured over the slide for one minute. Then the slide is rinsed with water. Gram's iodine acts as a mordant, binds to the dye to form bigger dye-iodine complexes in the cytoplasm
- ❖ **Step 3 (Decolorization):** Next step is pouring of few drops of decolorizer to the smear, e.g. acetone (for 1-2 sec) or ethyl alcohol (20-30 sec) or acetone alcohol (for 10 sec). Slide is immediately rinsed with water. Decolorizer removes the primary stain from gram-negative bacteria while the gram-positive bacteria retain the primary stain

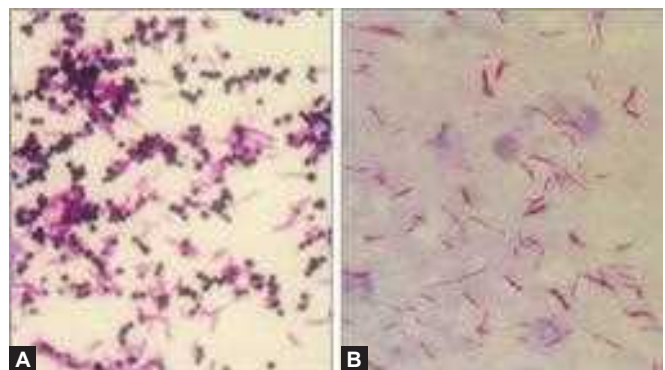
Note: Decolorization is the most crucial step of Gram staining. If the decolorizer is poured for more time, even gram-positive bacteria lose color (over decolorization) and if poured for less time, the gram-negative bacteria do not lose the color of primary stain properly (under decolorization).

- ❖ **Step 4 (Counter stain):** Secondary stains such as safranin or dilute carbol fuchsin is added for 30 seconds. It imparts pink or red color to the gram-negative bacteria. Alternatively, neutral red may also be used as counter stain, especially for gonococci. The slide is rinsed in tap water, dried, and then examined under oil immersion objective.

Interpretation of Gram Stain

Smear is examined under oil immersion objective (Fig. 3.3.2A).

- ❖ Gram-positive bacteria resist decolorization and retain the color of primary stain i.e. violet
- ❖ Gram-negative bacteria are decolorized and, therefore, take counterstain and appear pink.



Figs 3.3.2A and B: **A.** Gram staining demonstrating violet-colored gram-positive cocci in clusters and pink colored gram-negative bacilli in scattered arrangement; **B.** Acid-fast staining shows long slender straight or slightly curved beaded red acid-fast bacilli.

Source: A. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; B. Department of Microbiology, JIPMER, Puducherry (with permission).

Principle of Gram Staining

Though the exact mechanism is not understood, the following theories have been put forward.

- ❖ **pH theory:** Cytoplasm of gram-positive bacteria is more acidic, hence, can retain the basic dye (e.g. crystal violet) for longer time. **Iodine** serves as mordant, i.e. it combines with the primary stain to form a dye-iodine complex which gets retained inside the cell
- ❖ **Cell wall theory:** This is believed to be the most important postulate to describe the mechanism of Gram stain
 - Gram-positive cell wall has a thick peptidoglycan layer (50–100 layers thick), with tight cross linkages
 - The peptidoglycan itself is not stained; instead, it seems to act as a permeability barrier preventing loss of crystal violet. More so, alcohol is thought to shrink the pores of the thick peptidoglycan; hence large dye-iodine complexes are not able to penetrate this tightened peptidoglycan layer in a gram-positive bacteria
 - Gram-negative cell wall is more permeable thus allowing the outflow of crystal violet easily. This is attributed to:
 - ◆ The thin peptidoglycan layer in gram-negative cell wall which is not tightly cross linked
 - ◆ Presence of lipopolysaccharide layer in the cell wall of gram-negative bacteria, which gets disrupted easily by the decolorizer; forming larger pores, that allow the dye-iodine complexes to escape from the cytoplasm.

Modifications of Gram Staining

There are a few minor modifications of Gram stain which vary slightly from the method described earlier.

- ❖ **Kopeloff and Beerman's modification:** Primary stain and counter stain used are methyl violet and basic fuchsin respectively
- ❖ **Jensen's modification:** This method involves use of absolute alcohol as decolorizer and neutral red as counter stain. It is useful for meningococci and gonococci
- ❖ **Brown and Brenn modification:** This is used for Actinomycetes.

Uses of Gram Stain

- ❑ **To differentiate bacteria into gram-positive and gram-negative:** It is the first step towards identification of bacteria
- ❑ **For identification:** Gram staining from bacterial culture gives an idea to put the corresponding biochemical tests for further identification of bacteria
- ❑ **To start empirical treatment:** Gram stain from the specimen gives a preliminary clue about the bacteria present (based on the shape and Gram staining property of the bacteria) so that the empirical treatment with broad-spectrum antibiotics can be started early before the culture report is available
- ❑ **For fastidious organisms,** such as *Haemophilus* which takes time to grow in culture; Gram stain helps in early presumptive identification

Contd...

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- ❑ **Anaerobic organisms,** such as *Clostridium* do not grow in routine culture. Therefore, organisms detected in Gram stain, but aerobic culture-negative gives a preliminary clue to perform an anaerobic culture of the specimen
- ❑ **Yeasts:** In addition to stain the bacteria, Gram stain is useful for staining certain fungi such as *Candida* and *Cryptococcus* (appear gram-positive)
- ❑ **Quality of specimen:** Gram stain helps in screening the quality of the **sputum specimen** before processing it for culture. Presence of more pus cells and less epithelial cells indicates good quality specimen.

Acid-fast Stain

The acid-fast stain was discovered by Paul Ehrlich and subsequently modified by Ziehl and Neelsen. This staining is done to identify acid-fast organisms, such as *Mycobacterium tuberculosis* and others. Acid-fastness is due to presence of mycolic acid in the cell wall.

Ziehl-Neelsen Technique (Hot Method)

Smear Preparation

Smear measuring 2 × 3 cm in size is prepared in a new clean grease free scratch free slide from the yellow purulent portion of the sputum.

- ❖ The smear should neither be too thick nor too thin. When placed over a printed matter, the print should be readable through the smear
- ❖ Smear preparation should be done near a flame, as six inches around the flame is considered sterile zone (as heat coagulates the aerosols raised during the smear preparation).

Heat Fixation

The smear is air dried for 15–30 minutes and then heat fixed by passing over the flame 3–5 times for 3–4 seconds each time. Coagulation of the proteinaceous material in the sputum will facilitate fixing of the smear.

Procedure

Step 1 (Primary stain)

Smear is poured with strong carbol fuchsin (1%) for 5 minutes. Intermittent heating is done by flaming the underneath of the slide until the vapor rises. Heating helps in better penetration of the stain.

- ❖ Care must be taken to ensure that the smear does not dry out, to counteract drying more solution of stain is added to the slide and the slide reheated
- ❖ Rinse the slide with tap water, until all free carbol fuchsin stain is washed away. At this point, the smear on the slide looks red in color.

Step 2 (Decolorization)

It is done by pouring 25% sulfuric acid over the slide and allowing it to stand for 2–4 minutes. The slide is gently rinsed with tap water and tilted to drain off the water.

- ❖ A properly decolorized slide appears light pink. If the slide is still red, sulfuric acid is reapplied for 1–3 minutes and then rinsed gently with tap water
- ❖ The back of the slide is wiped clean with a swab dipped in sulfuric acid.

Step 3 (Counter staining)

0.1% methylene blue is poured onto the slide and left for 30 seconds. Then the slide is rinsed gently with tap water and allowed to dry.

- ❖ The slide is examined under the binocular microscope using 40× lens to select a suitable area and then examined under oil immersion field (100×)
- ❖ Contaminated materials/slide should be discarded in a jar containing 5% phenol.

Interpretation

Mycobacterium tuberculosis appears as long slender, straight or slightly curved and beaded, red colored acid-fast bacillus. Other non-acid fast organisms present in the smear and the background take up the counter stain and appear blue (Fig. 3.3.2B).

Modifications of Acid-Fast Staining

Hot method (Ziehl–Neelsen technique) is the most commonly done acid-fast staining technique. Other modifications are as follows:

- ❖ **Cold method (Kinyoun's method):** It is modification, where the intermittent heating is not required (described in Chapter 63)
- ❖ Acid-alcohol can be used as decolorizer alternatively
- ❖ Malachite green can be used as counter stain
- ❖ The concentration of sulfuric acid may vary depending on the acid-fastness of the structure to be demonstrated. More the content of mycolic acid in the cell wall, more is the acid-fastness, hence more is the percentage of sulfuric acid required for decolorization (Table 3.3.2).

Albert Stain

Albert stain is used to demonstrate the metachromatic granules of *Corynebacterium diphtheriae*.

Procedure

- ❖ Fixation: The smear is heat fixed

Acid-fast organisms/structures	Sulfuric acid (%) needed for decolorization
<i>Mycobacterium tuberculosis</i>	25%
<i>Mycobacterium leprae</i>	5%
<i>Nocardia</i>	1%
Acid-fast parasites such as <i>Cryptosporidium</i> , <i>Cyclospora</i> , <i>Cystoisospora</i> , <i>Microsporidia</i> *	0.5%
Bacterial spore	0.25–0.5%

*Microsporidia are now considered to be evolved from fungi.

- ❖ Smear is covered with Albert I stain for 5 minutes, then the excess stain is drained out
- ❖ Albert II (iodine solution) is added for 1 minute
- ❖ Slide is washed with water, blotted dry and examined under oil immersion field.

Composition

Composition of Albert stain includes:

- ❖ Albert I: Comprises of toluidine blue, malachite green, glacial acetic acid, alcohol (95% ethanol), and distilled water
- ❖ Albert II: Contains iodine in potassium iodide.

Interpretation

Corynebacterium diphtheriae appears as green colored bacilli arranged in Chinese letter or cuneiform pattern, with bluish black metachromatic granules at polar ends (Refer Fig. 60.2C, Chapter 60). These can be differentiated from diphtheroids which do not show granules and are arranged in palisade pattern. However, certain bacteria, such as *Corynebacterium xerosis* and *Gardnerella vaginalis* also possess metachromatic granules.

Other Microscopic Techniques

Other microscopic techniques include:

- ❖ **Dark-ground and phase-contrast microscopy**—for demonstration of spirochetes in genital specimens
- ❖ **Hanging drop preparation** for stool specimen—for demonstration of darting motility; gives a clue about *V. cholerae*.

OTHER METHODS OF DIRECT DETECTION

Antigen Detection

Various immunological methods such as latex agglutination test, immunochromatographic test are available which detect antigens in clinical specimens.

- ❖ The classical example includes detection of capsular antigen of pneumococci, meningococci, *H. influenzae* in CSF specimen
- ❖ Urinary antigen detection for pneumococci and *Legionella*
- ❖ Direct fluorescent antibody test—for detection of *T. pallidum* from tissue sections or exudates.

Details about these antigen detection methods are discussed in Chapter 12.

Molecular Diagnosis

Bacterial DNA or RNA can be directly detected in the clinical specimens by various molecular methods such as polymerase chain reaction (PCR). It is discussed in detail subsequently in this chapter.

CULTURE, IDENTIFICATION AND AST

Culture is the most common diagnostic method used for detection of bacterial infections. Specimens are inoculated on to various culture media and incubated. The colonies

grown are subjected to identification and antimicrobial susceptibility test (AST).

CULTURE MEDIA

A microbiological culture medium is a liquid or solid substance that contains nutrients to support the growth, and survival of microorganisms.

Constituents of Culture Media

The various constituents of culture media are as follows:

- ❖ **Water and electrolytes** (e.g. sodium chloride)
- ❖ **Peptone:** It is a complex mixture of partially digested proteins, obtained from various sources such as heart muscle, casein or fibrin, or soya
- ❖ **Agar:** It is used for solidifying the culture media, does not add nutritive value to the medium
 - **Source:** It is prepared from the cell wall of seaweeds and available commercially in powder form
 - **Preparation:** Agar powder is dissolved in water and subjected to sterilization by autoclave. When the temperature of the molten agar comes down to 45°C, it is poured into the Petri dishes and then allowed to set for 20 minutes
 - **Concentration:** It is used in concentration of 1–2% for solid medium, 0.5% for semisolid agar and 6% to inhibit *Proteus* swarming.
- ❖ **Meat extract:** It is a commercial preparation of highly concentrated meat stock, usually made from beef
- ❖ **Yeast extract** (prepared from Baker's yeast) and **malt extract** (contains maltose)
- ❖ **Blood and serum:** They are important components of enriched media; provide extra nutrition to fastidious bacteria. Usually 5–10% of sheep blood is used. Alternatively, horse, ox, or human blood can also be used.

Types of Culture Media

Bacteriological culture media can be classified in two ways.

A. Based on consistency, culture media are grouped into—liquid (or broth), semisolid and solid media.

B. Based on the method of growth detection, culture media are classified as:

1. **Conventional culture media:** They are prepared from nutrients, such as aqueous extract of meat, peptone,

etc. The bacterial growth is detected manually by visual inspection of turbidity or colony morphology. They are of various types based on their functional use or application

- Simple/basal media
- Enriched media
- Enrichment broth
- Selective media
- Differential media
- Transport media
- Anaerobic media.

2. **Automated culture media:** They are mainly available for blood and sterile body fluid culture. The growth is detected automatically by the equipment.

Conventional Culture Media

Simple/Basal Media

They contain minimum ingredients that support the growth of non-fastidious bacteria. Examples include—

- ❖ **Peptone water:** It contains peptone (1%) + NaCl (0.5%) + water (Fig. 3.3.3A)
- ❖ **Nutrient broth:** It is made up of peptone water + meat extract (1%). It is available in three forms: (1) meat extract, (2) meat infusion, (3) meat digest broth
- ❖ **Nutrient agar:** It is made up of nutrient broth + 2% agar (Fig. 3.3.3B)
- ❖ **Semisolid medium:** It is prepared by reducing the concentration of agar to 0.2–0.5 %.

Uses of Basal Media

The basal media are used for:

- ❑ Testing the non-fastidiousness of bacteria
- ❑ They serve as the base for the preparation of many other media
- ❑ Nutrient broth is used for studying the bacterial growth curve
- ❑ Nutrient agar is the preferred medium for:
 - Performing the biochemical tests, such as oxidase, catalase and slide agglutination test, etc.
 - To study the colony morphology
 - Pigment demonstration.
- ❑ Semisolid medium is used for: (1) demonstrating motility of the bacteria; motile bacteria spread throughout the semi-solid medium, making the medium hazy, (2) maintaining stock culture.



Figs 3.3.3A to D: A. Peptone water; B. Nutrient agar; C. Blood agar; D. Chocolate agar.

Source: A to D. Department of Microbiology, JIPMER, Puducherry (with permission).

Enriched Media

When a basal medium is added with additional nutrients, such as blood, serum or egg, it is called enriched medium. In addition to non-fastidious organisms, they also support the growth of fastidious nutritionally exacting bacteria. Examples include:

- ❖ **Blood agar:** It is prepared by adding 5–10% of sheep blood to the molten nutrient agar at 45°C (Fig. 3.3.3C). It is the most widely used medium in diagnostic bacteriology. Blood agar also tests the hemolytic property of the bacteria, which may be either: (1) partial or α (green) hemolysis and (2) complete or β -hemolysis (described subsequently in this chapter)
- ❖ **Chocolate agar:** It is the heated blood agar, prepared by adding 5–10% of sheep blood to the molten nutrient agar at 70°C, so that the RBCs will be lysed and the content of RBCs will be released, changing the color of the medium to brown (Fig. 3.3.3D). It is more nutritious than blood agar, and even supports certain highly fastidious bacteria, such as *Haemophilus influenzae* that does not grow on blood agar
- ❖ **Loeffler's serum slope:** It contains serum. It is used for isolation of *Corynebacterium diphtheriae*
- ❖ **Blood culture media:** They are also enriched media, used for isolating microorganisms from blood. They are available either as conventional or automated blood culture media (described later in this chapter).

Enrichment Broth

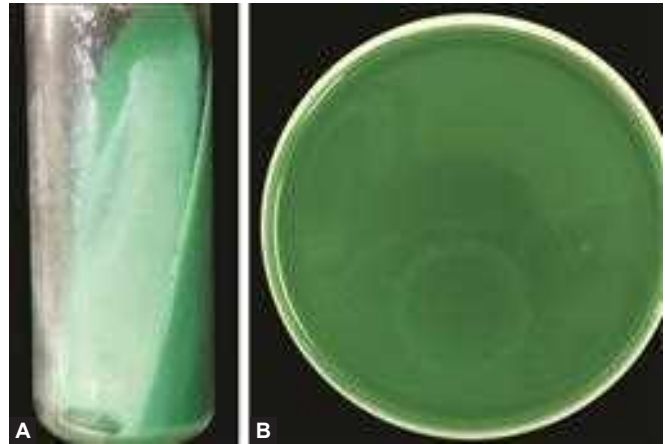
They are the liquid media added with some inhibitory agents which selectively allow certain organism to grow and inhibit others. This is important for isolation of the pathogens from clinical specimens which also contain normal flora (e.g. stool and sputum specimen). Examples for enrichment broth include:

- ❖ Tetrathionate broth—Used for *Salmonella* Typhi
- ❖ Gram-negative broth—Used for isolation of *Shigella*
- ❖ Selenite F broth—Used for isolation of *Shigella*
- ❖ Alkaline peptone water (APW)—Used for *Vibrio cholerae*.

Selective Media

They are solid media containing inhibitory substances that inhibit the normal flora present in the specimen and allow the pathogens to grow.

- ❖ **Lowenstein-Jensen (LJ) medium:** It is used for isolation of *Mycobacterium tuberculosis* (Fig. 3.3.4A)
- ❖ **Thiosulfate citrate bile salt sucrose (TCBS) agar:** It is used for isolation of *Vibrio* species (Fig. 3.3.4B)
- ❖ **DCA (deoxycholate citrate agar and XLD (xylose lysine deoxycholate) agar:** They are used for the isolation of enteric pathogens, such as *Salmonella* and *Shigella* from stool (Figs 3.3.5A and B)
- ❖ **Potassium tellurite agar (PTA):** It is used for isolation of *Corynebacterium diphtheriae*.



Figs 3.3.4A and B: A. Lowenstein–Jensen medium; B. TCBS agar.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Transport Media

They are used for the transport of the clinical specimens suspected to contain delicate organism or when delay is expected while transporting the specimens from the site of collection to the laboratory (Table 3.3.3). Bacteria do not multiply in the transport media, they only remain viable.

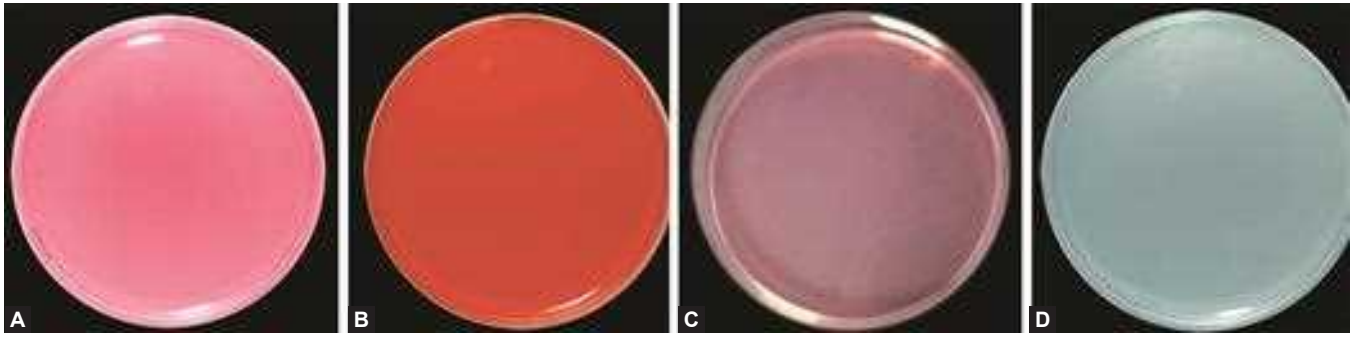
Differential Media

These media differentiate between two groups of bacteria by using an indicator, which changes the color of the colonies of a particular group of bacteria but not the other group.

- ❖ **MacConkey agar:** It is a differential and low selective medium, commonly used for the isolation of enteric gram-negative bacteria (Fig. 3.3.5C)
 - It differentiates organisms into LF or lactose fermenters (produce pink colored colonies, e.g. *Escherichia coli*) and NLF or non-lactose fermenters (produce colorless colonies, e.g. *Shigella*)
 - **Composition:** It contains peptone, lactose, agar, neutral red (indicator) and taurocholate
 - Most laboratories use combination of blood agar and MacConkey agar for routine bacterial culture.
- ❖ **CLED agar (cysteine lactose electrolyte-deficient agar):** This is another differential medium similar to

Table 3.3.3: Transport media used for common bacteria.

Organism	Transport media
<i>Neisseria</i>	Amies medium and Stuart's medium
<i>Vibrio cholerae</i>	<ul style="list-style-type: none"> • VR (Venkatraman-Ramakrishnan) medium • Autoclaved sea water • Cary Blair medium
<i>Shigella, Salmonella</i>	<ul style="list-style-type: none"> • Buffered glycerol saline • Cary Blair medium



Figs 3.3.5A to D: A. DCA; B. XLD agar; C. MacConkey agar; D. CLED agar.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

MacConkey agar, capable of differentiating between LF and NLF. It is used as an alternative to combination of blood agar and MacConkey agar, for the processing of urine specimens (Fig. 3.3.5D).

Anaerobic Culture Media

Anaerobic media contain reducing substances which take-up oxygen and create lower redox potential and thus permit the growth of obligate anaerobes, such as *Clostridium*. Examples are as follows:

- ❖ **Robertson's cooked meat (RCM) broth:** It contains chopped meat particles (beef heart), which provide glutathione (a sulfhydryl group containing reducing substance) and unsaturated fatty acids. It is the most widely used anaerobic culture medium (Fig. 3.3.6A). It is also used for maintenance of stock cultures
- ❖ **Other anaerobic media** include:
 - Thioglycollate broth (Fig. 3.3.6B)
 - Anaerobic blood agar
 - BHIS agar (Brain-heart infusion agar) with supplements (vitamin K and hemin)
 - Neomycin blood agar
 - Egg yolk agar
 - Phenyl ethyl agar
 - *Bacteroides* bile esculin agar (BBE agar).

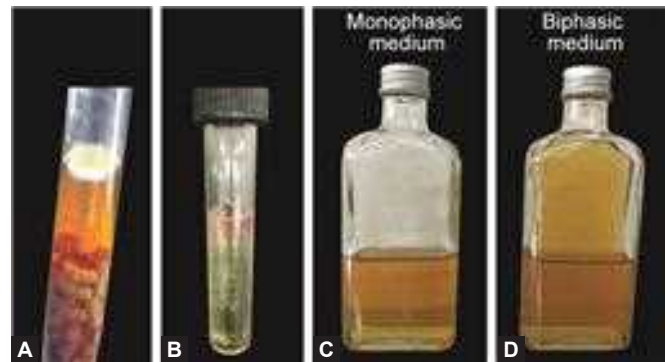
Blood Culture Media

Recovery of bacteria from blood is difficult as they are usually present in lesser quantity in the blood and many of the blood pathogens are fastidious. Therefore, enriched media are used for isolating microorganisms from blood. Blood culture media are available either as conventional or automated media.

Conventional Blood Culture Media

The conventional blood culture media are of two types.

1. Monophasic medium: It contains brain-heart infusion (BHI) broth (Fig. 3.3.6C)
2. Biphasic medium: It has a liquid phase containing BHI broth and a solid agar slope made up of BHI agar (Fig. 3.3.6D).



Figs 3.3.6A to D: A. Robertson's cooked meat medium; B. Thioglycollate broth; C. Brain-heart infusion broth; D. Biphasic medium (Brain-heart infusion broth/agar).

Source: A, C and D. Department of Microbiology, JIPMER, Puducherry; B. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

The recovery of organisms in the blood is enhanced by mixing the blood in the broth periodically. If any growth occurs, it can be detected by subcultures.

Disadvantages

In conventional blood culture, subcultures are made manually. This process can be performed less-frequently (once a day) as it is cumbersome.

- ❖ **From monophasic BHI broth**, subcultures are made onto blood agar and MacConkey agar periodically for 1 week. There is a higher risk of contamination due to opening of the cap of the bottle every time when subcultures are made
- ❖ **From biphasic BHI broth**, subcultures can be made just by tilting the bottles so that the broth runs over the agar slope. There is lower risk of contamination as it obviates the opening of the cap of the bottle.

Automated Blood Culture Techniques

Automated blood culture techniques have been in use since last two decades. They are revolutionary, offer several advantages over conventional blood cultures.

- ❖ **Continuous automated monitoring:** Following inoculation, the culture bottles are loaded inside the automated culture system

- The incubated bottles are periodically tilted automatically every 10 minutes, which allows mixing of blood with broth which fastens the recovery
 - Bottles are periodically monitored for the microbial growth once in every 10 minutes by the instrument. Once positive for microbial growth, the instrument gives a signal (producing beep or color change on the screen).
- ❖ **Composition:** Automated blood culture bottles contain:
- Tryptic soy broth and/or brain heart infusion broth (as enriched media) added with
 - Polymeric resin beads which adsorb and neutralize the antimicrobials present in blood specimen.
- ❖ **Specimens:** In addition to blood, these bottles can also be used for culture of bone marrow, sterile body fluids such as CSF, peritoneal, pleural and synovial fluid
- ❖ **More sensitive:** It gives a higher yield of positive cultures from clinical specimens
- ❖ **Rapid:** It takes less time than conventional methods
- ❖ **Less labor intensive,** as fully-automated.

Automated Systems

There are three automated systems commercially available.

1. **BacT/ALERT 3D** (Figs 3.3.7A and B): Its principle is based on colorimetric detection of growth. When bacteria multiply, they produce CO₂ that increases the pH, which in turn changes the color of a blue-green sensor present at the bottom of the bottle to yellow, that is detected by colorimetry
2. **BacT/ALERT VIRTUO** (bioMerieux) (Fig. 3.3.8): It is an advanced form of BacT/ALERT which offers several advantages such as (i) automatic loading and unloading of bottles, (ii) faster detection of growth, (iii) can determine the volume of blood present in the bottle



Figs 3.3.7A and B: A. BacT/ALERT automated blood culture system; B. BacT/ALERT blood culture bottle.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).



Fig. 3.3.8: BacT/ALERT VIRTUO automated blood culture system.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

3. **BACTEC** (BD Diagnostics): Its principle is based on fluorometric detection of growth; use an oxygen-sensitive fluorescent dye present in the medium
 - In an uninoculated medium, the large amount of dissolved oxygen present in the broth quenches the fluorescent dye
 - Later, actively dividing microorganisms consume the oxygen removing the quenching effect and allowing the fluorescence to be detected.

Note: There is an automated culture system available for culture of *Mycobacterium tuberculosis* from various pulmonary and extrapulmonary specimens; called as Mycobacteria Growth Indicator Tube (**MGIT**). This works on fluorometric principle of detection, similar to BACTEC.

Disadvantages

Automated culture methods do have several disadvantages like (1) high cost of the instrument and culture bottles, (2) inability to observe the colony morphology as liquid medium is used.

CULTURE METHODS

Culture methods involve inoculating the specimen on to appropriate culture media, followed by incubating the culture plates in appropriate conditions.

Selection of Media

The first step of a culture investigation is selection of appropriate media, which in turn depends up on the type of specimen to be processed. In general, combination of blood agar and MacConkey agar is commonly used for processing of most specimens. However, there are few specimens for which additional or alternative media are used (Table 3.3.4).

Table 3.3.4: Selection of media for various specimen types.	
Specimens	Recommended culture media
Exudate specimens*	Blood agar plus MacConkey agar
Sterile body fluids	Blood agar, plus MacConkey agar, plus chocolate agar or Automated blood culture bottles
Blood	Blood culture bottles (Conventional or automated)
Urine	Blood agar plus MacConkey agar CLED agar can be used alternatively
Stool	Selenite-F broth plus MacConkey agar plus DCA and/or XLD agar (if cholera is suspected- add TCBS agar)
Respiratory specimens	Blood agar, plus MacConkey agar, plus chocolate agar (if diphtheria is suspected - add LSS and PTA)

*Exudate specimens include pus, wound swab, aspirates, and tissue bits.

Abbreviations: CLED, cysteine lactose electrolyte deficient agar; DCA, deoxycholate citrate agar; XLD, xylose lysine deoxycholate; TCBS, thiosulfate-citrate-bile salts-sucrose agar; LSS, Loeffler serum slope; PTA, potassium tellurite agar.

Inoculation of the Specimens

Inoculation of the specimens onto the culture media is carried out with the help of bacteriological loops made up of platinum or nichrome wire (Fig. 3.3.9A).

- ❖ The inoculating loop is first heated in the Bunsen flame by making it red hot (Fig. 3.3.9B) and then made cool waiting for 10 seconds
- ❖ The entire process of bacteriological culture method should be carried out in a biological safety cabinet and wearing appropriate personal protective equipment such as gloves, laboratory coat or gown and mask (for respiratory specimens).

Biosafety Cabinet (BSC)

It is an enclosed, ventilated laboratory work station, used to protect the laboratory personnel while working with potential infectious clinical specimens.

- They are specially designed in a way that the air is blown into the cabinet away from the worker and then exhausted outside through a duct lined with HEPA filters (Fig. 3.3.10)
- There are various types of BSCs, depending upon air velocity and percentage of air recirculated. Most of the microbiology laboratories require Class 2A BSC. A higher class of BSCs may be required for certain high-risk pathogens.

Inoculation Methods

Inoculation methods are of two types.

1. Methods used for inoculating clinical specimens on to the culture media
2. Methods used for inoculating colonies on to various media for further processing.

Streak Culture

It is the most common inoculation method; used for the inoculation of the specimens on to the solid media. It is



Figs 3.3.9A and B: A. Bacteriological loop and straight wire; B. Flaming the loop (red hot).

Source: A. Department of Microbiology, JIPMER, Puducherry (with permission).



Fig. 3.3.10: Biological safety cabinet.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

also used for obtaining individual isolated colonies from a mixed culture of bacteria.

- ❖ **Streaking:** A loopful of the specimen is smeared onto the solid media to form round-shaped primary inoculum, which is then spread over the culture plate by streaking parallel lines to form the secondary, tertiary inoculum and finally a feathery tail end (Fig. 3.3.11A)
- ❖ **Intermittent heating:** The loop is flamed and cooled in between the different set of streaks to get isolated colonies on the final streaks (Fig. 3.3.11B). Obtaining isolated colonies is the prerequisite to perform tests for identification and AST.

Liquid Culture

Liquid culture is used for culture of specimens such as blood or body fluids, which are inoculated by directly adding the specimen in to the liquid medium or with the help of a syringe or pipette.

- ❖ **Bacterial growth** is detected by observing the turbidity in the medium. Some aerobic bacteria form surface pellicles (Fig. 3.3.12A)



Figs 3.3.11A to C: A. Streak culture (schematic representation); B. Isolated colonies grown by following streak culture; C. Lawn culture of a bacterial isolate to perform the antimicrobial susceptibility testing.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ **Uses:** Liquid cultures are useful for—(1) blood, or body fluids culture, (2) automated culture for mycobacteria (MGIT, i.e. mycobacteria growth indicator tube), (3) water analysis
- ❖ **Advantages:** Liquid cultures are preferable for culture of—(1) specimens containing small quantity of bacteria, (2) specimens (e.g. blood) containing antibiotics and other antibacterial substances, as they get neutralized by dilution in the medium, (3) It is also preferred when large yields of bacteria are required
- ❖ **Disadvantages:** (1) Liquid cultures do not provide a pure culture from a mixed inoculum, (2) there is no visible colonies, therefore unlike solid media, it does not give any preliminary clue about the bacteria.

Lawn or Carpet Culture

Lawn culture is useful to carry out antimicrobial susceptibility testing (AST) by disk diffusion method (Fig. 3.3.11C). Here, the uniform lawn of bacterial growth is obtained by either swabbing or flooding with a bacterial broth onto the culture plate (discussed in detail subsequently in this chapter).

Pour Plate Technique

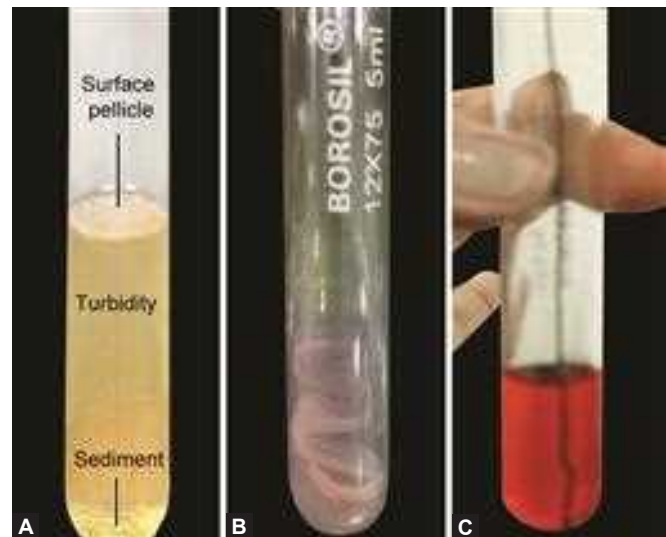
Seldom used for quantifying the bacterial load present in the specimens such as urine or blood. Here, serial dilutions of the specimen are added on to the molten agar. After being cooled and solidified, the Petri dishes are incubated and then the colony count is estimated.

Stroke Culture

This is carried out on agar slopes or slants by streaking the straight wire in a zigzag fashion (Fig. 3.3.12B). It is used for biochemical test such as urease test.

Stab Culture

It is made by stabbing the semisolid agar butt by a straight wire. It is used for motility testing using mannitol motility



Figs 3.3.12A to C: A. Liquid culture in test tube (turbidity indicates growth); B. Stroke culture; C. Stabbing with inoculation wire (stab culture).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

medium (Fig. 3.3.12C), and triple sugar iron agar test (here, both stroke and stab cultures are made).

Incubatory Conditions

Most of the pathogenic bacteria are aerobes or facultative anaerobes; grow best at 37°C, i.e. body temperature of human beings. Therefore, the inoculated culture plates are incubated at 37°C aerobically overnight in an incubator.

Bacteriological Incubator

It is an equipment used to incubate the culture plates, biochemical tests and AST plates (Fig. 3.3.13). The incubator maintains optimal temperature. Some incubators are specially designed to maintain other conditions, such as humidity and CO₂.



Fig. 3.3.13: Bacteriological incubator.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

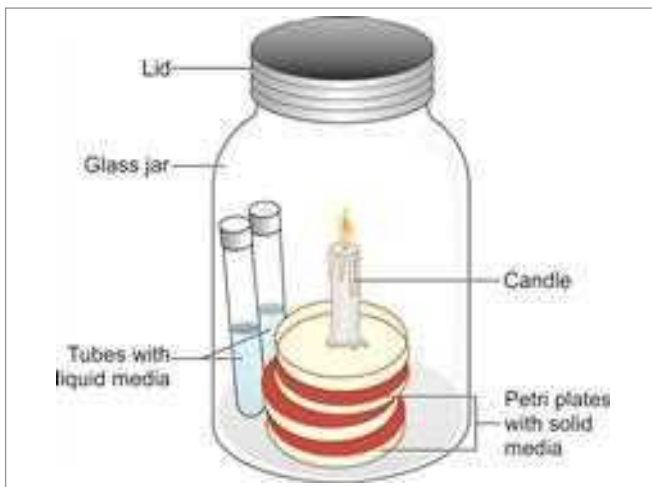


Fig. 3.3.14: Candle jar.

Other Incubatory Conditions

The incubatory conditions may vary depending upon the bacteria to be isolated.

- ❖ **For capnophilic bacteria:** Candle jar is used. Here, inoculated media are placed inside a jar, along with a lighted candle and then jar is sealed
 - The burning candle reduces oxygen to a point where the flame goes off (Fig. 3.3.14). This provides an atmosphere of approximately 3–5% CO₂
 - This is useful for capnophilic bacteria, such as *Brucella*, *Streptococcus*, pneumococcus and gonococcus.
- ❖ **For microaerophilic bacteria**, such as *Campylobacter* and *Helicobacter* require 5% oxygen for optimum growth
- ❖ **For obligate anaerobes**, anaerobic culture methods are used (see below).

Anaerobic Culture Methods

Obligate anaerobic bacteria can grow only in the absence of oxygen, hence for the growth of such bacteria, anaerobic environment is needed. The following are the methods used to create anaerobiosis.

Evacuation and Replacement

This involves evacuation of the air from jar and replacement with inert gas like hydrogen followed by removal of the residual oxygen by use of a catalyst. It is carried out either by:

- ❖ **Manual method** by using McIntosh and Fildes's anaerobic jar (Fig. 3.3.15A): It was the most popular method for creating anaerobiosis in the past, now not in use
- ❖ **Automated system (Anoxomat):** It automatically evacuates air and replaces by hydrogen gas from a cylinder (Fig. 3.3.15B)
 - The catalyst used to combust residual oxygen is a sachet containing aluminum pellets coated with palladium



Figs 3.3.15A to C: **A.** McIntosh and Fildes's anaerobic jar; **B.** Anoxomat anaerobic system; **C.** Anaerobic work station (Whitley pvt. Ltd.).

Source: A. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; B. Department of Microbiology, JIPMER, Puducherry; C. Dr Padmaja A Shenoy, Department of Microbiology, Kasturba Medical College, Manipal, Karnataka (with permission).

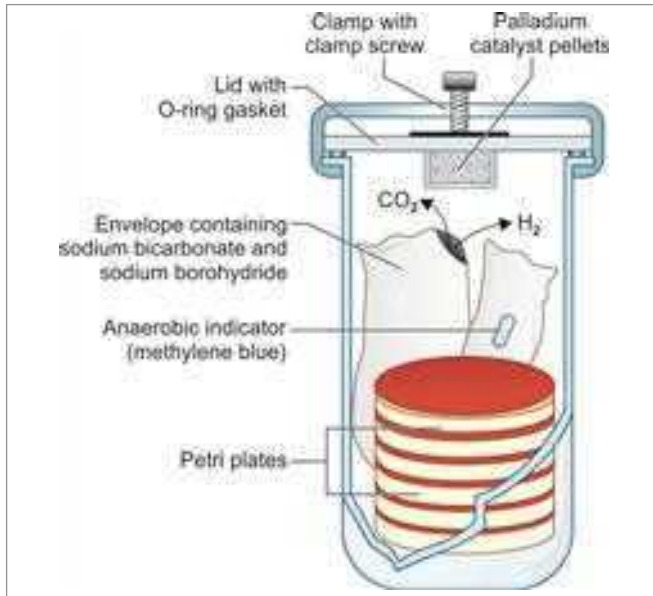


Fig. 3.3.16: GasPak anaerobic system.

- It is easier to operate than McIntosh jar method and claims to be highly effective for creating anaerobiosis.

Absorption of Oxygen by Chemical Methods

GasPak system (BD diagnostics) works on this principle. It is the most commonly used method for anaerobiosis, especially for laboratories with less sample load.

- ❖ Here, the oxygen is removed by chemical reactions, instead of evacuation and replacement technique used in Anoxomat
- ❖ It uses a sachet containing sodium bicarbonate and sodium borohydride which react chemically in presence of water, to produce hydrogen and CO₂ gas
- ❖ The traces of oxygen is removed by using the same catalyst used for Anoxomat (aluminium pellets coated with palladium) placed below the jar lid (Fig. 3.3.16)
- ❖ **Indicator of anaerobiosis:** The effectiveness of anaerobiosis can be checked by:
 - **Chemical indicator:** Reduced methylene blue remains colorless in anaerobic conditions, but turns blue on exposure to oxygen
 - **Biological indicator** using obligate aerobe such as *Pseudomonas*: Absence of its growth indicates that complete anaerobiosis has been achieved.

GENbag (bioMérieux): It consists of an airtight transparent bag with a generator sachet, which rapidly produces carbon dioxide and creates an anaerobic environment. Its application is similar to that of GasPak system.

Anaerobic Glove Box and Anaerobic Work Station

These systems provide facility for easy processing, incubation and examination of the specimens without exposure to oxygen (Fig. 3.3.15C).

Reducing Agents

Oxygen in culture media can be reduced by various reducing agents, such as glucose, thioglycollate, cooked meat pieces, cysteine and ascorbic acid. **Robertson cooked meat broth** is the most widely employed anaerobic culture medium which uses chopped meat particles (beef heart) as reducing agent (Fig. 3.3.6A).

Pre-reduced Anaerobically Sterilized (PRAS)

PRAS media are prepared entirely under oxygen-free conditions from initial sterilization to packaging in sealed foil packets.

Colony Morphology

After overnight incubation, the culture media are removed from the incubator and are examined under bright illumination. The appearance of bacterial colony on culture medium is characteristic for many organisms; which helps in their preliminary identification. The following features of the colony are studied.

- ❖ **Size**—in millimeters; e.g. pinhead size is characteristic of staphylococcal colony, whereas pinpoint size is characteristic of streptococcal colony
- ❖ **Shape**—circular or irregular
- ❖ **Consistency**—dry, moist or mucoid
- ❖ **Density**—opaque, translucent or transparent
- ❖ **Hemolysis on blood agar** (see below)
- ❖ **Color of the colony:** Colonies may be colored due to certain properties of the media or organisms. For example, pink colonies produced by lactose fermenters on MacConkey agar and black colonies by *Corynebacterium diphtheriae* on potassium tellurite agar due to the reduction of tellurite. Color of the colonies may also be due to pigment production by the bacteria
- ❖ **Pigment production:** Bacteria may produce two types of pigments
 1. **Diffusible pigments**, e.g. blue-green pigments produced by *Pseudomonas aeruginosa*
 2. **Non-diffusible pigments:** They do not diffuse into surrounding media, hence only the colonies are colored, not the surrounding media; e.g. *S. aureus* producing golden-yellow colonies.

Hemolysis on Blood Agar

Certain bacteria produce hemolysin enzymes that lyse the red blood cells surrounding the colonies on blood agar, forming a zone of hemolysis (Fig. 3.3.17). Hemolysis may be:

- **Partial or α hemolysis:** Partial clearing of blood around the colonies occurs with green discoloration of the surrounding medium; outline of the RBCs is intact (e.g. pneumococci, viridans streptococci)
- **Complete or β hemolysis:** Zone of complete clearing of blood around the colonies due to complete lysis of the RBCs (e.g. *Staphylococcus aureus* and *Streptococcus pyogenes*)
- **No hemolysis (γ hemolysis, a misnomer):** There is no color change surrounding the colony (e.g. *Enterococcus*)



Fig. 3.3.17: Hemolysis on blood agar.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Culture Smear and Motility Testing

The colonies grown on the culture media are subjected to Gram staining and motility testing by hanging drop method.

Hanging drop preparation is one of the most common and easiest method to demonstrate bacterial motility.

- ❖ A drop of bacterial broth is prepared on a coverslip and kept over a cavity slide
- ❖ Then the edge of the drop is focused under the microscope for demonstration of motile bacteria, as they usually migrate towards the edge to get more oxygen
- ❖ Hanging drop may give some clue about the identification, especially for gram-negative bacilli.

CULTURE IDENTIFICATION

Identification of bacteria from culture is made either by conventional biochemical tests or by automated identification systems.

Biochemical Identification

Based on the type of colony morphology and Gram staining appearance observed in culture smear, the appropriate biochemical tests are employed.

1. **Initially**, catalase and oxidase tests are done on all types of colonies grown on the media
2. **For gram-negative bacilli:** The following are the common biochemical tests done routinely, abbreviated as 'ICUT':
 - Indole test
 - Citrate utilization test
 - Urea hydrolysis test
 - Triple sugar iron test (TSI).
3. **For gram-positive cocci:** The useful biochemical tests are as follows:
 - Coagulase test (for *Staphylococcus aureus*)

- CAMP (Christie-Atkins-Munch-Petersen) test for group B *Streptococcus*
- Bile esculin hydrolysis test (for *Enterococcus*)
- Heat tolerance test (for *Enterococcus*)
- Inulin fermentation (for pneumococcus) and
- Bile solubility test (for pneumococcus)
- Antimicrobial susceptibility tests done for bacterial identification are as follows:
 - ♦ Optochin susceptibility test—done to differentiate pneumococcus (sensitive) from viridans streptococci (resistant)
 - ♦ Bacitracin susceptibility test—done to differentiate group A (sensitive) from group B (resistant) *Streptococcus*.

Some of the important biochemical tests are described below. Coagulase test and other biochemical reactions for gram-positive cocci are described in the respective chapters.

Catalase Test

When a colony of any catalase producing bacteria is mixed with a drop of hydrogen peroxide (3% H₂O₂) placed on a slide, effervescence or bubbles appear due to breakdown of H₂O₂ by catalase to produce oxygen (Fig. 3.3.18).

- ❖ Catalase test is primarily used to differentiate between *Staphylococcus* (catalase positive) from *Streptococcus* (catalase negative)
- ❖ It is also positive for members of the families Enterobacteriaceae, Vibrionaceae, Pseudomonadaceae, etc.

Oxidase Test

It detects the presence of cytochrome oxidase enzyme in bacteria, which catalyzes the oxidation of reduced cytochrome by atmospheric oxygen.

- ❖ When a filter paper strip or disk, soaked in oxidase reagent is smeared with a bacterial colony producing cytochrome oxidase enzyme, the smeared area turns deep purple within 10 seconds due to oxidation of the dye to form a purple colored compound indophenol blue
- ❖ Interpretation (Fig. 3.3.19A) and examples:
 - **Oxidase positive (deep purple):** Examples include *Pseudomonas*, *Vibrio*, *Neisseria*, *Bacillus*, *Haemophilus*, etc.
 - **Oxidase negative (no color change):** Examples include; members of family Enterobacteriaceae, *Acinetobacter*, etc.

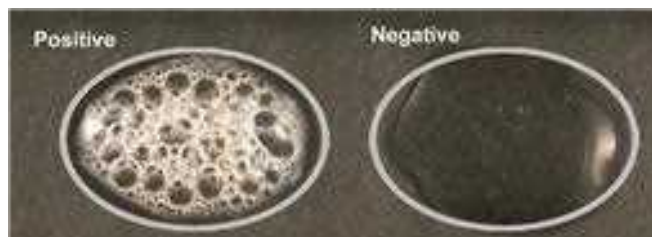
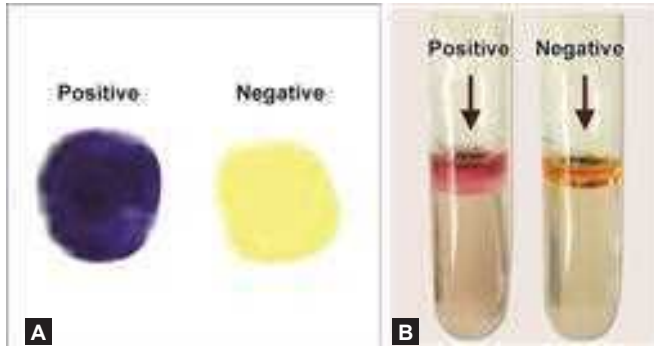


Fig. 3.3.18: Catalase test.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).



Figs 3.3.19A and B: A. Oxidase test; B. Indole test.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Indole Test

It detects the ability of certain bacteria to produce an enzyme tryptophanase that breaks down amino acid tryptophan present in the medium into indole.

- ❖ When Kovac's reagent is added to an overnight incubated broth of a bacterial colony, it complexes with indole to produce a cherry red color ring near the surface of the medium
- ❖ **Indole positive** (Fig. 3.3.19B): A red colored ring is formed near the surface of the broth. Examples include *Escherichia coli*, *Proteus vulgaris*, *Vibrio cholerae*, etc.
- ❖ **Indole negative** (Fig. 3.3.19B): Yellow colored ring is formed near the surface of the broth, e.g. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas*, *Salmonella*, etc.

Citrate Utilization Test

It detects the ability of a few bacteria to utilize citrate as the sole source of carbon for their growth, with production of alkaline metabolic products. Test is performed on Simmon's citrate medium. Citrate utilizing bacteria produce growth and a color change, i.e. original green color changes to blue (Fig. 3.3.20A)

- ❖ Citrate test is positive for *Klebsiella pneumoniae*, *Citrobacter*, *Enterobacter*, etc.
- ❖ The test is negative for *Escherichia coli*, *Shigella*, etc.

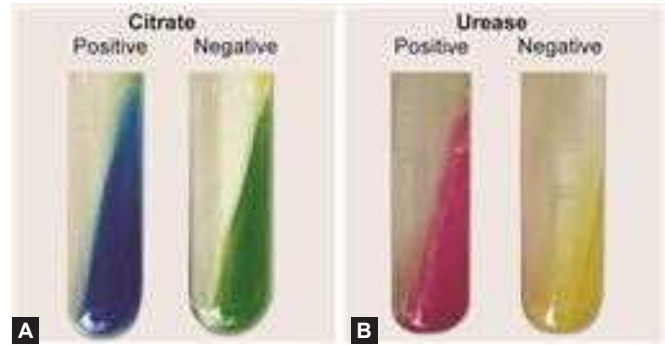
Urea Hydrolysis Test

Urease producing bacteria can split urea present in the medium to produce ammonia that makes the medium alkaline.

- ❖ Test is done on Christensen's urea medium, which contains phenol red indicator that changes to pink color in alkaline medium (Fig. 3.3.20B)
- ❖ **Urease test is positive for:** *Klebsiella pneumoniae*, *Proteus* species, *Helicobacter pylori*, *Brucella*, etc.
- ❖ **Urease test is negative for:** *Escherichia coli*, *Shigella*, *Salmonella*, etc.

Triple Sugar Iron (TSI) Agar Test

TSI is a very important medium employed widely for identification of gram-negative bacteria. TSI medium



Figs 3.3.20A and B: A. Citrate utilization test; B. Urea hydrolysis test.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

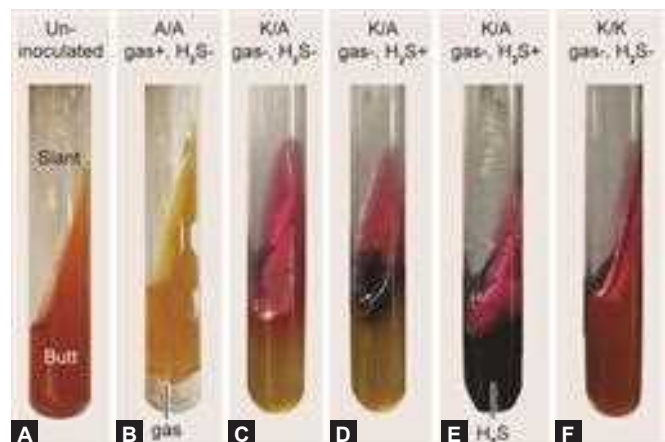
contains three sugars—glucose, sucrose and lactose in the ratio of 1:10:10 parts. Uninoculated TSI medium is red in color; has a slant and a butt (Fig. 3.3.21A). After inoculation, the medium is incubated at 37°C for 18–24 hours.

Interpretation

TSI detects three properties of bacteria, which includes fermentation of sugars to produce acid and/or gas and production of H_2S (Figs 3.3.21A to F and Table 3.3.5).

- ❖ **Acid production:** If acid is produced, the medium is turned yellow from red. Accordingly the organisms are categorized into three groups

1. **Nonfermenters:** They do not ferment any sugars, hence the medium (both slant and butt) remain red, producing Alkaline slant/Alkaline butt (K/K) reaction (Fig. 3.3.21F); e.g. *Pseudomonas* and *Acinetobacter*
2. **Glucose only fermenters:** They ferment only glucose and produce little acid only at the butt, whereas the slant remains alkaline giving rise to Alkaline slant/Acidic butt (K/A) reaction (Fig. 3.3.21C); e.g. *Salmonella* and *Shigella*
3. **≥ 2 sugars fermenters:** They ferment glucose and also ferment lactose and/or sucrose to produce large



Figs 3.3.21A to F: Triple sugar iron test.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Table 3.3.5: Various reactions in TSI with examples.	
Reactions in TSI	Examples
Acidic slant/acidic butt	≥2 sugars fermented (1) glucose, (2) lactose or/and sucrose
A/A, gas produced, no H ₂ S (Fig. 3.3.21B)	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>
Alkaline slant/acidic butt	Only glucose-fermenter group
K/A, no gas, no H ₂ S (Fig. 3.3.21C)	<i>Shigella</i>
K/A, no gas, H ₂ S produced (small amount) (Fig. 3.3.21D)	<i>Salmonella</i> Typhi
K/A, no gas, H ₂ S produced (abundant) (Fig. 3.3.21E)	<i>Proteus vulgaris</i>
K/A, gas produced, H ₂ S produced (abundant)	<i>Salmonella</i> Paratyphi B
K/A, gas produced, no H ₂ S	<i>Salmonella</i> Paratyphi A
Alkaline slant/alkaline butt	Non-fermenters group
K/K, no gas, no H ₂ S (Fig. 3.3.21F)	<i>Pseudomonas</i> , <i>Acinetobacter</i>

amount of acid so that the medium (both slant and butt) change to yellow giving rise to Acidic slant/Acidic butt (A/A) reaction (Fig. 3.3.21B); examples, *E. coli* and *Klebsiella*.

- ❖ **Gas production:** If gas is produced, the medium is lifted up or broken with cracks (Fig. 3.3.21B); examples, *E. coli* and *Klebsiella*
- ❖ **H₂S production:** If H₂S is produced, the medium changes color to black (Figs. 3.3.21D and E); examples, *Salmonella* Typhi and *Proteus vulgaris*.

Automated Systems for Bacterial Identification

Automated identification systems are revolutionary in diagnostic microbiology. They have several advantages—(i) produce faster result, (ii) can identify a wide range of

organisms with accuracy, which are otherwise difficult to identify (e.g. anaerobes) through conventional biochemical tests.

- ❖ **MALDI-TOF** (Matrix-assisted laser desorption/ionization time-of-flight), e.g. VITEK MS (bioMérieux): Refer the highlight box and Fig. 3.3.22 for details
- ❖ **VITEK 2** (bioMérieux) for automated identification and antimicrobial susceptibility test: Refer the highlight box and Figure 3.3.23 for details
- ❖ **Phoenix** (BD Diagnostics) for automated identification and antimicrobial susceptibility test
- ❖ **MicroScanWalkAway system** (Beckman Coulter) for automated identification and antimicrobial susceptibility test.

MALDI-TOF

MALDI-TOF technology (Matrix Assisted Laser Desorption Ionization Time-of-Flight) has revolutionized the identification of organisms in clinical microbiology laboratories.

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Fig. 3.3.23: VITEK 2 system with its panels (reagent cards) for identification and antimicrobial susceptibility test.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

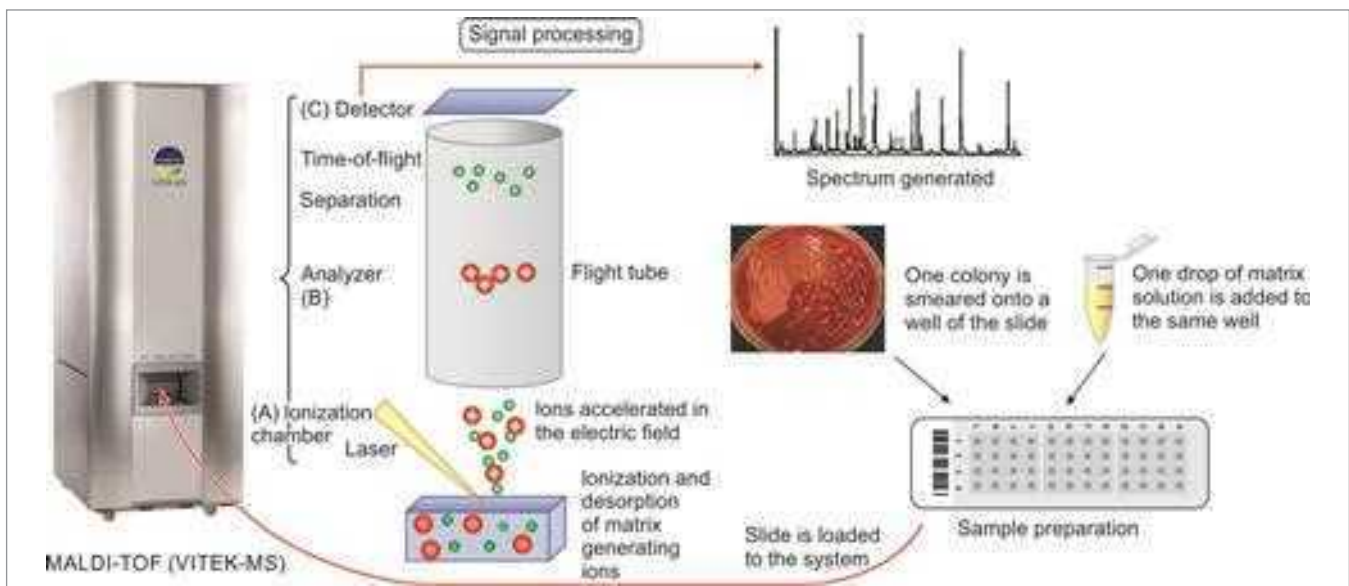


Fig. 3.3.22: MALDI-TOF and its working principle.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

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- It can identify bacteria, fungi, and mycobacteria with a turnaround time of few minutes and with absolute accuracy
- Two systems are commercially available: VITEK MS (bioMérieux) and Biotyper system (Bruker).

Principle (Fig. 3.3.22)

MALDI-TOF examines the pattern of ribosomal proteins present in the organism.

Sample preparation: The colony of an organism is smeared onto a well of the slide and one drop of matrix solution (composed of cyano-hydroxy-cinnamic acid) is added to the same well and mixed; then the slide is loaded in the system.

Steps after loading: Overall, mass spectrometry can be divided into three steps occurring in three chambers of the system.

1. **Ionization chamber:** Here, the wells are irradiated with the laser beam. The matrix absorbs the laser light causing desorption and ionization of bacterial ribosomal proteins, generating singly protonated ions
2. **Analyzer:** These ions are then accelerated into an electric field which directs them to the analyzer chamber. The analyzer (mass spectrometer) separates them according to their time-of-flight (TOF) in the flight tube. The smaller molecules travel faster, followed by the bigger, according to the mass to charge (m/z) ratio
3. **Detector:** It converts the received ion into an electrical current which is then amplified and digitized to generate a characteristic spectrum that is unique to a species due to its conserved ribosomal proteins. The test isolate is identified by comparing its spectrum with a known database.

VITEK 2 Automated System

VITEK 2 is the most widely used automated system in India; can perform both identification and antimicrobial susceptibility testing (AST) of bacteria and yeast. Principle of VITEK for identification is discussed below, VITEK for AST is discussed later in this chapter.

- It uses colorimetric reagent card containing 64 wells; each well contains an individual test substrate. Separate cards are available for gram-negative, gram-positive bacteria, fastidious bacteria and yeasts (Fig. 3.3.23)
- Substrates in the well measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis, etc. which helps in identification of the organism
- The reaction pattern obtained from the test organism is compared with the database and the identification is reported with a confidence level of matching (excellent matching to the unidentified organism)
- **Incubation:** The cards are incubated in the system at $35.5 \pm 1^\circ\text{C}$. The reading is taken once every 15 minutes by the optical system of the equipment, which measures the presence of any colored products of substrate metabolism (by advanced colorimetry method)
- The result of identification is usually available within 4–6 hours.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility test (AST) is the most important investigation carried out by a Microbiology laboratory.

- ❖ Bacteria exhibit great strain variations in susceptibility to antimicrobial agents. Therefore, AST plays a vital role to guide the clinician for tailoring the empirical antibiotic therapy to pathogen-directed therapy
- ❖ AST is performed only for pathogenic bacteria isolated from the specimen, and not for the commensal bacteria. For example, *E. coli* isolated from urine specimen should be subjected to AST, whereas *E. coli* isolated from stool is a commensal; hence, AST is not performed.

Classification of AST Methods

AST methods are classified into phenotypic and genotypic methods.

- ❖ The phenotypic methods are further grouped into-
 - Disk Diffusion Method, e.g. Kirby-Bauer's disk diffusion (DD) test
 - Dilution tests: Broth dilution and agar dilution methods
 - Epsilometer or E-test
 - Automated AST, e.g. Vitek, Phoenix and Microscan systems.
- ❖ Genotypic methods such as PCR detecting drug-resistant genes.

Disk Diffusion Method

Kirby-Bauer's disk diffusion (DD) test is the most widely used AST method. They are suitable for rapidly growing pathogenic bacteria; however, they are not suitable for slow growing bacteria. It is mostly performed from colony (called colony-DD), or performed directly from the specimens (called direct DD).

Procedure (Colony Disk Diffusion)

Antibiotic disks are impregnated on to a suitable medium lawn cultured with the test isolate.

- ❖ **Antibiotic disks:** Antibiotic disks are available commercially or prepared in-house. Sterile filter paper disks of 6 mm diameter are impregnated with standard quantity of antibiotic solution
- ❖ **Medium:** Mueller-Hinton agar (MHA) is the standard medium used for AST. For certain fastidious organisms such as *S. pyogenes* and *S. pneumoniae*, Mueller-Hinton blood agar (MHBA) containing 5% of sheep blood is used
- ❖ **Inoculum:** The inoculum is prepared by — (1) directly suspending the colony in the normal saline or (2) by inoculating into a suitable broth and incubating at 37°C for 2 hours
- ❖ **Turbidity:** The turbidity of the inoculum is adjusted to 0.5 McFarland opacity standard, which is equivalent to approximately 1.5×10^8 CFU/mL of bacteria
- ❖ **Lawn culture:** The broth is then inoculated on to the medium by spreading with sterile swabs
- ❖ **Disks impregnation:** After MHA plate is dried (3–5 min), the antibiotic disks are placed and gently pressed on its surface. Disks should be placed at least 24 mm (center to center) apart on the MHA plate. Ordinarily, maximum

up to 6 disks can be applied on a 100 mm plate (Fig. 3.3.24)

- ❖ **Incubation:** The plates are then incubated at 37°C for 16–18 hours and then interpreted.

Interpretation

The antibiotic in the disk diffuses through the solid medium, so that the concentration is highest near the site of application of the antibiotic disk and decreases gradually away from it

- ❖ Susceptibility to the drug is determined by the zone of inhibition of bacterial growth around the disk,

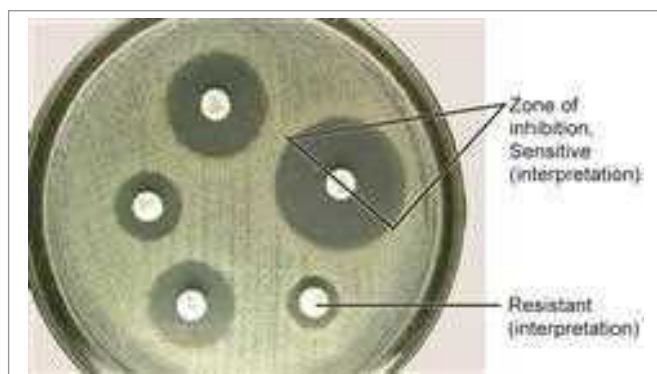


Fig. 3.3.24: Kirby–Bauer disk diffusion method.

Source: Department of Microbiology, Pondicherry Institute of Medical sciences, Puducherry (with permission).

which can be measured by using Vernier caliper (Fig. 3.3.25)

- ❖ The interpretation of zone size into sensitive, intermediate or resistant is based on the standard zone size interpretation chart, provided by CLSI or EUCAST guidelines (Table 3.3.6).

Note: CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) are international agencies, which provide guidelines for zone size interpretation, and are updated annually.

Direct Disk Diffusion Test

The direct DD (or direct susceptibility test, i.e. DST) test can be performed when results are required urgently and single pathogenic bacterium is suspected in the specimen (for positively-flagged blood culture bottle, sterile body fluids or urine).



Fig. 3.3.25: Vernier caliper.

Table 3.3.6: Commonly used disk concentrations and interpretation of disk diffusion test (as per CLSI 2020 guideline).

Breakpoints for Enterobacteriaceae (CLSI 2020)							
Antimicrobial agents	Disk strength (µg)	Zone diameter break points (mm)			MIC Breakpoints (µg/mL)		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ceftazidime	30	≥ 21	18-20	≤ 17	≤ 4	8	≥ 16
Ceftriaxone	30	≥ 23	20-22	≤ 19	≤ 1	2	≥ 4
Ciprofloxacin	5	≥ 26	22-25	≤ 21	≤ 0.25	0.5	≥ 1
Piperacillin-tazobactam	100/10	≥ 21	18-20	≤ 17	≤ 16	32-64	≥ 128
Amikacin	30	≥ 17	15-16	≤ 14	≤ 16	32	≥ 64
Meropenem	10	≥ 23	20-22	≤ 19	≤ 1	2	≥ 4
Colistin	-	-	-	-	-	≤ 2	≥ 4
Breakpoints for gram-positive organisms (CLSI 2020)							
Antimicrobial agents	Disk strength (µg)	Zone diameter break points (mm)			MIC Breakpoints (µg/mL)		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Cefoxitin (<i>S.aureus</i>)	30	≥ 22	-	≤ 21	≤ 4	-	≥ 8
Levofloxacin (<i>S.aureus</i>)	5	≥ 19	16-18	≤ 15	≤ 1	2	≥ 4
Cotrimoxazole (<i>S.aureus</i>)	1.25/23.75	≥ 16	11-15	≤ 10	≤ 2/38	-	≥ 4/76
Tetracycline (<i>S.aureus</i>)	30	≥ 19	15-18	≤ 14	≤ 4	8	≥ 16
Linezolid (<i>S.aureus</i>)	30	≥ 21	-	≤ 20	≤ 4	-	≥ 8
Vancomycin (<i>S.aureus</i>)	-	-	-	-	≤ 2	4-8	≥ 16
Ampicillin (<i>Enterococcus</i>)	10	≥ 17	-	≤ 16	≤ 8	-	≥ 16
Linezolid (<i>Enterococcus</i>)	30	≥ 23	21-22	≤ 20	≤ 2	4	≥ 8
Vancomycin (<i>Enterococcus</i>)	30	≥ 17	15-16	≤ 14	≤ 4	8-16	≥ 32

Abbreviation: CLSI, Clinical and Laboratory Standards Institute.

- ❖ Here, the specimen is directly inoculated uniformly on to the surface of an agar plate and the antibiotic disks are applied
- ❖ The results of the direct-DD test should always be verified by performing AST from the colony subsequently
- ❖ This test is of no use when mixed growth is suspected in the specimen, e.g. pus, stool, sputum, etc.

Dilution Tests

Here, the antimicrobial agent is serially diluted, each dilution is tested with the test organism for antimicrobial susceptibility test and the MIC is calculated.

- ❖ MIC (minimum inhibitory concentration) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation
- ❖ Depending upon whether the dilutions of the antimicrobial agent are made in agar or broth, there are two types of dilution tests.

Broth Dilution Method

It is of two types: macro broth dilution (performed in tubes) and micro broth dilution (performed in microtiter plate). The procedure of macro broth dilution is explained below.

- ❖ Serial dilutions of the antimicrobial agent in Mueller-Hinton broth are taken in tubes and each tube is inoculated with a fixed amount of suspension of the test organism. A control organism of known sensitivity should also be tested. Tubes are incubated at 37°C for 18 hours
- ❖ The MIC is determined by noting the lowest concentration of the drug at which there is no visible growth, i.e. broth appears clear (Fig. 3.3.26)
- ❖ The minimum bactericidal concentration (MBC) can be obtained by subculturing from each tube (showing no growth) onto a nutrient agar plate without any antimicrobial agent. The tube containing the lowest concentration of the drug that fails to show growth, on subculture, is the MBC of the drug for that test strain (Fig. 3.3.26).

Agar Dilution Method

Here, the serial dilutions of the drug are prepared in molten agar and poured into Petri dishes. The test strain is spot inoculated. This method is more convenient than broth dilution and has the added advantage of:

- ❖ Several strains can be tested at the same time by using the same plate
- ❖ It directly measures the MBC; there is no need of subculturing as it is done with broth dilution method.

Epsilon meter or E-test

This is a quantitative method of detecting MIC by using the principles of both dilution and diffusion of antibiotic into the medium.

- ❖ It uses an absorbent strip containing predefined gradient (serial dilution) of antibiotic concentration immobilized along its length

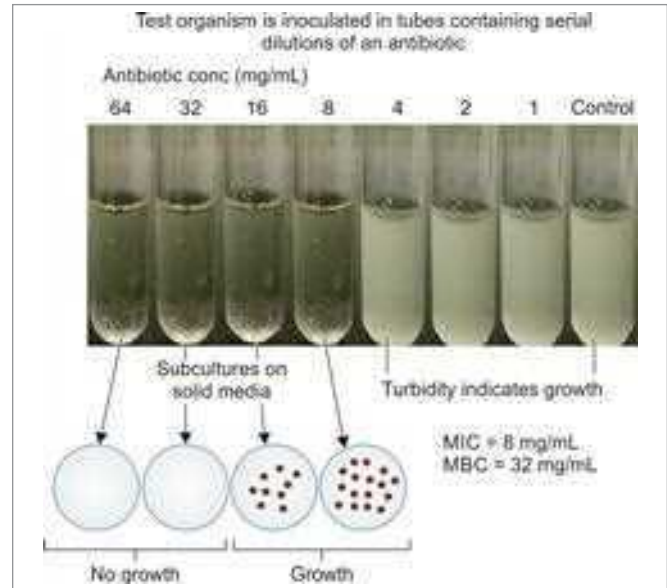


Fig. 3.3.26: Macro broth dilution method.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ It is applied to a lawn inoculum of a bacterium. Following incubation of the test organism, an elliptical zone of inhibition is produced surrounding the strip
- ❖ The antibiotic concentration at which the ellipse edge intersects the strip, is taken as MIC value (Fig. 3.3.27).

Automated Antimicrobial Susceptibility Tests

Several automated systems are available now, such as:

- ❖ VITEK 2 identification and antimicrobial sensitivity system (bioMerieux)
- ❖ Phoenix System (Becton Dickinson)
- ❖ Micro Scan Walk Away system.

Most systems are computer assisted and have sophisticated softwares to analyze the growth rates and determine the antibiotic susceptibility report. They work by the principle of micro broth dilution. They use commercially available panels that contain antibiotic solution in serial dilutions. They provide more rapid results compared with traditional methods.

VITEK 2 Automated System for AST

VITEK 2 is the most widely used automated AST system in India; can perform AST of bacteria and yeasts; whereas other automated AST systems can perform AST of bacteria only, not for yeasts.

- ❖ It works on the principle of microbroth dilution
- ❖ It uses a reagent card containing 64 wells, which contain doubling dilution of antimicrobial agents. The organism suspension (of 0.5 McFarland turbidity) is added to the wells (Figure 3.3.23 and Table 3.3.7)
- ❖ The cards are incubated in the system at 35.5 ± 1°C. The reading is taken once in every 15 minutes by the optical system of the equipment. It measures the presence of



Fig. 3.3.27: Epsilonometer or E-test.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

any turbidity (by nephelometry) which indicates the organism has grown in that antibiotic well

- ❖ The MIC is determined as the highest dilution of the antimicrobial agent which inhibits the growth of organism and there is no turbidity in the well
- ❖ The results are available within 8-10 hours for gram-negative bacilli and 16-18 hours for gram-positive cocci.

Role of MIC-based Methods

The clinical microbiology laboratory should perform a MIC-based method whenever possible. This is because the MIC-based methods are much superior to disk diffusion test for a number of reasons.

- ❖ **For confirming the AST results** obtained by disk diffusion tests, as they are more reliable and accurate than the latter
- ❖ AST for bacteria for which disk diffusion test is not standardized should only be performed by MIC testing
- ❖ For performing AST for slow growing bacteria, such as tubercle bacilli
- ❖ **To select the most appropriate antibiotic:** Lower is the MIC, better is the therapeutic efficacy. If >1 antimicrobial agents are found susceptible, then the antibiotic having the lowest MIC (when compared with the susceptibility breakpoint) should be chosen for therapy. This is better guided by calculating the **therapeutic index**; which is the ratio of susceptibility breakpoint divided by the MIC of the test isolate. It is discussed in detail in Chapter 26
- ❖ **MIC-guided therapy:** There are certain situations, where the antibiotic treatment is MIC-guided
 - Clinical conditions such as endocarditis, pneumococcal meningitis/pneumonia, etc.

Table 3.3.7: Antibiotic panel used in VITEK AST card for Enterobacteriaceae.

Enterobacteriaceae	Antimicrobial agent used in VITEK
First line	Ampicillin
First line	Amoxicillin- clavulanic acid
First line	Ciprofloxacin
First line	Ceftriaxone
First line	Ceftazidime
First line	Gentamicin
Second line	Cefoperazone-sulbactam
Second line	Piperacillin- tazobactam
Second line	Cefepime
Second line	Amikacin
Restricted	Meropenem
Restricted	Doripenem
Restricted	Ertapenem
Restricted	Imipenem
Restricted	Colistin
Restricted	Tigecycline

- Vancomycin for *S. aureus*: vancomycin should be avoided if MIC is >1µg/mL.

Molecular Methods (Detecting Drug-resistant Genes)

Molecular methods are available targeting specific drug resistant genes; for example:

- ❖ *mecA* gene for MRSA detection by PCR
- ❖ *van* gene detection for vancomycin resistant *S. aureus* (VRSA) and vancomycin resistant *Enterococcus* (VRE) by PCR
- ❖ GeneXpert for detection of rifampicin resistance (in *M. tuberculosis*) and line probe assay for detection of resistance to many anti-tubercular drugs.

Interpretation of AST

The result of AST (whether disk diffusion or MIC based methods) is always expressed in four interpretative categories.

- ❖ **Susceptible (S):** Indicates that the antibiotic is clinically effective when used in standard therapeutic dose
- ❖ **Intermediate (I):** Indicates that the antibiotic is not clinically effective when used in standard dose; but may be active when used in increased dose. Antibiotics reported as 'I' should be avoided for treatment if alternative agents are available
- ❖ **Susceptible dose dependent (SDD):** Indicates that the antibiotic will be clinically active only if given in increased dose. This category is available only for few agents such as cefepime for Enterobacteriaceae
- ❖ **Resistant (R):** Indicates that the antibiotic is NOT clinically effective when used in either standard dose or increased dose; and therefore should not be included in the treatment regimen.

Choice of Antibiotics to be Included in Panel

It is neither possible nor desirable to test the susceptibility against all the drugs. The panel of the drugs to be tested against an isolate depends upon various factors:

- ❖ **Clinically indicated:** Include only those antibiotics for testing which are clinically indicated either as a first-line agent or alternative-agent for the suspected infective syndrome and the organism isolated
- ❖ **Organism isolated,** for which the AST is going to be performed, and its **local resistance pattern** (as per hospital antibiogram)
- ❖ **Intrinsic resistance:** The antibiotics to which the isolate is intrinsically resistant must be excluded from the test panel
- ❖ **Antimicrobial agent:** Both oral and parenteral antibiotics should be included in the testing panel, as they can be administered based on clinical severity (i.e parenteral drugs for severe and oral for mild illness)
- ❖ **Locally available** antibiotic at the hospital
- ❖ **Site of action:** Only those antibiotics should be tested which are active in the site. For example, the following antibiotics should be EXCLUDED from the testing, depending upon the clinical specimen
 - **When testing for CSF isolate:** 1st- and 2nd-generation cephalosporins and cephamycins, clindamycin, macrolides, tetracyclines, fluoroquinolones and the antibiotics which are administered only by oral route should be excluded from testing
 - **When testing for urine isolates:** Antibiotics such as clindamycin, macrolide and chloramphenicol are excluded from testing
 - **When testing for respiratory isolates:** daptomycin is excluded from testing.
- ❖ **Predicting susceptibility:** Testing for an antibiotic (or a group) is not necessary, if their susceptibility can be predicted from the susceptibility result of another antibiotic. For example:
 - Ceftriaxone result can be predicted from that of cefotaxime for Enterobacteriaceae; therefore only one agent is tested and the result can be extrapolated to the other
 - For MRSA: Cefoxitin or oxacillin are considered as surrogate marker for MRSA, and therefore the susceptibility result can be extrapolated for all other beta lactams.

Selective or Cascade Reporting

Based on the spectrum of action and local resistance pattern, the antimicrobial agents tested for an organism can be grouped into (Table 3.3.7):

- ❖ **First-line antimicrobials:** These agents are narrow spectrum in action, resistance of the organism to these agents is usually high. This should also include the empirical antibiotics currently administered to the patient

- ❖ **Second-line antimicrobials:** Broad spectrum antimicrobials, resistance of the organism to these agents is usually moderate

- ❖ **Restricted antimicrobials:** Extended-spectrum antimicrobials, resistance of the organism to these agents is usually very low.

The clinical microbiology laboratory may test for the full antibiotic panel; however, should report only few of them in a selective manner.

- ❖ If the organism is found susceptible to all antimicrobial agents tested, only first-line agents are reported. The report of second-line and restricted antimicrobials is suppressed

- ❖ If the organism is found susceptible to second-line and restricted antimicrobial agents tested but resistant to first-line agents, then only the first- and second-line agents are reported. The report of restricted antimicrobials is suppressed.

This concept is called as **selective or cascade reporting;** which aims at encouraging the clinicians to use narrow spectrum antimicrobials if found susceptible and to reserve the overuse of broad and extended spectrum antimicrobials. Selective reporting is not applicable to organisms for which only a few antibiotics are available as therapeutic option and resistance is not very high; e.g. *Salmonella* or *Burkholderia pseudomallei*.

SEROLOGY

The serological tests play an important role in the diagnosis of various bacterial infections. These include detection of either antigen or antibody in the serum of the patient, by various immunological assays—precipitation, agglutination, ELISA, and rapid test. The detail of these methods is discussed in Chapter 12. The important serological tests used for diagnosis of bacterial infections include:

- ❖ Widal test for enteric fever
- ❖ Standard agglutination test for brucellosis
- ❖ Microscopic agglutination test and rapid diagnostic test for leptospirosis
- ❖ Weil-Felix test for rickettsial infections
- ❖ VDRL (venereal disease research laboratory) test and RPR (rapid plasma reagin) test for syphilis
- ❖ ELISA is available for various bacterial diseases such as chlamydial infections, brucellosis, *Mycoplasma pneumoniae*, leptospirosis and rickettsial infections, etc.

MOLECULAR METHODS

Molecular methods are broadly grouped into amplification based and non-amplification based methods.

Nucleic acid amplification techniques (NAATs) have been increasingly used in diagnostic microbiology. Various NAATs used are:

- ❖ Polymerase chain reaction (PCR)
- ❖ Real-time polymerase chain reaction (rt-PCR)

- ❖ Loop mediated isothermal amplification (LAMP)
- ❖ Automated PCR such as Biofire FilmArray
- ❖ Automated real-time PCR such as cartridge based nucleic acid amplification test (CBNAAT): Used for tuberculosis, described in Chapter 63.

Non-amplification molecular methods include DNA hybridization method e.g. line probe assay.

Polymerase Chain Reaction (PCR)

PCR is a technology in molecular biology used to amplify a single or few copies of a piece of DNA to generate millions of copies of DNA. It was developed by Kary B Mullis (1983) for which he and Michael Smith were awarded the Nobel prize in Chemistry in 1993.

Principle of PCR

PCR involves three basic steps.

1. **DNA extraction from the organism:** This involves lysis of the organisms and release of the DNA which may be done by various methods—boiling, adding enzymes (e.g. lysozyme,

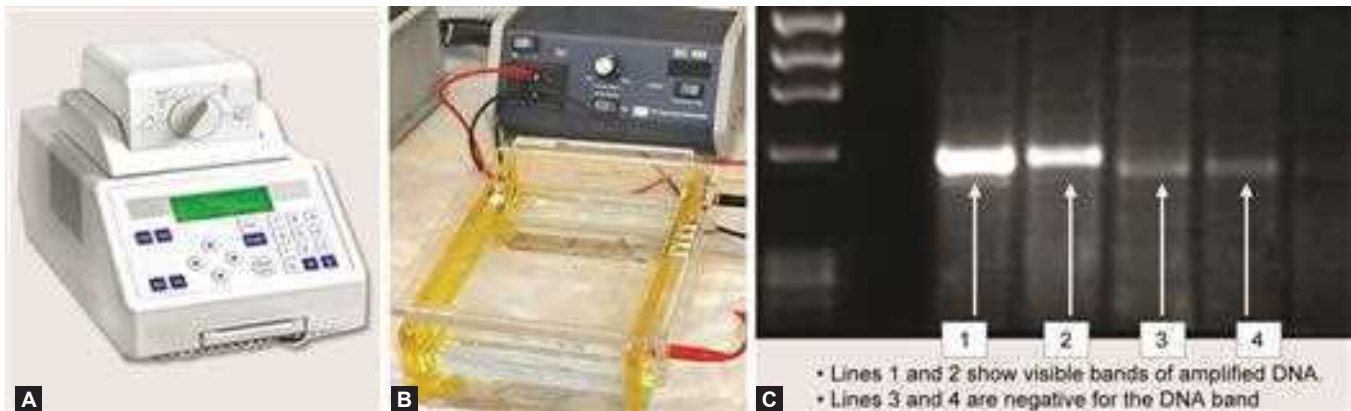
proteinase K), etc. DNA extraction kits are also available commercially.

2. **Amplification of extracted DNA:** This is carried out in a special PCR machine called thermocycler (Fig. 3.3.28A). The extracted DNA is subjected to repeated cycles (30–35 numbers) of amplification which takes about 3–4 hours. Each amplification cycle has three steps (Fig. 3.3.29).

- **Denaturation at 95°C:** This involves separation of the dsDNA into two separate single strands
- **Primer annealing (55°C):** Primer is a short oligonucleotide complementary to a small sequence of the target DNA. It anneals to the complementary site on the target ssDNA
- **Extension of the primer (72°C):** This step is catalyzed by Taq Polymerase enzyme which keeps on adding the free nucleotides to the growing end of the primer. Taq Polymerase is a special type of DNA polymerase (isolated from the plant bacterium *Thermus aquaticus*), capable of withstanding the high temperature of PCR reaction.

Contd..

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Figs 3.3.28A to C: A. Thermocycler machine (Eppendorf); B. Gel electrophoresis of amplified product; C. Visualization of amplified DNA under UV light.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

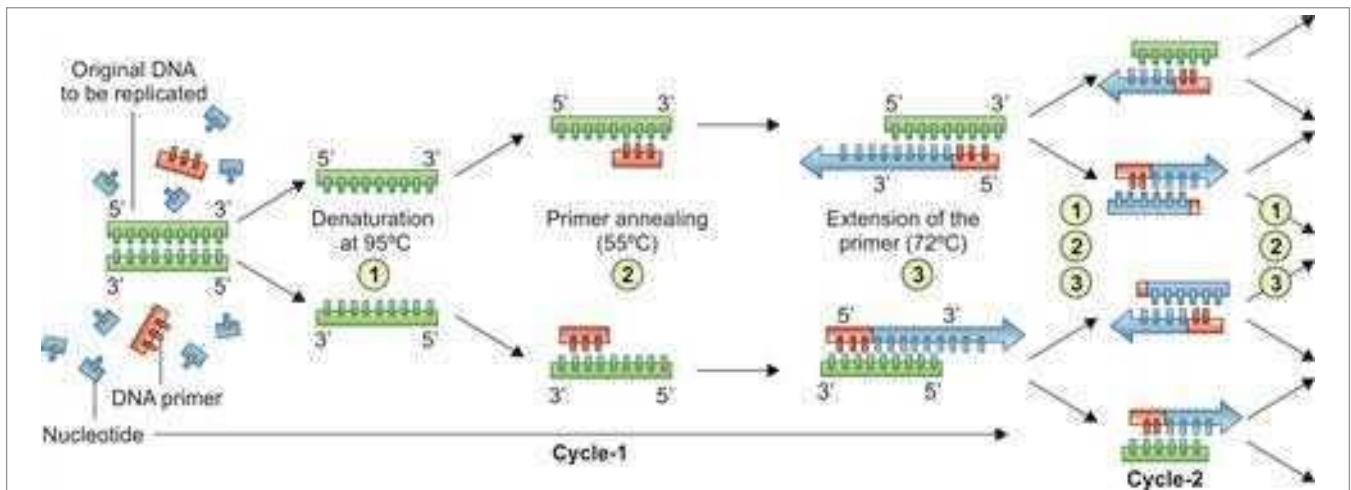


Fig. 3.3.29: Polymerase chain reaction cycle—3 basic steps of amplification.

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3. **Gel electrophoresis of amplified product:** The amplified DNA is electrophoretically migrated according to their molecular size by performing agarose gel electrophoresis (Fig. 3.3.28B). The amplified DNA forms clear band, which can be visualized under ultraviolet (UV) light (Fig. 3.3.28C).

Applications of PCR

PCR is now a common and often indispensable technique used in medical diagnostics and research laboratories for a variety of applications. It has the following advantages compared to the conventional culture methods:

- ❖ **More sensitive:** It can amplify very few copies of a specific DNA, so it is more sensitive
- ❖ **More specific:** Use of primers targeting specific DNA sequence of the organism makes the PCR assays highly specific
- ❖ PCR can be done to amplify the DNA of the organism: (1) either directly from the sample, or (2) to confirm the organism grown in culture
- ❖ PCR can also detect the organisms that are highly fastidious or noncultivable by conventional culture methods
- ❖ PCR can be used to detect the genes in the organism responsible for drug resistance (e.g. *mec A* gene detection in *Staphylococcus aureus*)
- ❖ Detects genetic diseases, such as sickle cell anemia, phenylketonuria, and muscular dystrophy.

Disadvantages of PCR

Conventional PCR detects only the DNA, but not the RNA (latter can be detected by reverse transcriptase PCR).

- ❖ **Qualitative, not quantitative:** Conventional PCR can only detect the presence or absence of DNA. It cannot quantitate the amount of DNA of the organism present in the sample. This is possible by real time PCR
- ❖ **Viability:** PCR cannot differentiate between viable or nonviable organisms. It only detects the presence of DNA in the sample which may be extracted from viable or nonviable organism
- ❖ **False-positive amplification:** It may occur due to contamination with environmental DNA. Hence, strict asepsis should be maintained in the PCR laboratory
- ❖ **False-negative:** The PCR inhibitors present in some specimens, such as blood, feces, etc. may inhibit the amplification of target DNA.

Modifications of PCR

1. **Reverse transcriptase PCR (RT-PCR):** Conventional PCR amplifies only the DNA. For amplifying RNA, RT-PCR is done
 - After RNA extraction, the first step is addition of reverse transcriptase enzyme that converts RNA into DNA. Then, the amplification of DNA and gel

documentation steps are similar to that described for conventional PCR

- It is extremely useful for detection of RNA viruses or 16S rRNA genes of the organisms.
2. **Nested PCR:** It is modification of PCR, where two rounds of PCR amplification are carried out by using two primers that are targeted against two different DNA sequences of same organism
 - The amplified products of the first round PCR is subjected to another round of amplification using a second primer which targets the same organism but a different DNA sequence
 - More sensitive: Double round of amplification yields high quantity of DNA
 - More specific: Use of two primers targeting two regions of DNA of the same organisms makes the test more specific
 - Application: Nested PCR is used for detection of *Mycobacterium tuberculosis* (targeting IS6110 gene) in samples
 - Disadvantage: There is more chance of contamination of the PCR tubes, which may lead to false-positive results.
 3. **Multiplex PCR:** It uses more than one primer which can detect many DNA sequences of several organisms in one reaction
 - Syndromic approach: Multiplex PCR is useful for the diagnosis of the infectious diseases that are caused by more than one organism
 - For example, for the etiological diagnosis of pyogenic meningitis, different primers targeting the common agents of pyogenic meningitis, such as pneumococcus, meningococcus and *H. influenzae* can be added simultaneously in the same reaction tube
 - Contamination issues: There is a risk of the reaction tubes being contaminated with environmental DNA.

Biofire FilmArray

Biofire FilmArray (bioMérieux) is a completely automated multiplex nested PCR system where all the steps from sample preparation to amplification, detection and analysis are performed automatically by the system; giving result in about one hour (Fig. 3.3.30).

- ❖ Four panels are available such as respiratory, gastrointestinal, meningitis-encephalitis and blood culture identification panels; each panel comprises of primers targeting 20–25 common pathogens infecting the respective systems
- ❖ Except for the blood panel which detects pathogens from positively flagged blood culture bottles, the other panels detect pathogen(s) directly from the specimen
- ❖ It has excellent sensitivity and specificity with turnaround time of 1 hour. However the higher cost of the panels limits its wide use.



Fig. 3.3.30: Biofire FilmArray system.

Source: BioMérieux (with permission).

Real-time PCR (rt-PCR)

It is based on PCR technology, which is used to amplify and simultaneously detect or quantify a targeted DNA molecule on a real-time basis. Reverse transcriptase real-time PCR formats can detect and quantify RNA molecules of the test organism in the sample on a real-time basis.

It uses a different thermocycler than the conventional PCR. It is very expensive, 5-10 times more than the cost of conventional PCR (Fig. 3.3.31).

Advantages: Real-time PCR has many advantages over a conventional PCR, such as:

- ❖ **Quantitative:** rt-PCR can quantitate the DNA or RNA present in the specimen; hence can be used for monitoring the disease progression in response to treatment, e.g. viral load monitoring in HIV or hepatitis B viral infection
- ❖ **Takes less time:** In rt-PCR, the amplification can be visualized simultaneously during the process of amplification unlike the conventional PCR where there is an extra-step of gel electrophoresis to detect the amplicons
- ❖ **Contamination rate** is extremely less
- ❖ **Sensitivity and specificity** of rt-PCR assays are much more than the conventional PCR.

Detection of amplification products of real-time PCR:

The detection of amplified nucleic acid in a real-time PCR reaction is carried out by using a variety of fluorogenic molecules which may be either nonspecific or specific.

- ❖ **Nonspecific methods:** They use SYBR green dye that stains any nucleic acid nonspecifically
- ❖ **Specific methods:** They use fluorescent labeled oligonucleotide probe which binds (i.e. hybridizes) only to a particular region of amplified nucleic acid. Three types of hybridization probes are commonly used:
 - TaqMan or hydrolysis probe



Fig. 3.3.31: Real time PCR.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- Molecular beacon
- Fluorescence resonance energy transfer (FRET) probe.

Post-amplification melting curve analysis is used for quantitation of the nucleic acid load. Analysis of result of Real time PCR (for influenza and COVID-19) has been explained in detail in Chapters 66 and 67 respectively.

Loop Mediated Isothermal Amplification (LAMP)

LAMP is an isothermal nucleic acid amplification technique. It provides several advantages over PCR.

- ❖ Amplification is carried out at a constant temperature of 60–65° (in contrast to alternating temperature cycles in PCR)
- ❖ **Polymerase with strand displacement ability:** The isothermal nature of LAMP assay is due to the use of specific DNA polymerase enzymes which have additional strand displacement capacity, e.g. polymerase derived from *Geobacillus stearothermophilus*
- ❖ **Cheaper and easy to perform:** It does not require thermocycler or gel electrophoresis
- ❖ More specific, as it uses 4 primers targeting different regions on the target gene
- ❖ **Detection:** Amplicons are detected directly by naked eyes either by turbidity or visual fluorescence detection (in contrast to gel documentation in PCR)
- ❖ **Uses:** LAMP assay has been approved for tuberculosis
- ❖ **Drawback:** It shows high false positive results due to cross contamination between the reaction tubes.

Nucleic Acid Probes

Nucleic acid probes are radiolabeled or fluorescent labelled pieces of single stranded DNA or RNA, which can be used for the detection of homologous nucleic acid by hybridization.

- ❖ Hybridization is the technique in which two single strands of nucleic acid come together to form a stable double stranded molecule
- ❖ There are two types of nucleic acid probes—DNA probes (hybridizes with DNA) and RNA probes (hybridizes with RNA)
- ❖ Nucleic acid probes are used to detect the specific nucleic acid from:
 1. Clinical samples directly *or*
 2. Following amplification of small quantity nucleic acid present in the clinical sample (e.g. in real time PCR) *or*
 3. From culture isolates.
- ❖ Following enzymatic digestion of the extracted nucleic acid—so that it detects only the specific DNA fragment from the mixture (e.g. in Southern blot)
- ❖ **Disadvantage:** For detection of nucleic acid from clinical specimens, probe-based methods have low sensitivity than amplification-based methods
- ❖ **Line probe assay** is a classic example of molecular test that uses nucleic acid probe technology. It is used for diagnosis of tuberculosis (described in Chapter 63).

MICROBIAL TYPING

Microbial typing refers to characterization of an organism beyond its species level.

Applications: Microbial typing is an important tool for hospital microbiologists and epidemiologists. It is used to determine the relatedness between different microbial strains of the same species and thereby it helps to:

- ❖ Investigate outbreaks: All isolates tracked in an outbreak should belong to a similar type
- ❖ Determine the source and routes of infections
- ❖ Trace cross-infection, i.e. transmission of healthcare-associated pathogens
- ❖ Differentiate virulent strains from avirulent strains of same species
- ❖ Differentiate between recurrence and infection with new strain
- ❖ Evaluate the effectiveness of control measures.

Classification: Typing methods are broadly classified as phenotypic and genotypic methods.

Characteristic of Typing Methods

A good typing method should have the following properties:

- ❖ **Typeability:** Ability of the method to type and generate a result for each isolate tested
- ❖ **Reproducibility:** Ability to produce similar results when tested repeatedly in different laboratories
- ❖ **Discriminative power:** Ability to generate distinct units of information making fine distinctions between the types at the subspecies level
- ❖ **Practicality:** Ease of use and interpretation, cost and affordability.

In general, genotypic methods are more reliable and have better reproducibility and discriminative power than phenotypic methods, however they are expensive.

Phenotypic Methods

Various phenotypic methods are as follows.

- ❖ **Bacteriophage typing:** Strains of an organism can be further differentiated into subspecies level based on their susceptibility to bacteriophages. Phage typing is obsolete now; was used in the past for the typing of—*Staphylococcus aureus*, *Salmonella* Typhi and *Vibrio* species, etc.
- ❖ **Bacteriocin typing:** Bacteriocin is an antibiotic like proteinaceous substance produced by one bacterium that inhibits other strains of the same or other closely related bacteria. Bacteriocin typing is based on the ability of a strain to produce particular bacteriocin which inhibits the growth of a set of selected indicator strains. It is done for—*Shigella sonnei* (colicin typing) and *Pseudomonas* (pyocin typing)
- ❖ **Biotyping:** It refers to intra-species classification based on different biochemical properties of the organism. It is used for:
 - *Corynebacterium diphtheriae*: It is classified into gravis, intermedius and mitis
 - *Vibrio cholerae* O1 is classified into two biotypes—(1) Classical and (2) El Tor.
- ❖ **Antibiogram typing:** It classifies the organism into different groups based on their resistance pattern to different antimicrobials. Since antimicrobial susceptibility testing is routinely done in any hospital, this typing system provides the first clue to a microbiologist about outbreaks occurring in a hospital
- ❖ **Serotyping:** It refers to a typing method based on the antigenic property of an organism. This is the most widely used and the most reliable phenotypic typing method. Serotyping is done for many organisms; important ones are given below
 - *Streptococcus* (Lancefield grouping, based on carbohydrate antigen)
 - Based on capsular antigen—For example, pneumococcus, meningococcus and *Haemophilus influenzae*
 - Based on somatic antigen—For example, *Escherichia coli*, *Shigella*, *Salmonella* and *Vibrio cholerae*.

Genotypic Methods

Restricted Fragment Length Polymorphism (RFLP)

1. **Digestion of DNA:** This is done by using two or more restriction enzymes which cleave the DNA from a bacterial strain at different sites so that multiple DNA fragments are generated
2. **Southern blot to detect DNA fragments:** The DNA fragments are separated by electrophoresis and transferred to a nitrocellulose membrane and then are detected by using specific DNA probes (Chapter 3.4).

The pattern of fragments generated by different strains tracked in an outbreak can be compared to know the relatedness between the strains.

Ribotyping

Ribotyping is a type of RFLP analysis which is done on chromosomal DNA coding for ribosomal RNA.

Pulse Field Gel Electrophoresis (PFGE)

PFGE is considered as a **gold standard** typing method. It is used for epidemiological investigation of pathogenic organisms. It comprises of the following steps.

- ❖ **Lysis:** First, the bacterial suspension is loaded into an agarose suspension. This is done to protect the chromosomal DNA from mechanical damage by immobilizing it into agarose blocks. Then the bacterial cell is lysed to release the DNA. The agarose-DNA suspension is also known as pulse mold
- ❖ **Digestion of DNA:** The bacterial DNA is treated with rare cutting restriction enzymes so that it yields the generation of less number of larger size DNA fragments (in contrast to frequent cutting restriction enzymes used in RFLP which produces a large number of smaller fragments)
- ❖ **Electrophoresis:** The larger pieces of DNA are subjected to pulse field gel electrophoresis by applying electric current and altering its direction at a regular interval (in contrast to the conventional agarose gel electrophoresis done to separate the smaller fragments where the current is applied in a single direction)
- ❖ **Analysis:** The fragments generated by PFGE of various strains obtained during an outbreak are compared manually or by computer software BioNumerics.

The drawbacks of PFGE are (1) it is labor intensive, (2) requires many days to perform the procedure, (3) requires

skilled personnel to interpret the results and (4) requires computer-assisted analysis of banding patterns.

Amplified Fragment Length Polymorphism (AFLP)

AFLP uses the principle of performing RFLP of the bacterial DNA followed by PCR.

- ❖ The genomic DNA is digested by restriction enzymes, followed by use of adaptors to ligate to the sticky ends of the restriction fragments
- ❖ PCR amplification of the restriction fragments is carried out by using primers complementary to both adaptor and restriction site sequences
- ❖ The amplified fragments are separated and visualized on denaturing polyacrylamide gels.

Sequencing-based Methods

The nucleotide sequence of a microbial gene can be obtained by specially designed equipment called *sequencer*. The variability within the sequences of particular genes can be used to determine the relatedness of bacteria. Sequence analysis can be done by two ways.

- ❖ **Nucleotide sequencing:** It is performed by Sanger sequencer. Sequencing can be done at one or multiple genes
 - *Sequencing at single gene:* This is useful for: (i) identification of organism, (ii) to find out polymorphism within the gene (called as single nucleotide polymorphism or SNP analysis)
 - *Sequencing at multiple genes* (called as multi-locus sequence typing or MLST).
- ❖ **Whole genome sequencing:** This is done an advanced sequencer called as next generation sequencer
 - It involves determining the complete DNA sequence of an organism's genome at a single time
 - Currently, it is largely been used as a research tool, in future the whole genome sequence data may serve as an important tool to guide therapeutic intervention.

EXPECTED QUESTIONS

I. Write short notes on:

1. Gram staining.
2. Selective media.
3. Anaerobic culture methods.
4. Automations in Microbiology.
5. Polymerase chain reaction.
6. Real time PCR.

II. Multiple Choice Questions (MCQs):

1. **Recommended transport medium for stool specimen suspected to contain *Vibrio cholerae* is:**
 - a. Buffered glycerol saline medium
 - b. Venkatraman-Ramakrishnan medium
 - c. Nutrient broth
 - d. Blood agar

2. Which is an enriched medium?

- a. Selenite F broth
- b. Peptone water
- c. MacConkey agar
- d. Chocolate agar

3. Agar concentration required to prepare nutrient agar is:

- a. 2%
- b. 6%
- c. 0.25%
- d. 0.5%

4. Robertson cooked meat broth is an example of:

- a. Enriched media
- b. Enrichment media
- c. Anaerobic media
- d. Nutrient media

5. Blood culture bottle contains:

- a. BHI broth
- b. Peptone water broth
- c. Selenite F broth
- d. Tryptic soy broth

6. The three components of PCR involves all, except:

- a. DNA extraction
- b. Amplification
- c. Gel documentation
- d. Blotting

Answers

1. b 2. d 3. a 4. c 5. a 6. d

General Bacteriology: Bacterial Genetics

CHAPTER 3.4

CHAPTER PREVIEW

- Principles of Bacterial Genetics
 - Bacterial DNA
 - Bacterial RNA
 - Polypeptide Synthesis
- Plasmid
- Mutation
 - Horizontal Gene Transfer
 - Transformation
 - Transduction
- Lysogenic Conversion
- Conjugation
- Transposition
- Gene Transfer by Artificial Methods

PRINCIPLES OF BACTERIAL GENETICS

Bacterial genetics deals with the study of heredity and gene variations seen in bacteria. All hereditary characteristics of the bacteria are encoded in their DNA (deoxyribonucleic acid). Bacterial DNA is present in chromosome as well in extrachromosomal genetic material as plasmid.

BACTERIAL DNA

Bacteria possess a single haploid chromosome, comprising of super coiled circular double stranded DNA of 1 mm length. The bacterial DNA lacks basic proteins. However, some bacteria have a linear chromosomal DNA and some have two chromosomes (e.g. *Vibrio cholerae*). Bacteria do not have a true nucleus; but the genetic material is located in an irregularly-shaped region called the nucleoid. There is no nuclear membrane or nucleolus.

Structure of DNA (Watson and Crick Model)

The bacterial DNA molecule is composed of two strands of complementary nucleotides that are coiled together in the form of a double helix (Fig. 3.4.1) as described first by Watson and Crick.

- ❖ **Each strand is composed of three elements:** It has a backbone of deoxyribose sugar and phosphate groups. The nitrogenous bases are attached to the sugar group. The terms nucleotide and nucleoside are often used to describe the components of the DNA strand—
 - Nucleoside = Sugar + nitrogenous base
 - Nucleotide = Sugar + nitrogenous base + phosphate.
- ❖ There are four nitrogenous bases:
 - Two purines—adenine (A) and guanine (G)
 - Two pyrimidines—thymine (T) and cytosine (C).
- ❖ **Pairing:** The two DNA strands are held together by hydrogen bonds occurring between the nitrogenous bases on the opposite strands. The pairing follows a specific rule—

- Adenine of one strand binds with thymine (A-T) of other strand by double hydrogen bonds
- Guanine of one strand binds with cytosine (G-C) of other strand by triple hydrogen bonds.
- ❖ Hence, in a molecule of DNA, the number of adenine molecules is equal to that of thymine, and the number of guanines is equal to cytosines
- ❖ The ratio of A + T to G + C is constant for each species but varies widely from one bacterial species to another.

DNA Replication

In eukaryotes, during DNA replication, the two strands of the double helix unwind from one another and separate. Each strand acts as template for a new DNA strand which is

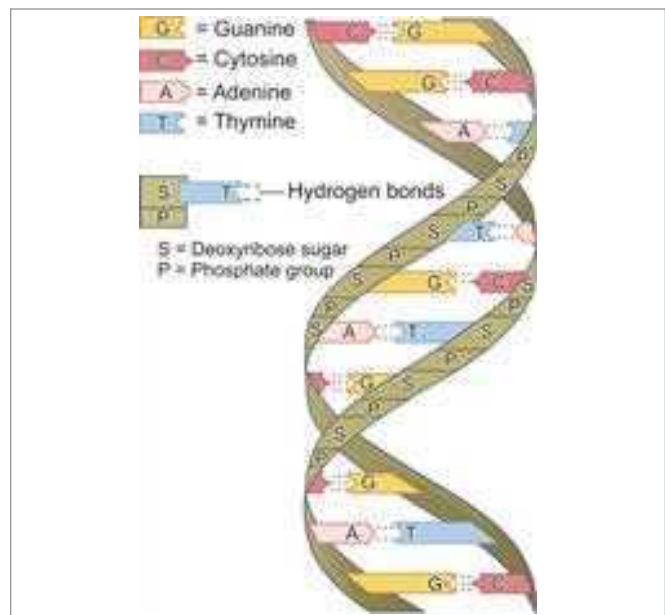


Fig. 3.4.1: Structure of DNA.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

synthesized through complementary base pairing—A with T, and G with C. In prokaryotic cells, DNA replication takes place in a similar way with some differences as follows:

- ❖ **Bidirectional replication:** Here, DNA helix is unwound at a region called replication fork, which is the site at which the DNA synthesis occurs and individual strands are replicated. It is seen in *E. coli*
- ❖ **Rolling-circle mechanism:** This pattern of DNA replication occurs during bacterial conjugation and during the reproduction of viruses.

The DNA replication in bacteria is catalyzed by several replication enzymes such as—

- ❖ **Helicase:** It is responsible for DNA unwinding
- ❖ **Topoisomerase** (e.g. DNA gyrase in *E. coli*): It relieves the tension generated by rapid unwinding by removing the super twists
- ❖ **DNA polymerase:** It forms a complementary strand synthesis by adding nucleotides to the growing end of the strand. It catalyzes the synthesis of DNA in the 5' to 3' direction while reading the DNA template in the 3' to 5' direction. DNA polymerase III plays the major role in replication, although it is probably assisted by polymerase I. It is thought that polymerases I and II participate in the repair of damaged DNA
- ❖ **DNA ligase:** It helps in the joining of the fragments.

BACTERIAL RNA

RNA (ribonucleic acid) is structurally similar to DNA, except for two differences.

- ❖ In sugar—ribose is present instead of deoxyribose and
- ❖ In nitrogenous base—uracil replaces thymine.

There are three different types of RNA in a cell, messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). The main function of RNA is protein synthesis.

POLYPEPTIDE SYNTHESIS

Gene is a segment of DNA that stores information for a particular polypeptide synthesis. The genetic information that is stored in DNA is transcribed into RNA and then translated to form the particular polypeptide.

Genetic Code

Codon: It is a sequence of three nucleotide bases present on mRNA that stores the information of an amino acid synthesis. It was discovered by Nirenberg and Khorana (1968).

- ❖ **Sense codons:** There are 64 codons, out of which 61 are sense codons, each directs the production of a single amino acid. As there are only 20 amino acids, so more than one codon exist for the same amino acid
- ❖ **Non-sense codons:** The remaining three codons (UGA, UAG, and UAA) do not code for any amino acids and are involved in the termination of translation; hence called as stop codons

- ❖ **Start codon:** It is the first codon of an mRNA from which the translation begins. The most common start codon is AUG which codes for methionine in eukaryotes and modified methionine [N-Formyl methionine (fMet)] in prokaryotes.

Anticodon: It is a set of three nucleotide bases present on tRNA that is complementary to the nucleotide bases of codon on mRNA.

Transcription

Transcription is a process, in which a particular segment of DNA is copied into RNA by the enzyme RNA polymerase. Since DNA acts as a template for synthesis of mRNA, therefore, the bases in mRNA are complementary to that of DNA.

Translation

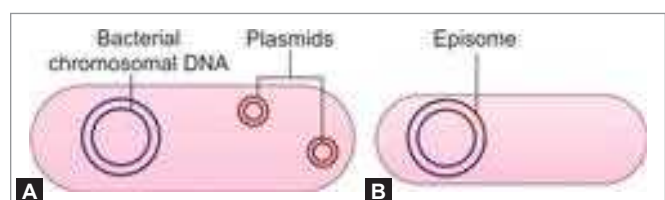
In translation, the mRNA transcribed from DNA is decoded by a ribosome to produce a specific amino acid chain, or polypeptide. It occurs in the cytoplasm of the bacteria and proceeds in four phases:

1. **Initiation:** The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon of mRNA
2. **Elongation:** The tRNA transfers an amino acid to the adjacent tRNA, corresponding to the next codon
3. **Translocation:** The ribosome then moves (translocates) to the next mRNA codon to continue the process, creating an amino acid chain
4. **Termination:** When a stop codon is reached, the ribosome releases the polypeptide.

PLASMID

Plasmids are the extrachromosomal ds circular DNA molecules that exist in a free state in the cytoplasm of bacteria (Fig. 3.4.2A) and also found in some yeasts.

- ❖ **Not essential:** Plasmids are not essential for life; bacteria may gain or lose plasmid during their lifetime
- ❖ **Numbers:** They may be present singly or in multiple numbers—up to 40 or even more per cell
- ❖ **Independent replication:** Plasmids are capable of replicating independently. They can behave as replicons, possessing an origin of replication and other genes that help in replication
- ❖ **Episome:** Sometimes, the plasmid may integrate with chromosomal DNA of bacteria and such plasmids are



Figs 3.4.2A and B: Plasmids.

called as episomes. They replicate along with bacterial chromosome (Fig. 3.4.2B)

- ❖ **Curing:** The process of eliminating the plasmids from bacteria is known as curing. It may occur spontaneously or may be induced by treatment of the host cells with substances that inhibit plasmid replication without affecting the host cell, such as acridine, radiations, thymine starvation, and growth at higher temperatures.

Classification of Plasmids

Plasmids can be classified in many ways:

1. **Based on ability to perform conjugation:**
 - Conjugative plasmids: Some plasmids have an ability to transfer themselves to other bacteria by means of conjugation. These are called self-transmissible or conjugative plasmids
 - Non-conjugative plasmids: They are also called as nontransmissible plasmids as they cannot transfer themselves.
2. **Based on compatibility between the plasmids,** they can be grouped into:
 - Compatible plasmids: Different plasmids can exist in a single bacterial cell only if they are compatible to each other
 - Incompatible plasmids: If two plasmids are not compatible, one or the other will be rapidly lost from the cell. They normally share the same replication or partition mechanisms, hence compete with each other.
3. **Based on function,** there are five main classes of plasmids:
 - a. **Fertility or F-plasmids:** They contain *tra*-genes, which code for the expression of sex pili that help in bacterial conjugation by forming the conjugation tube
 - b. **Resistance (R) plasmids:** They contain genes that code for resistance to various antibiotics
 - c. **Col plasmids:** They contain genes that code for bacteriocins (antibiotic-like protein substances produced by bacteria that can kill other bacteria)
 - d. **Virulence plasmids:** They code for certain virulence factors and toxins that help in bacterial pathogenesis. Examples include:
 - ◆ Heat labile and heat stable toxin of *E. coli*
 - ◆ Siderophore production
 - ◆ Adherence antigens (K88 plasmid in *E. coli*).
 - e. **Metabolic plasmids:** They enable the host in various metabolic activities:
 - ◆ Digestion of unusual substances, e.g. toluene and salicylate, camphor, etc.
 - ◆ Urease synthesis
 - ◆ Nitrogen fixation.

Plasmid as Vector

Plasmids by their ability to transfer DNA from one cell to another, they have become important vectors in genetic

engineering. Plasmids contain certain sites where genes can be inserted artificially by recombinant DNA technology. Such plasmids can be used for various purposes such as protein production, gene therapy, etc. (described later in this chapter).

GENE TRANSFER IN BACTERIA

Bacteria undergo genetic variation and acquire new gene through a mechanism called mutation. Following which, the newly acquired genes are transferred either vertically to their offsprings during cell division or horizontally to other bacteria in the surrounding.

MUTATION

Definition: Mutation is a random, undirected heritable variation caused by a change in the nucleotide sequence of the genome of the cell.

Mutation can involve any of the numerous genes present in bacterial chromosome or rarely plasmid. The frequency of mutation ranges from 10^{-2} to 10^{-10} per bacterium per division.

Mutations occur in one of the two ways:

1. **Spontaneous mutations:** Mutations that occur naturally in any dividing cells that arise occasionally without adding any mutagen
2. **Induced mutations:** These mutations on the other hand, are as a result of exposure of the organism to a **mutagen**, an agent capable of inducing mutagenesis. Examples of mutagens include—
 - Physical agents, e.g. ultraviolet (UV) radiations— cytosine and thymine are more vulnerable to UV rays
 - Chemical agents, e.g. alkylating agents, 5-bromouracil and acridine dyes.

Mutation is a natural event, taking place all the time, in all dividing cells. Most mutants go unrecognized as the mutation may be lethal or may involve some minor functions that may not be expressed. Mutation is best appreciated when it involves a function, which can be readily observed by experimental methods. For example, *E. coli* mutant that loses its ability to ferment lactose can be readily detected on MacConkey agar.

Mutation can affect any gene and hence may modify any characteristic of the bacterium, for example—

- ❖ Sensitivity to bacteriophages
- ❖ Loss of ability to produce capsule or flagella
- ❖ Loss of virulence
- ❖ Alteration in colony morphology
- ❖ Alteration in pigment production
- ❖ Drug susceptibility
- ❖ Biochemical reactions
- ❖ Antigenic structure.

The practical importance of bacterial mutation is mainly in the field of drug resistance and the development of live vaccines.

Classification of Mutation Types

Mutations may occur in two ways—

1. **Small-scale mutations:** They are more commonly seen in bacteria. Examples include (1) point mutations—occur at a single nucleotide, (2) addition or deletion of single nucleotide pair
2. **Large-scale mutations** occur in chromosomal structure: These include deletion or addition of several nucleotide base pairs or gene duplications.

Various types of mutations observed in bacteria are described in Table 3.4.1.

Detection and Isolation of Mutants

Mutation can be recognized both by genetic and phenotypic methods.

- ❖ **Gene sequencing:** It is the method of choice currently used
- ❖ **Phenotypic methods:** They are less commonly used now
 - **Fluctuation test:** It demonstrates spontaneous mutations in bacteria
 - **Replica plating method:** It is used to demonstrate auxotrophic mutants (does not grow in absence of a particular nutrient)
 - **Ames test:** It is used to test the carcinogenicity of a mutagen.

HORIZONTAL GENE TRANSFER

Horizontal gene transfer occurs in bacteria by several methods, such as:

- ❖ Transformation (uptake of naked DNA)
- ❖ Transduction (through bacteriophage)
- ❖ Lysogenic conversion
- ❖ Conjugation (plasmid mediated via conjugation tube).

TRANSFORMATION

Definition

Transformation is a process of random uptake of free or naked DNA fragment from the surrounding medium by a bacterial cell and incorporation of this DNA fragment into its chromosome in a heritable form.

Natural transformation has been studied so far only in certain bacteria—*Streptococcus*, *Bacillus*, *Haemophilus*, *Neisseria*, *Acinetobacter* and *Pseudomonas*.

Mechanism of Transformation

When bacteria lyse, they release large amounts of dsDNA into the surrounding environment. Their uptake depends up on the competency of the bacteria present in the surroundings.

Table 3.4.1: Types of mutations.

Forward mutations	
Substitutions at single nucleotide base pair	
At DNA Level	
Transition	It is a point mutation that changes a purine nucleotide to another purine (A ↔ G) or a pyrimidine nucleotide to another pyrimidine (C ↔ T)
Transversion	It refers to the substitution of a purine for a pyrimidine or vice versa in DNA, (C/T ↔ A/G)
At codon level	
Silent mutation	The new codon codes for the same amino acid, e.g. AGG ↔ CGG, both code for arginine
Neutral mutation	The new codon forms different but functionally equivalent amino acid: AAA (lysine) AGA (arginine)
Missense mutation	The new codon codes for a different amino acid
Nonsense mutation	The new codon is a stop codon which causes termination, e.g. CAG (Glutamine) ↔ UAG (stop)
Addition or deletion at single or many nucleotide base pairs	
Frame-shift mutation	Any addition or deletion of base pairs that is not a multiple of three results in a shift in the normal reading frame of the coded message forming new set of triplet codon. They are usually very deleterious and may lead to synthesis of nonfunctional proteins
Reverse mutations	
It is a second mutation that nullifies the effect of the first mutation and results in gaining back the function of the wild phenotype	
True reversion	<ul style="list-style-type: none"> • A true reverse mutation converts the mutant nucleotide sequence back to the wild-type sequence. • AAA (Lysine) forward mutation GAA (Glutamine) reverse mutation AAA (Lysine) (wild type) → (mutant) → (wild type)
Equivalent reversion	<ul style="list-style-type: none"> • Second mutation produces a different codon which codes for the same amino acid of wild type sequence • UCC (Serine) forward mutation GAA (Cystine) reverse mutation AAA (Serine) (wild type) → (mutant) → (wild type)
Suppressor mutation	It is a second mutation in a different gene that reverts the phenotypic effects of an already existing mutation

Competency for Transformation

Competent bacteria refers to the cells multiplying in log phase of cell division and expressing certain transformation promoting factors called competence factors.

- ❑ Bacteria expressing competence factors (e.g. *S. pneumoniae*) can uptake any DNA fragment irrespective of source
- ❑ But competence factors are not expressed by all bacteria that mediate transformation e.g. *Haemophilus influenzae*. In such case, the uptake of DNA occurs only from the closely related species.

The transformation frequency of very competent cells is around 10^{-3} for most genera. Steps involved in transformation are as follows (Fig. 3.4.3):

1. A long dsDNA fragment comes in contact with a competent bacterium and binds to DNA-binding protein present on its surface and then it is nicked by a nuclease
2. One strand is degraded by the recipient cell exonucleases
3. The other strand associates with a competence specific protein and is internalized, which requires energy expenditure
4. The single strand enters into the cell and is integrated into the host chromosome in place of the homologous region of the host DNA.

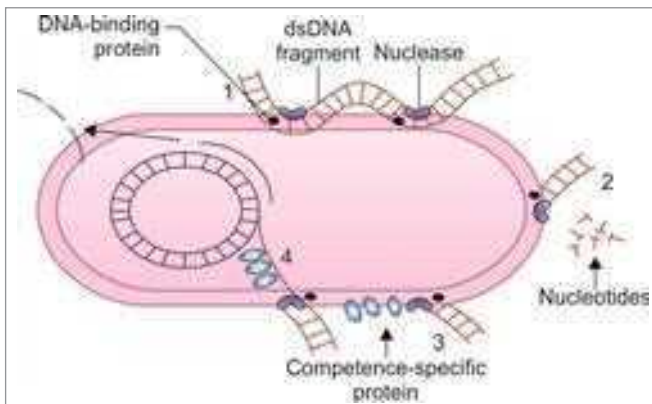


Fig. 3.4.3: Transformation.

Griffith Experiment

The famous Griffith experiment (1928) on mice using pneumococci strains provided the direct evidence of existence of transformation.

- ❖ Griffith found that mice died when they were injected with a mixture of live nonencapsulated pneumococci and heat killed capsulated pneumococci strains. However, neither of which separately proved fatal to mice (Fig. 3.4.4)
- ❖ He stated that the live nonencapsulated strains were transformed into the capsulated strains due to transfer of the capsular genes released from the lysis of the killed capsulated strains, which was confirmed later by Avery, Macleod and McCarty in 1944.

TRANSDUCTION**Definition**

Transduction is defined as transmission of a portion of DNA from one bacterium to another by a bacteriophage (bacteriophage is a virus that infects and multiplies inside the bacterium).

Mechanism of Transduction

During the transmission of bacteriophages from one bacterium to other, a part of the host DNA may accidentally get incorporated into the bacteriophage and then gets transferred to the recipient bacterium. This leads to acquisition of new characters by the recipient bacterium coded by the donor DNA.

Bacteriophages can perform two types of life cycle inside the host bacteria.

1. **Lytic or virulent cycle:** Bacteriophage multiplies in host cytoplasm, produces a large number of progeny phages, which subsequently, are released causing death and lysis of the host cell
2. **Lysogenic or temperate cycle:** In contrast to virulent cycle, here the host bacterium is unharmed. The

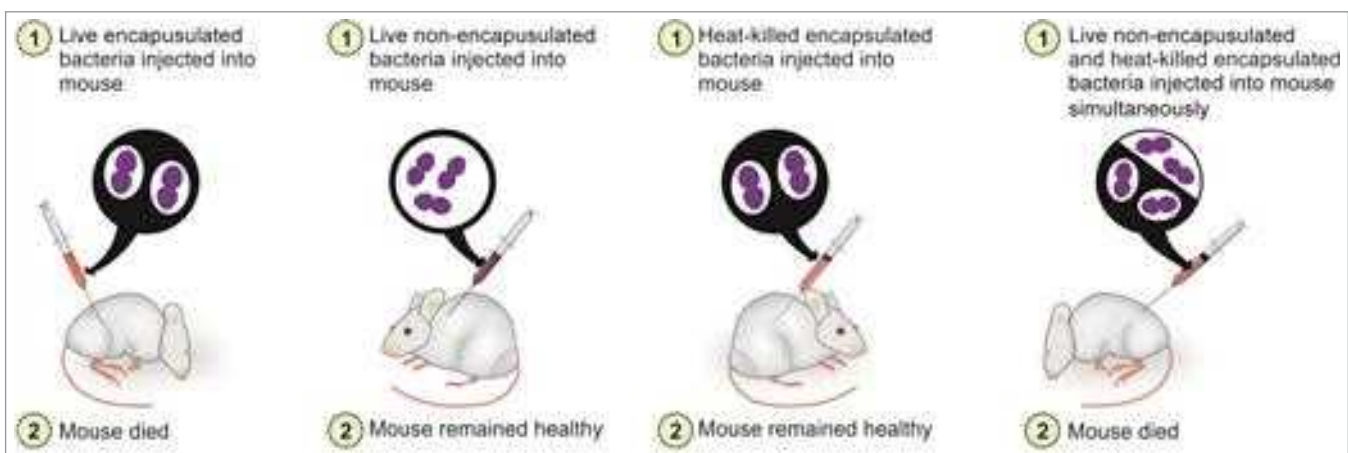


Fig. 3.4.4: Griffith experiment demonstrating transformation.

phage DNA remains integrated with the bacterial chromosome as the prophage, which multiplies synchronously with bacterial DNA. However, when the phage DNA tries to come out, it is disintegrated from host chromosome, comes out into the cytoplasm, and behaves as a lytic phage. It replicates to produce daughter phages, which are subsequently released by host cell lysis.

Types of Transduction

Transduction is of two types, either generalized or restricted.

Generalized Transduction

It involves transfer of any part of the donor bacterial genome into the recipient bacteria. Generalized transduction usually occurs as a result of defective assembly during the lytic cycle of virulent and some temperate phages.

- ❖ Packaging errors may happen occasionally due to defective assembly of the daughter phages. Instead of their own DNA, a part of host DNA may accidentally be incorporated into the daughter bacteriophages
- ❖ The resulting bacteriophage (called transducing phage) often injects the donor DNA into another bacterial cell but does not initiate a lytic cycle as the original phage DNA is lost
- ❖ The donor DNA may have three fates inside the recipient bacterium (Fig. 3.4.5):
 - **Abortive transduction:** About 70–90% of the transferred DNA is not integrated with the recipient bacterial chromosome, but often is able to survive and express itself. Such bacteria containing this non-integrated, transduced DNA are called abortive transductants
 - **Stable gene transfer:** The donor DNA gets integrated with recipient bacterial chromosome
 - **Unstable gene transfer:** In some cases, the donor DNA gets disintegrated by the host cell enzymes.

Restricted or Specialized Transduction

In contrast to generalized transduction, the restricted transduction is capable of transducing only a particular genetic segment of the bacterial chromosome that is present adjacent to the phage DNA.

It occurs as a result of defect in the disintegration of the lysogenic phage DNA from the bacterial chromosome.

- ❖ Restricted transduction has been studied intensively in the 'lambda' phage of *E. coli*
- ❖ When a prophage (i.e. lysogenic bacteriophage is integrated with the bacterial chromosome) leaves the host chromosome, portions of the bacterial chromosome present adjacent to the phage DNA may get wrongly excised along with it
- ❖ Such transducing phages carrying a part of bacterial DNA in addition to their DNA, when infecting another

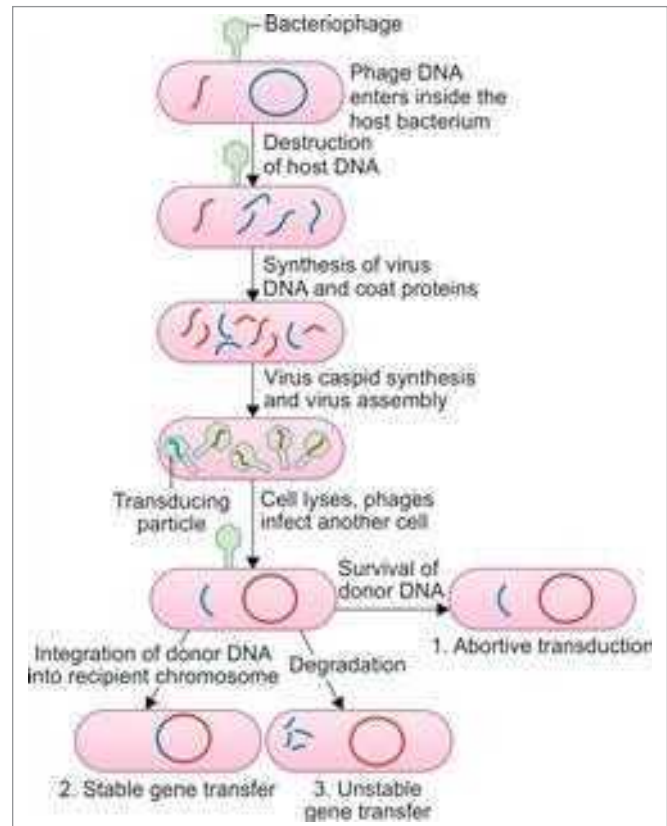


Fig. 3.4.5: Generalized transduction.

bacterium, the transfer of the donor DNA takes place in two ways (Fig. 3.4.6)

1. The entire transducing genome (i.e. phage DNA + donor DNA) acts as a prophage and gets integrated to the recipient's chromosome. This occurs if the recipient bacterium is already infected by another helper bacteriophage
2. A crossover between the donor DNA and a part of recipient DNA—leads to an integration of the donor DNA into the recipient chromosome and a part of recipient DNA into the phage DNA.

Role of Transduction

In addition to chromosomal DNA, transduction is also a method of transfer of episomes and plasmids.

- ❖ **Drug resistance:** Transduction may be a mechanism for the transfer of bacterial genes coding for drug resistance; for example, plasmid coded penicillin resistance in staphylococci
- ❖ **Treatment:** Transduction has also been proposed as a method of genetic engineering in the treatment of some inborn metabolic defects.

LYSOGENIC CONVERSION

During the temperate or lysogenic life cycle, the phage DNA remains integrated with the bacterial chromosome

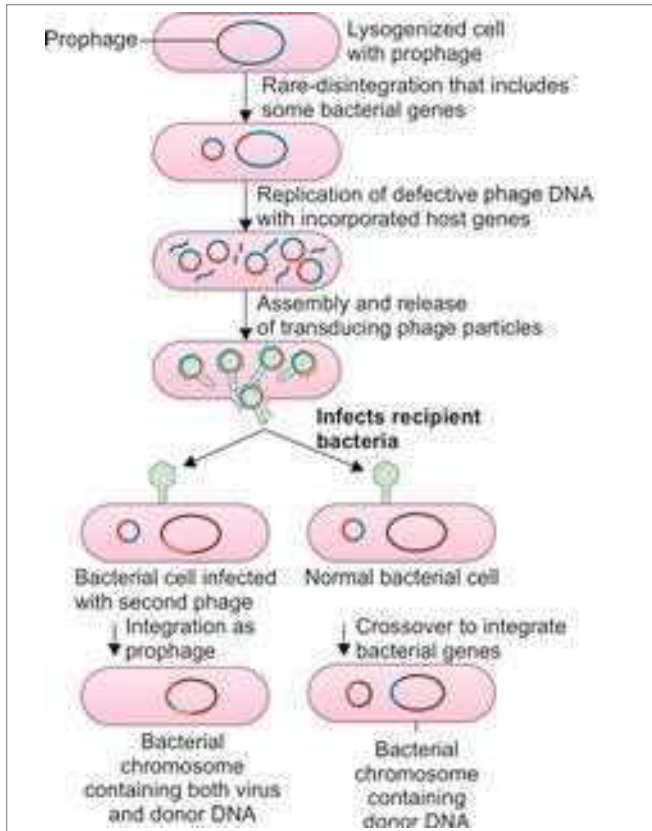


Fig. 3.4.6: Restricted transduction.

as a prophage, which multiplies synchronously with the bacterial DNA.

- ❖ The prophage acts as an additional chromosomal element which encodes for new characters and is transferred to the daughter cells. This process is known as lysogeny or lysogenic conversion
- ❖ **Imparts toxigenicity to the bacteria:** Phage DNA may be responsible for bacterial virulence by coding for their toxin production. For example, in *Corynebacterium diphtheriae*, the diphtheria toxin is coded by a lysogenic phage DNA which is integrated with the bacterial chromosome. Elimination of the phage from a toxigenic strain renders the bacterium nontoxigenic

Phage Coded Toxins

Bacterial toxins that are coded by lysogenic phages include:

- Diphtheria toxin
- Cholera toxin
- Verocytotoxin of *E. coli*
- Streptococcal pyrogenic exotoxin (SPE)—A and C
- Botulinum toxin C and D

- ❖ In lysogenic conversion, the phage DNA itself behaves as the new genetic element, in contrast to transduction where the phage acts only as a vehicle carrying bacterial genes.

CONJUGATION

Conjugation refers to the transfer of genetic material from one bacterium (donor or male) to another bacterium (recipient or female) by mating or contact with each other and forming the conjugation tube. It was discovered first by Lederberg and Tatum (1946).

F⁺ X F⁻ Mating

The F⁺ cell (also called as the donor or the male bacterium) contains a plasmid called F factor or fertility factor. The bacteria lacking the F factor are called as recipient or female bacteria or F⁻ cells.

- ❖ F factor is a conjugative plasmid; carries genes that encode for the formation of sex pilus (that helps in conjugation) and self plasmid transfer
- ❖ The F pilus brings the donor and nearby recipient cells close to each other and form a conjugation tube that bridges between the donor and recipient cells (Fig. 3.4.7A)
- ❖ During conjugation, the plasmid DNA replicates by the rolling-circle mechanism, and a copy moves to the recipient bacterium through the conjugation tube. Then, in the recipient, the entering strand is copied to produce complete F factor with ds DNA
- ❖ As a result, the recipient (F⁻) becomes (F⁺) cell and can in turn conjugate with other (F⁻) cells. Therefore, it is said that this character of maleness (F⁺) in bacteria is transmissible or infectious
- ❖ During F⁺ X F⁻ conjugation, chromosomal genes from donor bacterium may rarely be transferred along with F factor. Here, though the donor chromosomal gene may undergo recombination with the recipient chromosome; but with a lower frequency.

Hfr Conjugation

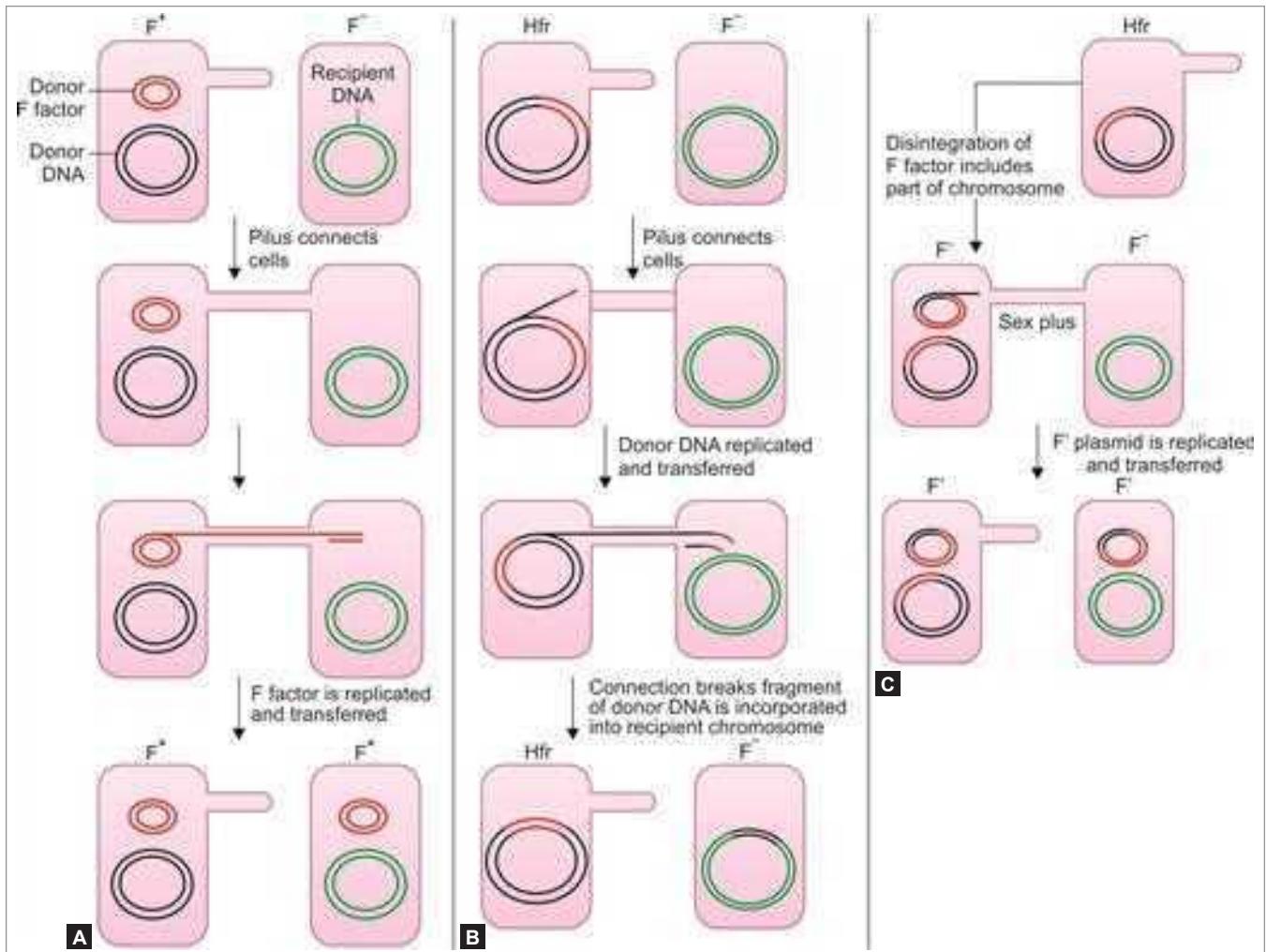
F factor being a plasmid, it may integrate with bacterial chromosome and behave as episome.

- ❖ Such donor cells are able to transfer chromosomal DNA to recipient cells with high frequency in comparison to F⁺ cells, therefore, named as Hfr cells (high frequency of recombination)
- ❖ During conjugation of Hfr cell with an F⁻ cell, only few chromosomal genes along with a part of the F factor get transferred. Connection between the cells usually breaks before the whole genome is transferred
- ❖ As the entire F factor does not get transferred, hence following conjugation, F⁻ recipient cells do not become F⁺ cells (Fig. 3.4.7B).

F' Conjugation

The conversion of a F⁺ cell into a Hfr cell is reversible.

- ❖ When the F factor reverts from the integrated to free-state, it may sometimes carry with it some chromosomal DNA from the adjacent site of its attachment. Such an F



Figs 3.4.7A to C: Bacterial conjugation: **A.** F⁺ X F⁻ mating; **B.** Hfr X F⁻ mating; **C.** F' X F⁻ mating.

factor carrying some chromosomal DNA is named as F' factor (F prime factor)

- ❖ When F' cell conjugates with a recipient (F⁻), it transfers the host DNA incorporated with it along with the F factor. The recipient becomes F' cell. This process is called sexduction (Fig. 3.4.7C).

Conjugation plays an important role in the transfer of plasmids coding for antibacterial drug resistance [resistance transfer factor (RTF), see the box in the preceding text] and bacteriocin production [Colicinogenic (Col) factor].

Colicinogenic (Col) Factor

The bacteriocin production in bacteria is plasmid coded which may be transferred by conjugation. Such plasmids are called as the col factors. Bacteriocins are the antibiotic-like substances produced by one bacterium that inhibit other bacteria. Bacteriocin produced by coliform bacteria are called as colicin. Bacteria other than coliforms also produce similar kind of substances e.g. pyocin

by *Pseudomonas*, diphthericin by *Corynebacterium diphtheriae*.

Role of Conjugation in Bacterial Drug Resistance

Conjugation is also an important method of transfer of plasmids coding for multiple drug resistance among bacteria.

- ❑ **R factor** (or the resistance factor) is a plasmid which has two components. (R factor = RTF + r determinants)
 - Resistance transfer factor (RTF): It is the plasmid responsible for conjugational transfer (similar to F factor)
 - Resistance determinant (r): R factor can have several r determinants and each r determinant coding for resistance to one drug.
- ❑ Sometimes, the R factor dissociates and both RTF and the r determinants exist as separate plasmids. In such cases, the resistance is not transferable though the host cell remains drug resistant
- ❑ In addition to r determinants, the RTF can also attach to other genes; for example, genes coding for enterotoxin and hemolysin production in some enteropathogenic *E. coli*. Mutational and transferrable drug resistance have been discussed in Chapter 3.5.

Fate of the Donor DNA

Following horizontal gene transfer, the donor DNA either gets degraded by host nucleases or may integrate with recipient chromosome by a method called **recombination**. The donor DNA integrates with the recipient chromosome either as a replacement piece (usually occurs in transformation) or as an extra piece (as seen in other methods).

TRANSPOSITION

Transposons or transposable elements are the bacterial genes that are capable of intracellular transfer between chromosome to chromosome, plasmid to plasmid, and chromosome to plasmid or vice versa and the process of such intracellular transfer of transposons is called as transposition. As transposons move around the genome in a cut-and-paste manner, they are also called **jumping genes** or **mobile genetic elements**.

- ❖ Transposition does not require any DNA homology between transposon and the site of insertion. It is, therefore, different from recombination
- ❖ Unlike plasmids, transposons are not self-replicating and are dependent on chromosomal or plasmid DNA for replication
- ❖ Transposons were first discovered in the 1940s by Barbara McClintock during her studies on maize genetics for which she won the Nobel prize in 1983
- ❖ Transposons are also discovered in the virus and in eukaryotic genome.

Types of Transposons

Insertion Sequence Transposon

The simplest form of transposon is an insertion sequence. It is about 1-2 kilo basepairs (kbp) in length and consists of a **transposase gene** (that helps in transposition) which is flanked at both the ends by **inverted repeat sequences** of nucleotides, i.e. nucleotide sequences complementary to each other but in the reverse order (Figs 3.4.8A and B).

Because of this feature, each strand of the transposon can form a single-stranded loop carrying the transposase gene, and a double stranded stem formed by hydrogen bonding between the terminal inverted repeat sequences.

Composite Transposon

They are larger transposons carrying additional genes, such as genes coding for antibiotic resistance or toxin production in the center and both the ends are flanked by insertion sequences that are identical or very similar in sequence (Fig. 3.4.8C).

GENE TRANSFER BY ARTIFICIAL METHODS

GENETIC ENGINEERING

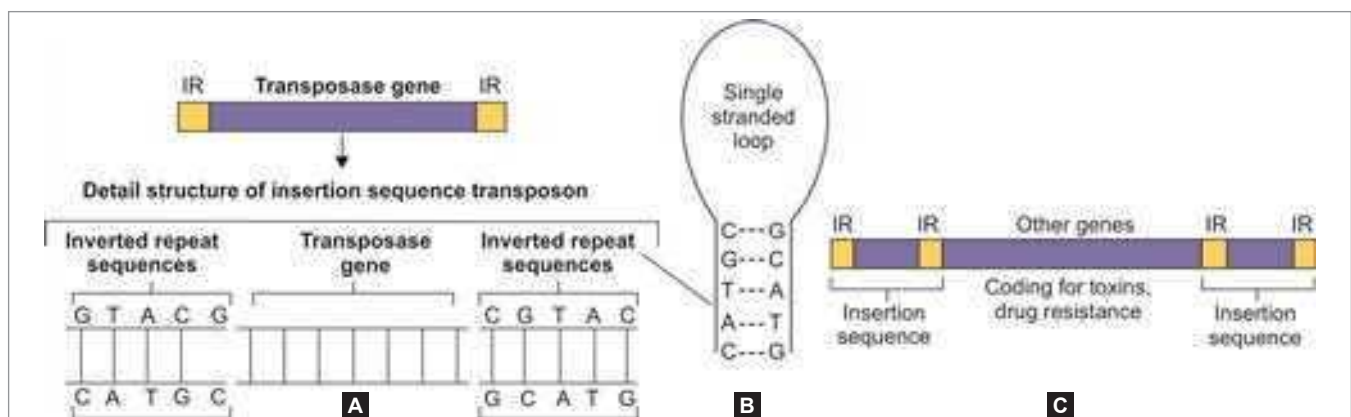
Genetic engineering refers to deliberate modification of an organism's genetic information by directly altering its nucleic acid genome. Genetic engineering is accomplished by a precise mechanism known as recombinant DNA technology.

The gene coding for any desired protein is isolated from an organism, and then inserted into suitable vector, which is then cloned in such a way that it can be expressed in the formation of specific (desired) protein.

Recombinant DNA Technology

The procedure of recombinant DNA technology involves the following steps:

1. **Treatment with restriction enzyme:** The DNA from the microorganism is extracted and then is cleaved by enzymes called restriction endonucleases to produce a mixture of DNA fragments
2. **Southern blot:** The fragment containing the desired gene is isolated from the mixture of DNA fragments. This is done by:
 - Electrophoresis: DNA fragments are electrophoretically separated by subjecting to agar gel electrophoresis



Figs 3.4.8A to C: Transposons: **A.** Insertion sequence transposon; **B.** Hairpin loop structure of transposon; **C.** Composite transposon.

- Transfer to nitrocellulose membrane: The separated DNA fragments are transferred from the gel to a nitrocellulose membrane
 - Detection of the desired gene: The DNA fragment containing the desired gene is detected adding a specific DNA probe, complementary to the gene of interest
 - Isolation: The band containing the desired gene is isolated by DNA extraction and then, is subjected to electrophoresis in a different gel.
3. **Recombination with a vector:** The isolated DNA fragment is annealed with a vector by DNA ligase enzyme
 4. **Introduction of the vector into bacteria:** The vector is introduced into bacteria usually by transformation (injecting by electroporation) and rarely by phage vector by transduction
 5. **Cloning:** Culture of the bacteria containing the desired gene followed by expression of the gene products, yields a large quantity of desired protein.

Applications of Genetic Engineering

- ❖ **Production of vaccines:** Preparation of certain vaccines is done by DNA recombination technology by producing the desired antigen that can be used as an immunogen in vaccine, against which the protective antibody will be produced, e.g. vaccines for hepatitis B and human papillomavirus
- ❖ **Production of antigens used in diagnostic kits:** The antigens used in diagnostic techniques for antibody detection (e.g. ELISA) are prepared by DNA recombinant technology
- ❖ **Production of proteins used in therapy:** Genetic engineering has also been used for the production of proteins of therapeutic interest. These include human growth hormone, insulin, interferons, interleukin-2, tumor necrosis factor, and factor VIII
- ❖ **Transgenic animals:** Recombinant DNA technology can be used to artificially introduce a foreign DNA

into the genome of animals. The process is called transfection and the recombinant animals produced in this way are named transgenic or genetically modified animals. Transgenic mice are available for a variety of biotechnological applications

- ❖ **Gene therapy:** Genetic diseases can be cured by replacing the defective gene by introducing the normal gene into the patient.

Vector

A vector is a small piece of DNA, into which a foreign DNA fragment can be inserted and that can be stably maintained in an organism and used for cloning purposes. There are four major types of vectors, such as: 1. Plasmids, 2. bacteriophages, 3. cosmids, 4. artificial chromosomes, such as bacterial/yeast artificial chromosomes.

BLOTTING TECHNIQUES

A blot, in molecular biology refers to a method of transferring DNA, RNA, or proteins, from the gel onto a carrier (e.g. nitrocellulose membrane), followed by their detection by using specific nucleic acid probes (for DNA or RNA detection) or enzyme immunoassay (for protein detection). There are various blotting techniques:

- ❖ Southern blot is used to detect DNA
- ❖ Northern blot is used to detect RNA
- ❖ Western blot is used to detect proteins
- ❖ Eastern blot: It is a modification of Western blot, used to analyze proteins for post-translational modifications using probes that may detect lipids, carbohydrate, phosphorylation or any other protein modification.

Southern blotting technique is described above, under genetic engineering. The methodology of Northern blot is similar to Southern blot, but uses a RNA probe to detect the specific RNA fragment. Western blot is described in Chapter 12.

EXPECTED QUESTIONS

I. Write essay on:

1. Name various methods of horizontal gene transfer. Discuss in detail about mechanism of conjugation.

II. Write short notes on:

1. Transformation.
2. Transposition.
3. Transduction.

III. Multiple Choice Questions (MCQs):

1. Mechanism of direct transfer of free DNA:

- a. Transformation
- b. Conjugation
- c. Transduction
- d. Transposition

2. Phage mediated transfer of DNA from one bacterium to another bacterium is known as:

- a. Transformation
- b. Transduction
- c. Transmission
- d. Conjugation

3. Horizontal transmission of 'R' factor is by:

- a. Transduction
- b. Transformation
- c. Conjugation
- d. Fusion

4. F factor carrying chromosomal DNA is called as:

- a. F factor
- b. Hfr
- c. RTF
- d. F' factor

Answers

1. a 2. b 3. c 4. d

General Bacteriology: Antimicrobial Agents and Antimicrobial Resistance

CHAPTER 3.5

CHAPTER PREVIEW

- Antimicrobial Agents
- Antimicrobial Resistance
- Acquired and Intrinsic
- Mutational and Transferable
- Mechanism of Resistance

ANTIMICROBIAL AGENTS

Antimicrobials are the agents that kill or inhibit the growth of microorganisms.

Classification

Antimicrobial agents are classified in various ways:

1. According to microorganisms against which they are used—antibacterial, antifungal, antiparasitic, antiviral agents. Only antibacterial agents are discussed here.
2. According to their ability to kill (ends with suffix cidal) or inhibit (ends with suffix static) the microorganism, e.g. bactericidal and bacteriostatic
3. According to the source:
 - Antibiotics: These are natural substances, produced by certain groups of microorganisms
 - Chemotherapeutic agents: These agents are chemically synthesized.

Note: Since many antibiotics and their analogues are now synthesized, antibiotics and chemotherapeutic agents are no more distinct terminologies. Therefore they should be addressed as a single entity, 'antimicrobial agents'
4. According to their site of action and usage:
 - Disinfectants destroy a wide range of microbes on non-living surfaces to prevent their spread
 - Antiseptics (which are applied to the living tissues and help to reduce infection), and
 - Antibiotics (which destroy microorganisms within the body).
5. According to the chemical structure and mechanism of action—the antimicrobial agents can be further divided into many classes, as described in Fig. 3.5.1 and Table 3.5.1. Though incorrect, the word 'antibiotics' is loosely used to describe antimicrobial agents.

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance refers to development of resistance to an antimicrobial agent by a microorganism. It can be of two types—acquired and intrinsic.

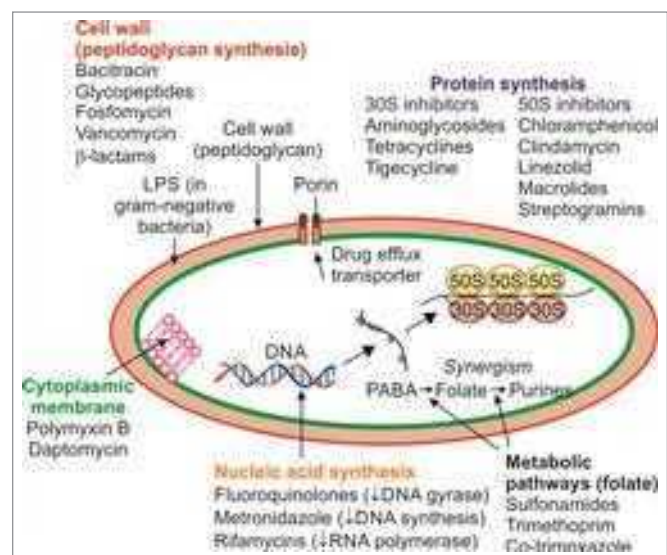


Fig. 3.5.1: Mechanism of action of antimicrobial agents.

Acquired Resistance

This refers to the emergence of resistance in bacteria that are ordinarily susceptible to antimicrobial agents, by acquiring the genes coding for resistance. Most of the antimicrobial resistance shown by bacteria belong to this category.

The emergence of resistance is a major problem worldwide in antimicrobial therapy. Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness, higher healthcare expenditures, and a greater risk of death.

- ❖ Overuse and misuse of antimicrobial agents is the single most important cause of development of acquired resistance
- ❖ The evolution of resistant strains is a natural phenomenon, which can occur among bacteria especially when an antibiotic is overused
- ❖ Use of a particular antibiotic poses selective pressure in a population of bacteria which in turn promotes resistant bacteria to thrive and the susceptible bacteria to die off (Fig. 3.5.2)

Table 3.5.1: Antimicrobial agents—classification, indication, and mechanism of resistance.

Class/mechanism	Drugs	Spectrum of activity	Mechanism of resistance
A. Inhibit Cell Wall Synthesis			
β-Lactam Antibiotics (Bactericidal: block peptidoglycan cross linking by inhibiting the transpeptidase enzyme, i.e. penicillin-binding protein)			
Penicillins			
Penicillin	Penicillin G Aqueous penicillin G Procaine penicillin G Benzathine penicillin G Penicillin V	Mostly gram-positive bacteria: <ul style="list-style-type: none"> • <i>Streptococcus pyogenes</i> • Pneumococcus • <i>Corynebacterium diphtheriae</i> (diphtheria) • <i>Clostridium tetani</i> (tetanus) • <i>Clostridium perfringens</i> (gas gangrene) • Meningococcal infection • Gonococcus (resistance has been reported) • <i>Treponema pallidum</i> (syphilis) 	<ol style="list-style-type: none"> 1. Drug inactivation (by producing β-lactamase enzyme): Seen in both gram-positive and gram-negative bacteria 2. Alteration of target site-PBP (penicillin-binding protein) is altered to PBP2a, seen in gram-positive bacteria 3. Decreased permeability as in gram-negative bacteria- due to altered outer-membrane porins
Penicillinase-resistant-penicillins	Cloxacillin, dicloxacillin, flucloxacillin, nafcillin, oxacillin and methicillin	Same as penicillin plus Penicillinase producing <i>Staphylococcus aureus</i>	
Aminopenicillins (extended spectrum)	Ampicillin Amoxicillin	Same as penicillin plus <ul style="list-style-type: none"> • <i>Enterococcus faecalis</i> • <i>Escherichia coli</i> • <i>Helicobacter pylori</i> • <i>Salmonella</i> (resistance reported) • <i>Shigella</i> (bacillary dysentery) 	
Antipseudomonal penicillins	Carbenicillin, Ticarcillin, piperacillin	Same as aminopenicillins plus <i>Pseudomonas aeruginosa</i>	
Cephalosporins			
1st generation	Cefazolin Cephalexin	<ul style="list-style-type: none"> • <i>Staphylococcus aureus</i> • <i>Staphylococcus epidermidis</i> • Some gram-negative bacteria like <i>Escherichia coli</i> and <i>Klebsiella</i> 	ESBL (extended spectrum β-lactamases)
2nd generation	Cefoxitin, cefotetan Cefaclor, Cefuroxime	<ul style="list-style-type: none"> • Same as 1st generation plus • ↑ Gram-negative activity • ↑ Anaerobic activity (cefoxitin and cefotetan) 	
3rd generation	Ceftriaxone Cefotaxime Ceftazidime	<ul style="list-style-type: none"> • Decreased activity against gram-positives compared to 1st, 2nd generations • ↑ Gram-negative activity • Some are active against <i>Pseudomonas</i> (Ceftazidime) • Ceftriaxone is active against pneumococci, meningococci and MSSA. 	
4th generation	Cefepime Cefpirome	<ul style="list-style-type: none"> • Good activity against gram-positive and negative bacteria including <i>Pseudomonas</i> 	
5th generation	Ceftobiprole Ceftaroline	<ul style="list-style-type: none"> • Same as 4th generation and MRSA (only β-lactam to be effective against MRSA) 	
β lactam + β lactamase inhibitors	<ul style="list-style-type: none"> • Ampicillin-sulbactam* • Amoxicillin-clavulanate* • Cefoperazone-sulbactam • Ceftazidime-avibactam • Ceftolozane-tazobactam • Piperacillin-tazobactam* • Meropenem-vaborbactam* 	<ul style="list-style-type: none"> • Same as spectrum of respective β-lactam drug plus active against β-lactamase producing bacteria • *Have excellent anaerobic coverage 	
Carbapenems	Imipenem Meropenem Doripenem Ertapenem	<ul style="list-style-type: none"> • Broadest range of activity against most bacteria, which include gram-positive cocci, Enterobacteriaceae, <i>Pseudomonas</i>, <i>Listeria</i>, anaerobes like <i>Bacteroides fragilis</i> and <i>Clostridioides difficile</i> • No action on MRSA and <i>Mycoplasma</i> 	<ol style="list-style-type: none"> 1. Produce carbapenemases 2. Efflux pump

Contd...

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Class/mechanism	Drugs	Spectrum of activity	Mechanism of resistance
Monobactam	Aztreonam	• Gram-negative rods	ESBL
Other cell wall inhibitors			
Glycopeptides (bactericidal: disrupt peptidoglycan cross-linkage)	Vancomycin Teicoplanin	Active against most gram-positive bacteria including MRSA (drug of choice), and for <i>Clostridioides difficile</i> infection (CDI)	Alteration of target (substitution of D-alanine—D-alanine side chain of peptidoglycan)
Fosfomycin	Fosfomycin	Inactivates the enzyme UDP-N-acetylglucosamine-3-enolpyruvyltransferase, also known as MurA; required for cell wall synthesis. Active against urinary tract pathogens; against both gram-positive and gram-negative bacteria such as <i>Enterococcus faecalis</i> , <i>Escherichia coli</i> , etc.	1. Alteration of target 2. Producing enzymes that inactivates fosfomycin
Bacitracin	Bacitracin	Topical gram-positive cocci infections	Not defined
B. Protein Synthesis Inhibition			
Anti-30S ribosomal subunit			
Aminoglycosides (bactericidal: irreversible binding to 30S)	Gentamicin Neomycin Amikacin Tobramycin Streptomycin	Aerobic gram-negative bacteria, such as— Enterobacteriaceae and some are active against <i>Pseudomonas</i> (gentamicin and amikacin) Often used for empirical therapy in adjunct with third generation cephalosporins in respiratory infections, meningitis and subacute bacterial endocarditis	1. Drug inactivation by aminoglycoside-modifying enzyme 2. Decreased permeability through gram-negative outer membrane 3. Decreased influx of drug
Tetracyclines (bacteriostatic: bind to 30S subunit of ribosome and block tRNA attachment)	Tetracycline Doxycycline Minocycline Demeclocycline	Rickettsiae, Chlamydiae, <i>Mycoplasma</i> , Spirochetes <i>Yersinia pestis</i> , <i>Brucella</i> , <i>Haemophilus ducreyi</i> , <i>Campylobacter</i> , <i>Vibrio cholerae</i>	1. Decreased intracellular drug accumulation (active efflux) 2. Ribosomal target site alteration
Glycylglycines (MOA, same as tetracycline)	Tigecycline	<i>Staphylococcus</i> , <i>Enterococcus</i> <i>Acinetobacter</i> , and <i>E. coli</i>	Active drug efflux pump
Anti-50S ribosomal subunit			
Chloramphenicol (bacteriostatic: binds to 50S ribosomal subunit and interfere with peptide bond formation)	Chloramphenicol	<i>Haemophilus influenzae</i> Pyogenic meningitis Brain abscess Anaerobic infection Enteric fever (<i>Salmonella</i>)—not used now due to development of resistance	1. Drug inactivation by producing chloramphenicol acetyltransferase enzyme 2. Altered membrane transport (active efflux)
Macrolides (bacteriostatic: binds 50S ribosomal subunit and prevent translocation of elongated peptide)	Erythromycin Azithromycin Clarithromycin	<i>Streptococcus</i> <i>Haemophilus influenzae</i> <i>Mycoplasma pneumoniae</i>	1. Alteration of ribosomal target 2. Active efflux of antibiotic
Ketolide (MOA, same as macrolide)	Telithromycin	Community acquired pneumonia (mild to moderate) by <i>S. pneumoniae</i>	Altered target (methylation of ribosomal binding site) Active drug efflux
Lincosamides (binds 50S subunit, blocks peptide bond formation)	Clindamycin Lincomycin	<i>S. aureus</i> (CA-MRSA, MSSA) Beta hemolytic streptococci <i>Actinomyces</i> , <i>Arcanobacter</i> , <i>Capnocytophaga</i> Anaerobic infection	Altered target (methylation of ribosomal binding site)
Oxazolidinones (Inhibit protein synthesis by binding to 50S)	Linezolid	Resistant gram-positives like MRSA	Alteration of target site
Streptogramins (Inhibit protein synthesis by binding to 50S)	Quinupristin Dalfopristin	<i>Streptococcus pyogenes</i> and <i>Staphylococcus aureus</i> skin infections MRSA infections VRE (Vancomycin resistant enterococci) infections	1. Alteration of target (dalfopristin) 2. Active efflux (quinupristin) 3. Drug inactivation (quinupristin and dalfopristin)

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Class/mechanism	Drugs	Spectrum of activity	Mechanism of resistance
Mupirocin (Inhibits isoleucyl-tRNA synthetase)	Mupirocin	Topical ointment is given for- Skin infections Nasal carriers of MRSA	Mutation of gene for target site protein
Fusidane (Prevents the turnover of elongation factor G from the ribosome)	Fusidic acid	<i>S. aureus</i> (oral and topical use)	Altered target
C. Nucleic Acid Synthesis Inhibitors			
DNA synthesis inhibitors			
Fluoroquinolones	Inhibit DNA gyrase (A subunit) and topoisomerase IV, thus inhibiting DNA synthesis		
Nalidixic acid		Coliform gram-negative bacilli	1. Alteration of target (mutation of DNA gyrase genes) 2. Poor transport across cell membrane
Fluoroquinolones 1st generation	Norfloxacin, ciprofloxacin, ofloxacin	Enterobacteriaceae: such as <i>E. coli</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Proteus</i> , <i>Yersinia</i>	
Fluoroquinolones 2nd generation	Levofloxacin, lomefloxacin, moxifloxacin, sparfloxacin	Others: <i>Neisseria</i> , <i>Haemophilus</i> , <i>Campylobacter</i> , <i>Vibrio cholerae</i> , <i>Pseudomonas</i> , <i>Staphylococcus aureus</i>	
Nitroimidazoles (Damage DNA)	Metronidazole, tinidazole, secnidazole	Anaerobic organisms Also active against protozoa such as <i>Entamoeba</i> , <i>Giardia</i> and <i>Trichomonas</i>	1. Decreased drug uptake 2. Active efflux 3. Decreased drug activation
Nitrofurantoin (Damages bacterial DNA)	Nitrofurantoin	Urinary tract infection (<i>E. coli</i> , <i>Klebsiella</i> , <i>Enterococcus</i>)	Altered drug activating enzyme
RNA synthesis inhibitors			
Rifamycins (Inhibits RNA polymerase)	Rifampicin, rifaximin	<i>M. tuberculosis</i> , <i>M. leprae</i> Nontuberculous mycobacteria <i>Staphylococcus aureus</i> Prophylaxis for <i>H. influenzae</i> meningitis Prophylaxis for meningococcal meningitis	Alteration of target (mutation of <i>rpoB</i> gene)
D. Mycolic Acid Synthesis Inhibitors			
Isonicotinic acid hydrazide (Inhibits mycolic acid synthesis)	Isoniazid (INH)	Tuberculosis Latent TB	Mutations in enzyme processing isoniazid into active metabolites (KatG enzyme)
E. Folic acid Synthesis Inhibitors			
<i>Bacteriostatic:</i> Competitively inhibit enzymes involved in two steps of folic acid biosynthesis			
PABA (para-amino-benzoic acid)	Folate synthase Sulfonamide blocks	Dihydrofolic acid	Dihydrofolate reductase Trimethoprim blocks
Antifolates (Sulfonamides and trimethoprim)	Sulfadiazine Sulfacetamide Co-trimoxazole (Trimethoprim + Sulfamethoxazole)	Sulfadiazine: Used topically in burn wound surface Co-trimoxazole is indicated in: • Urinary tract and respiratory tract infections- Active against <i>Serratia</i> , <i>Klebsiella</i> , <i>Enterobacter</i> • <i>Shigella</i> dysentery, <i>Vibrio cholerae</i> • <i>Toxoplasma gondii</i> , <i>Haemophilus ducreyi</i> • <i>Pneumocystis jirovecii</i>	Production of insensitive targets [dihydropteroate synthetase (sulfonamides) and dihydrofolate reductase (trimethoprim)] that bypass metabolic block
F. Antimicrobial agents that act on cell membrane			
Gramicidin (Forms pores)		Topical use against cocci (gram-positive and negative)	Not defined
Lipopeptides (Forms channel in cell membrane, leading to leakage)	Daptomycin	Bactericidal against gram-positive bacteria including VRE and MRSA	Not defined
Polymyxins (Binds to LPS and disrupt both outer and inner cell membrane)	Polymyxin B Colistin or Polymyxin E (systemic and inhalational use)	Gram-negative infections	1. Alteration of LPS 2. Efflux pump mediated

Abbreviations: MSSA, methicillin sensitive *Staphylococcus aureus*; MRSA, methicillin resistant *Staphylococcus aureus*; CA-MRSA, community acquired MRSA; ESBL, extended spectrum β -lactamases; VRE, vancomycin resistant *Enterococcus*.

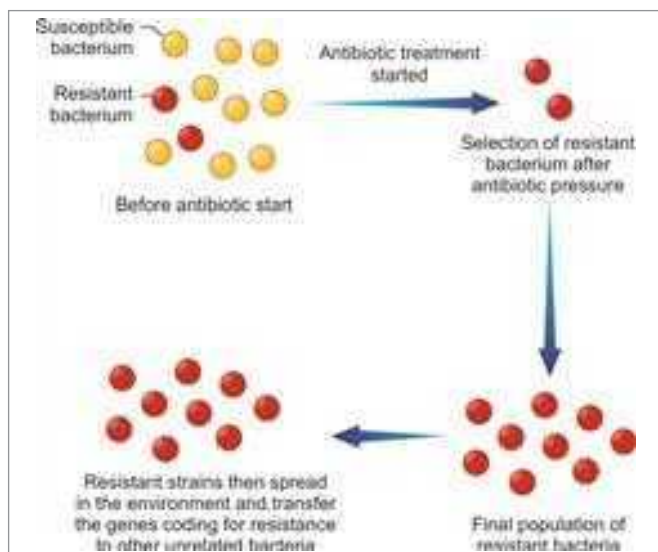


Fig. 3.5.2: Mechanism of development of acquired resistance.

- ❖ Thus the resistant bacterial populations flourish in areas of high antimicrobial use, where they enjoy a selective advantage over susceptible populations
- ❖ The resistant strains then spread in the environment and transfer the genes coding for resistance to other unrelated bacteria.

Other factors favouring the spread of antimicrobial resistance include—

- ❖ Poor infection control practices in hospitals, e.g. poor hand hygiene practices can facilitate the transmission of resistant strains
- ❖ Inadequate sanitary conditions
- ❖ Inappropriate food-handling
- ❖ Irrational use of antibiotics by doctors, not following antimicrobial susceptibility report
- ❖ Uncontrolled sale of antibiotics over the counters without prescription.

Intrinsic Resistance

It refers to the innate ability of a bacterium to resist a class of antimicrobial agents due to its inherent structural or functional characteristics, (e.g. gram-negative bacteria are resistant to vancomycin). This imposes negligible threat as it is a defined pattern of resistance and is non-transferable. However, the clinicians must be aware so as to exclude these antibiotics from therapy (Table 3.5.2).

Mutational and Transferable Drug Resistance

In presence of selective antibiotic pressure, bacteria acquire new genes mainly by two broad methods:

Mutational Resistance

Resistance can develop due to mutation of the resident genes.

- ❖ It is typically seen in *Mycobacterium tuberculosis*, developing resistance to anti-tubercular drugs

- ❖ Mutational drug resistance differs from transferable drug resistance in many ways (Table 3.5.3)
- ❖ Usually, it is a low level resistance, developed to one drug at a time; which can be overcome by using combination of different classes of drugs
- ❖ That is why multidrug therapy is used in tuberculosis using 4–5 different classes of drugs, such as isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin.

Transferrable Drug Resistance

In contrast, transferrable drug resistance is plasmid coded and usually transferred by conjugation or rarely by transduction, or transformation (refer Chapter 3.4).

- ❖ The resistance coded plasmid (called R plasmid) can carry multiple genes, each coding for resistance to one class of antibiotic
- ❖ Thus, it results in a high degree of resistance to multiple drugs, which cannot be overcome by using combination of drugs.

Mechanism of Antimicrobial Resistance

Bacteria develop antimicrobial resistance by several mechanisms.

1. Decreased Permeability across the Cell Wall

Certain bacteria modify their cell membrane porin channels; either in their frequency, size, or selectivity; thereby preventing the antimicrobials from entering into the cell. This resistance mechanism has been observed in many gram-negative bacteria, such as *Pseudomonas*, *Enterobacter* and *Klebsiella* species against drugs, such as imipenem, aminoglycosides and quinolones.

2. Efflux Pumps

Certain bacteria possess efflux pumps which mediate expulsion of the drug(s) from the cell, soon after their entry; thereby preventing the intracellular accumulation of drugs. This strategy has been observed in:

- ❖ *Escherichia coli* and other Enterobacteriaceae against tetracyclines, chloramphenicol
- ❖ Staphylococci against macrolides and streptogramins
- ❖ *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones.

3. By Enzymatic Inactivation

Certain bacteria can inactivate the antimicrobial agents by producing various enzymes, such as:

- ❖ **β -lactamase** enzyme production (observed in both gram-positive and gram-negative bacteria): It breaks down the β -lactam rings, thereby inactivating the β -lactam antibiotics (see the highlight box)
- ❖ **Aminoglycoside modifying enzymes** like (acetyltransferases, adenytransferases, and phosphotransferases, produced by both gram-negative and gram-positive bacteria)—they destroy the structure of aminoglycosides

Table 3.5.2: Intrinsic antimicrobial resistance.	
Organisms	Intrinsic resistance to the following antimicrobial agents
Enterobacteriaceae	Members of family Enterobacteriaceae are intrinsically resistant to antimicrobials specific for gram-positive organisms such as: clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), lipoglycopeptides (oritavancin, teicoplanin, and telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin, and azithromycin) Exceptions: <i>Salmonella</i> and <i>Shigella</i> spp. are susceptible azithromycin
<i>Klebsiella pneumoniae</i>	Same as for Enterobacteriaceae plus ampicillin and ticarcillin
<i>Citrobacter</i> species	Same as for Enterobacteriaceae plus ampicillin, first and second generation cephalosporins, cephamycins, amoxicillin-clavulanate and ampicillin-sulbactam
<i>Enterobacter</i> species	Same as for Enterobacteriaceae plus ampicillin, first generation cephalosporins, cephamycins, amoxicillin-clavulanate and ampicillin-sulbactam
Proteeae tribe	Same as for Enterobacteriaceae plus ampicillin, first and second generation cephalosporins, tetracyclines, tigecycline, nitrofurantoin and polymyxins (polymyxin B and polymyxin E or colistin)
<i>Salmonella</i> species	Same as for Enterobacteriaceae plus aminoglycosides, first and second generation cephalosporins
<i>Shigella</i> species	Same as for Enterobacteriaceae plus aminoglycosides, first and second generation cephalosporins, and cephamycins
<i>Serratia marcescens</i>	Same as for Enterobacteriaceae plus ampicillin, first and second generation cephalosporins, cephamycins, amoxicillin-clavulanate, ampicillin-sulbactam, nitrofurantoin and polymyxins (Polymyxin B and colistin)
<i>Yersinia enterocolitica</i>	Same as for Enterobacteriaceae plus ampicillin, ticarcillin, first generation cephalosporins and amoxicillin-clavulanate
Non-fermentative gram-negative bacteria (NF-GNB)	Non-fermentative gram-negative bacteria are intrinsically resistant to penicillin (i.e., benzyl penicillin), first and second generation cephalosporins, cephamycins, clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), linezolid, macrolides, quinupristin-dalfopristin, and rifampin
<i>Pseudomonas aeruginosa</i>	Same as for NF-GNB, plus ampicillin, ceftriaxone, amoxicillin-clavulanate, ampicillin-sulbactam, Ertapenem, tetracyclines, tigecycline, co-trimoxazole and chloramphenicol
<i>Acinetobacter baumannii</i>	Same as for NF-GNB, plus ampicillin, amoxicillin, amoxicillin-clavulanate, ertapenem, aztreonam, chloramphenicol and fosfomycin
<i>Stenotrophomonas maltophilia</i>	Same as for NF-GNB, plus ampicillin, amoxicillin, cefotaxime, ceftriaxone, cefepime, amoxicillin-clavulanate, aztreonam, imipenem, meropenem, ertapenem, polymyxins (polymyxin B and colistin), aminoglycosides, chloramphenicol and fosfomycin
<i>Burkholderia cepacia</i> complex	Same as for NF-GNB, plus ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, ertapenem, polymyxins (polymyxin B and colistin) and fosfomycin
Gram-positive bacteria	Gram-positive bacteria are intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid
<i>S. aureus</i>	Same as for other gram-positive bacteria
<i>Enterococcus</i> species	Same as for other gram-positive bacteria plus cephalosporins, aminoglycosides*, clindamycin and co-trimoxazole. <i>E. gallinarum</i> and <i>E. casseliflavus</i> are intrinsically resistant to vancomycin, in addition.

*Aminoglycosides such as gentamicin are effective against *Enterococcus*, when given along with cell-wall acting drugs like penicillin, ampicillin or vancomycin, due to synergistic effect (Chapter 76).

Table 3.5.3: Mutational vs transferable drug resistance.	
Mutational drug resistance	Transferable drug resistance
Resistance to one drug at a time	Multiple drugs resistance at the same time
Low-degree resistance	High-degree resistance
Resistance can be overcome by combination of drugs	Cannot be overcome by drug combinations
Virulence of resistance mutants may be lowered	Virulence not decreased
Resistance is not transferable to other organisms Spread to off-springs by vertical spread only	Resistance is transferable to other organisms Spread by: Horizontal spread (conjugation, or rarely by transduction/transformation)

❖ **Chloramphenicol acetyl transferase:** It is produced by members of Enterobacteriaceae; it destroys the structure of chloramphenicol.

4. By Modifying the Target Sites

Modification in the target sites of antimicrobial agent (which are within the bacteria) is a very important mechanism. It is observed in:

- ❖ **MRSA (Methicillin-resistant *Staphylococcus aureus*):** In these strains, the target site of penicillin i.e. penicillin binding protein (PBP) gets altered to PBP-2a. The altered PBP coded by a chromosomally coded gene *mec A*, do not sufficiently bind to β -lactam antibiotics and therefore prevent them from inhibiting the cell wall synthesis. (Described in detail in Chapter 51)
- ❖ β -lactam resistance in pneumococci is due to alteration of PBP to PBP2b.
- ❖ Streptomycin resistance in *Mycobacterium tuberculosis* is due to modification of ribosomal proteins or 16S rRNA
- ❖ Rifampicin resistance in *Mycobacterium tuberculosis*—due to mutations in RNA polymerase

- ❖ Quinolone resistance seen in many gram-positive bacteria, particularly *S. aureus* and *S. pneumoniae*— due to mutations in DNA gyrase enzyme.
- ❖ **Vancomycin resistance in enterococci (VRE):** These strains have a change in the target site of vancomycin (i.e. D-alanine D-alanine side chain of peptidoglycan) (Chapter 76).

Beta-lactamase Enzymes

β-lactamase enzymes are capable of hydrolyzing the β-lactam rings (the active site) of β-lactam antibiotics; thereby deactivating their antibacterial properties.

- They can be produced by both gram-positive and gram-negative organisms
- They are plasmid coded, and transferred from one bacterium to other mostly by conjugation, (except in *Staphylococcus aureus* where they are transferred by transduction).

Beta-lactamases are of various types:

- **Extended spectrum β-lactamases (ESBL):** Organisms producing ESBL enzymes are resistant to all penicillins, 1st, 2nd

Contd...

and 3rd generation cephalosporins and monobactams; however remain sensitive to carbapenems and cephamycins. The resistance can be overcome by use of β-lactam along with β-lactamase inhibitor (e.g. sulbactam or clavulanic acid)

- **AmpC beta-lactamases:** In addition to the antibiotics to which ESBL producers are resistant, AmpC beta-lactamase producers are resistant to cephamycins (e.g. cefoxitin and cefotetan). But they are sensitive to carbapenems. Resistance cannot be overcome by β-lactam + β-lactamase inhibitor combination (BL/BLI)
- **Carbapenamases:** These organisms are resistant to all those antibiotics to which AmpC beta-lactamase producers are resistant. In addition, they are also resistant to carbapenems. Resistance cannot be overcome by BL/BLI. Important carbapenemase enzymes are:
 - *Klebsiella pneumoniae* carbapenemase (KPC)
 - New Delhi metallo-beta-lactamase (NDM)

Routine detection of β-lactamase enzymes in the laboratory is not necessary, as it does not play a vital role in deciding the treatment. It is only necessary for epidemiological purposes.

Contd...

EXPECTED QUESTIONS

I. Write short notes on:

1. Mechanism of antibiotic resistance.
2. Mutational and transferable drug resistance.
3. Antimicrobial susceptibility testing method.

II. Multiple Choice Questions (MCQs):

1. MRSA is mediated due to

- a. Plasmid
- b. *mecA* gene
- c. Transposons
- d. None

2. All of the following antimicrobial agents act on cell membrane, except:

- a. Gramicidin
- b. Daptomycin
- c. Polymyxins
- d. Vancomycin

3. Fosfomycin—all are true, except:

- a. Inactivates the enzyme MurA
- b. Active against urinary tract pathogens
- c. Active against both gram-positive and gram-negative bacteria
- d. Resistance has not been reported yet

4. All of the following antimicrobial agents act on 50S ribosomal subunit, except:

- a. Aminoglycosides
- b. Macrolides
- c. Streptogramins
- d. Chloramphenicol

5. All of the following are examples of intrinsic antimicrobial resistance, except:

- a. Anaerobic bacteria-aminoglycosides
- b. *Pseudomonas*- carbapenems
- c. Aerobic bacteria-metronidazole
- d. Gram-negative bacteria-vancomycin

6. Extended spectrum β-lactamases (ESBL) producing organisms are resistant to all, except:

- a. All penicillins
- b. 3rd generation cephalosporins
- c. Monobactam
- d. Carbapenems

7. All of the following can be given for the treatment of Extended spectrum β-lactamases (ESBL) producing organisms, except:

- a. Carbapenems
- b. β-lactam/ lactamase inhibitor combination
- c. 3rd generation cephalosporins
- d. Aminoglycoside

8. Which of the following can be given for the treatment of carbapenemase producing organisms?

- a. Carbapenems
- b. β-lactam/lactam inhibitor combination
- c. 3rd generation cephalosporins
- d. Aminoglycoside

Answers

1. b 2. d 3. d 4. a 5. b 6. d 7. c 8. d

General Bacteriology: Pathogenesis of Bacterial Infections

CHAPTER 3.6

CHAPTER PREVIEW

- Mechanism of Bacterial Pathogenesis

MECHANISM OF BACTERIAL PATHOGENESIS

Ability of bacteria to produce disease or tissue injury is often referred to as two closely related but not synonymous term 'pathogenesis' and 'virulence'.

- ❖ 'Pathogenesis' is generally employed to refer to the ability of a microbial species to produce disease
- ❖ While the term 'virulence' is used more specifically to describe the relative degree of pathogenesis (tissue damage), which may vary between different strains of the same organism depending upon the expression of the virulence factors
- ❖ Virulence is a relative term; different strains of same species may exhibit varying degrees of virulence. Some strains are highly virulent; while some strains are low and some are avirulent (vaccine strains).

The virulence of a strain may undergo spontaneous or induced variation.

- ❖ **Exaltation:** Enhancement of virulence is known as exaltation, which can be induced experimentally by serial passage into susceptible hosts
- ❖ **Attenuation:** It refers to the reduction of virulence, which can be achieved by passage through unfavorable hosts, repeated cultures in artificial media, growth in high temperature or in the presence of weak antiseptics, desiccation or prolonged storage in culture.

Route of Transmission

Route of transmission of infection plays a crucial role in the pathogenesis of certain bacteria (Table 3.6.1).

- ❖ Some bacteria, such as streptococci, can initiate infection whatever be the route of entry
- ❖ Others can survive and multiply only when introduced by the optimal routes. *Vibrio cholerae* are infective orally but are unable to cause infection when introduced subcutaneously
- ❖ This difference is probably related to the modes by which different bacteria are able to initiate tissue damage and establish themselves.

Infective Dose

Infective dose of the bacteria is referred to as the minimum inoculum size that is capable of initiating an infection. Infective dose plays a major role in determining whether the disease is going to set in or not.

- ❖ **Low infective dose:** Certain organisms require a relatively small inoculum to initiate infection
 - *Shigella*: Very low (as low as 10 bacilli)
 - *Escherichia coli* O157: H7 (<10 bacilli)
 - *Campylobacter jejuni* (500 bacilli).
- ❖ **Large infective dose:** In contrast, bacteria with high infective dose can initiate the infection only when the inoculum size exceeds a particular critical size

Table 3.6.1: Mode of transmission of bacterial infections*.

Transmission	Bacterial agents/diseases
Contact	Multi-drug resistant organisms in hospitals such as <i>S. aureus</i> , <i>E. coli</i> , <i>Klebsiella</i> , etc. <i>Bacillus anthracis</i>
Droplet	Meningococcus <i>C. diphtheriae</i> Pneumococcus
Aerosol	<i>M. tuberculosis</i>
Ingestion	<i>Salmonella</i> and <i>Shigella</i> <i>Vibrio</i> and diarrheagenic <i>E. coli</i> <i>Campylobacter</i> Agents of food poisoning
Vector-borne	Rickettsiae <i>Borrelia</i>
Sexual	Gonococcus <i>Chlamydia trachomatis</i> <i>Treponema pallidum</i> <i>Haemophilus ducreyi</i>
Vertical	<i>Treponema pallidum</i>
Birth canal	<i>Listeria</i> <i>Streptococcus agalactiae</i>

*Rare modes of transmission of these bacterial agents have been excluded from the list for the ease of understanding.

- *Escherichia coli* (10^6 – 10^8 bacilli)
- *Salmonella* (10^2 – 10^5 bacilli)
- *Vibrio cholerae* (10^6 – 10^8 bacilli).

Infective dose varies depending upon the factors, such as:

- ❖ Virulence of the organism: Higher the virulence, lower is the infective dose
- ❖ Host's age and overall immune status
- ❖ The ability of the organism resisting the gastric acidity: *Shigella* has an ability to survive in gastric acidity, even a low infective dose can initiate the infection. In contrast *Vibrio* is extremely acid labile, hence requires a heavy inoculum to bypass the gastric barrier.

Adhesion

Adhesion of the bacteria to body surfaces is the initial event in the pathogenesis of the disease. It is mediated by specialized molecules called adhesins that bind to specific host cell receptors. Adherence prevents the bacteria from being flushed away in secretions and also facilitates bacterial invasion into the host cells.

- ❖ **Fimbriae or pili:** They are the most important adhesins present in some bacteria. They directly bind to the sugar residues (glycolipids or glycoproteins) on host cells
- ❖ **Non-pilus adhesins:** Apart from pili, there are other adhesins found in certain bacteria, such as M protein (*Streptococcus pyogenes*), lipoteichoic acid (gram-positive cocci), cell surface lectin (*Chlamydia*), etc.
- ❖ **Biofilm formation:** It is another mechanism by which certain bacteria mediate strong adherence to certain structures, such as catheters, prosthetic implants, and heart valves. Biofilm is a group of bacterial cells which stick to each other on a surface and are embedded within layer (called slime layer) of a self-produced matrix of extracellular polymeric substance called glycocalyx.

Invasion

Invasion refers to entry of bacteria into host cells, leading to spread within the host tissues.

- ❖ Highly invasive pathogens produce spreading or generalized lesions (e.g. streptococcal infections), while less invasive pathogens cause localized lesions (e.g. staphylococcal abscess)
- ❖ Some pathogens though capable of causing fatal diseases, lack invasiveness but remain confined to the site of entry and produce disease by elaborating a potent toxin, e.g. *Clostridium tetani*.

Important virulence factors that help in invasion include:

- ❖ Virulence marker antigen or invasion plasmid antigens in *Shigella*
- ❖ Enzymes: Invasion of bacteria is enhanced by many enzymes such as: hyaluronidase, collagenase, streptokinase, IgA proteases.

Antiphagocytic Factors

Bacteria are rapidly killed once they are ingested by phagocytes, such as polymorphonuclear cells (neutrophils) or macrophages. Some pathogens develop strategies to evade phagocytosis by several antiphagocytic factors, the most important ones being—

- ❑ **Capsule:** It prevents phagocytosis of bacteria by preventing the phagocytes from adhering to the bacteria. Capsules are produced by—
 - *Neisseria meningitidis*
 - *Streptococcus pneumoniae*
 - *Haemophilus influenzae*
 - *Klebsiella pneumoniae*.
- ❑ **Cell wall proteins** may help in invasion, such as—
 - Protein A of *Staphylococcus aureus* binds to IgG and prevents the activation of complement
 - M protein of *Streptococcus pyogenes*.
- ❑ **Cytotoxins:** Certain bacteria produce cytotoxins that interfere with chemotaxis or killing of phagocytes. For example, *S. aureus* produces hemolysins and leukocidins that lyse and damage RBCs and WBCs.

Intracellular Survival

Some organisms survive in intracellular environment. They are grouped into obligate and facultative intracellular organisms (Table 3.6.2). Once engulfed, they develop strategies that inhibit various steps of phagocytosis (see highlight box above and Table 3.6.3).

Toxins

Endotoxins

Endotoxins are the lipid A portion of lipopolysaccharide (LPS). They are present as an integral part of the cell wall of gram-negative bacteria. They are released from the bacterial surface by natural lysis of the bacteria and are responsible for various biological effects in the host (Fig. 3.6.1).

Table 3.6.2: Intracellular bacteria.

Facultative intracellular bacteria	Obligate intracellular
<i>Salmonella</i> Typhi, <i>Brucella</i>	<i>Mycobacterium leprae</i>
<i>Legionella</i> , <i>Listeria</i> , <i>Nocardia</i>	<i>Rickettsia</i>
<i>Neisseria meningitidis</i> , <i>Yersinia</i>	<i>Chlamydia</i>
<i>Mycobacterium tuberculosis</i>	<i>Coxiella burnetii</i>

Table 3.6.3: Mechanisms used by bacteria for intracellular survival.

Mechanism of intracellular survival	Organism
Inhibition of phagolysosome fusion	<i>Legionella</i> species <i>Mycobacterium tuberculosis</i> <i>Chlamydia</i> species
Resistance to lysosomal enzymes	<i>Salmonella</i> Typhimurium <i>Coxiella</i> species <i>Mycobacterium leprae</i>
Adaptation to cytoplasmic replication	<i>Listeria</i> , <i>Rickettsia</i> <i>Francisella tularensis</i>

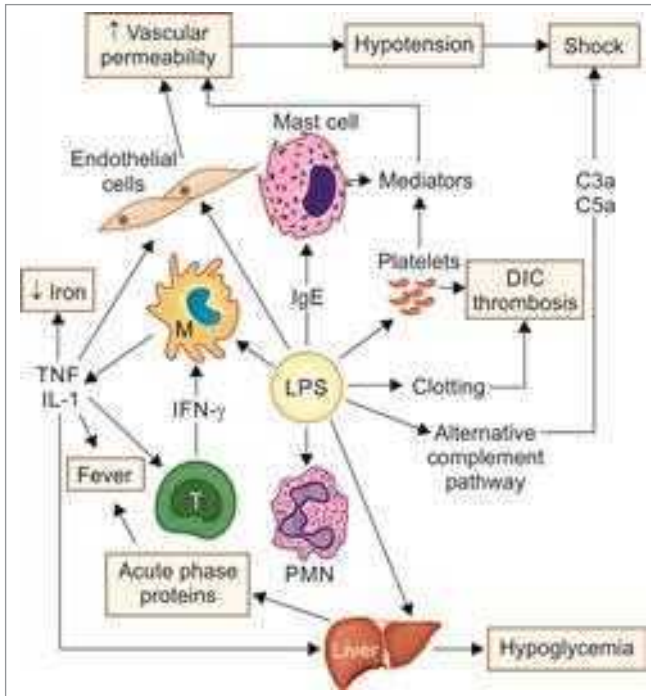


Fig. 3.6.1: Effects of bacterial endotoxins (cell wall lipopolysaccharide).

Abbreviations: DIC, disseminated intravascular coagulation; LPS, lipopolysaccharide; IL-1, interleukin-1; IFN- γ , interferon gamma; PMN, polymorphonuclear leukocyte; IgE, immunoglobulin E; T, T lymphocyte; M, macrophage.

- ❖ **Macrophage activation:** Endotoxin binds to specific receptors on macrophages and stimulates the release of acute-phase cytokines, such as interleukin (IL)-1, tumor necrosis factor- α , IL-6, nitric oxide and prostaglandins which cause fever and inflammation and activation of immune system (T cells and B cells)
- ❖ **Complement activation:** High concentrations of endotoxin can activate the alternative pathway of complement

→ release C3a and C5a → promote inflammatory cells chemotaxis, high grade fever, hypotension, shock produced by vasodilatation and capillary leakage

- ❖ **Endothelial activation:** Leads to \uparrow vascular permeability
- ❖ **Coagulation pathways activation:** It activates Hageman factor and other coagulation factors, leads to thrombosis, and disseminated intravascular coagulation (DIC)
- ❖ **Platelet activation:** Leads to release of mediators that cause \uparrow vascular permeability, thrombosis, and DIC
- ❖ **Mast cell activation:** Leads to release of mediators (e.g. histamine) that causes muscle contraction and \uparrow vascular permeability
- ❖ **In gram-negative septicemia:** Endotoxins are released in large quantity, causing high fever, petechiae (skin lesions resulting from the capillary leakage) and DIC which may result in shock and possibly death.

Exotoxins

They are heat labile proteins; secreted by certain species of both gram-positive and gram-negative bacteria and diffuse readily into the surrounding medium.

- ❖ **High potency:** Exotoxins are highly potent even in minute amounts. Botulinum toxin is the most potent, it has been estimated that 39.2 g of botulinum toxin would be sufficient to eradicate the entire humankind
- ❖ **Used for vaccine:** Exotoxins can be converted into toxoids by treatment with formaldehyde. Toxoids lack toxicity but retain antigenicity and thus induce protective immunity when used as vaccines
- ❖ **Specific action:** They are highly specific for a particular tissue, e.g. tetanus toxin for CNS. They have specific pharmacological activities (Table 3.6.4).

Exotoxins differ from endotoxins in several ways (Table 3.6.5).

Table 3.6.4: Bacterial exotoxins and their mechanism of action.

Organisms	Toxins (Exotoxins)	Mechanism
<i>Staphylococcus aureus</i>	Enterotoxin, Toxic shock syndrome toxin	Act as super antigen; stimulate T cell nonspecifically, to release large amounts of cytokines
<i>Streptococcus pyogenes</i>	Streptococcal pyrogenic exotoxin	
<i>Corynebacterium diphtheriae</i>	Diphtheria toxin (DT)	Inhibits protein synthesis (by inhibiting elongation factor-2)
<i>Bacillus anthracis</i>	Anthrax toxin	\uparrow cAMP in target cell, edema
<i>Clostridium perfringens</i>	α toxin, other major and minor toxins	Lecithinase and phospholipase activity → causes myonecrosis
<i>Clostridium tetani</i>	Tetanus toxin (tetanospasmin)	Decrease in neurotransmitter (GABA and glycine) release from the inhibitory neurons → spastic paralysis
<i>Clostridium botulinum</i>	Botulinum toxin (BT)	Decrease in neurotransmitter (acetyl choline) release from neurons → flaccid paralysis
<i>Escherichia coli</i> (diarrheagenic)	Heat labile toxin (LT)	Activation of adenylate cyclase → \uparrow cAMP in target cell → secretory diarrhea
	Heat stable toxin (ST)	\uparrow cGMP in target cell → secretory diarrhea
	Verocytotoxin	
<i>Shigella dysenteriae</i> type-1	Shiga toxin	Inhibit protein synthesis (by inhibiting ribosome)
<i>Vibrio cholerae</i>	Cholera toxin (CT)	Activation of adenylate cyclase → \uparrow cAMP in target cell → secretory diarrhea
<i>Pseudomonas</i>	Exotoxin-A	Inhibit protein synthesis (by inhibiting elongation factor-2)

Table 3.6.5: Differences between bacterial endotoxins and exotoxins.

Feature	Endotoxins	Exotoxins
Nature	Lipopolysaccharides	Proteins
Source	Part of cell wall of gram-negative bacteria	Secreted both by gram-positive and negative bacteria; diffuse into surrounding medium
Released by	Cell lysis, not by secretion	Actively secreted by the bacteria
Heat stability	Highly stable	Heat labile, destroyed at 60°C
Mode of action	↑IL-1 and TNF- α	Mostly enzyme like action
Effect	Nonspecific (fever, shock, etc.)	Specific action on particular tissues
Tissue affinity	No	Specific affinity for tissues
Fatal dose	Only large doses are fatal	More potent, even smaller doses—fatal
Antigenicity	Poorly antigenic	Highly antigenic
Neutralization by antibodies	Ineffective	Neutralized by specific antibodies
Used for vaccine	No effective vaccine is available using endotoxin	Toxoid forms are used as vaccine, e.g. tetanus toxoid

EXPECTED QUESTIONS

I. Write short notes on:

1. Various modes of transmission of infection.
2. Mechanisms of bacterial pathogenesis.
3. Differences between endotoxins and exotoxins.

II. Multiple Choice Questions (MCQs):

1. Chemical nature of endotoxin is:

- a. Protein
- b. Lipopolysaccharide
- c. Carbohydrate
- d. None

2. The following are exotoxins, except:

- a. Botulinum toxin
- b. Anthrax toxin
- c. Diphtheria toxin
- d. Lipid A portion of LPS

3. Obligate intracellular bacteria are all, except:

- a. *M. leprae*
- b. *Rickettsia*
- c. *Chlamydia*
- d. *M. tuberculosis*

4. The following have the intracellular survival strategy of inhibition of phagolysosome fusion, except:

- a. *Legionella* species
- b. *M. tuberculosis*
- c. *M. leprae*
- d. *Chlamydia* species

5. The following bacteria require large infective dose, except:

- a. *Escherichia coli*
- b. *Shigella*
- c. *Salmonella*
- d. *Vibrio cholerae*

Answers

1. b 2. d 3. d 4. c 5. b

General Bacteriology: Overview of Bacterial Infections

CHAPTER 3.7

CHAPTER PREVIEW

- Gram-positive cocci Infections
- Gram-negative cocci Infections
- Gram-positive bacilli Infections
- Gram-negative bacilli Infections
- Anaerobic Bacterial Infections
- Miscellaneous Bacterial Infections

This chapter gives an overview of various bacteria infecting man, which will help to understand the bacterial infections that are discussed in detail under the respective infective syndromes (Part-II) of this book.

Conventionally based on Gram stain, the bacterial infections can be grouped into infections caused by gram-positive cocci, gram-negative cocci, and gram-positive bacilli, gram-negative bacilli (Chapter 3.1, Table 3.1.4). There is another group comprising of miscellaneous bacteria that do not take up or poorly take up Gram stain.

GRAM-POSITIVE COCCI INFECTIONS

Gram-positive cocci are classified into two families—Micrococcaceae and Streptococcaceae, differentiated by the catalase test.

- ❖ Micrococcaceae are catalase positive, gram-positive cocci arranged in tetrads or clusters
 - Tetrads—*Micrococcus*, non-pathogenic
 - Clusters—*Staphylococcus*, pathogenic.
- ❖ Streptococcaceae are catalase negative gram-positive cocci, arranged in pairs or chains
 - Pairs—Enterococci, *Streptococcus pneumoniae* (pathogenic)
 - Short chains—Beta-hemolytic streptococci (pathogenic)
 - Long chains—Viridans streptococci (commensals, occasionally pathogenic).

Staphylococcus species (Chapter 51)

Among all staphylococci, *S. aureus* is the most virulent species, pathogenic to man. The other staphylococci, collectively designated as coagulase-negative staphylococci (CoNS) are usually harmless commensals and less virulent; but can occasionally cause infections, especially prosthetic device associated and surgical site infections.

Staphylococcus aureus

S. aureus is a pluripotent pathogen, responsible for both community and nosocomial acquired infections that may range from relatively milder skin and soft tissue infections to life-threatening systemic infections.

- ❖ **Virulence factors:** The pathogenic potential of *S. aureus* is due to expression of several virulence factors which include
 - Toxins such as hemolysins, exfoliative toxin, enterotoxin and toxic shock syndrome toxin
 - Extracellular enzymes such as coagulase.
- ❖ **Clinical manifestations:** Clinical spectrum of *S. aureus* include:
 - Skin and soft tissue infections such as folliculitis, furuncle, and cellulitis, etc.
 - Musculoskeletal infections such as osteomyelitis, septic arthritis and abscess
 - Respiratory tract infection such as pneumonia
 - Bacteremia and sepsis
 - Urinary tract infections (UTI)
 - Toxin-mediated infections such as scalded skin syndrome, food poisoning and toxic shock syndrome.
- ❖ **Laboratory diagnosis:** *S. aureus* is gram-positive cocci, arranged in clusters
 - Staphylococci are differentiated from streptococci by being catalase positive
 - *S. aureus* produces as golden-yellow pigmented colonies on nutrient agar and β -hemolytic colonies on blood agar
 - *S. aureus* is differentiated from CoNS by being coagulase positive.
- ❖ **MRSA:** Methicillin-resistant *S. aureus* is a resistant phenotype, has been increasingly reported over last few decades. It shows resistance to all beta-lactam antimicrobials and thus possesses a great therapeutic challenge
- ❖ **Treatment:** *S. aureus* is primarily treated by anti-staphylococcal penicillins such as cloxacillin. However, for MRSA infections, vancomycin is recommended for

serious infections and doxycycline or cotrimoxazole for non-serious infections.

Streptococcus species

Streptococci are catalase-negative, gram-positive cocci, arranged in pairs or chains. They can be classified based on hemolysis produced on sheep blood agar as:

- ❖ **α-hemolytic streptococci:** e.g. viridans streptococci and *S. pneumoniae*
- ❖ **β-hemolytic streptococci:** Based on the cell wall carbohydrate antigen, they can be typed into 20 Lancefield groups. Important pathogens include group A (*S. pyogenes*) and group B (*S. agalactiae*)
- ❖ **γ-hemolytic streptococci:** They produce non-hemolytic colonies, e.g. *Enterococcus*.

Streptococci are found as a part of normal flora, colonizing human intestine and respiratory tract. However, some are important human pathogens such as *S. pyogenes* and *S. agalactiae*, *Enterococcus* and *S. pneumoniae*.

Streptococcus pyogenes (Chapter 52)

S. pyogenes (group A *Streptococcus*) is one of the leading cause of pyogenic infections in humans.

- ❖ **Virulence factors:** The virulence factors of *S. pyogenes* responsible for majority of pathogenesis include
 - Cell wall antigen: e.g. M protein
 - Toxins: e.g. streptococcal pyrogenic exotoxin (SPE)
 - Various enzymes: e.g. streptokinase, streptodornase, hyaluronidase, etc.
- ❖ **Clinical manifestations:** *S. pyogenes* is associated with variety of suppurative and non-suppurative manifestations
 - **Suppurative manifestations** include sore throat (pharyngitis), scarlet fever, skin and soft-tissue infections (impetigo, cellulitis and necrotizing fasciitis, and toxic shock syndrome)
 - **Non-suppurative manifestations** are acute rheumatic fever and post-streptococcal glomerulonephritis. The underlying pathogenesis is due to *molecular mimicry*; where, the antibodies produced against previous streptococcal infections cross react with human tissues (heart or kidneys) to produce lesions.
- ❖ **Laboratory diagnosis:** *S. pyogenes* appears gram-positive cocci, arranged in short chain
 - *S. pyogenes* produces pin point colonies with wide zone of β-hemolysis on blood agar
 - *S. pyogenes* can be differentiated from *S. agalactiae* being bacitracin sensitive and CAMP test (Christie, Atkins, and Munch-Peterson) negative.
- ❖ **Treatment:** *S. pyogenes* is primarily treated by penicillin. Erythromycin can be given in case of penicillin allergy.

Streptococcus agalactiae (Chapter 52)

Streptococcus agalactiae (group B *Streptococcus*) colonizes female genital tract. It has been recognized as major cause

of neonatal meningitis and sepsis. Infections in pregnancy can lead to peripartum fever. It can be differentiated from group A *Streptococcus* by being CAMP test positive and bacitracin resistant. Penicillin is the drug of choice.

Enterococcus (Chapter 76)

Enterococci are part of normal flora of human intestine. *E. faecalis* and *E. faecium* are the common species infecting man.

- ❖ **Clinical manifestations:** Enterococci can cause various infections ranging from UTI, bacteremia and endocarditis, and intra-abdominal infections
- ❖ **Laboratory diagnosis:** They appear oval-shaped gram-positive cocci in pairs; produce non-hemolytic translucent colonies on blood agar and are identified by positive bile esculin test
- ❖ **Treatment:** Enterococci are treated with ampicillin ± gentamicin, vancomycin and fosfomycin.

Viridans streptococci (Chapter 28)

Viridans streptococci are commensals of mouth and upper respiratory tract. However occasionally they can cause infections such as dental caries, subacute bacterial endocarditis and suppurative infections. They appear as long chains of gram-positive cocci and produce minute α-hemolytic colonies on blood agar. They are usually susceptible to penicillin and vancomycin.

Streptococcus pneumoniae (Chapters 61, 71)

Streptococcus pneumoniae, commonly referred to as pneumococcus is the leading cause of lobar pneumonia, and otitis media in children and meningitis in all ages.

- ❖ **Clinical manifestations:** *S. pneumoniae* can cause both invasive infections such as pneumonia, blood stream infection, pyogenic meningitis and non-invasive infections such as otitis media and sinusitis
- ❖ **Laboratory diagnosis:** They appear capsulated gram-positive cocci in pair, lanceolate-shaped
 - On blood agar, they produce characteristic draughtsman or carrom coin shaped α-hemolytic colonies
 - They are identified by being bile soluble and susceptible to optochin.
- ❖ **Treatment:** *S. pneumoniae* responds well to penicillin-G. Ceftriaxone or vancomycin can be given in case of penicillin resistance
- ❖ **Vaccine:** There are two vaccines available for pneumococcus: (i) 23-valent pneumococcal polysaccharide vaccine (PPSV23), and (ii) pneumococcal conjugate vaccine (PCV13).

GRAM-NEGATIVE COCCI INFECTIONS

Neisseriae are gram-negative diplococci. Two species are pathogenic to humans; *N. meningitidis* and *N. gonorrhoeae*. Others species are commensals of oral cavity or genital tract.

Neisseria meningitidis (Chapter 71)

N. meningitidis (or meningococci) are capsulated gram-negative diplococci; lens-shaped.

- ❖ **Virulence factors** include polysaccharide capsule, endotoxin and outer membrane proteins
- ❖ **Clinical manifestations:** It can cause invasive diseases such as pyogenic meningitis and septicemia among susceptible individuals
- ❖ **Laboratory diagnosis:** It can be isolated from CSF or blood culture or from nasopharyngeal swabs. It is oxidase positive, ferments glucose and maltose
- ❖ **Treatment:** Third generation cephalosporins are the drug of choice
- ❖ **Vaccines:** Meningococcal capsular polysaccharide vaccine, conjugated capsular vaccine and recombinant vaccines are currently available.

Neisseria gonorrhoeae (Chapter 77)

Neisseria gonorrhoeae causes a sexually transmitted infection (STI), known as 'gonorrhea'.

- ❖ **Virulence factors** of *N. gonorrhoeae* include pili, and outer membrane protein
- ❖ **Clinical manifestations:** Gonorrhea commonly manifests as mucopurulent cervicitis (in females) and urethritis (in males). It can also cause conjunctivitis (ophthalmia neonatorum) in newborn
- ❖ **Laboratory diagnosis:** Urethral swab (for males) and endocervical swabs (for females) are the ideal specimens; which should be collected in charcoal-coated swabs (Stuart's transport medium)
 - They appear gram-negative intracellular kidney-shaped diplococci
 - For culture, selective media such as Thayer Martin medium and Modified New York City medium are used
 - It is oxidase-positive, and ferments only glucose, but not maltose.
- ❖ **Treatment:** Third generation cephalosporin such as ceftriaxone is the drug of choice. Both the sexual partners should be treated.

GRAM-POSITIVE BACILLI INFECTIONS

Corynebacterium species (Chapter 60)

Corynebacteria are club-shaped gram-positive bacilli. *C. diphtheriae* is the most important species pathogenic to man; other species called as *diphtheroids* are mainly skin commensals, occasionally can be pathogenic to man.

Corynebacterium diphtheriae

C. diphtheriae is the causative agent of **diphtheria**; a contagious disease of nasopharynx and skin, commonly affecting the unvaccinated children.

- ❖ **Virulence factors:** The pathogenesis of diphtheria is mediated by diphtheria toxin; which acts by inhibiting protein synthesis

- ❖ **Clinical manifestations:** The characteristic clinical feature of diphtheria is—presence of a tough leathery greyish white pseudomembrane, formed over the tonsils (respiratory diphtheria). The other manifestations are cutaneous diphtheria and less commonly, toxic systemic complications such as myocarditis and polyneuropathy
- ❖ **Laboratory diagnosis:** Throat swabs containing fibrinous exudates from pseudomembrane are the ideal specimens
 - **Microscopy:** Albert staining reveals characteristic green bacilli arranged in Chinese letter pattern with bluish-black metachromatic granules at the poles
 - **Culture:** Important culture media are Loeffler's serum slope (enriched media) and potassium tellurite agar (selective media, produces black colored colonies)
 - Toxin demonstration should be done following isolation.
- ❖ **Treatment:** Diphtheria should be treated at the earliest; regimen includes anti-diphtheritic serum and antibiotics such as penicillin
- ❖ **Vaccine:** The diphtheria vaccine (toxoid) is given under national immunization schedule as a combined vaccine along with pertussis and tetanus (DPT vaccine).

Bacillus species

Bacillus species are gram-positive spore bearing bacilli. Most of the species are laboratory contaminants; except *B. anthracis* (causes anthrax) and *B. cereus* (causes food poisoning, Chapter 40).

Bacillus anthracis (Chapter 55)

Bacillus anthracis is the causative agent of anthrax, an important zoonotic disease transmitted by occupational exposure to infected animals such as cattle and sheep. Anthrax in humans manifests either as cutaneous form (most common, characterized by black eschar called **malignant pustule**) or rarely pulmonary or intestinal form. In microscopy, *B. anthracis* appears as long chains of gram-positive bacilli with non-bulging spores, described as bamboo stick appearance. Ciprofloxacin or doxycycline are the drugs of choice.

Mycobacteria (Chapter 63)

Mycobacteria are acid-fast obligate aerobes. They can be classified into:

- ❖ **M. tuberculosis complex:** It is responsible for tuberculosis in man (Chapter 63)
- ❖ **M. leprae** (Hansen's bacillus): It causes leprosy a disease of social stigma; characterized by anesthetic skin lesions, bony deformities and disfigurement (Chapter 54)
 - **Microscopy** from the slit skin smears made from the lesions shows red acid fast bacilli arranged in cigar like bundles to form globi, found inside the foamy macrophages

- **Treatment** of leprosy includes multidrug regimen; consisting of dapsone, rifampicin and/or clofazimine given for longer duration (6 months to one year).
- ❖ **Nontuberculous mycobacteria (NTM):** These are diverse group, exist either as saprophytes or commensals or occasional opportunistic human pathogens, e.g. *M. kansasii* and *M. ulcerans*. They cause cutaneous and pulmonary infections and others (Chapters 55 and 63).

Mycobacterium tuberculosis (Chapter 63)

M. tuberculosis complex causes tuberculosis, which is one of the oldest disease of mankind, and is a major cause of death worldwide. It usually affects the lungs, although other organs are also involved.

- ❖ **Transmission:** *M. tuberculosis* is mainly transmitted by inhalation of droplet nuclei (aerosols), generated while coughing or sneezing of infected patients
- ❖ **Pathogenesis:** It is an obligate intracellular pathogen, survives inside the macrophage by inhibition of phagolysosome fusion. Host's cell-mediated immune response to *M. tuberculosis* is critical to contain the infection
- ❖ **Clinical forms:** Tuberculosis occurs both in pulmonary and extrapulmonary forms
 - **Pulmonary tuberculosis:** It is the most common type, usually infects children; characterized by fibrotic nodular lesions (Ghon focus) in lungs and hilar lymphadenopathy
 - **Extrapulmonary tuberculosis (EPTB)** results from hematogenous dissemination of tubercle bacilli to various organs. In HIV patients, the occurrence of EPTB is much higher. The common types of EPTB include tuberculous lymphadenitis, pleural and genitourinary tuberculosis and tuberculous meningitis.
- ❖ **Laboratory diagnosis:** The specimens collected depends upon the clinical forms. The common specimens are sputum (two, spot and early morning sample), pleural fluid, CSF, urine, etc.
 - **Acid-fast staining** (Ziehl-Neelsen and auramine phenol) of the clinical specimen shows long slender, beaded, acid-fast bacilli
 - **Culture** can be performed on conventional Lowenstein Jensen medium or automated culture systems e.g. MGIT (Mycobacteria growth indicator tube)
 - **Molecular methods:** Cartridge-based nucleic acid amplification test (e.g. GeneXpert) is an automated real-time PCR system, that has completely revolutionized the diagnosis of TB.
- ❖ **Treatment:** Multidrug regimen is recommended for TB; comprising of isoniazid, rifampicin, pyrazinamide and ethambutol. The treatment is longer (6 months) and under direct supervision
- ❖ **Resistance:** Failure to adhere to multidrug regimen is common, leading to emergence of drug resistance
- ❖ **Vaccine:** Bacillus Calmette-Guérin (BCG) is a live attenuated vaccine for tuberculosis, given at birth.

Miscellaneous Gram-positive bacilli

- ❖ **Actinomycetes** (Chapter 55): They are diverse group of gram-positive bacilli arranged in chains or branching filaments. Important genera include:
 - **Actinomyces:** They are anaerobe and non-acid fast; produce a clinical condition called actinomycosis, characterized by painless, slow growing mass with a cutaneous fistula in cervicofacial region
 - **Nocardia:** They are aerobe and acid fast; cause pulmonary infection and a subcutaneous infection called as actinomycetoma.
- ❖ **Listeria:** *L. monocytogenes* is a food-borne pathogen that can cause serious infections, particularly in neonates (neonatal meningitis and sepsis), pregnant women and elderly people (Chapter 71)
- ❖ **Erysipelothrix rhusiopathiae** (Chapter 55): It causes purplish-red itchy skin lesion called erysipeloid
- ❖ **Tropheryma whipplei:** It is the agent of Whipple's disease, characterized by malabsorption in intestine (Chapter 39).

GRAM-NEGATIVE BACILLI INFECTIONS

Enterobacteriaceae

Members of the family Enterobacteriaceae are gram-negative bacilli, non-fastidious, catalase positive and oxidase negative; glucose fermenters and nitrate reducers. Most of them are commensals in human intestine, called coliform bacilli (e.g. *Escherichia*, *Klebsiella* and *Proteus*); whereas some are enteric pathogens, e.g. *Shigella* and *Salmonella*. Based on lactose fermentation, the members can be grouped into:

- ❖ Lactose fermenters: e.g. *Escherichia* and *Klebsiella*
- ❖ Non-lactose fermenters: *Shigella*, *Salmonella*, *Proteus*, *Morganella*, *Providencia* and *Yersinia*.

Escherichia coli (Chapters 41, 76)

E. coli is a harmless commensal in human intestine. At the same time, it is one of the most common pathogen encountered clinically and has been associated with various clinical infections.

- ❖ **UTI:** It is caused by uropathogenic *E. coli* (UPEC). It is the most common cause of UTI in all age group
- ❖ **Diarrhea:** It is caused by diarrheagenic *E. coli*. There are six pathotypes of diarrheagenic *E. coli*; elaborating several enterotoxins such as heat labile toxin, heat stable toxin and verocytotoxin
- ❖ **Other infections:** *E. coli* can cause various pyogenic infections such as abdominal infections (e.g. bacterial peritonitis), pneumonia, meningitis (especially in neonates), wound and soft tissue infections such as cellulitis and infection of ulcers and wounds, especially in patient with diabetic foot
- ❖ **Laboratory diagnosis:** It produces lactose-fermenting pink colonies on MacConkey agar, which is subsequently identified by various biochemical reactions

❖ **Treatment:** The primary agents preferred for treatment include cephalosporins, quinolones, co-trimoxazole, etc. However, as majority of *E. coli* infections, especially acquired in hospitals are multi-drug resistant (MDR); the second-line antimicrobial agents such as carbapenems, amikacin or β -lactam/ β -lactamase inhibitor combinations can also be given as primary agent.

Klebsiella pneumoniae (Chapter 61)

Similar to *E. coli*, *Klebsiella pneumoniae* can cause UTI, lobar pneumonia, meningitis (in neonates), septicemia, pyogenic infections such as abscesses and wound infections. Similar to *E. coli*, *K. pneumoniae* is also lactose fermenter; however, it differs in being non-motile, capsulated, and produces mucoid colonies. Treatment for *K. pneumoniae* is same as for *E. coli*.

Other *Klebsiella* species include:

- ❖ ***Klebsiella granulomatis*:** It causes a genito-ulcerative disease called, granuloma inguinale (Chapter 77)
- ❖ ***K. rhinoscleromatis* and *K. ozaenae*:** Produce infections of nasal cavity, called rhinoscleroma and atrophic rhinitis respectively.

Shigella (Chapter 41)

Shigella is the causative the agent of bacillary dysentery; characterized by passage of loose stool mixed with blood and mucus. It comprises of four species—*S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.

- ❖ **Transmission:** Infection occurs by ingestion through contaminated fingers (most common), food, water or rarely flies. Risk factors include overcrowding, poor hygiene and children less than 5 years
- ❖ **Laboratory diagnosis** includes isolation of organism from diarrheic stool specimen using enrichment medium such as selenite F medium and selective media such as DCA (Deoxycholate citrate agar) or XLD (Xylose lysine deoxycholate) agar; followed by identification by using appropriate biochemical reactions
- ❖ **Treatment** of shigellosis includes fluid replacement and antimicrobials such as ciprofloxacin.

Salmonella (Chapter 30)

Salmonellae are classified into typhoidal and non typhoidal serotypes.

- ❖ **Typhoidal *Salmonella*** includes serotypes *S. Typhi* and *S. Paratyphi A, B, and C*. They are restricted to human hosts; in whom they cause enteric fever (typhoid/paratyphoid fever)
- ❖ **Non-typhoidal *Salmonella* (NTS):** The remaining serotypes can colonize the intestine of several animals. They also infect humans causing food-borne gastroenteritis and rarely bacteremia (Chapter 41).

Enteric Fever (Chapter 30)

Enteric fever presents with various manifestations such as fever (with step ladder pattern), headache, chills, rashes

(rose spots), abdominal pain, hepatosplenomegaly, and relative bradycardia.

- ❖ **Pathogenesis:** *Salmonella* is transmitted by ingestion of contaminated water and food
- ❖ **Laboratory diagnosis** of enteric fever includes blood culture by using conventional or automated blood culture systems (e.g. BacT/ALERT) and serological test detecting antibodies (by Widal test)
- ❖ **Treatment** for enteric fever includes ceftriaxone, ciprofloxacin, and azithromycin.

Other Members of *Enterobacteriaceae*

- ❖ ***Enterobacter*:** *Enterobacter* species are similar to *Klebsiella* in clinical manifestations and also in most of the biochemical reactions except being motile. *E. aerogenes* and *E. cloacae* are the most commonly isolated species from the clinical specimens (Chapter 61)
- ❖ ***Proteae*:** Tribe Proteae comprises of three genera: *Proteus*, *Morganella* and *Providencia* (Chapter 76)
 - Although they are saprophytes and commensals; they can also cause opportunistic infections such as urinary tract infections, wound and soft tissue infections, septicemia and nosocomial outbreaks
 - Somatic antigen of certain non-motile *Proteus* strains (called X strains) can be used to detect cross-reacting heterophile antibodies in sera of patients suffering from rickettsial infections (Weil-Felix reaction).
- ❖ ***Serratia*:** *S. marcescens* is usually a saprophyte in the environment, typically produces a red non-diffusible pigment called prodigiosin. However, the hospital strains of *S. marcescens* are often non-pigmented and multiple drug-resistant and are associated with various nosocomial infections (Chapter 61)
- ❖ ***Yersinia pestis*:** It is the causative agent of plague, a fulminant systemic zoonosis; transmitted from rodents by arthropod vector, the rat flea (Chapter 81)
 - **Epidemiology:** Plague was one of the greatest killer known to mankind; caused several pandemics in the ancient days producing millions of deaths. In India, Surat epidemic (in 1994) has witnessed more than 6000 suspected plague cases with 60 deaths
 - **Clinical forms:** Human plague occurs in three clinical forms—(1) bubonic (most common form, characterized by enlarged and tender regional lymph nodes), (2) pneumonic and (3) septicemic.
- ❖ **Yersiniosis:** Infections due to other *Yersinia* species such as *Y. enterocolitica* or *Y. pseudotuberculosis* are called yersiniosis. They are enteropathogenic, cause gastroenteritis, terminal ileitis and mesenteric adenitis (Chapter 41).

Vibrio species (Chapter 42)

Vibrio cholerae is the causative agent of an acute diarrheal disease called cholera.

- ❖ **Epidemiology:** World has witnessed several cholera pandemics in the past; resulting in several thousands

of deaths. Currently, it occurs as sporadic and limited outbreaks

- ❖ **Pathogenesis** of *V. cholerae* is due to a potent enterotoxin, called cholera toxin
- ❖ **Clinical manifestations:** Cholera manifests as painless watery diarrhea, described as rice water stool. Severe dehydration can result in renal failure
- ❖ **Laboratory diagnosis** of cholera includes demonstration of actively motile bacilli (darting motility) in watery stool specimen, followed by isolation of the bacilli in selective medium such as thiosulfate citrate bile salts sucrose (TCBS) agar
- ❖ **Treatment:** Fluid replacement is the mainstay of treatment. Antibiotics such as macrolides can be given in severely dehydrated patients.

The *Vibrio* species other than *V. cholerae* that grow in higher salt concentration are called **halophilic *Vibrio***; examples include *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*. They cause intestinal and extraintestinal manifestations (Chapter 42).

Non-fermenting Gram-negative bacilli (Chapter 65)

Non-fermenters do not ferment any carbohydrates, but utilize them oxidatively. Important human pathogens are as discussed below.

Pseudomonas aeruginosa

P. aeruginosa is a major pathogenic species, causing infections among hospitalized patients and in patients with cystic fibrosis.

- ❖ The **pathogenesis** is greatly attributed to its ability to develop widespread resistance to multiple antibiotics and disinfectants, and producing a number of virulence factors
- ❖ **Clinical manifestations** include various healthcare associated infections such as ventilator associated pneumonia, surgical site infections, nosocomial bacteremia, burn wound infection, etc.
- ❖ **Laboratory diagnosis:** *P. aeruginosa* is an oxidase positive, pigment producing (blue-green, diffusible), non-fermenting gram-negative bacillus
- ❖ **Treatment:** Antimicrobial agents with good anti-pseudomonal action such as ceftazidime, piperacillin or carbapenems are the preferred therapeutic options.

Acinetobacter species

They are saprophytic bacilli, can cause wide-spread healthcare associated infections especially in patients with underlying diseases and immunosuppression. The spectrum of infections and treatment are similar to that of *Pseudomonas*.

Burkholderia species

- ❖ *B. cepacia* inhabits moist hospital environment and intravenous fluids; can cause fatal respiratory infections

and septicemia in hospitalized patients with underlying diseases and immunosuppression

- ❖ *B. pseudomallei*: It is the causative agent of **melioidosis**; which presents in various clinical forms ranging from acute localized infection, subacute pulmonary infection, bloodstream infection and chronic suppurative infection
 - It is bipolar stained on Gram staining, intrinsically resistant to polymyxin B and grows on selective media such as Ashdown's medium
 - Treatment is given for longer duration; agents of choice are meropenem, ceftazidime and cotrimoxazole.

Fastidious Gram-negative bacilli

Fastidious gram-negative bacilli include *Haemophilus*, *Bordetella* and *Brucella*.

Haemophilus species (Chapter 61)

Haemophilus species are pleomorphic gram-negative bacilli that require special growth factors (X or V or both).

- ❖ *H. influenzae*: It is the most pathogenic species; causes pneumonia and meningitis in children. It is diagnosed by demonstrating its ability to grow on blood agar, adjacent to *S. aureus* streak line; a property described as **satellitism**. Ceftriaxone is given for treatment. Vaccination with Hib conjugate vaccine (*H. influenzae* type b) is recommended in children (Chapters 61, 71)
- ❖ *H. ducreyi*: It is the causative agent of soft chancre (chancroid); a sexually transmitted infection characterized by painful genital ulcers and enlarged tender inguinal lymph nodes (bubo) (Chapter 77)
- ❖ **HACEK group** (Chapter 28): They represent a group of highly fastidious gram-negative bacilli, which are found as normal commensal of the oral cavity, but can cause serious infections such as endocarditis. They include—*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.

Bordetella pertussis (Chapter 64)

B. pertussis is the causative agent of whooping cough; a highly contagious toxin-mediated disease, characterized by paroxysmal cough ending in a high pitched inspiratory sound described as “whoop”. The disease can be prevented by vaccination of children—by whole cell pertussis vaccine (as DPT vaccine), given under national immunization schedule.

Brucella species (Chapter 32)

It causes a highly contagious zoonotic febrile illness called undulant fever or brucellosis; manifests as a triad of fever, arthralgia and hepatosplenomegaly. Diagnosis involves isolation of the bacilli in blood culture or detection of antibodies by serological tests such as standard agglutination test. Treatment comprises of doxycycline, in combination with rifampicin or streptomycin.

Miscellaneous Gram-negative bacilli

- ❖ **Campylobacter:** *C. jejuni* causes inflammatory diarrhea/dysentery, abdominal pain and fever. It is a curved gram-negative rod; isolation can be done by incubating in microaerophilic conditions. (Chapter 43)
- ❖ **Helicobacter pylori** (Chapter 43): It is a curved gram-negative rod that colonizes the stomach
 - It is associated with the pathogenesis of acute gastritis, peptic ulcer disease and gastric carcinoma
 - Urea breath test and biopsy urease test are the preferred diagnostic modalities
 - Treatment includes a triple-drug regimen, comprising of omeprazole, clarithromycin and metronidazole; given for 7-14 days.
- ❖ **Legionella** (Chapter 62): *L. pneumophila* is fastidious, pleomorphic gram-negative short rod, associated with two clinical syndromes—(i) Pontiac fever is an acute, milder flu-like self-limited illness and (ii) Legionnaires' disease (a severe form of interstitial pneumonia)
 - Laboratory diagnosis includes isolation of the organism in buffered charcoal, yeast extract (BCYE) agar or urinary antigen detection
 - Macrolides (especially azithromycin) and respiratory quinolones are now the antibiotics of choice.
- ❖ **Gardnerella vaginalis:** It causes profuse watery vaginal discharge—a condition called bacterial vaginosis (Chapter 77)
- ❖ **Streptobacillus moniliformis:** It causes a zoonotic systemic illness transmitted by rodents; called **rat bite fever**. This condition is also caused by another gram-negative bacillus called *Spirillum minus* (Chapter 81).

ANAEROBIC BACTERIAL INFECTIONS

The obligate anaerobic bacteria infecting man can be grouped into spore bearing (e.g. *Clostridium*) and non-sporing anaerobes (Chapter 53).

Clostridium species (Chapter 53)

Clostridia are gram-positive bacilli with bulging spores, commonly found as saprophytes in soil and commensals in the intestine of man and animals. However, few members can cause a variety of infections in humans.

- ❖ **Clostridium perfringens:** It is the causative agent of **gas gangrene**, a rapidly spreading edematous myonecrosis. The pathogenesis is due to its invasiveness and liberation of variety of toxins including α -toxin (lecithinase), which is principal virulence factor (Chapter 53)
- ❖ **Clostridium tetani:** It produces a powerful neurotoxin *tetanospasmin*, which mediates an acute disease manifested by skeletal muscle spasm and autonomic nervous system disturbance; called as **tetanus** (Chapter 72)
- ❖ **Clostridium botulinum:** It also produces a powerful neurotoxin *botulinum toxin*, which causes flaccid paralysis of voluntary muscles. **Botulism** occurs as three

clinical types; food-borne, wound, and infant botulism (Chapter 40)

- ❖ **Clostridioides difficile:** It is responsible for a unique colonic disease—pseudomembranous colitis, which occurs almost exclusively in association with prolonged antimicrobial use; therefore, called as antibiotic-associated diarrhea (Chapter 43).

Non-sporing Anaerobes (Chapter 53)

Non-sporing anaerobes are often a part of the normal flora of mouth, GIT and genital tract of man and animals. Many of these bacteria (e.g. *Bacteroides*) have also been recognized as an important cause of human infections. *Bacteroides fragilis* is recognized as the most common commensal in human intestine; it is also the most frequent anaerobe isolated from clinical specimens.

MISCELLANEOUS BACTERIAL INFECTIONS

Spirochetes

Spirochetes are thin, flexible, elongated spirally coiled helical bacilli; e.g. *Treponema*, *Borrelia* and *Leptospira* (Chapter 32).

Treponema pallidum (Chapter 77)

Treponema pallidum is the causative agent of a sexually transmitted infection called as syphilis.

- ❖ **Clinical stages:** The clinical course of syphilis passes through four clinical stages
 - **Primary syphilis:** It is characterized by painless, firm, non-suppurative genital ulcers and lymphadenopathy (usually inguinal)
 - **Secondary syphilis:** It presents as skin rashes, mucosal patches and condylomata lata
 - **Late syphilis:** It occurs years later, is associated with CVS, and CNS manifestations.
- ❖ **Laboratory diagnosis:** It is mainly diagnosed by detection of:
 - Non-specific antibodies: e.g. venereal disease research laboratory (VDRL) test or rapid plasma regain (RPR) test
 - Specific antibody: e.g. *T. pallidum* hemagglutination (TPHA) test
 - Dark ground microscopy can also be performed to detect the organism in superficial lesions.
- ❖ **Treatment:** Penicillin is the drug of choice for treating all stages of syphilis. Doxycycline can be used alternatively in case of penicillin allergy.

Nonvenereal Treponema species (Chapter 55)

Endemic or nonvenereal treponematoses are caused by three close relatives of *T. pallidum*; producing primary mucocutaenous lesions in non-genital sites (e.g. extremities, oral mucosa).

- ❖ *T. pertenue:* Causes yaws
- ❖ *T. endemicum:* Causes endemic syphilis
- ❖ *T. carateum:* Causes pinta.

Borrelia species (Chapter 32)

Most of the species of *Borrelia* occur as commensals on the buccal and genital mucosa. Few are pathogenic to man, such as:

- ❖ *B. recurrentis* causes epidemic relapsing fever
- ❖ *B. burgdorferi* is the agent of Lyme disease
- ❖ *B. vincentii* causes Vincent's angina in association with fusiform bacilli (Chapter 59).

Leptospira interrogans (Chapter 32)

L. interrogans is the causative agent of leptospirosis; a zoonotic disease transmitted, by direct contact with urine of infected animals such as rodents.

- ❖ **Clinical manifestations:** Produces two types of illnesses
 - The majority (90%) of leptospirosis cases present as mild anicteric febrile illness
 - Few cases (10%) progress to severe form hepatorenal hemorrhagic syndrome or **Weil's disease**; characterized by icterus, high grade fever, hemorrhagic manifestations and impaired renal functions.
- ❖ **Laboratory diagnosis:**
 - *Dark ground microscopy* of clinical specimens such as blood or CSF reveals spirally coiled bacilli (tightly) with hooked ends
 - *Isolation* can also be performed on media such as Ellinghausen-McCullough-Johnson-Harris (EMJH) medium
 - *Antibody against specific serovars* can be detected by microscopic agglutination test (MAT), which serves as the gold standard reference method for diagnosis of leptospirosis.
- ❖ **Treatment:** Oral doxycycline is given for mild leptospirosis; whereas severe cases are treated with penicillin.

Rickettsiae and Related Genera (Chapter 31)

Rickettsiaceae comprise of two genera—*Rickettsia* and *Orientia*; both possess the following properties:

- ❖ They are obligate intracellular organisms
- ❖ They are not cultivable in artificial media, although they can grow in cell lines, or by animal and egg inoculation
- ❖ They are transmitted by arthropod vectors, such as tick, mite, flea or louse.

The various members of Rickettsiae are:

- ❖ *R. prowazekii*: It is the causative agent of epidemic typhus, transmitted by louse
- ❖ *R. typhi*: It causes endemic typhus, transmitted by flea
- ❖ *R. rickettsii*: It is the causative agent of Rocky Mountain spotted fever, transmitted by tick
- ❖ *R. conorii*: It causes Indian tick typhus, transmitted by tick

- ❖ *R. akari*: It is the causative agent of rickettsialpox, transmitted by mite

- ❖ *Orientia tsutsugamushi*: It is the causative agent of scrub typhus, transmitted by mite.

For all Rickettsiae, the final target site is the endothelial cells. Clinically the rickettsial infections may manifest as combination of one or more of the following features—fever, rashes, headache, myalgia, eschar, lymphadenopathy, etc. Doxycycline is the drug of choice in majority of the rickettsial infections.

Other genera related to Rickettsia are:

- ❖ *Ehrlichia*: It produces an acute febrile illness called ehrlichiosis, transmitted by ticks. It infects leukocytes such as granulocytes, monocytes; producing intracellular inclusions, called as **morula**
- ❖ *Coxiella burnetii*: It causes **Q fever**, transmitted by inhalational mode; characterized by atypical pneumonia, hepatitis and on chronic stage, produces endocarditis
- ❖ *Bartonella*: They are the causative agent of Carrion's disease (*B. bacilliformis*), trench fever (*B. quintana*), and cat-scratch disease (*B. henselae*).

Laboratory diagnosis of rickettsial infection includes: (i) Weil Felix test—here, rickettsial antibodies are detected by using non-specific cross reacting *Proteus* antigens or (ii) specific antibody detection test (e.g. by indirect immunofluorescence test and ELISA).

Chlamydiae (Chapter 77)

Chlamydiae are obligate intracellular bacteria that cause a spectrum of diseases in man infecting eye, genital organs and lungs. The following are pathogenic species to man.

- ❖ *Chlamydia trachomatis*: It has 19 serovars, causes various infections to man such as trachoma (a type of chronic keratoconjunctivitis), genital chlamydiasis, inclusion conjunctivitis, infant pneumonia and a sexually transmitted infection called LGV (lymphogranuloma venereum) (Chapter 77)
- ❖ *Chlamydophila psittaci*: It is a pathogen of birds. Infection in man can range from mild influenza-like syndrome to fatal atypical pneumonia (Chapter 62)
- ❖ *Chlamydophila pneumoniae*: It is an exclusively human pathogen, causes atypical pneumonia (Chapter 62).

Mycoplasma (Chapter 62)

Mycoplasmas are the smallest microbes capable of free-living in the environment. They lack rigid cell wall and therefore, are resistant to cell wall acting antibiotics such as beta-lactams.

- ❖ *M. pneumoniae* is the pathogenic species, which is the causative agent of **primary atypical pneumonia** (community acquired)
 - Laboratory diagnosis involves detection of antibodies or isolation of the organism in specific culture media
 - Macrolides are the drug of choice.
- ❖ Urogenital mycoplasmas include *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum*. They cause urethritis (Chapter 77).

EXPECTED QUESTIONS**I. Write short notes on:**

1. Gram-positive cocci infections.
2. Gram-negative cocci infections.
3. Gram-positive bacilli infections.
4. Gram-negative bacilli infections.

General Virology and Overview of Viral Infections

CHAPTER 4

CHAPTER PREVIEW

- General Properties of Viruses
 - Morphology of Virus
 - Classification
- Viral Replication
- Pathogenesis
- Laboratory Diagnosis
- Treatment
- Immunoprophylaxis
- Overview of Viral Infections

GENERAL PROPERTIES OF VIRUSES

Viruses are the smallest unicellular organisms that are obligate intracellular. Viruses are the most primitive microorganisms infecting man. They differ from bacteria and other prokaryotes in many ways.

Viruses Differ from Bacteria as:

- They are obligate intracellular
- They possess either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), but never both
- Filterable: They are smaller than bacteria, can be passed through the bacterial filters
- They cannot be grown on artificial cell free media (However, they can grow in experimental animals, embryonated eggs or tissue culture)
- They multiply by a complex method, but not by binary fission as seen in bacteria
- Viruses do not have a proper cellular organization
- They do not have cell wall or cell membrane or cellular organelles including ribosomes
- They lack the enzymes necessary for protein and nucleic acid synthesis
- They are not susceptible to antibacterial antibiotics.

MORPHOLOGY OF VIRUS

The entire virus particle called as virion, comprises of a **nucleic acid** (DNA or RNA) surrounded by a protein coat called as **capsid**, together known as the nucleocapsid. Some viruses also have an outer **envelope** (Fig. 4.1).

Nucleic Acid

Viruses have only one type of nucleic acid, either DNA or RNA but never both. Accordingly, they are classified as DNA viruses and RNA viruses. The nucleic acid may be single or double stranded, circular or linear, segmented or unsegmented.

- ❖ Most DNA viruses possess dsDNA, except parvoviruses, which have ssDNA
- ❖ RNA viruses possess ssRNA, except:
 - Reoviruses (e.g. rotaviruses)—possess dsRNA
 - Retroviruses—possess two copies of ssRNA.
- ❖ The nucleic acid of most viruses are unsegmented; except bunyavirus, influenza virus, rotavirus and arenaviruses which have multiple segments of RNA.

Capsid

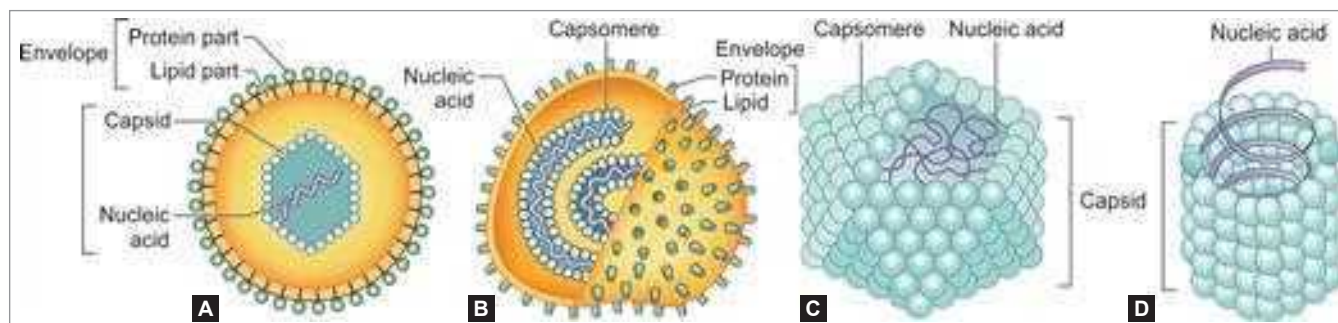
Capsid is composed of a number of repeated protein subunits (polypeptides) called capsomeres. Functions of capsid include:

- ❖ It protects the nucleic acid core from the external environment, e.g. nucleases
- ❖ In non-enveloped viruses, it initiates the first step of viral replication by attaching to specific receptors on the host cells, thus facilitating the entry of the virus
- ❖ It is antigenic and specific for each virus.

Symmetry

Depending upon the arrangement of capsomeres surrounding the nucleic acid, three types of symmetry are described.

1. **Icosahedral (cubical) symmetry:** The capsomeres are arranged as if they lay on the faces of an icosahedron; such viruses have a rigid structure. All DNA viruses (except poxviruses) and most of the RNA viruses have icosahedral symmetry (Figs 4.1A and C)
2. **Helical symmetry:** The capsomeres are coiled surrounding the nucleic acid in the form of a helix or spiral. Such viruses are often flexible. Example include few RNA viruses such as—myxoviruses, rhabdoviruses, filoviruses, bunyaviruses, etc. (Figs 4.1B and D)
3. **Complex symmetry:** Poxviruses do not have either of the above symmetry, but they possess a complex symmetry.



Figs 4.1A to D: Structure and symmetry of virus: **A.** Enveloped virus with icosahedral nucleocapsid; **B.** Enveloped virus with helical nucleocapsid; **C.** Non-enveloped virus with icosahedral nucleocapsid; **D.** Non-enveloped virus with helical nucleocapsid.

Envelope

Certain viruses possess an envelope surrounding the nucleocapsid. Envelope is lipoprotein in nature.

- ❖ The lipid part is derived from the host cell membrane and the protein part is virus coded, made up of subunits called **peplomers**, which project as spikes on the surface of the envelope
- ❖ Some viruses may have more than one kind of peplomers, e.g. influenza viruses possess hemagglutinin and neuraminidase peplomers
- ❖ Enveloped viruses are more susceptible to heat and lipid solvents like ether
- ❖ Peplomers are antigenic. They can also bind to specific receptors on the host cells, thus facilitating the entry of the virus.

Most Viruses are Enveloped Except

- ❑ Non-enveloped DNA viruses—parvovirus, adenovirus and papovavirus
- ❑ Non-enveloped RNA viruses—picornavirus, reovirus, calicivirus, hepatitis A virus and hepatitis E virus.

Size of the Viruses

Viruses are extremely small, vary from 20–400 nm in size. Smallest virus is parvovirus (20 nm) and largest being poxvirus (400 nm) (Fig. 4.2). Because of the small size,

viruses can pass through bacterial filters and they cannot be visualized under light microscope.

Shapes of the Viruses

Most of the animal viruses are **roughly spherical** with some exceptions.

Shapes of the Viruses

- ❑ **Rabies virus:** Bullet-shaped
- ❑ **Ebola virus:** Filamentous
- ❑ **Poxvirus:** Brick-shaped
- ❑ **Adenovirus:** Space vehicle-shaped
- ❑ **Rotavirus:** Wheel-shaped
- ❑ **Tobacco mosaic virus:** Rod-shaped

Viruses Differ from Viroids, Prions and Virusoids

The entire viral particle is called as virion; comprising of protein capsid coat and nucleic acid. There are some incomplete viral particles such as viroid, prion and virusoid.

- ❑ **Viroids** comprise of naked, circular, small ssRNA without a capsid. They are mostly restricted to plants. They depend on host enzymes for replication. Hepatitis D virus in humans is similar to viroids
- ❑ **Prions** consist of abnormal infectious protein molecules without nucleic acid
 - They are highly resistant to physical and chemical agents

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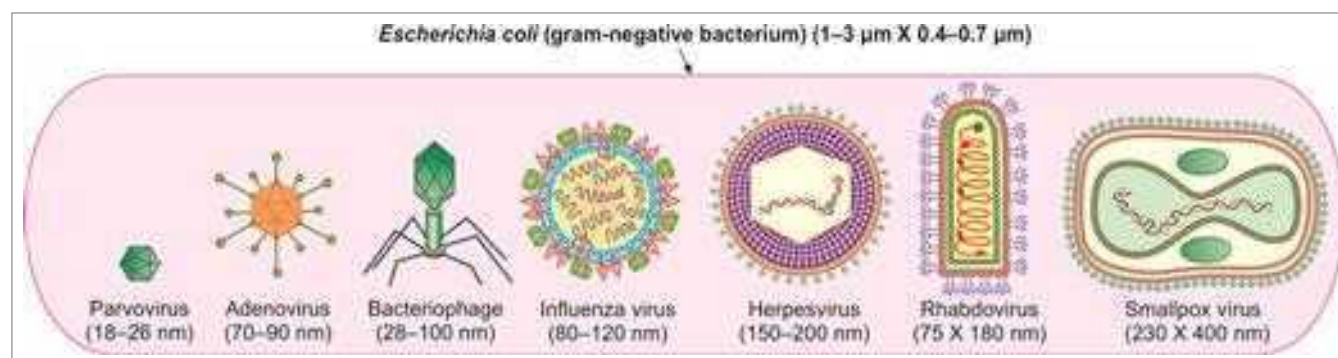


Fig. 4.2: Comparison of sizes of various viruses with that of *Escherichia coli*.

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- They produce neurodegenerative condition of brain in humans (having long incubation period in years) called prion disease (described in Chapter 74).

NOMENCLATURE AND CLASSIFICATION

International Committee on Taxonomy of Viruses (2000) had proposed a classification for viruses (Table 4.1).

- ❖ Viruses are grouped into families (ending with the suffix '*viridae*') on the basis of morphology, genome structure, and strategies of replication
- ❖ Viruses infecting humans belong to 24 families, out of which important ones are listed below (Table 4.1)
- ❖ Most of the families are further classified into genera (ending with the suffix '*-virus*') based on physicochemical or serological differences

Table 4.1: Classification of viruses.					
Family	Nucleic acid	Envelope	Symmetry	Size (nm)	Representative viruses
DNA viruses					
DNA					
Herpesviridae	ds, linear	Yes	Icosahedron	150–200	<ul style="list-style-type: none"> • Herpes simplex virus (HSV)-1 • Herpes simplex virus (HSV)-2 • Varicella-zoster virus • Epstein-Barr virus (EBV) • Cytomegalovirus (CMV) • Human herpesvirus 6, 7 and 8
Hepadnaviridae	ds, circular, incomplete	Yes	Icosahedron	40–48	<ul style="list-style-type: none"> • Hepatitis B virus
Parvoviridae	ss, linear	Absent	Icosahedron	18–26	<ul style="list-style-type: none"> • Parvovirus B19
Papovaviridae	ds, circular	Absent	Icosahedron	45–55	<ul style="list-style-type: none"> • Human papillomavirus • JC virus and BK virus
Poxviridae	ds, linear	Yes	Complex	230 x 400	<ul style="list-style-type: none"> • Variola (smallpox) • Molluscum contagiosum virus
Adenoviridae	ds, linear	Absent	Icosahedron	70–90	<ul style="list-style-type: none"> • Human adenovirus
RNA viruses					
RNA					
Picornaviridae	ss, +ve sense	Absent	Icosahedron	28–30	<ul style="list-style-type: none"> • Poliovirus • Coxsackievirus • Echovirus • Enterovirus • Rhinovirus • Hepatitis A virus
Caliciviridae	ss, +ve sense	Absent	Icosahedron	27–40	<ul style="list-style-type: none"> • Norwalk virus • Hepatitis E virus
Togaviridae	ss, +ve sense	Yes	Icosahedron	50–70	<ul style="list-style-type: none"> • Rubella virus • Eastern equine encephalitis virus • Western equine encephalitis virus
Flaviviridae	ss, +ve sense	Yes	Icosahedron	40–60	<ul style="list-style-type: none"> • Yellow fever virus • Dengue virus • St Louis encephalitis virus • West Nile virus • Hepatitis C virus
Coronaviridae	ss, +ve sense	Yes	Helical	120–160	<ul style="list-style-type: none"> • Coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2 (the agent of COVID-19)
Rhabdoviridae	ss, -ve sense	Yes	Helical	75 x 180	<ul style="list-style-type: none"> • Rabies virus • Vesicular stomatitis virus
Filoviridae	ss, -ve sense	Yes	Helical	80 x 1000	<ul style="list-style-type: none"> • Marburg virus • Ebola virus
Paramyxoviridae	ss, -ve sense	Yes	Helical	150–300	<ul style="list-style-type: none"> • Parainfluenza virus • Mumps virus • Measles virus • Respiratory syncytial virus (RSV) • Nipah virus • Hendra virus • Newcastle disease virus • Metapneumovirus

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Family	Nucleic acid	Envelope	Symmetry	Size (nm)	Representative viruses
Orthomyxoviridae	ss, -ve sense, 7 or 8 segments	Yes	Helical	80–120	• Influenza viruses- A, B, C and D
Bunyaviridae	ss, -ve sense, 3 circular segments	Yes	Helical	80–120	• Hantavirus • California encephalitis virus • Sandfly fever virus
Arenaviridae	ss, -ve sense 2 circular segments	Yes	Helical*	50–300	• Lymphocytic choriomeningitis virus • Lassa fever virus • South American hemorrhagic fever virus
Reoviridae	ds, 10–12 segments	Absent	Icosahedron	60–80	• Rotavirus • Reovirus • Colorado tick fever virus
Retroviridae	Two identical copies of +ve sense ss linear RNA	Yes	Icosahedron (spherical)	80–110	• HTLV (Human T Lymphotropic virus) • HIV (Human immunodeficiency virus)

*Doubtful

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ds, double stranded; ss, single stranded; +ve, positive; -ve, negative; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, middle east respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

- ❖ Some families (Poxviridae, Herpesviridae, Parvoviridae and Paramyxoviridae) have subfamilies, which in turn consist of genera.

VIRAL REPLICATION

Viruses do not undergo binary fission (seen in bacteria), but undergo a complex way of cell division. Replication of viruses passes through seven sequential steps:

Attachment → Penetration → Uncoating → Biosynthesis → Assembly → Maturation → Release.

1. Adsorption/Attachment

It is the first and the most specific step of viral replication. It involves receptor interactions. The viruses have attachment sites on their envelopes or capsid proteins that bind to the complementary receptor sites present on the host cell surface.

- ❖ **HIV:** Viral surface glycoprotein gp 120 binds to CD4 molecules on the host cells
- ❖ **Influenza:** Viral hemagglutinin (an envelope protein) binds specifically to glycoprotein receptors present on the surface of the respiratory epithelium.

2. Penetration

After attachment, the virus particles penetrate into the host cells either by:

- ❖ **Phagocytosis (or viropexis):** It occurs through receptor mediated endocytosis resulting in the uptake of virus particles within the endosomes of the host cytoplasm
- ❖ **Membrane fusion:** Some enveloped viruses (e.g. human immunodeficiency virus or HIV) enter by fusion of their envelope proteins with the plasma membrane of the host cell so that only the nucleocapsid enters into the

cytoplasm, whereas the viral envelope remains attached to the host cell membrane

- ❖ **Injection of nucleic acid:** Bacteriophages (viruses that infect bacteria) cannot penetrate the rigid bacterial cell wall, hence only the nucleic acid is injected; while the capsid remains attached to the cell wall.

3. Uncoating

By the action of lysosomal enzymes of the host cells, the viral capsid gets separated and the nucleic acid is released into the cytoplasm. This step is absent for bacteriophages.

4. Biosynthesis

In this step, the following viral components are synthesized:

- ❖ Nucleic acid
- ❖ Capsid protein
- ❖ Enzymes required for various stages of viral replication
- ❖ Regulatory proteins to shut down the host cell metabolism.

Site of Nucleic Acid Replication

- ❑ In DNA viruses, the DNA replication occurs in the nucleus except in poxviruses, which synthesize DNA in the cytoplasm
- ❑ In RNA viruses, the RNA replication occurs in the cytoplasm except in retroviruses and orthomyxoviruses, which synthesize RNA in the nucleus.

DNA Viruses

Biosynthesis of DNA viruses involves the following basic steps (Fig. 4.3):

- ❖ Transcription of parental DNA to form early messenger RNA (mRNA)
- ❖ Early mRNA undergoes translation to produce early non-structural proteins
- ❖ **Viral DNA replication:** Early non-structural proteins shutdown the host metabolism and help in the replication of parental DNA to form copies of progeny DNA

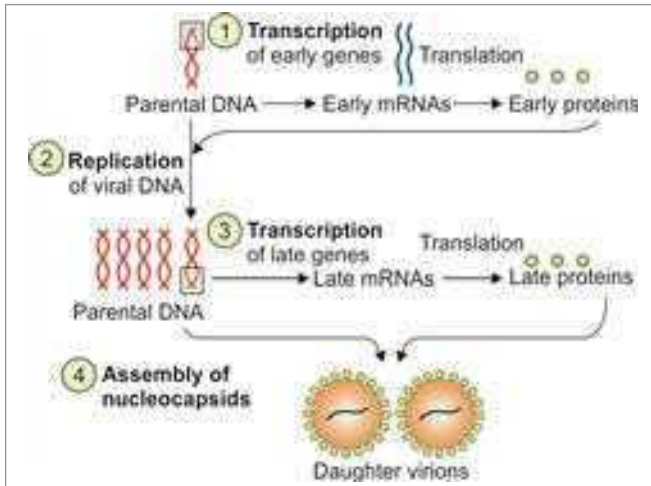


Fig. 4.3: Steps of DNA virus biosynthesis.

- ❖ Progeny DNA undergoes transcription to form late mRNA, which are further translated to form late structural proteins (i.e. capsids and envelope proteins).

Minor Differences in Replication Among DNA Viruses

- ❖ **Site:** DNA replication occurs in nucleus (except in poxviruses), whereas the mRNA transcription and protein translation take place in the cytoplasm
- ❖ The linear dsDNA of herpesviruses becomes circular inside the host cell and then replicates by **rolling circle mechanism**
- ❖ **Hepadnaviruses** (e.g. Hepatitis B virus) contain partially dsDNA that is first converted to fully dsDNA by viral polymerases (discussed in detail in Chapter 48).

RNA Viruses

The process of biosynthesis varies among RNA viruses depending on whether the genomic RNA is positive/negative sense and single/double-stranded.

Type I (Positive-sense ssRNA Viruses)

In most RNA viruses, their RNA has the same polarity as mRNA, hence, they can directly translate to form early proteins (Fig. 4.4A).

- ❖ **Synthesis of progeny RNA:** Early proteins have RNA polymerase activity that direct replication (transcription) of (+) ssRNA → (-) ssRNA → (+) ssRNA
- ❖ Late proteins are formed by translation of (+) ssRNA.

Type II (Negative-sense ssRNA Viruses)

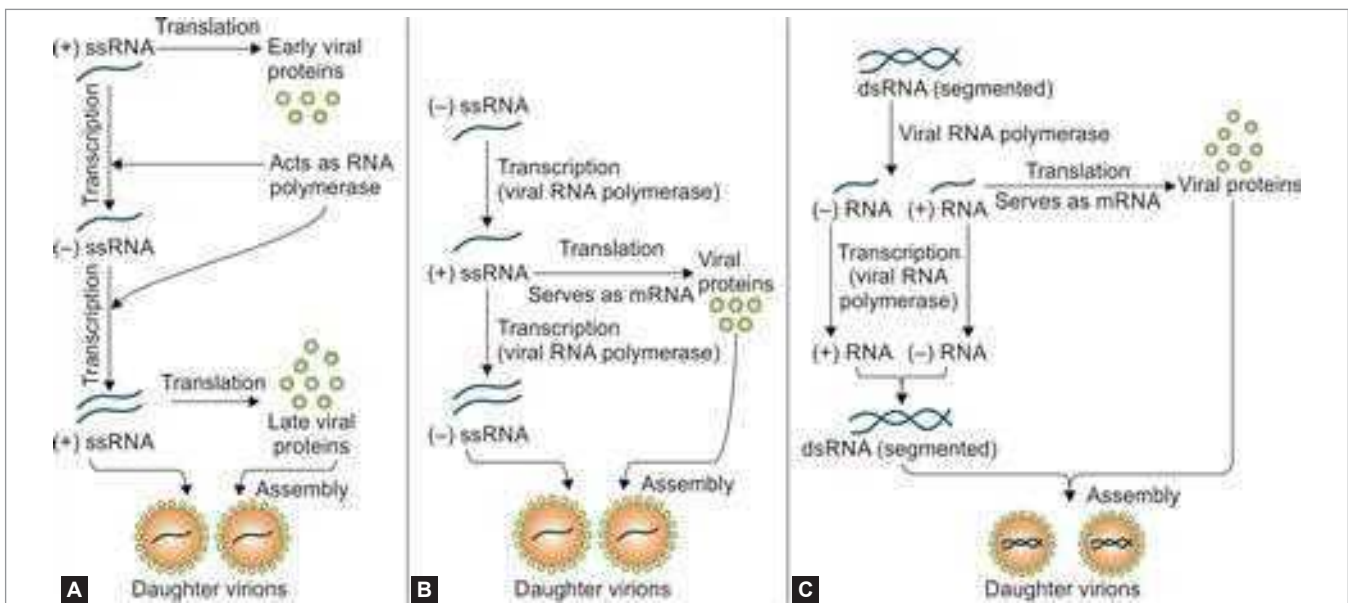
Myxoviruses and rabies virus have negative sense ssRNA. Their RNA polarity is opposite to that of mRNA, hence, they cannot directly translate into proteins (Fig. 4.4B).

- ❖ The (-) ssRNA transcribes into (+) ssRNA first, using viral RNA polymerases
- ❖ Then the (+) ssRNA translates to form proteins and also it acts as template and undergoes replication to form copies of (-) ssRNA.

Type III (Double-stranded RNA Viruses)

Reoviruses (e.g. rotavirus) have dsRNA which is usually segmented with each segment coding for one polypeptide (Fig. 4.4C).

- ❖ The (+) strand RNA can act as mRNA and undergoes translation to form proteins by using viral enzymes
- ❖ Both the (+) and (-) strands serve as templates for the synthesis of complementary strands to form the duplex.



Figs 4.4A to C: Replication of: A. Positive-sense ssRNA virus; B. Negative-sense ssRNA virus; C. dsRNA virus.

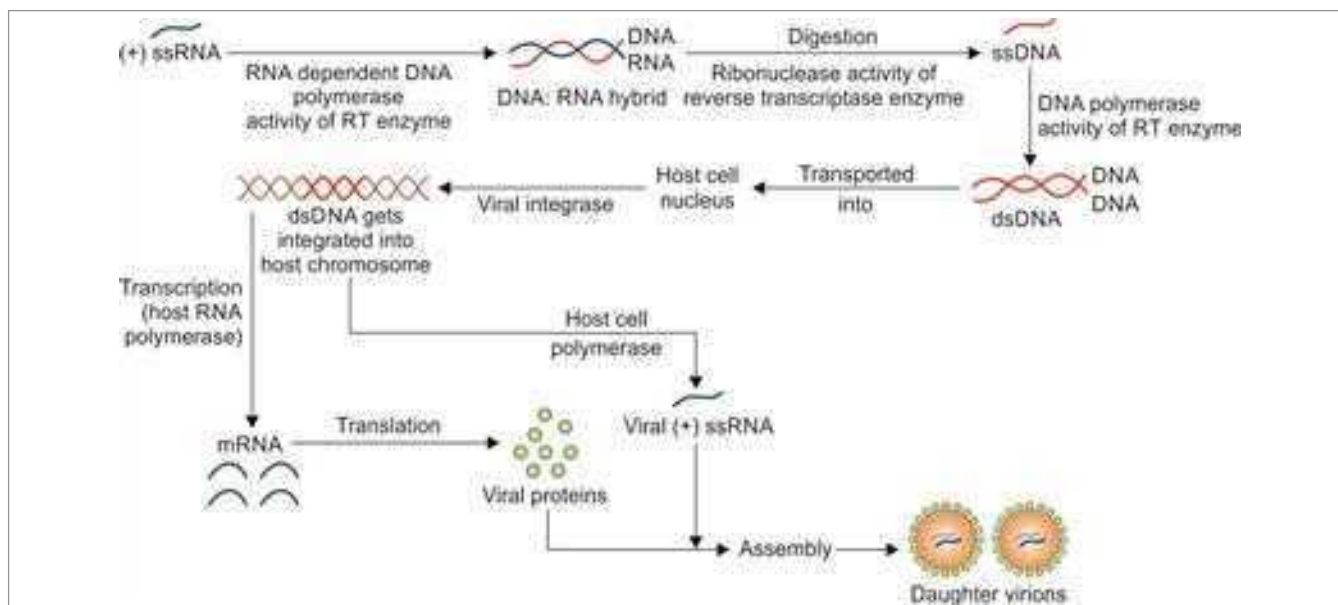


Fig. 4.5: Replication retroviruses (e.g. HIV).

Type IV (Retroviruses)

HIV and other retroviruses possess two copies of linear non-segmented (+) ssRNA and enzymes such as reverse transcriptase (RT) and integrase (Fig. 4.5).

- ❖ After entry into the host cell, the ssRNA gets reverse transcribed to form ssDNA by viral RT which acts as RNA dependent DNA polymerase. DNA : RNA hybrid is formed
- ❖ Reverse transcriptase has also ribonuclease activity by which it digests the RNA from DNA:RNA hybrid
- ❖ The resulting ssDNA is converted to dsDNA by the DNA polymerase activity of the same RT enzyme
- ❖ dsDNA is transported to the nucleus where it gets integrated into the host chromosome by viral integrase
- ❖ The integrated DNA serves as a template for the production of mRNA (which are translated into proteins) and genomic progeny RNA.

5. Assembly

Viral nucleic acid and proteins are packaged together to form progeny viruses (nucleocapsids). Assembly may take place in the host cell nucleus or cytoplasm.

- ❖ DNA viruses are assembled in the nucleus except hepadnaviruses and poxviruses (in cytoplasm)
- ❖ RNA viruses are assembled in the cytoplasm.

6. Maturation

Following assembly, maturation of daughter virions take place either in the host cell nucleus or cytoplasm or membranes (Golgi or endoplasmic reticulum or plasma membrane).

7. Release

Release of daughter virions occur either by:

- ❖ **Lysis of the host cells:** As shown by non-enveloped viruses and bacteriophages

- ❖ **Budding:** It is shown by enveloped viruses. During budding, they acquire a part of the host cell membrane to form the lipid part of their envelopes; subsequently on which viral glycoproteins are inserted.

Eclipse phase: It is defined as ‘interval between the penetration of the virus into the host cell till the appearance of first infectious virus progeny particle.’

- ❖ During this period, the virus cannot be demonstrated inside the host cell
- ❖ The duration of eclipse phase is about 15 to 30 minutes for bacteriophages and 15–30 hours for most of the animal viruses.

Defective Viruses (Dependoviruses)

Such viruses are genetically defective. They cannot perform all the steps of viral replication by themselves, but they need a second helper virus, which can supplement the genetic deficiency. Examples of defective viruses are:

- ❖ Hepatitis D virus (requires the help of hepatitis B virus)
- ❖ Adeno-associated satellite viruses (require the help of adenoviruses).

VIRAL GENETIC MODIFICATIONS

Similar to other living objects, viruses also follow laws of genetics. Several properties of viruses (e.g. virulence, antigenicity, capsid production) are under genetic control. The viruses show genetic modifications by two principal methods—(1) mutations and (2) interactions between viral genes or their gene products (proteins).

Mutation

Mutations occur during every viral infection, at a frequency of 10^{-4} to 10^{-8} mutations per base pair per generation. However, mutation becomes evident only if it induces

some readily observable property or leads to survival or death of the virus. The mutants may be of various types as described in bacteria (Chapter 3.4). A special class of mutant is seen among viruses called **conditional lethal mutant** which has great applications in virology.

Conditional lethal mutant can grow only in specific conditions called permissive conditions, but cannot grow in other conditions.

Temperature-sensitive mutant (*ts* mutant) is a type of conditional lethal mutant that can grow at a low (permissive) temperature (28–31°C), but not at higher (restrictive) temperature (37°C). *ts* mutants have been used for the preparation of live viral vaccines (e.g. *ts* influenza vaccine).

Interactions Between Viral Genes

When two or more virus particles infect the same host cell, there occurs a variety of interactions, both genetic and non-genetic.

Genetic Recombination

It occurs between two different but related viruses of the same family infecting a host cell simultaneously.

- ❖ The two viruses exchange segments of nucleic acids between them so that a hybrid (recombinant virus) results
- ❖ Such hybrids possess new genes not found in both the parent viruses, are genetically stable and able to replicate.

Reassortment

It is a type of recombination seen in segmented RNA viruses such as influenza, rota, bunya, and arena viruses.

- ❖ When two strains of influenza virus infect a host cell, gene exchanges take place between the RNA segments resulting in production of reassortants
- ❖ Reassortment is probably the most important method by which the pandemic strains of the influenza virus originate in nature (e.g. H1N1 strain in 2009).

Viral Interference

When two viruses infect a host cell or a cell line, sometimes it leads to inhibition of one of the virus, called viral interference

- ❖ Interference does not occur with all viral combinations; many viruses may infect and multiply together in a host cell
- ❖ **Oral Polio Vaccine (OPV)** is a classical example where viral interference is seen. OPV serotypes interfere with the spread of wild poliovirus, thus played a crucial role in control of polio outbreaks.

PATHOGENESIS OF VIRAL INFECTIONS

Most of the viral infections progress through the following steps inside the human body:

- ❖ Transmission (entry into the body)
- ❖ Primary site replication
- ❖ Spread to secondary site
- ❖ Manifestations of the disease.

Transmission

Viruses enter into the human body through various routes (Table 4.2).

Table 4.2: Transmission and spread of viruses.

Mode of transmission	Produce local infection at the portal of entry	Spread to distant sites from the portal of entry
Respiratory route (probably the most common route)	Produce respiratory infection <ul style="list-style-type: none"> • Influenza virus • Parainfluenza virus • Respiratory syncytial virus (RSV) • Rhinovirus • Adenovirus • Coronaviruses such as SARS-CoV-2 (the agent of COVID-19) • Herpes simplex virus (HSV) (rare) 	<ul style="list-style-type: none"> • Measles virus • Mumps virus • Rubella virus • Varicella-zoster virus • Cytomegalovirus (CMV) • Parvovirus • Smallpox virus
Oral route	Produce gastroenteritis <ul style="list-style-type: none"> • Rotavirus • Adenovirus-40,41 • Calicivirus • Astrovirus 	<ul style="list-style-type: none"> • Poliovirus • Coxsackie virus • Hepatitis viruses—A and E • Cytomegalovirus • Epstein-Barr virus (EBV)
Cutaneous route	Produce skin lesions <ul style="list-style-type: none"> • Herpes simplex virus-1 (HSV-1) • Human papillomavirus (HPV) • Molluscum contagiosum virus 	<ul style="list-style-type: none"> • Herpes simplex virus
Vector bite	—	Arboviruses such as: <ul style="list-style-type: none"> • Dengue virus (<i>Aedes</i>) • Chikungunya virus (<i>Aedes</i>) • Japanese encephalitis virus (<i>Culex</i>) • Yellow fever virus and Zika virus (<i>Aedes</i>) • Kyasanur Forest disease virus (Tick)

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Mode of transmission	Produce local infection at the portal of entry	Spread to distant sites from the portal of entry
Animal bite	—	Rabies virus
Sexual route	Produce genital lesions <ul style="list-style-type: none"> • Herpes simplex virus-2 (HSV-2) • Human papillomavirus (HPV) 	<ul style="list-style-type: none"> • Hepatitis B, C and rarely D viruses • Human immunodeficiency virus (HIV)
Blood transfusion	—	<ul style="list-style-type: none"> • Hepatitis B, C and rarely D viruses • HIV • Parvovirus
Injection	—	<ul style="list-style-type: none"> • Hepatitis B, C and rarely D viruses • HIV
Transplacental route	Produce congenital manifestations in fetus <ul style="list-style-type: none"> • Rubella virus • Cytomegalovirus • Herpes simplex virus • Varicella-zoster virus • Parvovirus 	Transmitted through placenta to fetus, without congenital manifestations <ul style="list-style-type: none"> • Measles virus • Mumps virus • Hepatitis B, C and rarely D viruses • HIV
Conjunctival route	<ul style="list-style-type: none"> • Adenovirus • Enterovirus 70 • Coxsackie virus A-24 • Herpes simplex virus • Varicella-zoster virus 	—

Primary Site of Replication

- ❖ Some viruses are restricted to the portal of entry where they multiply and produce local diseases. They spread locally over the epithelial surfaces, but there is no viremia or spread to distant sites. They have a shorter incubation period and shorter duration of immunity
- ❖ On the other hand, most viruses multiply locally to initiate a silent local infection, which is followed by the spread via lymphatics to regional lymph nodes (most viruses) or via blood (e.g. poliovirus) or via neuronal spread to reach central nervous system or CNS (e.g. rabies virus).

Spread of Virus (Fig. 4.6)

- ❖ **Primary viremia:** Viruses spread to the blood stream either from the primary sites or from the lymph nodes. In blood, viruses may remain as free in plasma or may be cell-associated in lymphocytes or macrophages
- ❖ **Secondary site replication:** Viruses are then transported to the reticuloendothelial system (bone marrow, endothelial cells, spleen and liver) where further multiplication takes place. Secondary sites are called as the central foci for viral multiplication
- ❖ **Secondary viremia:** From the spleen and liver, viruses spillover into the blood stream leading to *secondary viremia* which results in the onset of non-specific symptoms
- ❖ **Target organs:** Via the bloodstream, they reach the target organs (lung, brain, skin, etc.). Certain viruses (e.g. rabies) affecting brain, there is no viremia. Instead, virus reaches the target organ via neuronal spread
- ❖ **Tropism** of the viruses for specific organs (Table 4.3) determines the pattern of systemic illness (e.g. hepatitis viruses have tropism for hepatocytes, and produce hepatitis as the primary disease). Tropism in turn

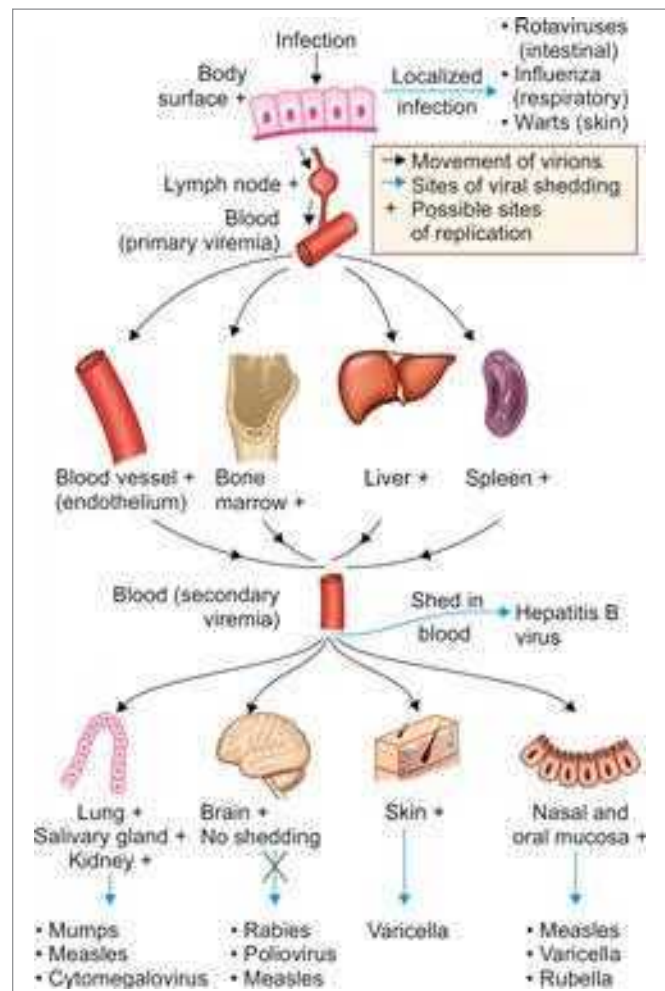


Fig. 4.6: Pathogenesis of viral infections.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

Table 4.3: Cell tropism of human viruses.

Cell type associated	DNA viruses	RNA viruses
Lymphocytes	Epstein-Barr virus Cytomegalovirus Hepatitis B virus JC virus BK virus	Mumps Measles Rubella HIV
Monocytes-macrophages	Cytomegalovirus	Poliovirus, HIV, Measles
Neutrophils	—	Influenza virus
Red blood cells	Parvovirus B19	Colorado tick fever virus
None (free in plasma)	—	Togavirus Picornavirus

depends on the presence of host cell receptors specific for the viruses.

Virus Shedding

Shedding of infectious virus is a necessary step to maintain viruses in the environment or hosts. Shedding may occur at various stages of the disease.

- ❖ **Portal of entry** is the site for shedding for those viruses that produce local infection, e.g. influenza virus is shed in respiratory secretions
- ❖ **Blood:** The viruses that spread through vector bite (arboviruses) or by blood transfusion or needle pricks (hepatitis B) are shed in blood
- ❖ **Near the target tissue/organ:** Skin (varicella-zoster), salivary gland (mumps), kidney (CMV)
- ❖ **No viral shedding:** Humans are the dead-end for certain viruses infecting central nervous system, such as rabies.

Manifestations of Viral Infections

Incubation Period

The incubation period is the time interval between the entry of the virus into the body and appearance of the first clinical manifestation. Therefore, incubation period depends on the distance between the site of entry and the target organ.

- ❖ It is shorter if the virus produces lesions near to the site of entry, e.g. influenza virus
- ❖ It is longer if the target organ is much far from the site of entry, e.g. poliovirus and rabies virus
- ❖ **Exception:** There are many exceptions to this rule. For example, both dengue and hepatitis B virus are blood borne, but they greatly vary in incubation periods (4–5 days and 30–180 days respectively). This is because there are many other factors that affect the incubation period such as host immune response, nature of the virus, etc.

Clinical Manifestations

Persons infected with viruses develop either an inapparent (subclinical) infection or apparent (clinical) infection; the latter may be acute, subacute or chronic depending upon

the onset of illness. The symptoms developed depend upon the target body sites where the virus multiplies.

- ❖ Respiratory viruses such as influenza and coronaviruses produce upper and lower respiratory tract infections
- ❖ Gastroenteritis may be produced by viruses such as rotavirus and norovirus
- ❖ Hemorrhagic fever may be a manifestation of viruses such as dengue, Ebola virus, etc.
- ❖ Neurotropic viruses can produce meningitis (enteroviruses) or encephalitis (rabies, Japanese encephalitis)
- ❖ Exanthematous rashes are produced by a number of viruses including measles, varicella-zoster, etc.
- ❖ Hepatitis viruses produce hepatitis and jaundice.

Viral Pathogenesis at Cellular Level

At the cellular level, the virus can produce three types of infections in a host cell which in turn depends on the nature of the virus and the cell infected.

Failed Infection (Abortive Infection)

It occurs if the virus infects the host cells which are non-permissive (i.e. absence of surface receptors or machineries to support viral replication).

Cell Death (Cytocidal or Lytic Infection)

Viruses adopt different mechanisms to induce host cell death such as:

- ❖ Inhibition of host cell DNA by herpesvirus
- ❖ Inhibition of host cell protein synthesis by poliovirus
- ❖ **Fusion (syncytia formation):** Glycoproteins of some enveloped viruses (paramyxoviruses, herpesviruses, and retroviruses) are expressed on host cell surface which triggers the fusion of neighboring cells to form multinucleated giant cells called syncytia, that allows the virus to spread from cell to cell and escape antibody neutralization
- ❖ **Immune mediated lysis:** The expression of viral antigens on the host cell surface can lead to binding of antibodies followed by complement or natural killer (NK) cell mediated lysis.

Infection without Cell Death

Infection without cell death may occur in two ways.

1. Steady State Infection

The virus and host cell enter into a peaceful coexistence, both replicating independently without any cellular injury. Such persons develop either inapparent (subclinical) infection or apparent (clinical) infections; which may be acute, subacute or chronic infection.

2. Persistent Viral Infection

The virus undergoes a period of latency which may be of various types:

- ❖ **Latent infection with periodic exacerbations:** Seen with members of Herpesviridae family

- ❖ **Cell transformation:** Oncogenic viruses such as hepatitis B virus or Epstein-Barr virus or human papillomavirus induce host cell transformation and the transformed cells divide indefinitely leading to tumor production
- ❖ **Latency in HIV infection:** Viral genome gets integrated with host cell chromosome and undergoes long period of clinical latency
- ❖ **Latency in slow virus infection:** Slow viruses have an unusual long incubation period (years)
- ❖ **Persistent tolerant infection:** The classical example is lymphocytic choriomeningitis virus infecting mice. Here, the host is immunologically tolerant to the virus, does not show any immune response, but the virus is readily demonstrable in the tissues. Disease sets in when the tolerance is interrupted.

Morphological Changes in the Host Cells

Certain viruses induce characteristic changes in the host cells (e.g. **inclusion body**), which can be detected by histopathological staining.

Inclusion Body

They are the aggregates of virions or viral proteins and other products of viral replication that confer altered staining property to the host cell.

Role in Laboratory Diagnosis

Inclusion bodies are characteristic of specific viral infections. They have distinct size, shape, location and staining properties by which they can be demonstrated in virus infected cells under the light microscope.

Location

They may be present either in the host cell cytoplasm or nucleus or both (Table 4.4).

- ❑ **Intracytoplasmic inclusion bodies:** They are generally acidophilic and can be seen as pink structures when stained with Giemsa or eosin methylene blue stains (e.g. most poxviruses and rabies)
- ❑ **Intranuclear inclusion bodies:** They are basophilic in nature. Cowdry (1934) had classified them into:
 - Cowdry type A inclusions: They are variable in size and have granular appearance
 - Cowdry type B inclusions: They are more circumscribed, amorphous or hyaline spheres; multiple in number.
- ❑ **Both intracytoplasmic and intranuclear inclusions.**

LABORATORY DIAGNOSIS OF VIRAL DISEASES

Laboratory diagnosis of viral infections is useful for the following purposes:

- ❖ **To start antiviral drugs** for those viral infections for which specific drugs are available such as herpes, CMV, HIV, influenza and respiratory syncytial virus (RSV)
- ❖ **Screening of blood donors** for HIV, hepatitis B and hepatitis C helps in the prevention of transfusion-transmitted infections

Table 4.4: Inclusion bodies and viruses producing them.

Intracytoplasmic inclusion bodies
<ul style="list-style-type: none"> • Negri bodies—rabies virus • Paschen body—variola virus • Guarneri bodies—vaccinia virus • Bollinger bodies—fowlpox virus • Molluscum bodies—molluscum contagiosum virus • Perinuclear cytoplasmic body—reovirus
Intranuclear inclusion bodies
Cowdry type A inclusions
<ul style="list-style-type: none"> • Torres body—yellow fever virus • Lipschultz body—herpes simplex virus
Cowdry type B inclusions
<ul style="list-style-type: none"> • Poliovirus • Adenovirus
Intracytoplasmic and intranuclear inclusion bodies
<ul style="list-style-type: none"> • Owl's eye appearance—cytomegalovirus • Measles virus

- ❖ **Surveillance purpose:** To assess the disease burden in the community by estimating the prevalence and incidence of viral infections
- ❖ **For outbreak or epidemic investigation,** e.g. influenza epidemics, dengue outbreaks—to initiate appropriate control measures
- ❖ **To start post-exposure prophylaxis** of antiretroviral drugs to the health care workers following needle stick injury (Chapter 25)
- ❖ **To initiate certain measures:** For example,
 - If rubella is diagnosed in the first trimester of pregnancy, termination of pregnancy is recommended
 - If newborn is diagnosed to have hepatitis B infection, then immunoglobulins (HBIG) should be started within 12 hours of birth.

LABORATORY DIAGNOSIS

Viral diseases

1. Direct Demonstration of Virus

- ❑ Electron microscopy
- ❑ Immunoelectron microscopy
- ❑ Fluorescent microscopy
- ❑ Light microscopy:
 - Histopathological staining: To demonstrate inclusion bodies
 - Immunoperoxidase staining.

2. Detection of Viral Antigens

By various formats such as ELISA, direct IF, ICT, flow through assays.

3. Detection of Specific Antibodies

- ❑ Conventional techniques such as HAI, neutralization test and CFT
- ❑ Newer diagnostic formats such as ELISA, ICT, flow through assays.

4. Molecular Methods to Detect Viral Genes

- ❑ Nucleic acid probe—for detection of DNA or RNA by hybridization

Contd...

Contd...

L **LABORATORY DIAGNOSIS****Viral diseases**

- ❑ PCR—for DNA detection by amplification
- ❑ Reverse transcriptase-PCR—for RNA detection
- ❑ Real time PCR—for DNA quantification
- ❑ Real time RT-PCR—for RNA quantification.

5. Isolation of Virus by

- ❑ Animal inoculation
- ❑ Embryonated egg inoculation
- ❑ Tissue cultures: Organ culture, explant culture, cell line culture (primary, secondary and continuous cell lines).

Direct Demonstration of Virus**Electron Microscopy**

Detection of viruses by electron microscopy (EM) is increasingly used nowadays. Specimens are negatively stained by potassium phosphotungstate and scanned under EM.

- ❖ **Shape:** Viruses can be identified based on their distinct appearances; for example:
 - Rabies virus—bullet-shaped
 - Rotavirus—wheel-shaped
 - Coronavirus—petal-shaped peplomers
 - Adenovirus—space vehicle-shaped
 - Astrovirus—star-shaped peplomers.
- ❖ **Direct detection from specimens:** This is useful for viruses that are difficult to cultivate; e.g. rotavirus, hepatitis A and E viruses from feces and CMV from urine
- ❖ **Virus detection from tissue culture:** EM can also be used for detection of viral growth in tissue cultures
- ❖ **Drawbacks:** EM is highly expensive, has low sensitivity with a detection threshold of 10^7 virions/mL. The specificity is also low.

Immuno Electron Microscopy

The sensitivity and specificity of EM can be improved by adding specific antiviral antibody to the specimen to aggregate the virus particles which can be centrifuged. The sediment is negatively stained and viewed under EM.

Fluorescent Microscopy

Direct immunofluorescence (Direct-IF) technique is employed to detect viral particles in the clinical samples.

- ❖ **Procedure:** Specimen is mounted on slide, stained with specific antiviral antibody tagged with fluorescent dye and viewed under fluorescent microscope
- ❖ **Clinical applications:**
 - Diagnosis of rabies virus antigen in skin biopsies, corneal smear of infected patients
 - Syndromic approach: Rapid diagnosis of respiratory infections caused by influenza virus, rhinoviruses, respiratory syncytial virus, adenoviruses and herpesviruses can be carried out by adding specific antibodies to each of these viruses
 - Detection of adenovirus from conjunctival smears.

Light Microscopy

Light microscopy is useful in the following situations.

- ❖ **Inclusion bodies:** Histopathological staining of tissue sections may be useful for detection of inclusion bodies which helps in the diagnosis of certain viral infections (see Table 4.4)
- ❖ **Immunoperoxidase staining:** Tissue sections or cells coated with viral antigens are stained using antibodies tagged with horseradish peroxidase following which hydrogen peroxide and a coloring agent (benzidine derivative) are added. The color complex formed can be viewed under a light microscope.

Detection of Viral Antigens

Various formats are available for the detection of viral antigens in serum and other samples such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic test (ICT), flow through assays, enzyme-linked fluorescence assay (ELFA), etc. Some important antigen detection tests include:

- ❖ HBsAg and HBeAg antigen detection for hepatitis B virus infection from serum
- ❖ NS1 antigen detection for dengue virus infection from serum
- ❖ SARS-CoV-2 antigen (nucleocapsid protein) detection in nasopharyngeal swabs by immunochromatographic assay
- ❖ p24 antigen detection for HIV infected patients from serum
- ❖ Rotavirus antigen detection from diarrheic stool
- ❖ CMV specific pp65 antigen detection in peripheral blood leukocyte.

Detection of Viral Antibodies

Antibody detection from serum is one of the most commonly used method in diagnostic virology. Appearance of IgM antibody or a four-fold rise of titer of IgG antibody indicates recent infection; whereas the presence of IgG antibody (without a recent rise) indicates chronic or past infection. Various techniques available are described below:

Techniques such as ELISA, ELFA, ICT, flow through assays are widely used for antibody detection against most of the viral infections, for example:

- ❖ Anti-HBc, Anti-HBs and Anti-HBe antibodies in serum for hepatitis B infection
- ❖ Anti-Hepatitis C antibodies in serum
- ❖ Antibodies against HIV-1 and HIV-2 antigens from serum
- ❖ Anti-Dengue IgM/IgG antibodies from serum.

Molecular Methods

Advent of molecular techniques has eased the diagnosis of viral infections. They are more sensitive, specific and yield quicker results than culture.

- ❖ **Polymerase chain reaction (PCR)** is the simplest molecular assay; used to detect viral DNA in clinical specimens (e.g. HSV DNA in CSF)

- ❖ **Reverse transcriptase-PCR (RT-PCR)** is used for the detection of RNA viruses (e.g. HIV RNA in blood). After RNA extraction, the viral RNA is reverse transcribed into DNA, which is then subjected to amplification similar to that followed in PCR
- ❖ **Multiplex PCR formats** are available that can simultaneously detect genes of common organisms responsible for a clinical syndrome; for example, multiplex PCR for upper respiratory syndromes simultaneously detecting genes of adenovirus, influenza virus, parainfluenza virus and respiratory syncytial virus in respiratory specimen
- ❖ **BioFire FilmArray:** It is an automated nested multiplex PCR commercially available. It has various syndrome specific panels such as respiratory, gastrointestinal, meningitis-encephalitis; each panel comprises of primers targeting 20–25 common pathogens infecting the respective systems
- ❖ **Real time-PCR (rt-PCR):** It is revolutionized the diagnostic virology; considered as the gold standard method for diagnosis of several viral infections such as influenza, COVID-19, etc. It has several advantages over conventional PCR such as—
 - Quantifying viral nucleic acid in the samples, hence used to monitor the treatment response and estimating viral load, e.g., HIV, hepatitis B virus
 - Takes lesser time than PCR
 - More sensitive and specific than PCR.

Isolation of Virus

Viruses cannot be grown on artificial culture media. They are cultivated by animal inoculation, embryonated egg inoculation or tissue cultures.

- ❖ Being labor-intensive, technically demanding and time consuming, virus isolation is not routinely used in diagnostic virology
- ❖ The specimen should be collected properly and transported immediately to the laboratory. Refrigeration is essential during transportation as most viruses are heat labile. Type of specimen collected depends on the virus suspected.

Animal Inoculation

Because of the ethical issues related to use of animals, animal inoculation is largely restricted only for research purpose.

- ❖ **Research use:** To study viral pathogenesis or viral oncogenesis or for viral vaccine trials
- ❖ **Diagnostic use:** Primary isolation of certain viruses which are difficult to cultivate otherwise; such as arboviruses and coxsackieviruses.

Egg Inoculation

Embryonated eggs were first used for viral cultivation by Good pasture in 1931. Subsequently, this technique was widely used in the past. However, with the advent of better

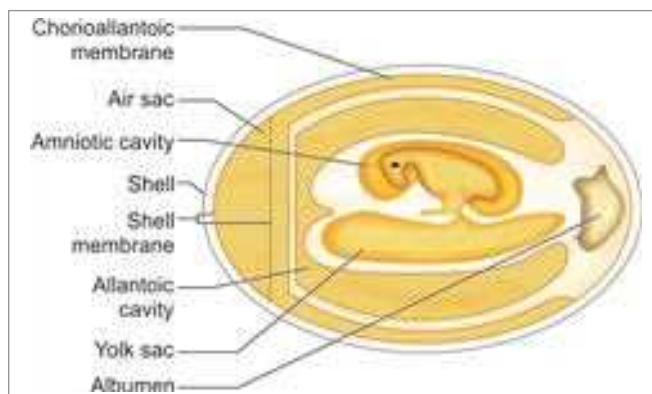


Fig. 4.7: Schematic diagram of embryonated egg.

diagnostic techniques, the use of egg inoculation in viral diagnostics is greatly limited now. Embryonated hen's egg has four sites that are specific for the growth of certain viruses (Fig. 4.7).

- ❖ **Yolk sac inoculation:** Used for arboviruses (e.g. JE virus) and some bacteria such as *Rickettsia*, *Chlamydia* and *Haemophilus ducreyi*
- ❖ **Amniotic sac:** Used for the isolation of influenza virus
- ❖ **Allantoic sac:** It is a larger cavity, hence is used for better yield of viral vaccines such as influenza vaccine, yellow fever (17D) vaccine
- ❖ **Chorioallantoic membrane:** Used for the isolation of poxviruses (e.g. vaccinia and variola). They produce visible lesions over the chorioallantoic membrane called as pocks.

Tissue Culture

Enders, Weller, and Robbins (1949) used tissue cultures of non-neural origin to cultivate poliovirus and that was the turning point following which tissue culture technique was widely used in diagnostic virology.

Tissue culture can be of three types:

1. **Organ culture:** It was previously used for certain fastidious viruses that have affinity to specific organs; for example, tracheal ring culture for isolation of coronavirus
2. **Explant culture:** Fragments of minced tissue can be grown as 'explants', e.g. adenoid explants used for adenoviruses. This method is obsolete now
3. **Cell line culture:** This is the only isolation method which is in use now. The preparation of cell lines and the types of cell lines have been described below.

Preparation of the Cell Lines

Tissues are completely digested by treatment with proteolytic enzymes (trypsin or collagenase), followed by mechanical shaking so that the components are completely dissociated into individual cells.

- ❖ **Viral growth medium:** The cells are then washed, counted, and suspended in viral growth medium which contains balanced salt solution added with essential amino acids, vitamins, fetal calf serum and antibiotics



Fig. 4.8: Tissue culture flask.

- ❖ **Tissue culture flasks:** The viral growth medium containing cells are dispensed in tissue culture flasks (Fig. 4.8)
- ❖ **Monolayer sheet formation:** On incubation, the cells adhere to the glass surfaces of the flask and then they divide to form a confluent monolayer sheet of cells within a week covering the floor of the tissue culture flask
- ❖ **Incubation:** Tissue culture flasks are incubated horizontally in presence of CO₂, either as a stationary culture or as a roller drum culture. Rolling of the culture bottle in *roller drums* provides better aeration which is useful for isolation of fastidious viruses (e.g. rotavirus).

Types of Cell Lines

The cell line cultures can be classified into three types based on their origin, chromosomal characters, and maximum number of cell divisions that they can undergo.



Fig. 4.9: Human lung fibroblast cell line (Normal).

Source: American Type Culture Collection (ATCC), USA (with permission).

1. **Primary cell lines:** They are derived from normal cells freshly taken from the organs and cultured
 - They are capable of very limited growth in culture, maximum up to 5–10 divisions
 - They maintain a diploid karyosome
 - Common examples include:
 - ♦ Monkey kidney cell line—useful for isolation of myxoviruses, enteroviruses and adenoviruses
 - ♦ Human amnion cell line
 - ♦ Chick embryo cell line.
2. **Secondary or diploid cell lines:** They can divide maximum up to 10–50 divisions before they undergo senescence (death). They are also derived from the normal host cells and they maintain the diploid karyosome. Common examples include:
 - Human fibroblast cell line for recovery of CMV (Fig. 4.9)
 - MRC-5 and WI-38 (human embryonic lung cell strain): Used for preparation of various viral vaccines (rabies, chickenpox, MMR vaccines) and virus isolation (e.g. HSV).
3. **Continuous cell lines** (see the box below).

Continuous Cell Lines

They are derived from cancerous cell lines, hence are immortal (capable of indefinite growth). They also possess altered haploid chromosome.

They are easy to maintain in the laboratories by serial subculturing for indefinite divisions. This is the reason why continuous cell lines are the most widely used cell lines.

Common examples include (Figs 4.10A to C)

- HeLa cell line (Human carcinoma of cervix cell line)
- HEp-2 cell line (Human epithelioma of larynx cell line)—widely used for RSV, adenoviruses and HSV
- KB cell line (Human carcinoma of nasopharynx cell line)
- McCoy cell line (Human synovial carcinoma cell line)—useful for isolation of viruses, as well as *Chlamydia*
- Vero cell line (Vervet monkey kidney cell line)—used for rabies vaccine production
- BHK cell line (Baby hamster kidney cell line).



Figs 4.10A to C: Continuous cell lines (normal, uninfected): **A.** HeLa cell line; **B.** Vero cell line; **C.** HEp-2 cell line.

Source: American Type Culture Collection (ATCC), USA (with permission).

Detection of Viral Growth in Cell Cultures

Following methods are used to detect the growth of the virus in cell cultures.

Cytopathic Effect (CPE)

It is defined as the morphological change produced by the virus in the cell line detected by a light microscope.

Cytopathic viruses: Not all, but few viruses can produce CPE and those are called as cytopathic viruses. The type of CPE is unique for each virus and that helps for their presumptive identification (Table 4.5).

Other Methods to Detect Viral Growth

Other methods to detect viruses in the cell line include:

- ❖ **Detection of viral antigens** on the surface of infected cells by direct immunofluorescence assay
- ❖ **Viral genes** detection by using PCR
- ❖ **Electron microscopy**, demonstrating the viruses in infected cell lines.

Shell Vial Technique

For viruses such as CMV which take several weeks for cytopathic effect to develop, shell vial technique can be followed for early growth detection (1–2 days).

- ❖ It involves centrifugation of cell culture (mixed with the specimen) to enhance the cell contact and viral replication, followed by
- ❖ Detection of early viral antigen in the infected cells by direct fluorescence technique.

TREATMENT OF VIRAL DISEASES

Unlike most bacteria, viruses are obligate intracellular and they use host machinery and enzymes for replication. Viral chemotherapy therefore was considered impracticable, as it was believed that it would inhibit cellular metabolism. Nevertheless, intense research made it possible to develop various antiviral drugs that can inhibit various steps of viral replication by selectively targeting viral machineries without affecting host enzymes and without being toxic to host cells. However, the antiviral drugs are limited and not available against most of the viral diseases. Drugs

currently approved for various viral diseases are listed in Table 4.6.

Interferons (IFNs)

IFNs- α , β have antiviral action; produced by many cell types such as macrophages (IFN- α) and fibroblasts (IFN- β). INF- γ does not have antiviral action; produced by T helper cells, which are component of CMI.

- ❖ **Mechanism of action:** IFNs have *no direct action* on viruses; but they induce the host cells to produce certain proteins, which in turn inhibit host cell protein synthesis
- ❖ **Innate immunity:** IFNs are part of innate immunity. Therefore, unlike antibodies they are nonspecific in action; produced quickly following infection (within hours) and do not have immunological memory
- ❖ **Inducers:** Several agents including certain RNA viruses can induce IFN synthesis; e.g. togaviruses, vesicular stomatitis virus, Sendai virus and New Castle disease virus, dsRNA, bacterial endotoxin, synthetic polymers, etc.
- ❖ **Preparations of IFNs:** Available in two forms.
 - **Human IFNs:** Prepared commercially by DNA recombinant technology
 - **Pegylated IFNs:** They are the IFN- α linked to polyethylene glycol. This linkage results in slower absorption, decreased clearance, and more sustained serum concentration; hence they can be administered once a week.
- ❖ **Application:** IFN- α are used in the following clinical conditions
 - Topically—used in rhinovirus infection, genital warts and herpetic keratitis
 - Systemically—used in chronic hepatitis B, C and D infections
 - In addition to antiviral action, IFN have anti-proliferative action; and therefore can be used to treat **cancers** such as hairy cell leukemia and Kaposi's sarcoma and **autoimmune diseases** such as multiple sclerosis.

Table 4.5: Viral cytopathic effects (CPE).

Types of cytopathic effect (CPE)	Virus
Rapid crenation and degeneration of the entire cell sheet	Enteroviruses
Syncytium or multinucleated giant cell formation	Measles, RSV, HSV
Diffuse rounding and ballooning of the cell line	HSV
Cytoplasmic vacuolations	SV 40 (Simian vacuolating virus-40)
Large granular clumps resembling bunches of grapes	Adenovirus

Abbreviations: RSV, respiratory syncytial virus; HSV, herpes simplex virus.

IMMUNOPROPHYLAXIS FOR VIRAL DISEASES

Viral Vaccines (Active Immunization)

Since viral antigens are potent immunogens, viral vaccines confer prolonged and effective immunity. Vaccines for viral infections may be available either in live, killed or in subunit forms. For certain viruses, both live and killed vaccines are available (Table 4.7).

Killed Viral Vaccines

Killed vaccines are available for various viruses (Table 4.7).

- ❖ **Preparation:** They are prepared by inactivating viruses with heat, phenol, formalin or beta propiolactone. Ultraviolet irradiation is not recommended because of the

Table 4.6: Commonly used antiviral drugs, their mechanism of action and spectrum of action.

Antiviral drugs	Mechanism of action	Active against
Anti-herpesvirus drugs		
Acyclovir, valacyclovir, penciclovir	Inhibit viral DNA polymerase	HSV1>HSV2>VZV and EBV
Famciclovir	Inhibit viral DNA polymerase	HSV, VZV and HBV
Ganciclovir, valganciclovir	Inhibit viral DNA polymerase	CMV and EBV
Cidofovir	Inhibit viral DNA polymerase	HSV and CMV
Foscarnet	Inhibit viral DNA polymerase	HSV and CMV (including resistant strains)
Fomivirsen	Inhibit mRNA of CMV	CMV (including resistant strains)
Docosanol (topical)	Inhibit the fusion of the human host cell with envelope of herpes virus	HSV (recurrent herpes labialis)
Trifluridine (topical)	Inhibits viral DNA polymerase	Herpes keratitis (eye drops)
Anti-influenza virus drugs		
Oseltamivir, zanamivir	Neuraminidase inhibitor	H1N1 flu, Avian flu, Seasonal flu
Amantadine, rimantadine	Matrix protein inhibitor	Seasonal flu
Anti-hepatitis drugs		
Telbivudine, tenofovir, lamivudine, adefovir, entecavir	Nucleoside analogues	For hepatitis B infection
Interferon alfa (2a and 2b**)	Indirectly inhibits viral protein synthesis	For hepatitis B and C infection
Grazoprevir, paritaprevir, simeprevir	NS3/4A (proteases) inhibitors	For hepatitis C infection
Dasabuvir, sofosbuvir	NS5B (polymerases) inhibitors	For hepatitis C infection
Daclatasvir, ledipasvir, velpatasvir	NS5A inhibitors	For hepatitis C infection
Ribavirin*	Nucleoside inhibitor	For hepatitis C infection
Anti-retroviral therapy (ART)- Refer Chapter 33, Tables 33.5 and 33.6		

*Ribavirin has action against influenza, parainfluenza, respiratory syncytial virus, HCV, and HIV-1.

**Intra-lesional injection of interferon alfa-2b may be used for treatment of condylomata acuminata.

Abbreviations: HSV, herpes simplex virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; NtRTI, nucleotide reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitors.

risk of multiplicity reactivation. For example, rabies vaccine

- ❖ **Advantages:** They are more stable and are considered safe when given in immunodeficiency or in pregnancy
- ❖ **Disadvantages:** Killed vaccines are associated with more adverse side effects due to reactogenicity, which can be reduced to some extent by purification of viruses.

Subunit Vaccines

In subunit vaccines, only a particular antigen of the virus is incorporated (Table 4.7).

- ❖ **Preparation:** Subunit vaccines are prepared by DNA recombinant technology. The gene coding for the desired antigen is integrated into bacteria or yeast chromosome. Replication of the bacteria or yeast yields a large quantity of desired antigens, e.g. hepatitis B vaccine
- ❖ Unlike killed vaccines, there is no local side effects associated with subunit vaccines.

Live Vaccines

Live vaccines are available for various viruses (Table 4.7).

- ❖ **Preparation:** Most of the live vaccines are prepared by attenuation by serial passages. (Exception is smallpox vaccine where the naturally occurring vaccinia viruses were used for vaccination)
- ❖ **Advantages:** Live vaccines provide a stronger and long-lasting immunity, mimicking immunity produced after natural infection. They are administered as a single dose (except OPV)
- ❖ **Disadvantages:** Live vaccines are risky in immunodeficiency or in pregnancy. They are less stable than killed vaccines.

Passive Immunization (Immunoglobulin)

Passive immunization is indicated when an individual is immunodeficient or when early protection is needed (i.e. for post-exposure prophylaxis). However, as there is no memory cells involved, passive immunization has no role in the prevention of subsequent infections.

- ❖ Previously used horse derived immunoglobulins were less effective with more side effects due to local hypersensitivity reactions; hence, they are now replaced by human immunoglobulins

Table 4.7: Vaccines for viral infections.		
	Examples	Derived from
Inactivated vaccine		
Rabies neural vaccine	Semple vaccine	Sheep brain derived, inactivated with phenol
	BPL vaccine	Sheep brain derived, beta propiolactone inactivated
	Infant mouse brain vaccine	Neural tissue of newborn mice
Rabies Non-neural vaccine	PCEC (purified chick-embryo cell) vaccine	Chicken fibroblast cell line
	HDC (human diploid cell) vaccine	Human fetal lung fibroblast cell line (WI-38 and MRC-5)
	Purified Vero cell (PVC) vaccine	Vero cell line
Kyasanur Forest Disease (KFD)	Killed KFD vaccine	Formalin-inactivated chick embryo vaccine
Subunit vaccine		
Hepatitis B	HBsAg (Hepatitis B surface antigen)	Yeast (recombinant DNA technology)
Papilloma	L1 protein	Yeast (recombinant DNA technology)
Both live and inactivated vaccines		
Poliovirus	Live Oral Polio Vaccine (OPV)	Monkey kidney cell line
	Killed Injectable Polio Vaccine (IPV)	Monkey kidney cell line
Japanese B encephalitis	SA 14-14-2 / Kolar- 821564XY strain (killed)	Vero cell culture-derived vaccine
	SA 14-14-2 strain (live)	Primary hamster kidney cell line
Influenza	Killed vaccine	Embryonated chicken egg
	Live attenuated (intranasal)	Embryonated chicken egg
Yellow fever	17D live attenuated	Embryonated chicken egg
	Dakar strain (killed)	Mouse brain derived
Hepatitis A	Inactivated	Human fetal lung fibroblast cell line (WI-38 and MRC-5)
	Live attenuated	Human diploid cell line (H2 and L-A-1)
Live attenuated vaccine		
Mumps	Jeryl-Lynn strain	Embryonated eggs and chicken embryo fibroblast cell line
Measles	Edmonston-Zagreb Strain	Chicken embryo fibroblast cell line
Rubella	RA 27/3 Strain	Human fetal lung fibroblast cell line (WI-38 and MRC-5)
Chickenpox	Oka strain of varicella-zoster	Human fetal lung fibroblast cell line (WI-38 and MRC-5)
Smallpox	Live vaccinia virus	Calf lymph
Rotavirus	Live attenuated	Vero cell line
Adenovirus	Live	Human fetal lung fibroblast cell line (WI-38 and MRC-5)

- ❖ Currently, human immunoglobulins are available for many viral infections such as mumps, measles, hepatitis B, rabies and varicella-zoster.

Combined Immunization

Simultaneous administration of vaccine and immunoglobulin in post-exposure prophylaxis is extremely useful. It is recommended for:

- ❖ Hepatitis B (neonates born to HBsAg positive mothers or for unvaccinated people following exposure)
- ❖ Rabies (for exposures to severe class III bites).

OVERVIEW OF VIRAL INFECTIONS

This division of the chapter gives an overview of various viruses infecting man, which will help to understand the viral infections that are discussed in detail under the respective infective syndromes (Part-II) of this book. The

DNA viruses (herpesviruses and others) are discussed first, followed by RNA viruses.

HERPESVIRUSES

Herpesviruses (Chapter 56) are group of DNA viruses that possess a unique property of establishing latent or persistent infections in their hosts and later on undergoing periodic reactivation. Based upon the site of latency, they can be further grouped into three subfamilies.

- ❖ **α-herpesviruses:** They undergo latency in neurons. Examples include Herpes simplex virus, Varicella-zoster virus)
- ❖ **β-herpesviruses:** They undergo latency in glands and kidneys. Example includes cytomegalovirus
- ❖ **γ-herpesviruses:** They undergo latency in lymphoid tissues. Example includes Epstein-Barr virus.

Herpes Simplex Virus (Chapter 56)

Herpes simplex viruses (HSV) are of two distinct types; HSV-1 and HSV-2.

- ❖ **Pathogenesis:** Infection is transmitted through abraded skin or mucosa; oropharyngeal contact is common for HSV-1, whereas sexual contact is common for HSV-2. They invade the local nerve endings and migrate to dorsal root ganglia where they undergo latency (HSV-1 in trigeminal ganglia and HSV-2 in sacral ganglia)
- ❖ **Clinical manifestation:** HSV are extremely widespread and can cause a spectrum of diseases—involving skin, mucosa and various organs- oral-facial mucosal lesions (vesicular lesions), CNS infections (e.g. encephalitis, Chapter 74), cutaneous lesions, genital lesions (multiple vesicular ulcers, Chapter 77), and ocular lesions (e.g. corneal ulcers, Chapter 78). Transmission of infection during birth can lead to neonatal herpes
- ❖ **Laboratory diagnosis include:**
 - Demonstration of inclusion bodies in the Giemsa staining of scraping obtained from the lesions (Tzanck smear preparation)
 - Viral isolation in various cell lines to demonstrate characteristic cytopathic effect
 - Detection of viral antigen (direct immunofluorescence) or specific antibody (ELISA) or HSV DNA (PCR).
- ❖ **Treatment:** Specific antiviral drugs such as acyclovir are effective for HSV infections.

Varicella-zoster Virus (Chapter 56)

Varicella-zoster virus (VZV) produces vesicular eruptions (rashes) on the skin and mucous membranes in the form of two clinical entities:

1. **Chickenpox:** It is characterized by generalized diffuse bilateral vesicular rashes which occur following primary infection, usually affecting children. Infection during pregnancy can cause congenital malformations in fetus (Chapter 79)
2. **Zoster or shingles:** It occurs following reactivation of latent VZV, present in the trigeminal ganglia that occurs mainly in adult life. Vesicular rashes are unilateral and segmental (confined to the skin innervated by a single sensory ganglion). Laboratory diagnosis and treatment are similar as for HSV.

Cytomegalovirus (Chapter 79)

Cytomegalovirus (CMV) causes an array of clinical syndromes such as congenital and perinatal infections, severe infection in immunocompromised and transplant recipients. It can also cause mononucleosis like syndrome in adults following blood transfusion.

- ❖ **Laboratory diagnosis** include detection of inclusion bodies (with owl's eye appearance), virus isolation

in human fibroblasts cell line, antibody detection (by ELISA), antigen detection (e.g. pp65 antigen) and molecular methods (by PCR)

- ❖ **Treatment:** CMV does not respond to acyclovir. Drugs such as ganciclovir, valganciclovir and foscarnet and cidofovir are used for the treatment of CMV infections.

Epstein-Barr Virus (Chapter 68)

EBV is transmitted by oropharyngeal contact through infected salivary secretions.

- ❖ **Pathogenesis:** They infect the B-lymphocytes, infected B cells become immortalized and produce large number of polyclonal immunoglobulins. In response to this, the bystander CD8 T lymphocytes are stimulated and appear atypical
- ❖ **Clinical manifestations:**
 - **Infectious mononucleosis:** It is characterized by pharyngitis, cervical lymphadenopathy and atypical lymphocytosis
 - **EBV associated malignancies** such as Burkitt's lymphoma and nasopharyngeal carcinoma, Hodgkin's and non-Hodgkin's lymphoma (Chapter 80).
- ❖ **Laboratory diagnosis:**
 - Detection of nonspecific heterophile antibody to sheep RBC antigens (by Paul Bunnell test)
 - Detection of specific anti-EBV antibodies.

Less Common Herpesviruses (Chapter 56)

- ❖ Human herpesvirus (HHV) 6 and 7 infect T-lymphocytes. HHV-6 produce an exanthematous disease called as sixth disease, (exanthem subitum or roseola infantum)
- ❖ Human herpesvirus 8 infect B-lymphocytes, can cause Kaposi's sarcoma in immunocompromised individuals (Chapter 80).

OTHER DNA VIRUSES

Parvoviruses (Chapter 56)

Parvoviruses are the smallest animal viruses infecting humans, responsible for a common childhood exanthema called erythema infectiosum (fifth disease); characterized by rashes on the face, described as slapped cheek appearance. They infect RBC precursors to cause non-immune hydrops fetalis and aplastic anemia.

Papillomaviruses (Chapter 80)

Human papillomavirus (HPV) produces an array of infections ranging from benign lesions (e.g. warts, epidermodysplasia verruciformis) to malignant neoplasia (e.g. carcinoma of cervix and other genital mucosa, larynx or esophagus). It has several serotypes, of which 16 and 18 have high malignant potential. Nine valent and bivalent vaccines are available for prevention of HPV infections.

Polyomaviruses

Family Polyomaviridae have several genera infecting animals; the important human pathogens being JC virus and BK virus. Both are named after the initials of the patients in whom they were described first. They have oncogenic potential in vitro, but rarely in vivo.

- ❖ **JC (John Cunningham) virus:** Causes Progressive Multifocal Leukoencephalopathy (PML); a slow virus disease infecting brain (Chapter 74)
- ❖ **BK virus:** Causes nephropathy in transplant recipients. It differs from JC virus, by its ability to grow in a wide range of cell lines and is less oncogenic (Chapter 76)
- ❖ **Merkel cell virus:** Causes Merkel cell carcinoma, a rare but highly aggressive skin cancer
- ❖ **SV40 virus** (Simian vacuolating 40 virus): It is not pathogenic to man.

Adenoviruses (Chapter 68)

Adenoviruses infect and replicate in the epithelial cells and produce various infections – for example, infection of respiratory tract (upper respiratory tract infection, pneumonia), intestine (infantile diarrhea), eyes (conjunctivitis) and bladder (cystitis).

Poxviruses (Chapter 56)

Poxviruses are the largest among all the viruses infecting man. They are the only DNA viruses that possess double stranded DNA and replicate in the cytoplasm.

- ❖ **Smallpox virus (*Variola*):** It is the agent of a highly contagious severe exanthematous disease ‘smallpox’; which was the first infectious disease to be eradicated from the world. The rashes were typically deep seated, appeared in one stage and centrifugally distributed (extremities were affected first). The introduction of live-attenuated *Vaccinia* vaccine was one of the reasons of its successful eradication
- ❖ **Mulloscum contagiosum virus** is another poxvirus that infects human. It produces pink pearly wart-like lesions, umbilicated with characteristic dimple at the center.

Bacteriophages

Bacteriophages are the viruses that infect bacteria. It was first described by Twort and d’Herelle (1917).

Morphology

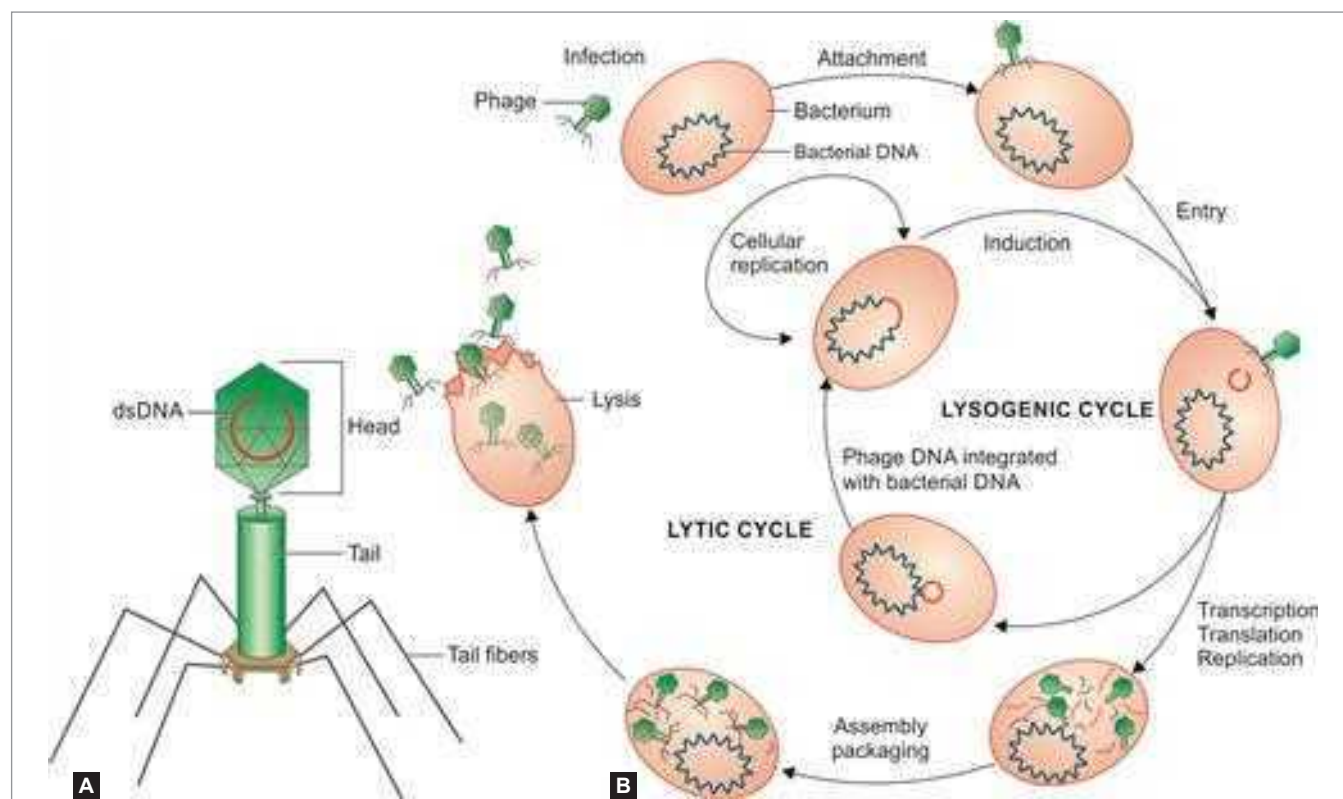
Bacteriophages are **tadpole-shaped**; measure about 28–100 nm in size. They possess a hexagonal head (capsid) with tail ending with tail fibers and a dsDNA enclosed inside capsid (Fig. 4.11A).

Life Cycle

Bacteriophages exhibit two different types of life cycles—(i) virulent or lytic cycle and (ii) temperate or lysogenic cycle.

Lytic Cycle (seen in Virulent Phages)

It resembles with the replication of other DNA viruses; consists of the following steps (Fig. 4.11B).



Figs 4.11A and B: A. Morphology of bacteriophage; B. Life cycle of a bacteriophage (Lytic and lysogenic cycles).

- ❖ **Adsorption:** The phages come into contact with bacterial cells by random collision and attach to specific receptors on bacterial cell wall by means of tail fibers
- ❖ **Penetration:** Phage acts as a syringe, injects the dsDNA into the bacterial cell
 - Lysozyme present on tail tip makes a hole on the bacterial cell wall
 - The empty phages remain outside, attached to bacterial cell wall as 'ghosts', hence there is no uncoating step needed, as seen with other viruses.
- ❖ **Biosynthesis:** Phage components such as dsDNA and capsid proteins are synthesized
- ❖ **Maturation and assembly:** Phage DNA, head and tail proteins are assembled to form infective daughter virions
- ❖ **Release** of the daughter phages occurs by lysis of the bacterial cell which is mediated by phage enzymes
- ❖ **Duration of eclipse phase** is about 15–30 minutes. It is the interval between entry of phage DNA and the appearance of first infectious phage particle inside the host cell. During this period, phages are not detected.

Lysogenic Cycle (seen in Temperate Phages)

After the entry into bacteria, the temperate phage DNA gets integrated into the bacterial chromosome (Fig. 4.11B).

- ❖ The integrated phage genome is called the **prophage**
- ❖ The bacterium that carries a prophage is known as a **lysogenic bacterium**
- ❖ **Lysogeny:** The prophage behaves like a segment of the host chromosome and replicates synchronously with it. This phenomenon is called lysogeny
- ❖ **Lysogenic conversion:** During the integrated state, the phage DNA confers certain new properties to the bacteria (e.g. provides gene for toxin synthesis)
- ❖ **Lysogenic to lytic interconversion:** Temperate phages remain integrated into the bacterial chromosome. But when they want to come out, they get excised from bacterial chromosome, then transform to lytic phages, multiply in the cytoplasm and are released by lysis.

Significance/Uses of Bacteriophages

Bacteriophages have been used for various purposes.

- ❖ **Phage typing:** It was previously used for classifying bacteria beyond species level (e.g. for *S. aureus*). Now with advent of better methods, phage typing is less commonly used
- ❖ **Used in treatment (Phage therapy):** Lytic phages can kill the bacteria, therefore may be used for treatment of bacterial infections such as post-burn and wound infections
- ❖ **Used as a cloning vector:** Bacteriophages have been used as cloning vectors in recombinant DNA technology
- ❖ **Transfer drug resistance:** Temperate phages can transfer bacterial genes from one bacterium to another by **transduction** (e.g. transfer of plasmids coding for β -lactamases)

- ❖ **Code for toxins:** The phage genomes code for the following bacterial toxins—diphtheria toxin, cholera toxin, shiga toxin, botulinum toxin C and D and streptococcal pyrogenic toxin A and C.

MYXOVIRUSES

Myxoviruses (Chapter 66) are a group of viruses that bind to mucin receptors on the surface of RBCs. They are divided into two families:

- ❖ **Orthomyxoviridae:** They possess segmented RNA; e.g. influenza virus
- ❖ **Paramyxoviridae:** They possess non-segmented RNA; e.g. parainfluenza virus, mumps, measles, Nipah, Hendra and respiratory syncytial virus.

Influenza Virus

Influenza viruses are one of the major cause of morbidity and mortality and have been responsible for several epidemics and pandemics of respiratory diseases in the last two centuries, caused by various serotypes; of which the latest pandemic was caused by H1N1 serotype in 2009.

- ❖ **Types:** Based on hemagglutinin (HA) and neuraminidase (NA) antigen, Influenza comprises of four types (A to D). Influenza type A is further divided into various subtypes
- ❖ **Antigen variation:** Influenza virus has a unique property of undergoing frequent antigen variations; which may be either a minor genetic variation (antigenic drift) or a major genetic change (antigen shift). This accounts for occurrence of its pandemics, frequent epidemics and seasonal outbreaks
- ❖ **Seasonal flu:** The currently circulating strains causing seasonal flu are influenza A/H1N1, A/H3N2 and influenza B
- ❖ **Clinical manifestations:** Majority of individuals develop mild flu-like symptoms such as chills, headache, and dry cough, followed by high-grade fever, myalgia and anorexia. Minor cases can develop secondary bacterial pneumonia
- ❖ **Laboratory diagnosis** includes detection of viral RNA in nasopharyngeal swabs by real-time reverse transcriptase PCR
- ❖ **Treatment:** Specific antiviral agents given for treatment of influenza are neuraminidase inhibitors (e.g. oseltamivir) or matrix protein M2 inhibitor (e.g. amantadine)
- ❖ **Vaccine:** Both live attenuated and injectable vaccines are available for influenza.

Parainfluenza Viruses

Human parainfluenza viruses are one of the major cause of respiratory tract disease in young children; producing various infections such as mild common cold syndrome, croup (laryngotracheobronchitis), pneumonia or bronchiolitis.

Mumps Virus

Mumps virus is the most common cause of parotid gland enlargement in children. In severe cases, it can also cause orchitis and aseptic meningitis. It is transmitted through the respiratory route via droplets, saliva, and fomites. Live attenuated vaccine is available for the prevention of mumps.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a major respiratory pathogen of young children and is the most common cause of bronchiolitis in infants.

Measles Virus (Chapter 56)

Measles is an acute, highly contagious childhood disease, characterized by fever and respiratory symptoms, followed by, appearance of Koplik's spots on buccal mucosa and maculopapular rash. Rarely, post measles complications may occur such as giant cell pneumonitis, subacute sclerosing panencephalitis (SSPE, Chapter 74). It can be prevented by administration of a live attenuated vaccine, given at nine months of birth.

Nipah and Hendra Viruses (Chapter 74)

They cause an emerging viral infection (encephalitis); may be transmitted to humans after direct contact with infected bats, pigs, or persons. Latest outbreak of Nipah encephalitis occurred in Kerala, India in 2018.

RUBELLA VIRUS

Similar to measles, rubella (Chapter 56) is another important agent of childhood exanthema. In addition, it is highly teratogenic and can cause congenital malformations affecting organs such as eye, ear and heart in fetus (congenital rubella syndrome, Chapter 79). Live attenuated vaccine is available for rubella; recommended for children and young women.

CORONAVIRUSES

Human coronaviruses (Chapter 67) are widespread and produce mild upper respiratory tract infection. Three exceptions are SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome coronavirus) and SARS-CoV-2; which are geographically restricted, transmitted from man to man and have produced outbreaks of severe respiratory disease with higher mortality.

COVID-19 (Chapter 67)

SARS-CoV-2 has emerged in 2019-2020 in China and then rapidly spread to other part of the World causing a catastrophic global pandemic known as COVID-19 (coronavirus disease-2019).

- ❖ **Epidemiology:** Till August 2020, over 2.5 crore cases have been reported worldwide with >8.5 Lakh deaths.

Although >200 countries were effected, the worst affected are USA, India, Brazil and Russia

- ❖ **Clinical manifestations:** It produces milder respiratory illness with lower mortality (5%) than SARS-CoV and MERS-CoV. However, occasionally it can proceed to pneumonia, acute respiratory distress syndrome and death
- ❖ **Diagnosis:** Definitive diagnosis is made by detection of viral RNA by real time RT-PCR in throat swab or nasopharyngeal swab
- ❖ **Treatment:** There is no effective drug or vaccine has been developed yet, although intense research is ongoing
- ❖ **Control measures:** Many countries have implemented control measures such as social distancing, lockdown, hand hygiene and use of mask.

PICORNAVIRUSES

Picornaviruses include two major groups of human pathogens: enteroviruses and rhinoviruses.

- ❖ Enteroviruses are transmitted by feco-oral route. They include polioviruses, coxsackieviruses and others (Chapter 73)
- ❖ Rhinoviruses are transmitted by respiratory route and cause common cold (Chapter 68).

Poliovirus

Poliovirus, the causative agent of polio (also known as poliomyelitis).

- ❖ **Pathogenesis:** It is transmitted by feco-oral route and then spreads to CNS/spinal cord by hematogenous route
- ❖ **Clinical manifestation:** Majority of infections are asymptomatic; rarely progresses to aseptic meningitis and paralytic poliomyelitis
- ❖ **Vaccine:** Killed injectable and live oral polio vaccines are available for use
- ❖ **Eradication:** Poliomyelitis is now at the verge of eradication. This is attributed to the extensive immunization program being conducted globally.

Coxsackievirus

Coxsackieviruses produce a variety of clinical illnesses in humans, such as aseptic meningitis, hand-foot-and-mouth disease, conjunctivitis, myocarditis, pericarditis and pancreatitis.

ARBOVIRUSES

Arboviruses (arthropod-borne viruses) are a diverse group of RNA viruses that are transmitted by blood-sucking arthropods (insect vectors) from one vertebrate host to another.

- ❖ There are several hundred arboviruses that exist in the world and all are believed to be endemic in animals. However, only about 100 are human pathogens
- ❖ They cause various type of manifestations such as hemorrhagic fever (Chapter 34) and encephalitis (Chapter 74).

India: The following are the important arboviruses prevalent in India.

- ❖ **Dengue virus** (Chapter 34): Dengue is the most common arboviral infection prevalent in India
 - *Aedes aegypti* is the principal vector followed by *Aedes albopictus*
 - It presents in three clinical stages: dengue fever, dengue hemorrhagic fever and dengue shock syndrome
 - It is diagnosed by detection of NS1 antigen, IgM antibody by ELISA and viral RNA by reverse transcriptase PCR.
- ❖ **Chikungunya virus** (Chapter 34): It is transmitted by *Aedes aegypti* mosquito. It is a re-emerging viral infection; mainly presents as fever and arthritis; rarely hemorrhagic fever
- ❖ **Kyasunar forest disease (KFD) virus:** It is transmitted by ticks. It causes hemorrhagic fever. The disease is rare, confined to the Shimoga and nearby districts of Karnataka (Chapter 34)
- ❖ **Japanese B encephalitis** (Chapter 74): It is transmitted by *Culex tritaeniorhynchus*. Pigs are the amplifier host. It is the leading cause of viral encephalitis in Asia, including India. Both live-attenuated and inactivated vaccines are available for human use.

Arboviruses outside India: The arboviral infections prevalent in other countries, but not in India are enlisted in Chapter 34. The important ones are discussed in detail.

- ❖ **Yellow fever virus:** Endemic in West Africa; transmitted by *Aedes aegypti*. It causes hepatitis (Chapter 48)
- ❖ **Zika virus:** Endemic in Brazil; transmitted by *Aedes aegypti*. It causes mild febrile illness in adults and also can cause congenital infection (Chapter 79).

RABIES VIRUS

Rabies virus causes a rapidly progressive, acute infectious disease of the CNS in humans and animals (Chapter 74).

- ❖ **Transmission:** Rabies is transmitted from another infected animal bite (most common being dog)
- ❖ **Clinical manifestation:** From the site of bite, it spreads to CNS via neuronal route. It produces an acute neurologic phase, which may be either an encephalitic or paralytic type
 - The encephalitic type presents as hyperexcitability, autonomic dysfunction and hydrophobia
 - Patients gradually develop coma and eventually leads to death. Recovery and survival are extremely rare.
- ❖ **Laboratory diagnosis** includes detection of rabies viral antigen from hair follicles at the nape of the neck by direct IF test, antibody detection and viral RNA detection by RT PCR. Postmortem diagnosis of rabies can be done by detection of characteristic inclusion bodies (Negri bodies) in histopathological staining of brain tissue

- ❖ **Management:** Post-exposure prophylaxis can be provided by local wound care and administration of anti-rabies immunoglobulin and vaccine.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS); the biggest threat to mankind in last three decades (Chapter 33).

- ❖ **Transmission:** HIV is transmitted by sexual route (most commonly); vertical (mother to fetus), parenteral (blood transfusion) and percutaneous (needle prick injury)
- ❖ **Pathogenesis:** Following transmission, HIV infects CD4 T cells and where the unique viral enzyme 'reverse transcriptase' converts viral RNA to DNA, which then integrates with host DNA and undergoes latency
- ❖ **Clinical manifestations:** The typical course of HIV infection includes the following stages—acute retroviral syndrome, followed by asymptomatic stage (clinical latency), then progresses into persistent generalized lymphadenopathy, and symptomatic HIV infection. Finally, the patients move towards the advanced end stage called AIDS, where a number of opportunistic infections set in secondary to profound immune suppression
- ❖ **Laboratory diagnosis** includes detection of antibodies (by rapid tests or ELISA), p24 antigen (by ELISA), or viral RNA (by RT-PCR). In India, the strategy recommended by national AIDS control organization (NACO) is used for HIV diagnosis
- ❖ **Treatment:** Antiretroviral Therapy (ART) is advocated for treatment of HIV infected patients. The NACO recommended first-line ART regimen in adults includes combination of tenofovir, lamivudine and efavirenz (TLE regimen). Refer Tables 33.5 and 33.6.

HEPATITIS VIRUSES

Hepatitis viruses (Chapter 48) are heterogeneous group of viruses that are taxonomically diverse (belong to different families) but all are hepatotropic; and produce similar clinical illness such as fever, nausea, vomiting, and jaundice. There are five important hepatitis viruses (A to E); all are RNA viruses except HBV which is a DNA virus. They can be divided into two groups based on the route of transmission.

- ❖ **Hepatitis A and E viruses:** They are transmitted by feco-oral route. The clinical course has an abrupt onset. The disease is self-limiting with a good prognosis. They do not have carrier stage, or chronicity or oncogenic potential
- ❖ **Hepatitis B, C and D viruses:** They are transmitted by percutaneous, sexual or vertical routes. The clinical course has an insidious onset and variable prognosis. The disease may progress to carrier stage, chronic hepatitis, cirrhosis or hepatocellular carcinoma
- ❖ **Laboratory diagnosis** of viral hepatitis includes detection of various viral markers such as:
 - Detection of viral antigens (e.g. HBsAg for HBV) or

- Detection of antibodies [anti-HAV, anti-HBc and anti-HBs (for HBV), anti-HCV and anti-HEV] or
- Detection of viral nucleic acid (by PCR).
- ❖ **Treatment:** HAV and HEV infections are self-limiting, do not require specific treatment. Antiviral drugs are available for HBV and HCV infections; for example,
 - Tenofovir and telbivudine for HBV
 - Interferon, ribavirin for HCV
- ❖ **Vaccine:** Vaccines are available for HAV and HBV.

MISCELLANEOUS RNA VIRUSES

Rodent-borne Viruses (Chapter 34)

Rodent-borne viruses are transmitted from rodents to man by contact with infected body fluids or excretions. Major rodent-borne viruses include:

- ❖ **Hantaviruses:** They cause two categories of manifestations—hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome
- ❖ **Arenaviruses:** They cause various diseases such as South American hemorrhagic fever.

Filoviruses (Chapter 34)

Filoviruses are the long filamentous viruses; example include Ebola virus and Marburg virus. They cause

hemorrhagic fever, associated with very high mortality. They are transmitted by close contact with blood or other body fluids or secretions of infected animals or man.

Slow Viruses and Prions (Chapter 74)

Slow virus diseases and prions are a group of neurodegenerative conditions affecting both humans and animals, characterized by: long incubation period, predilection for CNS and a strong genetic predisposition.

- ❖ **Conventional slow viruses:** Examples include—subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy
- ❖ **Unconventional transmissible agents**—termed as '*prion disease*', characterized by spongiform encephalopathies due to deposition of abnormal prion proteins in neural tissues.

Viral Gastroenteritis (Chapter 44)

Viral etiology accounts for the most of the acute infectious gastroenteritis worldwide; most commonly occurs among children. Several enteric viruses can cause acute gastroenteritis in humans, most common being **rotavirus**. Others include adenovirus (type 40 and 41), calicivirus and astrovirus.

EXPECTED QUESTIONS

I. Write essay on:

1. Discuss in detail laboratory diagnosis of viral infections.

II. Write short notes on:

1. Replication of viruses.
2. Methods of detecting viral growth in cell cultures.
3. Interferons.
4. Inclusion bodies.
5. Viral vaccines.

III. Multiple Choice Questions (MCQs):

1. Which of the following virus is enveloped?

- a. Poliovirus
- b. Adenovirus
- c. Herpesvirus
- d. Parvovirus B19

2. All of the following are RNA viruses, except:

- a. Enterovirus
- b. Human adenoviruses
- c. Coxsackievirus
- d. Hepatitis A virus

3. All of the following viruses are transmitted by respiratory route, except:

- a. Influenza virus
- b. Rotavirus
- c. Respiratory syncytial virus
- d. Rhinovirus

4. All of the following are continuous cell lines, except:

- a. HeLa cell line
- b. Chick embryo cell line
- c. HEp-2 cell line
- d. KB cell line

5. Suckling mice are used for isolation of:

- a. Coxsackievirus
- b. HIV

- c. Hepatitis B virus
- d. Poliovirus

6. All of the following are intracytoplasmic inclusion bodies, except:

- a. Negri bodies
- b. Molluscum bodies
- c. Cowdry type A inclusions
- d. Guarnieri bodies

7. Which of the following vaccine is a killed vaccine?

- a. Mumps vaccine
- b. Measles vaccine
- c. Rubella vaccine
- d. IPV

8. The largest virus in size is:

- a. Herpes simplex virus
- b. Hepatitis B virus
- c. Poxvirus
- d. Adenovirus

9. The smallest virus in size is:

- a. Picornaviruses
- b. Parvovirus
- c. Hepatitis D virus
- d. Adenovirus

10. Which of the following DNA virus is single-stranded?

- a. Parvovirus
- b. Hepatitis B virus
- c. Adenovirus
- d. Poxvirus

11. Which of the following RNA virus has double stranded RNA?

- a. Retroviruses
- b. Hepatitis C virus
- c. Rotavirus
- d. Influenza virus

Answers

1. c 2. b 3. b 4. b 5. a 6. c 7. d 8. c 9. b 10. a 11. c

General Parasitology and Overview of Parasitic Infections

CHAPTER 5

CHAPTER PREVIEW

- General Parasitology
 - Life Cycle of Parasites
 - Laboratory Diagnosis and Treatment of Parasitic Diseases
- Overview of Parasitic Infections
 - Protozoan infections
 - Helminthic infections

GENERAL PARASITOLOGY

Parasite is a living organism, which lives in or upon another organism (host) and derives nutrients directly from it, without giving any benefit to the host. Medical Parasitology deals with the study of animal parasites, which infect and produce diseases in human beings. Parasites may be classified as:

- ❖ **Ectoparasites:** They inhabit the surface of the body of the host without penetrating into the tissues (e.g. fleas, mites or ticks). They serve as important vectors transmitting the pathogenic microbes. The infection produced by these parasites is called as infestation (e.g. scabies)
- ❖ **Endoparasites:** These are the parasites, that live within the body of the host (e.g., *Leishmania*). Invasion by the endoparasite is called as infection.

The endoparasites are further classified into protozoans and helminths.

- ❖ **Protozoa:** They are unicellular eukaryotic cells that perform all the physiological functions. Although like bacteria they are unicellular, they are considered as lower eukaryotes, as they possess cellular organelles and metabolic pathways, similar to that of eukaryotes
- ❖ **Helminths:** They are elongated flat or round worm-like parasites measuring few millimeters to as long as few meters. They are eukaryotic multicellular and bilaterally symmetrical.

Medically important protozoans and helminths are listed in Table 5.1.

LIFE CYCLE OF PARASITES

The life cycle of parasites depends upon three factors: host, mode of transmission and infective form (Tables 5.2 and 5.3)

- ❖ **Host:** It is as an organism, which harbors the parasite and provides the nourishment and shelter (Tables 5.2 and 5.3)
 - Hosts can be classified into *definitive host* (where the parasite undergoes sexual cycle) or *intermediate host* (where the parasite undergoes asexual cycle)

Table 5.1: Medically important protozoans and helminths.

Medically important protozoans
Amoeba <ul style="list-style-type: none">● <i>Entamoeba histolytica</i>● Free-living amoebae: <i>Naegleria</i>, <i>Acanthamoeba</i>, <i>Balamuthia</i>
Flagellates <ul style="list-style-type: none">● Intestinal Flagellate: <i>Giardia</i>● Genital Flagellate: <i>Trichomonas</i>● Hemoflagellates: <i>Leishmania</i> and <i>Trypanosoma</i>
Apicomplexa <ul style="list-style-type: none">● Malaria parasites and <i>Babesia</i>● Opportunistic coccidian parasites : <i>Toxoplasma</i>, <i>Cryptosporidium</i>, <i>Cyclospora</i>, <i>Cystoisospora</i> and <i>Sarcocystis</i>
Miscellaneous Protozoa : <i>Balantidium coli</i> , <i>Blastocystis</i> and <i>Microsporidia</i> *
Medically important helminths
Cestodes <i>Diphyllobothrium</i> , <i>Taenia</i> , <i>Echinococcus</i> , <i>Hymenolepis</i> , <i>Dipylidium</i> and others
Trematodes or Flukes <i>Schistosoma</i> , <i>Fasciola</i> , <i>Clonorchis</i> , <i>Opisthorchis</i> , <i>Fasciolopsis</i> , <i>Paragonimus</i> and others
Intestinal Nematodes <i>Trichuris</i> , <i>Enterobius</i> , Hookworm, <i>Strongyloides</i> , <i>Ascaris</i> and others
Somatic Nematodes Filarial nematodes, <i>Dracunculus</i> and <i>Trichinella</i>

*Microsporidia are now considered to be evolved from fungi.

Note: Ectoparasite infestation (scabies); discussed in Annexure 7.

- Depending upon the number of hosts involved, the life cycle of the parasite may be direct (simple) or indirect (complex) (Tables 5.2 and 5.3)
 - ◆ In *direct/simple life cycle*, the parasite requires only one host to complete its development
 - ◆ In *indirect/complex life cycle*, the parasite requires two/three hosts (one definitive host and another one or two intermediate host/s) to complete its development.
- ❖ **Infective form:** It is the morphological form of the parasite which is transmitted to man

Table 5.2: Life cycle of various protozoan parasites.

Protozoa	Host		Mode of transmission	Infective form
	Definitive	Intermediate		
<i>Entamoeba</i> and <i>Giardia</i>	Man	-	Ingestion	Cysts (quadrinucleated)
<i>Trichomonas vaginalis</i>	Man	-	Sexual	Trophozoites
<i>Leishmania</i> species	Man	Sandfly	Vector-borne	Promastigotes
<i>Trypanosoma cruzi</i>	Man	Reduviid bugs	Vector-borne	Trypomastigotes
<i>Trypanosoma brucei</i>	Man	Tsetse fly	Vector-borne	Trypomastigotes
<i>Plasmodium</i> spp.	<i>Anopheles</i>	Man	Vector-borne	Sporozoites
<i>Toxoplasma gondii</i>	Cat	Man	Ingestion, blood transfusion, vertical	Tissue cysts, oocysts, tachyzoites
<i>Cryptosporidium</i>	Man	-	Ingestion, autoinfection	Oocysts (sporulated)
<i>Cyclospora</i> , <i>Cystoisospora</i>	Man	-	Ingestion	Oocysts (sporulated)

Table 5.3: Life cycle of various helminthic parasites.

Helminths	Host		Mode of transmission	Infective form
	Definitive	Intermediate		
Cestodes				
<i>Taenia solium</i> (intestinal taeniasis)	Man	Pig	Ingestion	Cysticercus larvae
<i>Taenia solium</i> (cysticercosis)	Man	Man	Ingestion, autoinfection	Embryonated eggs
<i>Taenia saginata</i>	Man	Cattle	Ingestion	Cysticercus larvae
<i>Echinococcus granulosus</i>	Dog	Man*	Ingestion	Embryonated eggs
<i>Hymenolepis nana</i>	Man	-	Ingestion, autoinfection	Embryonated eggs
<i>Diphyllobothrium latum</i>	Man	1 st - Cyclops, 2 nd - Fish	Ingestion	Plerocercoid larvae
Trematodes				
<i>Schistosoma</i> species	Man	Snail	Skin penetration	Cercaria larvae
<i>Fasciola hepatica</i> <i>Fasciolopsis buski</i>	Man	1 st - Snail 2 nd - Aquatic plants	Ingestion	Metacercaria larvae
<i>Paragonimus</i> spp.	Man	1 st - Snail, 2 nd - Crab	Ingestion	Metacercaria larvae
<i>Clonorchis</i> spp., <i>Opisthorchis</i> spp.	Man	1 st - Snail, 2 nd - Fish	Ingestion	Metacercaria larvae
Intestinal nematodes				
<i>Ascaris</i> , <i>Trichuris</i>	Man	-	Ingestion	Embryonated eggs
<i>Enterobius</i> spp.	Man	-	Ingestion, autoinfection	Embryonated eggs
Hookworm	Man	-	Skin penetration	L ₃ filariform larvae
<i>Strongyloides stercoralis</i>	Man	-	Skin penetration, autoinfection	L ₃ filariform larvae
Somatic nematodes				
Filarial worms	Man	Mosquito and flies	Vector-borne	L ₃ filariform larvae
<i>Dracunculus medinensis</i>	Man	Cyclops	Ingestion	L ₃ larvae
<i>Trichinella spiralis</i>	Man/Pig	-	Ingestion	L ₁ larvae

*Man acts as an accidental intermediate host for *Echinococcus granulosus*.

- ❖ **Mode of transmission:** Parasites may be transmitted by various modes such as ingestion, skin penetration, vector-borne, sexual, vertical, blood transfusion, and autoinfection.

Autoinfection

Few intestinal parasites may infect the same person by contaminated hand (external autoinfection) or by reverse peristalsis (internal autoinfection). It is observed in some parasitic infections such as *Cryptosporidium parvum*, *Taenia solium*, *Enterobius vermicularis*, *Strongyloides stercoralis* and *Hymenolepis nana*.

LABORATORY DIAGNOSIS OF PARASITIC DISEASES

Laboratory diagnosis plays an important role in establishing the specific diagnosis of various parasitic infections. Following are the techniques used in the diagnosis of parasitic infections.

Examination of Feces

As many parasites inhabit in the intestinal tract, stool examination is the most common diagnostic technique used for the diagnosis of parasitic infections.

Specimen Collection

Stool specimens should be collected in a wide-mouthed, clean, leak-proof, screw capped containers and should be handled carefully to avoid acquiring infection from organisms present in the stool (Fig. 5.1).

- ❖ **Timing:** Specimen should be collected before starting anti-parasitic drugs and closer to the onset of symptoms
- ❖ **Frequency:** At least three stool specimens collected on alternate days (within 10 days) are adequate to make the diagnosis of intestinal parasitic diseases (except for intestinal amoebiasis, for which six specimens may be recommended)
- ❖ **When to examine:** Liquid stool specimens should be examined within 30 minutes, semisolid stools within 1hr (as on storage, trophozoites may disintegrate or become non-motile) and formed stools up to 24 hours after collection
- ❖ **For monitoring response to therapy:** Repeat stool examination can be done 3 to 4 weeks after the therapy for intestinal protozoan infection, and 5–6 weeks for *Taenia* infection
- ❖ **If delay in transport:** Fecal specimens should be kept at room temperature; preservatives (e.g. 10% formalin) can be used to maintain the morphology of the parasitic cysts and eggs
- ❖ **Specimens other than stool:**
 - **Perianal Swabs** (cellophane tape or NIH swab): Useful for detecting eggs of *Enterobius vermicularis* deposited on the surface of perianal skin. It is also used for eggs of *Schistosoma mansoni* and *Taenia* species
 - **Duodenal Contents:** It is very useful for the detection of small intestine parasites like, *Giardia intestinalis* and larva of *Strongyloides stercoralis*. Duodenal fluid can be collected by endoscopy or by Entero-test.

Macroscopic Examination

Macroscopic examination of stool may provide clue about various parasitic infections.

- ❖ **Mucoid bloody stool:** Found in acute amoebic dysentery, intestinal schistosomiasis, and invasive balantidiasis



Fig. 5.1: Sample container for stool.

- ❖ **Color:** Dark red stool indicates upper gastrointestinal tract (GIT) bleeding and a bright red stool is suggestive of bleeding from lower GIT
- ❖ **Frothy pale offensive stool** (containing fat) is usually found in giardiasis.

Stool Consistency

In liquid stool, trophozoites are usually found; whereas in semi-formed stool both trophozoites and cysts are found and the cysts are mainly found in formed specimens (Fig. 5.2). Exceptions to this general statement include:

- ❖ Coccidian oocysts, microsporidian spores, helminths eggs that can be found in any type of fecal specimen
- ❖ In cryptosporidiosis, oocysts load is higher in liquid stool
- ❖ Tapeworm proglottids and adult worms of *Enterobius* and *Ascaris* are occasionally found in the stool.

Microscopic Examination

Microscopic examination includes direct wet mount examination and permanent staining methods.

Direct Wet Mount (Saline and Iodine Mount)

Drops of saline and Lugol's iodine are placed on left and right halves of the slide respectively (Fig. 5.3).

- ❖ A small amount of feces (~2 mg) is mixed with a stick to form a uniform smooth suspension. If more or less fecal material has been taken for the stool wet mount, the chance of finding stool parasites decreases

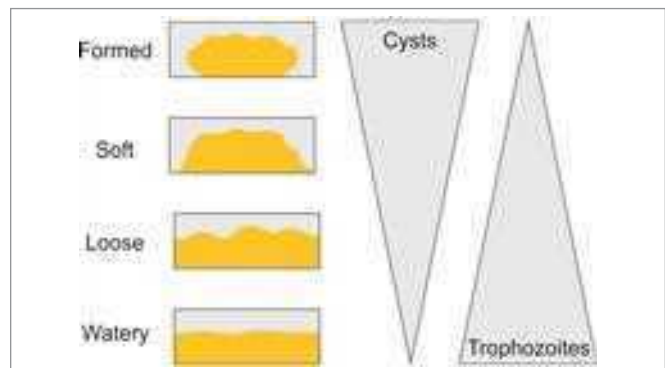


Fig. 5.2: Relative frequency of trophozoites and cysts in stool specimens with various consistencies.



Fig. 5.3: Saline and iodine wet mount.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

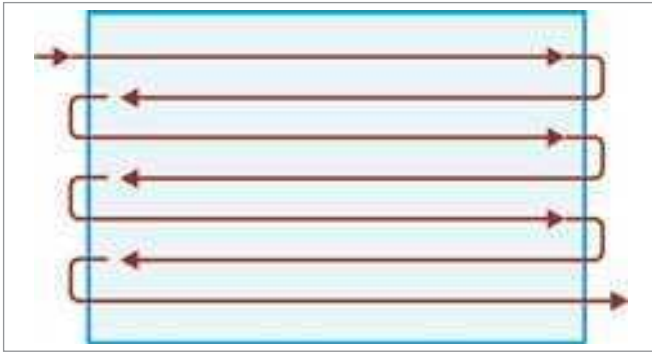
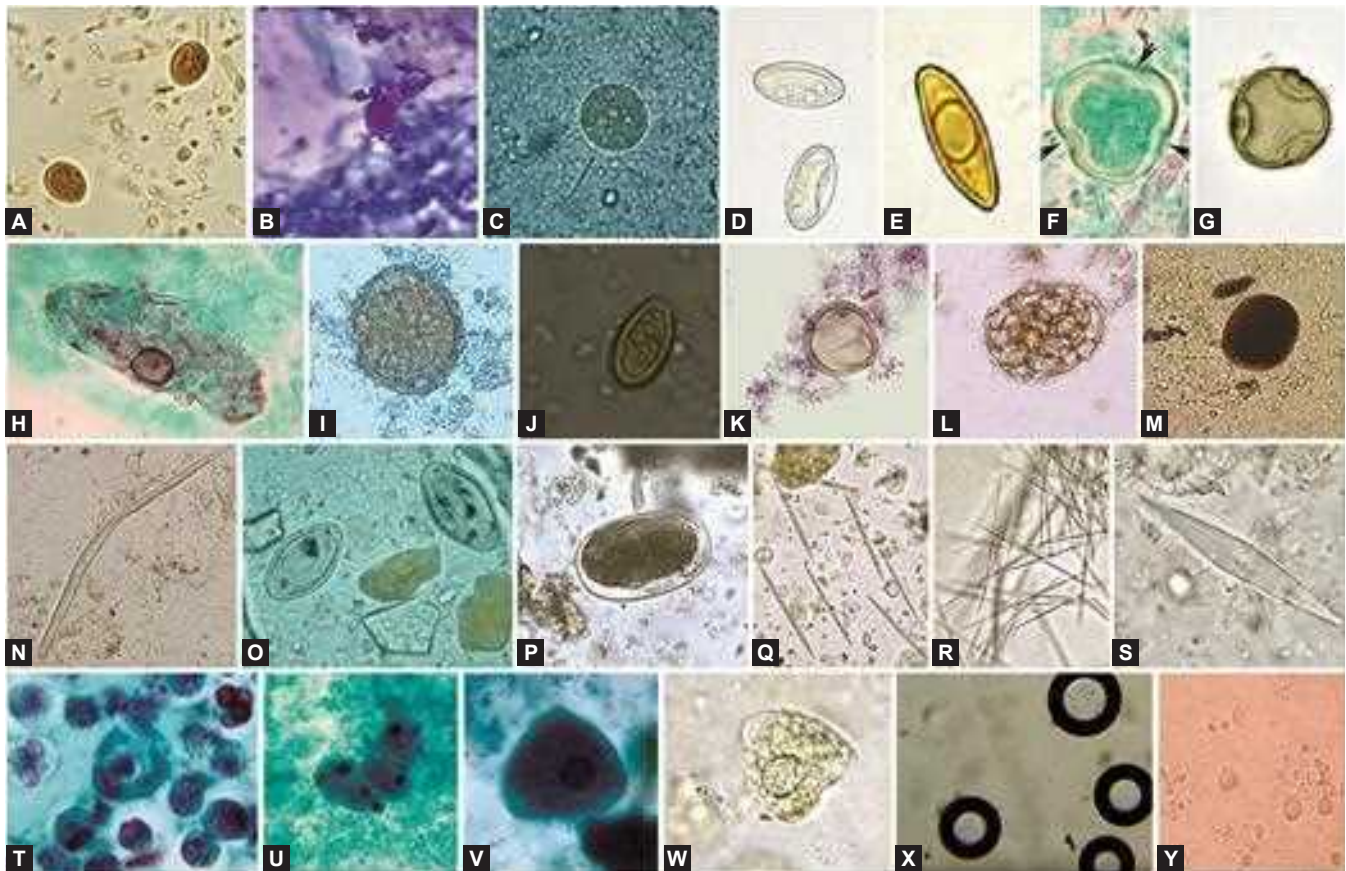


Fig. 5.4: Method of screening of slide during wet mount examination of stool.

- ❖ **Screening area:** The entire coverslip preparation should be examined in a zigzag fashion, first under low power and then under high power objective, before reporting a negative result (Fig. 5.4)
 - ❖ **Motility:** If a finding is suspected to be a trophozoite, then examination for at least 15 seconds should be allowed to detect motility. Motility can be stimulated by—application of heat by placing a hot penny on the edge of a slide or tapping on the coverslip or increasing the intensity of the light source.
- The following are the structures that can be visualized by microscopic examination of stool specimen, which may be confused with various protozoan trophozoites, cysts or helminthic eggs and larvae (Fig. 5.5).
- ❖ **Normal constituents:** These include plant fiber, starch cells (stains blue-black with iodine), muscle fibers, animal hair, pollen grains, yeast cells, bacteria, epithelial cells, fat globules, and air bubbles, etc.



Figs 5.5A to Y: Normal constituents and artifacts found in the stool in wet mount examination: **A and B. Yeast cell** resembling: (A) *Giardia* cyst; (B) *Cryptosporidium* oocyst; **C to E. Fungal spore** resembling: (C) Cyst of *Entamoeba*; (D and E) *Cystoisospora* oocyst; **F to K. Pollen grain** resembling: (F) *Blastocystis* (Trichrome staining); (G) *Blastocystis* (saline mount); (H) Protozoan trophozoite; (I) *Ascaris* egg; (J) *Clonorchis* egg; (K) *Taenia* egg; **L and M. Plant cell** resembling: (L) Helminth eggs; (M) Hookworm egg; **N. Plant hair** resembling *Strongyloides* larva; **O. Diatoms; P. Mite egg** resembling hookworm egg; **Q to S. Crystals:** (Q and R) Pineapple juice crystals and kiwi crystals; (S) Charcot-Leyden crystals; **T to W. Human cells** resembling trophozoites: (T) White blood cells; (U) Macrophages; (V and W) Epithelial cell; **X. Air bubbles; Y. Fat globules.**

Source: A to E, I to V. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta; F to H, W. Swierczynski G, Milanese B. Atlas of Human Intestinal Protozoa Microscopic Diagnosis; X and Y. Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ **Cellular elements:** Like pus cells (in inflammatory diarrhea), red blood cells (RBC) (in dysentery) may be present
- ❖ **Charcot-Leyden crystals** (diamond-shaped): They are the breakdown products of eosinophils and may be seen in the stool or sputum of patients with parasitic diseases such as amoebic dysentery, ascariasis, and allergic diseases like bronchial asthma (sputum).

Saline Mount

Saline mount is useful in the detection of trophozoites and cysts of protozoan parasites, and eggs and larvae of helminths. It has the following advantages than iodine mount.

- ❖ Motility of trophozoites and larvae in acute infection can be demonstrated
- ❖ Bile staining property can be appreciated—bile stained eggs appear golden brown and non-bile stained eggs appear colorless
- ❖ In stool specimen with preservatives, directly the wet mount can be prepared without using saline.

Iodine Mount

- ❖ **Advantages:** Nuclear details of protozoan cysts, helminthic eggs and larvae are better visualized, compared to saline mount
- ❖ **Disadvantages:** (i) Iodine immobilizes and kills the parasites, hence motility of the trophozoites and helminthic larvae cannot be appreciated, (ii) Bile staining property cannot be appreciated.

Non-bile Stained Eggs

Eggs of most of the intestinal parasites when they pass through intestine are stained by bile. The exceptions being *Enterobius*, hookworm and *Hymenolepis nana*; these eggs are non-bile stained.

Permanent Stained Smear

Permanent stained smears are required for accurate detection of protozoan cysts and trophozoites by staining their internal structures. Commonly used methods are:

- ❖ Iron-hematoxylin stain
- ❖ Trichrome stain
- ❖ Modified acid-fast stain—this is useful for coccidian parasites such as *Cryptosporidium*, *Cyclospora* and *Cystoisospora*.

Concentration Techniques

If the parasite output is low in feces (egg, cysts, trophozoites and larvae) and direct examination may not be able to detect the parasites, then the stool specimens need to be concentrated. These methods are also useful in epidemiological analysis and for assessing the treatment response. The eggs, cysts and larvae are recovered after concentration procedures; however, the trophozoites get destroyed.

Commonly used concentration techniques are:

- ❖ **Sedimentation techniques:** The eggs and cysts settle down at the bottom of the tube because they have

greater density than the suspending medium following centrifugation. Example includes formalin-ether concentration technique

- The sensitivity of detecting ova or cysts increases by 8–10 folds
- The size and shape of the parasitic structures are maintained.

- ❖ **Flotation techniques:** It involves suspending the specimen in a medium (e.g. **saturated salt solution**) of greater density, so that the helminthic eggs and protozoan cysts float on the surface of the solution. The disadvantage is, it cannot be used for concentration of the parasites that do not float in saturated salt solution such as—unfertilized eggs of *Ascaris*, larva of *Strongyloides*, *Taenia* eggs and operculated eggs of trematodes.

Other flotation methods are:

- Zinc sulphate flotation concentration technique
- Sheather's sugar flotation technique (useful for *Cryptosporidium*, *Cystoisospora* and *Cyclospora*)

Various morphological forms of parasites seen in stool specimens are enlisted in Table 5.4.

Egg Counting (Egg Quantification) Methods

The intensity of intestinal helminthic infection (especially *Trichuris*, *Ascaris* and hookworm) can be estimated by egg counting in feces; which can be performed by various egg counting methods:

- ❖ Direct smear counting method of Beaver
- ❖ Kato-Katz thick film method
- ❖ Stoll's method or dilution egg counting method

Examination of Blood

Blood examination is useful in the diagnosis of infection caused by blood parasites like *Plasmodium*, *Trypanosoma*, *Leishmania*, *Babesia*, *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa* and *Mansonella*.

Table 5.4: Morphological forms of parasites seen in stool specimens.

Morphological form	Parasites
Trophozoite and cyst	<i>Entamoeba histolytica</i> <i>Giardia lamblia</i>
Adult worm	<i>Ascaris lumbricoides</i> <i>Enterobius vermicularis</i>
Adult worm segments	<i>Taenia</i> species <i>Diphyllobothrium latum</i>
Egg	<i>Diphyllobothrium latum</i> <i>Taenia</i> species <i>Hymenolepis nana</i> <i>Schistosoma</i> species <i>Fasciola hepatica</i> <i>Fasciolopsis buski</i> <i>Ascaris lumbricoides</i> Hookworm <i>Enterobius vermicularis</i> <i>Trichuris trichiura</i>
Larva	<i>Strongyloides stercoralis</i>

Various methods of examination of blood include:

1. **Direct wet mount examination:** It is useful for detection of malaria parasites and microfilariae in lymphatic filariasis
2. **Examination of blood smears:** Thin smear and thick smears are examined after staining with various Romanowsky stains such as Leishman's stain, Giemsa stain, Field's stain and Jaswant Singh and Bhattacharjee (JSB) stain. This is the most common method of microscopic examination of peripheral blood, useful for most of the blood parasites
3. **Quantitative buffy coat (QBC):** This involves collection of the blood in a capillary tube coated internally with acridine orange stain, centrifugation and then examination of the buffy coat region under fluorescence microscopy. This is extremely useful for the detection of the malaria parasites and microfilariae
4. **Concentration of blood:** This is useful for detection of microfilariae from the blood specimen.

Various concentration methods are:

- Sedimentation technique
- Cytocentrifugation (cyospin)
- Knott concentration
- Gradient centrifugation
- Membrane filtration.

Microscopic Examination of Other Specimens

Microscopic examination of various specimens (other than stool) can also be performed to demonstrate different morphological forms of the parasites (Table 5.5).

Immunodiagnostic Methods

Immunodiagnostic methods involve detection of parasite specific antibodies in serum, and detection of circulating parasitic antigen in the serum. These methods are useful when:

- ❖ Parasites are detected only during the early stages of the disease
- ❖ Parasites occur in very small numbers
- ❖ Parasites reside in the internal organs and morphological identification is not possible
- ❖ When other techniques like culture are time consuming.

Antibody Detection Tests

Antibodies are detected in various parasitic infections; mainly from serum, sometime from other specimens such as CSF (neurocysticercosis) or pleural fluid (paragonimiasis). The parasitic diseases where antibody detection methods are useful are:

- ❖ **Amoebic liver abscess:** ELISA, detecting antibodies in serum against 170 kDa of lectin antigen
- ❖ **Visceral leishmaniasis:** Detecting antibodies in serum to rK-39 antigen by immunochromatographic test (ICT)
- ❖ **Toxoplasmosis:** (i) Sabin-Feldman dye test—a complement mediated neutralization test, which detects

Table 5.5: Various morphological forms of parasites seen in different specimens other than stool.

Specimen	Morphological form	Parasite
Peripheral blood smear	Ring form, schizont, gametocyte	<i>Plasmodium</i> spp.
	Amastigote	<i>Leishmania</i> spp.
	Trypomastigote	<i>Trypanosoma</i> spp.
	Microfilaria	Filarial nematodes*
Bone marrow, liver, lymph node, splenic aspirate	Tachyzoite	<i>Toxoplasma gondii</i>
	Amastigote	<i>Leishmania donovani</i>
Liver aspirate	Trophozoite	<i>Entamoeba histolytica</i>
Lymph node aspirate	Trypomastigote	<i>Trypanosoma</i> spp.
Lymph node biopsy	Adult worm	<i>Wuchereria bancrofti</i> <i>Brugia malayi</i>
CSF	Trophozoite	<i>Naegleria fowleri</i> <i>Acanthamoeba</i> spp.
	Trypomastigote	<i>Trypanosoma</i> spp.
Urine	Trophozoite	<i>Trichomonas vaginalis</i>
	Microfilaria	<i>Wuchereria bancrofti</i>
Sputum	Egg	<i>Schistosoma haematobium</i>
	Adult worm	<i>Paragonimus</i> spp.
	Egg	<i>Paragonimus</i> spp.
Duodenal aspirate	Larva (migrating)	<i>Ascaris lumbricoides</i> <i>Strongyloides</i> spp. Hookworm
	Trophozoite	<i>Entamoeba histolytica</i>
	Larva	<i>Strongyloides stercoralis</i>
Corneal scrapings	Trophozoite	<i>Acanthamoeba</i> spp.
Skin	Amastigote	<i>Leishmania</i> spp.
	Microfilaria	<i>Onchocerca volvulus</i>
	Larva in skin ulcer fluid	<i>Dracunculus medinensis</i>
Muscle tissue	Encysted larva	<i>Trichinella spiralis</i>
	Cysticercus cellulosae	<i>Taenia solium</i>
Perianal area	Egg	<i>Enterobius</i> spp.

*Filarial nematodes found in the peripheral blood smears are *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa*, *Mansonella* spp.

antibodies, (ii) Detection of specific IgM or IgA or IgG antibodies by ELISA

- ❖ **Cysticercosis:** (i) ELISA, detecting antibodies against purified glycoprotein antigens, (ii) Western blot, detecting antibodies against highly specific 50–13 kDa lentil lectin-purified seven glycoprotein (LLGP) antigenic fractions
- ❖ **Hydatid disease:** (i) ELISA, detecting antibodies against B2t or 2B2t antigen, (ii) DIGFA (Dot immunogold filtration assay)
- ❖ **Lymphatic filariasis:** (i) Flow-through assay, detecting antibodies against recombinant filarial antigen

(WbSXP-1), (ii) *Brugia* Rapid, detecting antibodies against Recombinant *B. malayi* antigen (Bm-14).

Antigen Detection Tests

The antigen detection methods are available for various parasitic diseases.

- ❖ **Amoebiasis:** ELISA, detecting 170 kDa of lectin antigen in blood and stool
- ❖ **Triage parasite panel:** It is an ICT, that simultaneously detects three antigens in stool:
 - *Giardia* (alpha-1 giardin antigen)
 - *E. histolytica/E. dispar* (29 kDa Ag)
 - *Cryptosporidium* (isomerase Ag).
- ❖ **Malaria:** ICT format available detecting:
 - Histidine rich protein-2 (Pf. HRP 2)—*P. falciparum* specific
 - Parasite lactate dehydrogenase (pLDH) and aldolase—common to all species.
- ❖ **Lymphatic filariasis:** ELISA and ICT formats are available detecting filarial antigens by using monoclonal Ab against Og4C3 and AD12 antigens.

Molecular Methods

Molecular methods most frequently used in diagnostic parasitology include:

- ❖ Polymerase chain reaction (PCR) and real time PCR have been developed for most of the parasitic infections
- ❖ LAMP assay: It has been developed for visceral leishmaniasis and malaria
- ❖ BioFire FilmArray: It is an automated multiplex nested PCR. The gastrointestinal panel can simultaneously detect 22 enteric pathogens, including 4 parasites—*E. histolytica*, *G. lamblia*, *Cryptosporidium*, *Cyclospora*.

Other Diagnostic Modalities

- ❖ **Culture techniques:** The culture techniques have been described for various parasites such as amoeba, *Trichomonas*, malaria parasite, *Leishmania* and nematode larvae. They are not routinely done in diagnostic parasitology; but useful in research and teaching purposes
- ❖ **Imaging techniques:** Being noninvasive methods, imaging techniques such as the X-ray, ultrasound (USG), computed tomography (CT) and magnetic resonance imaging (MRI) are extensively used for various space occupying parasitic infections such as amoebic liver abscess, cysticercosis and hydatid disease
- ❖ **Intradermal skin tests:** Positive intra-dermal skin tests are suggestive of past exposure. As they remain positive for longer duration, they cannot differentiate between old and recent infection
 - Skin tests showing immediate hypersensitivity—available for hydatid disease (Casoni's test), filariasis, schistosomiasis, ascariasis, strongyloidiasis and trichinellosis (Bachman test)

- Skin tests showing delayed hypersensitivity—available for leishmaniasis (Montenegro test), trypanosomiasis and toxoplasmosis

- ❖ **Xenodiagnostic technique:** Here the arthropod vectors are fed on infected patients; 2-4 weeks following which the vectors are examined for presence of parasites. This test is seldom used for the diagnosis of Chagas' disease
- ❖ **Animal inoculation methods:** Mainly useful for research purpose; not routinely used in diagnosis of parasitic infections. The animal models are specific for different parasites; for example, mice for *Toxoplasma*, and *Trypanosoma*, hamsters for *Leishmania* and rats for *Toxoplasma*, *Trypanosoma* and *Trichinella*.

TREATMENT OF PARASITIC DISEASES

Treatment of parasitic diseases primarily is based on chemotherapy and in some cases by surgery.

- ❖ **Anti-parasitic drugs:** Various chemotherapeutic agents are used for the treatment and prophylaxis of parasitic infections (Table 5.6)
- ❖ **Surgical management:** It is useful for the management of parasitic diseases like cystic echinococcosis, neurocysticercosis, etc.

OVERVIEW OF PARASITIC INFECTIONS

This division of the chapter gives an overview of various parasites infecting man, which will help to understand the parasitic infections that are discussed in detail under the respective infective syndromes (Part-II) of this book.

PROTOZOAN INFECTIONS

The protozoa are though unicellular, they belong to lower eukaryotes as they possess cellular organelles and metabolic pathways, similar to that of eukaryotes.

- ❖ More than two lakhs protozoa are named, but only about 80 species belonging to nearly 30 genera infect human beings, out of which majority are harmless
- ❖ Only few are considered as human pathogens such as amoebae, flagellates (*Giardia*, *Trichomonas*, *Leishmania* and *Trypanosoma*), *Plasmodium*, coccidian parasites and others (Table 5.1).

Classification

Various classification schemes were used for protozoans.

- ❖ The traditional classification of Levine et al., (1980): It was popular before, now not in use
- ❖ Molecular Classification (2000): This is the current method followed to classify protozoa. It is based on ribonucleic acid and protein sequences of the parasites
 - Also called as Cavalier and Smith's six kingdoms classification, where all living creatures are classified into six kingdoms

Protozoa	Anti-parasitic agents
<i>Entamoeba histolytica</i>	Metronidazole, tinidazole Diloxanide furoate
<i>Giardia</i> species	Metronidazole, tinidazole
<i>Trichomonas</i>	Metronidazole, tinidazole
<i>Trypanosoma cruzi</i>	Benznidazole, nifurtimox
<i>Trypanosoma brucei</i>	Pentamidine, suramin
<i>Leishmania donovani</i>	Amphotericin B, Antimonials Paromomycin, Miltefosine
<i>Plasmodium</i> species	Chloroquine, Quinine, Artemisinin derivative Primaquine, Mefloquine Sulfadoxine-pyrimethamine Lumefantrine
<i>Cryptosporidium</i>	Nitazoxanide
<i>Cyclospora</i>	Cotrimoxazole
<i>Cystoisospora</i>	Cotrimoxazole
<i>Toxoplasma</i>	Cotrimoxazole, spiramycin
Microsporidia	Albendazole
<i>Balantidium coli</i>	Tetracycline
Helminths	
Cestodes	Praziquantel Niclosamide, Albendazole
Trematodes	Praziquantel Triclabendazole for <i>Fasciola hepatica</i>
Intestinal nematodes	Mebendazole, albendazole Pyrantel pamoate Ivermectin (for <i>Strongyloides</i>)
Filarial nematodes	Diethylcarbamazine (DEC) Albendazole, Ivermectin

- Protozoans belong to Kingdom Protozoa, which comprises of 11–13 phyla, of which six phyla contain parasites that infect man (Table 5.7)
- Microsporidia, previously considered as protozoa are now classified under the Kingdom Fungi.

Amoeba

Amoebae (meaning “change”) are a single-celled protozoa that constantly change their shape; which is due to presence of an organ of locomotion called as “pseudopodium”. They comprise of intestinal amoebae and free-living amoebae.

Intestinal amoebae (Chapter 45)

Entamoeba histolytica is the most important pathogenic intestinal amoeba. Most other species of *Entamoeba* are harmless commensals in human intestine; e.g. *Entamoeba coli*.

Entamoeba histolytica

E. histolytica is worldwide in distribution, but more common in tropical and subtropical countries.

- ❖ **Morphological forms:** *E. histolytica* has three stages— (1) trophozoite, (2) precyst and (3) cyst
- ❖ **Life cycle:** Man is the only host. Infection is transmitted by ingestion of contaminated food or water containing the infective form; mature quadrinucleated cysts. Cysts develop into trophozoites which are the pathogenic form; invade the intestinal mucosa. Trophozoites transform back into cysts in the large intestine and are liberated in feces
- ❖ **Clinical manifestations:** Majority of the infected patients are asymptomatic carriers. In few cases, they produce amoebic dysentery; which may further lead to extraintestinal invasion of trophozoites into liver leading to amoebic liver abscess

Table 5.7: Molecular classification of protozoans (2000).

Kingdom	Subkingdom	Phylum	Class	Order	Genus	
Protozoa	Archezoa	Metamonada (intestinal and genital flagellates)	Trepomonadea	Diplomonadida	<i>Giardia</i> , <i>Enteromonas</i>	
			Retortamonadea	Retortamonadida	<i>Retortamonas</i> , <i>Chilomastix</i>	
			Trichomonadea	Trichomonadida	<i>Trichomonas</i> , <i>Pentatrichomonas</i> , <i>Dientamoeba</i>	
	Neozoa	Amoebozoa (amoebae)	Archamoebae	Euamoebida	<i>Entamoeba</i> , <i>Endolimax</i> , <i>Iodamoeba</i>	
			Amoebaea	Acanthopodida	<i>Acanthamoeba</i> , <i>Balamuthia</i>	
		Percolozoa (flagellated amoeba)	Heterolobosea	Schizopyrenida	<i>Naegleria</i>	
		Euglenozoa (blood and tissue flagellates)	Kinetoplastidea	Trypanosomatida		<i>Leishmania</i> <i>Trypanosoma</i>
				Coccidea	Eimeriida	<i>Eimeria</i> , <i>Toxoplasma</i> , <i>Cryptosporidium</i> <i>Cyclospora</i> , <i>Cystoisospora</i> , <i>Sarcocystis</i>
					Haemosporida	<i>Plasmodium</i>
		Ciliophora (ciliates)	Litostomatea	Piroplasmida		<i>Babesia</i>
Trichostomatia				<i>Balantidium</i>		

Adapted from: Topley and Wilson's Microbiology and Microbial Infections, 10th edition and Patrick Murray's Manual of Clinical Microbiology, 11th Edition, ASM Press.

- ❖ **Laboratory diagnosis:** Amoebic dysentery can be diagnosed by stool microscopy demonstrating characteristic cysts and trophozoites. Amoebic liver abscess can be diagnosed by antibody detection or molecular diagnosis by PCR
- ❖ **Treatment:** Metronidazole is the drug of choice for both intestinal and extraintestinal amoebiasis.

Free-living amoebae (Chapter 75)

Free-living amoebae are small, freely living, widely distributed in soil and water and can cause opportunistic infections in humans. Important human pathogenic free-living amoebae are:

- ❖ *Naegleria fowleri*: It is the causative agent of primary amoebic meningoencephalitis (PAM)
- ❖ *Acanthamoeba* species: It causes granulomatous amoebic encephalitis (GAE) and amoebic keratitis in contact lens wearers
- ❖ *Balamuthia* species: It also causes GAE.

Flagellates

This group of parasites bear flagella as the organ of locomotion. Flagella are slender, long and thread-like extension of cytoplasm. Its intracellular portion is called as **axoneme**. Flagella arise from kinetoplast (made up of copies of mitochondrial DNA).

Intestinal Flagellates (Chapter 45)

Giardia lamblia is a flagellated parasite that harbors in duodenum and jejunum. It is worldwide in distribution, considered as one of the most common parasite causing intestinal disease and diarrhea. It causes malabsorption of fats and frothy diarrhea in children. Diagnosis includes detection of oval shaped cysts and pear shaped motile trophozoites in stool microscopy. Metronidazole is the drug of choice.

Dientamoeba fragilis is an intestinal flagellate, differs from others as it bears internal flagellum (therefore considered as ameobo-flagellate). It is a harmless commensal, occasionally has been reported in association with mucous diarrhea

Genital Flagellates: *Trichomonas vaginalis* (Chapter 77)

Trichomonas vaginalis is the most common parasitic cause of sexually transmitted infection (STI). It produces vulvovaginitis; characterized by thin profuse foul smelling purulent vaginal discharge, strawberry appearance of vaginal mucosa and vaginal pH >4.5. Laboratory diagnosis includes detection of jerky motile pear shaped trophozoites in saline wet mount examination of freshly collected specimens such as vaginal secretions and urethral discharge. Metronidazole is the preferred agent of choice. Both the sexual partners must be treated simultaneously.

Hemoflagellates (Chapter 36)

Flagellated protozoa that are found in peripheral blood circulation include *Leishmania* and *Trypanosoma* (Fig. 5.6).

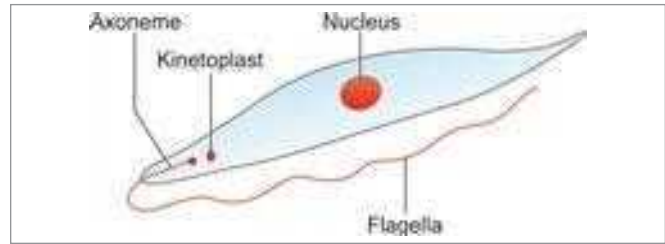


Fig. 5.6: Hemoflagellate (schematic diagram).

Leishmania (Chapter 36)

Leishmania donovani causes visceral leishmaniasis or kala-azar. It is transmitted by sand fly.

- ❖ **Clinical manifestations:** Kala-azar is characterized by pentad of fever, hepatosplenomegaly, weight loss, and hypergammaglobulinemia and pancytopenia. Hyperpigmentation may be seen in Indian cases
- ❖ **Laboratory diagnosis** includes demonstration of Leishman Donovan (LD) bodies in the stained specimens such as splenic and bone marrow aspirate, detection antibodies against rk39 antigen by ICT
- ❖ **Treatment:** Liposomal amphotericin B and pentavalent antimonials are the agents given for kala-azar.

Other human pathogenic species of *Leishmania* can cause old world cutaneous leishmaniasis and new world leishmaniasis (Chapter 57).

Trypanosoma (Chapter 36)

There are two medically important *Trypanosoma* species infecting humans. Both are not found in India.

- ❖ *Trypanosoma cruzi* is the agent of Chagas' disease; a disease prevalent in South America, transmitted by reduviid bug
- ❖ *Trypanosoma brucei* is the agent of African sleeping sickness; transmitted by tsetse fly.

Malaria Parasite (Chapter 35)

Malaria is the most lethal parasitic disease of humans—caused by different species of *Plasmodium*; such as *P. falciparum* (the most common and most lethal species worldwide), *P. vivax*, *P. ovale*, and *P. malariae*.

- ❖ **Life cycle:** Humans are the intermediate host and female *Anopheles* mosquitoes are the definitive host. Man gets infection by the bite of the vector, transmitting the sporozoites (infective form)
- ❖ **Clinical manifestations:** Comprise of benign malaria and malignant tertian malaria
 - **Benign malaria** is milder in nature, can be caused by all species. It is characterized by a triad of febrile paroxysm, anemia and splenomegaly. The febrile paroxysms are intermittent and depending on the infecting species every fourth day (72 hour cycle for *P. malariae*) and every third day (48 hour cycle for other three species)
 - **Malignant tertian malaria** is caused exclusively by *P. falciparum*, characterized by various complications,

such as cerebral malaria, black water fever, pernicious malaria, etc.

- ❖ **Laboratory diagnosis** includes detection of various parasitic forms (ring forms, schizonts and gametocytes) in peripheral blood smear examination, quantitative buffy coat examination (QBC) and detection of malaria parasite antigens by ICT
- ❖ **Treatment:** Chloroquine is given for vivax malaria; whereas falciparum malaria is treated with artemisinin, lumefantrine or sulfadoxine/pyrimethamine. Primaquine is given for prevention of relapse of malaria.

Opportunistic Coccidian Parasites

Opportunistic coccidian parasites include *Toxoplasma*, *Cryptosporidium*, *Cyclospora*, and *Cystoisospora*; all can cause opportunistic infections in HIV (human immunodeficiency virus) infected patients

Toxoplasma (Chapter 75)

Toxoplasma gondii is an obligate intracellular parasite that causes opportunistic infections in immunocompromised persons and congenital infection in fetus.

- ❖ **Life cycle:** Definitive hosts are cat and other felines; whereas man acts as intermediate host. Man gets infection by ingestion of tissue cysts from uncooked meat (common route), or ingestion of sporulated oocysts from contaminated food or water, or by blood transfusion or transplacental route
- ❖ **Clinical manifestation:** In immunocompetent host, it causes lymphadenopathy; whereas, in immunocompromised host, it can cause encephalitis. Intrauterine infection can lead to congenital toxoplasmosis in fetus
- ❖ **Laboratory diagnosis** includes detection of tachyzoites in blood and tissue cysts in tissue biopsy. Specific antibodies can be detected by various methods, such as Sabin-Feldman dye test and ELISA

- ❖ **Treatment:** Pyrimethamine plus sulfadiazine (immunocompetent patients) or co-trimoxazole (AIDS patients) or spiramycin (in pregnancy) are used for treatment of toxoplasmosis.

Other Coccidian Parasites (Chapter 45)

Other coccidian parasites such as *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* can cause opportunistic infections in HIV infected patients producing profuse watery diarrhea. They can be diagnosed by detection of acid fast oocysts in stool microscopy.

HELMINTHIC INFECTIONS

Helminths are elongated flat or round worm-like parasites measuring few millimeters to meters. They are eukaryotic multicellular and bilaterally symmetrical. They belong to two phyla:

1. **Platyhelminths** (flat worms)— include cestodes (tapeworms) and trematodes (flukes)
2. **Nemathelminths**— include intestinal and tissues nematodes.

In general, helminths exist in three morphological forms—(1) adult form (or the worm), (2) larvae and (3) eggs.

Differences between cestodes, trematodes and nematodes have been depicted in Table 5.8 and classification of helminths based on habitat is given in Table 5.9.

Cestodes (Chapter 46)

Cestodes are long, segmented, flattened dorsoventrally, tape like worms hence also called as tapeworms. Medically important cestodes are grouped into intestinal and tissue/somatic cestodes (Table 5.9).

Diphyllobothrium latum (Chapter 46)

Diphyllobothrium latum is the longest tapeworm infecting man.

Table 5.8: Differences between cestodes, trematodes and nematodes (Refer Table 5.9 for examples).

Properties	Cestodes	Trematodes	Nematodes
Shape	Tape-like and segmented	Leaf-like and unsegmented	Elongated, cylindrical and unsegmented
Head end	Suckers present, some have attached hooklets	Suckers present No hooklets	No sucker, no hooklets. Some have well developed buccal capsule
Alimentary canal	Absent	Present but incomplete	Complete from mouth to anus
Body cavity	Absent	Absent	Present
Sexes	Monoecious	Monoecious (except schistosomes)	Diecious
Life cycle	Require two hosts; except <i>Hymenolepis</i> (one host) and <i>Diphyllobothrium</i> (three hosts)	Require three hosts; except <i>Schistosomes</i> (require two hosts)	Require one host (except filarial worms and <i>Dracunculus</i> —require two hosts)
Larva forms	Most cestodes have one larval stage such as—cysticercus (<i>Taenia</i>), hydatid cyst (<i>Echinococcus</i>), cysticeroid (<i>Hymeolepis</i>) <i>Diphyllobothrium</i> —has three larval stages; coracidium, proceroid and plerocercoid	Five larval stages: Cercaria, metacercaria, reidia, miracidium and sporocyst <i>Schistosoma</i> —3 stages; no metacercaria and reidia stages	Four larval stages (L ₁ to L ₄)—known in various names such as rhabditiform larva (L ₁), filariform larva (L ₃), etc.

Table 5.9: Classification of helminths based on habitat.		
Cestodes	Trematodes	Nematodes*
Intestinal cestodes <ul style="list-style-type: none"> • <i>Diphyllobothrium</i> • <i>Taenia solium</i> and <i>T. saginata</i>- cause intestinal taeniasis • <i>Hymenolepis nana</i> • <i>Dipylidium caninum</i> 	Blood flukes (Schistosomes) <ul style="list-style-type: none"> • <i>Schistosoma haematobium</i> • <i>S. mansoni</i> • <i>S. japonicum</i> Hepatic flukes <ul style="list-style-type: none"> • <i>Fasciola hepatica</i> • <i>Clonorchis sinensis</i> • <i>Opisthorchis</i> 	Intestinal nematodes <ul style="list-style-type: none"> • <i>Trichuris trichiura</i> • <i>Enterobius vermicularis</i> • Hookworm: <i>Necator</i> and <i>Ancylostoma</i> • <i>Ascaris lumbricoides</i> • <i>Strongyloides</i>
Somatic/tissue cestodes <ul style="list-style-type: none"> • <i>Taenia solium</i>- causes cysticercosis • <i>T. multiceps</i> • <i>Echinococcus</i> • <i>Spirometra</i> 	Intestinal flukes <ul style="list-style-type: none"> • <i>Fasciolopsis buski</i> Lung flukes <ul style="list-style-type: none"> • <i>Paragonimus westermani</i> 	Tissue nematodes A. Filarial nematodes <ul style="list-style-type: none"> • <i>Wuchereria</i> • <i>Brugia malayi</i> • <i>Loa loa</i> • <i>Onchocerca</i> • <i>Mansonella</i> species B. Others: Trichinella, Dracunculus

*Apart from this list, there are a number of nematodes of lower animals that occasionally infect humans, which are discussed briefly under individual infective syndromes.

- ❖ **Life cycle:** It requires three hosts to complete its life cycle-1) man (definitive host); 2) *Cyclops* (first intermediate host) and 3) fish (second intermediate host). Man gets infected by ingestion of fish containing plerocercoid larva
- ❖ **Clinical manifestations:** It causes malabsorption of vitamin B₁₂, leading megaloblastic anemia
- ❖ **Diagnosis** is by demonstration of operculated eggs in stool microscopy.

Taenia

Taenia species cause two types of manifestations in humans—intestinal taeniasis and cysticercosis. Two important pathogenic species are *T. saginata* and *T. solium*.

Intestinal Taeniasis (Chapter 46)

It is caused by both *T. saginata* and *T. solium*. Man is the definitive host; whereas cattle and pigs serve as intermediate hosts for *T. saginata* and *T. solium* respectively. Man acquires infection by ingestion of contaminated undercooked beef or pork containing infective form (cysticercus larva).

- ❖ Clinically, intestinal taeniasis presents with symptoms such as mild abdominal pain, nausea, loss of appetite and change in bowel habit
- ❖ Diagnosis includes detection of eggs in stool microscopy.

Cysticercosis (Chapter 75)

It is caused only by *T. solium*. Man serves both as definitive and intermediate host. Man acquires infection by

ingestion of food and water contaminated with eggs or by autoinfection.

- ❖ **Clinically**, cysticercosis is characterized by deposition of larvae (*Cysticercus cellulosae*) forming cysts in various organs such as CNS, eye or muscle
- ❖ **Diagnosis** includes detection of specific antibodies in serum or CSF; or radiological diagnosis such as CT scan or MRI.

Echinococcus (Chapter 49)

Echinococcus granulosus is the etiological agent of hydatid disease in man, affecting liver.

- ❖ **Life cycle:** Dogs are the definitive host; man and other herbivores such as sheep act as intermediate host. Man acquires infection by ingestion of food or water contaminated with dog's feces containing *E. granulosus* eggs
- ❖ **Clinically**, hydatid disease presents as deposition of hydatid cysts in various organs causing space occupying lesions; most common organ affected being liver
- ❖ **Laboratory diagnosis** includes radiological diagnosis (USG) and histopathological examination to demonstrate cyst wall and attached brood capsules
- ❖ **Treatment** consists of drugs such as albendazole and semiconservative surgery called as PAIR (puncture, aspiration, injection and re-aspiration).

Hymenolepis nana (Chapter 46)

Hymenolepis nana is the smallest cestode infecting man. It is the most common tapeworm infection throughout the world with higher prevalence seen among children.

- ❖ **Life cycle:** Man is the only host. Man acquires the infection by ingestion of food and water contaminated with eggs or by autoinfection
- ❖ **Clinical manifestations:** *H. nana* infection manifests as anorexia, abdominal pain, headache, dizziness and diarrhea with mucus
- ❖ **Laboratory diagnosis** is by demonstration of detection of the characteristic non-bile stained, round to oval eggs in stool microscopy.

Cestode infections are usually treated with anti-parasitic drugs such as praziquantel, niclosamide or albendazole. Surgical management may be needed for neurocysticercosis or hydatid disease.

Trematodes or Flukes (Chapter 46)

Trematodes are unsegmented, flat and leaf-like.

- ❖ **Classification:** Trematodes are classified based on their habitat; each produce a distinct clinical syndrome
 - **Blood flukes:** e.g. *Schistosoma* (resides in venous plexus)
 - ♦ *S. haematobium* (Chapter 76)—resides in venous plexuses of bladder, may cause carcinoma bladder

- ♦ *S. mansoni* and *S. japonicum* (Chapter 46)—reside in venous plexuses of GIT, may cause various GI symptoms including dysentery.
- **Hepatic flukes** (Chapter 49): e.g.
 - ♦ *Fasciola hepatica* and *F. gigantica*: It resides in liver, may cause hepatomegaly
 - ♦ *Clonorchis* species and *Opisthorchis* species: They reside in bile duct and may produce carcinoma of bile duct.
- **Intestinal flukes**: e.g. *Fasciolopsis buski*. It resides in intestine, may cause various GI symptoms (Chapter 46)
- **Lung flukes**: e.g. *Paragonimus westermani*. It resides in lungs, may produce endemic hemoptysis (Chapter 69).
- ❖ **Life cycle** of trematodes require three hosts; except for *Schistosoma* which needs two hosts
 - **Hosts**: Man is the definitive host; snail is the first intermediate host. The second intermediate host is aquatic plants for *Fasciola hepatica* and *Fasciolopsis*; carb for *Paragonimus* and fish for *Clonorchis* and *Opisthorchis*. *Schistosoma* does not have a second intermediate host
 - **Transmission**: Man acquires infection by ingestion of second intermediate host infected with metacercaria larva (infective form); except for *Schistosoma* where skin penetration by cercaria larva is the mode of transmission.
- ❖ **Laboratory diagnosis** of trematodes includes detection of characteristic eggs in clinical specimens. Eggs of all trematodes are characteristically operculated, except for *Schistosoma*, where the eggs are non-operculated, but bear a characteristic spine
- ❖ **Treatment**: Praziquantel is the drug of choice for most of the trematode infections, except for *F. hepatica* where triclabendazole is the agent of choice.

Intestinal Nematodes (Chapter 46)

Intestinal nematodes include small intestinal nematodes—*Ascaris*, hookworm and *Strongyloides*; and large intestinal nematodes such as *Trichuris* and *Enterobius*. Based on the mode of transmission, they can be grouped into:

- ❖ *Trichuris*, *Enterobius* and *Ascaris* are transmitted by ingestion of contaminated food and water, containing embryonated eggs
- ❖ Hookworm and *Strongyloides* are transmitted by skin penetration by third-stage filariform larvae
- ❖ In addition to this, *Enterobius* and *Strongyloides* show autoinfection
- ❖ Pulmonary migratory phase may be seen with *Ascaris*, hookworm and *Strongyloides*.

The important features of intestinal nematodes are:

- ❖ ***Trichuris trichiura***: It is also called as whipworm, produces dysentery and rectal prolapse. It is diagnosed

by detection of characteristic barrel-shaped bile-stained eggs, with mucus plugs at both the poles; in stool microscopy

- ❖ ***Ascaris lumbricoides***: It is commonly called as roundworm, produces GI symptoms, malnutrition, growth retardation, Loeffler's pneumonia, and may cause complications such as intestinal obstruction and intussusception. It is diagnosed by the detection of characteristic bile-stained fertilized and unfertilized eggs in stool microscopy
- ❖ ***Enterobius vermicularis***: It is commonly called as pinworm, produces perianal pruritus (which worse at night). It is diagnosed by detection of characteristic planoconvex non-bile stained eggs in perianal swabs
- ❖ **Hookworm**: *Ancylostoma duodenale* and *Necator americanus* are the common two hookworms pathogenic to man. It produces GI symptoms and iron deficiency anemia. It is diagnosed by the detection of characteristic non-bile stained, oval segmented eggs with four blastomeres in stool microscopy
- ❖ ***Strongyloides stercoralis***: It produces GI symptoms and cutaneous larva migrans called larva currens. In severe cases, it can cause hyperinfection syndrome affecting CNS. It is diagnosed by the detection of characteristic rhabditiform larvae in stool microscopy.

All intestinal nematodes are treated with albendazole or mebendazole; except for *Strongyloides*, where ivermectin is the drug of choice.

Somatic Nematodes

Somatic or tissue nematodes include filarial nematodes, *Dracunculus* and *Trichinella*.

Filarial Nematodes (Chapter 37)

Different filarial worms reside in various body sites; and are transmitted by various vectors.

- ❖ *Wuchereria bancrofti* and *Brugia malayi* reside in the lymphatic system
- ❖ *Loa loa*, *Onchocerca volvulus*, and *Mansonella streptocerca* are found in skin, subcutaneous tissue (Chapter 57)
- ❖ *Mansonella ozzardi* and *Mansonella perstans* reside in body cavity (Chapter 57).

Lymphatic filariasis manifests as acute adenolymphangitis and at a later stage may develop complications such as hydrocele, and elephantiasis. It is diagnosed by detection of microfilaria in peripheral blood smear, or detection of filarial antigens or antibodies (by ELISA or ICT) in patient's serum. Treatment involves diethylcarbamazine, albendazole or ivermectin.

Dracunculus medinensis (Chapter 57)

Dracunculiasis, also called Guinea-worm disease; once prevalent, now eradicated from many parts of the world, including India. Men get infected when they drink water

that contain cyclops infected with guinea worm larvae. It produces painful cutaneous blisters.

Trichinella spiralis (Chapter 57)

Trichinellosis is a zoonotic infection, acquired from ingestion of raw uncooked pork, containing infective L₁ larvae. It

clinically presents as profuse watery diarrhea, followed by myalgia due to deposition of encysted larvae in muscles.

Apart from these above mentioned list, there are a number of nematodes of lower animals that occasionally infect humans. They are discussed briefly under individual infective syndromes.

EXPECTED QUESTIONS

I. Write short notes on:

1. Stool concentration techniques.
2. Serodiagnosis in parasitic diseases.
3. Classify protozoan infections.
4. Classify helminthic infections.

II. Multiple Choice Questions (MCQs):

1. Advantages of saline mount are all, except:

- a. Useful in the detection of trophozoites and cysts of protozoan parasites and eggs and larvae of helminths
- b. Nuclear details of cysts and helminthic eggs and larvae are better visualized
- c. Motility of trophozoites and larvae can be seen in acute infection
- d. Bile staining property can be appreciated

2. Flotation technique is useful for the detection of:

- a. Fertilized eggs of *Ascaris lumbricoides*
- b. Larva of *Strongyloides*

c. *Taenia* eggs

d. Operculated eggs of trematodes

3. Which of the following protozoa has two hosts in its life cycle?

- a. *Entamoeba histolytica*
- b. *Giardia*
- c. *Plasmodium*
- d. *Trichomonas*

4. Tetranucleated cysts are infective form for:

- a. *Entamoeba histolytica*
- b. *Leishmania donovani*
- c. Malaria parasite
- d. Hookworm

5. Which of the following trematode is transmitted by skin penetration:

- a. *Fasciola*
- b. *Clonorchis*
- c. *Paragonimus*
- d. *Schistosoma*

Answers

1. b 2. a 3. c 4. a 5. d

General Mycology and Overview of Fungal Infections

CHAPTER 6

CHAPTER PREVIEW

- General Mycology
 - Classification
 - Laboratory Diagnosis
 - Treatment
- Overview of Fungal Infections
 - Superficial Mycoses
 - Subcutaneous Mycoses
 - Systemic Mycoses
- Opportunistic Mycoses
- Other Mycoses

GENERAL MYCOLOGY

Medical mycology is the branch of medical science that deals with the study of medically important fungi. The name 'fungus' is derived from Greek 'mykes' meaning mushroom (a type of edible fungus). Fungi differ from bacteria and other eukaryotes in many ways.

- ❖ Fungi are eukaryotic and they possess all the eukaryotic cell organelles such as mitochondria
- ❖ They possess a rigid cell wall, composed of chitin, β -glucans and other polysaccharides
- ❖ Fungal cell membrane contains ergosterol instead of cholesterol
- ❖ Fungi may be unicellular or multicellular
- ❖ They lack chlorophyll and divide by asexual and/or sexual means by producing spores.

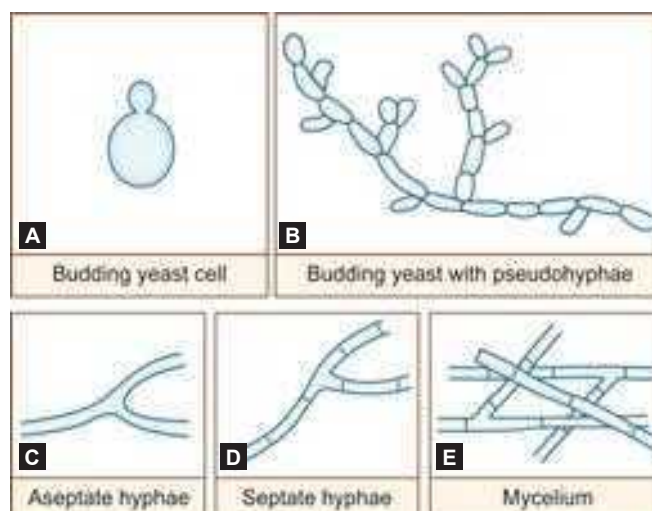
CLASSIFICATION OF FUNGI

Morphological Classification

Based on the morphological appearance, there are four main groups of fungi given as follows (Fig. 6.1):

1. **Yeast:** They grow as round to oval cells that reproduce by an asexual process called **budding** in which cells form protuberances which enlarge and eventually separate from the parent cells. Examples include:
 - *Cryptococcus neoformans* (pathogenic)
 - *Saccharomyces cerevisiae* (non-pathogenic).
2. **Yeast-like:** In some yeasts (e.g. *Candida*), the bud remains attached to the mother cell, elongates and undergoes repeated budding to form chains of elongated cells known as **pseudohyphae**. They can be differentiated from true hyphae as they have constrictions at the septa
3. **Molds:** They grow as long branching filaments of 2–10 μm width called **hyphae**
 - Hyphae are either septate (i.e. form transverse walls) or nonseptate (there are no transverse walls and they are multinucleated, i.e. coenocytic)

- Hyphae grow continuously and form a branching tangled mass of growth called **mycelium**
 - Based on the growth pattern in culture medium, the mycelia may be categorized into two types:
 - i. **Aerial mycelium:** It is the part of the mycelium which projects above the surface of the culture medium
 - ii. **Vegetative mycelium:** It is the part of the mycelium that grows on the surface of the culture medium.
 - Molds reproduce by formation of different types of sexual and asexual spores
 - Examples of true molds include—Dermatophytes, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, etc.
4. **Dimorphic fungi:** They exist as molds (hyphal form) in the environment at ambient temperature (25°C) and as yeasts in human tissues at body temperature (37°C). Several medically important fungi are thermally dimorphic such as:
- *Histoplasma capsulatum*



Figs 6.1A to E: Morphological forms of fungi.

- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Paracoccidioides brasiliensis*
- *Penicillium marneffeii*
- *Sporothrix schenckii*.

Taxonomical Classification

Based on the production of sexual spores, the Kingdom Fungi has been divided into four medically important phyla. They are as follows:

1. Phylum zygomycota: They are lower fungi, produce sexual spores known as **zygospores** and possess aseptate hyphae, e.g. *Rhizopus* and *Mucor*
2. Phylum ascomycota: They produce sexual spores known as **ascospores** and possess septate hyphae, e.g. *Aspergillus*
3. Phylum basidiomycota: They produce sexual spores known as **basidiospore** e.g. *Cryptococcus*
4. Phylum deuteromycota (**Fungi imperfecti**): In majority of the medically important fungi, the sexual state is either absent or unidentified yet. Hence, they are traditionally grouped as fungi imperfecti.

Types of fungal spores produced are given in Table 6.1.

LABORATORY DIAGNOSIS OF FUNGAL DISEASES

The laboratory diagnosis of fungal diseases comprises of the following:

Specimen Collection

It depends on the site of infection such as skin scraping, hair, nail, sputum, etc. For systemic mycoses, blood sample may also be collected. Cerebrospinal fluid (CSF) is collected for cryptococcal meningitis.

Microscopy

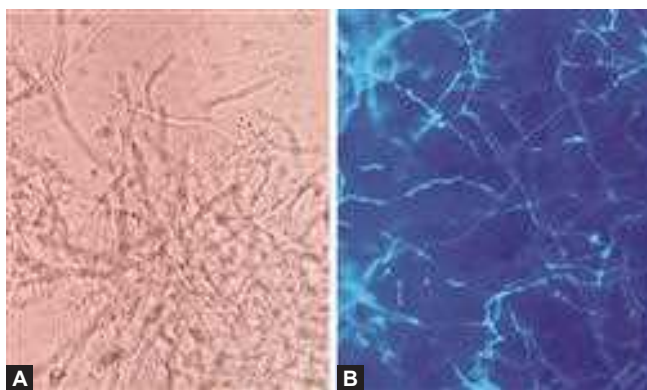
Fungal elements can be detected in the clinical specimens by direct microscopic examination of material from the lesion.

- ❖ **Potassium hydroxide (KOH) preparation:** Keratinized tissue specimens such as skin scrapings and plucked hair samples are treated with 10% KOH which digests the keratin material so that the fungal hyphae will be clearly seen under the microscope. Heat the slide gently over the flame and leave it aside for 5–10 minutes before examination (Fig. 6.2A)
 - About 10% is the usual concentration of KOH used
 - About 20–40% KOH is needed for the specimens such as nail that otherwise takes a longer time to dissolve
 - Biopsy specimens as they take longer time to dissolve, are usually dissolved in 10% KOH in a test tube and examined after overnight incubation
 - Glycerol (10%) can be added to prevent drying
 - DMSO (dimethyl sulfoxide) can be added to help in tissue digestion

Table 6.1: Types of fungal spores.

Sexual spore	Observed in
Zygosporos	Zygomycetes
Ascospores	<i>Aspergillus</i>
Basidiospores	<i>Cryptococcus</i>
Asexual spore	Observed in
Vegetative asexual spore	
Arthrospore	<i>Coccidioides</i> and <i>Trichosporon</i>
Blastospore	<i>Candida</i>
Chlamydo-spore	<i>Candida albicans</i>
Aerial asexual spore	
Conidiospore or conidia	<i>Aspergillus</i>
Sporangiospore	Zygomycetes
Microconidia	Dermatophytes
Macroconidia	Dermatophytes

- Caution should be maintained while interpretation of hyphae, which may be confused with collagen fiber, cotton fiber or hair present in the clinical specimens.
- ❖ **Gram stain:** It is useful in identifying the yeasts (e.g. *Cryptococcus*) and yeast like fungi (e.g. *Candida*). They appear as gram-positive budding yeast cells
- ❖ **India ink and nigrosin stains:** They are used as negative stains for demonstration of capsule of *Cryptococcus neoformans* (Fig. 75.14A)
- ❖ **Calcofluor white stain:** It is more sensitive than other stains; binds to cellulose and chitin of fungal cell wall and fluoresce under UV light (Fig. 6.2B)
- ❖ **Histopathological stains:** They are useful for demonstrating fungal elements from biopsy tissues. This is useful for detecting invasive fungal infection
 - Periodic acid schiff (PAS) stain: It is the recommended stain for detecting fungi. PAS positive fungi appear magenta/deep pink, whereas the nuclei stain blue
 - Gomori methenamine silver (GMS) stain: It is used as an alternative to PAS for detecting fungi.



Figs 6.2A and B: Fungal hyphae in: **A.** KOH mount; **B.** Calcofluor white stain mount.

Source: A. Dr Sherly Antony, Pushpagiri Medical College, Thiruvalla, Kerala; B. Department of Microbiology, JIPMER, Puducherry (with permission).

It stains both live and dead fungi, as compared to PAS which stains only the live fungi. GMS stains the polysaccharide component of the cell wall. Fungi appear black whereas the background tissue takes pale green color

- Mucicarmine stain: It is used for staining the carminophilic cell wall of *Cryptococcus* and *Rhinosporidium*
- Masson fontana stain: It is used for pigmented (or pheoid) fungi
- Hematoxylin and Eosin (H and E) stain.
- ❖ **Lactophenol cotton blue (LPCB):** It is used to study the microscopic appearance of the fungal isolates grown in culture. It contains:
 - Phenol acts as disinfectant
 - Lactic acid preserves the morphology of fungi
 - Glycerol prevents drying
 - Cotton blue stains the fungal elements blue.

Culture

Fungal culture is frequently performed for isolation and correct identification of the fungi.

Culture Media

- ❖ **Sabouraud's dextrose agar (SDA):** It is the most commonly used medium in diagnostic mycology. It contains peptone (1%), dextrose (4%) and has a pH of 5.6. This may not support some pathogenic fungi
- ❖ **Neutral SDA (Emmons' modification):** It differs from original SDA in having neopeptone (1%) and dextrose (2%) and pH of 7.2
- ❖ **Corn meal agar and rice starch agar:** They are the nutritionally deficient media used for stimulation of chlamydospore production
- ❖ **Brain heart infusion (BHI) agar and blood agar:** They are the enriched media, used for growing fastidious fungi like *Cryptococcus* and *Histoplasma*
- ❖ **Niger seed agar and bird seed agar:** They are used for the selective growth of *Cryptococcus*
- ❖ **CHROMagar Candida medium:** It is used as a selective as well as a differential medium for speciation of *Candida*.

Culture Condition

- ❖ **Temperature:** Most of the fungi grow well at 25–30°C except the dimorphic fungi that grow at both 25°C and 37°C
- ❖ **BOD incubators** (biological oxygen demand): It is a special incubator used in diagnostic mycology, which is capable of maintaining low temperature
- ❖ **Incubation time:** Culture plates should be incubated for 2–3 weeks
- ❖ **Antibiotics** such as cycloheximide (actidione), chloramphenicol and gentamicin can be added to the culture media to inhibit bacterial growth.

Culture Identification

The correct identification of the fungus is based on the macroscopic appearance of the colonies grown on culture and microscopic appearance (LPCB mount of colonies).

Macroscopic Appearance of the Colony

- ❖ **Rate of growth:**
 - Rapid growth (<5 days): It is seen in saprophytes, yeasts and agents of opportunistic mycoses
 - Slow growth (1–4 weeks): It is observed in dermatophytes, agents of subcutaneous and systemic mycoses.
- ❖ **Pigmentation:** It can be seen on the reverse side of the culture media
- ❖ **Texture:** It refers to how the colony would have felt if allowed to touch. It may be of various types such as—glabrous (waxy/leathery), velvety, yeast-like, cottony or granular/powdery
- ❖ **Colony topography:** Colony surface may be rugose (radial grooves), folded, verrucose or cerebriform (brain-like).

Microscopic Appearance of Fungi

- ❖ **Teased mount:** A bit of fungal colony is teased out from the culture tube and the LPCB mount is made on a slide and viewed under microscope. If proper teasing is not done, then the intact morphology may not be identified properly. Identification is based on the following:
 - Nature of hyphae (such as septate or aseptate, hyaline or phaeoid, narrow or wide) and
 - Type of sporulation (conidia or sporangiospores).
- ❖ **Slide culture:** Though this is a tedious procedure, it gives the most accurate *in situ* microscopic appearance of the fungal colony. A sterile slide is placed on a bent glass rod in a sterile petri dish. Two square agar blocks measuring around 1 cm² (smaller than the coverslip) are placed on the slide. Bit of the fungal colony is inoculated onto the margins (at the center) of the agar block. Then the coverslip is placed on the agar block and the petri dish is incubated at 25°C. After sufficient growth occurs, LPCB mounts are made both from the coverslip and the underneath slide (Fig. 6.3)
- ❖ **Cellophane tape mount:** The impressions are taken by placing the cellophane tape on the colonies present on the surface of SDA plate, then LPCB mount is made from the cellophane tape. This is easy to perform than slide culture and *in-situ* fungal morphology is also maintained.

Other Methods of Identification

- ❖ **For Candida:** Germ tube test, Dalmau plate culture, carbohydrate fermentation and carbohydrate assimilation tests are done
- ❖ **For dermatophytes:** Hair perforation test, dermatophyte test medium and dermatophyte identification medium are used



Fig. 6.3: Slide culture technique.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ Urease test can be done for the fungi that produce urease enzyme, e.g. *Cryptococcus*.

Immunological Methods

These tests are available to detect the antibody or antigen from serum and/or other body fluids.

- ❖ **Antibody detection** can be done by ELISA, immunodiffusion test, agglutination test, and complement fixation test (CFT)
- ❖ **Antigen detection:** Various fungal antigens can be detected in clinical specimens such as blood, CSF, urine, etc.
 - **Cryptococcal capsular antigen** from CSF by Latex agglutination test
 - Detection of *Aspergillus* specific **galactomannan antigen** in patient's sera or urine (by ELISA)
 - **β-d-Glucan assay**, detecting β-d-Glucan antigen in blood by ELISA: It is a marker of invasive fungal infections, raised in all invasive fungal infections, except for zygomycosis, blastomycosis and cryptococcosis.
- ❖ **Immunohistochemistry:** It refers to detecting antigens (e.g. proteins) on the cells of a tissue section by using fluorescent tagged antibodies that bind specifically to the antigens. It is useful in deep mycoses.

Automations

Automated identification system such as MALDI-TOF and VITEK are revolutionary in accurate identification of yeasts and to some extent molds.

Molecular Methods

Polymerase chain reaction (PCR) and its modifications such as multiplex PCR, nested PCR and the most advanced real time PCR and DNA sequencing methods have been

developed for accurate identification of fungi from culture as well as from the specimens.

TREATMENT OF FUNGAL INFECTIONS

Antifungal agents, their mechanisms of action and their use have been described in Table 6.2.

OVERVIEW OF FUNGAL INFECTIONS

Although more than 25,000 species of fungi are known, most of them are saprophytes in soil and decaying plant materials. Only few are medically important. Fungal infections (or mycoses) can be categorized into the following clinical types (Table 6.3).

SUPERFICIAL MYCOSES

Superficial mycoses (Chapter 58) are the fungal infections involving the skin, hair, nail and mucosa. Examples include:

- ❖ **Tinea versicolor:** It is caused by lipophilic fungus *Malassezia furfur*, which is characterized by flat-round scaly hypopigmented to hyperpigmented patches of skin
- ❖ **Tinea nigra:** It is characterized by painless, black, non-scaly patches present on palm and sole; caused by a black-colored yeast like fungus *Hortaea werneckii*
- ❖ **Piedra:** It is characterized by nodule formation on the hair shaft, which may be either black in color (caused by *Trichosporon beigelii*) or white in color (caused by *Piedraia hortae*)
- ❖ **Dermatophytoses** (or tinea or ringworm): It is the most common superficial mycoses affecting skin, hair and nail; caused by a group of related fungi (called dermatophytes) that are capable of infecting keratinized tissues; producing annular pruritic scaly lesions. These include:
 - *Trichophyton* species: Infect skin, hair and nail
 - *Microsporum* species: Infect skin and hair
 - *Epidermophyton* species: Infect skin and nail.

SUBCUTANEOUS MYCOSES

Subcutaneous mycoses (Chapter 58) are the mycotic infections of the skin, subcutaneous tissue and sometimes bone, resulting from inoculation of saprophytic fungi of soil or decaying matter. They are mainly confined to the tropics and subtropics. Examples include:

- ❖ **Mycetoma:** Mycetoma, also known as Maduramycosis or Madura foot is a chronic, slowly progressive granulomatous infection of the skin and subcutaneous tissues; clinically manifested as a triad of swelling, discharging sinuses and presence of granules in the discharge. Mycetoma is of two types:
 - Eumycetoma—caused by fungal agents such as *Madurella mycetomatis*
 - Actinomycetoma—caused by bacterial agents such as *Nocardia*.

Table 6.2: Antifungal agents, their mechanisms of action and uses.			
Class	Drug	Use	Mechanisms
Antifungal antibiotics			
Polyenes	Amphotericin B	Systemic mycoses (all invasive fungal infections)	Bind to ergosterol and disrupts fungal cell membranes
	Nystatin, hamycin	Topical use (skin infection)	
Echinocandins	Caspofungin, micafungin, anidulafungin	Systemic mycoses mainly due to <i>Candida</i> and <i>Aspergillus</i>	Inhibits β -glucan synthesis in fungal cell wall
Benzofurans	Griseofulvin	Dermatophytoses	Disrupts mitotic spindle by binding to fungal cell tubulin
Synthetic antifungal agents			
Azoles	Imidazoles: Clotrimazole, miconazole, ketoconazole, oxiconazole	Topical use (except, ketoconazole can be used for both topical and systemic use)	Inhibits ergosterol synthesis of fungi
	Triazoles		
	Fluconazole	Systemic mycoses (invasive fungal infections) <i>Cryptococcus</i> and <i>Candida</i> (except <i>C. krusei</i> , <i>C. glabrata</i>)	
	Itraconazole	Sporothrix, chromoblastomycosis, dermatophytes, <i>Cryptococcus</i> , <i>Candida</i> (except <i>C. krusei</i> , <i>C. glabrata</i>) and <i>Trichosporon</i>	
	Voriconazole	All invasive fungal infections except mucormycosis	
	Posaconazole and isavuconazole	All invasive fungal infections including mucormycosis	
Antimetabolite	Flucytosine (5-FC) (converted to fluorouracil inside body)	Systemic mycoses Used in combination with amphotericin B.	Fluorouracil inhibits thymidylate synthetase, thus inhibiting DNA synthesis
Allylamines	Terbinafine	Topical use	Inhibits ergosterol synthesis
Other topical agents	Tolnaftate, Benzoic acid, Whitfield's ointment, Undecylenic acid, Ciclopirox olamine		

- ❖ **Sporotrichosis** also known as Rose Gardner's disease is caused by a thermally dimorphic fungus, *Sporothrix schenckii*. It presents as subcutaneous noduloulcerative lesions
- ❖ **Chromoblastomycosis**: It refers to slow growing chronic subcutaneous lesions caused by group of dematiaceous or phaeoid fungi (i.e. darkly pigmented fungi) that produce a characteristic morphology called sclerotic body. Agents include: *Fonsecaea*, *Phialophora*, *Cladosporium* and *Rhinocladiella*
- ❖ **Phaeohyphomycosis**: Chronic subcutaneous lesions; caused by dematiaceous fungi such as *Alternaria*, *Bipolaris*, *Curvularia*, *Exophiala* and *Cladophialophora*
- ❖ **Rhinosporidiosis**: It is caused by *Rhinosporidium seeberi*. It produces large friable polyps in the nose (most common site), conjunctiva and rarely in other sites.

SYSTEMIC MYCOSES

Systemic mycoses (Chapter 38) is characterized by involvement of multiple organs. Mostly they are caused by saprophytic fungi, which spread by inhalation of spores leading to pulmonary infection. From lungs, they disseminate to cause various systemic manifestations. These agents are thermally dimorphic, include—*Histoplasma*, *Blastomyces*, *Coccidioides* and *Paracoccidioides*.

OPPORTUNISTIC MYCOSES

They are caused by the fungi that are normally found as human commensals or environmental contaminants; but can act as human pathogens in presence of opportunities such as low immunity. Various opportunistic mycoses are as follows:

- ❖ **Candidiasis** (Chapter 38): Candidiasis is the most common fungal disease in humans, affecting the skin, mucosa, and various internal organs; caused by *Candida*, a yeast like fungus that produces pseudohyphae
 - Various species include *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, etc.
 - Candidiasis is common in presence of various predisposing factors such as patients on steroid or immunosuppressive drugs, post-transplantation, malignancy, HIV infection.
- ❖ **Cryptococcosis** (Chapter 75): It is caused by a capsulated yeast called *Cryptococcus neoformans*, which is capable of producing potentially fatal meningitis in HIV infected people. Negative staining (e.g. modified India ink stain) of CSF specimen is usually performed to demonstrate the capsule, which appears as refractile delineated clear space surrounding the round budding yeast cells against the black background.
- ❖ **Zygomycosis** (Chapter 69): It represents group of life-threatening infections caused by aseptate fungi; belongs to the phylum Zygomycota. They include *Rhizopus*, *Mucor*, *Rhizomucor*, *Lichtheimia*, etc.

Table 6.3: Classification of fungal diseases.	
Fungal disease	Agents
Superficial mycoses*	
Tinea versicolor	<i>Malassezia furfur</i>
Tinea nigra	<i>Hortaea werneckii</i>
Piedra	<i>Trichosporon beigeli</i> , <i>Piedraia hortae</i>
Dermatophytosis	<i>Trichophyton</i> , <i>Microsporum</i> , <i>Epidermophyton</i>
Subcutaneous mycoses	
Mycetoma	<i>Madurella mycetomatis</i> , <i>Pseudallescheria boydii</i> , Others
Sporotrichosis	<i>Sporothrix schenckii</i>
Chromoblastomycosis	<i>Phialophora verrucosa</i> , <i>Fonsecaea pedrosoi</i>
Phaeohyphomycosis	<i>Exophiala</i> , <i>Cladophialophora</i> and others
Rhinosporidiosis	<i>Rhinosporidium seeberi</i>
Systemic mycoses*	
Histoplasmosis	<i>Histoplasma capsulatum</i>
Blastomycosis	<i>Blastomyces dermatitidis</i>
Coccidioidomycosis	<i>Coccidioides immitis</i>
Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>
Opportunistic mycoses	
Candidiasis	<i>Candida albicans</i> and other species
Cryptococcosis	<i>Cryptococcus neoformans</i>
Zygomycosis	Mucorales <ul style="list-style-type: none"> • <i>Rhizopus</i> • <i>Mucor</i> • <i>Absidia</i> (new name <i>Lichtheimia</i>) Entomophthorales** <ul style="list-style-type: none"> • <i>Basidiobolus ranarum</i> • <i>Conidiobolus coronatus</i>
Aspergillosis	<i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i>
Penicilliosis	<i>Penicillium marneffei</i> (new name <i>Talaromyces marneffei</i>) Other <i>Penicillium</i> species
Pneumocystosis	<i>Pneumocystis jirovecii</i>
Fusariosis	<i>Fusarium</i> species
Others	
Mycotoxicoses	Fungi producing toxins (Chapter 40)
Microsporidiosis	<i>Encephalitozoon cuniculi</i> and others (Previously parasite; recently re-classified under fungi)

*Superficial and systemic manifestations are also seen in candidiasis, cryptococcosis, aspergillosis and zygomycosis.

**Entomophthorales cause subcutaneous infection.

- **Risk factors:** It is common in presence of risk factors such as diabetic ketoacidosis
- **Clinical forms:** Rhinocerebral mucormycosis is the most common form; starts as eye and facial pain, may progress to cause orbital cellulitis, proptosis and vision loss. It can also affect other organs such as lungs, GIT, brain, etc.
- **Laboratory diagnosis** includes culture on SDA producing white cottony woolly colonies and LPCB mount of the colonies showing typical broad aseptate hyaline hyphae. Some species like *Rhizopus* shows a unique root-like growth arising from hyphae called **rhizoid**.
- ❖ **Aspergillosis** (Chapter 69): It refers to the invasive and allergic diseases caused by a hyaline mold named *Aspergillus*; important species being—*A. fumigatus*, *A. flavus* and *A. niger*
 - **Risk factors:** Glucocorticoid use and profound neutropenia are the important risk factors
 - **Clinical forms:** Pulmonary aspergillosis is the most common form. Other forms include invasive sinusitis, cardiac, cerebral, ocular aspergillosis, etc.
 - **Laboratory diagnosis:** LPCB mount of the colonies grown on SDA reveals typical narrow septate hyaline hyphae.
- ❖ **Penicilliosis** (Chapter 69): *Penicillium* species produce various opportunistic infections such as endophthalmitis, otomycosis, keratitis and onychomycosis. *Penicillium marneffei* is the only dimorphic species of *Penicillium*, which causes opportunistic infection (wart-like skin lesions) in HIV-infected patients
- ❖ **Pneumocystosis** (Chapter 69): It refers to plasma cell pneumonia due to *Pneumocystis jirovecii*, which typically occurs in HIV infected patients.

OTHER MYCOSES

- ❖ **Mycotoxicoses:** Mycotic poisoning can be classified into two varieties:
 1. **Mycotoxicoses** (Chapter 40): It occurs following consumption of food contaminated by toxins liberated by certain fungi; e.g. aflatoxin by *Aspergillus flavus* which causes carcinoma liver
 2. **Mycetism** (Chapter 40): It refers to toxic effects produced by, eating poisonous fleshy fungi; usually different types of mushrooms.
- ❖ **Microsporidiosis:** Microsporidia were previously placed under parasites; now taxonomically re-classified under fungi. They comprise of several species (e.g. *Encephalitozoon cuniculi* and others), which cause gastrointestinal, musculoskeletal and ocular infections (Chapters 45, 58 and 78).

EXPECTED QUESTIONS

I. Write short notes on:

1. Laboratory diagnosis of fungal infections.
2. Dimorphic fungi.

II. Multiple Choice Questions (MCQs):

1. All are yeast or yeast-like fungi except:
 - a. *Candida*
 - b. *Trichosporon*
 - c. *Cryptococcus*
 - d. *Trichophyton*

2. Fungi which do not have sexual stage:

- a. Zygomycota
- b. Ascomycota
- c. Basidiomycota
- d. Fungi imperfecti

3. Fungal agent that does not infect nail:

- a. *Trichophyton*
- b. *Epidermophyton*
- c. *Microsporum*
- d. *Candida albicans*

Answers

1. d 2. d 3. c

CHAPTER PREVIEW

■ Microbiology of Normal Flora

■ Role of Normal Flora

INTRODUCTION

Normal microbial flora (also called “indigenous microbiota”) refers to the diverse group of microbial population that every human being harbors on his/her skin and mucous membranes.

- ❖ In humans, the normal flora is located in various sites such as gastrointestinal tract (GIT), respiratory tract, genitourinary tract and skin
- ❖ Although there are many species of normal flora, these microbes typically fall into one of the two categories: resident flora and transient flora.

Resident Flora

These organisms are life-long members of the body’s normal microbial community.

- ❖ They are very closely associated with a particular area. When disturbed, they again re-establish themselves. For example, *Escherichia coli* is a resident flora of the intestine
- ❖ They do not cause harm; rather they have beneficiary effect on the host (described later).

Transient Flora

The transient flora consists of microorganisms that inhabit the body surface or mucous membranes temporarily for a short interval.

- ❖ Transient flora do not produce disease as long as the resident flora remains intact. If the resident flora gets disturbed due to any reasons, transient flora may colonize and produce disease, e.g. pneumococcus and meningococcus in nasopharynx
- ❖ In hospitals, patients may acquire many resistant organisms as transient flora from the healthcare workers and hospital environment. For examples, MRSA (Methicillin-resistant *Staphylococcus aureus*) in nose and skin, multidrug resistant gram-negative organisms such as *Klebsiella*, *Escherichia coli*, *Pseudomonas*, *Acinetobacter* in respiratory tract
- ❖ In contrast to resident flora, they can be easily eliminated from the body surface by following proper hand hygiene and other infection control practices.

MICROBIOLOGY OF NORMAL FLORA

The resident microbial flora is more or less constant for a given area of the body at a given age.

- ❖ Humans acquire the normal flora soon after the birth and then continue to harbor until death
- ❖ Although life is possible without normal flora (e.g. germ free experimental animals), but they certainly have a definite role in maintaining health and normal functions of their host
- ❖ The presence of the normal microbial flora in a given body site depends upon various factors such as: Local temperature, moisture, pH, environmental flora (hospital or community), immunity, and anatomical site (skin or mucosa)
- ❖ Most of the normal flora predominantly contain bacteria and to a less extent some fungi and parasites. The existence of viruses as normal flora is recently gained importance (see the highlight box below)

Human Viral Microbiota

The human viral microbiota or virome is the collection of viruses in and on the human body. Viruses may get integrated into the human genome as proviruses. As viruses evolve rapidly, there is constant change in the human virome.

- ❑ Every human being has a unique virome, which may get affected by age, life style, diet, pre-existing immunity, geographic location and seasonal variation
 - ❑ With the advancement of deep sequencing technique, it is now possible to gather information about human viral microbiota
 - ❑ Many viruses colonize the human skin, called as skin virome; examples include human papillomavirus and bacteriophages that infect the commensal skin bacteria such as staphylococci.
- ❖ The total population of normal flora in humans is roughly about 10^{14} bacteria; which is more than the total number of cells (10^{13}), present in the human body
 - ❖ Overall, anaerobic flora dominates over aerobes; the ratio of anaerobic/aerobic bacteria varies depending upon the body site (Table 7.1)

Table 7.1: Microbiology of normal flora.					
Anatomical site	Total bacteria/g or mL	Anaerobic/aerobic ratio	Anaerobic normal bacterial flora (common)	Aerobic normal bacterial flora, commensal fungi and parasites	
Mouth				Predominant	Less predominant
Saliva	10 ⁸ –10 ⁹	1:1	<ul style="list-style-type: none"> Anaerobic cocci <i>Actinomyces</i> <i>Fusobacterium</i> <i>Bifidobacterium</i> <i>Prevotella</i> Spirochetes 	Viridans streptococci	Commensal parasites in oral cavity <ul style="list-style-type: none"> <i>Trichomonas tenax</i> <i>Entamoeba gingivalis</i>
Tooth surface	10 ¹⁰ –10 ¹¹	1:1			
Gingiva	10 ¹¹ –10 ¹²	10 ³ :1			
Nasopharynx				Predominant	Less predominant
			<ul style="list-style-type: none"> <i>Prevotella</i> species Anaerobic cocci <i>Fusobacterium</i> 	<ul style="list-style-type: none"> Streptococci (α and non-hemolytic) <i>Neisseria</i> (non-pathogenic species) Diphtheroids <i>Staphylococcus epidermidis</i> 	<ul style="list-style-type: none"> <i>Haemophilus</i> Meningococcus Pneumococcus <i>Staphylococcus aureus</i> Gram-negative rods Yeasts
Gastrointestinal tract				Predominant	Less predominant
Stomach	0–10 ⁵	1:1	<i>Lactobacillus</i>	<i>Helicobacter pylori</i>	
Jejunum/ileum	10 ⁴ –10 ⁷	1:1	<ul style="list-style-type: none"> Anaerobic cocci <i>Bacteroides fragilis</i> <i>Fusobacterium</i> <i>Bifidobacterium</i> <i>Prevotella</i> <i>Clostridium</i> 	<ul style="list-style-type: none"> Enterobacteriaceae and other gram-negative rods Enterococci Streptococci (α and non hemolytic), <i>S. agalactiae</i> 	<ul style="list-style-type: none"> Diphtheroids <i>Candida</i> species <i>Staphylococcus aureus</i> Commensal <i>Entamoeba</i> species* Commensal intestinal flagellates**
Terminal ileum and colon	10 ¹¹ –10 ¹²	10 ³ :1			
Female genital tract				Predominant	Less predominant
Vagina	10 ⁷ –10 ⁹	10:1	<ul style="list-style-type: none"> Anaerobic cocci <i>Lactobacillus</i> <i>Prevotella</i> <i>Bifidobacterium</i> <i>Clostridium</i> 	<ul style="list-style-type: none"> <i>Corynebacterium</i> species <i>Lactobacillus</i> species Streptococci (α, non-hemolytic and <i>S. agalactiae</i>) <i>Neisseria</i> (non-pathogenic species) 	<ul style="list-style-type: none"> Enterococci Enterobacteriaceae and other gram-negative rods <i>S. epidermidis</i> <i>Candida</i> species
Skin				Predominant	Less predominant
	10 ² –10 ³	3:2	<ul style="list-style-type: none"> <i>Propionibacterium</i> Anaerobic cocci 	<ul style="list-style-type: none"> <i>Staphylococcus epidermidis</i> Diphtheroids <i>Micrococcus</i> species <i>Neisseria</i> (Non-pathogenic species) Streptococci (α and non hemolytic) 	<ul style="list-style-type: none"> <i>Staphylococcus aureus</i> <i>Candida</i> species <i>Acinetobacter</i> species

*Commensal *Entamoeba* species are *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmanni* (all colonize the large intestine).

**Commensal intestinal flagellates are *Pentatrichomonas hominis*, *Retortamonas intestinalis*, *Chilomastix mesnili* and *Enteromonas hominis*.

❖ GIT is the predominant site of normal flora, where the most common flora is *Bacteroides fragilis* (anaerobic flora). Among aerobes, *Escherichia coli* is the most common. The microbiological profile of the normal flora in various sites of human body is given in Table 7.1.

ROLE OF NORMAL FLORA

Various microorganisms present as the normal flora have different relationship with the host.

- ❖ They may have beneficiary effect on the host or;
- ❖ They may be harmful to the host (if enter into a wrong site, cause endogenous infection), or;
- ❖ They may exist as commensals (inhabiting the host for long periods without causing detectable harm or benefit).

Beneficial Effects

The normal microbial flora has several beneficial effects to the host (Fig. 7.1):

- ❖ **Prevent colonization of pathogen:** By competing for attachment sites or for essential nutrients
- ❖ **Synthesize vitamin:** Human enteric bacteria secrete several vitamins such as vitamin K and B complex (e.g. vitamin B12) in excess; which can be absorbed by the host as nutrient
- ❖ **Waste produced antagonize other bacteria:** Normal flora may inhibit or kill other nonindigenous organisms by producing a variety of waste substances such as:
 - Fatty acids and peroxides
 - Lactic acid: Lactobacilli present as normal flora in vagina of adult females maintain the acidic pH by

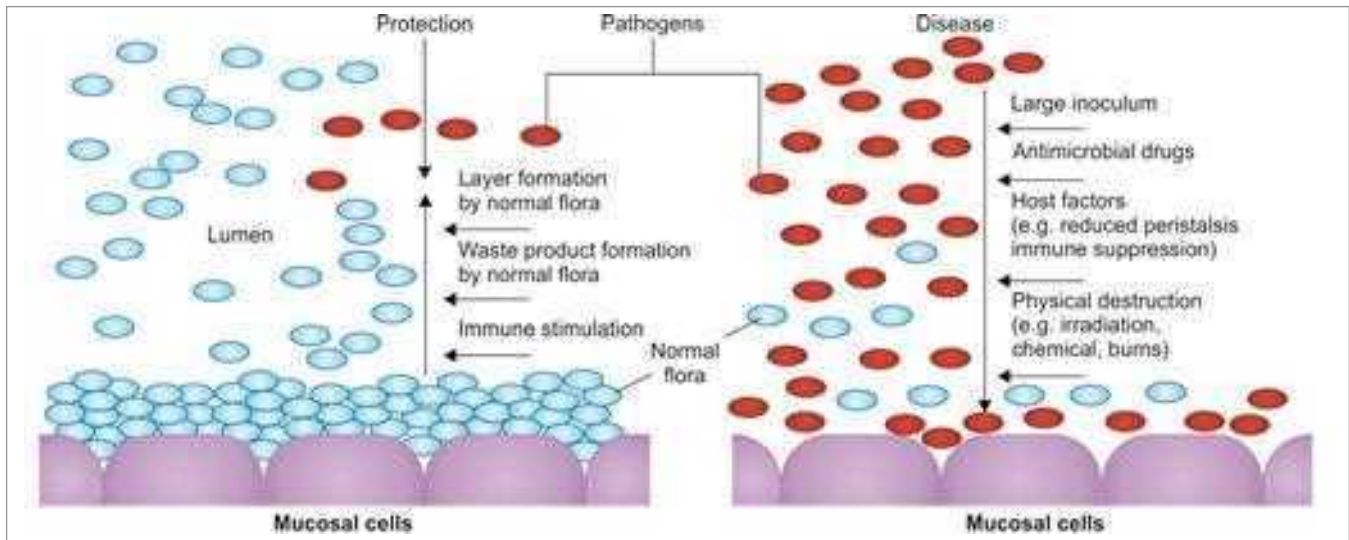


Fig. 7.1: Beneficial effect of normal flora in health and its disruption leading to disease.

producing lactic acid, thereby prevent the growth of pathogenic bacteria

- **Bacteriocin:** Some bacterial flora may produce bacteriocin or colicins which are antibiotic-like substances that can inhibit or kill other bacteria.
- ❖ **Immune stimulation:** Normal microbiota being foreign to the host stimulates the host's immune system
 - **Development of lymphatic tissues:** Immune stimulation helps in development of lymphatic tissues in the local sites (e.g. Peyer's patches in intestine)
 - **Stimulate antibody production:** The antigens of the normal microbiota stimulate the host immune system to produce antibodies that cross-react with pathogens having related or shared antigens and prevent their entry.
- ❖ **Prevent allergic diseases (Hygiene hypothesis):** It states that a lack of early childhood exposure to symbiotic microorganisms (e.g. gut flora or probiotics), infectious agents and parasites increases susceptibility of the individual to allergic diseases by suppressing the natural development of the immune system
- ❖ **Complement activation:** The endotoxins liberated by the gram-negative population of normal flora may help the defense mechanisms of the body by triggering the alternative complement pathway.

Disturbed Normal Flora Promote Infection

When the composition of normal flora is disturbed, it facilitates pathogenic organisms to enter and cause disease. Several mechanisms by which the normal flora is disturbed are as follows (Fig. 7.1):

- ❖ **Injudicious use of broad spectrum antimicrobial agent:** It may completely suppress the normal flora thus permitting the pathogen (exogenous and endogenous)

to take the upper hand and cause infection. For example, *Clostridioides difficile*, causing pseudomembranous colitis

- ❖ **Host factors** such as immune suppression, reduced peristalsis may promote the pathogen to grow
- ❖ **Physical destruction** of the normal flora by irradiations, chemicals, burns, etc.
- ❖ If the **inoculum size** of the entering pathogen is high then it can dominate over the normal flora
- ❖ **Minor trauma** in mouth (e.g. by dental procedure, chewing or vigorous brushing) can promote passage of small numbers of bacteria (e.g. viridians streptococci) transiently into bloodstream, which can cause bacterial endocarditis.

Harmful Effects

Normal flora may produce the following harmful effects, out of which the first two are significant.

- ❖ **May be agents of disease:** Members of the normal flora may cause various endogenous disease (Table 7.2)
 - When the host immunity is lowered, the transient flora may invade and produce disease, e.g. gram-negative organisms (*E. coli*) colonizing the respiratory tract can cause pneumonia
 - If they enter a wrong site or tissue (e.g. blood, sterile body cavities)—then even the resident flora can produce disease. For example, *E. coli* which is a resident flora of intestine may cause urinary tract infection if enters into urinary tract.
- ❖ **Transfer to susceptible hosts:** Some human pathogens that are members of the normal flora for one host can produce disease if transferred to another host. For example, the pathogens that colonize the upper respiratory tract (such as meningococcus, pneumococcus, etc.) can produce disease in susceptible hosts

Diseases produced by normal flora	Anatomical site from which the flora is transferred
Urogenital infections including UTI	Intestinal flora such as <i>Escherichia coli</i> , <i>Klebsiella</i> , <i>Proteus</i>
Endocarditis	Oral flora (Viridans streptococci)
Dental caries and periodontal disease	Oral flora (<i>Streptococcus mutans</i>)
Peritonitis, abdominal infection	Intestinal flora
Pneumonia	Transient respiratory flora
Septicemia	From any site

- ❖ **Bacterial synergism:** Bacterial vitamins and growth factors produced by members of the normal flora may promote the growth of the potential pathogens
- ❖ **Contribute to the drug resistance of pathogens:** Some members of normal flora produce enzymes such as beta-lactamases which destroy the beta-lactam antibiotics; thus indirectly contribute to the drug resistance of pathogens that are otherwise susceptible to the drug
- ❖ **Competition for host nutrients:** Bacteria in GIT absorb some of the host's nutrients for their survival.

Probiotics

The term "Probiotics" is defined as the live microorganisms (part of normal flora) which, when administered in adequate amounts, confer a health benefit to the host.

- ❖ They are extremely useful in the conditions where the normal intestinal flora is suppressed
- ❖ Probiotics are commercially available in the form of capsule or sachet, consisting of mixture of some important beneficiary bacteria and yeast of human

intestinal flora such as *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, etc.

Probiotics are found to have beneficiary role in the following conditions/diseases:

- ❖ **To treat various forms of GIT conditions like:**
 - Gastroenteritis due to any cause
 - Antibiotic-associated diarrhea
 - Lactose intolerance
 - Irritable bowel syndrome and colitis
 - Necrotizing enterocolitis
 - *Helicobacter pylori* infection.
- ❖ Reducing serum cholesterol level by breaking down bile in the gut, thus inhibiting its reabsorption
- ❖ Reducing blood pressure (by producing ACE inhibitor-like peptides during fermentation)
- ❖ Immune function restoration and preventing infections
- ❖ Modulate inflammatory and hypersensitivity responses, hence can be given in allergic disorders, eczema and atopic dermatitis
- ❖ Bacterial vaginosis (restoring the acid pH of vagina by lactic acid-producing bacteria).

The live organisms contained in probiotics must remain live to have their action on the large intestine. More so, they have to compete with the existing flora to get themselves established. They can exert their beneficiary effect only after that. Hence, nowadays, instead of probiotics, another related preparation called prebiotics is being increasingly used.

Prebiotics

In contrast to probiotics, prebiotics are the dietary non-digestible fibers which when administered, stimulate the growth and activity of commensal microorganisms and thereby exert beneficiary effect to the host indirectly.

EXPECTED QUESTIONS

I. Write short notes on:

1. Resident flora and transient flora.
2. Beneficial effects of the normal flora.
3. Harmful effects of the normal flora.
4. Probiotics.

II. Multiple Choice Questions (MCQs):

1. **The most common commensal in human intestine is:**
 - a. *Bacteroides fragilis*
 - b. *Escherichia coli*
 - c. *Klebsiella pneumoniae*
 - d. *Lactobacillus*

2. Which of the following is not a component of commercially available probiotic?

- a. *Bifidobacterium*
- b. *Lactobacillus*
- c. *Bacteroides fragilis*
- d. *Saccharomyces*

3. Which of the following is not a commensal in human female genital tract?

- a. *Lactobacillus* species
- b. *Streptococcus agalactiae*
- c. *Neisseria* (non-pathogenic species)
- d. *Trichomonas vaginalis*

Answers

1. a 2. c 3. d

CHAPTER PREVIEW

- Infection and Related-terminologies
- Epidemiological Patterns
- Epidemiological Indicators
- Eradication and Elimination
- Epidemiological Determinants of Disease Causation

The epidemiology branch of infectious disease deals with the distribution and determinants of infection-related health state in specified populations, and its application to the control of the disease.

INFECTION AND RELATED-TERMINOLOGIES

Following the entry of the microorganism into the body, it may lead to either infection or colonization; both the terms need to be distinguished.

- ❖ **Infection:** It is a process in which a pathogenic organism enters, establishes itself, multiplies and invades the normal anatomical barrier of the host resulting in disease. When infection becomes apparent results in clinical manifestation and it is referred to as ‘infectious disease’
- ❖ **Colonization:** Here, the pathogenic organism enters, multiplies but does not invade, and neither causes disease nor elicits a specific immune response
 - Colonizers are different from normal flora. They have pathogenic potential and may invade and cause disease in another host or the same host later
 - However, commensals are limited to a particular anatomical site, e.g. intestine, respiratory and genital tract. When they enter through other routes, they may behave as pathogens. For example, *Escherichia coli* is a normal flora in the intestine, but it causes infection when enters through respiratory or urinary tract.
- ❖ **Infestation:** It refers to inhabiting the surface of the body of the host by certain larger parasites called as **ectoparasites**; without penetrating into the tissues
 - They act as important vectors to transmit the pathogenic microbes—such as louse, fleas, mites and ticks, etc. (Annexure 7)
 - Some of these ectoparasites may penetrate into the host causing disease; examples *Sarcoptes scabiei* causing scabies (Annexure 7).

The infectious diseases can also be classified based on being developed in community or in hospital.

- ❖ **Healthcare-associated infection (HAIs):** Defined as, the new infections acquired in a healthcare facility

(HCF) by a patient after 48 hours of admission, which was neither present nor incubating at the time of admission. As the site of HCF has increasingly shifted from inpatient hospital care based to the ambulatory setting such as outpatient services, the relevance of traditional terminologies such as “hospital-associated or nosocomial” infections has diminished (Chapter 21)

- ❖ **Community-associated infections:** Refers to the infections which developed in the community or within 48 hours of admission to a HCF.

EPIDEMIOLOGICAL PATTERNS

Infectious diseases that are capable of directly transmitting to man from another man, animal or environment (air, water, food, etc.) are called as communicable disease. The spread of communicable diseases in the community may occur in several epidemiological patterns—outbreak, epidemic, pandemic, hyperendemic and sporadic.

- ❖ **Outbreak** is a sudden rise in the number of cases in a limited geographic area. If it’s not quickly controlled, an outbreak can become an epidemic. Examples include:
 - Cholera outbreak in Bengaluru in 2020, affecting 17 people
 - Nipah virus encephalitis outbreak in Kerala in 2018, resulting in 18 cases with 16 deaths.
- ❖ **Epidemic:** If the infection occurs at a much higher rate than usual in a particular geographical area, it is known as epidemic. It usually affects a large number of people within a community, population, or region. If it is not quickly contained, it can result into pandemic. The classical examples include:
 - SARS epidemic in China in 2003
 - Ebola epidemic in Africa in 2014
 - Zika epidemic in Brazil in 2015.
- ❖ **Pandemic:** Infection that spreads rapidly to large areas of the world is known as pandemic. Examples include:
 - COVID-19 pandemic in 2020 affecting >200 countries
 - Influenza pandemics: Several flu pandemic occurred so far including H1N1 pandemic in 2009

- Cholera: Seven pandemics of cholera have occurred so far in the past
- Plague: Three pandemics occurred so fast in the past. The second pandemic (black death) occurred in 14th century, killed over 50 million of mankind
- HIV pandemic: Occurs throughout the world since its discovery, claimed 32 million deaths so far.
- ❖ **Endemic:** When a disease occurs at a persistent, usually low level in a certain geographical area, it is called as endemic. India is endemic for several diseases such as typhoid fever, cholera, filariasis, malaria, etc.
- ❖ **Hyperendemic:** When a disease occurs at persistent high level in a geographical area, it is known as **hyperendemic**. Examples include:
 - India is hyperendemic for hepatitis A and E
 - India was hyperendemic for polio until 1990. With constant immunization effort, India was declared polio-free since 2014.
- ❖ **Sporadic:** Infections occur at irregular intervals or only in a few places; scattered or isolated. Example, several sporadic cases of cholera occur in India every year.

The same disease may occur in several epidemiological patterns over time. For example, H1N1 when introduced first time in the World in 2009 has resulted in a pandemic, affecting several countries including India. Since then India became endemic to H1N1. It occurs as sporadically throughout the year with peak (resulting in several outbreaks) during the winter season.

EPIDEMIOLOGICAL INDICATORS

Morbidity Indicators

- ❖ **Incidence rate:** It is defined as number of new cases occurring in a defined population during a specified period of time (usually taken as one year)

$$\frac{\text{Number of new cases in a year}}{\text{Population at-risk during the same period}} \times 1000$$

- ❖ **Prevalence rate:** It refers to all current cases (old and new) existing at a given point of time. It is more useful for diseases that have a longer course such as tuberculosis, HIV, hepatitis B, etc.

$$\frac{\text{Number of all current cases (old + new) existing at a given point of time}}{\text{Estimated population at the same point of time}} \times 100$$

For example, prevalence of global HIV at the end of 2018 is 0.8% (per 100 population).

Mortality Indicators

- ❖ **Mortality rate:** The total number of deaths due to a disease in a given time (usually a year) as compared to mid-year population

$$\frac{\text{Number of deaths due to TB in a year}}{\text{Mid-year population}} \times 1000$$

- ❖ **Case fatality ratio:** It is the number of deaths due to a disease compared to total number of cases occurred during the same time-frame.

$$\frac{\text{Number of deaths due to COVID-19 in 2020}}{\text{Total number of COVID-19 cases in 2020}} \times 100$$

Indicators During an Epidemic or Outbreak

The following indicators are used to measure the burden and the transmission potential of a disease during an epidemic or outbreak.

- ❖ **Overall attack rate:** It is the incidence rate (expressed in percentage) during an epidemic

$$\frac{\text{Number of H1N1 new cases during winter season in 2020}}{\text{Population at-risk during the same period}} \times 100$$

- ❖ **Secondary attack rate:** It is the number of exposed persons developing into disease within the range of incubation period following exposure to a primary case.

$$\frac{\text{Number of cases among contacts of primary cases}}{\text{Total number of contacts}} \times 100$$

Example: Consider an outbreak of shigellosis in which 18 persons in 18 different households developed the disease. If the population of the community was 1,000, then the overall attack rate was $18/1,000 \times 100 = 1.8\%$. If the 18 households comprised of 86 persons, then the total susceptible contact will be $86 - 18 = 68$. One incubation period later, if 17 persons in the same households of “primary” cases developed shigellosis, then the secondary attack rate = secondary cases/no. of susceptible contacts $\times 100 = 17/68 \times 100 = 25.0\%$

- ❖ **Basic reproduction number (R_0):** It is the average number of secondary cases produced by a primary case in a given population

- For example, if there are five measles cases, each infected 12, 10, 14, 13 and 11 number of new cases; then the R_0 is the total no. of secondary cases/total no. of primary cases, i.e. $60/5 = 12$
- If R_0 for COVID-19 in a population is 2.3, then we would expect each new case of COVID-19 to produce 2.3 new secondary cases.

ERADICATION AND ELIMINATION

Eradication, elimination and control of an infectious disease are related terminologies with distinct differences.

Eradication

It refers to the complete and permanent worldwide reduction to ‘zero new cases’ of the disease through deliberate efforts. If a disease has been eradicated, no further control measures are required.

- ❖ Smallpox was the only disease to be eradicated from the whole world (in 1980)

- ❖ Polio is on the verge of eradication. Most countries including India have already declared polio-free except Pakistan, Nigeria and Afghanistan
- ❖ Guinea worm disease also has been eradicated from most of the world including India, except few countries from sub-Saharan Africa.

Elimination

It refers to the 'reduction to zero' (or a very low defined target rate) of new cases in a defined geographical area. Elimination requires continued measures to prevent re-establishment of disease transmission. The diseases which attained elimination in India include neonatal tetanus and leprosy.

Control

It refers to the reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts. However, continued intervention measures are still required to maintain the reduction, e.g. diarrhoeal diseases.

EPIDEMIOLOGICAL DETERMINANTS OF DISEASE CAUSATION

The **Epidemiological Triad** is one of the traditional, but simplest model for depicting causation of infectious disease. The triad consists of an external **agent**, a susceptible **host**, and an **environment** that brings the host and agent together.

AGENT FACTORS

It refers to the infectious microorganism such as a virus, bacterium, parasite, or fungus that is responsible for the causation of the disease.

Table 8.1: Epidemiological determinants of disease causation.

Agent
<ul style="list-style-type: none"> • Organism's pathogenicity • Infective dose • Source and reservoir: Human or animal • Mode of transmission: Contact, inhalation, ingestion, vector-borne, vertical transmission • Infectivity or communicability • Secondary attack rate and R_0 factor
Host
<ul style="list-style-type: none"> • Age, gender, and race • Underlying disease, pregnancy, etc. • Underlying immune status and nutritional status • Occupational status • Personal practices: Hygiene and sexual practices • Genetic make-up
Environment
<ul style="list-style-type: none"> • Seasonality • Resistance to disinfectants • Effective antimicrobial agents • Soil, moisture, oxygen, rainfall

- ❖ Generally, the agent must be present for disease to occur; however, presence of that agent alone is not always sufficient to cause the disease.
- ❖ A variety of agent-related factors influence whether the exposure to an organism will result into disease (Table 8.1).

Organism's Pathogenicity

Pathogenicity refers to the ability of the organism to cause disease. Not all, but only the pathogenic organisms are capable of producing the disease. This is because they express various virulence factors that allow the organism to become established in a host and maintain the disease state.

Infective Dose

Organism must be present in sufficient quantity for the causation of the disease. Infective dose is the minimum inoculum size that is capable of initiating an infection.

- ❖ **Low infective dose:** e.g. *Shigella*, *Cryptosporidium parvum*, *E. coli* O157: H7, *Giardia* and *Campylobacter jejuni*. They require small inoculum to initiate infection
- ❖ **Large infective dose:** In contrast, organisms with high infective dose can initiate the infection only when the inoculum size exceeds a particular critical size. Examples, include *Salmonella* and *Vibrio cholerae*.

Source and Reservoir

The starting point for the occurrence of an infectious disease is known as a source or/and reservoir of infection. They are not always synonymous.

- ❖ **Source:** It refers to a person, animal, or object from which the microorganism is transmitted to the host
- ❖ **Reservoir:** It is the natural habitat in which the organism lives, multiplies. It may be a person, animal, arthropod, plant, soil or substance (or combination of these) on which the organism is dependent for its survival; where it reproduces in such a way that it can be transmitted to susceptible hosts.

The term source and reservoir may be the same for many organisms but are not always synonymous. For example,

- ❖ In tetanus infection, the reservoir and source of the agent (*Clostridium tetani*) are same, i.e. the soil
- ❖ In hookworm infection, the reservoir is man, but the source of infection is the soil contaminated with the larva of hookworm
- ❖ In typhoid fever, the reservoir may be a case or carrier, but the source of infection is usually contaminated food and water.

Thus, the term 'source' refers to the immediate source of infection and may or may not be a part of reservoir.

The reservoir (and/or source) may be of three types.

Human Reservoir

By far the most important reservoir and/or source of infection for humans is man himself. Man is often described

as his own enemy because most of the infectious diseases are contracted from human sources. The diseases that can be spread from one person to another are called **communicable diseases**. Human sources may be either cases or carriers.

- ❖ **Cases or patients:** They are the persons in a given population identified as having a particular disease
- ❖ **Carrier:** It refers to the persons/animals who harbor the infectious agent in the absence of any clinical symptoms and shed the organism from the body via contact, air or secretions
 - It results due to inadequate treatment or immune response, which leads to incomplete elimination of the organism from the body
 - Though, carriers are less infectious than cases, but are more dangerous as they often go undetected and continue to transmit the infection for a long period.

Types of Carriers

Carriers can be of various types:

- ❑ **Incubatory carriers** are those who shed the organism during the incubation period of the disease. This usually occurs in the last few days of incubation period, e.g. measles, mumps, polio, diphtheria, pertussis, hepatitis B, influenza, etc.
- ❑ **Healthy carriers** refer to the subclinical cases who develop into carriers without suffering from overt disease, e.g. polio, cholera, salmonellosis, diphtheria, meningococcal meningitis, etc.
- ❑ **Convalescent carrier** is the one who has recovered from the disease and continues to harbor and shed the pathogen from his body.

Depending on the duration of carriage:

- ❑ **Temporary carriers:** They shed the organisms for less than six months. Incubatory, healthy and convalescent carriers are actually the types of temporary carriers
- ❑ **Chronic carriers:** They shed the organisms for indefinite period, e.g. in hepatitis B, typhoid fever, malaria, gonorrhoea, etc.

Depending on the source:

- ❑ Contact carrier is a person who acquires the pathogen from a patient
- ❑ Paradoxical carrier refers to a carrier who acquires the pathogen from another carrier.

Animal Reservoir

The source of infection may sometime be animals and birds. The disease and the infections which are transmitted to man from vertebrates are called zoonoses. Common examples include:

- ❖ **From animals:** Rabies (from dog), leptospirosis (from rodents), influenza (from pigs), etc.
- ❖ **Birds** may be source of infection for various diseases like influenza, *Chlamydothyla psittaci* infection (psittacosis), histoplasmosis, etc.
- ❖ **Amplifying host:** It refers to the vertebrate reservoir in which the organism multiplies exponentially, e.g. pigs in Japanese B encephalitis.

Non-living Things as Reservoir

Soil and inanimate matter can also act as reservoir/source of infection, for example, soil may harbor the agents of tetanus, anthrax and some intestinal helminths such as *Trichuris*, hookworm and *Ascaris*.

Mode of Transmission

Microorganisms may be transmitted from the reservoir or source to a susceptible host in different ways.

Contact

This is the most common mode of transmission. Infection may be transmitted by direct or indirect contact.

- ❖ **Direct contact** is via skin and mucosa of an infected person, e.g. through an unclean hand, kissing, or sexual contact. Organisms transmitted by direct contact include agents of common cold, skin and eye infections and agents of sexually transmitted infections (STIs), such as HIV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Treponema pallidum*, etc.
- ❖ **Indirect contact** is through the agency of fomites, which are inanimate objects, such as clothing, toys, etc. These may be contaminated by a pathogen and act as a vehicle for its transmission, e.g. face towels shared by various persons may lead to spread of trachoma.

Inhalation

Inhalational route is the second most common mode of transmission. Transmission through respiratory route occurs either through droplets or aerosols.

Droplet Transmission

Respiratory droplets are large-particles (>5 µm in size) generated during coughing, sneezing or talking.

- ❖ Transmission via large droplets requires close contact (<3 feet), as the droplets do not remain suspended in the air and generally travel only for short distances
- ❖ Along with droplet mode, there is also possibility of contact transmission as droplets may fall on surfaces and fomites present within 1 meter. People can subsequently acquire infection when they touch the infected surfaces or fomites and then touch their nose, eyes or mouth
- ❖ Agents transmitted through droplets include:
 - Bacterial agents/diseases: Diphtheria, *H. influenzae*, meningococcus, pertussis, pneumonic plague, *Mycoplasma pneumonia*, streptococcal pharyngitis
 - Viral agents/diseases: COVID-19, influenza, viral hemorrhagic fever (e.g. Ebola), mumps, parvovirus B19, rhinovirus, rubella, adenovirus.

Aerosol Transmission

Aerosols are small-particles (<5 µm) generated by an infectious person during coughing, sneezing, talking or while performing certain aerosol generating procedures

(e.g. intubation). These smaller droplets remain suspended in air for long period of time and may disperse to a distant place along the air current.

Infectious agents that are transmitted through aerosols include:

- ❖ *Mycobacterium tuberculosis*
- ❖ Measles virus
- ❖ Varicella (chickenpox and zoster).
- ❖ Smallpox (variola) virus.

Ingestion

Infectious agents can be transmitted by ingestion mode, either through contaminated water or food. Food-borne infections occur mostly through carriers engaged in handling or preparation of food and contaminating the foodstuffs. The water supply may get contaminated with the feces of the patients or carriers. Examples include:

- ❖ Intestinal infections like cholera, dysentery, diarrheagenic *E. coli* and intestinal parasitic infections and viral agents of gastroenteritis, such as rotavirus
- ❖ Extraintestinal infections: In this type of infections, pathogens are transmitted by enteric route but produce disease manifestations elsewhere—*Salmonella* Typhi (typhoid fever), hepatitis A and E viruses, poliovirus, *Echinococcus granulosus* (causing hydatid disease) and *Taenia solium* (causing cysticercosis).

Inoculation

Pathogens, in some instances, may be inoculated directly into the skin or tissues of the host:

- ❖ **Animal bite**—for example, rabies virus is inoculated directly by the bite of a rabid animal
- ❖ **Inoculated directly into tissue**—Spores of *Clostridium tetani* present in the soil, get deposited directly into the host tissues following severe wounds leading to tetanus.

Transmission of Blood-borne Infections

Blood-borne infections, such as hepatitis B, hepatitis C and HIV may be transmitted by:

- ❖ Needle prick and other sharp injuries
- ❖ Blood transfusion
- ❖ Intravenous drug abuse (contaminated needles).

Vector Borne

Arthropod vectors, such as mosquitoes, flies, fleas, ticks, mite and lice are the vectors that transmit many diseases (Annexure 7). Vectors may be of two types:

- ❖ **Mechanical vectors:** These carry the microorganisms (do not multiply) and transmit them to the eatables
- ❖ **Biological vectors:** The pathogen multiplies in the body of the vector, often undergo part of a development cycle in it, such vectors are named as biological vectors (e.g. female *Anopheles* mosquito in malaria; *Culex* mosquito in filariasis).

Extrinsic incubation period: After the entry of pathogen into the vector, the time required for the vector to become infective is called extrinsic incubation period.

Vertical Transmission

It refers to the transmission of infection from mother to the fetus. It may be categorized into:

- ❖ **Transplacental transmission:** Infection transmitted via the placental barrier can lead to abortion, miscarriage or stillbirth. If babies are born, they suffer from congenital malformations; such infections are known as teratogenic infections. The pathogens causing congenital infections are abbreviated as '**TORCH**':
 - *Toxoplasma gondii*
 - Others (*Treponema pallidum*, varicella-zoster virus, parvovirus, zika virus)
 - Rubella virus
 - Cytomegalovirus
 - Herpes simplex virus.
- ❖ **Transmission via the birth canal** without causing congenital malformation in the baby, Examples include: Group B *Streptococcus*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, *Listeria* and viruses (e.g. Hepatitis B, C and HIV).

Infectivity or Communicability

It refers to ability of an infectious agent to transmit from one person to another. **Period of communicability** or period of infectivity is the time during which an infectious agent may be transferred directly or indirectly from an infected person to another person.

- ❖ Chickenpox: From -2 to +5 days of onset of rash (until all lesions are crusted)
- ❖ Mumps: From -2 to +5 days of onset of parotitis
- ❖ Measles: From -4 to +4 days of onset of rash
- ❖ Rubella : From -1 to +1 week of onset of rash
- ❖ Influenza: From -1 to +5-7 days of onset of symptoms
- ❖ COVID-19: From -2 to +10 days of onset of symptoms.

Secondary Attack Rate and R_0 Factor

- ❖ Agents with higher secondary attack rate can rapidly infect their contacts (e.g. measles, mumps, rubella, diphtheria, pertussis, etc.)
- ❖ Similarly, agents with higher basic reproduction number (R_0) can transmit faster and more efficiently, e.g. influenza and COVID-19.

HOST FACTORS

Host refers to the human who can get the disease. A variety of factors intrinsic to the host, sometimes called risk factors, can influence an individual's exposure, susceptibility, or response to a causative agent.

- ❖ **Age:** Most viral infections are common at extremes of age, i.e. childhood and old age. However, sexually transmitted viral infections are common among young adults

- ❖ **Gender:** Most infections are either equally distributed or common in males. Sex differences in infectious diseases in humans may be due to social, behavioural and sexual practices and may also be due to genetic factors
 - Males have a greater exposure risk to infections transmitted in work environments than females
 - Women are at greater risk of acquiring HIV and gonorrhoea from sexual intercourse with an infected partner, as compared to men.
- ❖ **Pregnancy:** Certain diseases are common in pregnancy such as transplacental infections (e.g. CMV, rubella) or infection through birth canal such as *S. agalactiae* infection
- ❖ **Host immune status:** Low immunity predisposes to many infections, such as CMV. On the other hand, certain infections require an adequate host immune response (e.g. dengue hemorrhagic fever)
- ❖ **Prior immunity:** Prior immunity to the agent due to vaccination or past infection can protect the individual from further infection. Some viral infections such as smallpox, chickenpox, measles, mumps, and rubella provide lifelong immunity
- ❖ **Nutritional status:** Malnutrition lowers the host immunity and thus predisposes to many viral infections, e.g. measles. However, in certain viral infections such as dengue, malnutrition has a paradoxical effect
- ❖ **Underlying comorbid disease:** People with diabetes, immunodeficiency disorders or receiving steroid therapy are more prone to acquire various infections
- ❖ **Occupational status:** Sometimes, infectious diseases are more common in certain occupations; example, zoonotic diseases such as anthrax are common among butchers, abattoirs and farmers
- ❖ **Racial susceptibility:** Sometimes, innate immunity is confined to a particular race; may be absent in other communities. For example, Negroes of America are more susceptible to tuberculosis than the Whites
- ❖ **Sexual practices:** People with multiple sex partners, men who have sex with men are more prone to develop various sexually-transmitted infections such as HIV
- ❖ **Hygiene:** Poor hygiene, poor sanitation, over-crowding, etc. predispose for several diseases such as acute diarrheal illness and typhoid fever
- ❖ **Genetic makeup:** Certain individuals are more prone to develop some microbial infections. This depends on the genetic makeup of the individual.

ENVIRONMENTAL FACTORS

Environmental factors play an important role in disease causation.

- ❖ **Seasonality:** Many diseases are common in winter such as influenza and meningococcal meningitis; whereas vector-borne diseases such as malaria, dengue are more common in rainy season which parallels with mosquito breeding. Leptospirosis is more common in rainy season
- ❖ **Disinfectants:** The organisms which are more resistant to the action of disinfectant can survive in the environment for longer. This is particularly important in hospital environment where the multidrug resistant organisms such as *Pseudomonas*, *Acinetobacter* and *Klebsiella*, etc. are widely prevalent
- ❖ **Soil:** Damp, sandy or friable soil with vegetation is suitable for certain soil-transmitted helminths such as hookworm, *Ascaris* and *Trichuris* than clay soil
- ❖ **Moisture:** Moisture is necessary for the survival of most microbes as dryness is rapidly fatal
- ❖ **Presence of vectors:** Vector-borne diseases have a strict geographical preponderance, depending upon the presence of vector (Annexure 7)
 - Malaria (*Anopheles*) is more common in Africa and India than the rest of the world
 - Arboviruses are geographically restricted; for example, dengue (*Aedes*) and Japanese encephalitis (*Culex*) are common in India.

EXPECTED QUESTIONS

I. Write short notes on:

1. Epidemiological patterns.
2. Epidemiological indicators.
3. Types of carriers.
4. Droplet versus aerosol transmission.

II. Multiple Choice Questions (MCQs):

1. **If the infection occurs at a much higher rate than usual in a particular geographical area, it is known as:**
 - a. Epidemic
 - b. Pandemic
 - c. Hyperendemic
 - d. Sporadic
2. **All current cases (old and new) existing at a given point of time is referred to as:**

- a. Incidence rate
- b. Prevalence rate
- c. Case fatality ratio
- d. Overall attack rate

3. Organisms with low infective dose include all except:

- a. *Shigella*
- b. *Cryptosporidium parvum*
- c. *Giardia*
- d. *Vibrio cholerae*

4. Aerosol transmission occurs in all except:

- a. *Mycobacterium tuberculosis*
- b. Pneumococcus
- c. Measles virus
- d. Varicella (chickenpox and zoster)

Answers

1. a 2. b 3. d 4. b

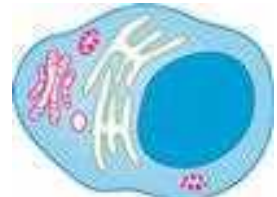
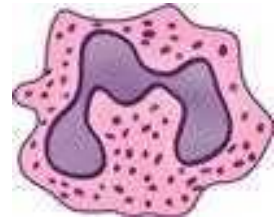
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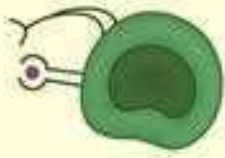
SECTION

2

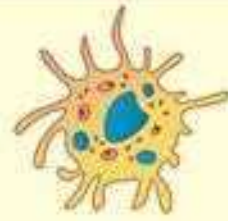
SECTION OUTLINE

9. Immunity (Innate and Acquired)
10. Antigen
11. Antibody
12. Antigen–antibody Reaction
13. Complement
14. Components of Immune System: Organs, Cells and Products
15. Immune Responses: Cell-mediated and Antibody-mediated
16. Hypersensitivity
17. Autoimmunity
18. Immunodeficiency Disorders
19. Transplant and Cancer Immunology
20. Immunoprophylaxis

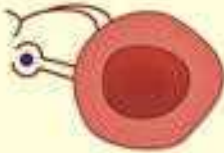




CD₄ T_H cell



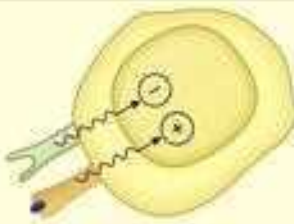
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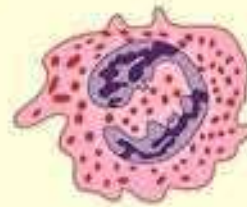
CD₈ T_C cell



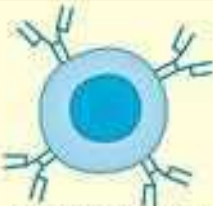
Follicular dendritic cell



NK cell



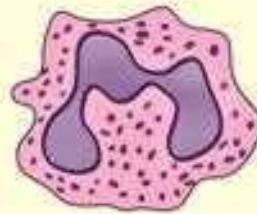
Eosinophil



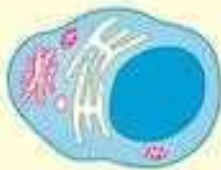
Memory B cell



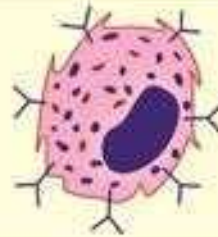
B cell



Neutrophil



Plasma cell



Mast cell



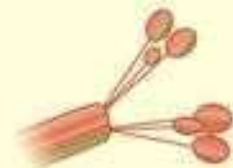
Macrophage



Target cell



Antigen-presenting cell



Complement

Immunity (Innate and Acquired)

CHAPTER PREVIEW

- Innate Immunity
- Acquired or Adaptive Immunity
 - Active Immunity
 - Passive Immunity
- Bridges Between Innate and Acquired Immunity
- Other Types of Immunity
 - Local (or Mucosal) Immunity
- Herd Immunity
- Adoptive Immunity

The term “immunity” (Latin word “*immunitas*,” meaning freedom from disease) is defined as the resistance offered by the host against microorganism(s) or any foreign substance(s). Immunity can be broadly classified into two types:

1. Innate immunity—present right from birth
2. Acquired/adaptive immunity—acquired during the course of the life.

INNATE IMMUNITY

Innate immunity is the inborn resistance against infections that an individual possesses right from the birth, due to his genetic or constitutional makeup.

Innate immunity has certain unique properties by which it can be differentiated from acquired immunity (Table 9.1).

- ❖ **Acts in minutes:** Innate immunity is the **first line of host defense** against infections; occurs immediately after the microbial entry
- ❖ **Prior microbial exposure is not required:** Innate immunity is independent of prior exposure to the microbes; present even before the first entry of the microorganism
- ❖ **Diversity is limited:** Innate immunity is active only against a limited repertoire of antigens; in contrast to acquired immunity which is more varied and involves specialized immune responses
- ❖ **Non-specific:** Cells of innate immunity are non-specific in their action; can be directed against any microbial antigen(s)
- ❖ **No memory:** Innate immunity does not have a memory component. Response to a repeat infection is identical to the primary response.

MECHANISMS OF INNATE IMMUNITY

Receptor Interaction

Following the exposure to microorganisms, several mediators of innate immunity are recruited to the site

of infection (Table 9.1). The first step that takes place is **attachment**, which involves binding of the surface molecules of microorganisms to the receptors on the cells of innate immunity.

Microbial Surface Molecules

They are the repeating patterns of conserved molecules which are common to most microbial surfaces; called **Microbes-associated molecular patterns (MAMPs)**. Examples of MAMPs include peptidoglycan, lipopolysaccharides (LPS), teichoic acid and lipoproteins present on bacterial surface.

Pattern Recognition Receptors (PRRs)

These are the molecules present on the surface of host cells (e.g. phagocytes) that recognize MAMPs. They are generally conserved regions, encoded by germ line genes.

- ❖ **Toll-like receptors (TLRs)** are classical examples of pattern recognition receptors, named after the fruit fly (*Drosophila*); the main receptor for inducing innate immunity
- ❖ Signals generated following binding of TLRs to MAMPs activate transcription factors that stimulate expression of genes encoding cytokines and enzymes, which are involved in several antimicrobial activities of cells of innate immunity.

Components of Innate Immunity

There are several mediators of innate immunity. They exert antimicrobial activities by various mechanisms as described below. Some of these mediators are not purely part of innate immunity; they often act as bridge between innate and acquired immunity (e.g. complements and macrophages).

Anatomical and Physiological Barriers

- ❖ Anatomical barriers such as skin and mucosal surfaces have a spectrum of antimicrobial activities (Table 9.2)

Table 9.1: Differences between innate and acquired immunity.

Innate immunity	Acquired/Adaptive immunity
Resistance to infection that an individual possesses from birth	Resistance to infection that an individual acquires during his lifetime
Immune response occurs in minutes	Immune response occurs in days
Prior exposure to the antigen is not required	Develops following the antigenic exposure
Diversity is limited, acts through a restricted set of reactions	More varied and specialized responses
Immunological memory responses are absent	Immunological memory responses are present
Microbial antigen: Innate immunity develops against antigens that are shared by many microbes (called microbes-associated molecular patterns)	Microbial antigen: Acquired immunity develops against antigens that are specific for each microbes
Host cell receptors of innate immunity (called pattern recognition receptors) are non-specific, e.g. Toll-like receptor	Host cell receptors are specific, e.g. T cell receptors and B cell immunoglobulin receptors
Components of innate immunity <ul style="list-style-type: none"> Anatomical barriers such as skin and mucosa Physiological barriers (e.g. body temperature) Phagocytes (neutrophils, macrophages and monocytes) Natural killer (NK) cells Other classes of lymphocytes: <ul style="list-style-type: none"> γδ T cells, NK-T cells B-1 cells and marginal-zone B cells Mast cells, macrophages and dendritic cells Complement pathways—alternative and mannose binding pathways Fever and inflammatory responses Normal resident flora Cytokines: TNF-α, interleukins (IL-1, IL-6, IL-8, IL-12, IL-16, IL-18), IFN-α, β and TGF-β Acute phase reactant proteins (APRs) 	Components of acquired immunity <ul style="list-style-type: none"> T cell B cell Classical complement pathway Cytokines (IL-2, IL-4, IL-5, IFN-γ)
	Types of acquired immunity <p>It can be classified in two ways:</p> <ul style="list-style-type: none"> Active and passive immunity Artificial and natural immunity

Abbreviations: TNF, tumor necrosis factor; TGF-β, transforming growth factor-β; IFN, interferon; IL, interleukin.

- ❖ Physiological barriers that contribute to the innate immunity are the body temperature, pH and various soluble secretory products of mucosa (Table 9.2).

Phagocytes

Phagocytes such as **neutrophils, macrophages** including **monocytes** are the main components of innate immunity. They are rapidly recruited to the site of infection. Phagocytosis involves three sequential steps—(1) engulfment of microbes and subsequent hosting in phagosome, (2) fusion of lysosome with phagosome to form phagolysosome and (3) microbial killing (described in Chapter 14).

Natural Killer (NK) Cells

They are a class of lymphocytes that kill virus infected cells and tumor cells. NK cell mediated mechanism of killing microbes is described in Chapter 15.

Other Rare Classes of Lymphocytes

T and B lymphocytes are the chief mediators of acquired immunity. However, there are several rare types of lymphocytes that share the features of both acquired and innate immunity (Described in detail in Chapter 14), e.g.

- ❖ **γδ T cells** (also called intraepithelial lymphocytes): They are present in epithelial lining of skin and mucosa

- ❖ **NK-T cells:** They are present in epithelium and lymphoid organs
- ❖ **B-1 cells:** They are found mostly in the peritoneal cavity and mucosal tissues
- ❖ **Marginal-zone B cells:** They are present at the edges of lymphoid follicles of spleen.

Mast Cells

They are present in the epithelial lining of the respiratory and other mucosa.

- ❖ They are activated by microbial products binding to toll-like receptors or by IgE antibody dependent mechanism, following which;
- ❖ They release abundant cytoplasmic granules rich in histamine, prostaglandins and cytokines that initiate inflammation and proteolytic enzymes that results in killing of bacteria.

Dendritic Cells

They respond to microbes by producing numerous cytokines that initiate inflammation. They also serve as vehicle in transporting the antigen(s) from the skin and mucosal sites to lymph nodes where they present the antigen(s) to T cells. Hence, dendritic cells serve as a bridge between innate and acquired immunity.

Table 9.2: Role of barriers in innate immunity.	
Anatomical barrier	Function
Skin barrier	
	<ul style="list-style-type: none"> • Mechanically prevents entry of microbes • Produces sebum containing antimicrobial peptides and fatty acids • Killing of microbes by intraepithelial lymphocytes
Mucosal barrier	
Mucous membrane	Prevents entry of microbes mechanically and by producing mucus which entraps microbes
Cilia	Cilia present in the lower respiratory tract propel the microbes outside
Normal flora	Intestinal and respiratory mucosa are lined by normal flora
Physiological barrier	
Temperature	Normal body temperature inhibits the growth of some microbes
Low pH	Gastric acidity inhibits most of the microbes
Secretory products of mucosa	
Saliva	Enzymes in saliva damage the cell wall and cell membrane of bacteria
Tears	Contains lysozyme that destroys the peptidoglycan layer in bacterial cell wall
Gastric juice	HCl kills microbes by its low pH
Trypsin	Hydrolyses bacterial protein
Bile salts	Interfere with bacterial cell membrane
Fatty acids	Denature the bacterial proteins
Spermine	Present in semen, inhibits growth of gram-positive bacteria
Lactoferrin	Binds to iron, thus interferes with acquisition of iron by bacteria

Complement Pathways

Alternative and mannose binding pathways are the chief mediators of innate immunity.

- ❖ Alternative complement pathway is activated in response to bacterial endotoxin whereas the mannose binding pathway is stimulated by mannose carbohydrate residues on bacterial surface.
- ❖ Following activation, the complements mediate various biological functions such as (refer Chapter 13):
 - Lysis of the target microbes (by forming pores on the microbial surfaces)
 - Stimulate inflammation (by secreting inflammatory mediators)
 - Stimulate acquired immunity: Complements are another bridge between innate and acquired immunity.

Inflammatory Response

Inflammation is defined as the biological response of vascular tissues to harmful stimuli, such as microorganisms

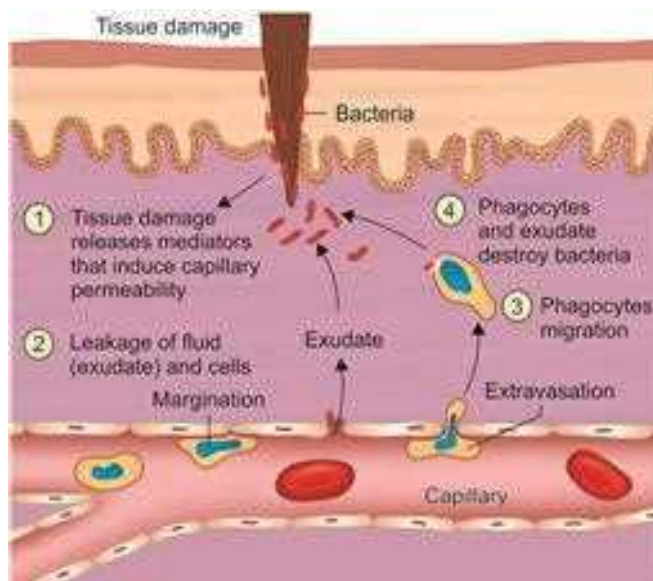


Fig. 9.1: Major events in an inflammatory response.

or other foreign substances. The major events that take place during an inflammatory response following a microbial entry are as follows (Fig. 9.1):

- ❖ **Vasodilation** due to release of vasoactive substances from the damaged tissues
- ❖ **Leakage** of plasma proteins through blood vessels
- ❖ **Recruitment** of phagocytes (e.g. neutrophils) to the site of inflammation—phagocytes undergo the following steps—(1) margination (adherence to the endothelium), (2) rolling on endothelium, (3) extravasation (moves out of the blood vessels), (4) chemotactic migration to the inflammation site
- ❖ **Engulfment** of microbes and dead material by the phagocytes
- ❖ **Destruction** of the microbes.

Inflammation is not always protective in nature, sometime, it may produce injurious consequences to host tissues in the form of **hypersensitivity reactions**.

Normal Resident Flora

Normal resident flora lining intestinal, respiratory and genital tract exert several antimicrobial activities. (described in Chapter 7)

- ❖ They compete with the pathogens for nutrition
- ❖ They produce antibacterial substances.

Cytokines

In response to the microbial antigens, dendritic cells, macrophages, and other cells secrete several cytokines that mediate many of the cellular reactions of innate immunity such as:

- ❖ Tumor necrosis factor- α (TNF- α)
- ❖ Interleukin-1 (IL-1), IL-6, IL-8, IL-12 and IL-16
- ❖ Interferons (IFN- α , β) and
- ❖ Transforming growth factor (TGF- β).

Acute Phase Reactant Proteins (APRs)

They are the proteins synthesized by liver at steady concentration, but their synthesis either increases or decreases exponentially during acute inflammatory conditions. Though liver is the primary site, APRs can also be synthesized by various other cells such as endothelial cells, fibroblasts, monocytes and adipocytes.

- ❖ **Positive APRs:** They are the proteins whose levels increase during acute inflammation. Examples include:
 - Serum amyloid A
 - C-Reactive protein
 - Complement proteins—complement factors (C1–C9), factor B, D, and properdin
 - Coagulation protein, e.g. fibrinogen, von Willebrand factor
 - Proteinase inhibitors, e.g. α -1 antitrypsin
 - α 1 acid glycoprotein
 - Mannose binding protein
 - Haptoglobin
 - Metal binding proteins, e.g. ceruloplasmin.
- ❖ **Negative APRs:** They are the proteins whose levels are decreased during acute inflammation; thus creating a negative feedback that stimulates the liver to produce positive APRs. Examples of negative APRs include albumin, transferrin and antithrombin
- ❖ **Role of APRs:** They have a wide range of activities that contribute to the host defense
 - APRs have various antimicrobial and anti-inflammatory activities (e.g. complement factors)
 - Metal binding proteins can chelate various metals such as iron, copper, etc. making them unavailable for the bacteria.

C-Reactive Protein (CRP)

C-reactive protein is an example of APR that rise in acute inflammatory conditions including bacterial infections. It belongs to beta globulin family.

- ❑ CRP is so named because it precipitates with C- carbohydrate (polysaccharide) antigen of pneumococcus. However, it is not an antibody against the C-carbohydrate antigen of pneumococcus; it is non-specific, can be raised in any inflammatory conditions
- ❑ It is one of the most common markers of acute inflammation, used in most diagnostic laboratories.
- ❑ Following an injury or inflammation, the CRP level starts increasing by 6 hr, doubles every 8 hr, reaches its peak by 48 hr.

CRP Level

The normal level of CRP is less than 0.2 mg/dL. However, it increases by several folds in acute inflammatory conditions.

- ❑ **Insignificant increase** of CRP (<1 mg/dL): It occurs in conditions such as heavy exercise, common cold, and pregnancy
- ❑ **Moderate increase** (1–10 mg/dL): It occurs in conditions such as bronchitis, cystitis, malignancies, pancreatitis, myocardial infarction

Contd...

- ❑ **Marked increase** of CRP (>10 mg/dL): It occurs in conditions such as acute bacterial infections, major trauma and systemic vasculitis.

CRP Can be Detected by

- ❑ Precipitation method using C-carbohydrate antigen (obsolete, not in use now)
- ❑ Latex (passive) agglutination test using latex particles coated with anti-CRP antibodies
 - It is the most widely used method employed worldwide
 - Detection limit of CRP by latex agglutination test is 0.6 mg/dL.

Highly Sensitive CRP (hs-CRP) Test

Minute quantities of CRP can be detected by various methods (e.g. nephelometry, enzyme immunoassays). This is useful in assessing the risk to cardiovascular diseases.

ACQUIRED OR ADAPTIVE IMMUNITY

Acquired immunity is defined as the resistance against the infecting foreign substance that an individual acquires or adapts during the course of his life.

Acquired immunity has unique properties by which it can be differentiated from innate immunity (see Table 9.1).

- ❖ **Mediators: T cells and B cells** are the chief mediators of acquired immunity. Other mediators include:
 - Classical complement pathway
 - Antigen presenting cells
 - Cytokines (IL-2, IL-4, IL-5).
- ❖ **Response occurs in days:** Acquired immunity involves activation of T and B cells against the microbial antigens; which takes several days to weeks to develop, following the microbial entry
- ❖ **Requires prior microbial exposure:** Acquired immunity develops only after the exposure to the microbes. It is not present prior to the first contact with the microbes
- ❖ **Specific:** Acquired immunity is highly specific; directed against specific antigens that are unique to the microbes
- ❖ **Memory present:** Acquired immunity does have a memory component. A proportion of T and B cells become memory cells following primary contact of the microbe, which play an important role when the microbe is encountered subsequently
- ❖ **Diversity is wide:** Acquired immunity though takes time to develop, is active against a wide range of repertoire of antigens
- ❖ **Host cell receptors** of acquired immunity are specific for a particular microbial antigen
 - Examples include T cell receptors and B cell immunoglobulin receptors
 - They are encoded by genes produced by somatic recombination of gene segments.

Types of acquired immunity: Acquired immunity can be classified in two ways:

1. Active and passive immunity.
2. Artificial and natural immunity.

Contd...

ACTIVE IMMUNITY

Active immunity is the resistance developed by an individual towards an antigenic stimulus.

- ❖ Here, the host's immune system is actively involved in response to the antigenic stimulus; leading to the production of immunologically active T cells, B cells and production of specific antibodies
- ❖ Active immunity may be induced naturally or artificially
 - **Natural active immunity** occurs following exposure to a microbial infection (e.g. measles virus infection)
 - **Artificial active immunity** develops following exposure to an immunogen by vaccination (e.g. measles vaccine). Vaccines are discussed in Chapter 20.
- ❖ As host's immune apparatus is actively involved, active immunity often fails to develop when the host is immunocompromised
- ❖ **Long-lasting:** Active immunity usually lasts for longer periods, but the duration varies depending on the type of pathogen
 - It may last life long, e.g. following certain viral infections such as chickenpox, measles, smallpox, mumps and rubella
 - It may last for short duration, e.g. following influenza virus infection
 - It may last for as long as the microbe is present. Once the disease is cured, the patient becomes susceptible to the microbe again. This is called **premunity** or concomitant immunity. It is seen following some microbial infections like spirochetes and *Plasmodium*
 - Active immunity may not be protective at all, e.g. *Haemophilus ducreyi*, the patient may develop genital lesions following reinfection even while the primary infection is active.

Types of immune response in active immunity vary depending on the microbial exposure that occurs for the first time (called primary immune response) and subsequent time (called secondary immune response).

Primary Immune Response

When the antigenic exposure occurs for the first time, the following events take place:

- ❖ **Latent or lag period:** Active immunity develops only after a latent period following the antigenic exposure, which corresponds to the time required for the host's immune apparatus to become active
- ❖ **Effector cells:** Majority of activated T and B cells against the antigenic stimulus become effector T and B cells
 - Effector T cells—helper T cells and cytotoxic T cells
 - Effector B cells include plasma cells.
- ❖ **Memory cells:** A minor proportion of stimulated T and B cells become memory cells, which are the key cells for secondary immune response
- ❖ **Antibody surge:** Effector B cells produce antibodies (mainly IgM type). Antibodies appear in the serum in slow and sluggish manner; reach peak, maintain the

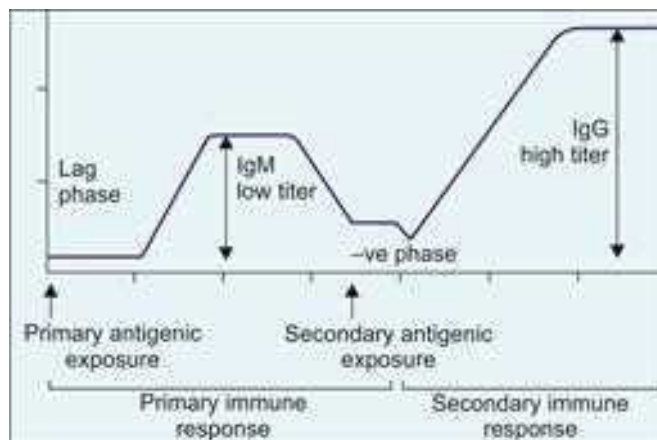


Fig. 9.2: Primary and secondary immune responses.

level for a while and then fall down. Finally, a low titer of baseline antibodies may be maintained in the serum (Fig. 9.2).

Secondary Immune Response

When the same antigenic exposure occurs subsequently, the events which take place are as follows (Fig. 9.2).

- ❖ **Latent period** is either absent or of short duration. This is because memory cells become active soon after the antigenic exposure
- ❖ **Negative phase:** At the onset of secondary immune response, there may be a negative phase during which the antibody level may become lower than it was before the antigenic stimulus. This is because the exposed antigen combines with the pre-existing antibody and thus the antibody level in serum falls down
- ❖ **Antibody surge:** Secondary antibody response is prompt, powerful, long-lasting and mainly of IgG type. Hence, it is said that, the booster doses of vaccines are more effective than the first dose.

The differences between primary and secondary immune response are tabulated in Table 9.3.

PASSIVE IMMUNITY

Passive immunity is defined as the resistance that is transferred passively to a host in a "readymade" form without active participation of the host's immune system.

- ❖ Passive immunity can also be induced naturally or artificially
 - **Natural passive immunity** involves the IgG antibody transfer from mother to fetus across the placenta
 - **Artificial passive immunity** develops following readymade transfer of commercially prepared immunoglobulin (e.g. Rabies immunoglobulin).
- ❖ Passive immunity plays a very important role in:
 - Immunodeficient individuals (as host's immune apparatus is not effective) and;
 - Post-exposure prophylaxis; when an immediate effect is warranted

Table 9.3: Differences between primary and secondary immune response.

Primary immune response	Secondary immune response
Immune response against first antigenic challenge	Immune response against subsequent antigenic challenge
Slow, sluggish (appear late) and short lived	Prompt, powerful and prolonged (long lasting)
Lag period is longer (4–7 days)	Lag period is absent or short (1–3 days)
No negative phase	Negative phase may occur
<ul style="list-style-type: none"> • Antibody produced in low titer and is of IgM type • Antibodies are more specific but less avid 	<ul style="list-style-type: none"> • Antibody produced in high titer and is of IgG type • Antibodies are less specific but more avid
Antibody producing cells—Naive B cells	Antibody producing cells—Memory B cells
Both T dependent and T independent antigens are processed	Only T dependent antigens are processed

- ❖ Passive immunity **develops faster**; there is no lag phase or negative phase
- ❖ There is no **immunological memory** as the memory cells are not involved
- ❖ **Booster doses are not effective:**
 - As memory component is absent, the effect produced following subsequent immunoglobulin administration is same as the effect produced after the primary dose
 - In certain situations, the booster doses of an immunoglobulin may be less effective because of its immunological clearance, which is mediated by the antibodies produced against the first dose of immunoglobulin.

The differences between active and passive immunity are listed in Table 9.4.

BRIDGES BETWEEN INNATE AND ACQUIRED IMMUNITY

The innate and acquired immunity do not work independently; rather they function in a highly interactive and collaborative manner, increasing each other's efficiency and producing a combined response, which is more effective than either branch could produce by itself. Certain immune components play important roles in both types of immunity and are considered as bridges between innate and acquired immunity. Examples include:

- ❖ **Macrophages** and **dendritic cells** belong to innate immune system, but as antigen presenting cells, they present the antigenic peptides to T cells. More so, cytokines secreted from macrophages (interleukin-1) are also involved in T cell activation
- ❖ **ADCC** (antibody dependent cell-mediated cytotoxicity) is a type of cell-mediated immune response (CMI)

Table 9.4: Differences between active and passive immunity.

Active immunity	Passive immunity
Produced actively by host immune system	Immunoglobulins received passively
Induced by: <ul style="list-style-type: none"> • Infection (natural) • Vaccination (artificial) 	Acquired by: <ul style="list-style-type: none"> • Mother to fetus IgG transfer (natural) • Readymade antibody transfer (artificial)
Long lasting	Lasts for short time
Lag period present	No Lag period
Memory present	No memory
Booster doses are useful	Subsequent doses are less effective
Negative phase may occur	No negative phase
Not useful in immunodeficient individuals	Useful in immunodeficient individuals

described in Chapter 15, which involves both innate and adaptive components. Cells of innate immunity such as NK cell, eosinophils and neutrophils destroy the target cells which are coated with specific antibodies

- ❖ **Complements:** Although principally part of innate immunity, the complement pathways can be activated by both innate and antibody-mediated mechanisms. The classical pathway is activated by the target cells coated with specific antibodies. However, activation of alternative and mannose binding pathways do not require help of antibodies (Chapter 13)
- ❖ **Cytokines** secreted from cells of innate immunity can activate cells of adaptive immunity and vice versa. For example, IL-1 secreted from macrophage activates helper T cells and interferon- γ secreted by helper T cell can activate macrophage
- ❖ **Rare classes of lymphocytes** such as $\gamma\delta$ T cells, NK-T cells, B-1 cells and marginal-zone B cells: These cells have many characteristics that place them in the border of innate and acquired immunity
 - They function in the early defense against microbes as part of innate immunity
 - Although their receptors are encoded by somatic recombination of genes (similar to that of classical T and B cells), these receptors have limited diversity
 - They possess a memory phenotype in contrast to the property of innate immunity.

OTHER TYPES OF IMMUNITY

Local (or Mucosal) Immunity

Local or mucosal immunity is the immune response that is active at the mucosal surfaces such as intestinal or respiratory or genitourinary mucosa.

- ❖ It is mediated by a type of IgA antibody called secretory IgA, which prevents the entry of microbes at the local site itself

- ❖ Local immunity can only be induced by natural infection or by live vaccination (but not by killed vaccines)
- ❖ **Example:** Following administration of live oral polio vaccine (OPV) or following infection with poliovirus; secretory IgA antibodies are synthesized and coated on intestinal mucosa which prevent subsequent poliovirus infections. Such immunity does not develop following injectable killed polio vaccine (IPV).
- Occurrence of clinical and subclinical cases in the herd
- On-going immunization program
- Herd structure, i.e. type of population involved
- Type of pathogen—herd immunity may not be strong in a community against all the pathogens.
- ❖ Herd immunity develops following effective vaccination against some diseases like:
 - Diphtheria and pertussis vaccine
 - Measles, mumps and rubella (MMR) vaccine
 - Polio (oral polio vaccine)
 - Smallpox vaccine.

Herd Immunity

Herd immunity is defined as the overall immunity of a community (or herd) towards a pathogen.

- ❖ Herd immunity plays a vital role in preventing epidemic diseases. If the herd immunity is good, that means large population of the community are immune towards a pathogen. Hence, epidemics are less likely to occur and eradication of the disease may be possible
- ❖ Elements that contribute to the development of a strong herd immunity are:

Adoptive Immunity

Adoptive immunity is a special type of cell-mediated immune response (CMI) which develops following injection of immunologically competent T-lymphocytes known as **transfer factor**. It is useful for treatment when the CMI is low, e.g. in lepromatous leprosy.

EXPECTED QUESTIONS

I. Write essay on:

1. Define immunity. Describe in detail about the properties and mediators of innate immunity.

II. Write short notes on:

1. Herd immunity.
2. Differences between innate and acquired immunity.
3. Differences between active and passive immunity.

III. Multiple Choice Questions (MCQs):

1. Which is not a mediator of innate immunity?

- a. T cells
- b. NK cell
- c. B-1 cell
- d. Neutrophil

2. Which of the following about innate immunity is wrong?

- a. Immune response occurs in minutes
- b. Non-specific
- c. First line of defense
- d. Need prior contact with the antigen

3. Which of the following about active immunity is correct?

- a. No lag phase
- b. Booster doses are useful
- c. Useful in immunodeficient people
- d. No memory cells

4. Primary immune response—the correct statement is:

- a. Involves IgG

- b. Memory B cell are the antibody producing cells
- c. No lag period
- d. Slow and sluggish

5. Innate immunity differs from acquired immunity in all, except:

- a. Present from birth
- b. Diversity is limited
- c. Immunological memory absent
- d. Prior antigen exposure is must

6. Toll-like receptor is a component of:

- a. Innate immunity
- b. Acquired immunity
- c. Active immunity
- d. Passive immunity

7. Which of the following cytokine is a component of innate immunity?

- a. IL-1
- b. IL-2
- c. IL-4
- d. IL-5

8. Which among the following is a component of innate immunity?

- a. T cells
- b. B cells
- c. Mast cells
- d. IFN- γ

9. Acute phase reactant proteins are all, except:

- a. Serum amyloid A
- b. C-reactive protein
- c. Fibrinogen
- d. Coagulase

10. Negative acute phase reactant proteins are all, except:

- a. Albumin
- b. α -1 acid glycoprotein
- c. Transferrin
- d. Antithrombin

Answers

1. a 2. d 3. b 4. d 5. d 6. a 7. a 8. c 9. d 10. b

CHAPTER PREVIEW

- Antigen and Hapten
- Antigen-Host Relationship
- Factors Influencing Immunogenicity
- Biological Classes of Antigens

ANTIGEN

Antigen is defined as any substance that satisfies two distinct immunologic properties—immunogenicity and antigenicity.

1. **Immunogenicity:** It is the ability of an antigen to induce immune response in the body (both humoral and/or cell mediated)
 - B cells + antigen → effector B cells (plasma cell) + memory B cells
 - T cells + antigen → effector T cells (helper T cell or cytotoxic T cell) + memory T cells.
2. **Antigenicity (immunological reactivity):** It is the ability of an antigen to combine specifically with the final products of the above two responses (i.e. antibodies and/or T cell-surface receptors).

The substance that satisfies the first property, i.e. immunogenicity (inducing specific immune response) is more appropriately called “**immunogen**” rather than using the word “antigen”.

All molecules having immunogenicity property, also show antigenicity, but the reverse is not true (e.g. haptens—which are antigenic, but not immunogenic).

Epitope

Epitope or antigenic determinant is the smallest unit of antigenicity.

- ❖ It is defined as a small area present on the antigen comprising of few (four to five) amino acids or monosaccharide residues, that is capable of sensitizing T and B cells and reacting with specific site of T cell receptor or an antibody
- ❖ The specific site of an antibody that reacts with the corresponding epitope of an antigen is called **paratope**.

Epitopes may be grouped into two types:

1. **Sequential or linear epitope:** It presents as a single linear sequence of few amino acid residues
2. **Conformational or non-sequential epitopes** are found on the flexible region of complex antigens having tertiary structures. They are formed by bringing together the

surface residues from different sites of the peptide chain during its folding into tertiary structure.

In general, T cells recognize sequential epitopes, while B cells bind to the conformational epitopes.

HAPTEN

Haptens are low molecular weight molecules that **lack immunogenicity** (cannot induce immune response) but **retain antigenicity or immunological reactivity** (i.e. can bind to their specific antibody or T cell receptor). Haptens can become immunogenic when combined with a larger protein molecule called **carrier**.

Haptens may be classified as complex or simple:

- ❖ **Complex haptens** contain two or more epitopes; they can react with specific antibodies and the hapten-antibody complex can be visualized by various methods such as precipitation reaction
- ❖ **Simple haptens** usually contain only one epitope (univalent). Such haptens can bind to the antibodies but the hapten antibody complex cannot be visualized, as it is believed that precipitation reaction to occur, it requires the antigen to have at least two or more epitopes.

ANTIGEN-HOST RELATIONSHIP

Based on the antigen-host relationship, antigens can be grouped into two groups as follows:

1. **Self or autoantigens:** They belong to the host itself; hence they are not immunogenic. Host’s immune system does not react to its own antigen, which is due to exhibiting a mechanism called **immunological tolerance** (Chapter 17). However, sometimes, the self-antigens are biologically altered (e.g. as in cancer cells) and can become immunogenic
2. **Non-self or foreign antigens:** They are immunogenic and are of three types based on their phylogenetic distance to the host
 - **Alloantigens** are species specific. Tissues of all individuals in a species contain species-specific antigens

- **Isoantigens** are type of antigens which are present only in subsets of a species, e.g. blood group antigens and histocompatibility antigens. The histocompatibility antigens are highly specific as they are unique to every individual of a species
- **Heteroantigens:** Antigens belonging to two different species are called heteroantigens, e.g. antigens of plant or animal or microorganisms, etc. A **heterophile antigen** is a type of heteroantigen that exists in unrelated species (explained below).

Heterophile Antigens

Heterophile antigens are a type of heteroantigens that are present in two different species; but they share epitopes with each other. Antibody produced against antigen of one species can react with the other and vice versa.

Diagnostic Application

Heterophile antigens can be used in various serological tests. Antibody against one antigen can be detected in patient's serum by employing a different antigen which is heterophile (cross reactive) to the first antigen. For example:

- **Weil-Felix reaction** is done for typhus fever. Antibodies against rickettsial antigens are detected by using cross reacting *Proteus* antigens
- **Paul-Bunnell test** is done for infectious mononucleosis (caused by Epstein-Barr virus). Here, sheep red blood cell (RBC) antigens are used to detect cross-reacting antibodies in patient's sera
- **Cold agglutination test** and *Streptococcus* MG test are done for primary atypical pneumonia. Here, antibodies against *Mycoplasma pneumoniae* are detected by using human O blood group RBCs and *Streptococcus* MG antigens respectively.

FACTORS INFLUENCING IMMUNOGENICITY

There are various factors that influence immunogenicity of an antigen.

- ❖ **Size of the antigen:** Larger is the size (>10,000 Dalton molecular weight, e.g. hemoglobin); more potent is the molecule as an immunogen
- ❖ **Chemical nature of the antigen:** Proteins are stronger immunogens than carbohydrates followed by lipids and nucleic acids
- ❖ **Susceptibility of antigen to tissue enzymes:** Only substances that are susceptible to the action of tissue enzymes are immunogenic. Degradation of the antigen by the tissue enzymes produces several immunogenic fragments having more number of epitopes exposed. Molecules that are not susceptible to tissue enzymes such as polystyrene latex composed of **D-amino acids** are not antigenic; while polypeptides consisting of **L-amino acids** are antigenic as they are degradable by tissue enzymes
- ❖ **Structural complexity:** Polymers made up of at least two or more amino acids are immunogenic. Complex proteins containing 20 amino acids and with four levels of structural organization are strongly immunogenic; e.g. hemoglobin
- ❖ **Foreignness to the host:** This is one of the key factor which determines immunogenicity. Higher is the phylogenetic distance between the antigen and the host; more is the immunogenicity
 - Self-antigens are not immunogenic; whereas, hetero-antigens and alloantigens are immunogenic—the degree of immunogenicity increases with the distance, e.g. plant antigens are more immunogenic than animal antigens to humans
 - Isoantigens are not immunogenic to those individuals who possess these antigens; but for other individuals they are immunogenic.
- ❖ **Genetic factor:** Different individuals of a given species show different types of immune responses towards the same antigen. This is believed to be due to the genetic differences between the individuals
 - Responders are the individuals who produce antibody faster
 - Slow responders are the individuals who produce antibody slowly and may need repeated antigenic exposures
 - Non-responders are the individuals who do not produce antibody in spite of repeated antigenic exposures.
- ❖ **Optimal dose of antigen:** An antigen is immunologically active only in the optimal dose range. A too little dose fails to elicit immune response and a too large dose leads to development of immunological tolerance (Chapter 17)
- ❖ **Route of antigen administration:** In general, the immune response is better induced following parenteral administration of an antigen; however it also affects the type of antibody produced
 - Immunoglobulin A (IgA) are better induced following oral administration of antigens
 - Inhalation of pollen antigens induces IgE synthesis; whereas the same antigens given parenterally, elicits IgG antibodies
 - **Site of injection** may influence immunogenicity: The hepatitis B vaccine is more immunogenic following deltoid injection than gluteal injection. This may be due to the paucity of antigen presenting cells (APCs) in gluteal fat.
- ❖ **Repeated doses of antigens:** Sometimes to generate an adequate immune response, repeated doses of antigens over a period of time may be needed. However, after certain doses of antigens, no further increase in antibody response is seen
- ❖ **Multiple antigens:** When two or more antigens are administered simultaneously, the effects may vary. The antibody response to one or the other antigen may be equal or diminished (due to antigenic competition)

or enhanced (due to adjuvant like action, see below highlight box)

- ❖ **Effect of prior administration of antibody:** The immune response against a particular antigen is suppressed if its corresponding antibody was administered prior to that
 - The primary immune response is more susceptible to get suppressed than the secondary immune response
 - **Therapeutic application:** In Rh negative women carrying an Rh positive fetus, the anti-Rh globulin is administered immediately following delivery (within 72 hours) which prevents the Rh sensitization in Rh negative women by a negative feedback mechanism.

Adjuvant

The term “adjuvant” refers to any substance that enhances the immunogenicity of an antigen. They are usually added to vaccines to increase the immunogenicity of the vaccine antigen.

Examples of Adjuvant Activity

- ❑ **Alum** (aluminum hydroxide or phosphate)
- ❑ **Mineral oil** (liquid paraffin)
- ❑ **Freund’s incomplete adjuvant:** It is a water-in-oil emulsion containing a protein antigen in the aqueous phase
- ❑ **Freund’s complete adjuvant** is the mixture of Freund’s incomplete adjuvant and suspension of killed tubercle bacilli in the oil phase
- ❑ **Lipopolysaccharide (LPS)** fraction of gram-negative bacilli, e.g. LPS of *Bordetella pertussis* acts as an excellent adjuvant for diphtheria and tetanus toxoids. This explains the reason for using combined immunization for diphtheria, pertussis and tetanus in the form of DPT vaccine
- ❑ **Other bacteria or their products:**
 - *Mycobacterium bovis*
 - Toxoid (diphtheria toxoid and tetanus toxoid act as adjuvant for *Haemophilus influenzae*—type b vaccine).
- ❑ **Nonbacterial products:** Such as silica particles, beryllium sulfate, squalene and thiomersal.

Mechanism of Adjuvant Action

Adjuvants act through the following steps:

- ❑ **Delaying the release of antigen:** Adjuvant on mixing, precipitate the antigen which is then released slowly from the site of administration, thus prolonging the antigenic exposure
- ❑ **By activating phagocytosis:** The adjuvant-antigen precipitate is of larger size, thus increases the likelihood of phagocytosis. The MDP (muramyl dipeptide) component of tubercle bacilli can activate the macrophages directly
- ❑ **By activating T_H cells:** Activated macrophages release interleukin-11(IL-11) and express higher level of MHC-II; thus promoting helper T (T_H) cell activation which in turn activates B cells to produce specific antibodies.

BIOLOGICAL CLASSES OF ANTIGENS

Depending on the mechanisms of inducing antibody formation, antigens are classified as T cell dependent (TD) and T cell independent (TI) antigens (Table 10.1).

Table 10.1: Differences between T-independent antigens and T-dependent antigen.

T-independent antigen	T-dependent antigen
Structurally simple—LPS, capsular polysaccharide, flagella	Structurally complex—protein in nature
Dose dependent immunogenicity	Immunogenic over wide range of doses
No memory	Memory present
No antigen processing	Antigen processing step is needed
Slowly metabolized	Rapidly metabolized
Activate B cells polyclonally	Activate B cells monoclonally
Activate both mature and immature B cells	Activate mature B cells only
B cells stimulated against T-independent antigen do not undergo <ul style="list-style-type: none"> • Affinity maturation • Class switch over 	B cells stimulated against T-dependent antigen undergo <ul style="list-style-type: none"> • Affinity maturation • Class switch over
Antibody response is restricted to IgM and IgG3	Antibodies of all classes can be produced

Abbreviations: LPS, lipopolysaccharides; Ig, immunoglobulin.

T-dependent (TD) Antigens

Most of the normal antigens are T cell dependent, they are processed and presented by antigen-presenting cells (APCs) to T cells which leads to T cell activation. The activated T cells secrete cytokines that in turn stimulate the B cells to produce antibodies.

T-independent (TI) Antigens

There are a few antigens such as **bacterial capsule, flagella** and **LPS** (lipopolysaccharide) that do not need the help of T cells and APCs. They directly bind to immunoglobulin receptors present on B cells and stimulate B cells polyclonally. It leads to increased secretion of non-specific antibodies (i.e. hypergammaglobulinemia).

Detailed mechanism of B cell activation against TD antigen is given in Chapter 15.

Superantigens

Superantigens are the third variety of biological class of antigens, recently described in the last decade. The unique feature of superantigens is, they can activate T cells directly without being processed by antigen-presenting cells (APCs).

- ❖ The variable β region of T cell receptor ($v\beta$ of TCR) appears to be the receptor for superantigens
- ❖ They directly bridge non-specifically between major histocompatibility complex (MHC)-II of APCs and T cells (Fig. 10.1)
- ❖ Non-specific activation of T cells leads to massive release of cytokines known as “cytokine storm,” which include inflammatory mediators such as interferon γ , IL-1, IL-6, TNF- α , and TNF- β

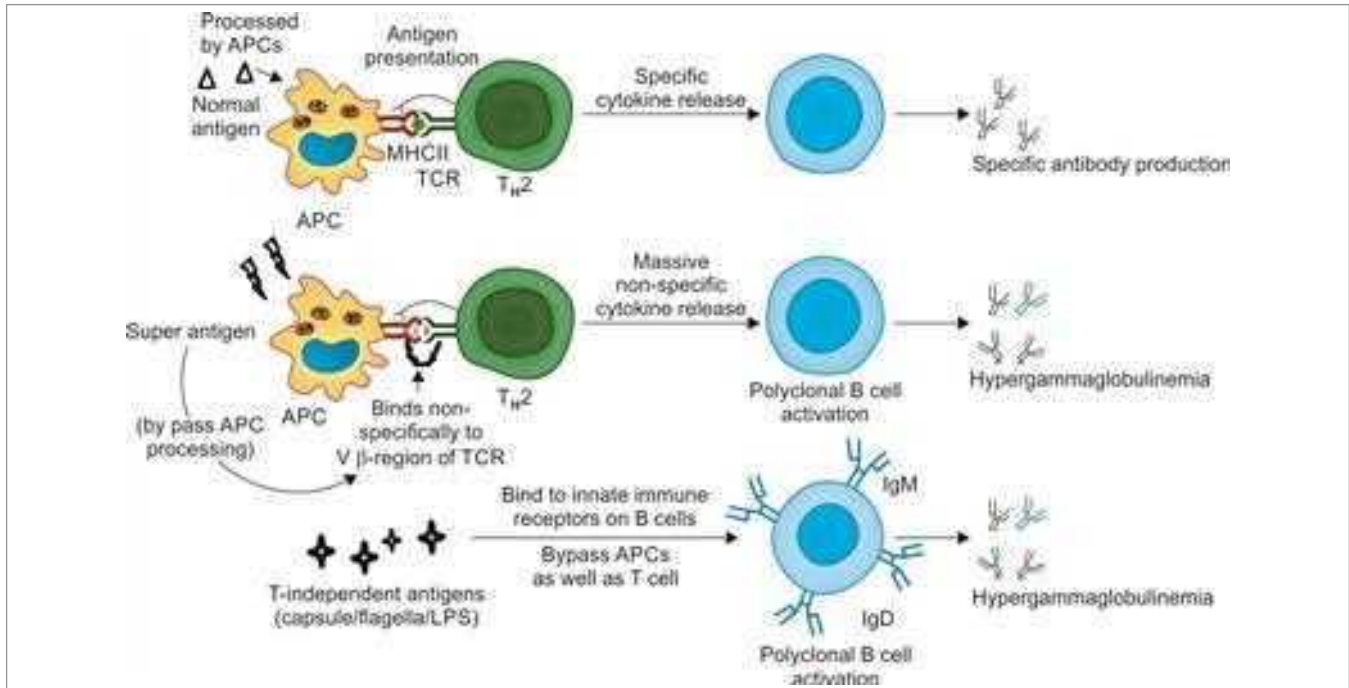


Fig. 10.1: Mechanism of action of superantigens, T-dependent and T-independent antigens.

Abbreviations: APCs, antigen-presenting cells; TCR, T cell receptor; MHC, major histocompatibility complex.

- ❖ They in turn can activate B cell polyclonally, which leads to increased secretion of non-specific antibodies (hypergammaglobulinemia).

Examples of Superantigens

Various products of microorganisms behave as superantigens; the most important being staphylococcal and streptococcal toxins (Table 10.2).

Diseases Associated with Superantigens

Superantigens can cause a number of diseases.

- ❖ Toxic shock syndrome
- ❖ Food poisoning
- ❖ Scalded skin syndrome
- ❖ Rare conditions such as—atopic dermatitis, Kawasaki syndrome, psoriasis, acute disseminated encephalomyelitis.

Table 10.2: Superantigens.

Bacterial superantigen

Staphylococcal toxin:

- Toxic shock syndrome toxin-1 (TSST-1)
- Exfoliative toxin
- Enterotoxins

Streptococcal pyrogenic exotoxin (SPE)-A and C

Mycoplasma arthritidis mitogen-I

Yersinia enterocolitica

Yersinia pseudotuberculosis

Viral superantigen

Epstein-Barr virus associated superantigen

Cytomegalovirus associated superantigen

Rabies nucleocapsid

HIV encoded superantigen (nef- negative regulatory factor)

Fungal superantigen

Malassezia furfur

EXPECTED QUESTIONS

I. Write short notes on:

1. Heterophile antigens.
2. Adjuvant.
3. Superantigens.

II. Multiple Choice Questions (MCQs):

1. Superantigen causes:

- a. Enhancement of phagocytosis
- b. Polyclonal activation of B cells

- c. Antigen presentation by macrophage
- d. Activation of complement

2. Which part of the bacteria is most antigenic?

- a. Protein
- b. Carbohydrate
- c. Lipid
- d. Nucleic acid

3. Which of the following antigen is T-dependent?

- a. Bacterial capsule
- b. Flagella
- c. Lipopolysaccharide
- d. Exotoxin

Answers

1. b 2. a 3. d

CHAPTER PREVIEW

- Structure of Antibody
- Immunoglobulin Classes
- Antigenic Determinants of Immunoglobulins
- Monoclonal Antibody
- Antibody Diversity

Antibody or immunoglobulin is a specialized glycoprotein, produced from activated B cells (plasma cells) in response to an antigen, and is capable of combining with the antigen that triggered its production.

- ❖ It was found that (*A. Tiselius, 1939*) when the serum is subjected to electrophoresis, the serum proteins are separated into four fragments—albumin, globulin α , β and γ (Fig. 11.1). Antibodies are located in the γ -globulin fraction. Because they immunologically react with the antigen, they were given the name as immunoglobulin
- ❖ Both the terms, immunoglobulin (Ig) and antibody are used interchangeably, representing the physiological and functional properties of the molecule respectively
- ❖ Immunoglobulin (Ig) constitutes 20–25% of total serum proteins
- ❖ There are five classes (or isotypes) of immunoglobulins recognized—IgG, IgA, IgM, IgD and IgE.

STRUCTURE OF ANTIBODY

An antibody molecule is a ‘Y-shaped’ heterodimer, composed of four polypeptide chains (Fig. 11.2).

- ❖ Two identical light (L) chains, of molecular weight 25,000 Da each and
- ❖ Two identical heavy (H) chains each having molecular weight 50,000 Da or more.

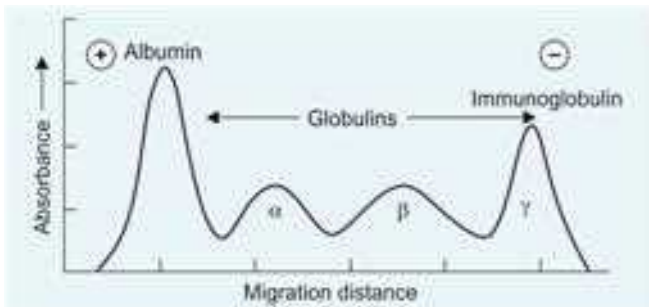


Fig. 11.1: Electrophoresis of human serum proteins.

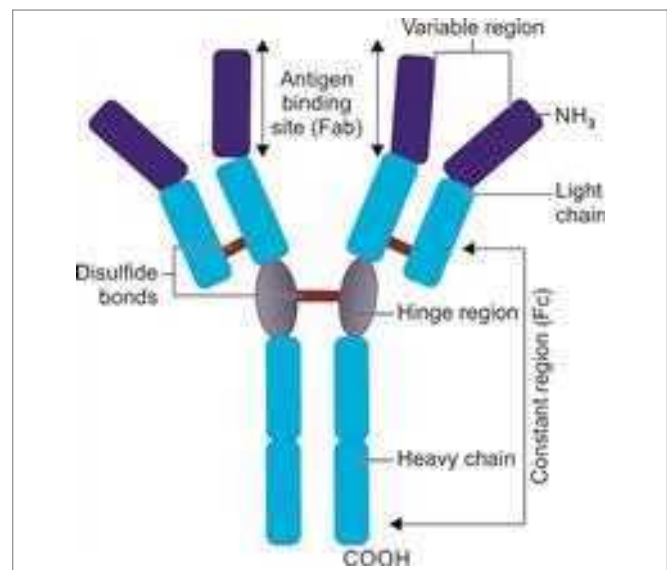


Fig. 11.2: General structure of antibody.

H and L Chain

All four H and L chains are bound to each other by disulfide bonds, and by noncovalent interactions, such as salt linkages, hydrogen bonds, and hydrophobic bonds.

- ❖ All the chains have two ends—an amino terminal end (NH_2) and a carboxyl terminal end (COOH)
- ❖ There are five classes of H chains and two classes of L chains
- ❖ The five classes of H chains are structurally and antigenically distinct; each is designated by the Greek letters γ , α , μ , δ and ϵ
- ❖ The five classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE) are classified based on the amino acid sequences of the heavy chains (Table 11.1)
- ❖ The L chains are of two types—kappa (κ) and lambda (λ), named after Korngold and Lipari who originally described them
 - In humans, 60% of the light chains are kappa and 40% are lambda type (ratio 3:2)

Immunoglobulin class	Heavy chain type
IgG	γ (gamma)
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	ϵ (epsilon)

- Both the light chains of an antibody molecule are of same type, either κ or λ , but never both.
- L chains are composed of 214 amino acids; whereas the number of amino acids in the heavy chain varies—ranging from 446 (in α chain) to 576 (in μ chain).

Variable and Constant Regions

Each H and L chain comprises of two regions—variable and constant region, depending upon whether the amino acid sequences of the regions show variable or uniform pattern among different antibodies.

Variable Region

The first 110 amino acid residues near the amino terminal (NH_3) of both L and H chains constitute the variable region—designated as V_L and V_H , respectively. It represents the antigen binding site of the antibody.

- Hypervariable region:** Within the variable region, there are some zones (**hot spots**) that show relatively higher variability in the amino acid sequences. Such zones are called as hypervariable regions or complementarity determining regions (CDRs). They form the antigen—binding site. There are three hot spots in the L and four in the H chain, respectively
- Paratope:** The site on the hypervariable regions that make actual contact with the epitope of an antigen is called as paratope.

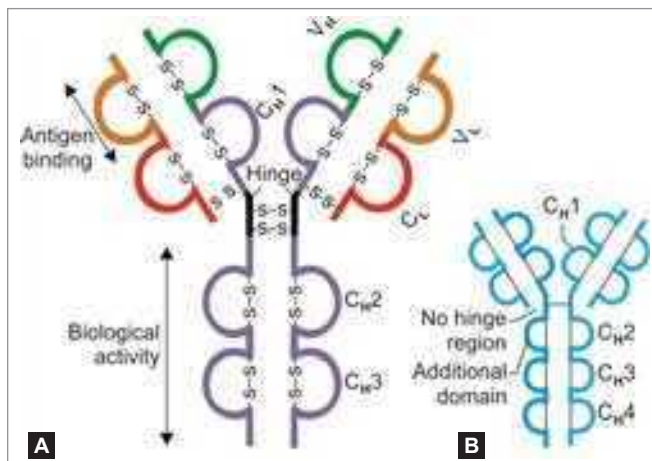
Constant Region

It constitutes the remaining part of an Ig molecule other than that of variable region. The length of the constant regions is approximately 104 amino acids for light chain, 330 amino acids for γ , α and δ heavy chains and 440 amino acids for μ and ϵ heavy chains. The amino acid sequence of constant region shows uniform pattern. A single antibody molecule has two identical heavy chains and two identical light chains; H_2L_2 .

Heavy and Light Chain Domains

Heavy (H) and light (L) chains are further folded into domains, each containing about 110 amino acid residues. Within the domain, a loop like structure of 60 amino acids is present which is formed due to an intrachain disulfide bond. The number of domains in each chain varies:

- Light chain contains one variable domain (V_L) and one constant domain (C_L)



Figs 11.3A and B: Immunoglobulin domains: **A.** Ig with γ , δ and α heavy chains; **B.** Ig with μ and ϵ heavy chains.

- Heavy chains possess one variable domain (V_H) and 3 or 4 numbers of constant domain (C_H):
 - Heavy chains γ , α and δ have three constant domains- C_{H1} , C_{H2} and C_{H3} (Fig. 11.3A)
 - Heavy chains μ and ϵ have four constant domains- C_{H1} to C_{H4} (Fig. 11.3B).

Hinge Region

In heavy chain (γ , α , and δ), the junction formed between C_{H1} and C_{H2} domain constitutes the hinge region (Fig. 11.3A).

- This region is rich in proline and cysteine. The hinge region is flexible, allowing the Ig molecule to assume different positions, thus helps the antibody in reaching towards the antigen
- In IgE and IgM, the ϵ and μ heavy chains do not have hinge region; instead, their constant region has an additional domain (C_{H4}) (Fig. 11.3B)
- The hinge region is sensitive to various enzymatic digestions.

Enzymatic Digestion

When an immunoglobulin molecule is subjected to enzymatic digestion, it generates various fragments (Fig. 11.4).

- Papain digestion:** Papain cleaves the Ig molecule at a point above the disulfide bridge of hinge region; resulting in three fragments each having a sedimentation coefficient of 3.5 Svedberg (S):
 - Two Fab fragments:** Soluble fragments which bind to the antigen (Fab for antigen binding fragment) and
 - Fc fragment:** An insoluble fraction which gets crystallized in the cold (Fc for crystallizable fragment).
- Pepsin digestion:** Pepsin cleaves the Ig molecule at a point below the disulfide bridge of hinge region; resulting in formation of:
 - One F (ab')₂ fragment:** A fragment having a sedimentation coefficient of 5S; composed of two Fab subunits bound together

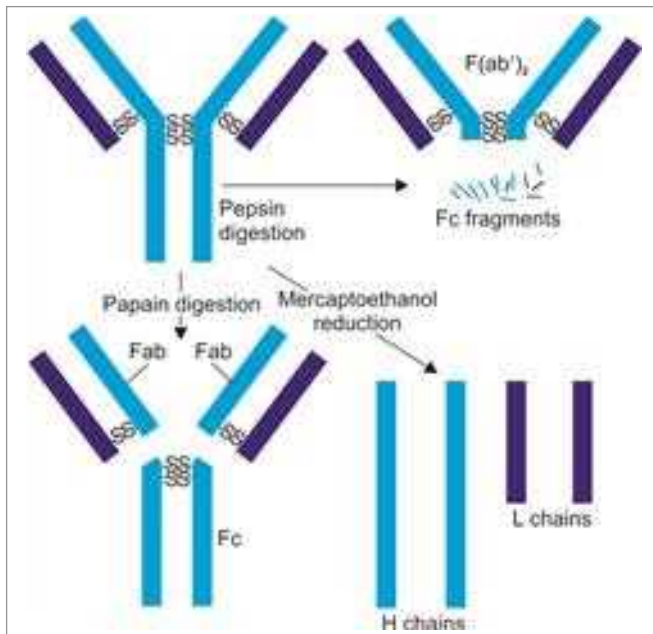


Fig. 11.4: Enzymatic digestion of immunoglobulin.

- **Many smaller fragments:** Due to digestion of Fc portion by pepsin into smaller fragments.
- ❖ **Mercaptoethanol** reduction of Ig molecule—generates four fragments (two H and two L chains) as it cleaves only disulfide bonds sparing the peptide bonds.

FUNCTIONS OF IMMUNOGLOBULINS

Antigen Binding (by Fab Region)

Binding to the antigen is the primary function of an antibody which can result in protection of the host.

- ❖ The Fab fragment bears the variable region and is involved in interaction with the antigen
- ❖ The valency of an antibody refers to the number of Fab regions it possesses. Thus, a simple monomeric antibody molecule has a valency of two.

Effector Functions (by Fc Region)

Most of the times, the binding of an antibody to its antigen does not result in any direct biological effect. Rather, variety of secondary “effector functions” are produced; mediated by Fc region of the antibody. These effector functions include:

- ❖ **Fixation of complement:** Antibody coating the target cell binds to complement through its Fc receptor which leads to complement mediated lysis of the target cell
- ❖ **Binding to various cell types:** Phagocytes, lymphocytes, platelets, mast cells, NK cell, eosinophils and basophils bear Fc receptors (FcR) that bind to Fc region of immunoglobulins. This binding can activate the cells to perform some biological functions (described with individual immunoglobulins and also in Chapter 15). Some immunoglobulins (e.g. IgG) also bind to receptors on placental trophoblasts, which results in transfer of IgG across the placenta.

IMMUNOGLOBULIN CLASSES

Based on five types of heavy chains, there are five classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE). Each class can also exist as two types due to presence of different light chain type—kappa or lambda. IgG and IgA are further divided into subclasses (four for IgG and two for IgA) due to minor differences in amino acid sequences in constant region of heavy chains. Important properties of different Ig classes are summarized in Table 11.2.

Immunoglobulin G (IgG)

It constitutes about 70–80% of total Ig in the body.

- ❖ Among all Ig, IgG has maximum daily production, longest half-life of 23 days and highest serum concentration
- ❖ **IgG has four subclasses:** IgG1, IgG2, IgG3 and IgG4; all differ from each other in the amino acid sequences of the constant region of their γ -heavy chain
- ❖ The subclasses vary in their biological functions, length of hinge region and number of disulfide bridges. IgG3 has longest hinge region with 11 interchain disulfide bonds.

Functions of IgG

- ❖ IgG can **cross placenta**; hence provide immunity to the fetus and newborn. Among subclasses, IgG2 has the poorest ability to cross placenta
- ❖ **Complement fixing:** Fc region of IgG can bind to complement factors; thus activates the classical pathway of complement system. The complement-fixing ability of subclasses varies—IgG3> IgG1> IgG2. IgG4 does not fix complements
- ❖ **Phagocytosis:** IgG1 and IgG3 bind to Fc receptors present on phagocytes (macrophages, neutrophils) with high affinity and enhance the phagocytosis (opsonization) of antigen bound to them. IgG2 has an extremely low affinity for Fc receptors of phagocytes
- ❖ It mediates precipitation and neutralization reactions
- ❖ IgG plays a major role in neutralization of toxins as it can easily diffuse into extravascular space
- ❖ IgG is raised after a long time following infection and represents chronic or past infection (recovery)
- ❖ **Coagglutination:** IgG subclasses (except IgG3) mediate coagglutination reaction by binding to protein-A of *S. aureus* (refer Chapter 12).

Immunoglobulin M (IgM)

Among all immunoglobulins, IgM has highest molecular weight, and maximum sedimentation coefficient (19S). It is present only in intravascular compartment, not in body fluids or secretions.

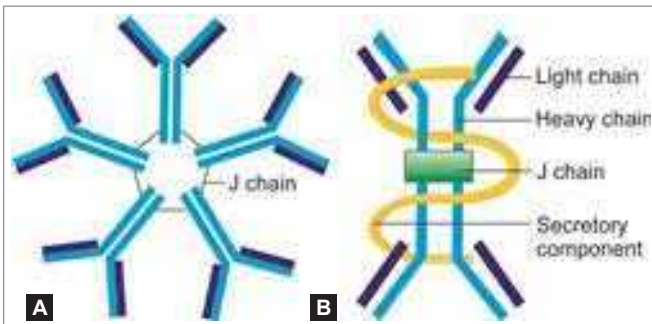
IgM exists in both monomeric and pentameric forms:

- ❖ When present as membrane-bound antibody on B cells, it exists in monomeric form
- ❖ When present in secreted form, it is pentameric in nature; i.e. five IgM monomeric units are joined with each other

Table 11.2: Properties of various immunoglobulins.

Property	IgG	IgA	IgM	IgD	IgE
Usual form	Monomer	Monomer, dimer	Monomer, pentamer	Monomer	Monomer
Valency	2	2 or 4	2 or 10	2	2
Other chains	None	J chain, secretory component	J chain	None	None
Subclasses	G1, G2, G3, G4	A1, A2	None	None	None
Molecular weight (kDa)	150	150–600	900	150	190
Serum level (mg/mL)	9.5–12.5	IgA 1–3.0 IgA 2–0.5	1.5	0.03	0.0003
% of total serum Ig	75–85%	10–15%	5–10%	0.3%	0.019%
Half-life, days	23*	6	5	3	2.5
Daily production (mg/kg)	34	24	3.3	0.4	0.0023
Intravascular distribution (%)	45	42	80	75	50
Sedimentation coefficient (S)	7	7	19	7	8
Complement activation					
Classical	++ (IgG3>1>2)	–	+++	–	–
Alternative	–	+	–	–	–
Binds to Fc receptors of phagocytes	++	–	? **	–	–
Placental transfer	Yes (except IgG2)	–	–	–	–
Mediates coagglutination	Yes (except IgG3)	–	–	–	–
Mucosal transport	–	Yes	–	–	–
Mast cell degranulation	–	–	–	–	yes
Marker for B cells	–	–	+	+	–
Heat stability	+	+	+	+	–

*Half-life of IgG3 is 8 days; **?- Questionable



Figs 11.5A and B: A. Pentameric IgM; B. Dimeric IgA.

(by J chain) to form a complete IgM pentamer having 10 Fab regions and 10 valencies (Fig. 11.5A).

Functions of IgM

- ❖ **Acute infection:** IgM is the first antibody to be produced following an infection; represents acute or recent infection. It is also called as primary immune response antibody
- ❖ **Complement fixing:** It is the most potent activator of classical complement pathway due to multiple complement binding sites (5 Fc regions) present in IgM pentamer
- ❖ It is also present on B cell surface in monomeric form and serves as **B cell receptor** for antigen binding

- ❖ It acts as an **opsonin**; binds to antigen which is then easily recognized and removed. IgM is 500–1000 times more potent in opsonization than IgG
- ❖ **Fetal immunity:** It is the first antibody to be synthesized in fetal life (20 weeks); thus provides immunity to the fetus. Presence of IgM in fetus or newborn indicates intrauterine infection (as it cannot cross placenta), and its detection is useful in the diagnosis of congenital infections
- ❖ **Protection against intravascular organisms:** IgM being intravascular, is responsible for protection against blood invasion by microorganisms. IgM deficiency is often associated with septicemia
- ❖ **Mediates agglutination:** IgM is about 20 times more effective in bacterial agglutination than IgG.

Immunoglobulin A (IgA)

IgA is the second most abundant class of Ig next to IgG, constituting about 10–15% of total serum Ig. It exists in both monomeric and dimeric forms (Fig. 11.5B).

Serum IgA

- ❖ IgA in serum is predominantly in monomeric form
- ❖ Functions: Serum IgA interacts with the Fc receptors expressed on immune effector cells, to initiate various functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), degranulation of immune cells, etc.

Secretory IgA

Secretory IgA is dimeric in nature; the two IgA monomeric units are joined by **J chain**. In addition, there is another joining segment present between two IgA molecules called **secretory component**.

- ❖ **Location:** Secretory IgA is the predominant antibody found in body secretions like milk, saliva, tears, intestinal and respiratory tract mucosal secretions
- ❖ The secretory component is derived from poly-Ig receptor present on the serosal surfaces of the epithelial cells
- ❖ **Function:** The secretory IgA mediates **local or mucosal immunity**; provides protection against pathogens by cross-linking bigger antigens with multiple epitopes and preventing their entry through the mucosal surface
 - It is effective against bacteria like *Salmonella*, *Vibrio*, *Neisseria*, and viruses like polio and influenza
 - Breast milk is rich in secretory IgA and provides good protection to the immunologically immature infant's gut.

Secretory IgA

The dimeric secretory IgA is synthesized by plasma cells situated near mucosal epithelium. The J chain is also produced in the same cell.

Whereas, the secretory component is synthesized by the mucosal epithelial cells.

- It is derived from the poly-Ig receptor present on the basolateral surfaces of the epithelial cells
- It helps the dimeric IgA to cross the epithelial surface to reach the lumen
- It also protects IgA from denaturation by bacterial proteases produced by intestinal flora.

Subclasses of IgA

Depending upon the amino acid sequences in the constant region of heavy chain, IgA exists as two isotypes:

1. IgA1 is the dominant subclass in serum. Serum IgA comprises of ~90% IgA1 and 10% IgA2
2. IgA2 is present in higher concentration in secretions than in serum (ranging from 10% to 20% in nasal and male genital secretions, 40% in saliva, to 60% in colonic and female genital secretions)
 - IgA2 lacks the disulfide bonds between the heavy and light chains
 - Polysaccharide antigens tend to induce more IgA2 synthesis than protein antigens.

Immunoglobulin E (IgE)

Among all Ig, IgE is having the lowest serum concentration, shortest half-life and minimum daily production. It is also the only heat labile antibody (inactivated at 56°C in one hour). It has affinity for the surface of tissue cells (mainly mast cells) of the same species (homocytotropism). It is mainly extravascular in distribution.

Functions of IgE

- ❖ IgE is highly potent and mediate **type I hypersensitivity** reactions by binding to the mast cells causing degranulation. IgE response is seen in various allergic conditions, such as asthma, anaphylaxis, hay fever, etc. (Described in detail in Chapter 16)
- ❖ IgE is elevated in **helminthic infections**. By coating on the surface of eosinophils, IgE stimulates the release of the mediators on to the surface of helminths by a process known as antibody mediated cellular cytotoxicity or ADCC (Chapter 15).

Immunoglobulin D (IgD)

IgD is found as membrane Ig on the surface of B cells and acts as a B cell receptor along with IgM. It has the highest carbohydrate content among all the immunoglobulin. No other function of IgD is known so far.

ANTIGENIC DETERMINANTS OF IMMUNOGLOBULINS

Since antibodies are glycoproteins, they can themselves function as potent immunogens, having a number of antigenic determinants which can induce antibody responses in hosts other than the parent host. It is observed that the entire Ig molecule is not immunogenic, but it contains antigenic determinants at specific sites. Based on the location of antigenic determinants, the Ig molecules are divided into isotypes, idiotypes and allotypes (Fig. 11.6).

Isotypes

The five classes of Ig (IgG, IgA, IgM, IgD and IgE) and their subclasses are called as isotypes; they vary from each other in the amino acid sequences of the constant region of their heavy chains. Such variation is called as isotypic variation.

- ❖ Isotypes that are present in all members of a given species are similar in nature
- ❖ Hence, antibody against isotypes can be produced by injecting the Ig from one species into another.

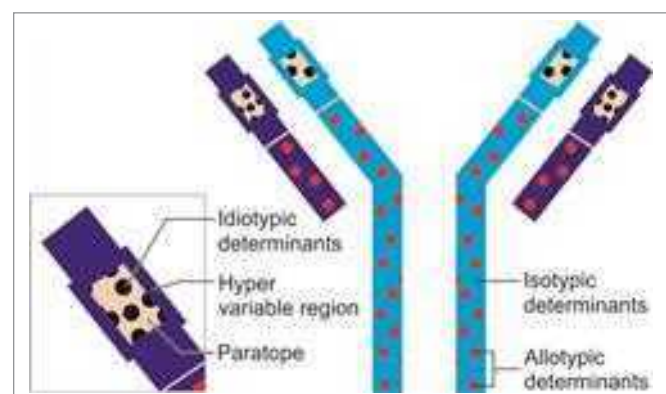


Fig. 11.6: Antigenic determinants of immunoglobulins.

Idiotypes

The unique amino acid sequence present in paratope region (in V_H and V_L regions) of one member of a species acts as antigenic determinant to other members of the same species.

- ❖ Such antigenic determinants are called as idiotypes and the sum total of idiotypes on an Ig molecule constitutes its idiotypes (Fig. 11.6)
- ❖ Such variation between immunoglobulins due to differences in the amino acid sequences of the variable region is called as idiotype variation
- ❖ Idiotypes in an individual arise continuously from mutations (somatic hypermutations) in the genes of variable region. Hence, idiotypes may act as foreign to the host itself; however, do not evoke autoimmune response because they are present in small numbers.

Allotypes

The antigenic determinants present in the isotype genes in the constant region of H and L chains, encoded by multiple alleles are called as allotypes.

- ❖ Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the allele genes
- ❖ Hence, allotypes are present in the constant region of Ig molecules of the same class, in some, but not all, members of a species
- ❖ The sum of the individual allotypic determinants displayed by an antibody determines its allotype
- ❖ Allotypes differ in sequence of 1–4 amino acid from one another
- ❖ **Allotype systems:** To date, three systems of allotypic markers have been characterized for humans:
 - For kappa light chain (Km system)—has three Km allotypes
 - For γ heavy chain (Gm system)—has 25 Gm types
 - For α heavy chain (Am system).

Antibody to allotype determinants can be produced by injecting antibodies containing these determinants from one member to another within a given species. Anti allotype specific antibodies may also be developed following blood transfusion or by maternal passage of IgG into the fetus.

ABNORMAL IMMUNOGLOBULINS

In addition to the five classes of normal antibodies, other structurally similar proteins are seen in sera of patients and sometimes even in healthy individuals.

Bence Jones Proteins

They are produced in a neoplastic condition of plasma cells called multiple myeloma.

- ❖ This condition is also called as light chain disease as the cancerous plasma cells produce excess of light

chains (Bence Jones proteins) which are accumulated in patient's serum and excreted in urine

- ❖ Such proteins have a unique property of getting coagulated at 50°C and redissolving again at 70°C.

Other abnormal immunoglobulins include:

- ❖ **Waldenstrom's Macroglobulinemia:** It is a B cell lymphoma, producing excess IgM
- ❖ **Heavy chain disease:** It is characterized by an excessive production of heavy chains that are short and truncated
- ❖ **Cryoglobulinemia:** Seen in multiple myeloma and hepatitis C infection.

MONOCLONAL ANTIBODY

Monoclonal antibodies (mAb) are defined as the antibodies derived from a single clone of plasma cell; all having the same antigen specificity, i.e. produced against a single epitope of an antigen.

Polyclonal vs Monoclonal Nature of Antibody

When an antigen having multiple epitopes enters the body, each epitope may stimulate one clone of B cells producing one type of antibody. Hence the resultant antibody mixture present in serum is said to be **polyclonal**, i.e. contains mixture of antibodies derived from different clones of B cells.

However, when only one clone of B cell is stimulated by a single epitope of an antigen and then is allowed to proliferate and produce antibodies; such antibodies are referred to as **monoclonal** antibodies (mAb).

Production of mAb (Hybridoma Technique)

Monoclonal antibodies are produced by Hybridoma technique, developed by G Kohler and C Milstein (1975), for which they were awarded Nobel Prize in 1984.

Principle

A clone of B cell stimulated against a single epitope of antigen is fused with an immortal cell, e.g. myeloma cell (capable of multiplying indefinitely) to produce a hybridoma cell. This hybridoma cell has two unique properties:

1. Produces monoclonal antibody of same antigen specificity (due to B cell component)
2. Multiplies indefinitely producing clone of identical cells (due to immortal myeloma cell component).

Procedure

The steps of hybridoma technique are as follows (Fig. 11.7):

- ❖ **Mouse splenic B cells:** The mouse is injected with an antigen containing the desired epitope. After an interval, the mouse splenic B cells are obtained which are activated against the epitope of the antigen injected
- ❖ **Myeloma cells** are used as a source of immortal cells. They are cancerous plasma cells. They closely resemble mouse B cells; hence are compatible for fusion. However, myeloma cells also have the capacity to produce their

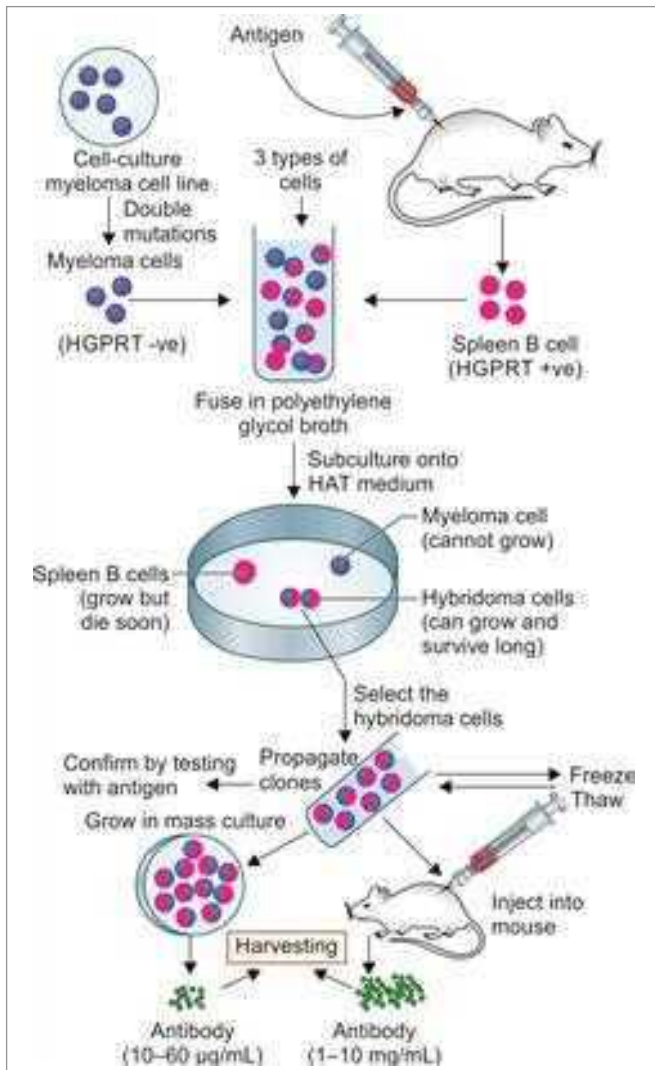


Fig. 11.7: Hybridoma technology.

Abbreviation: HGPRT, hypoxanthine guanine phosphoribosyl transferase.

own antibodies. Hence myeloma cells are genetically modified with two mutations (double mutated myeloma cells), so that they lose the ability to produce their own antibody but retain immortal property

- ❖ **Fusion:** The mouse splenic B cells and mutated myeloma cells are fused in polyethylene glycol broth. In the reaction chamber, three types of cells will be generated:
 1. Unfused myeloma cells
 2. Unfused mouse splenic B cells
 3. Fused hybridoma cells.
- ❖ **Purification** (by subculturing on HAT media): The next step is to remove the unwanted unfused cells and to propagate the clone of hybridoma cells. This is carried out by subculturing the cells in reaction chamber onto a special medium called HAT medium
- ❖ **HAT medium:** It contains hypoxanthine, aminopterin and thymidine
 - Purine synthesis in mammalian cell (e.g. splenic B cell) occurs by either *de novo* or salvage pathways

- Aminopterin blocks the *de novo* pathway so that the cell has to perform the salvage pathway to synthesize purines for its survival
- Salvage pathway requires two important enzymes- HGPRT (hypoxanthine guanine phosphoribosyl-transferase) and thymidine kinase
- So any cell (e.g. myeloma cell) that lacks HGPRT cannot grow on HAT medium.

❖ **Fate of three types of cells on HAT media:**

1. Unfused splenic B cells: They can grow, but do not survive long as they are not immortal
2. Unfused myeloma cells: They cannot grow as they lack HGPRT enzyme to perform the salvage pathway of purine synthesis
3. Hybridoma cells: They can grow and survive long.

❖ **Selection of individual hybridoma cells:** If the original antigen used has multiple epitopes, many B cells would fuse with myeloma cells to produce a mixture of hybridoma cells each having specificity for one epitope

- The medium containing hybridoma cells is then diluted into multi-well plates to such an extent that each well contains only one cell
- The hybridoma cells producing the desired mAb are selected by radioimmunoassay or ELISA techniques using the specific antigen fragments, and are selectively proliferated.

❖ **Maintenance of mAb:** The selected hybridoma cells can be maintained in two ways:

1. Hybridoma cell is cultured to generate a clone of identical cells; producing pure form of monoclonal antibodies at a concentration of 10–60 µg/mL
2. Alternatively the desired hybridoma cell is injected into the peritoneal cavity of mouse where it can multiply and produce mAb in ascitic fluid at a concentration of 1–10 mg/mL. Such mAb obtained from mouse ascitic fluid and serum may not be in pure form, is often mixed with other antibodies; hence, it is purified by chromatography or by immunoprecipitation test.

Types of Monoclonal Antibodies

The above mentioned procedure would yield mAb whose 100% amino acids are mouse derived. The problem of mouse mAb is that, the mouse proteins being foreign; can induce immune response in humans producing human anti-mouse antibodies (HAMA); that in turn eliminate the mAb faster from the body. Hence mouse derived monoclonal antibodies are not the best for human use. Since the discovery of hybridoma technique, various modifications have been attempted to produce mAb by recombining human and mouse proteins (Fig. 11.8).

- ❖ **Mouse mAb:** It contains 100% mouse derived proteins
- ❖ **Chimeric mAb:** It is prepared by recombination of 34% mouse proteins (variable region) and 66% human proteins (constant region)

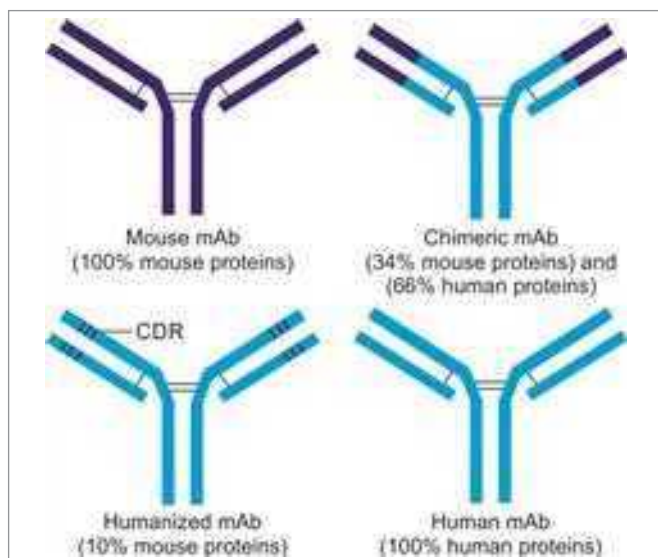


Fig. 11.8: Types of monoclonal antibodies.

- ❖ **Humanized mAb:** Here, only the antigen binding site (i.e. CDR—complementarity determining region) is mouse derived (10%) and the remaining part of mAb is human derived
- ❖ **Human mAb:** It contains 100% human derived amino acids. It is the best accepted mAb in humans.

Applications of Monoclonal Antibodies

- ❖ **Isolation and purification:** Monoclonal antibodies can be used to purify individual molecule from a mixture when they are present in low concentration, e.g. interferon and coagulation factor VIII
- ❖ **Identification of cells and clones:** For example T_H and T_C cells are identified by using anti-CD4 and anti-CD8 mAb
- ❖ **Diagnostic reagents:** The widest application of mAb is detection of antigen. The antigen detection kits employ various mAb tagged with detection molecules, such as fluorescent dye or enzyme to detect the specific antigens in the clinical specimen by using various formats like ELISA, rapid tests, etc. Examples include detection of hepatitis B surface antigen, serotyping of streptococci, etc.
- ❖ **Monitoring** of proteins and drug levels in serum
- ❖ **Passive immunity:** For post-exposure prophylaxis against various infections, mAb targeting specific antigens of the infecting organism can be administered. Examples include—immunoglobulins against hepatitis B, rabies, and tetanus

- ❖ **Therapeutic use:** Monoclonal antibodies are used in the treatment of various inflammatory and allergic diseases and cancers (Table 11.3)
- ❖ **Used as immunotoxin:** mAb conjugated with bacterial/chemical toxins (e.g. diphtheria toxin) can be used to kill the target cells such as cancer cells. Here, mAb against surface receptors helps in binding to the target cells and the toxin helps in target cell killing
- ❖ **Used as enzymes:** **Abzyme** is a monoclonal antibody with catalytic activity.

ANTIBODY DIVERSITY

Antibody diversity is a mechanism by virtue of which human immune system is capable of producing vast number of antibodies (10^8 or even more) corresponding to various epitopes of different antigens. There are several postulates which explain the mechanism of antibody diversity.

- ❖ **Multiple chromosomes:** Ig chains are coded on different chromosomes; H chain on chromosome 14, whereas light chain kappa and lambda on chromosome 2 and 22 respectively
- ❖ **Multiple genes exist for each segment** (V_H , C_H , V_L and C_L) of Ig chain. For example, there are 51 V_H genes known to exist in nature and out of which any one gene would code for V_H chain of an Ig. In this way, there are many possible combinations of joining of the Ig gene segments.

Table 11.3: Therapeutic uses of monoclonal antibodies.

Monoclonal antibody	Targeted against	Used in treatment of
Suppress immune system		
Adalimumab and infliximab	TNF- α	Rheumatoid arthritis Crohn's disease
Omalizumab	IgE	Asthma
Daclizumab	IL-2 receptor	Rejection of kidney transplants
Muromonab	CD3	
Anticancer		
Trastuzumab	HER-2	Breast cancer
Rituximab	CD20	Lymphoma
Inhibit angiogenesis		
Bevacizumab	VEGF (vascular endothelial growth factor)	Colorectal cancers
Abciximab	Platelet receptor GpIIb/IIIa	Coronary artery disease

Note:

- ❑ Mouse mAb ends with suffix 'umab or onab'
- ❑ Chimeric mAb ends with suffix 'ximab'
- ❑ Humanized mAb ends with suffix 'zumab'
- ❑ Human mAb ends with suffix 'mumab'

EXPECTED QUESTIONS

I. Write essay on:

1. Define antibody. Describe in detail about the structure and functions of various types of antibodies.
2. Monoclonal antibodies and their applications.

II. Multiple Choice Questions (MCQs):

1. Which antibody crosses placenta?
 - a. IgA
 - b. IgG
 - c. IgE
 - d. IgM

2. What is the total valencies of IgM?

- a. 10
- b. 5
- c. 2
- d. 1

3. Which antibody is elevated in acute infection?

- a. IgA
- b. IgG
- c. IgE
- d. IgM

4. Which antibody mediates mucosal immunity?

- a. IgA
- b. IgG
- c. IgE
- d. IgM

Answers

1. b 2. a 3. d 4. a

Antigen–Antibody Reaction

CHAPTER 12

CHAPTER PREVIEW

- General Properties
- Conventional Immunoassays
 - Precipitation Reaction
 - Agglutination Reaction
- Newer Techniques
 - ELISA
 - Enzyme-linked Fluorescent Assay
 - Immunofluorescence Assay
- Chemiluminescence
- Western Blot
- Rapid Tests

The antigen–antibody reaction is a bimolecular association where the antigen and antibody combine with each other specifically and in an observable manner similar to an enzyme–substrate interaction, the only difference is, it does not lead to an irreversible alteration in either antibody or in antigen.

GENERAL PROPERTIES OF ANTIGEN–ANTIBODY REACTIONS

Antigen (Ag)–antibody (Ab) reactions are characterized by the following general properties:

Specific

Ag–Ab reaction involves specific interaction between epitope of an antigen with the corresponding paratope of its homologous antibody. Exception is the cross reactions which may occur due to sharing of epitopes among different antigens. In such cases, antibody against one antigen can cross react with a similar epitope of a different antigen.

Noncovalent Interactions

The union of antigen and antibody requires formation of large number of non-covalent interactions between them such as:

- ❖ Hydrogen bonds
- ❖ Electrostatic interactions
- ❖ Hydrophobic interactions
- ❖ Van der Waals forces.

Strength

The strength or the firmness of the association is influenced by the affinity and avidity of the antigen–antibody interaction.

Affinity

It refers to the sum total of noncovalent interactions between a single epitope of an antigen with its corresponding paratope present on antibody.

Avidity

It is a term used to describe the affinities of all the binding sites when multivalent antibody reacts with a complex antigen carrying multiple epitopes.

- ❖ The total strength (i.e. avidity) would be much higher than the individual affinity at each binding site, but lower than the sum of all affinities. This difference is primarily due to geometry of Ag–Ab binding
- ❖ The geometry of the multivalent antibody gets stretched when it reacts with a complex antigen, as it has to reach and accommodate all the epitopes, thus resulting in less optimal binding interactions
- ❖ Avidity is a better indicator of strength of an antigen–antibody reaction. Avidity of an antibody can compensate for its low affinity. For example, IgM has a low affinity than IgG, but it is multivalent (10 valencies), therefore has a much higher avidity. Hence, it can bind to an antigen more effectively than IgG
- ❖ Avidity increases with time: Though IgG has a low avidity initially, IgG produced in later part of infection will have stronger avidity. This property is used in IgG avidity test; described later in this chapter.

Diagnostic Use

Because Ag–Ab reactions are specific and observable, they are extensively used in the laboratories for the diagnosis of infectious diseases. The diagnostic tests based on Ag–Ab reactions are called as immunoassays. Most immunoassays are also called serological tests as they are performed using serum samples. However, other samples can also be used such as urine, CSF, etc. Immunoassays can be broadly categorized into two types:

1. **Antigen detection assays:** Detect antigens in patient's sample by employing specific antibody
2. **Antibody detection assays:** Detect antibodies in patient's sample by employing specific antigen.

Qualitative vs Quantitative Immunoassays

Immunoassays can be performed by both qualitative and quantitative methods.

Qualitative Assays

Here, the undiluted specimen containing the antibody is directly mixed with the suspension of antigen or vice versa. The result is read as ‘positive’ or ‘negative’ based on presence or absence of antigen or antibody in the clinical specimen. The exact amount of antigen or antibody present in the specimen cannot be estimated.

Quantitative Assays

When the qualitative test turns positive, the exact amount of antibody in serum can be estimated by serial dilution of the patient’s serum and mixing each dilution of the serum with a known quantity of antigen. The measurement of antibody is expressed in terms of titer.

- ❖ The **antibody titer** of a serum is the highest dilution that shows an observable reaction with the antigen
- ❖ Antigen titer can also be measured in the sera in similar fashion by testing the series of diluted sera against known quantity of antibody.

The problem with qualitative test is that if the number of antigen or antibody molecules in the reaction are disproportionate to each other and if either antigen or antibody are present in higher quantity, then the antigen antibody reaction does not take place optimally and often the result turns negative (false-negative). To rule out a false negative result, it is ideal to test a series of diluted sera (quantitative test), instead of just testing once on an undiluted serum. Quantitative tests are more reliable as they can differentiate between true negative and false-negative results. This can be explained by Marrack’s Lattice hypothesis.

Marrack’s Lattice Hypothesis

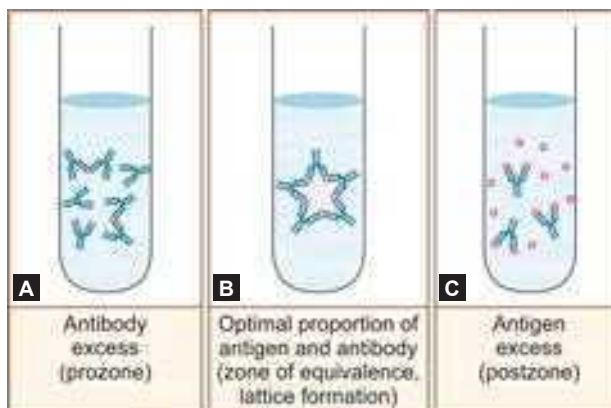
When the sera containing antibody is serially diluted (in normal saline), gradually the antibody level decreases. When a fixed quantity of antigen is added to such a set of test tubes containing serially diluted sera, then it is observed that the Ag-Ab reaction occurs at its best only in the middle test tubes where the amount of antigen and antibody are equivalent to each other (*zone of equivalence*). The Ag-Ab reaction is weak or fails to occur when the number of antigen and antibodies are not proportionate to each other (Figs 12.1A to C).

- In the earlier test tubes, *antibodies are excess*, hence the Ag-Ab reaction does not occur: This is called as **prozone phenomenon**
- In the later test tubes, *antigen is excess*, hence the Ag-Ab reaction fails to occur: This is called as **postzone phenomenon**.

Marrack (1934) proposed the **lattice hypothesis** to explain this mechanism. According to this concept the multivalent antigens combine with bivalent antibodies in varying proportions, depending on the antigen antibody ratio in the reacting mixture (Figs 12.1A to C).

- Ag-Ab reaction optimally occurs when a large lattice is formed consisting of alternating antigen and antibody molecules. This is possible only in the zone of equivalence
- In the zones of antibody or antigen excess (prozone/post zone), the lattice does not enlarge, due to inhibition of lattice formation by the excess antibody or antigen respectively.

Contd...



Figs 12.1A to C: A. Prozone; B. Zone of equivalence; C. Postzone.

Contd...

Lattice hypothesis was described first for precipitation reaction, but it also holds true for agglutination and other techniques of Ag-Ab reactions.

The prozone phenomenon is of great importance in clinical serology, as sera rich in high titer of antibody may sometimes give a false-negative result, unless serial dilutions of sera are tested.

Evaluation of Immunoassays

Evaluation of the performance of any diagnostic test including immunoassays can be done by calculating various statistical measures. Among all, sensitivity and specificity are the two most important statistical parameters.

Sensitivity is defined as ability of a test to identify correctly all those who have the disease, i.e. true-positives.

Sensitivity is calculated as = $\frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$

Specificity is defined as ability of a test to identify correctly all those who do not have disease, i.e. true negatives.

Specificity is calculated as = $\frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$

TYPES OF ANTIGEN–ANTIBODY REACTIONS

The antigen–antibody reactions used in diagnostic laboratories are based on various techniques which are broadly classified as conventional techniques and newer techniques (Table 12.1).

CONVENTIONAL IMMUNOASSAYS

PRECIPITATION REACTION

Definition

When a **soluble antigen** reacts with its antibody in the presence of optimal temperature, pH and electrolytes (NaCl), it leads to formation of the antigen–antibody complex in the form of:

- ❖ **Insoluble precipitation band** when gel or agar containing medium is used (called immunodiffusion) or

Table 12.1: Types of antigen–antibody reactions.

Conventional techniques	
• Precipitation reaction	• Complement fixation test
• Agglutination reaction	• Neutralization test
Newer techniques	
• Enzyme-linked immunosorbent assay (ELISA)	
• Enzyme-linked fluorescent assay (ELFA)	
• Immunofluorescence assay (IFA)	
• Chemiluminescence-linked immunoassay (CLIA)	
• Immunohistochemistry	
• Rapid tests	
➢ Lateral flow assay (Immunochromatographic test)	
➢ Flow through assay	
• Western blot	

❖ **Insoluble floccules** when liquid medium is used (called flocculation test).

Note: The results of immunodiffusion can be obtained quicker by carrying out the test in presence of electric current. This modification is called as **immuno-electrophoresis**. It has two variants such as countercurrent Immuno-electrophoresis (CIEP) and rocket electrophoresis.

Clinical Applications

Earlier, precipitation reactions were one of the widely used serological tests. However with the advent of simple and rapid newer techniques their application is greatly reduced. There are only limited situations where precipitation reaction is still in use; discussed below.

Slide Flocculation Test (for Syphilis)

It is used for serodiagnosis of syphilis, a sexually transmitted disease caused by *Treponema pallidum*.

- ❖ **Procedure:** When a drop of antigen is mixed with a drop of patient's serum (containing antibody) on a slide, then the precipitates formed remain suspended as floccules
- ❖ **Examples** include VDRL (Venereal Disease Research Laboratory) and RPR (Rapid Plasma Reagin) tests. Refer Chapter 77 for detail.

Elek's Gel Precipitation Test (Detecting Diphtheria Toxin)

The *Corynebacterium diphtheriae* strain isolated is streaked on to a medium containing a filter paper soaked with diphtheria antitoxin. (refer Chapter 60 for detail).

- ❖ If the strain is toxigenic, it produces the toxin, which diffuses in the agar, meets with the antitoxin and produces arrow-shaped precipitation band (Fig. 60.5)
- ❖ This test can also be used to know the relatedness between the strains isolated during an outbreak.

AGGLUTINATION REACTION

Definition

When a **particulate** or **insoluble** antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

❖ **Advantage:** Agglutination is more sensitive than precipitation test and the clumps are better visualized and interpreted as compared to bands or floccules. Hence, agglutination tests are widely used even in today's modern era of diagnosis

❖ **Applications:** Agglutination reactions are classified as direct, indirect (passive) and reverse passive agglutination reactions. All these agglutination tests are performed either on a slide, or in tube or in card or sometimes in microtiter plates.

Direct Agglutination Test

Here, the antigen directly agglutinates with the antibody.

Slide Agglutination

It is usually performed to confirm the identification and serotyping of bacterial colonies grown in culture. It is also the method used for blood grouping and cross matching.

Bacterial colony is mixed with a drop of saline on a slide to form a uniform smooth milky white suspension



To this, a drop of the antiserum (serum containing appropriate antibody) is added and the slide is shaken thoroughly (manually or by rotator) for few seconds



A positive result is indicated by visible clumping with clearing of the suspension (Fig. 12.2)



If the milky white suspension remains unchanged, indicates a negative result (Fig. 12.2)

Tube Agglutination

This is a quantitative test done for estimating antibody in serum. The **antibody titer** can be estimated as the highest dilution of the serum which produces a visible agglutination.

A fixed volume of a particulate antigen suspension is added to an equal volume of serial dilutions of a serum sample (containing appropriate antibody) in test tubes



Positive test indicates agglutination (clump formation at the bottom of the tube with clearing of the supernatant)



Negative test indicates agglutination has not occurred (Ag suspension forms button at the bottom of the tube)

Tube agglutination is routinely used for the serological diagnosis of various diseases, such as:

- ❖ Typhoid fever (Widal test): It detects antibodies against both H (flagellar) and O (somatic) antigens of *Salmonella* Typhi
 - H antigen–antibody clumps appear as loose fluffy clumps
 - O antigen–antibody clumps appear as chalky white granular dense deposits.
- ❖ Acute brucellosis (Standard agglutination test)

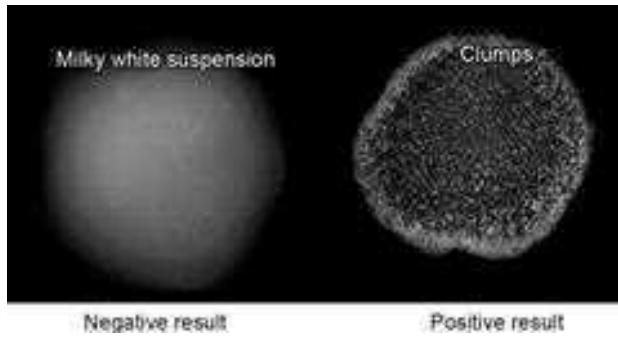


Fig. 12.2: Slide agglutination test.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ Coombs antiglobulin test (explained later in this chapter)
- ❖ Heterophile agglutination tests:
 - Typhus fever (Weil Felix reaction)
 - Infectious mononucleosis (Paul Bunnell test)
 - *Mycoplasma pneumonia* (Cold agglutination test).

Microscopic Agglutination

Here, the agglutination test is performed on a microtiter plate and the result is read under a microscope. The classical example is microscopic agglutination test (MAT) done for leptospirosis.

Indirect or Passive Agglutination Test (for Antibody Detection)

As agglutination test is more sensitive and better interpreted than precipitation test, attempt has been made to convert a precipitation reaction into an agglutination reaction. This is possible by coating the soluble antigen on the surface of a carrier molecule (e.g. RBC, latex or bentonite), so that the antibody binds to the coated antigen and agglutination takes place on the surface of the carrier molecule.

Indirect Hemagglutination Test (IHA)

It is a passive agglutination test where RBCs are used as carrier molecules. IHA was used widely in the past, but is less popular at present.

Latex Agglutination Test (LAT) for Antibody Detection

Here, polystyrene latex particles (0.8–1 μm in diameter) are used as carrier molecules which are capable of adsorbing several types of antigens. For better interpretation of result, the test is performed on a black color card.

- ❖ Drop of patient's serum (containing antibody) is added to a drop of latex solution coated with the antigen and the card is rotated for uniform mixing
- ❖ Positive result is indicated by formation of visible clumps (Fig. 12.3). LAT is one of the most widely used tests at present as it is very simple and rapid
- ❖ It is used for detection of ASO (antistreptolysin O antibody).

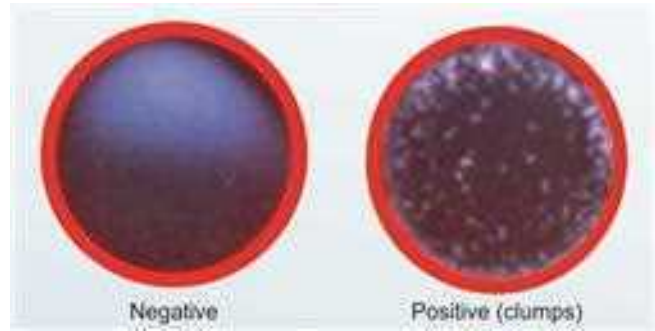


Fig. 12.3: Passive (latex) agglutination test.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Reverse Passive Agglutination Test (for Antigen Detection)

In this test, the antibody is coated on a carrier molecule which detects antigen in the patient's serum.

- ❖ **Reverse passive hemagglutination assay (RPHA):** Here, the RBCs are used as carrier molecules. RPHA was used in the past for detection of hepatitis B surface antigen (HBsAg); now obsolete
- ❖ **Latex agglutination test for antigen detection:** It is used widely for detection of CRP (C reactive protein), RA (rheumatoid arthritis factor), capsular antigen detection in CSF (for pneumococcus, meningococcus and *Cryptococcus*) and streptococcal grouping
- ❖ **Coagglutination test:** Here, *Staphylococcus aureus* (protein A) acts as carrier molecule. This test was used in the past to detect antigen from clinical specimens; now obsolete.

Hemagglutination Test

It refers to the agglutination tests that use RBCs as source of antigen. Hemagglutination tests are of two types: direct (described below) and indirect (or IHA, obsolete now).

Direct Hemagglutination Test

Serum antibodies directly agglutinate with surface antigens of RBCs to produce a matt. Examples include:

- ❖ **Paul Bunnell test:** It employs sheep RBCs as antigens to detect Epstein-Barr virus antibodies in serum. The test is performed in tubes
- ❖ **Cold agglutination test:** It uses human RBCs as antigens to detect *Mycoplasma* antibodies in serum. Test is performed in tubes
- ❖ **Blood grouping** (ABO and Rh grouping)
- ❖ **Coombs test or antiglobulin test:** It is performed to diagnose Rh incompatibility by detecting Rh antibody from mother's and baby's serum
 - Rh incompatibility is a condition when a Rh negative mother delivers a Rh positive baby (Rh Ag +ve). During birth, some Rh Ag +ve RBCs may pass from fetus to the maternal circulation and may induce Rh Ab formation in the mother, which may affect future Rh positive pregnancies

- **Rh antibodies** are **incomplete** or **blocking** antibodies of IgG type. They can cross placenta and bind to Rh Ag on fetal RBCs, but does not result in agglutination; instead, they block the sites on fetal RBCs
- This reaction can be visualized by Coombs test, which is carried out by adding **Coombs reagent**. It contains antibody to human IgG, which can bind to Fc portion of Rh Ab bound on RBCs, resulting in visible agglutination.

Viral Hemagglutination Test

In strict sense, it is not an antigen-antibody reaction. The hemagglutinin antigens (HA) present on surface of some viruses (hemagglutinating viruses, e.g. influenza virus) can agglutinate with the receptors present on the surface of RBCs.

Technical Issues in Agglutination Reactions

Two main problems pertaining to agglutination are prozone phenomenon and blocking antibody; both can cause false-negative agglutination test.

- ❖ **Prozone phenomenon:** Serum containing excess antibodies may fail to agglutinate with its antigen. This can be obviated by serial dilution of the serum and testing the antigen with each dilution of the serum sample
- ❖ **Blocking antibodies:** They are incomplete IgG antibodies. When they bind to antigens, they themselves cannot produce a visible agglutination; however, they can block the sites on antigens, thus prevent binding of any other antibodies to the antigens. Such blocking antibodies may be detected by performing the test in hypertonic (4%) saline or more reliably by adding antiglobulin or Coombs reagent.

COMPLEMENT FIXATION TEST

Complement fixation test (CFT) detects the antibodies in patient's serum that are capable of fixing with complements. It was once very popular, now is almost obsolete.

Applications

- ❖ CFT was widely used for detection of complement fixing antibodies in *Rickettsia*, *Chlamydia*, *Mycoplasma* infections and some viral infections, such as arboviral infections
- ❖ Complements are also used for various other serological tests such as: *Treponema pallidum* immobilization test for syphilis and Sabin-Feldman dye test for *Toxoplasma*.

NEUTRALIZATION TEST

Neutralization tests are also less commonly used in modern days. Various examples are as follows:

- ❖ **Viral neutralization test:** It detects the presence of neutralizing antibody in patient's serum. When the serum is mixed with a live viral suspension and poured onto a cell line, specific serum antibody neutralizes

the surface antigen, making the virus unable to infect a cell line

- ❖ **Plaque inhibition test:** This is done for bacteriophages
- ❖ **Toxin-antitoxin neutralization test:** Examples include
 - **Schick test:** It is a diphtheria toxin-antitoxin neutralization test
 - **Nagler's reaction:** It is used for detection of α -toxin of *Clostridium perfringens*
 - **ASO test:** Antistreptolysin O antibody was detected before by neutralization method; however, it is now replaced by latex agglutination.
- ❖ **Hemagglutination inhibition (HAI) test:** Antibodies in patient's sera can agglutinate with the hemagglutinin antigens present on the surfaces of some viruses. This test was used in the past for the diagnosis of various viral diseases, e.g. influenza.

NEWER TECHNIQUES

The newer techniques use a detector molecule to label antibody or antigen which in turn detects the corresponding antigen or the antibody in the sample by producing a visible effect. Most of the newer techniques use this common principle, but they differ from each other by the type of labeled molecule used and the type of visible effect produced (Table 12.2).

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA is an immunoassay that detects either antigen or antibodies in the specimen, by using enzyme-substrate-chromogen system for detection.

Principle of ELISA

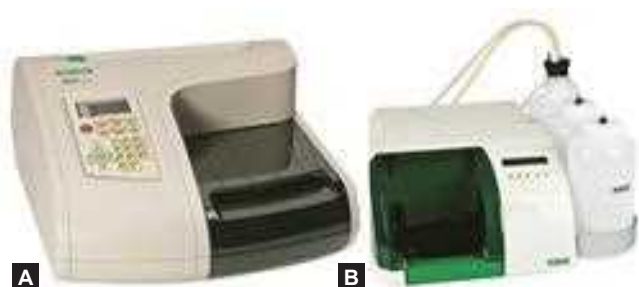
ELISA is so named because of its two components:

- ❖ **Immunsorbent:** Here, an absorbing material is used (e.g. polystyrene, polyvinyl) that specifically absorbs the antigen or antibody present in serum
 - ❖ **Enzyme** is used to label one of the components of immunoassay (i.e. antigen or antibody).
- Substrate-chromogen system:** A substrate-chromogen system is added at the final step of ELISA.
- ❖ The enzyme reacts with the substrate, which in turn activates the chromogen to produce a color
 - ❖ The classical example is, horseradish peroxidase used as enzyme which reacts with its substrate (hydrogen peroxide), that in turn activates the chromogen (tetramethyl benzidine) to produce a color
 - ❖ The color change is detected by spectrophotometry in an ELISA reader. Intensity of the color is directly proportional to the amount of the detection molecule (Ag or Ab) present in test serum.

(Ag-Ab complex)-enzyme + substrate → activates the chromogen → color change → detected by spectrophotometry (ELISA reader, Fig. 12.4A)

Table 12.2: Immunoassays and the types of molecule used for labeling .			
Abbreviation	Immunoassay method	Molecules used for labeling	Type of visible effect
ELISA	Enzyme-linked immunosorbent assay	Enzyme-substrate-chromogen complex	Color change is detected by spectrophotometer
ELFA	Enzyme-linked fluorescent assay	Enzyme-substrate	Fluorometric detection
IFA	Immunofluorescence assay	Fluorescent dye	Emits light, detected by fluorescence microscope
CLIA	Chemiluminescence-linked immunoassay	Chemiluminescent compounds	Emits light, detected by luminometer
IHC	Immunohistochemistry	Enzyme or fluorescent dye	Color change (naked eye) or fluorescence microscope
WB	Western blot	Enzyme	Color band (naked eye)
Rapid tests	Immuno chromatographic test	Colloidal gold or silver	Color band (naked eye)
	Flow-through assay	Protein A conjugate	Color band (naked eye)

Note: Radioimmunoassay (RIA) is used for quantitative detection of hormones, drugs or microbial antigens. Because of the radiohazard associated, its use is reduced.



Figs 12.4A and B: A. ELISA reader (Biorad); B. ELISA washer.

Source: Biorad Pvt. Ltd (with permission).

Procedure of ELISA

ELISA is performed on a microtiter plate containing 96 wells (Fig. 12.5), made up of polystyrene, polyvinyl or polycarbonate material.

- ❖ ELISA kits are commercially available; contain all necessary reagents (such as enzyme conjugate, dilution buffer, substrate/chromogen, etc.)
- ❖ The procedure involves a series of steps done sequentially. At each step, a reagent is being added, and then incubated, followed by washing of the wells [manually or by an automated ELISA washer (Fig. 12.4B)].

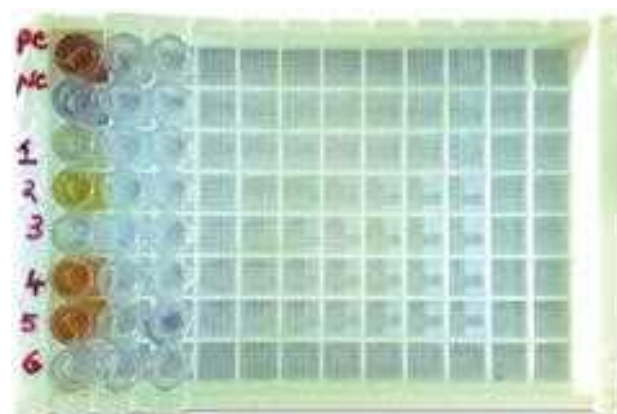
Types of ELISA

There are several types of ELISA, which differ from each other in their principles.

Direct ELISA

It is used for detection of antigen in test serum. Here, the primary antibody (targeted against the serum antigen) is labeled with the enzyme.

- ❖ **Step 1:** Wells of microtiter plate are empty, not precoated with Ag or Ab
- ❖ **Step 2:** Test serum (containing antigen) is added into the wells. Antigen becomes attached to the solid phase by passive adsorption
- ❖ **Step 3:** After washing, the enzyme-labeled primary antibodies (raised in rabbits) are added
- ❖ **Step 4:** After washing, a substrate–chromogen system is added and color is measured.



PC: Positive control; NC: Negative control
Interpretation: Sample No. 2, 4 & 5: Positive and
Sample No. 1, 3 and 6: Negative

Fig. 12.5: ELISA for HBsAg.

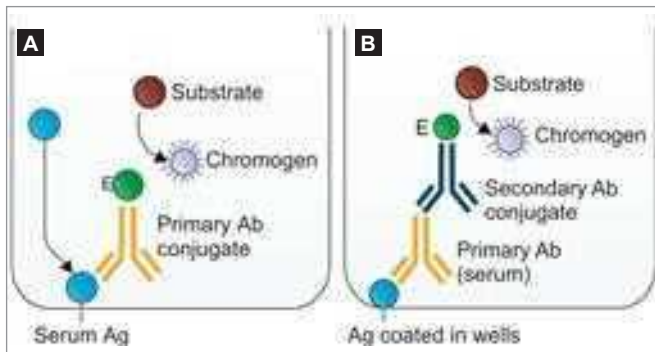
Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Well + Ag (test serum) + primary Ab-Enzyme + substrate-chromogen → Color change (Fig. 12.6A)

Indirect ELISA

It is used for detection of antibody or less commonly antigen in serum. It differs from the direct ELISA in that the secondary antibody is labeled with enzyme instead of primary antibody. The secondary antibody is an anti-species antibody, e.g. anti-human Ig (an antibody targeted to Fc region of any human Ig). Indirect ELISA for antibody detection is described below (Fig. 12.6B).

- ❖ **Step 1:** The solid phase of the wells of microtiter plates are precoated with the Ag
- ❖ **Step 2:** Test serum (containing primary Ab specific to the Ag) is added to the wells. Ab gets attached to the Ag coated on the well
- ❖ **Step 3:** After washing, enzyme-labeled secondary Ab (anti-human immunoglobulin) is added
- ❖ **Step 4:** After washing, a substrate-chromogen system is added and color is developed.



Figs 12.6A and B: **A.** Direct ELISA (for antigen detection); **B.** Indirect ELISA (for antibody detection).

Wells are coated with Ag + primary Ab (test serum) + secondary Ab-Enzyme + substrate- chromogen → development of color (Fig. 12.6B)

Sandwich ELISA

It detects the antigen in test serum. It is so named because the antigen gets sandwiched between a capture antibody and a detector antibody (Fig. 12.7A).

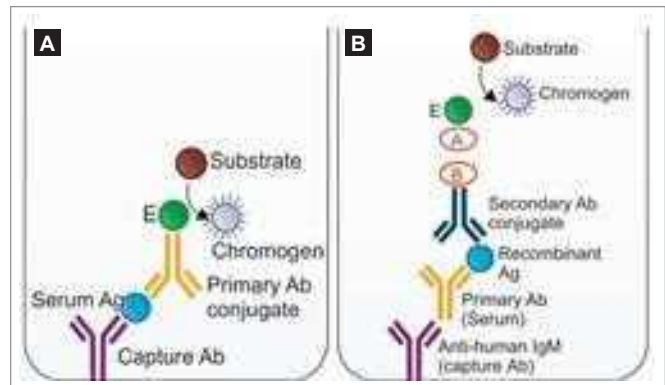
- ❖ **Step 1:** The microtiter well is precoated with the capture antibody (monoclonal Ab raised in rabbit) targeted against the test antigen
- ❖ **Step 2:** The test serum (containing antigen) is added to the wells. Ag gets attached to the capture antibody coated on the well
- ❖ **Step 3:** After washing, an enzyme labeled primary 'detector antibody' specific for the antigen is added. The detector antibody can be same as the capture antibody
- ❖ **Step 4:** After washing, a substrate-chromogen system is added and color is developed.

Wells coated with capture Ab + Ag (test serum) + primary Ab-enzyme + substrate-chromogen → color (Fig. 12.7A)

IgM Antibody Capture (MAC) ELISA

This is an enzymatically amplified sandwich-type immunoassay. This format of ELISA is widely used for dengue, Japanese encephalitis and West Nile virus, scrub typhus, leptospirosis, toxoplasmosis, etc. (Fig. 12.7B).

- ❖ It is based on capturing primary IgM Ab (in test serum) on a microtiter plate pre-coated with anti-human-IgM Ab, followed by addition of recombinant antigen (e.g. dengue antigen)
- ❖ Subsequently, enzyme labelled secondary antibody specific for the antigen is added, followed by addition of substrate-chromogen system
- ❖ The use of avidin-biotin system helps in amplifying the signal generated between enzyme-antibody complex, thus increases the sensitivity of the assay.



Figs 12.7A and B: **A.** Sandwich ELISA (for antigen detection); **B.** IgM antibody capture (MAC) ELISA.

Wells coated with capture anti-IgM Ab + IgM Ab (test serum) + recombinant antigen + secondary Ab-biotin + avidin-enzyme + substrate-chromogen → color (Fig. 12.7B)

Competitive ELISA

Competitive ELISA is so named because, antigen in test serum competes with another antigen of the same type coated on well to bind to the primary antibody.

- ❖ **Step 1:** Primary antibody is first incubated in a solution with a serum sample containing the test antigen
- ❖ **Step 2:** This antigen-antibody mixture is then added to the microtiter well precoated with the same type of antigen
- ❖ **Step 3:** The free antibodies bind to the antigen coated on the well. More the test antigens present in the sample, lesser free antibodies will be available to bind to the antigens coated onto well
- ❖ **Step 4:** After washing (to remove free antibodies and antigens), enzyme-conjugated secondary antibody is added
- ❖ **Step 5:** After washing, a substrate-chromogen system is added and color is developed. Intensity of the color is inversely proportional to the amount of antigen present in the test serum (Fig. 12.8).

The competitive ELISA can also be used for the detection of antibody in serum. More so, different formats of competitive ELISA are available such as direct, indirect and sandwich formats. The example given above is an indirect competitive ELISA format used for antigen detection (Fig. 12.8).

ELISPOT Test

It is modification of ELISA that allows the quantitative detection of cells producing antibodies (plasma cells) or cytokines (e.g. lymphocytes or macrophages). ELISPOT is currently used in IGRA (interferon gamma assay), for diagnosis of latent tuberculosis; where the sensitized T cells capable of producing the IFN- γ are measured.

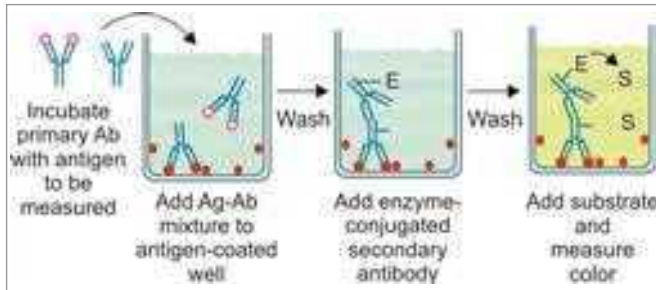


Fig. 12.8: Competitive ELISA for antigen detection.

IgG Avidity ELISA

This test is useful for differentiating recent from past infection.

- Usually, recent and past infections are differentiated by presence of IgM and IgG antibodies respectively. However, in situations where both the antibodies are present, IgG avidity test is useful to differentiate recent from past infection.
- **Principle:** Avidity of an antibody indicates how firmly it is bound with its antigen. Avidity reflects the maturity of the antibodies; which usually increases with time. Therefore, detection of low avidity IgG indicates recent infection, whereas in past infection high avidity IgG will be detected.
- **Applications:** IgG avidity ELISA is available for diagnosis of following infections—rubella, CMV, VZV, toxoplasmosis, EBV, HIV, viral hepatitis and West Nile virus infection.

Advantages of ELISA

ELISA is the method of choice for detection of antigens/antibodies in serum in modern days, especially in big laboratories as large number of samples can be tested together using the 96 well microtiter plate.

- ❖ It is economical, takes 2–3 hours for performing the assay
- ❖ ELISA has a high sensitivity; that is why, it is commonly used for performing screening test at blood banks and tertiary care sites
- ❖ Its specificity used to be low. But now, with use of more purified recombinant and synthetic antigens, and monoclonal antibodies, ELISA has become more specific.

Disadvantages of ELISA

- ❖ In small laboratories having less sample load, ELISA is less preferred than rapid tests as the latter can be performed on individual samples
- ❖ It takes more time (2–3 hours) compared to rapid tests which take 10–20 minutes
- ❖ It needs expensive equipment such as ELISA washer and reader.

Applications of ELISA

ELISA can be used both for antigen and antibody detection.

- ❖ ELISA used for antigen detection: Hepatitis B [hepatitis B surface antigen (HBsAg) and precore antigen (HBeAg)], NS1 antigen for dengue, etc.

- ❖ ELISA can also be used for antibody detection against hepatitis B, hepatitis C, HIV, dengue, EBV, HSV, toxoplasmosis, leishmaniasis, etc.

ENZYME-LINKED FLUORESCENT ASSAY (ELFA)

It is an modification of ELISA, differs from ELISA in two ways: (i) automated system, all steps are performed by the instrument itself, (ii) Ag-Ab-enzyme complex is detected by fluorometric method. VIDAS and miniVIDAS (bioMérieux) are commercially available systems based on ELFA technology (Fig. 12.9).

- ❖ **Procedure:** The solid phase receptacle (Fig. 12.9) present in reagent strip (equivalent to wells in microtiter plate in ELISA) serves as the solid phase; which is either coated with capture antigen (for antibody detection) or antibody (for antigen detection)

- The conjugate used here is either an antigen or antibody labeled with enzyme alkaline phosphatase and the substrate used is 4-methyl-umbelliferyl phosphate
- Following Ag-Ab-enzyme conjugate complex formation; the excess of enzyme conjugate are washed out
- Then the conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm
- The intensity of the fluorescence depends on the concentration of alkaline phosphatase present which in turn depends upon the amount of analyte (target Ag or Ab) in the sample.

- ❖ **Advantages:** It has many advantages over ELISA: (i) an automated system, (ii) easy to perform and user friendly, (iii) less contamination chance, (iv) gives quantitative results and (v) more sensitive and specific

- ❖ **Disadvantages:** (i) Expensive, (ii) can run only 12–24 number of tests at a time, (iii) can run 2–4 types of tests at a time

- ❖ **Use:** It can be used to detect numerous parameters
 - *Infectious diseases:* Markers of hepatitis viruses and HIV (Ag and Ab), Ab to TORCH infection, measles, mumps, varicella, *H. pylori* and antigen to *C. difficile*, Rotavirus, etc.



Fig. 12.9: miniVIDAS system and reagent strip (first well is solid phase receptacle coated with Ag or Ab and other wells contain various reagents).

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- **Other uses:** Biomarkers (e.g. procalcitonin), hormones (e.g. thyroid), tumor markers, cardiac markers and screening for allergy.

IMMUNOFLUORESCENCE ASSAY (IFA)

It is a technique similar to ELISA, but differs by some important features:

- ❖ Fluorescent dye is used instead of enzyme for labeling of antibody
- ❖ It detects cell surface antigens. It is also used to detect antibodies bound to cell surface antigens, unlike ELISA which detects free antigen or antibody.

Principle

Fluorescence refers to absorbing high energy-shorter wavelength ultraviolet light rays by the fluorescent compounds and in turn emitting visible light rays with a low energy-longer wavelength.

- ❖ The fluorescent dye is used to conjugate the antibody and such labeled antibody can be used to detect the antigens or antigen-antibody complexes on the cell surface
- ❖ The fluorescent compounds commonly used is fluorescein isothiocyanate (FITC).

Types

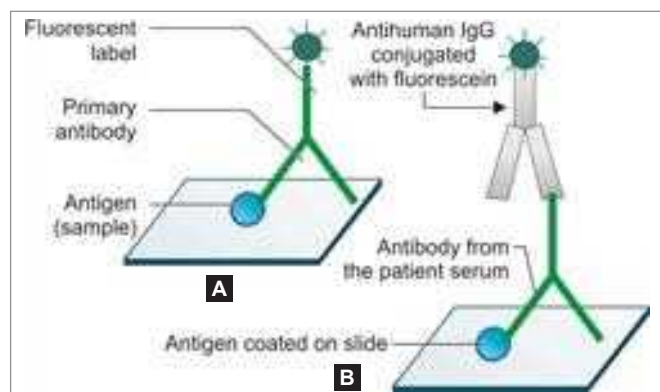
Direct Immunofluorescence Assay

- ❖ **Step 1:** Sample containing cells carrying surface antigens is smeared on a slide
- ❖ **Step 2:** Primary antibody specific to the antigen, tagged with fluorescent dye is added
- ❖ **Step 3:** Slide is washed to remove the unbound antibodies and then viewed under a fluorescence microscope (Fig. 12.10A).

Indirect Immunofluorescence Assay

This detects antibodies in sample. Slides smeared with cells carrying known antigens are commercially available.

- ❖ **Step 1:** Test serum containing primary antibody is added to the slide



Figs 12.10A and B: Immunofluorescence assay: A. Direct; B. Indirect.

- ❖ **Step 2:** Slide is washed to remove the unbound antibodies. A secondary antibody (antihuman antibody conjugated with fluorescent dye) is added
- ❖ **Step 3:** Slide is washed and then viewed under a fluorescence microscope (Fig. 12.10B).

Applications: Immunofluorescence assay has various applications, such as:

- ❖ Detection of autoantibodies (e.g. antinuclear antibody) in autoimmune diseases
- ❖ Detecting microbial antigens, e.g. rabies antigen in corneal smear
- ❖ Detection of viral antigens in cell lines inoculated with the specimens.

Flow Cytometry

Flow cytometry is a laser-based technology that quantitatively analyses and separates the cells as they pass through the laser beam. Flow cytometry can be used to analyze multiple parameters of cells (e.g. leukocytes) such as cell counting, cell sorting, analysis of size, shape, granularity, DNA or RNA content of a cell, etc. Important applications include:

- ❖ CD4 T cell count in HIV infected patients
- ❖ Detection of leukocytes with specific markers for the diagnosis of various lymphomas.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry refers to the process of detecting antigens (e.g. proteins) in cells of a tissue section by exploiting the principle of using labeled antibodies binding specifically to the antigens in biological tissues.

- ❖ It can be based on principles of ELISA or IFA:
 - The antibody (directed against tissue antigen) is conjugated to an enzyme, such as peroxidase, that can catalyse a color-producing reaction (immunoperoxidase staining)
 - Alternatively, the antibody can also be tagged to a fluorescent dye.
- ❖ It is widely used in the diagnosis of abnormal cells (e.g. tumor cells).

CHEMILUMINESCENCE-LINKED IMMUNOASSAY (CLIA)

Chemiluminescence refers to the emission of light (luminescence), as a result of a chemical reaction. The principle of CLIA is similar to that of ELISA; however, the chromogenic substance is replaced by chemiluminescent compounds (e.g. luminol and acridinium ester) that generate light during a chemical reaction (luxogenic). The light (photons) can be detected by a photomultiplier, also called as luminometer (Fig. 12.11).

(Ag-Ab complex)-enzyme (e.g. HRP) + chemiluminescent substrate (e.g. luminol and acridinium ester) → product+ light (photons) → detected by luminometer or photomultiplier.



Fig. 12.11: Chemiluminescence system (CLIA) and its principle.
Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Advantages of CLIA

CLIA claims to be 10 times more sensitive than ELISA.

- ❖ CLIA can be further modified by using an enhancer that potentiates the chemical reaction. This gives CLIA an overall improvement of 200 folds over ELISA
- ❖ Most samples have no ‘background’ signal, i.e. luminol compounds do not themselves emit light
- ❖ Measurement of chemiluminescence is not a ratio unlike the measurement of fluorescence (IFA) and or color (ELISA)
- ❖ Individual specimens can be tested in CLIA in contrast to ELISA which is preferred for testing multiple samples at a time.

Applications

CLIA has limited applications in diagnostic microbiology compared to ELISA. Currently, it is available for detection of antigens or antibodies against various infections such as hepatitis viruses, HIV, TORCH infections and biomarkers such as procalcitonin.

WESTERN BLOT

Western blot detects specific proteins (antibodies) in a sample containing mixture of antibodies each targeted against different antigens of same microbe.

- ❖ It is so named for its similarity to Southern blot (detects DNA fragments) and Northern blot (detects mRNAs)
- ❖ **Eastern blot** is the latest addition to the list; it is a modification of Western blot, which detects the carbohydrate epitopes present on proteins or lipids.

Procedure

Western blot comprises of three basic components as follows:

- ❖ **SDS PAGE:** This is a method which separates complex protein antigen mixture into individual fragments by treating with a strong denaturing detergent called **SDS** (sodium dodecyl sulfate). Then this mixture is subjected to **PAGE** (polyacrylamide gel electrophoresis), which separates the antigen fragments according to their molecular weight
- ❖ **NCM blotting:** Here, the antigen fragments in the gel are transferred (blotted) to a nitrocellulose membrane

(NCM) sheet by placing the gel over the NCM sheet and then passing the electric current

- ❖ **Enzyme immunoassay:** NCM strip is then treated with patient’s sample containing **antibodies**. Individual serum antibodies will bind to the respective antigen fragments present on NCM (thus making western blot **highly specific**), which can be subsequently detected by adding enzyme-linked anti-human Ig. Substrate/ chromogen is added for the development of colored bands.

Applications

Western blot has an excellent specificity. Hence, it is often used as a supplementary test to confirm the result of ELISA or other immunoassays having higher sensitivity. Western blot formats are available to detect antibody in various diseases such as HIV, Lyme’s disease, Herpes simplex virus infection, cysticercosis, hydatid disease and toxoplasmosis.

RAPID TESTS

Rapid tests are revolutionary in the diagnosis of infectious diseases. They are very simple to perform (one step method), rapid (takes 10–20 minutes), require minimal training, do not need any sophisticated instruments.

- ❖ These tests are also called **Point-of-care** (POC) tests, because unlike ELISA and other immunoassays, the POC tests can be performed independent of laboratory equipment and deliver instant results
- ❖ Two principles of rapid tests are available—lateral flow assay and flow through assay
- ❖ Both the formats are available for the diagnosis of various diseases such as malaria, hepatitis B, hepatitis C, HIV, leptospirosis, *Helicobacter pylori*, syphilis, etc.

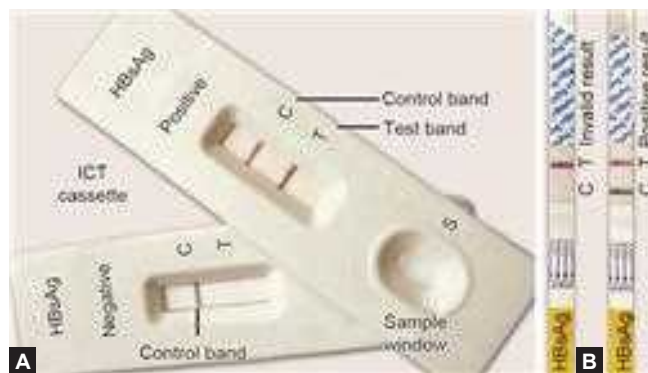
Immunochromatographic Test (Lateral Flow Assay)

Immunochromatographic test (ICT) is based on lateral flow technique. It is widely used in diagnostic laboratories because of its simplicity, low-cost and rapidity. It can be used for both antigen and antibody detection in sample. Principle of antigen detection method is described below.

Principle of ICT (Antigen Detection)

The test system consists of a nitrocellulose membrane (NCM) and an absorbent pad. Two formats are available: cassette or strip (Figs 12.12A and B). The NCM is coated at two places in the form of lines—a test line, coated with monoclonal antibody targeted against the test antigen and a control line, coated with anti-species immunoglobulin. Specific Ab against the target Ag labelled with chromogenic marker (specific Ab tagged with *colloidal gold* or *silver*, a visually detectable marker) is infiltrated in the absorbent pad lining the sample window.

- ❖ The sample (serum) containing the test antigen is added to sample well; it reacts with antibody labeled with chromogenic marker (*colloidal gold or silver*, a visually detectable marker)
- ❖ Both 'Ag-specific Ab-colloidal gold complex' as well as the 'free colloidal gold labeled Ab' move laterally along the nitrocellulose membrane
- ❖ **Test band:** At the test line, the Ag-labeled Ab complex is immobilized by binding to the monoclonal Ab in the test line to form a colored band (Fig. 12.12)
- ❖ **Control band:** The free colloidal gold labeled Ab can move further and binds to the anti-human Ig to form a color control band. If the control band is not formed, then the test is considered invalid irrespective of whether the test band is formed or not (Fig. 12.12).



Figs 12.12A and B: ICT for HBsAg detection:
A. Cassette format; B. Strip format.

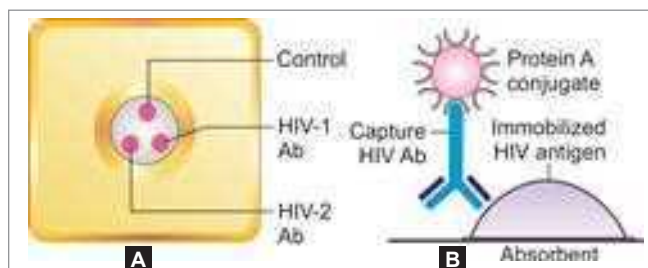
Source: Department of Microbiology, Pondicherry Institute of Medical sciences, Puducherry (with permission).

Flow-through Assay

Flow-through tests are another type of rapid diagnostic assays which differ from ICT in two aspects: (1) protein A is used for labeling antibody instead of gold conjugate and (2) the sample flows vertically through the nitrocellulose membrane (NCM) as compared to lateral flow in ICT.

Flow-through tests can be used for both antigen and antibody detection. HIV TRIDOT test is a classical example (described below, Fig. 12.13A). It detects antibodies to HIV-1 and 2 separately in patient's serum.

- ❖ The test system is in a cassette format, consisting of a NCM and an absorbent pad. The NCM is coated at three regions- two test regions coated with HIV-1 and 2 antigens and a third control region coated with antihuman Ig
- ❖ Sample and buffer reagents are added sequentially from the top following which they pass through the membrane and excess fluid is absorbed into the underlying absorbent pad
- ❖ As the patient's sample passes through the membrane, HIV antibodies, if present bind to the immobilized antigens (Fig. 12.13B)



Figs 12.13A and B: Flow-through assays: A. HIV TRI-DOT assay for HIV 1 and 2 antibodies detection; B. Principle of HIV TRI-DOT.

- ❖ **Test dots:** Protein-A conjugate (present in buffer) binds to the Fc portion of the HIV antibodies to give distinct pinkish purple DOT(s), separately for HIV-1 and 2 antibodies
- ❖ **Control dot:** Irrespective of whether the HIV antibodies are present or not, protein-A can bind to any IgG present in serum and the IgG-protein A complex can further bind to the antihuman Ig at the control line to give a pinkish purple DOT.

EXPECTED QUESTIONS

I. Write essay on:

1. Enumerate the properties and types of antigen-antibody reactions. Describe in detail about the principle, types, and applications of ELISA?
2. Describe in detail about the principle, types, and applications of agglutination reaction?

II. Write short notes on:

1. Indirect immunofluorescence assay.
2. Immunochromatographic test.
3. Chemiluminescence immunoassay (CLIA).

III. Multiple Choice Questions (MCQs):

1. Prozone phenomenon is due to:
 - a. Excess antigen

- b. Excess antibody
- c. Hyperimmune reaction
- d. Both antigen and antibody excess

2. All are agglutination reactions, except:

- a. VDRL test
- b. Standard agglutination test
- c. Widal test
- d. Paul Bunnell test

3. The following methods of diagnosis utilize labeled antibodies, except:

- a. ELISA
- b. CLIA
- c. Precipitation test
- d. Immunofluorescence

Answers

1. b 2. a 3. c

CHAPTER PREVIEW

- General Properties
- Complement Pathways
 - Effector Functions of Complement
- Regulation of Complement Pathways
- Complement Deficiencies

GENERAL PROPERTIES

The term 'complement' (C) represents a group of proteins normally found in serum in inactive form, but when activated they augment the immune responses. They constitute about 5% of normal serum proteins and their level does not increase following either infection or vaccination. Complements have the following general properties:

- ❖ **Bind to Fc region of antibody:** The effector function of complement is mediated by binding with Fc portion of antibody. The binding of complement to an antibody is described by various terms as, **fixing** or **consumption** (as it disappears from serum following binding)
- ❖ **Role of antigen:** The classical pathway of complements do not bind to free antibodies but they can only fix to those antibodies which are bound with antigens. However fixation of complement is not influenced by the nature of antigens, but only by the class of antibody
- ❖ **Species nonspecific:** Complements are present in the sera of all mammals, birds, amphibians and fish. Complements from one species can react with antibodies from other species, though the efficiency decreases with increase in taxonomic distance
- ❖ **Heat labile:** Complements get denatured by heating the serum at 56°C for 30 minutes. Such serum with lost complement activity is called **inactivated serum**.

Complement Components

The complement system comprises of about 30 serum proteins grouped into complement components, the properdin system and the regulatory proteins.

- ❖ The complement components are named by numerals. There are nine components; C1 to C9. C1 has three subunits—C1q, C1r and C1s
- ❖ The properdin system and the regulatory proteins are named by letter symbols, e.g. factor-B.

Synthesis

Liver is the major site for synthesis of complement proteins. Other minor sites include blood monocytes, tissue

macrophages, and epithelial cells of GIT and genitourinary tract.

Complement Activation

All the complement proteins are synthesized in inactive form (e.g. zymogens) and are activated by proteolysis.

- ❖ Complements have two unequal fragments (large and small fragment)
- ❖ The larger fragments are usually designated as 'b' (e.g. C3b) and the smaller fragments are designated as 'a' (e.g. C3a). An exception is C2a which is larger fragment
- ❖ During proteolysis, the smaller fragment is removed exposing the active site of the larger fragment
- ❖ The larger fragment participates in the cascade reaction of complement pathway and the smaller fragment diffuses away to mediate other functions
- ❖ **Cascade reaction:** The fragments of complements interact in a definite sequential manner with a cascade like effect, which leads to formation of complex. Such complex having enzymatic activity is designated by putting a bar over the number or symbol (e.g. $\bar{C}bBb$).

COMPLEMENT PATHWAYS

There are three pathways of complement activation:

1. **Classical pathway:** This is an antibody dependent pathway. Pathway is triggered by the antigen-antibody complex formation
2. **Alternative pathway:** This is an antibody independent pathway, triggered by the antigen directly
3. **Lectin pathway:** This is a recently described pathway. It resembles classical pathway, but it is antibody independent.

Stages of complement activation

There are four main stages in the activation of any of the complement pathways.

1. Initiation of the pathway
2. Formation of C3 convertase
3. Formation of C5 convertase
4. Formation of membrane attack complex (MAC).

All the three pathways (Fig. 13.1) differ from each other in their initiation till formation of C3 convertase. Then, the remaining stages are identical in all the pathways.

Classical Pathway

Classical pathway is antibody dependent. However, not all antibodies can bind to complements of classical pathway. Decreasing order of ability of antibodies to fix complement is—IgM (most potent) > IgG3 > IgG1 > IgG2. The other classes of antibodies do not fix complements. C_H2 domain on IgG, C_H4 on IgM participate in complement binding. The classical pathway begins with activation of C1 and binding to antigen-antibody complex.

Initiation

The first step is the binding of C1 to the antigen-antibody complex (Fig. 13.1).

- ❖ The first binding portion of C1 is C1q, which reacts with the Fc portion of IgM or IgG bound to antigen
- ❖ C1q is a hexamer having six globular heads each acting as a combining site
- ❖ Effective activation of classical pathway begins only when C1q is attached to the Fc portion of antibody by at least two of its globular binding sites
 - IgM being pentameric, has five Fc regions, hence one molecule of IgM can initiate the pathway
 - Whereas IgG is monomeric, therefore two IgG molecules are needed to initiate the process. Hence, IgM is much efficient stimulator of classical pathway.
- ❖ C1q binds in presence of calcium ions, which in turn activates sequentially C1r followed by C1s.

Formation of C3 Convertase

Activated C1s acts as an esterase (C1s esterase), which can cleave C4 to produce C4a (an anaphylatoxin), and C4b which binds to C1 and participates further in complement cascade.

- ❖ C14b in the presence of magnesium ions cleaves C2 into C2a, which remains linked to complement complex, and C2b (has kinin like activity), which is released outside
- ❖ C14b2a is referred to as C3 convertase of the classical pathway.

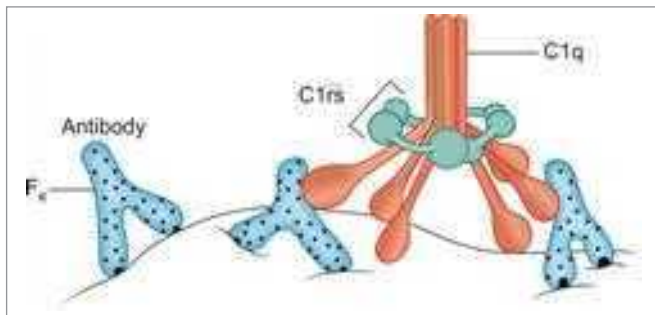


Fig. 13.1: Activation of C1 (initiation step of classical complement pathway).

Formation of C5 Convertase

C3 convertase hydrolyses many C3 molecules into two fragments: C3a (an anaphylatoxin) and C3b which remains attached to C14b2a to form C14b2a3b complex, which acts as C5 convertase of classical pathway.

Formation of Membrane Attack Complex

This phase begins with C5 convertase cleaving C5 into C5a (an anaphylatoxin, released into the medium) and C5b, which continues with the cascade.

- ❖ C5b is extremely labile, gets stabilized by binding soon with C6 and C7 to form C5b67 followed by addition of C8
- ❖ The hydrophobic regions on C7 and C8 help in penetration into the target cell membrane
- ❖ This inserted membrane complex (C5b678) has a catalytic property to bind to C9 molecule and then it polymerizes the C9 into a tubular channel of 10 nm diameter
- ❖ Penetration of C9 causes formation of channels or pores on the target cell membrane
- ❖ Each tubular channel behaves hydrophobic outside, but hydrophilic inside; thus allowing free passage of ions and water into the cell leading to cellular swelling and lysis
- ❖ Because C5b6789 destroys the target cell by attacking the cell membrane; it is called membrane attack complex (MAC) and the process of cytolysis is referred to as complement-mediated cytotoxicity (Figs 13.2 and 13.3).

Alternative Pathway

Alternative pathway (Fig. 13.3) is independent of antibody; hence is considered as a part of innate immunity. It also goes through the four stages; but differs from the classical pathway in first two stages. Unlike the classical pathway which involves all complement components from C1 to C9; in alternative pathway three complement components C1, C4 and C2 are not involved. Instead, it requires three other complement proteins present in serum named factor B, factor D and properdin.

Initiation

The alternative complement cascade is initiated by various cell surface constituents that are foreign to the host, e.g. bacterial endotoxin (Table 13.1).

The first complement component to be involved in alternative pathway is free C3 in the serum. C3 hydrolyzes spontaneously, to generate:

- ❖ C3a which diffuses out and

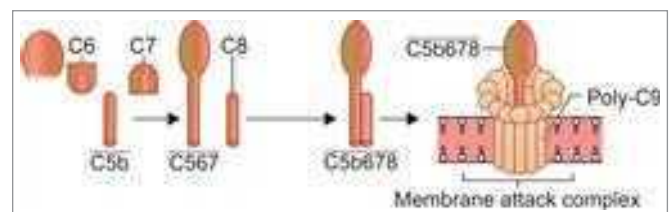


Fig. 13.2: Formation of membrane attack complex.

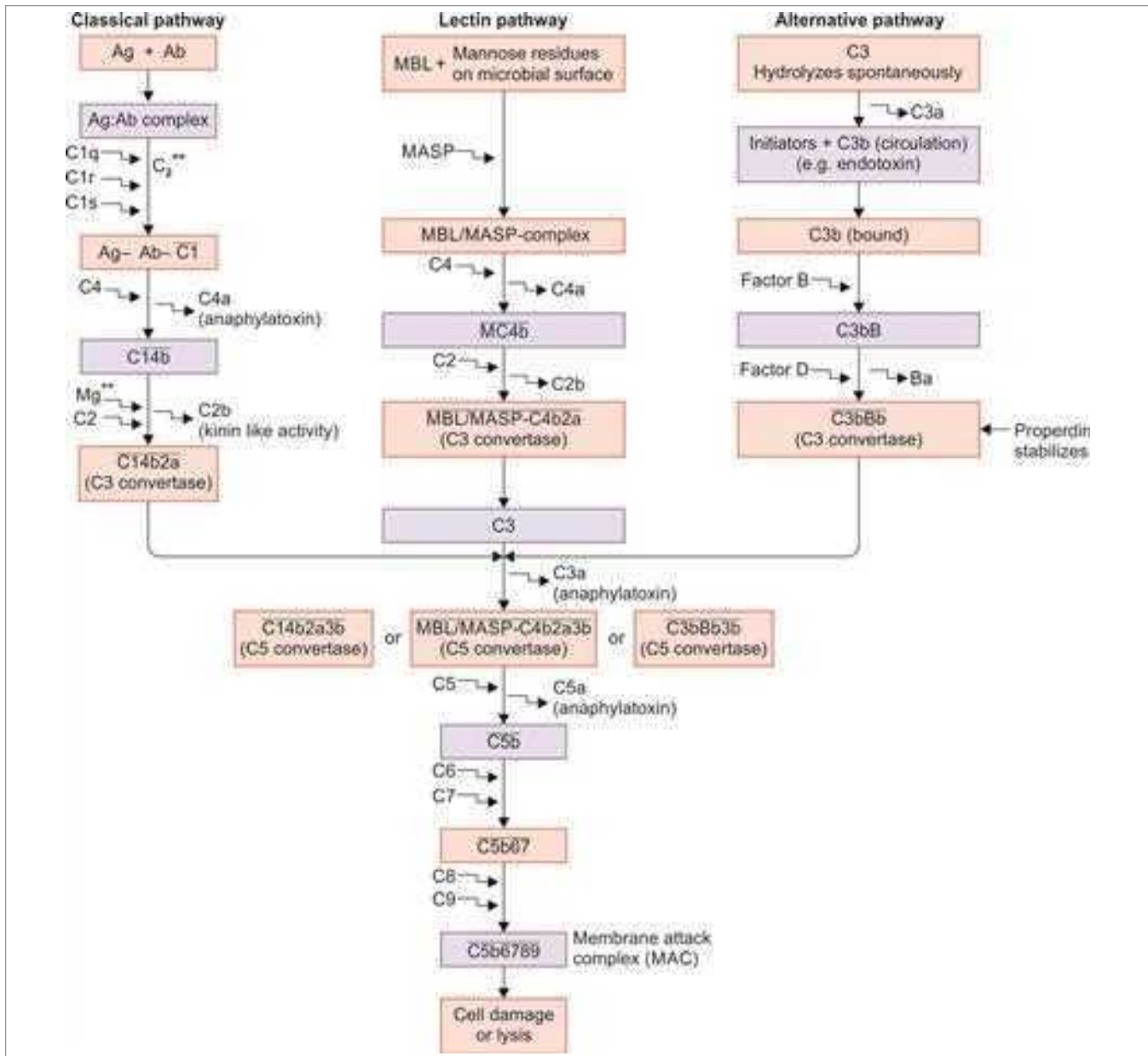


Fig. 13.3: The complement pathways.

Abbreviations: MBL, mannose binding lectins; MASP, MBL-associated serine protease.

Table 13.1: Initiators of alternative pathway.	
Antigens from pathogen	Nonmicrobial initiators
Endotoxin or LPS (lipopolysaccharide) from gram-negative bacteria	Human antibodies in complexes— IgA, IgD
Teichoic acid from gram-positive bacteria	Tumor cells
Fungal cells—Yeast cells	Cobra venom factor
Parasites like trypanosomes	Heterologous RBCs from mouse, rabbit and chicken
Virus-infected cells	Anion polymer like dextran sulfate
	Pure carbohydrates like agar and inulin

- ❖ C3b fragment which attaches to foreign cell surface antigen.

Formation of C3 Convertase

- ❖ In the next step, **Factor B** binds to C3b coated foreign cells
- ❖ **Factor D**, another alternative pathway complement factor, acts on factor B, and cleaves it into Ba (diffuses out) and Bb (remains attached)
- ❖ C3bBb is also called C3 convertase of alternative pathway
- ❖ C3bBb has a very short half-life of 5 minutes. If it is stabilized by another complement protein called properdin its half-life is increased to 30 minutes.

The remaining two stages, i.e. formation of C5 convertase and formation of membrane attack complex are identical to that of classical pathway.

Lectin Pathway

Lectin pathway is another complement pathway of innate immunity described recently, that works independent of antibody.

- ❖ It is mediated through lectin proteins of the host that interact with mannose residues present on microbial surface; hence the name lectin pathway
- ❖ Among the four stages, the first stage differs from classical pathway
- ❖ Lectin pathway involves all complement components used for classical pathways except C1 (i.e. from C2 to C9); Instead of C1, host lectin protein called mannose binding lectins mediate the first 'initiation' stage (see Fig. 13.3).

Initiation

Antigens that activate lectin pathway are the mannose carbohydrate residues of glycoproteins present on microbial surfaces.

- ❖ A specific host lectin protein called mannose binding lectins (MBL) bind to mannose residues on microbial surface
- ❖ MBL is an acute phase reactant protein, similar to C1q in structure
- ❖ After binding of MBL to microbial surface, another host protein called MBL-associated serine protease (MASP) gets complexed with MBL
- ❖ MASP is similar or C1r and C1s and mimics their functions
- ❖ The remaining three stages are similar to the classical pathway
- ❖ The MBL-MASP complex cleaves C4 which in turn splits C2 and the MBL/MASP-C4b2a acts as C3 convertase.

Important differences between the three complement pathways are summarized in Table 13.2.

EFFECTOR FUNCTIONS OF COMPLEMENT

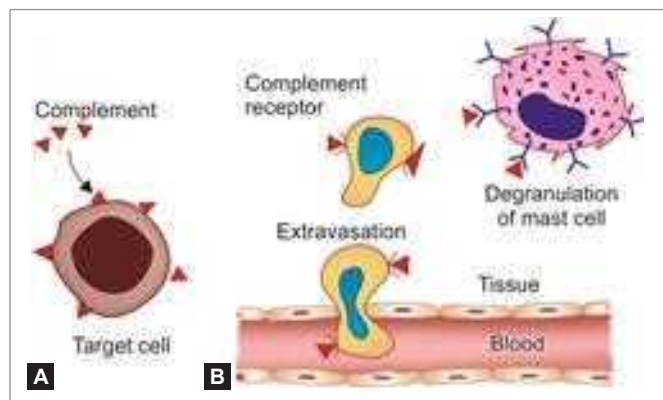
The membrane attack complex (MAC) and other complement by-products produced during the activation of complement pathways augment the immune response in many ways; which are collectively called as the effector functions of complement products. The functions are as follows:

1. **Target cell lysis by MAC:** As already explained, the MAC makes pores or channels in the target cell membrane; thereby allows the free passage of various ions and water into the cell leading to cell swelling, lysis and death. Bacteria, enveloped viruses, damaged cells, tumor cells, etc. are killed by this mechanism, commonly referred to as complement-mediated cell lysis (Fig. 13.4A)

Table 13.2: Differences between the three complement pathways.

Features	Classical pathway	Alternative pathway	Lectin pathway
Activator (initiator)	Antigen-antibody complex	Endotoxin IgA, IgD, cobra venom, Nephritic factor	Carbohydrate residue of bacterial cell wall (mannose binding protein) that binds to host lectin antigen
First complement activated	C1	C3b	C4
C3 convertase	C14b2a	C3bBb	MBL/MASP-C4b2a
C5 convertase (C3 convertase + 3b)	C14b2a3b	C3bBb3b	MBL/MASP-C4b2a3b
Complement levels in the serum	All C1-C9: Low	• C1, C4, C2: Normal • Others: Low	• C1: Normal • Others: Low
Immunity	Acquired	Innate	Innate

2. **Inflammatory response:** Complement by-products such as C3a, C4a and C5a are called anaphylatoxins. They bind to surface receptors of mast cells and induce their degranulation leading to release of histamine and other inflammatory mediators. They cause vasoconstriction, and increased vascular permeability (Fig. 13.4B)
3. **Opsonization:** C3b and C4b act as major opsonins that coat the immune complexes and particulate antigens. Phagocytic cells express complement receptors (CR1, CR3 and CR4) for complement components (C3b, C4b), and are able to bind to complement coated antigens and enhance phagocytosis (Fig 13.5). C5a augments this process by enhancing the CR1 expression on phagocytes by 10 folds
4. **Removing the immune complexes from blood:** C3b plays an important role in removing immune complexes from the blood. C3b bound immune complexes are



Figs 13.4A and B: A. Complement mediated cell lysis; **B.** Activation of inflammatory response.

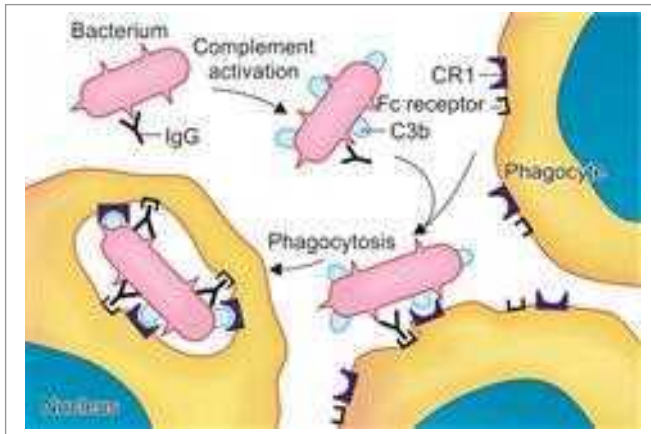


Fig. 13.5: Complement-mediated opsonization.

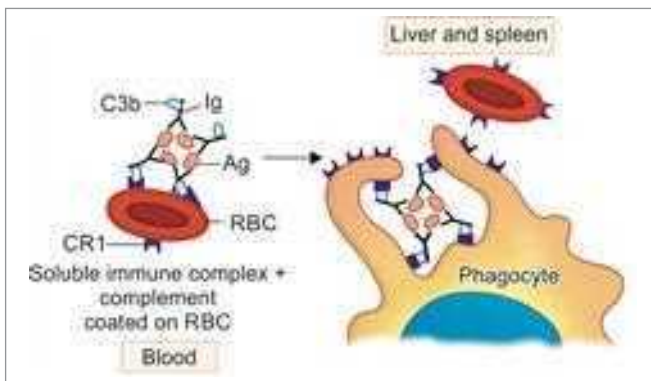


Fig. 13.6: Clearance of immune complexes.

recognized by complement receptor CR1 present on RBCs. Immune complexes bound to RBCs are taken to liver and spleen where they are phagocytosed after being separated from the RBCs (Fig. 13.6)

5. **Viral neutralization:** Complements play a crucial role in neutralization of the viruses; which occurs by many ways—
 - Complements coated on virus surfaces neutralize the viral infectivity by blocking their attachment sites
 - C3b mediated opsonization of viral particles
 - Lysis of the enveloped viruses either by activation of classical pathway (most viruses) or some time by alternative or lectin pathways (by some viruses like Epstein-Barr virus, rubella virus, etc.).

COMPLEMENT RECEPTORS

Complement receptors (CRs) play an important role in mediating the activities of complement products as well as in regulating their activities.

- ❖ There are many complement receptors (**CR1 to CR5**), which are distributed on various cell types and bind to specific ligands to mediate specific function
- ❖ For example, **CR2** is present on B cells and is involved in humoral immune response. It also acts as receptor for Epstein-Barr virus.

EVASION OF COMPLEMENT SYSTEM BY MICROORGANISMS

In order to escape from the complement mediated effector mechanisms, microorganisms can develop various counter mechanisms to evade the complement system (Table 13.3).

REGULATION OF COMPLEMENT PATHWAYS

Complement system are antigen non-specific; capable of attacking microorganisms as well as host cells. Hence, several regulatory mechanisms have evolved to restrict complement activity only to the designated target cells. There are a series of regulatory proteins, which inactivate various complement components at different stages.

For example:

- ❖ **C1 inhibitor (or C1 esterase inhibitor):** It is a soluble glycoprotein, inhibits the action of C1q by splitting C1qrs into C1rs and C1q. Thus, the whole classical pathway is inhibited
- ❖ **DAF (Decay accelerating factor):** It is CD55 molecule present on cell membrane, accelerates dissociation of C3 convertase; thus inhibiting all three pathways.

COMPLEMENT DEFICIENCIES

Complement deficiency associated diseases fall into two categories; diseases associated with—(1) complement protein deficiencies and (2) complement regulator protein deficiencies (Table 13.4).

Table 13.3: Mechanisms of microbial evasion of complement system.

Mechanisms	Examples
Shown by gram-negative bacteria	
Long polysaccharide side chain of bacteria can prevent membrane attack complex (MAC) insertion	<i>Escherichia coli</i> <i>Salmonella</i>
Noncovalent interactions between bacterial cell wall components can prevent MAC insertion	<i>Neisseria gonorrhoeae</i>
Elastases destroy C3a and C5a	<i>Pseudomonas</i>
Shown by gram-positive bacteria	
Thick peptidoglycan cell wall prevents MAC insertion	<i>Staphylococcus</i> <i>Streptococcus</i>
Bacterial capsule forms a physical barrier between C3b and CR1 interaction	<i>Streptococcus pneumoniae</i>
Shown by other microbes	
Proteins mimicking complement regulatory proteins	Vaccinia virus, Herpes simplex virus, Epstein-Barr virus, <i>Trypanosoma cruzi</i> , <i>Candida albicans</i>

Table 13.4: Complement deficiency diseases.

Complement deficiencies	Pathway(s) involved	Disease/pathology
Complement protein deficiencies		
C1, C2, C3, C4	C1, C2, C4—classical pathway C3—common deficiency	Systemic lupus erythematosus (SLE), glomerulonephritis and pyogenic infections
Properdin, Factor D	Alternative pathway	<i>Neisseria</i> and pyogenic infection
Membrane attack complex (C5-C9)	Common deficiency	Disseminated <i>Neisseria</i> infection
Complement regulatory protein deficiencies		
C1 esterase inhibitor	Overactive classical pathway	Hereditary angioneurotic edema
DAF (Decay accelerating factor) and CD59	Deregulated C3 convertase Increased RBC lysis	PNH (Paroxysmal nocturnal hemoglobinuria)

EXPECTED QUESTIONS

I. Write essay on:

1. What is complement? Explain in detail about classical complement pathway. List various effector functions of complement.

II. Write short notes on:

1. Alternative complement pathway.
2. Various mechanisms of microbial evasion of complement system.
3. Complement deficiency diseases.

III. Multiple Choice Questions (MCQs):

1. **C-3 convertase in alternative complement pathway is:**
 - a. C14b2a
 - b. C3bBb
 - c. MBL/MASP-C4b2a
 - d. C3b
2. **Which of the following acts as an anaphylatoxin?**
 - a. C3a b. C3b
 - c. C4b d. C2a
3. **Endotoxin acts by:**
 - a. Classical pathway
 - b. Lectin pathway
 - c. Alternative pathway
 - d. None

4. Disseminated *Neisseria* infection is commonly associated with deficiency of:

- a. Properdin
- b. Factor D
- c. C1 inhibitor deficiency
- d. Membrane attack complex (MAC)

5. Complement (classical pathway) is best fixed by:

- a. IgA b. IgD
- c. IgE d. IgM

6. Decreasing order of IgG in complement fixation:

- a. IgG1>IgG2>IgG3>IgG4
- b. IgG4>IgG3>IgG2>IgG1
- c. IgG3>IgG1>IgG2>IgG4
- d. IgG2>IgG1>IgG3>IgG4

7. Early complement deficiency is a predisposing factor for all, except:

- a. Systemic lupus erythematosus (SLE)
- b. Disseminated *Neisseria* infection
- c. Glomerulonephritis
- d. Pyogenic infections

8. C1 esterase inhibitor deficiency leads to:

- a. Paroxysmal nocturnal hemoglobinuria
- b. Hereditary angioneurotic edema
- c. Immune complex disease
- d. Recurrent pyogenic infections

Answers

1. b 2. a 3. c 4. d 5. d 6. c 7. b 8. b

Components of Immune System: Organs, Cells and Products

CHAPTER 14

CHAPTER PREVIEW

- Lymphoid Organs—Central and Peripheral Lymphoid Organs
- Lymphoid Cells—T Cells, B Cells and NK Cells
- Other Cells of Immune System
- Major Histocompatibility Complex
- Cytokines

Immune system comprises of lymphoid organs, cells of immune system (lymphoid cells and other cells) and their soluble products called cytokines (Table 14.1).

LYMPHOID ORGANS

CENTRAL LYMPHOID ORGANS

Bone Marrow

Almost all the cells in blood have originated from pluripotent hematopoietic stem cells of bone marrow and the process is called hematopoiesis.

- ❖ In early fetal life, hematopoiesis occurs in liver; gradually the stem cells migrate to bone marrow. By birth, the stem cells occupy most of the bone marrow space of large bones
- ❖ As the individual ages, hematopoietic activity in large bones decreases and after puberty hematopoiesis is mostly confined to axial bones such as pelvis, vertebrae, sternum, skull and ribs
- ❖ The progenitor T and B cells originate in bone marrow. Further development of B cells occurs in bone marrow

itself, whereas the progenitor T cells migrate to thymus for further proliferation.

Thymus

Thymus is the site of proliferation and maturation of T cells.

Development

Thymus is developed in the embryonic life (third month) from third/fourth pharyngeal pouch. It is highly active at birth, continues to grow for many years, reaches its peak size at puberty, and then it degenerates.

Structure

Thymus has two lobes surrounded by a fibrous capsule. Septa arising from capsule divide thymus into lobules, and each lobule is differentiated into an outer cortex and an inner medulla (Fig. 14.1).

Cortex is densely populated and contains:

- ❖ **Thymocytes:** Lymphocytes of thymus are called as thymocytes. The cortical thymocytes are immature and many in number

Table 14.1: Structure of immune system.

<ul style="list-style-type: none"> ● Lymphoid organs: Consist of central and peripheral lymphoid organs <ul style="list-style-type: none"> ➢ Central or primary lymphoid organs, e.g. thymus and bone marrow: They host the development of immune cells (hematopoiesis) ➢ Peripheral or secondary lymphoid organs, e.g. lymph node, spleen, and mucosa-associated lymphoid tissue (MALT)
<ul style="list-style-type: none"> ● Lymphoid cells: Consist of lymphocytes such as T cells, B cells and NK cells
<ul style="list-style-type: none"> ● Other cells of immune system: Include phagocytes, such as macrophage and microphages (neutrophil, eosinophil and basophil), dendritic cells, mast cells and platelets
<ul style="list-style-type: none"> ● Cytokines: They are the soluble products secreted from various cells of immune system. They include interleukins, interferons, tumor necrosis factors, colony-stimulating factors, etc.

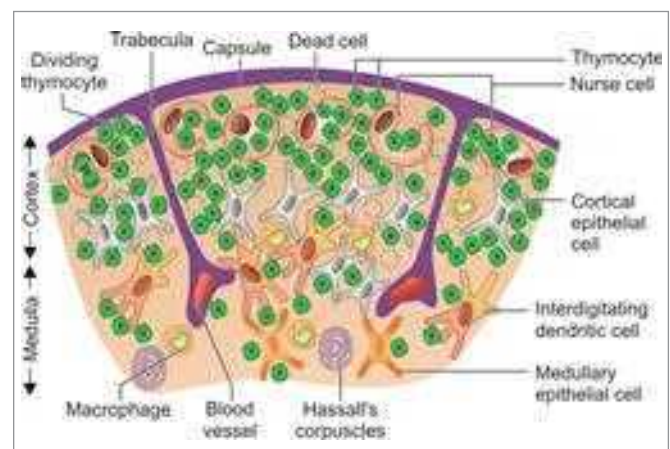


Fig. 14.1: Thymus (cross-section of a portion, schematic).

- ❖ Cortical epithelial cells and
- ❖ Nurse cells (specialized epithelial cells with long membrane extensions that surround many thymocytes).

Medulla is sparsely populated and contains:

- ❖ **Thymocytes:** Medullary thymocytes are relatively more mature and fewer in number
- ❖ Medullary epithelial cells
- ❖ Interdigitating dendritic cells and
- ❖ **Hassall's corpuscles:** They are concentric layers of degenerating epithelial cells.

Thymic Hormones

Several thymic hormones such as thymulin, thymopoietin and thymosin are produced from the epithelial cells of thymus. They are believed to attract the precursor T cells (progenitor T cells) from bone marrow.

Maturation of T Cells

The cell-to-cell interaction between thymocytes and thymic stromal cells (including epithelial cells, dendritic cells and macrophages) and the effect of thymic hormones help in maturation of T cells in thymus. (Maturation of T cells is described in detail later in this chapter).

Central Tolerance

One very interesting fact is that only 2–5% of the developing T cells become mature and released out from thymus; remaining T cells are destroyed as they are either not capable of recognizing major histocompatibility complex (MHC) or are believed to be self-reacting in nature.

- ❖ Destruction of such self-reacting T cells prevents development of autoimmunity (immune response against self-antigens)
- ❖ Such tolerance to self-antigens mediated by thymus that occurs in embryonic life is called as central tolerance.

Defect in Thymus

Any defect in thymus leads to defect in maturation of T lymphocytes that in turn results in severe life-threatening cell-mediated immunodeficiency disorders.

- ❖ **DiGeorge syndrome:** It is an immunodeficiency disorder in man, characterized by congenital aplasia of thymus (refer Chapter 18)
- ❖ **Nude mice:** Mice with congenital absence of thymus are called as nude mice.

PERIPHERAL LYMPHOID ORGANS

Lymph Node

Lymph nodes are small bean-shaped organs; they occur in clusters or in chains, distributed along the length of lymphatic vessels. They act as physiological barriers; filter the microbial antigens carried to lymph node by activating the T and B cells.

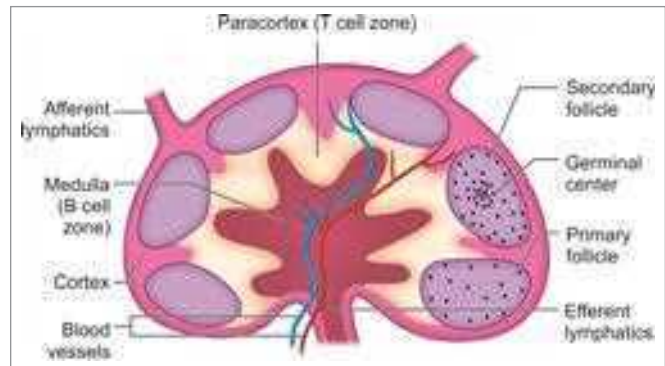


Fig. 14.2: Lymph node.

Structure

Lymph node is divided into three parts—(1) cortex (2) medulla (both are B cell areas) and (3) paracortex (T cell area). It bears the lymphatic and blood vessels. Cortex is surrounded by a capsule and intervened by trabeculae (Fig. 14.2).

- ❖ **Cortex:** It contains lymphoid follicles that are composed of mainly B cells and few special type of dendritic cells (called follicular dendritic cells). Lymphoid follicles are mainly of two types
 1. **Primary lymphoid follicles:** They are found before the antigenic stimulus. They are smaller in size and mainly contain the resting B cells
 2. **Secondary lymphoid follicles:** Following contact with an antigen, the resting B cells start dividing and become activated. The activated B cells differentiate rapidly into plasma cells (which produce antibodies) and memory B cells (which become activated on subsequent antigenic exposure). Follicles become larger in size and are called secondary lymphoid follicles. They have two areas:
 - ♦ The central area called **germinal center**; contains dividing B cells of various stages. It has two zones—light and dark zones. It is the site where activation of B cells takes place (described in detail in Chapter 15)
 - ♦ The peripheral zone called **mantle area**; contains activated B cells.
- ❖ **Paracortical area:** It is present in between cortex and medulla. It is the **T cell area** of lymph node; rich in naive T cells. In addition, it also contains macrophages and interdigitating dendritic cells, which trap the antigens and present to T cells
- ❖ **Medulla:** It is the innermost area of lymph node, rich in B-lymphocytes; mainly plasma cells.

Spleen

Spleen is the largest secondary lymphoid organ. It acts as physiological barrier similar to lymph node in clearing the microbial antigens through the stimulation of T and B cells.

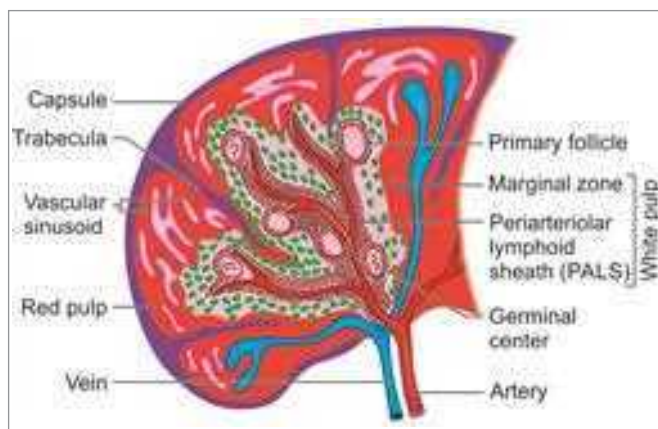


Fig. 14.3: Spleen (cross-section showing red pulp and white pulp area).

Structure

Spleen is situated below the diaphragm on left side of the abdomen. Adult spleen measures about 5-inch in length and weighs 150 g. It is divided into two compartments—(1) central white pulp and (2) outer red pulp, surrounded by capsule and intervened by trabeculae (Fig. 14.3).

- White pulp:** It is the central densely populated area, which contains T cells and B cells. It has two parts:
 - Periarteriolar lymphoid sheath (PALS):** It is T cell area, rich in T cells; surrounds the branches of the splenic artery
 - Marginal zone:** It is located peripheral to the PALS and is populated by B cell lymphoid follicles (primary and secondary) and macrophages.
- Red pulp:** It is the area that surrounds the sinusoids. It is filled with red blood cells (RBCs). The older and defective RBCs are destroyed here.

Defect in Spleen

As spleen is the site of destruction of most of the microbes, functional or structural abnormalities of spleen or splenectomy, especially in children, often leads to an increased incidence of bacterial sepsis caused primarily by capsulated bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*.

Mucosa-associated Lymphoid Tissue (MALT)

The mucous membranes lining the intestine, respiratory, and urogenital tract (total surface area of about 400 m²) are the major sites of entry for most pathogens. Hence, defense mechanisms are needed in the mucosal sites to prevent the microbial entry.

- ❖ Group of lymphoid tissues lining these mucosal sites are collectively known as MALT
- ❖ Structurally, MALT may be arranged in two types:
 1. Loose clusters of lymphoid cells (usually found in the lamina propria of intestinal villi)
 2. Lymphoid tissues arranged as organized structures such as tonsils, appendix and Peyer's patches).

MALT in Intestinal Mucosa

Lymphoid tissues lining the intestinal mucosa are the best studied MALT. They are present in different layers of wall.

- ❖ **Submucosa** contains Peyer's patches. Peyer's patch is a nodule of 30–40 lymphoid follicles (both primary and secondary follicles similar to that of lymph node)
- ❖ **Lamina propria** contains loose clusters of lymphocytes (B cells, plasma cells, T helper cells) and macrophages
- ❖ **Epithelial layer** contains few specialized lymphocytes called intraepithelial lymphocytes (IELs) and modified epithelial cells (called M cells)
 - **Intraepithelial lymphocytes (IELs)** are the $\gamma\delta$ T cells. The actual function of such T cells is not known, they may encounter the lipid antigens that enter through the intestinal mucosa
 - **M cells:** Described in the box below
 - **Secretory IgA:** These are the dimeric IgA antibodies that are present in the submucosa as well as in the lining epithelium. They prevent the microbial entry at the mucosal sites (local immunity).

M Cells

They are specialized flattened epithelial cells that do not have microvilli; instead they bear deep invaginations or pockets in the basolateral side that contain B cells, T cells and macrophages (Fig. 14.4B).

- ❑ M cells act as the portal of entry of a number of microbes such as *Salmonella*, *Shigella*, *Vibrio* and poliovirus
- ❑ Invading microbes are taken-up by M cells (by endocytosis), then transported in a vesicle and are delivered to the basolateral pockets
- ❑ T cells, B cells and macrophages in the underlying lymphoid follicles are activated following contact with the microbe
- ❑ B lymphocytes in MALT once activated at a site by antigenic exposure, migrate to other parts of the intestine, secrete the dimeric IgA, and thus extend the local immunity (Fig. 14.4A).

Cutaneous-associated Lymphoid Tissue

Similar to MALT, skin also contains a few loose lymphocytes and specialized antigen presenting cells in epidermis called **Langerhans cells**.

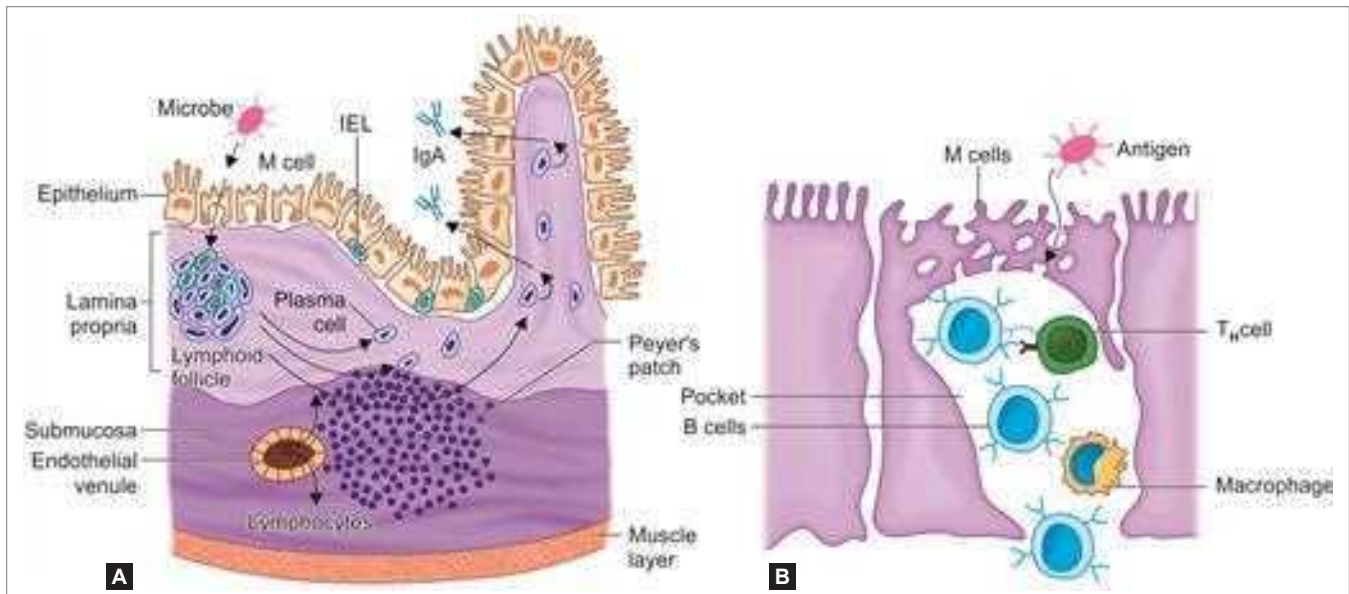
LYMPHOID CELLS

Cells of immune system comprise of lymphoid cells or lymphocytes and other cells such as phagocytes (e.g. macrophages and granulocytes), etc.

Lymphocytes are the major components of cells of immune system. There are approximately 10¹¹ lymphocytes in the body, accounting for 20–40% of the total white blood cells (WBCs) in blood and 99% of the cells in the lymph (Table 14.2).

CD molecules

Cluster of differentiation (CD) molecules are cell surface markers useful for the identification of cells of immune system. They have numerous functions, often act as surface



Figs 14.4A and B: A. MALT; B. Structure of M cell.

Table 14.2: Distribution of lymphocytes (%) in organs/blood.

Tissues	T cell	B cell	NK cell
Bone marrow	5–10	80–90	5–10
Thymus	99	<1	<1
Lymph node	70–80	20–30	<1
Spleen	30–40	50–60	1–5
Peripheral blood	70–80	10–15	10–15

receptors or some CD proteins may help in cell adhesion. As of 2015, CD molecules of humans are numbered up to 364; important examples are CD4 and CD8 molecules—expressed by helper T cells and cytotoxic T cells respectively.

TYPES OF LYMPHOCYTES

Based on function and cell membrane structure, lymphocytes can be of three types—(1) T lymphocytes, (2) B lymphocytes and (3) natural killer (NK) cells.

The T and B lymphocytes can also be classified into naive lymphocytes and lymphoblasts.

Naive Lymphocytes

They are resting B and T lymphocytes that have not interacted with any antigen (unprimed lymphocytes).

- ❖ They are also known as small lymphocytes, as they are small in size (6 μm); having thin rim of cytoplasm, larger nucleus with dense chromatin; fewer mitochondria, ribosomes, and lysosomes
- ❖ They generally have a short-life span (1–3 months).

Lymphoblasts

When the naive cells interact with antigen in the presence of certain cytokines (e.g. interleukin-7), become activated and transform into lymphoblasts, which eventually differentiate into effector cells or memory cells.

- ❖ **Effector cells** function in various ways to eliminate antigen

- They have short-life span (few days to few weeks)
- They are large lymphocytes (15 μm in size), having wider rim of cytoplasm with more organelles
- Antibody producing plasma cells are classical example of effector B cells; whereas effector T cells include helper T cells and cytotoxic T cells.

- ❖ **Memory cells:** They remain dormant like naive cells but are capable of transforming into effector cells rapidly on subsequent antigenic challenge

- They have a longer life span; providing long-term immunity against many pathogens
- They look like small lymphocytes but can be distinguished from naive cells by the presence or absence of certain surface markers (Table 14.3).

T LYMPHOCYTES

T cells constitute 70–80% of blood lymphocytes. Unlike B cells, they do not have microvilli on their surface. They bear specialized surface receptors called T cell receptors (TCR).

T Cell Receptor

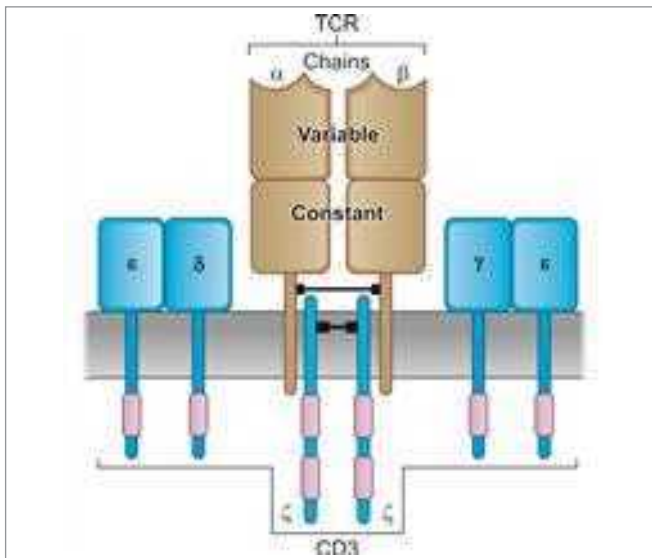
The T cell receptors (TCR) of T cells are equivalent to the surface immunoglobulins (B cell receptors) of the B cells. Their main function is antigen recognition. Unlike B cell receptor which binds to antigen directly, TCR does not recognize antigen by itself. It can only respond to an antigen which is processed and presented by the antigen presenting cells, such as macrophages.

TCR–CD3 Complex

Most T cell receptors (95%) comprise of two chains (α and β) which in turn have three regions—(1) extracellular domain, (2) transmembrane domain, and (3) cytoplasmic

Table 14.3: Differences between naive, effector and memory cells.

	Naive cell	Effector cell	Memory cell
Location (present mostly in)	Secondary lymphoid organs, e.g. lymph nodes, spleen	Inflamed tissues and mucosal surfaces	Both the locations of naive and effector cell
Cell cycle	Dormant (G0 phase)	Active	Dormant (G0 phase)
Morphology	Small lymphocyte	Large lymphocyte	Small lymphocyte
Life span	Short	Short	Long
Function	Transforms to effector cell on primary exposure to antigen, occurs slow due to lag period	Eliminate antigen	Transforms to effector cell on secondary exposure to antigen, occurs fast without lag period
Surface markers			
CD127 (IL-7R)	High	Low	High
CD45 isoform	CD45RA	CD45RO	CD45RO
CD25 (IL-2R α) on T cells	No	Yes	Yes
CD27 on B cells	No	Yes	Yes
B cells producing Ig types and their affinity	IgM and IgD Low affinity	IgG, IgA, IgE High affinity	IgG, IgA, IgE, IgM and IgD High affinity

**Fig. 14.5:** Structure of T cell receptor - CD3 complex.

tail. The extracellular domain of each polypeptide chain has 2 regions (variable and constant region). About 5% of TCRs do not have α/β chains, instead they bear γ/δ chains. TCR is active only when both the chains (α and β) complex with CD3 molecule (Fig. 14.5).

- ❖ The **variable region** of α and β chains of TCR bind to the presented antigens. They are polymorphic in nature. Rearrangement of α and β genes during T cell development can produce large number of different combinations of TCRs. Each TCR is capable of recognizing a particular epitope of an antigen
- ❖ The **CD3 complex** consists of three pairs of polypeptide chains— $\xi\xi$ (zeta-homodimer), $\delta\epsilon$ (delta-epsilon heterodimer) and $\gamma\epsilon$ (gamma-epsilon heterodimer)
- ❖ Following binding of antigen to α and β chains of TCR, a signal is generated that is transmitted through the CD3 complex leading to activation of T cells.

T Cell Development

The major events of T cell maturation take place in thymus, in contrast to bone marrow for B cells.

- ❖ The progenitor T cells are originated from the bone marrow (or liver in fetal life) and then migrate to thymus through bloodstream
- ❖ Developing T cells in the thymus (collectively called as thymocytes) pass through series of stages that are marked by characteristic changes in their cell surface markers
- ❖ Most of the development events take place in the cortex of thymus, under the influence of thymic stromal cells which secrete thymic hormones and lymphopoietic growth factor IL-7.

The sequence of events of T cell development is as follows (Fig. 14.6):

- ❖ **Double negative (DN) T cells:** T cell precursors after entering into the thymus transform into double negative T cells ($CD4^- CD8^-$). These cells are so called because, they do not express the surface markers of mature T cells, i.e. CD4 and CD8 molecules. DN T cells first express CD3 molecule and then undergo further development
 - Five percent of T cell precursors carrying TCR $\gamma\delta$ develop into mature $\gamma\delta$ T cells
 - The remaining (95%) of the cells express TCR $\alpha\beta$ and subsequently express both CD4 and CD8 molecules to become double positive (DP) T cells.
- ❖ **Double positive (DP) T cells ($CD4^+ CD8^+$):** They are immature T cells, carrying both CD4 and CD8 molecules on their surface. They further undergo one of the following fate:
 - **Positive selection:** The 5% of DP T cells, whose $\alpha\beta$ receptors are capable of recognizing their MHC molecules are positively selected. This results in MHC restriction

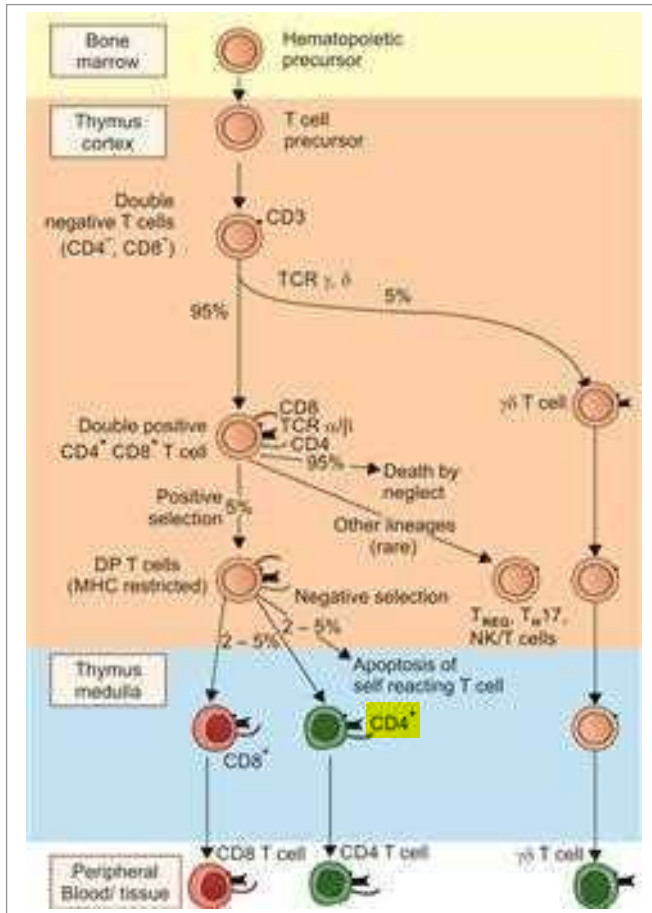


Fig. 14.6: T cell development.

- **Death by neglect:** Majority of DP cells (95%) fail positive selection because they do not specifically recognize their MHC molecules
- **Negative selection:** The survived cells that undergo positive selection (5%) are MHC restricted. However, some of these surviving cells (2–5%) react to the self-antigens and therefore, they are selected to be killed by apoptosis and removed (negatively selection)
- The remaining double positive T cells (2–5%) having $\alpha\beta$ type TCR selectively shut off the expression of either CD4 or CD8 molecules and eventually become single positive mature T cells ($CD4^+/CD8^-$ or $CD4^-/CD8^+$).
- ❖ **Mature T cells** (e.g. $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells) acquire thymus specific antigens, then are released into the circulation and migrate to the peripheral lymphoid organs where they respond to the antigenic stimulus.

Types of T Cells

Effector T Cells

There are two types of effector T cells—(1) $CD4^+$ helper T cells and (2) $CD8^+$ cytotoxic T cell.

- ❖ **Helper T cells:** Helper T cells (T_H) possess CD4 molecules as surface receptors. They recognize the antigenic

peptides that are processed by antigen presenting cells and presented along with MHC-II molecules (major histocompatibility complex)

- Following antigenic stimulus, the helper T cells differentiate into either of the two types of cells— (1) T_H1 and (2) T_H2 subset; each secrete specific cytokines which modulate the cellular and humoral immune responses respectively (for detail, refer Chapter 15)
- **T_H17 cells:** Recently a third subset of T helper cells called T_H17 cell has been discovered. It produces IL-17 and IL-22, and is primarily involved in recruiting neutrophils. They contribute to the pathogenesis of many autoimmune inflammatory diseases such as rheumatoid arthritis and others.
- ❖ **Cytotoxic T cells:** In contrast to T_H cells, cytotoxic T cells (T_C) possess CD8 molecules and recognize the intracellular antigens (e.g. viral antigens or tumor antigens) that are processed by any nucleated cells and presented along with MHC-I. In general, T_C cells are involved in destruction of virus infected cells and tumor cells (for detail, refer Chapter 15).

Rare Subtypes of T Cells

- ❖ **Regulatory T cells (T_{REG} cells):** The T_{REG} cells (formerly known as suppressor T cells) are a subpopulation of T cells which regulate the immune system
 - They provide tolerance to self-antigens (known as peripheral tolerance), and thus prevent the development of autoimmune disease
 - Surface markers: T_{REG} cells possess surface markers such as CD4, CD25 and Foxp3 (a forkhead family transcription factor)
 - Deficiency of Foxp 3 receptors leads to a severe form of autoimmune disease known as Immune dysregulation, Polyendocrinopathy, Enteropathy X-linked (**IPEX syndrome**).
- ❖ **$\gamma\delta$ T cells:** $\gamma\delta$ T cells represent a small subset of T cells (5%) that possess a distinct TCR composed of γ and δ chains; instead of α/β chains. They lack both CD4 and CD8 molecules
 - They differ from the conventional $\alpha\beta$ T cells by the fact that they do not require antigen processing and MHC presentation of peptides
 - They are part of innate immunity as the $\gamma\delta$ receptors exhibit limited diversity for the antigen
 - They are usually found in the gut mucosa, within a population of lymphocytes known as IELs
 - The function of $\gamma\delta$ T cells is not known, they may encounter the lipid antigens that enter through the intestinal mucosa.

B LYMPHOCYTES

B lymphocytes are the mediators of humoral immunity; constitutes 10–15% of blood lymphocytes. They are named after their site of maturation (**b**ursa of Fabricius in birds)

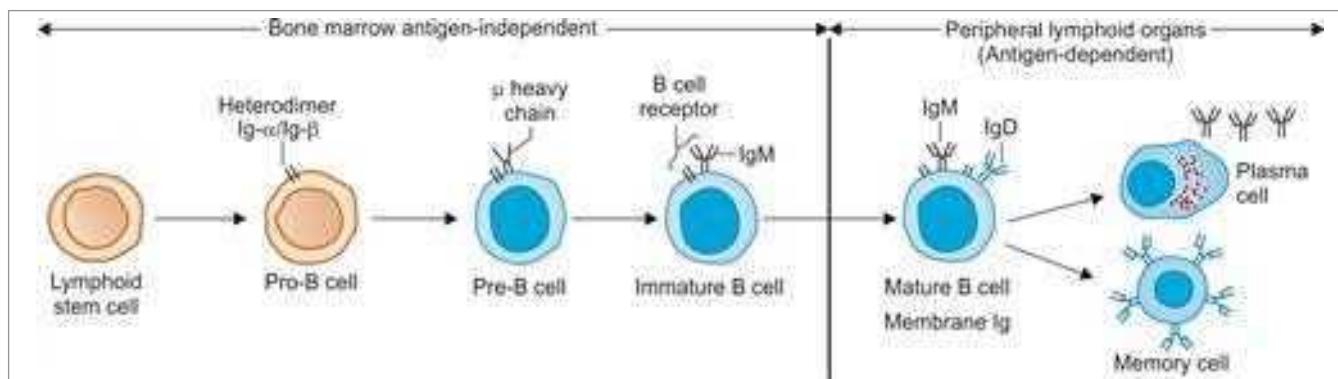


Fig. 14.7: B cell development.

and bone marrow in humans and other mammals). B cells proliferate through various stages, first in bone marrow, then in peripheral lymphoid organs (Fig. 14.7).

Development of B Cells in Bone Marrow

Initial stages of B cell proliferation occur in bone marrow; independent of exposure to antigen.

- ❖ **Pro-B Cells (Progenitor B Cells):** They are the earliest bone marrow cells of B cell lineage. They do not produce immunoglobulins (Ig) but express a heterodimer $Ig\alpha/Ig\beta$ that forms a part of the B cell receptor (BCR) in future
- ❖ **Pre-B Cells (Precursor B Cells):** Pro-B cells differentiate into pre-B cells by expressing μ heavy chain
- ❖ **Immature B Cells:** Pre-B cells proliferate into immature B cells by expressing light chain
 - **B cell receptor:** Heavy chain μ and its light chain join to form complete IgM molecule. IgM is then complexed with heterodimer $Ig\alpha/Ig\beta$ on the B cell surface to form B cell receptor (Fig. 14.8)
 - **Tolerance:** Some of the immature B cells are capable of reacting to self-antigens. Tolerance to those B cells are essential for prevention of autoimmunity. Following contact with a self-antigen, the tolerance is developed either by:
 - ♦ *Receptor editing:* A process by which the Ig genes coding light chains are rearranged so that a different (edited) B cell receptor is produced which no longer reacts to self-antigen or
 - ♦ *Negative selection:* By apoptosis of self-reacting immature B cells in spleen.

Development of B Cells in Peripheral Lymphoid Organs

Immature B cells migrate from bone marrow to peripheral lymphoid organs (lymph node and spleen) where they transform into mature B cells following contact with appropriate antigen.

Mature or Naive B Cells

Most mature B cells (95%) belong to the follicular B cell type and produce surface receptor IgD in addition to IgM.

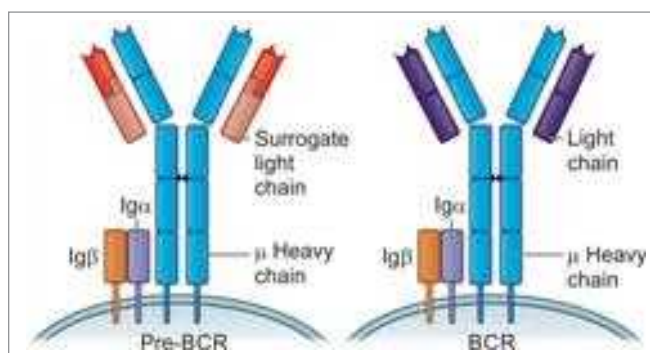


Fig. 14.8: Structure of B cell receptor (BCR) and pre-BCR.

They play an important role in humoral immune response (described in detail in Chapter 15).

- ❖ Following antigenic stimulus, the mature B cells transform into activated B cells (lymphoblasts) which further differentiate into either effector B cells, i.e. plasma cells (majority) or memory B cells
- ❖ **Plasma cells** (antibody secreting cells): They are oval, large (15 μm size), with an eccentrically oval nucleus containing large blocks of peripheral chromatin (cartwheel appearance) and the cytoplasm containing abundant organelles. They have a short life span of two or three days.
- ❖ However, there are few rare mature B cell types such as B-1 cell and marginal zone B cells which have limited diversity and are components of innate immunity.
- ❖ **B-1 cells:** They are found mostly in the peritoneal cavity, coated by surface markers IgM (natural antibodies) and CD5 molecules, but lack IgD
- ❖ **Marginal-zone B cells:** They are present at the edges of lymphoid follicles of spleen and are produced in response to the polysaccharide antigens.

B cells are the main components of humoral immunity; produce five classes of antibodies, which in turn have various biological functions (described in Chapters 11 and 15).

Differences between T cell and B cell are given in Table 14.4.

Property	T cell	B cell
Origin	Bone marrow	Bone marrow
Maturation	Thymus	Bone marrow
Peripheral blood	70–80% of total lymphocytes	10–15% of total lymphocytes
Antigen recognition receptors	T cell receptors complexed with CD3	B cell receptor-surface IgM or IgD complexed with Iga/Igβ
CD markers	CD 3, 4, 8	CD19, 21, 24
Thymus specific Ag	Present	Absent
Microvilli on the surface	Absent	Present

NATURAL KILLER CELLS

Natural killer (NK) cells are large granular lymphocytes that constitute 10–15% of peripheral blood lymphocytes. They are derived from a separate lymphoid lineage. Similar to cytotoxic T cells, NK cells also are involved in destruction of virus infected cells and tumor cells (described in Chapter 15).

OTHER CELLS OF IMMUNE SYSTEM

Macrophage

Macrophages were first described by Russian scientist Metchnikoff (1883) who suggested that the monocyte-macrophage system plays a vital role in host defense by performing two important functions— (1) phagocytosis and (2) antigen presentation.

Monocytes/macrophages originate from bone marrow, from a separate lineage, i.e. from the granulocyte-monocyte progenitor cells.

Monocytes: They are present in blood; they are the largest blood cells measuring 12–20 μm size. They do not divide and have an average transit time of 8 hours in blood; then they migrate to tissues.

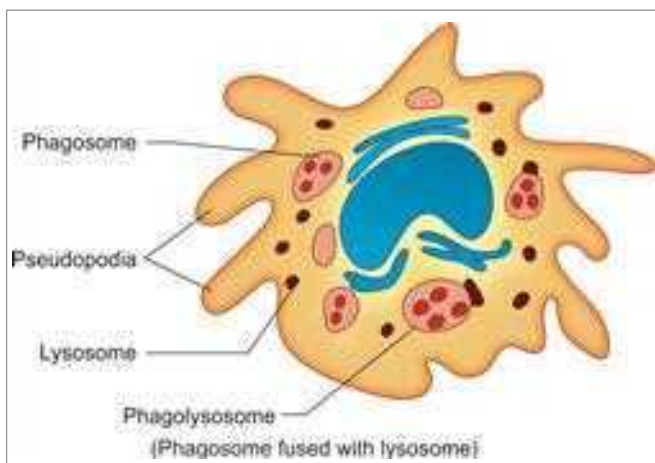


Fig. 14.9: Macrophage.

Macrophages: When monocytes migrate to tissues, they transform into macrophages (Fig. 14.9). Macrophages differ from monocytes in the following:

- ❖ 5–10 folds larger than monocytes
- ❖ Contain more lysozymes and cell organelles
- ❖ Produce more lytic enzymes and cytokines
- ❖ Possess greater phagocytic activity
- ❖ Have a longer life in tissues (months to years).

Most macrophages are motile, travel by amoeboid movement throughout the tissues and are called as free or wandering macrophages. While, some reside in particular tissue, become non-motile and are called fixed macrophages. Macrophages in various tissues are designated by different names (Table 14.5).

Secretory Products of Macrophages

Activated macrophages in turn produce a number of secretory products which mediate various functions (Table 14.6).

Functions of Macrophage

- ❖ **Phagocytosis:** Macrophages are the principle cells involved in phagocytosis. Macrophages from various tissues are together called as the mononuclear phagocyte system (or previously known as the reticuloendothelial system). They also remove old dying cells from the body. The steps involved in phagocytosis are as follows (Fig. 14.10):
 - **Recognition:** Attachment of the microbe to the receptors present on the surface of macrophage, such as toll like receptors or immunoglobulin G (IgG)
 - **Engulfment:** Microbe is ingested with subsequent formation of a phagocytic vacuole (phagosome)
 - **Fusion** of lysosome with phagosome to form phagolysosome
 - **Killing or degradation** of the ingested microbes which is accomplished largely by both:
 - ♦ Oxygen independent killing—degradation by lysosomal enzymes

Body sites	Macrophage designation
Peripheral blood	Monocytes
Tissues	Macrophages
Liver	Kupffer cells
Brain	Microglial cells
Kidney	Mesangial cells
Lungs	Alveolar macrophages
Bone	Osteoclasts
Inflammation site	Epithelioid cells, multinucleated cell (Langhans giant cells)
Connective tissues	Histiocytes
Placenta	Hofbauer cell
Lymphoid follicle	Tingible body macrophage

Secretory products	Examples
Enzymes	Lysozyme, acid hydrolases, elastases, Phosphatases, lipases, collagenases
Free radicals	Reactive oxygen intermediates <ul style="list-style-type: none"> • Superoxide anion (O_2^-) • Hydroxyl radicals (OH) • Hydrogen peroxide (H_2O_2) • Hypochlorite anion (ClO^-) Reactive nitrogen intermediates <ul style="list-style-type: none"> • Nitric oxide (NO) • Nitrogen dioxide (NO_2) • Nitrous acid (HNO_2)
Cytokines	<ul style="list-style-type: none"> • Interferon α, β • Interleukins (IL-1, IL-6, IL-8, IL-12) • Tumor necrosis factor- α (TNF- α)
Growth factors	<ul style="list-style-type: none"> • Colony-stimulating factors (CSF) • Platelet-derived growth factor (PDGF) • Platelet-activating factor (PAF) • Transforming growth factor β (TGF-β)
Coagulation factors	<ul style="list-style-type: none"> • Factor V, VII, IX, X • Prothrombin
Complement factors	C5, C8 properdin, factor B, D, I

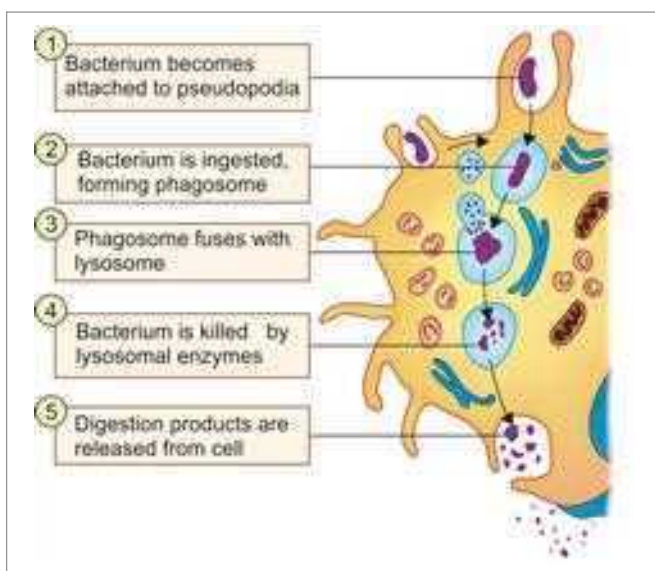


Fig. 14.10: Mechanism of phagocytosis.

- ♦ Oxygen dependent killing by generating free radicals (Table 14.6).
- ❖ **Antigen presentation:** Macrophages also promote adaptive immunity, by acting as antigen presenting cells (APCs). Macrophages capture the antigen, process into smaller antigenic peptides and present the antigenic peptides along with the MHC class II molecules to the helper T cells; thus facilitating helper T cell activation
- ❖ **Activated macrophages:** On exposure to certain cytokines such as interferon- γ , macrophages become activated. The activated macrophages have greater

phagocytic ability and produce many cytokines that act against intracellular bacteria, virus infected cells and tumor cells. They also express higher level of MHC class II, hence can act as efficient APCs

- ❖ **Secretory products** of macrophages have various biological functions (Table 14.6):
 - Interleukin 1 (IL-1): It promotes inflammatory responses, fever, and activate helper T cells
 - IL-6 and TNF- α : They promote innate immunity, (inflammation and fever) and eliminate the pathogens
 - Interferon α and β : They have antiviral activity
 - TNF- α : It lyses the tumor cells (antitumor activity)
 - Growth factors, such as CSF (colony-stimulating factor)—promote hematopoiesis
 - Following tissue injury, various mediators are secreted from macrophage; which help in tissue repair and scar formation.

Dendritic Cells

Dendritic cells are specialized antigen presenting cells of immune system (Fig. 14.11).

- ❖ **Naming:** They possess long membranous cytoplasmic extensions resembling dendrites of neurons; hence, they are named as dendritic cells
- ❖ **Origin:** Dendritic cells originate from bone marrow, but the pathway is uncertain. They either develop as a separate lineage from stem cells or may originate from the macrophage lineage
- ❖ **Types:** Dendritic cells are widely distributed; present in various tissues (Table 14.7)
- ❖ **Function:** Dendritic cells are nonphagocytic in nature. They are the most efficient APCs; their main function is to capture, process and present the antigenic peptides on their cell surface to the helper T cells
 - They carry high level of MHC class II and co-stimulatory B7 molecules
 - They act as messengers between the innate and the adaptive immune systems.
- ❖ **Follicular dendritic cells:** They are present in lymphoid follicles. They differ from other dendritic cells by the fact

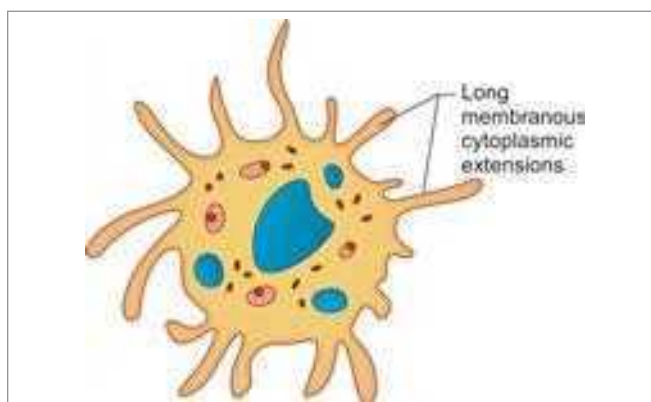


Fig. 14.11: Dendritic cell.

Types of dendritic cells	Site	Function
Langerhans cells*	Skin and mucosa	Antigen presentation, express high MHC-II and B7 molecules
Interstitial dendritic cells	Organs (lungs, liver, spleen, etc.)	
Interdigitating dendritic cells	Thymus	
Circulating dendritic cells	Blood and lymph	
Follicular dendritic cells	Lymph nodes	<ul style="list-style-type: none"> • B cells maturation, and differentiation • MHC-II and B7 molecules absent • Coated with Ag-Ab complex

*Langerhans cell is a dendritic cell; whereas Langerhans giant cell is a macrophage.

that they recognize antigen-antibody complex rather than antigen alone. They do not act as APCs and do not express MHC class II or B7 molecules (for detail refer Chapter 15).

Granulocytic Cells

Granulocytes (e.g. neutrophils, eosinophils and basophils) are a category of white blood cells, characterized by the presence of granules in their cytoplasm. They differ from each other by cell morphology and cytoplasmic staining and function.

Neutrophils

They have a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes. They are often called as polymorphonuclear leukocyte (PMN) because of their multilobed nucleus.

- ❖ Their cytoplasm is heavily granular; contains several granules such as myeloperoxidase, lysozyme, defensins, elastase, gelatinase, etc.
- ❖ Neutrophils constitute 50–70% of the circulating white blood cells (WBCs). However, the level is greatly increased in presence of infection under the influence of certain cytokines, such as IL-8
- ❖ They are the principal phagocytes of innate immunity; the mechanism of microbial killing is similar to that of macrophages, i.e. both by oxygen dependent and independent mechanisms.

Eosinophils

They have a bilobed nucleus and a granular cytoplasm that stains red with the acid dye eosin.

- ❖ They are also phagocytic, constitute only 1–3% of total leukocytes, but the number is greatly increased in certain allergic conditions and helminthic infections
- ❖ Interleukin-5 is believed to be the eosinophil chemotactic factor.

Basophils

They are nonphagocytic granulocytes that contain several secreting granules. They have a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye, methylene blue. They resemble mast cells in their function. Granules are rich in histamine and other mediators that play a major role in certain allergic responses.

Mast Cells

Mast cells are present in various body sites, such as skin, connective tissues of various organs, and mucosa (respiratory and intestinal). Like circulating basophils, mast cells also contain cytoplasmic granules rich in histamine and other active substances and play an important role in the development of certain allergic (type I hypersensitivity) reactions.

MAJOR HISTOCOMPATIBILITY COMPLEX

The major histocompatibility complex (MHC) is a group of genes coding for a set of host cell surface molecules that bind to peptide fragments derived from pathogens and display them on the host cell surface for recognition by the appropriate T cells.

- ❖ These are present in almost all the human cells, but first discovered on the surface of leukocytes; hence in humans, the MHC coded proteins are also called as human leukocyte antigens (HLA)
- ❖ MHC molecules serve as a unique identification marker for every individual as the genetic sequence of MHC genes is different for every individual
- ❖ Following transplantation of a graft, the recipient mounts an immune response against the graft's MHC molecule and vice versa. Greater the difference of the MHC gene sequence between the graft and the recipient, greater is the immune response and greater is the rejection of the graft
- ❖ The acceptance or rejection of the graft is directly dependent on the MHC molecules of the graft and the recipient. As the MHC molecules determine the compatibility between the graft and host tissues, they are named as **histocompatibility antigens**.

MHC GENES AND THEIR PRODUCTS

In humans, HLA complex coding for MHC proteins are located in **short arm of chromosome-6**. The HLA complex extends over 4000 kbp length covering >100 genes. The genes are clustered in three regions named as MHC region-I, II and III (Fig. 14.12).

MHC Region-I

It is about 2000 kbp in length, comprises of three class I genes called HLA-A, HLA-B and HLA-C genes which code for HLA-A, HLA-B and HLA-C proteins respectively,

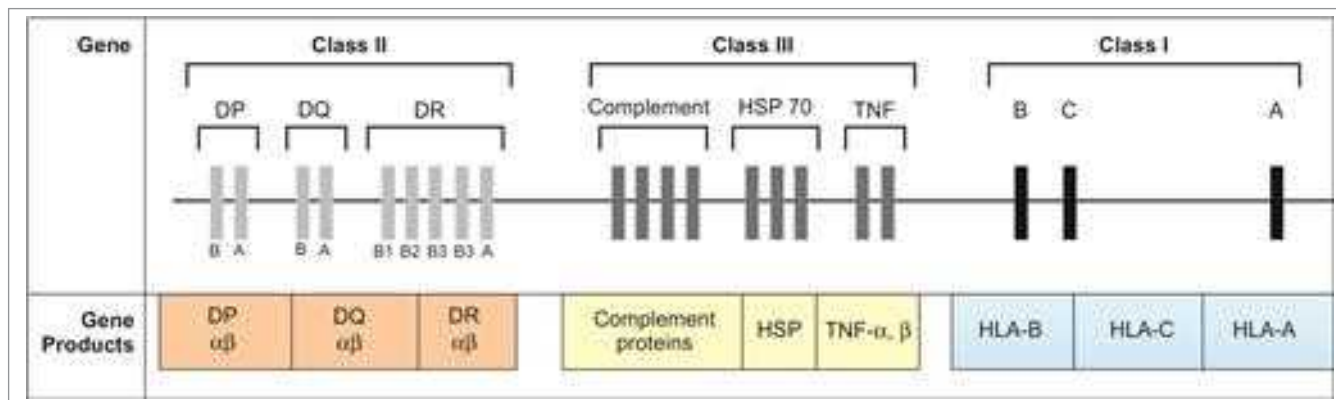


Fig. 14.12: Structure of human MHC complex.

each one is capable of forming the α -chain of MHC class I molecules.

- ❖ MHC-I proteins are located on the surface of all nucleated cells (except sperm cells) and platelets. They are absent in RBCs
- ❖ They present the peptide antigen to CD8 T cells.

MHC Region-II

It spans over 1000 kbp length; comprises of three regions— (1) DP, (2) DQ and (3) DR genes encoding DP, DQ and DR proteins respectively, each one is capable of forming the α and β -chain of MHC class II molecules. In addition, MHC II region also contains certain other non-classical genes, such as DO, DM, LMP and TAP (transporter associated with antigen processing) that help in antigen processing and presentation.

- ❖ MHC-II proteins are located on the surface of antigen presenting cells
- ❖ They present the peptide antigen to CD4 T cells.

MHC Region-III

It is also 1000 kbp in length. It is not involved in antigen presentation, instead it carries genes that code for complement factors (C2, C4, C3 convertase, factor B and properdin), heat shock protein (HSP70) and tumor necrosis factor (TNF- α and β) and steroid 21-hydroxylases.

STRUCTURE OF MHC MOLECULE (FIG. 14.13)

MHC Class I Molecule

It is composed of α chain (glycoprotein, 45kDa) coded by HLA class I genes and $\beta 2$ microglobulin (non-glycosylated 12 kDa protein, encoded by a non MHC gene from chromosome 15).

- ❖ The α chain is folded further and organized into three extracellular globular domains— $\alpha 1$, $\alpha 2$ and $\alpha 3$ (each containing 90 amino acids) and a cytoplasmic tail
- ❖ The association of $\beta 2$ microglobulin with α chain is necessary for the expression of MHC I molecules on to the cell surface. In **Daudi cells** (a type of human B cell tumor cell which are not able to produce $\beta 2$ microglobulin),

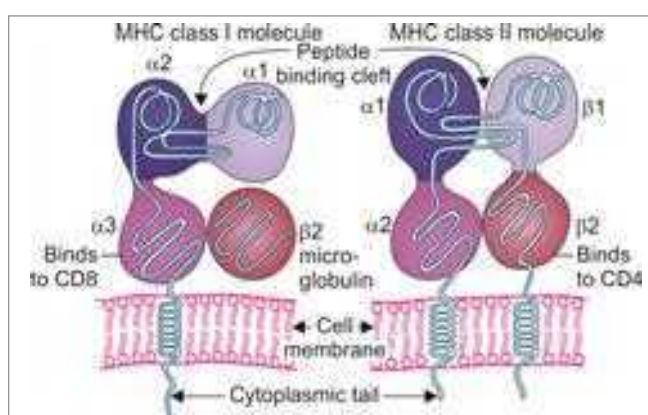


Fig. 14.13: Structure of MHC molecules.

it is observed that they synthesize MHC-I but do not express them on cell surface.

Role of MHC Class I Molecules

- ❖ The antigen peptide binding groove of class I MHC molecule (i.e. the site, where the antigen peptide binds) is formed by the cleft between $\alpha 1$ and $\alpha 2$ domains
- ❖ The $\alpha 3$ domain binds to CD8 molecule present on cytotoxic T cells during antigen presentation.

MHC Class II Molecule

It comprises of one α chain (33 kDa) and one β chain (28 kDa). The α and β chains in turn consist of two domains each—(1) $\alpha 1$ and $\alpha 2$ and (2) $\beta 1$ and $\beta 2$, respectively and cytoplasmic tails.

- ❖ The antigen peptide binding groove is formed by the cleft between $\alpha 1$ and $\beta 1$ domains
- ❖ The $\beta 2$ domain interacts with CD4 molecule of helper T cells during antigen presentation.

Differences between MHC class I and II molecules are described in Table 14.8.

Regulation of MHC Expression

There are several regulatory mechanisms that control the expression of MHC genes in different cell types.

Table 14.8: Differences between MHC class I and MHC class II molecules.

	MHC class I	MHC class II
Present on	All nucleated cells (except sperms) and platelets	Antigen presenting cells (APCs)
Peptide antigen is	Presented to CD8 T cells	Presented to CD4 T cells
Nature of peptide antigen	Endogenous or intracellular (viral/tumor antigen)	Exogenous
Peptide antigen (size)	8–10 amino acid long	13–18 amino acid long
Antigen presentation pathways	Cytosolic pathway	Endocytic pathway
Peptide-binding site	$\alpha 1/\alpha 2$ groove	$\alpha 1/\beta 1$ groove
CD4 or CD8 binding site	$\alpha 3$ binds to CD8 molecules on T_C cells	$\beta 2$ binds to CD4 on T_H cells

- ❖ **Transcription factors:** MHC genes have promoter sequences at their 5' end which are regulated by certain transcription factors such as **CIITA**, and **RFX** (both bind to MHC II promoter genes and increase their transcription). Defects in CIITA, and RFX cause one of the form of **Bare lymphocyte syndrome**
- ❖ **Cytokines** also influence MHC expression
 - IFN- γ activates both MHC-I and II promoter genes
 - IL-4 increases expression of class II MHC molecules on resting B cells.
- ❖ **Corticosteroid and prostaglandins** decrease the expression of MHC II molecules
- ❖ **In many viral infections**, the viral antigens inhibit various components of MHC-I (e.g. adenovirus proteins inhibit TAP, cytomegalovirus proteins inhibit $\beta 2$ microglobulin). As a result, MHC-I expression is suppressed.

MHC and Disease Susceptibility

Many HLA alleles have been associated with increased susceptibility to certain diseases (Table 14.9). The relative risk of occurrence of the disease in presence of the identified allele varies. For example, HLA B27 is strongly associated with ankylosing spondylitis (90 times higher risk than those not expressing HLA B 27).

SOLUBLE PRODUCTS OF LYMPHOID CELLS

CYTOKINES

Definition

Cytokines are chemical substances which serve as messengers, mediating interaction and communication between the various cells of immune system.

Major Classes of Cytokines

Present nomenclature of cytokines includes all the compounds that were known earlier by various names, such as:

Table 14.9: Diseases associated with certain HLA alleles.

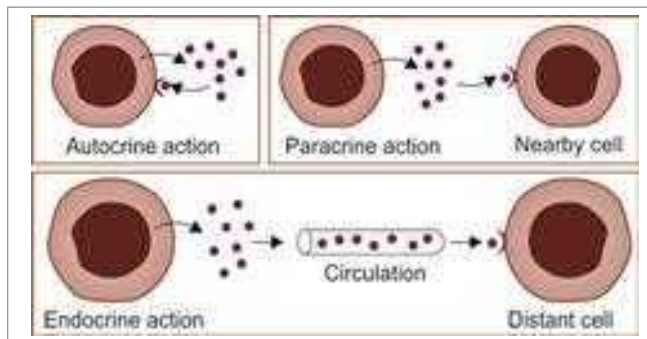
HLA allele	Associated disease
HLA B27	Ankylosing spondylitis, Reactive arthritis (<i>Yersinia</i> , <i>Salmonella</i> , gonococcus) and Reiter's syndrome
DR-2	Multiple sclerosis, Goodpasture's syndrome
DR-3	Myasthenia gravis, systemic lupus erythematosus
DR-3/DR-4	Insulin-dependent diabetes mellitus
DR-4	Rheumatoid arthritis
A3/B14	Hereditary hemochromatosis

- ❖ **Lymphokines**—produced by lymphocytes
- ❖ **Monokines**—produced by monocytes and macrophages
- ❖ **Interleukins**—produced by WBCs and acting on the same or different WBCs
- ❖ **Chemokines**—involved in chemotaxis and other leukocyte behavior.

Properties of Cytokines

Cytokines are comparable to growth factors and hormones in many ways such as all of them act at very low concentration (picomoles) and through specific receptors. However, there are some differences also.

- ❖ Growth factors are produced constitutively while cytokines are inducible, i.e. produced only after the activation of their cells of origin
- ❖ Hormones have mostly endocrine effects; whereas cytokines have broad range of effects, which include (Fig. 14.14):
 - Autocrine effect—that acts on the same cell
 - Paracrine effect—that acts on the adjacent cell
 - Endocrine effect—that acts on a cell present at a distant site.
- ❖ Unlike hormones and growth factors which work mostly independently, cytokines can work together and there are various types of interactions occurring between cytokines:
 - **Pleiotropy and redundancy effect:** Pleiotropy refers to same cytokine having different actions on different target cells, whereas redundancy implies to different cytokines producing the same effect on the same target cell (Fig. 14.15)

**Fig. 14.14: Action of cytokines on target cell.**

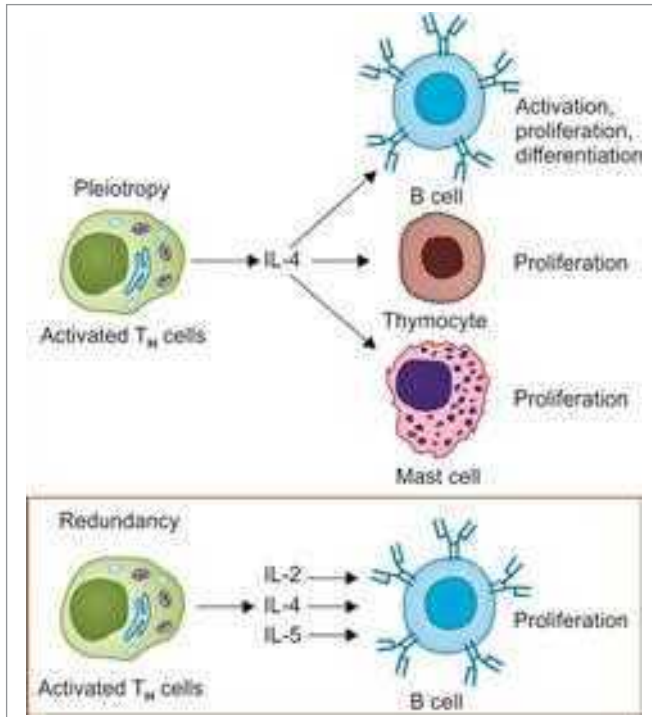


Fig. 14.15: Pleiotropy and redundancy effect of cytokines.

- **Synergy and antagonism effect:** Two cytokines may augment each other's action producing a larger effect (synergism) or may oppose each other's action (antagonism)
- **Cascade effect:** It refers to a series of effects mediated by different cytokines. One cytokine acts on a target cell to produce another cytokine which in turn acts on another target cell and so on.

Structure of Cytokines

Cytokines are glycoproteins with molecular weight less than 30 kDa. Most cytokines display high degree of α -helix structure but no β -structure. Cytokines characterized so far belong to one of the four groups—(1) the hematopoietin family, (2) the interferon family, (3) the chemokine family, or (4) the tumor necrosis factor family; each interacting with a separate class of cytokine receptors present on target cell surface.

Functions of Important Cytokines

Though cytokines are secreted by a wide variety of cells, the major producers are T_H cells and macrophages. Cytokines produce a range of overlapping functions on the target cells/tissues, which can be broadly categorized into two groups:

1. Promote development of cellular and humoral responses of adaptive immunity:
 - Interferon- γ
 - Cytokines such as IL-2, IL-4, IL-5.
2. Cytokines promote various responses of innate immunity:
 - Induction of inflammatory responses—by IL-1, IL-8, TNF- α
 - Regulation of hematopoiesis—by colony-stimulating factors, IL1, IL-3, IL-7, IL-9, IL-11, etc.
 - Antiviral activity—By interferon- α and β
 - Antitumor activity—By TNF- α and β
 - Pyrogenic activity—By TNF- α , IL-1 and IL-6.

The functions of individual cytokines are summarized in Table 14.10. Cytokines of cellular and humoral immune responses are also discussed in Chapter 15.

Table 14.10: Sources and functions of cytokines.		
Cytokine	Cytokine secreting cells	Target cells and functions
Interleukins (IL)		
IL-1	Produced by all nucleated cells, but principal sources are APCs, such as macrophages, monocytes, dendritic cell, B cells and endothelial cell	<ul style="list-style-type: none"> • T_H cells—IL-1 produced by APCs stimulates T_H cells activation and proliferation <ul style="list-style-type: none"> ➢ Promotes IL2 secretion by T_H cells ➢ Induces IL-2 receptor expression on T_H cells ➢ Induces \uparrowMHC-II expression on APCs • B cell—promotes B cell development and maturation • Liver—induces synthesis of acute phase reactant proteins • Hypothalamus—induction of fever • Macrophage and neutrophil activation—\uparrowexpression of ICAM
IL-2	T_H 1 cells	Induces proliferation activated T_H cells, T_C cells and some NK cells (Previously called as T cell growth factor)
IL-3	T_H cell, NK cell, mast cell	<ul style="list-style-type: none"> • Stimulates hematopoiesis (acts as multi-CSF) • Mast cell degranulation—\uparrow histamine secretion
IL-4	T_H 2 cells	<ul style="list-style-type: none"> • T_H cells—promotes T_H 2 cell activity and inhibits T_H 1 cell • B cell—promotes B cells activation and proliferation and induces B cell class switch over to produce IgE, IgG4, IgG1; previously called as B cell growth factor • Macrophage and APCs—induce \uparrowMHC-II expression
IL-5	T_H 2 cells	Promotes eosinophil growth and differentiation
IL-6	T_H 2 cells, macrophages	IL-1 and TNF like effects (synergistic effect) Promotes B cell proliferation and antibody production

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Cytokine	Cytokine secreting cells	Target cells and functions
IL-7	Bone marrow/thymic stromal cells	Serves as a growth factor for T cell and B cell precursors
IL-8	Macrophages, endothelial cells	Attracts neutrophils, NK cells, eosinophils and basophils
IL-9	T _H cells	Hematopoietic and thymopoietic effects
IL-10	T _H 2 cells	Reduces cytokine production by T _H 1 cell
IL-11	Bone marrow stromal cells	<ul style="list-style-type: none"> Hematopoietic effect (B cell and platelet development) Liver—induces synthesis of acute phase reactant protein
IL-12	Macrophages	Promote T _H 1 cell induction and inhibit T _H 2 activity; promotes CMI responses NK cell stimulatory factor
IL-13	T _H 2 cells	Mimic IL-4 function
IL-17	CD4 ⁺ activated memory T _H cell	Initiates and maintains inflammation
Interferons (IFN)*		
IFN-α	Leukocytes	Antiviral activity
IFN-β	Fibroblasts	Antiviral activity
IFN-γ	T _H and T _C cells, NK cells	<ul style="list-style-type: none"> Macrophage—activates the resting macrophages into activated macrophage B cells—activate B cells to produce IgG Promotes inflammation of delayed type of hypersensitivity (along with TNF-β) T_H 2 cell—inhibits T_H 2 cell proliferation
Tumor necrosis factors (TNF)		
TNF-α	Macrophage	<ul style="list-style-type: none"> IL-1 like effect Tumor cells—promote vascular thrombosis and tumor necrosis Inflammatory cells—induce cytokine secretion Induces lipolysis, causes extensive weight loss associated with chronic inflammation
TNF-β	T _H 1 cell and T _C cell	Tumor cells—similar effect like TNF-α Macrophage—enhance phagocytic activity
Colony-stimulating factor (CSF)		
GM-CSF	Fibroblasts, endothelium, T cells, macrophages	Macrophage and granulocyte growth stimulation
G-CSF	Bone marrow stromal cells, macrophages	Granulocyte growth stimulation
M-CSF	Fibroblasts, endothelium	Macrophage growth stimulation
Others		
TGF-β	Macrophages, mast cells T and B cells, platelet	<ul style="list-style-type: none"> Inhibit T and B cell proliferation and hematopoiesis Promotes wound healing Promotes class switching of B cells to the IgA class

*Interferons are discussed in detail in Chapter 4.

Abbreviations: ICAM-1, intercellular adhesion molecule 1; TGF-β, transforming growth factor-β; APC, antigen presenting cell.

Cytokines and Diseases

Pathogenesis of many diseases is characterized by increased expression of cytokines or their receptors. Common examples include the following:

- ❖ **Septic shock** due to gram-negative bacteria, such as *Escherichia coli* or *Neisseria meningitidis* is mediated by the endotoxins released by bacteria that stimulate macrophages to produce IL-1 and TNF-α
- ❖ **Toxic shock syndrome** is caused by the toxin released from *Staphylococcus aureus*, which activates T cells nonspecifically leading to massive cytokine release and that in turn activates macrophages to release large quantities of IL-1 and TNF-α
- ❖ **Cancers:** Several malignancies have been associated with ↑IL-6, e.g. cervical cancer, bladder cancer, etc.

- ❖ **Chaga's disease** is caused by a parasite named *Trypanosoma cruzi* which is shown to inhibit the IL-2α receptor, thus blocking the IL-2 action → leading to inhibition of T_H1 activity → immunosuppression
- ❖ Cytokine storm (see highlighted box).

Cytokine Storm

It is a condition, where the cytokines are produced in excess leading to **hypercytokinemia** which can cause significant damage to body tissues and organs.

- Normally, the production of cytokines is kept in check by the body. However, in some instances, the reaction becomes uncontrolled, and too many immune cells are activated in a single place

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- The precise reason for this is not entirely understood but may occur in a number of infectious and noninfectious diseases, including graft versus host disease (GVHD), acute respiratory distress syndrome (ARDS) in influenza and COVID-19, sepsis, Ebola, smallpox, and systemic inflammatory response syndrome (SIRS).

Cytokines used in Therapy

Cytokines offer great promise for the treatment of a number of diseases. Many strategies have been followed to create a

cytokine or anticytokine state in the body depending on the need. The strategies are:

- ❖ **Use of cytokines (e.g. interferons) as drug:**
 - Interferon- α is used for the treatment of hepatitis B, hepatitis C, hairy cell leukemia, multiple myeloma and chronic myeloid leukemia (CML)
 - Interferon- β is used for the treatment of multiple sclerosis
 - Interferon- γ is used for the treatment of chronic granulomatous disease.
- ❖ **Cytokine-toxin conjugates** are used to destroy the target cells; here the cytokines help in binding to the target cells so that the toxin can act on them.

EXPECTED QUESTIONS

I. Write essay on:

1. Describe in detail about the structure and function of various lymphoid organs and cells of immune system.

II. Write short notes on:

1. Major histocompatibility antigen.
2. Cytokines.
3. Development of T cells.
4. Development of B cells.

III. Multiple Choice Questions (MCQs):

1. T cell area of lymph node is:

- a. Cortex
- b. Medulla
- c. Paracortical area
- d. All of the above

2. Which one of the following cytokine induces fever?

- a. IL-2
- b. IL-1
- c. IL-4
- d. IL-5

3. All of these are antigen presenting cells (APCs), except:

- a. T cells
- b. B cells
- c. Dendritic cells
- d. Macrophage

4. Cell type which lacks HLA antigen is:

- a. Monocyte
- b. Thrombocyte
- c. Neutrophil
- d. Red blood cell

5. Interferon gamma is secreted by:

- a. Macrophage
- b. Fibroblasts
- c. Activated T cell
- d. Neutrophils

6. All of the following are example of peripheral or secondary lymphoid organs, except:

- a. Bone marrow
- b. Lymph node
- c. Spleen
- d. MALT

7. Which of the following represents the T cell area of spleen?

- a. Periarterial lymphoid sheath (PALS)
- b. Marginal zone
- c. Red pulp
- d. All of the above

8. Defect in spleen predisposes to all of the following infection, except:

- a. *Staphylococcus aureus*
- b. *Streptococcus pneumoniae*
- c. *Neisseria meningitidis*
- d. *Haemophilus influenzae*

9. M cells act as the portal of entry of following microbes, except:

- a. *Salmonella*
- b. *Shigella*
- c. *E. histolytica*
- d. Poliovirus

10. The phenomenon of receptor editing occurs during:

- a. B cell development
- b. T cell development
- c. NK cell development
- d. Macrophage development

11. Which type of dendritic cells help in B cell development?

- a. Interstitial
- b. Interdigitating
- c. Circulating
- d. Follicular

Answers

1. c 2. b 3. a 4. d 5. c 6. a 7. a 8. a 9. c 10. a 11. d

Immune Responses: Cell-mediated and Antibody-mediated

CHAPTER 15

CHAPTER PREVIEW

- Antigen Presentation
- Helper T Cells (Activation and Differentiation)
- Cell-mediated Immune Response
- Humoral/Antibody-mediated Immune Response

INTRODUCTION

Immune response refers to the highly coordinated reaction of the cells of immune system and their products. It has two arms (Fig. 15.1).

Humoral or Antibody-mediated Immune Response (AMI)

It provides protection to the host by secreting *antibodies*; that can bind and neutralize microbial antigens circulating free or present on the surface of the host cells and in the extracellular spaces, but have no role against intracellular antigens. If antibodies were the only agents of immune response, pathogens that manage to evade them by being in the intracellular environment would have escaped the immune response. Nevertheless, this is not the case.

Cell-mediated Immune Response (CMI)

It plays a crucial role in providing protection against intracellular microbes as well as tumor cells. Although CMI is mainly T cell mediated (especially cytotoxic T cells); however, various other effector cells such as natural killer (NK) cells, macrophages, granulocytes are also components of CMI.

CMI and AMI are Interdependent

CMI and AMI cannot work individually, but they are highly dependent on each other (Fig. 15.1). Cytokines released from T cells stimulate B cells to produce antibodies. Similarly, many effector cells of CMI such as macrophages and NK cells use antibodies as receptors to recognize the target cells for killing.

CMI also regulates the humoral immunity by releasing cytokines from activated T cells that stimulate the B cells to transform into antibody secreting plasma cells.

There are certain initial events that must take place before the induction of either CMI or AMI. These events are common to both CMI and AMI, and they occur irrespective

of the type of immune response that will follow. These events include:

- ❖ Antigen presentation to helper T cells
- ❖ Activation and differentiation of helper T cells into either T_H1 or T_H2 subsets.

Helper T (T_H) cells are the central key that regulate the type of immune response that is going to occur. Activated helper T cells differentiate into either T_H1 or T_H2 subsets. Induction of T_H1 cells secrete cytokines that stimulate cell mediated response, whereas if T_H2 cells are differentiated, they secrete certain cytokines that in turn induce the B cells to produce antibodies.

ANTIGEN PRESENTATION

For induction of immune responses, recognition of antigens by T cells is essential. T cells cannot recognize the native and free antigens, but they do so only after the antigen is processed into smaller antigenic peptides containing specific epitopes which are subsequently combined with MHC molecules (class I or II) and presented on the host cell surface.

Antigen-presenting Cells (APCs)

Although antigen presentation refers to presentation of antigenic peptide to both T_H (helper T cells) and T_C (cytotoxic T cells) by complexing with MHC-II and I respectively; however, antigen-presenting cells (APCs) in strict sense implies only to those cells (e.g. dendritic cell, macrophage, etc.) that present the antigenic peptide along with MHC class II to T_H cells (Table 15.1).

Cells presenting antigenic peptides along with MHC class I molecules to T_C cells are not included under APCs. These cells are usually virus infected cells or tumor cells. They are often referred to as **target cells** as the activated T_C cells cause lysis of these cells.

Dendritic cells, macrophages and B cells are the major APCs and are called **professional APCs**. There are some

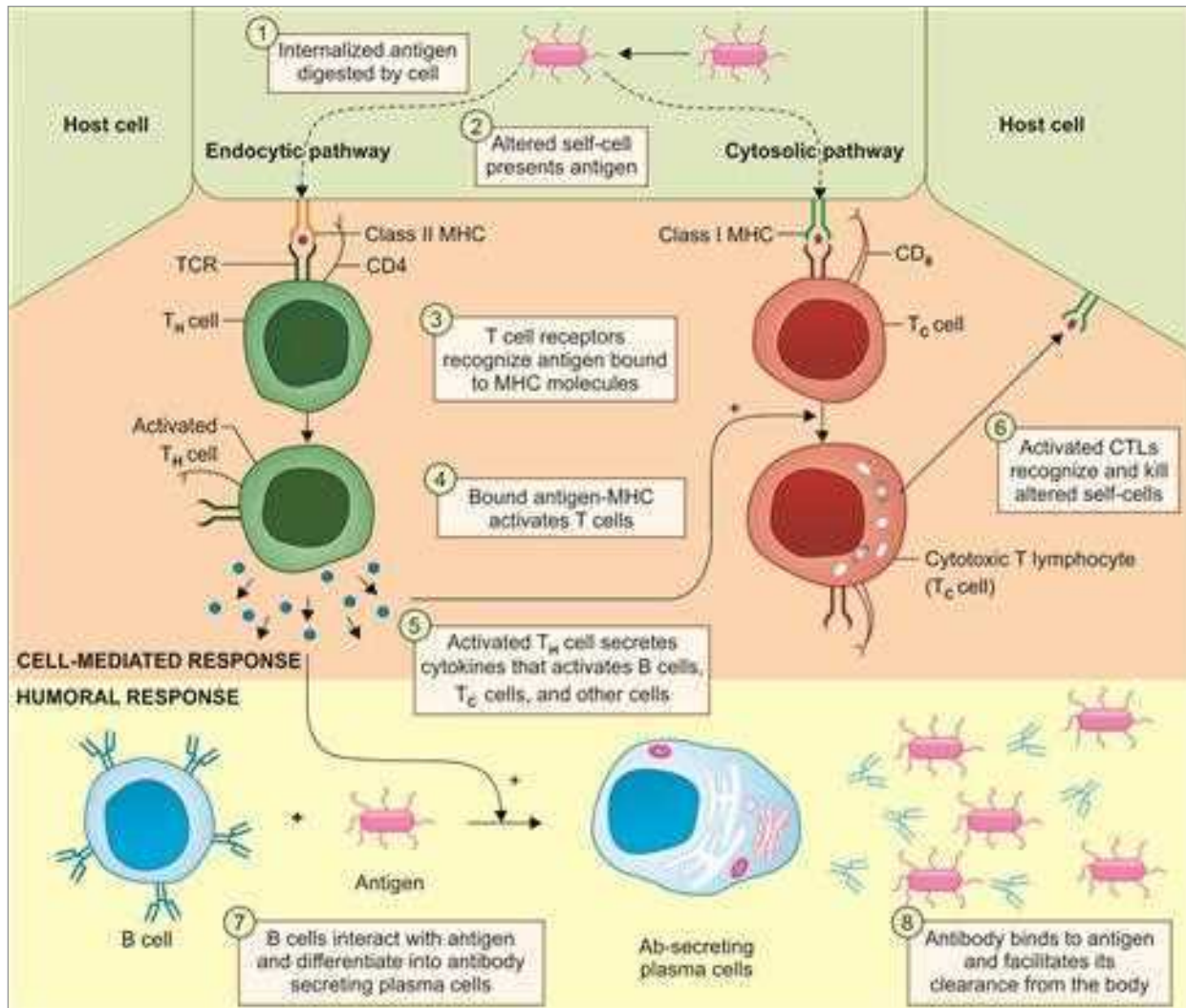


Fig. 15.1: Overview of immune response.

Abbreviations: MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte; Ab, antibody.

Professional APCs	Nonprofessional APCs
Dendritic cells	Fibroblasts (skin)
Macrophages	Thymic epithelial cells
B cells	Pancreatic beta cells
	Vascular endothelial cells
	Glial cells (brain)
	Thyroid epithelial cells

other non-professional cells that can occasionally present antigens to helper T cells (Table 15.1).

Antigen Processing Pathways

For induction of immune response (both CMI and AMI), antigens must be presented to T_H cells. In addition, for CMI induction, antigen presentation to T_C cells is essential. Two well defined pathways are used by the immune system

Property	Cytosolic pathway	Endocytic pathway
Antigen processed	Endogenous	Exogenous
Ag is complexed with	MHC I molecules	MHC II molecules
Antigen is presented to	T _C cells	T _H cells

for this purpose. They differ from each other in their mechanism and target antigen, as described below (Table 15.2):

- 1. Cytosolic pathway:** Here, the endogenous (intracellular) antigens such as viral antigens and tumor antigens are processed and presented along with MHC class I molecules to CD8 T cells
- 2. Endocytic pathway:** In this pathway, the exogenous antigens (extracellular microbes and their products, e.g. toxins) are processed and complexed with MHC class II molecules and presented to T_H cells. The cells

involved in endocytic pathway include the APCs such as macrophages, dendritic cells and B cells.

HELPER T CELLS (ACTIVATION AND DIFFERENTIATION)

Helper T cell (T_H) activation and differentiation is the central event that regulates both the components of immune response; CMI and AMI.

Activation of Helper T Cells

Signal Generation

Activation of T_H cells requires generation of three specific signals (Fig. 15.2).

1. **Antigen-specific signal:** It involves binding of antigenic peptide present in the groove of MHC-II on APCs to TCR (T cell receptor) present on surface of T_H cells. CD4 molecules of T_H cells also interact with $\beta 2$ domain of MHC-II
2. **Costimulatory signal:** It involves binding of CD28 molecule on T_H cells to B7 molecules on APCs
3. **Cytokine signal:** APCs (macrophages) secrete interleukin-1 (IL-1) which acts on T_H cells.

Signal Transduction

Following induction of signal, its transmission is essential for T_H cell activation. Signal transduction is initiated at CD4 molecule which interacts with CD3 complex, which in turn transmit the signal leading to activation of T_H cells.

Differentiation of Helper T Cells

Activated T_H cells secrete increased amount of IL-2 as well as IL-2 receptor (IL2R or CD25). IL-2 binds to its receptors on the same T_H cell and also on other T_H cells and induces the naive T_H cells to proliferate and differentiate. T_H cells get activated and become lymphoblast cells which

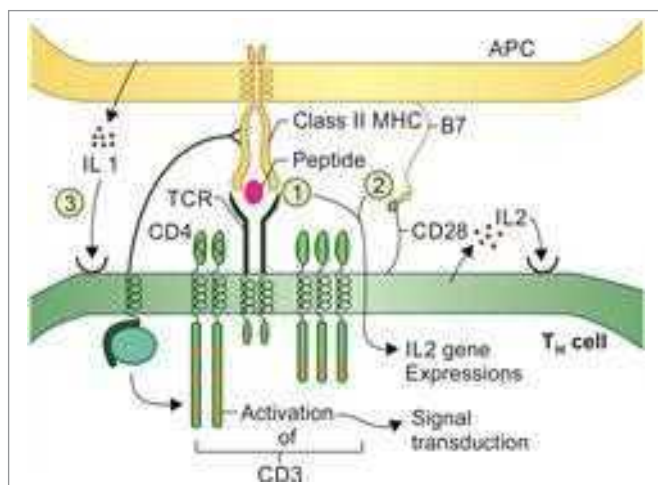


Fig. 15.2: Activation of T_H cell by interacting with APC.

Abbreviations: APC, antigen-presenting cells; MHC, major histocompatibility complex; TCR, T cell receptor.

subsequently differentiate into memory and effector T_H cells.

Effector T_H Cells

They are derived either from the naive T_H cells or pre-existing memory T_H cells following antigenic stimulus. They are short lived (few days to weeks). They further **differentiate into either T_H1 or T_H2** subsets. This differentiation is very crucial as they secrete distinct cytokines that further mediate specific functions.

Cytokines secreted by T_H1 cell stimulate cytotoxic T cells and induce cell mediated immune response; while cytokines secreted by T_H2 cell stimulate B cells producing different classes of antibodies (humoral immune responses). **IL12** secreted by macrophage plays an important role in the differentiation of T_H cells. It promotes T_H1 subset proliferation.

- ❖ **T_H1 cells** produce IL-2, interferon- γ (IFN- γ) and tumor necrosis factor- β (TNF- β); each has specific function (Table 15.3)
- ❖ **T_H2 cells** secrete IL-4, IL-5, IL-6, IL-10 and IL-13. They activate the B cells to transform into plasma cells which in turn secrete antibodies (Table 15.3).

Memory T Cells

They are derived from activated T_H cell. They have longer life span (months to years). They are in resting stage, but following subsequent antigenic stimulus, they become activated and differentiated into effector T_H cells. They express CD45RO isoform of common leukocyte antigen CD45, as compared to naive T cells which express CD45RA.

Table 15.3: Role of cytokines secreted by T_H1 and T_H2 cells.

T_H1 cytokines and their functions	
IL-2	<ul style="list-style-type: none"> • Promotes activation of T_H and T_C cells • Activates NK cells to become LAK cells
IFN- γ	<ul style="list-style-type: none"> • Activates the resting macrophages into activated macrophages • Activates B cells to produce IgG • Promotes inflammation of delayed type of hypersensitivity (along with TNF-β) • Inhibits T_H2 cell proliferation
TNF- β	Enhances phagocytic activity of macrophage
T_H2 cytokines and their functions	
IL-4	<ul style="list-style-type: none"> • Inhibits T_H1 cell differentiation • Stimulates B cells to produce IgE and also IgG4 and IgG1
IL-5	<ul style="list-style-type: none"> • Enhances proliferation of eosinophils • Both IL-4 and IL-5 together provide protection against helminthic infections and also mediate allergic reaction
IL-6	Promotes B cell proliferation and antibody production
IL-10	Inhibits T_H1 cell differentiation

Abbreviations: LAK cell, lymphokine-activated killer cells; IFN, interferons; IgG, immunoglobulin G; TNF, tumor necrosis factor; NK, natural killer.

CELL-MEDIATED IMMUNE RESPONSE

The term cell-mediated immune response (CMI) refers to destruction of cells carrying intracellular microbes and other abnormal cells, such as tumor cells by various specific and nonspecific cells of immune system, of which the most important is cytotoxic T (T_c) cells.

Role of CMI

CMI mediates the following immunological functions:

- ❖ Provides immunity against microbes residing in intracellular milieu:
 - For obligate intracellular organisms, CMI remains the only effective immune response. Examples include all viruses, some bacteria (*Mycobacterium*, *Chlamydia* and *Rickettsia*), some parasites (*Plasmodium*, *Leishmania*, *Trypanosoma* and *Cryptosporidium*) and some fungi (*Pneumocystis*)
 - For facultative intracellular organisms, humoral immunity is active as long as the organism is extracellular. Once they come to intracellular milieu, CMI takes the leading role. Examples include Bacteria like *Listeria*, *Salmonella* and *Yersinia* and fungi such as *Histoplasma* and *Cryptococcus*.
- ❖ Provides immunity against tumor cells and other damaged and altered cells
- ❖ Mediates delayed hypersensitivity (type IV hypersensitivity)
- ❖ Plays key role in transplantation immunity and graft-versus-host (GVH) reaction.

Effector Cells of CMI

CMI can be mediated by both antigen specific and nonspecific effector cells (Table 15.4). They perform their function by direct killing of the target cells (e.g. virus infected cells or tumor cells).

- ❖ The most important mediator of CMI is cytotoxic T cell which is antigen specific
- ❖ However, many other nonspecific effector cells such as macrophages, NK cells, neutrophils, and eosinophils also contribute to CMI
- ❖ Although CMI has many features distinct from humoral immune response but it is not completely independent. The nonspecific effector cells use antibodies as receptors to recognize the target cells for killing.

Effector cells of CMI	Antigen specificity
Cytotoxic T cells	Specific
NK cells	Nonspecific
Cells performing ADCC (NK cells, macrophages, neutrophil and eosinophils)	Nonspecific

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; NK, natural killer.

Cytotoxic T Lymphocytes

CD8 cytotoxic T lymphocytes (CTL or T_c) are the principal effector cells of CMI, involved in the destruction of target cells such as virus infected host cells and tumor cells. Naive T_c cells (or CTL precursors) respond to viral or tumor peptide antigens which are processed by the target host cells (by cytosolic pathway) and presented along with MHC class I molecules. Activated T_c in turn secretes cytotoxic enzymes that lyse the target cells.

Activation of CTL

Generation of activated CTL from naive T_c cells requires induction of at least three signals (Fig. 15.3):

1. **Antigen-specific signal:** It is induced by binding of TCR-CD3 complex of naive T_c cells to MHC I-peptide complex of target cells. CD8 of T_c cells also interacts with $\alpha 3$ domain of MHC-I
2. **Costimulatory signal:** CD28 of naive T_c cells interacts with B7 molecule on target cells
3. **Third signal:** IL-2 (secreted by T_H1 cell) acts on high-affinity IL-2 receptor on T_c cells.

Following induction, the transmission of signal occurs in a way similar to that described for T_H cells.

Functions of CTL (Target Cell Lysis)

The activated T_c cells produce two types of lethal enzymes; called perforins and granzymes.

- ❖ **Perforins** produce pores in the target cell membrane; through which granzymes are released inside
- ❖ **Granzymes** are serine proteases; they induce cell death by apoptosis through caspase pathway.

Natural Killer Cells

Natural killer cells are large granular lymphocytes that constitute 10–15% of peripheral blood lymphocytes.

- ❖ They are derived from a separate lymphoid lineage. NK cells are cytotoxic, but antigen nonspecific
- ❖ They are part of innate immunity, act as first line of defense and do not require prior contact with the antigen. NK cells act against virus infected cells and tumor cells till the T_c cells are activated and take over the function.

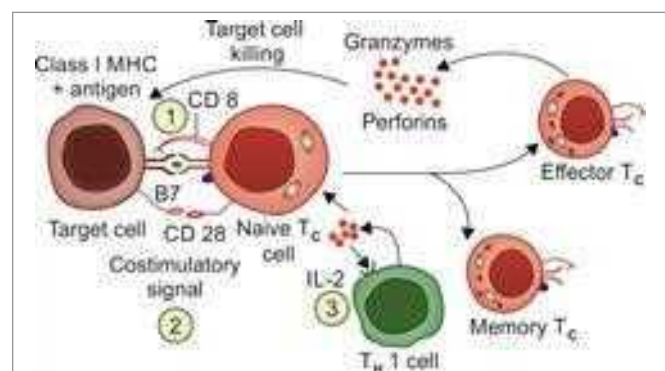


Fig. 15.3: Activation and differentiation of T_c cells.

Property	NK cells	T _C cells
Surface markers	CD16 and CD56	CD3, CD8
MHC restriction	No	MHC-I restricted
Memory	No	Yes
Immunity	Part of innate immunity	Part of acquired immunity
Target cell	Virus infected cells Tumor cells	Same as NK cells
Mechanism of destruction	Perforins and granzymes (constitutive)	Same as NK cells (inducible)
Immune response	CMI	CMI

Abbreviations: MHC, major histocompatibility complex; CMI, cell-mediated immunity.

However, they differ from T_C cells in many other aspects (Table 15.5) such as:

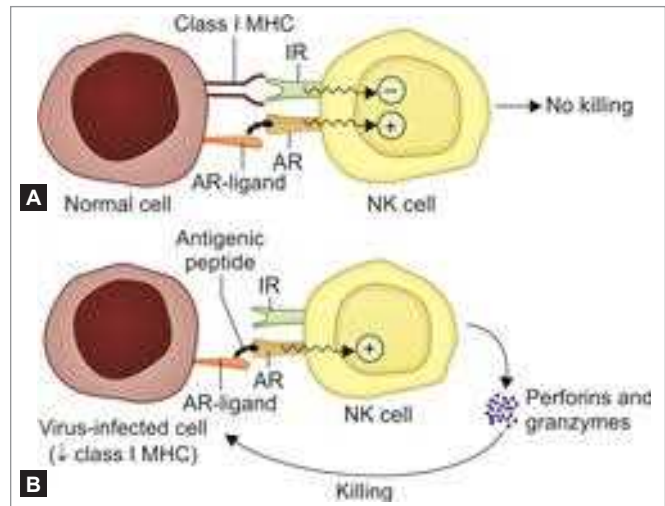
- ❖ **Natural killer cell markers:** NK cells lack the T cell markers such as CD3, CD4 or CD8 molecules (hence are also called null cells), instead possess specific surface markers such as CD16 and CD56
- ❖ **No MHC restriction:** NK cells can recognize the ligands (antigens) without MHC presentation
- ❖ **Innate immunity:** NK cells are part of innate immunity; they do not require the prior exposure to microbial antigen
- ❖ **No memory:** NK cells do not differentiate into memory cells.

Mechanism of NK Cell-mediated Cytotoxicity

Receptor Interaction

Natural killer cells are not MHC restricted. They directly recognize certain ligands (e.g. glycoproteins) present on the surface of altered host cells like virus-infected cells or tumor cells. However, such ligands are also present on normal cells. Still, NK cells are capable of distinguishing normal host cells from the altered cells (Figs 15.4A and B). This is mediated by two types of receptors present on NK cell surface (**theory of opposing-signals model**).

- ❖ **Activation receptors** (e.g. NKR-P1, CD16): When these receptors are engaged with ligands present on the target cells; NK cells become activated
- ❖ **Inhibitory receptors** (such as C-type lectin inhibitory receptors): They recognize a part of MHC I molecule (HLA-E) which is present on the surface of all normal nucleated cells
 - Binding of inhibitory receptors to MHC-I molecules generates an inhibitory signal that suppresses the NK cells even if they are bound to the activation receptors. This is because the inhibitory signal is the dominant signal and hence it overrides the activation signal
 - However, in virus infected cells and tumor cells, the MHC-I expression is remarkably reduced. In such cases, there would not be any inhibitory signal.



Figs 15.4A and B: NK cell-mediated cytotoxicity:

A. In normal cell; **B.** In virus infected cell.

Abbreviations: AR, activation receptor; IR, inhibition receptor.

Hence, binding of activation receptor to its ligand leads to activation of NK cells.

Target Cell Destruction

Mechanism of target cell lysis by NK cells is similar to that of T_C cells, i.e. via secreting perforins and granzymes. Perforins form pores on target cells, through which granzymes enter and lyse the target cells (Fig. 15.4B). The only difference is that, the enzymes are constitutively expressed in NK cell cytoplasm (i.e. they are cytotoxic all the time, even without exposure to the antigen).

Alternative Mechanisms of NK Cell Activity

- ❖ NK cells respond to IL-12 produced by macrophages and secrete IFN- γ , which in turn activates the macrophages. Then, the activated macrophages phagocytose and kill the microbes
- ❖ NK cells also mediate their function via ADCC (described below).

Antibody-dependent Cell-mediated Cytotoxicity (ADCC)

A number of nonspecific cytotoxic cells express receptors (FcR) on their surface that can bind to the Fc region of any immunoglobulin.

- ❖ Following contact with a target cell coated with an antibody, these FcR bearing cells can bind to Fc portion of the antibody coated on the target cells, and subsequently cause lysis of the target cell
- ❖ Although these cytotoxic cells are nonspecific for the antigen, the specificity of the antibody directs them towards the specific target cells. This type of cytotoxicity is referred to as **antibody-dependent cell-mediated cytotoxicity (ADCC)**.

ADCC is exhibited by various cells such as NK cells, macrophages, monocytes, neutrophils, and eosinophils.

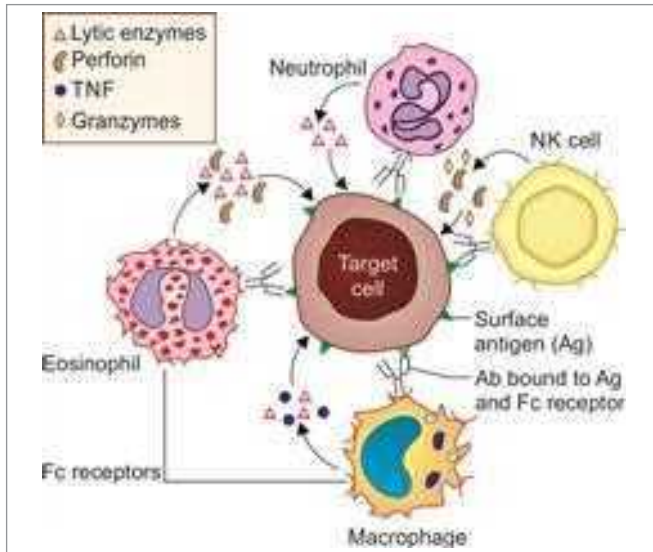


Fig. 15.5: Cytotoxic factors released by various cells in ADCC.

They release various cytotoxic factors into the target cells like perforins, granzymes, lytic enzymes, free radicals, TNF, etc. (Fig. 15.5). However, there is no complement dependent cytolytic activity.

- ❖ NK cells secrete perforins, and granzymes. Neutrophils release lytic enzymes
- ❖ Eosinophils can release lytic enzymes and perforins; play an important role in providing immunity against helminths
- ❖ Macrophages produce lytic enzymes and TNF.

Assessment/Detection of CMI

There are several methods for detection of CMI.

- ❖ The mixed-lymphocyte reaction (MLR) is an in vitro system for assaying T cell proliferation in a cell-mediated response
- ❖ Cell-mediated lympholysis (CML) is another in vitro assay for testing the cytotoxic function of effector cells of CMI
- ❖ The graft-versus-host (GVH) reaction in experimental animals provides an in vivo system for studying cell-mediated cytotoxicity.

HUMORAL/ANTIBODY-MEDIATED IMMUNE RESPONSE

Antibody-mediated immune response (AMI) provides protection to the host by **secreting antibodies**; that prevent invasion of microbes present on the surface of the host cells and in the extracellular environment, but has no role against intracellular microbes. AMI occurs through the following three sequential steps:

1. Activation of B cells following contact with the microbial antigen (B cells act as APCs)
2. Proliferation and differentiation of B cells into effector cells (antibody producing plasma cells) and memory cells

3. Effector function: Production of antibodies by plasma cells which in turn counter act with the microbes in many ways, such as neutralization, opsonization, complement activation, etc.

Activation of B Cells

Antigens that activate B cells fall into two categories.

1. Most antigens are **thymus-dependent (TD)**; they activate B cells indirectly via activation of T cells. TD antigens are processed by APCs, presented to T_H cells following which the activated T_H cells secrete cytokines that in turn activate the B cells
2. The thymus independent (TI) antigens (e.g. bacterial capsule) are not processed by APC. They can directly activate B cells without the help of T cell induced cytokines (for details refer Chapter 10).

TD antigens induced activation of B cell is described below.

Antigen Presentation of B Cells to Activated T_H Cells

The first and foremost step that occurs is recognition of microbial antigen (TD antigen) by B cell membrane immunoglobulin receptors (mIg) followed by receptor-mediated endocytosis of antigen. Then the antigen is processed into smaller antigenic peptides that are presented in complex with MHC-II to activated T_H cells (by endocytic pathway). This leads to induction of three signals.

Signal Induction

The naive B cells are in the resting stage. Activation requires induction of three signals (Fig. 15.6).

1. **Signal 1:** It is induced by the cross linking of IgM on B cell membrane with the microbial antigen
2. **Signal 2:** It is an additional signal provided by binding of CD40 on B cell with CD40L (ligand) on activated T_H cells
3. **Signal 3:** It is usually a cytokine stimulus. Cytokines produced by the activated T_H cells bind to specific cytokine receptor on B cells.

Signal Transduction

Following induction of signal, its transmission is essential for B cell activation.

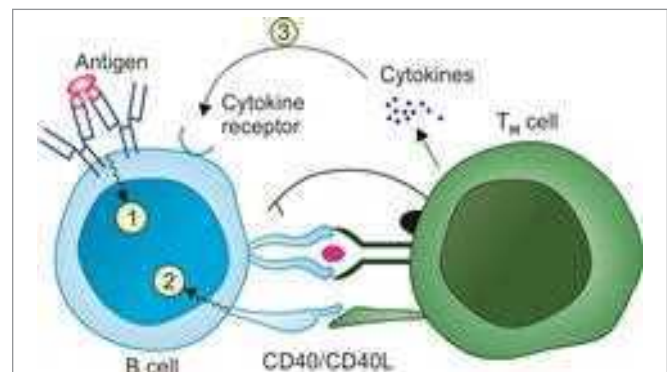


Fig. 15.6: Antigen presentation of B cells to activated T_H cells and signal induction

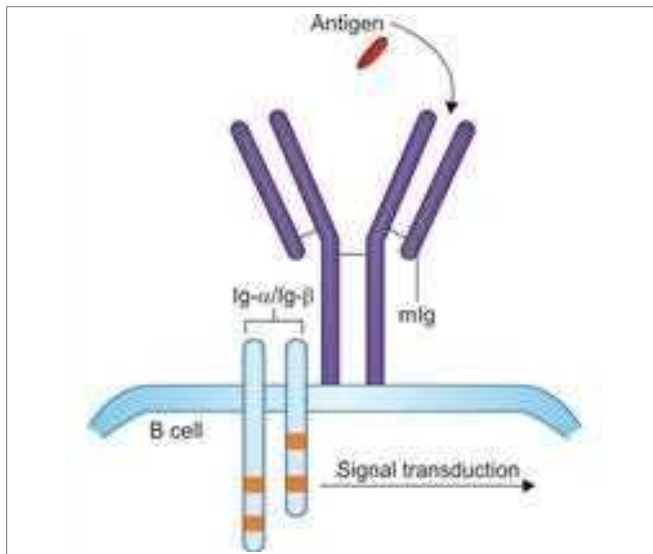


Fig. 15.7: B cell signal transduction.

- ❖ Signal transduction is initiated by the B cell receptor (BCR). The BCR comprises of two parts (Fig. 15.7)
 1. Antigen-binding membrane Ig
 2. Ig- α /Ig- β heterodimer.
- ❖ Following antigen cross linkage to membrane Ig, the Ig- α /Ig- β heterodimer is activated and in turn transmits the signal, ultimately leading to activation of B cells.

Proliferation and Differentiation of B Cells

As described in Chapter 14, the naive B cells, released from bone marrow go and house in the B cell areas of peripheral lymphoid organs (e.g. cortex of lymph node and marginal zone of spleen). There, the naive B cells are organized to form primary lymphoid follicles.

- ❖ Following the antigenic exposure, the naive B cells are activated and then they proliferate
- ❖ Eventually, the primary lymphoid follicles transform into secondary lymphoid follicles
- ❖ Secondary lymphoid follicles bear a germinal center which in turn has two areas; dark zone and light zone. Events occurring in the secondary lymphoid follicles are as follows.

Events in the Dark Zone of Germinal Center (Fig. 15.8)

The activated B cells differentiate into larger dividing cells called **centroblasts**, which further transform into smaller non dividing cells called **centrocytes** by expressing membrane Ig.

- ❖ Centroblasts express the membrane Ig by undergoing a type of mutation called **somatic hypermutations**. These are point mutations arising due to insertion or deletion in the variable region of Ig gene
- ❖ This results in alteration of the membrane Ig affinity by which it binds with the corresponding antigen. Thus, the resultant centrocytes would bear membrane Ig with altered affinity

- ❖ Because somatic hypermutations occur randomly; they generate membrane Ig with both high and low affinity
 - The centrocytes with low affinity membrane Ig undergo apoptosis and then are phagocytosed by special type of macrophages found in lymphoid follicles called **tingible body macrophages**
 - The centrocytes with high affinity membrane Ig are allowed to survive, following which they migrate to the light zone. The process of enhancement of affinity of membrane Ig for antigen binding is called **affinity maturation**.

Events in the Light Zone of Germinal Center (Fig. 15.8)

- ❖ **Binding of centrocytes to follicular dendritic cells:** The centrocytes with high affinity membrane Ig undergo

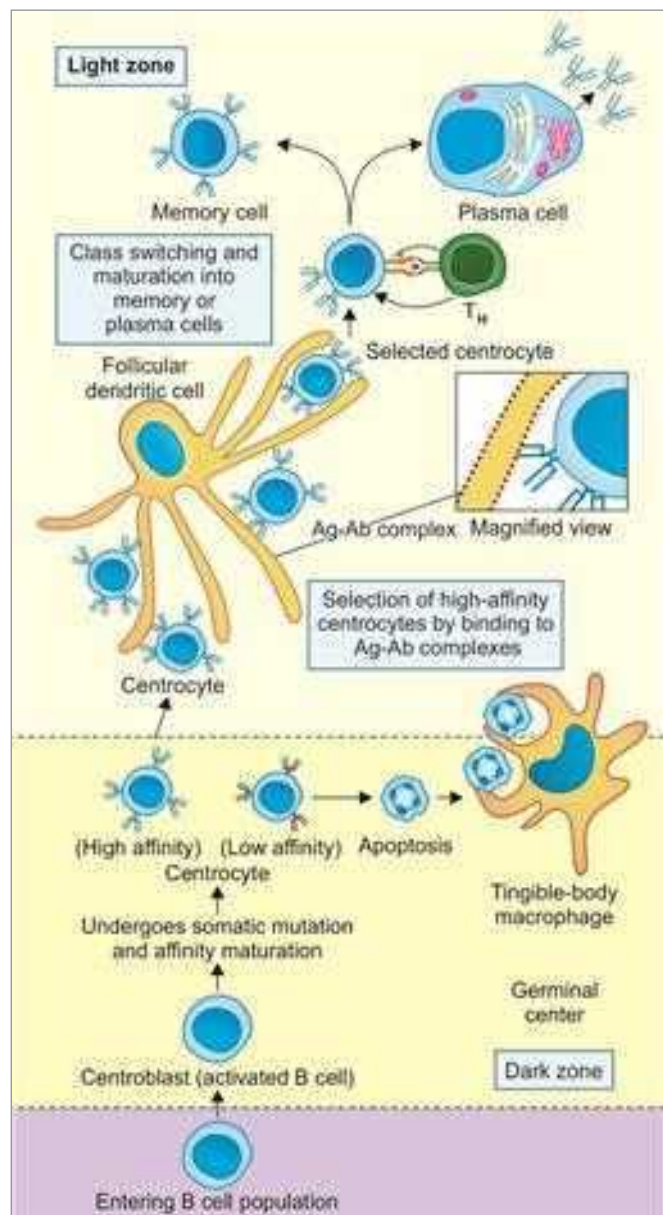


Fig. 15.8: Differentiation of B cells in secondary lymphoid follicles.

maturation by binding to a special type of dendritic cell called **follicular dendritic cell** (see box below). Then the mature centrocytes undergo class switch over

Follicular Dendritic Cells

The follicular dendritic cells (FDC) are special type of dendritic cells which differ from the other types of dendritic cells in various ways.

- ❑ They do not act as APCs and do not express MHC class II. Instead, they bear Fc receptors that recognize Ag-Ab complex
- ❑ Consequently, the antigen is unable to move and is retained in the lymphoid follicle for prolonged periods so that the centrocytes can come and bind to the antigens present in Ag-Ab complex
- ❑ This allows the FDCs to interact with the centrocytes which results in the selection of the centrocytes with high affinity membrane Ig.

- ❖ **Class switch over:** Early in the immune response, IgM is the predominant immunoglobulin secreted by the B cells. But as the maturation progresses, the same B cells undergo a phenomenon called **class switch over** to produce Ig of other classes (Fig. 15.8)
 - Class switch over occurs in the light zone of lymphoid follicles, where the positively selected centrocytes interact with activated T_H cells and receive a cytokine signal for class switching
 - Binding of cytokines produced by T_H cells to cytokine receptors present on centrocytes surface induces class switch over
 - Different cytokines induce production of different classes of Ig by switching mechanism (Table 15.6).
- ❖ **Differentiation of centrocytes into plasma cells and memory cells:**
 - After undergoing class switch over, the selected centrocytes further undergo differentiation into effector cells (plasma cells) and memory cells in the light zone of germinal center
 - Plasma cells are large antibody-secreting cells; produce secretory Ig enormously, but do not synthesize membrane Ig. They do not have MHC-II molecules and do not undergo further class switch over
 - Memory cells bear high affinity membrane Ig molecules of all classes as compared to naive B cell that bear only low affinity IgM or IgD membrane Ig. They are long lived cells which respond to the secondary antigenic stimulus.

Table 15.6: Cytokines secreted by T_H cells and the respective Ig class/subclass they induce.

Cytokine(s)	Ig class produced
IFN- γ	IgG2a or IgG3
IL-5 + TGF- β	IgA or IgG2b
IL-4	IgE or IgG1 or IgG4
IL-2, 4, 5	IgM
IL-4, 5, 6 + IFN- γ	IgG

Effector Functions of AMI

Antibodies secreted from plasma cells mediate a number of biological functions through their Fc portions that bind to Fc receptors (FcRs) expressed by many cell types.

- ❖ **Promotes opsonization:** FcRs present on phagocyte surface recognize antibody coated microbes, bind to them and that leads to enhanced phagocytosis (Fig. 15.9)
- ❖ **Transcytosis:** Poly-Ig receptors are expressed on the inner (basolateral) surface of epithelial cells (facing the blood). They bind to dimers of IgA and multimers of IgM antibodies and transfer them through the cell to their apical (outer) surface and into the lumen of an organ (e.g. the intestine). This is a process referred to as **transcytosis** and is responsible for the accumulation of antibodies in the lumen of the organ (Fig. 15.10)
- ❖ **Mediates mucosal immunity:** Transcytosis of IgA to gut lumen provides mucosal immunity by neutralizing the microbes at local mucosal sites
- ❖ **Activates complement-mediated inflammation and cytolysis:** Antigen antibody complex activates the classical complement pathway (Fig. 15.11). The final

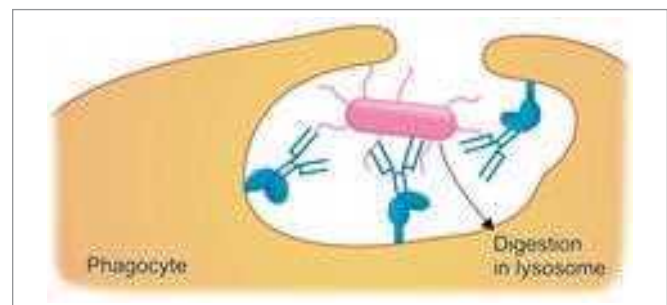


Fig. 15.9: Opsonization of bacteria and phagocytosis.

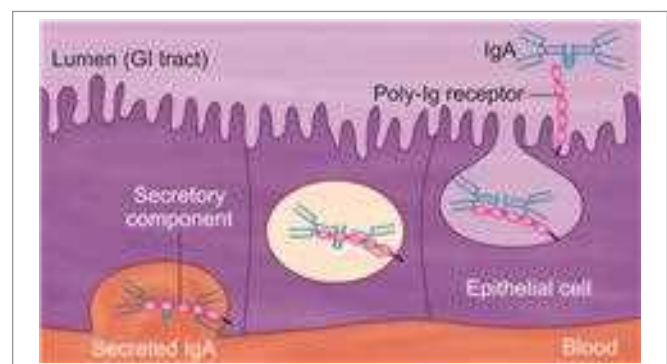


Fig. 15.10: Transcytosis of dimeric IgA.

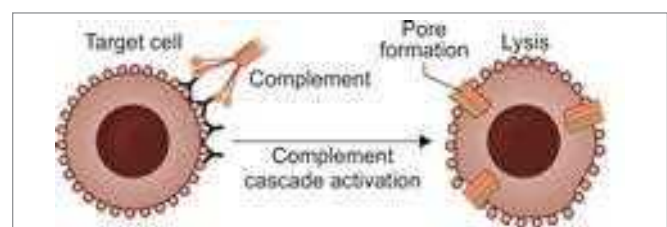


Fig. 15.11: Complement-mediated cytolysis.

complement factors (C5-C9), also called **membrane attack complex** which has lethal activity by forming pores on the target cells

- ❖ **Promotes ADCC:** Though ADCC is principally cell mediated (described under CMI section); antibodies

direct the cells to reach to the target cells. ADCC is important to provide immunity against:

- Helminths (eosinophil-IgE mediated)
- Tumor cells and virus infected cells (NK cell-IgG mediated).

EXPECTED QUESTIONS

I. Write essay on:

1. Describe in detail about the mechanism of cell-mediated immune response.
2. Describe in detail about the mechanism of antibody-mediated immune response.

II. Write short notes on:

1. Antigen presentation.
2. ADCC.

III. Multiple Choice Questions (MCQs):

1. Cell-mediated immunity is by virtue of:

- a. NK cell
- b. Eosinophil
- c. Cytotoxic T cells
- d. All above

2. Macrophages are major source of:

- a. IL-1
- b. IL-5
- c. IL-7
- d. IFN- γ

3. Perforins are produced by:

- a. Plasma cells
- b. Suppressor T cells
- c. Cytotoxic T cells
- d. Memory helper T cells

4. Professional antigen-presenting cells (APCs) include all, except:

- a. Dendritic cells
- b. Macrophages
- c. Fibroblasts (skin)
- d. B cells

5. Cytosolic pathway of antigen presentation—all are true, except:

- a. Endogenous antigens processed
- b. Antigen is complexed with MHC I molecule
- c. Antigen is presented to T_c cells
- d. Occurs only in antigen presenting cells

6. T_H2 cells secrete all the following cytokines, except:

- a. IL-2
- b. IL-4
- c. IL-5
- d. IL-6

Answers

1. d 2. a 3. c 4. c 5. d 6. a

CHAPTER PREVIEW

- Definition and Classification
- Type I Hypersensitivity Reaction
- Type II Hypersensitivity Reaction
- Type III Hypersensitivity Reaction
- Type IV Hypersensitivity Reaction

The purpose of immune response is to eliminate the foreign antigens that have entered into the host. In most instances, immune response leads to only a subclinical or localized inflammatory response which just eliminates the antigen without causing significant damage to the host. However, at times, this response becomes abnormal; leads to exaggerated inflammatory response which causes extensive tissue damage or sometimes even death.

HYPERSENSITIVITY REACTIONS

Definition

The term hypersensitivity or allergy refers to the injurious consequences in the sensitized host, following subsequent contact with specific antigens.

Gell and Coombs Classification

Following an antigen contact, hypersensitivity may occur immediately or after a few days. It may result from

abnormality of either humoral or cell-mediated immune response. Based on the above two features, Gell and Coombs classified hypersensitivity reactions into four types (Table 16.1).

Immediate Hypersensitivity Reactions

These reactions occur immediately, within few minutes to few hours of antigen contact, as a result of abnormal exaggerated humoral response (antibody mediated). This can be further classified into three types based on the type of effector mechanisms:

1. **Type I hypersensitivity reaction:** It is IgE-mediated, which causes mast cell degranulation following a contact with soluble antigen
2. **Type II hypersensitivity reaction:** It is IgG (or rarely IgM) mediated, which causes complement activation or antibody-dependent cellular cytotoxicity (ADCC) in response to cell surface bound antigens
3. **Type III hypersensitivity reaction:** It is immune complex-mediated; which are formed due to interaction

Table 16.1: Features of various types of hypersensitivity reactions.

	Type I	Type II	Type III	Type IV
Immune response altered	Humoral	Humoral	Humoral	Cell mediated
Immediate or delayed	Immediate	Immediate	Immediate	Delayed
Duration between appearance of symptoms and antigen contact	2–30 minutes	5–8 hour	2–8 hours	24–72 hours
Antigen	Soluble	Cell surface bound	Soluble	Soluble or bound
Mediator	IgE	IgG	Ag-Ab complex	T _{DTH} cell
Effector mechanism	Mast cell degranulation	<ul style="list-style-type: none"> • ADCC • Complement-mediated cytotoxicity 	Complement activation and inflammatory response	Macrophage activation leads to phagocytosis or cell cytotoxicity
Desensitization to the allergen	Easy, but short-lasting	Easy, but short-lasting	Easy, but short-lasting	Difficult, but sustained
Typical manifestations	<ul style="list-style-type: none"> • Anaphylaxis • Asthma • Atopic dermatitis 	<ul style="list-style-type: none"> • Transfusion reactions • Rh incompatibility • Hemolytic anemia 	<ul style="list-style-type: none"> • Arthus reaction • Serum sickness • Glomerulonephritis • Rheumatoid arthritis 	<ul style="list-style-type: none"> • Tuberculin test • Granuloma formation in tuberculosis, leprosy, etc. • Contact dermatitis

Abbreviation: ADCC, antibody- dependent cellular cytotoxicity.

between soluble antigen and antibody (usually IgG), resulting in an abnormal inflammatory response.

Delayed Hypersensitivity Reaction

Delayed hypersensitivity reaction occurs after few days of antigen contact, as a result of abnormal cell-mediated immune response. This is also called type IV hypersensitivity reaction. It is mediated by a specific subset of T_H cells called delayed hypersensitivity T cells or T_{DTH} cell.

TYPE I HYPERSENSITIVITY REACTION

The hallmark of type I hypersensitivity reaction is production of IgE by sensitized B cells following a contact with an allergen which in turn induces mast cell degranulation. The pharmacologically active mediators released from these granules cause vasodilation, vascular and smooth muscle contraction and increased vascular permeability. These changes ultimately lead to localized response (called **atopy**) and systemic response (called **anaphylaxis**).

Allergens

Allergens are foreign antigens that induce allergy. List of allergens is given in Table 16.2.

Experiments to Demonstrate Type I Reaction

Several experiments were conducted in the past to demonstrate type I hypersensitivity reactions; out of which, the most popular was P-K Reaction.

P-K Reaction

K Prausnitz and H Kustner (1921) were the first to demonstrate that antibody in the serum responsible for the allergy, and named it as P-K antibody or reaginic antibody; which later was known as IgE (in 1960), after its discovery. The experiment was as follows.

- ❖ Serum from an allergic person is injected intradermally into a nonallergic individual. Later when the appropriate allergen is injected at the same site, a wheal and flare reaction is developed at the site

Table 16.2: Common allergens associated with type I hypersensitivity reaction.	
Allergen types	Examples
Food	Nuts, egg, peas, sea food, beans, milk
Plants and pollens	Rye grass, rag weed
Proteins	Foreign serum, vaccines
Drugs	Penicillin, sulfonamides, local anesthetics and salicylates
Insect bite products	Venom of bee, wasp, ant, cockroach calyx and dust mites
Others	Mold spores, animal hair and dander

- ❖ The **wheal and flare response** occurs in three stages, as follows:

1. Begins with the appearance of an erythematous area at the site of injury, followed by
2. Development of a flare (erythema) surrounding the site
3. Finally, a wheal (swelling and congestion) forms at the site as fluid leaks under the skin from the surrounding capillaries.

Mechanism of Type I Hypersensitivity

Type I hypersensitivity reaction occurs through two phases; the sensitization and effector phases, both occurring with an interval of 2–3 weeks (Fig. 16.1).

Sensitization Phase

This occurs when an individual is exposed for the first time to the sensitizing or priming dose of an allergen.

- ❖ Sensitization is most effective when the allergen is introduced parenterally, but may occur by any route, including ingestion or inhalation
- ❖ In susceptible individuals, very minute doses can be sufficient to sensitize the host

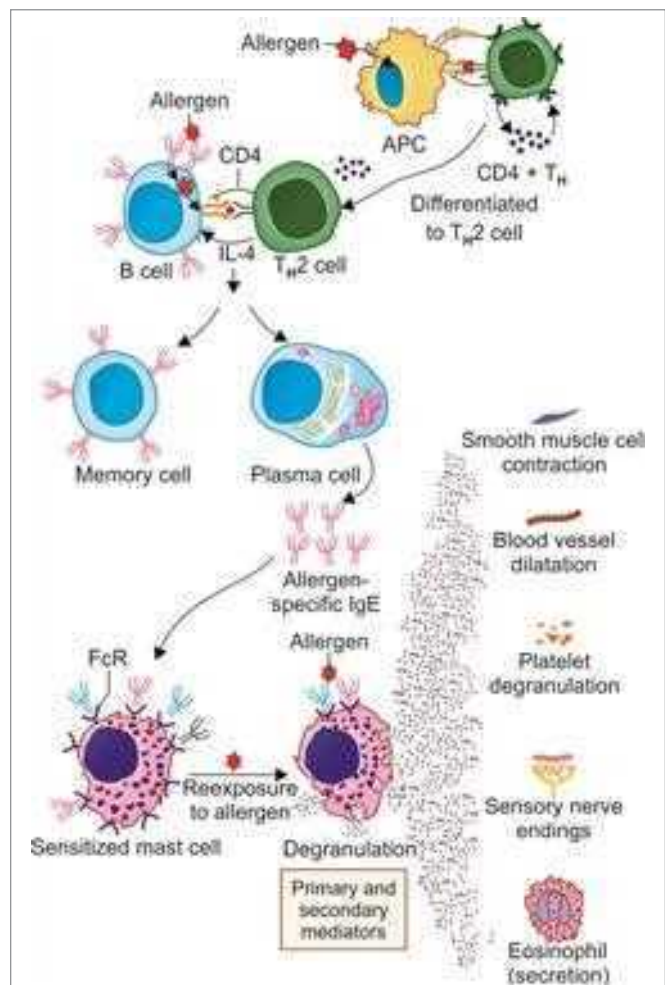


Fig. 16.1: Mechanism of type I hypersensitivity reaction.

- ❖ The allergen is processed by the antigen presenting cells and the antigenic peptides are presented to the CD4 helper T cells
- ❖ Activated T_H cells are differentiated into T_H2 cells which in turn secrete interleukin 4 (IL-4)
- ❖ IL-4 induces the B cells to differentiate into IgE producing plasma cells and memory cells. Many molecules of IgE with specificities against various epitopes of the allergen may be produced
- ❖ Secreted IgE migrate to the target sites, and coat on the surface of mast cells and basophils. Fc region (the C_H3 and C_H4 domains) of IgE binds to high affinity Fc receptors (e.g. FcεR1) present on mast cell surface
- ❖ Such sensitized mast cells (coated with IgE) will be waiting for interaction with the subsequent antigenic challenge.

Effector Phase

When the same allergen is introduced subsequently (shocking dose), it directly encounters with the Fab region of IgE coated on mast cells.

- ❖ IgE cross linkage initiates degranulation: Allergen bound to IgE triggers the mast cells (and basophils) activation and degranulation. Granules in turn release a number of pharmacologically active chemical mediators that lead to the various manifestations of type-1 reaction
- ❖ The memory B cells further differentiate into plasma cells that produce IgE
- ❖ **Degranulation in two phases:** Mast cells and basophils undergo degranulation in two phases
 1. Primary mediators: The preformed chemical mediators which are already synthesized by mast cells, are immediately released, e.g. histamine and serotonin (Table 16.3)
 2. Secondary mediators: The mast cells synthesize them following stimulation by allergen and release, e.g. prostaglandins and leukotrienes (Table 16.3).
- ❖ **Pharmacological actions:** The chemical mediators perform several pharmacological actions, such as bronchial and other smooth muscle contraction, increased vascular permeability and vasodilation (Table 16.3)
- ❖ **Symptoms:** These actions in combinations, produce symptoms such as breathlessness, hypotension and shock leading to death at times.

Manifestations of Type I Reaction

Manifestations are grouped into immediate and late.

Immediate Manifestations

Systemic Anaphylaxis

It is an acute medical emergency condition, characterized by severe dyspnea, hypotension, and vascular collapse leading to death at times.

- ❖ It occurs within minutes of exposure to allergen and unless treated promptly, may lead to fatality

Table 16.3: Mediators of type I hypersensitivity.

Primary mediators	Action
Histamine, heparin and serotonin	↑Vascular permeability ↑Smooth-muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases	Bronchial mucous secretion Degradation of blood-vessel and basement membrane
Secondary mediators	Action
Platelet-activating factor	Platelet aggregation and degranulation; Contraction of pulmonary smooth muscles
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	↑Vascular permeability; contraction of pulmonary smooth muscles
Prostaglandins	↑Vasodilation; Contraction of pulmonary smooth muscles Platelet aggregation
Bradykinin	↑Vascular permeability; smooth-muscle contraction
Cytokines (IL-1 and TNF-α)	Systemic anaphylaxis; ↑ Expression of cell adhesion molecules (CAMs) on venular endothelial cells

- ❖ Allergens: Wide range of allergens have been shown to trigger anaphylaxis in susceptible humans, including the venom (from bee, wasp, and ant stings); drugs (such as penicillin, insulin), antitoxins, seafood and nuts
- ❖ Epinephrine (adrenalin) is the drug of choice for systemic anaphylactic reactions.

Localized Anaphylaxis (Atopy)

Here, the reaction is limited to a specific target tissue or organ, mostly the epithelial surfaces at the entry sites of allergen. These allergies afflict more than 20% of people. They almost always run in families (i.e. inherited) and are collectively called **atopy**. Examples include:

- ❖ **Allergic rhinitis (or hay fever):** It is the most common atopic disorder, affecting 10% of the population. This results from exposure to airborne allergens with the conjunctiva and nasal mucosa leading to appearance of various symptoms such as ↑watery secretions of the conjunctiva, nasal mucosa, and upper respiratory tract, as well as sneezing and coughing
- ❖ **Asthma:** It is the second most common atopic manifestation. It differs from hay fever in involvement of lower respiratory mucosa, resulting in contraction of the bronchial smooth muscles and airway edema, ↑mucus secretion; all together leading to bronchoconstriction and dyspnea. The stimulus may or may not be an allergen. Accordingly, asthma can be classified as:

- Allergic asthma: It is induced by air-borne or blood-borne allergens, such as pollens, dust, fumes, insect products, or viral antigens
- Intrinsic asthma: It is independent of allergen stimulation; induced by exercise or cold.
- ❖ **Food allergy:** Various foods also can induce localized anaphylaxis in atopic individuals. The food allergens (e.g. nuts, egg, seafood, etc.) can either stimulate the mast cells lining gut mucosa to cause GI symptoms such as diarrhea and vomiting or may be carried in the blood stream to distant sites (e.g. when the allergen is deposited on skin, causes local wheal and flare like reaction called **atopic urticaria** (or hives)
- ❖ **Atopic dermatitis (allergic eczema):** It is an inflammatory disease of skin that is frequently associated with young children with family history of atopy. It often develops during infancy, manifested as erythematous skin eruptions which are filled with pus. The skin lesions have an increased response of T_H2 cells and eosinophils
- ❖ **Drug allergy:** Various drugs (such as penicillin, sulfonamides, etc.) may produce type I hypersensitivity responses which may be either local reactions or even sometimes produce systemic anaphylaxis.

Late Manifestations

The immediate phase of type 1 reaction is followed, 4–6 hours later, by an inflammatory response. This phase lasts for 1–2 days and leads to tissue damage.

- ❖ **Mediators:** They are released in acute phase along with cytokines (IL-3, IL-5, IL-8), ECF and NCF; induce recruitment of various inflammatory cells, such as neutrophils, eosinophils, macrophages, and lymphocytes, etc. Among the infiltrates, eosinophils and neutrophils predominate; each accounting for 30% of the total inflammatory cells influx
- ❖ **Eosinophil influx:** It is favored by ECF (eosinophil chemotactic factor), IL-5 and GM-CSF. Eosinophils express Fc receptors for IgG and IgE and thus bind directly to antibody-coated allergens. This in turn causes release of toxic granules from eosinophils which contribute to the chronic inflammation of the bronchial mucosa that characterizes persistent asthma
- ❖ **Neutrophil infiltration:** It is induced by NCF (neutrophil chemotactic factor), and other cytokines such as IL-8. Activated neutrophils release various mediators which further potentiate inflammatory tissue damage and thickening of basement membrane.

Factors Influencing Type I Hypersensitivity

1. Genetic Makeup

Host genetic factors play an important role in mounting an immune response against an allergen.

- ❖ Some individuals mount a normal response where as some mount an exaggerated immune response. Allergen to one individual may not be allergic to other individual

- ❖ There are several gene loci identified which encode proteins that are involved in the regulation of immune responses towards the allergens
- ❖ It is also observed that if both the parents are allergic there is 50% chance that the child will be allergic and when only one parent is allergic, the chance of the child being allergic drops down to 30%.

2. Allergen Dose

The dose of the allergen has a definite impact on the type of immune response produced. It is observed that repeated small doses of allergen induce a persistent IgE response in mice; while higher dosage leads to transient IgE response with a shift towards IgG response.

3. T_H1 vs T_H2 Response

The balance between T_H1 and T_H2 response determines the response of an individual towards an allergen.

- ❖ T_H1 response produces cytokine interferon- γ , which is inhibitory to type I hypersensitivity; whereas T_H2 response induced cytokine IL-3, IL-4 and IL-5 promotes IgE-mediated allergic response
- ❖ Hence, accordingly atopic and non-atopic individuals would demonstrate a predominant T_H2 and T_H1 response to an allergen respectively.

Detection of Type I Hypersensitivity

Skin Prick Test

Small amounts of suspected potential allergens are introduced at different skin sites either by intradermal injection or by superficial scratching.

- ❖ If a person is already sensitized to the allergen, a local wheal and flare response develops within 30 minutes at the inoculation sites (Fig. 16.2)
- ❖ Advantage: Skin test is relatively inexpensive and allows screening of a large number of allergens at one go
- ❖ Disadvantage: It may occasionally sensitize the individual to new allergens and in some rare cases may induce late-phase reaction or even systemic anaphylactic shock.

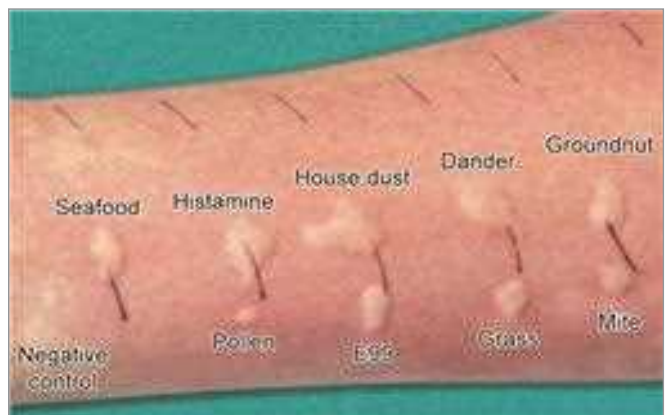


Fig. 16.2: Skin testing by intradermal testing of allergens into forearm.

Total Serum IgE Antibody

Quantitative detection of total serum IgE is performed by various formats such as enzyme immunoassay or radiometric assay called radioimmunosorbent test (RIST, now not in use).

Allergen-specific IgE

Detection of allergen-specific IgE is more specific than total IgE detection. Various test formats are available.

- ❖ **Multiplex immunoblot assay:** Uses a nitrocellulose strip coated with 54 allergens
- ❖ **Fluoro-enzyme immunoassay (FEIA):** Commercially available as ImmunoCAP assay
- ❖ **Automated immunoassay system** (Hytec 288 Plus system)
- ❖ **Anti-CCD absorbent IgE assay:** Detects IgE after absorbing (removing) the nonspecific anti-CCD IgE which are produced against the cross-reactive carbohydrate determinants (CCD) present on the allergens
- ❖ **RAST:** Earlier, a radiometric assay called RAST (Radioallergosorbent test) was in use.

TREATMENT

Type I hypersensitivity reaction

- ❑ **Avoidance of contact with known allergens:** The first and foremost step is identification and avoidance of contact with known allergens such as dusts, house pets, allergic food, etc. However, it is not practically possible to avoid all allergens especially airborne allergens, such as pollens
- ❑ **Hyposensitization:** Repeated exposure to increased subcutaneous doses of allergens can reduce or eliminate the allergic response to the same allergen
 - This occurs probably due to either (1) a shift of IgE response towards IgG or (2) a shift of T_H2 response towards T_H1 response, which secrete $IFN-\gamma$ that in turn can suppress the IgE response
 - Here, the IgG acts as blocking antibody because it competes with IgE for binding to the allergen. The IgG-allergen immunocomplex can be removed later by phagocytosis.
- ❑ **Monoclonal anti-IgE:** Humanized monoclonal anti-IgE can bind and block the IgE; but useful only if the IgE is not already bound to high affinity Fc receptors
- ❑ **Drugs:** Several drugs are useful in suppressing type 1 response through various mechanisms (Table 16.4).

Table 16.4: Drugs used in type I hypersensitivity.

Drugs	Mechanism of action
Antihistamines	Block H1 receptors on target cells; hence antagonize the effects of histamine released
Epinephrine (adrenaline)	Stimulates cAMP production in mast cells; thereby prevents mast cell degranulation Also it causes bronchial smooth muscle relaxation and ionotropic effect
Cortisone	Blocks conversion of histidine to histamine and stimulates cAMP levels in mast cells
Theophylline	Prolongs high cAMP levels in mast cells
Cromolyn sodium	Blocks Ca^{2+} influx into mast cells

Abbreviation: cAMP, cyclic adenosine monophosphate.

TYPE II HYPERSENSITIVITY REACTION

In type II reactions, the host injury is mediated by **antibodies** (IgG or rarely IgM) which interact with various types of antigens, such as:

- ❖ Host cell surface antigens (e.g. RBC membrane antigens like blood group and Rh antigens)
- ❖ Extracellular matrix antigens or
- ❖ Exogenous antigens absorbed on host cells (e.g. a drug coating on RBC membrane).

After Ag-Ab binding occurs, the Fc region of antibody initiates the type II reactions by the following three broad mechanisms (Figs 16.3A and B).

Complement-dependent Reactions

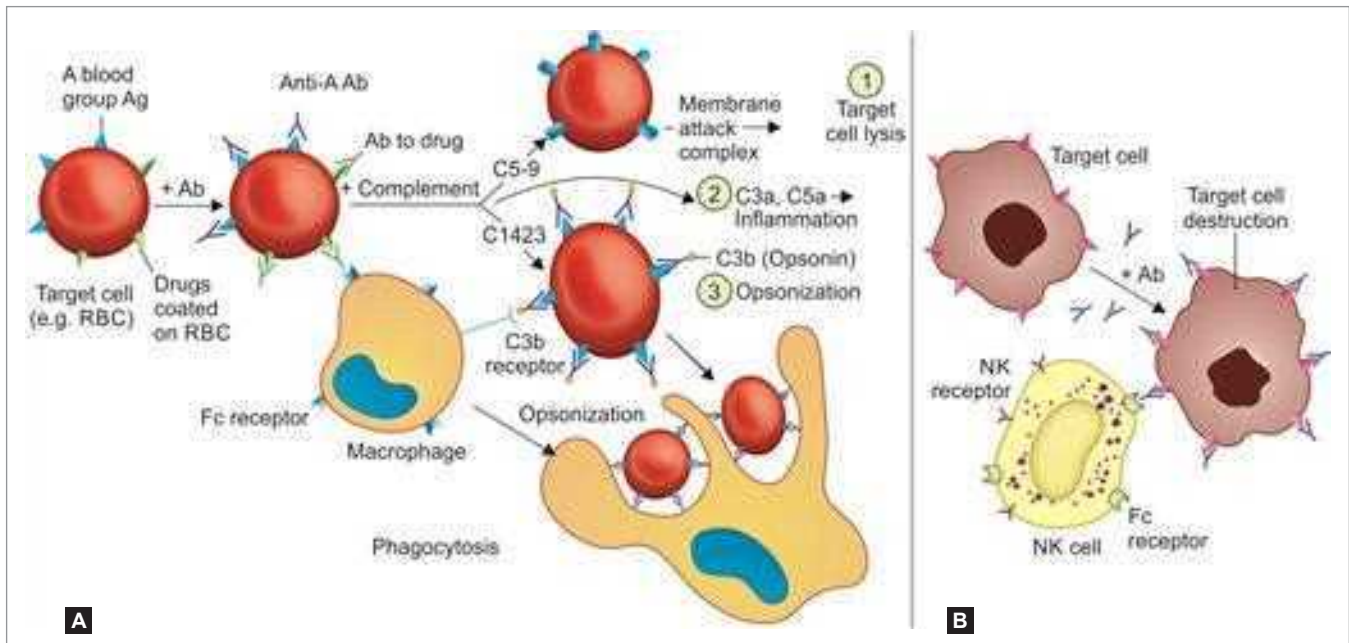
The Fc region of antibody (bound with antigen) can activate the classical pathway of complement system. Activation of classical pathway leads to host cell injury which is mediated by the following three mechanisms (Fig. 16.3A).

1. **Complement-dependent cytolysis:** The membrane attack complex (C5-C9) formed by the activation of classical pathway can produce pores which lead to lysis of the target cells
2. **Complement-dependent inflammation:** The by-products of complement pathways such as C3a and C5a are chemoattractants; hence can induce inflammatory response leading to tissue injury
3. **Opsonization:** By-products of complement pathway, such as C3b and C4b act as opsonins. They deposit on the target cells. Phagocytes, such as macrophage and neutrophil can engulf such C3b and C4b coated target cells via complement receptors.

Complement Mediated Type II Reactions

Antibody-dependent complement mediated type II hypersensitivity is observed in various clinical conditions such as:

- ❑ **Transfusion reaction (ABO incompatibility):** RBCs from an incompatible donor are destroyed after being coated with recipient antibodies directed against the donor's blood group antigens (Fig. 16.3A)
- ❑ **Erythroblastosis fetalis (Rh incompatibility):** Rh negative mother having anti-Rh antibodies due to prior exposure to Rh positive blood (due to previous pregnancy or blood transfusion), can cross the placenta and cause destruction of Rh-positive fetal RBCs
- ❑ **Autoimmune hemolytic anemia, agranulocytosis, or thrombocytopenia:** All these result due to production of autoantibodies to individual's own membrane antigens of RBCs/granulocytes/platelets respectively
- ❑ **Drug-induced hemolytic anemia:** Drug or its metabolic products may get adsorbed onto RBC membrane. If antibodies are formed against the drug, these antibodies will bind with the adsorbed drug on RBC surface and lead to complement activation and lysis of RBCs. For example, following quinine therapy used for malaria (resulting in black water fever) and penicillin therapy (Fig. 16.3A)
- ❑ **Pemphigus vulgaris** (autoantibodies against desmosomal proteins that lead to disruption of epidermal intercellular junctions).



Figs 16.3A and B: Different mechanisms of antibody mediated type II hypersensitivity reactions: **A.** Complement-dependent reactions; **B.** Antibody-dependent cellular cytotoxicity (ADCC).

Abbreviations: RBC, red blood cell; NK, natural killer.

Antibody-dependent Cellular Cytotoxicity (ADCC)

IgG antibodies can coat on the target cells by interacting with the surface antigens through Fab region. The Fc portion of IgG in turn binds to Fc receptors on various effector cells such as NK cells which result in destruction of the target cells (Fig. 16.3B).

- ❖ ADCC is involved in destruction of the targets that are too large to be phagocytosed, e.g. parasites, tumors or graft rejection
- ❖ Although ADCC is typically mediated by IgG antibodies, in certain instances (e.g. eosinophil-mediated killing of parasites) IgE antibodies are used.

Autoantibody Mediated (Antibody-dependent Cellular Dysfunction or ADCD)

In this condition, the host produces certain autoantibodies which bind and disturb the normal function of human self-antigens.

- ❖ **Anti-receptor Ab:** Antibodies may be directed against human receptors, resulting in either inhibition or excessive activation of the receptors leading to host injury
 - **Activation of receptor, e.g. Graves' disease:** Here, the autoantibodies produced are called LATS (long-acting thyroid stimulators), which stimulate the thyroid cells to upregulate the production of thyroid hormones
 - **Inhibition of receptor, e.g. myasthenia gravis:** In this condition, anti-acetylcholine (ACh) receptor antibodies are produced; which block the ACh receptors, leading to profound muscular weakness.

❖ Other examples of ADCC:

- Goodpasture syndrome (antibody produced against type IV collagen)
- Pernicious anemia (antibody directed against intrinsic factor)
- Rheumatic fever (antibody against streptococcal antigens cross reacting with heart)
- Myocarditis in Chagas disease.

TYPE III HYPERSENSITIVITY REACTION

Type III hypersensitivity reactions develop as a result of excess formation of immune complexes (Ag-Ab complexes) which initiate an inflammatory response through activation of complement system leading to tissue injury (Fig. 16.4).

- ❖ **Antigen involved:** Immune complexes can involve exogenous antigens such as bacteria and viruses or endogenous antigens such as DNA
- ❖ **Removal of immune complexes:** Mere formation of immune complexes does not result in type III hypersensitivity reaction
 - Under normal circumstances, the immune complexes are rapidly cleared by activation of complement system
 - Immune complexes coated with complements are either directly phagocytosed by macrophages/monocytes or are bound to RBCs and carried to liver and spleen where they are phagocytosed.
- ❖ However, in some situations, the immune system may be exposed to excess dose of antigen over long period of time such as in chronic infection, autoimmune diseases,

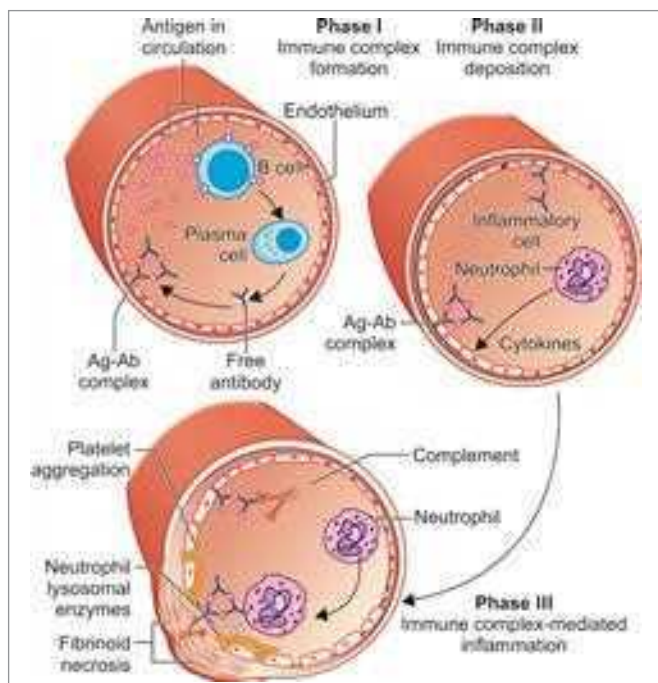


Fig. 16.4: Mechanism of systemic type III hypersensitivity reaction.

and repeated exposure to environmental pollutants. This leads to formation of excessive immune complexes.

Soluble vs Insoluble Immune Complexes

Balance between level of antigen and antibody decides the nature of immune complex that is going to be formed.

- ❖ In case of antibody excess or antigen-antibody equivalence, immune complexes formed are large and insoluble; which tend to localize near the site of antigen administration to produce a localized type III reaction
- ❖ However, in situations when the antigen is in excess (particularly monovalent antigens), small soluble complexes are formed which tend to travel through blood and get deposited in various sites producing a generalized type III reaction.

Mechanism of Tissue Injury

Classical Complement Activation

The Ag-Ab-immune complexes stimulate the classical pathway of complement; the products of which mediate the tissue injury in type III reaction.

- ❖ **Anaphylatoxin:** Complement by-products C3a and C5a being anaphylatoxins, induce localized mast cell degranulation with consequent increase in vascular permeability
- ❖ **Chemoattractant:** C3a and C5a also act as chemoattractants, causing recruitment of neutrophils to the site of immune complex deposition
- ❖ **Role of neutrophils:** Neutrophils attempt to phagocytose the large immune complexes, but fail in doing so. Instead, they release large number of lytic enzymes from the

secretory granules (through **frustrated phagocytosis**) which causes extensive tissue damage.

Platelet Activation

Immune complexes bind to the Fc receptors on platelets leading to their activation. Platelet aggregation (leads to microthrombi formation) and vasoactive amines released from activated platelets, both together cause tissue ischemia leading to further tissue damage.

Activation of Hageman Factor

Activation of Hageman factor leads to activation of kinin, which in turn causes vasodilatation and edema.

Types of Type III Hypersensitivity Reaction

Type III reactions are either localized or generalized.

Localized or Arthus Reaction

Arthus reaction is defined as localized area of tissue necrosis due to vasculitis resulting from acute immune complex deposition at the site of inoculation of antigen.

The reaction is produced experimentally (NM Arthus, 1903) by injecting an antigen into the skin of a previously immunized animal, e.g. rabbit (i.e. excess of preformed antibodies against the injected antigen are already present in the circulation). The circulating antibodies bind with the antigen in the dermis and form immune complexes. These immune complexes fix the complement, resulting in localized immune complex mediated inflammatory response called **Arthus reaction**.

In humans, localized Arthus reaction is seen in some situations, such as:

- ❖ **In skin:** (1) following insect bites or (2) during allergic desensitization treatment wherein repeated injections of the same antigen is given for long periods
- ❖ **In lungs,** following inhalation of bacteria, fungi, spores or proteins may produce intrapulmonary lesions. Examples include conditions causing extrinsic allergic alveolitis, such as:
 - Farmer's lung: It develops following inhalation of actinomycetes (*Saccharopolyspora* species) from mouldy hay
 - Bird-Fancier's disease: This develops following inhalation of serum proteins in dust derived from dried pigeon's feces.

Generalized or Systemic Type III Reactions

The pathogenesis of systemic immune complex disease can be divided into two phases:

1. **Formation of small sized soluble Ag-Ab complexes** in the circulation, which occurs following the entry of a large dose of antigen into the body
2. **Induces inflammatory reaction:** Deposition of the immune complexes in various tissues, thus initiating

Table 16.5: Diseases associated with generalized type III hypersensitivity reactions.

Connective tissue disorders: Result due to autoantibodies forming immune complexes with self-antigens
<ul style="list-style-type: none"> • SLE (systemic lupus erythematosus): Anti-DNA Ab • Rheumatoid arthritis: Ab against human immunoglobulin • PAN (polyarteritis nodosa)
Parasitic diseases: Resulting from immune complex deposition
<ul style="list-style-type: none"> • Nephrotic syndrome in <i>Plasmodium malariae</i> • Katayama fever in schistosomiasis • African trypanosomiasis
Bacterial diseases: Resulting from immune complex deposition
<ul style="list-style-type: none"> • <i>Streptococcus pyogenes</i>: Post-streptococcal glomerulonephritis • <i>Mycobacterium leprae</i> (Lepra reaction type 2)
Viral diseases: With immune complex deposition
<ul style="list-style-type: none"> • Hepatitis B (arthritis) • Hepatitis C (arthritis) • Infectious mononucleosis (Epstein-Barr virus) • Dengue (arthritis)
Others:
<ul style="list-style-type: none"> • Hyperacute graft rejection • Subacute bacterial endocarditis • Serum sickness

an inflammatory reaction in various sites throughout the body such as; blood vessels (vasculitis), glomerular basement membrane (glomerulonephritis), and synovial membrane (arthritis). This has been linked to the pathogenesis of various diseases (Table 16.5).

Serum Sickness

This is another historical example of type III reaction. This condition is not seen nowadays, it was seen in the past, following serum therapy, i.e. administration of foreign serum, e.g. horse anti-tetanus serum, to treat tetanus cases.

- ❖ The horse serum proteins being foreign can induce antibody formation in the host, leading to generation of large number of immune complexes
- ❖ Typically, after 7–8 days, the individuals begin to show various manifestations which are collectively called **serum sickness**. The symptoms include fever, weakness, vasculitis, edema, erythema and rarely lymphadenopathy and glomerulonephritis
- ❖ It subsides gradually once the immune complexes are cleared and free antibodies accumulate.

TYPE IV HYPERSENSITIVITY REACTION

Type IV hypersensitivity reactions differ from other types in various ways:

- ❖ It is delayed type (occurs after 48–72 hours of antigen exposure)
- ❖ It is cell-mediated; characteristic cells called **T_{DTH} cells** (delayed type of hypersensitivity T cells) are the principal mediators of type IV reactions
- ❖ Tissue injury occurs predominantly due to activated macrophages.

Mechanism of Type IV Reactions

Type IV hypersensitivity reactions occur through two phases—(1) sensitization and (2) effector phases (Figs 16.5A and B).

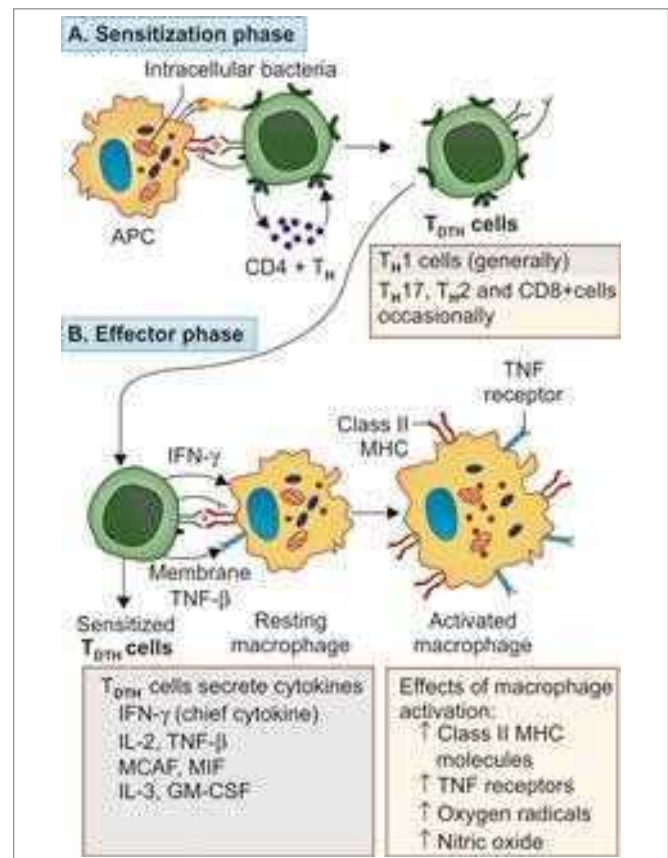
Sensitization Phase

This is the initial phase of 1–2 weeks occurring following antigenic exposure (Fig. 16.5A).

- ❖ During this period, the antigen presenting cells (APCs) process and present the antigenic peptides along with MHC-II to the helper T cells. T_H cells are differentiated to form T_{DTH} cells
- ❖ Most T_{DTH} cells are derived from T_H1 cells; but occasionally other T cells, such as CD8⁺ T cells and CD4⁺ T_H17 can also act as T_{DTH} cells.

Effector Phase

The T_{DTH} cells, on subsequent contact with the antigen, secrete variety of cytokines which attract and recruit various inflammatory cells (e.g. macrophages) at the site of DTH reaction (Fig. 16.5B).



Figs 16.5A and B: Mechanism of delayed type hypersensitivity: **A.** Sensitization phase; **B.** Effector phase.

Abbreviations: APCs, antigen presenting cells; MHC, major histocompatibility complex; TNF, tumor necrosis factor; IFN, interferons; DTH, delayed type hypersensitivity; MCAF, monocyte chemotactic and activating factor; MIF, migration inhibitory factor; GM-CSF, granulocyte monocyte colony stimulating factor.

Cytokines Secreted from T_{DTH} Cells

- **Interferon- γ** : It is the key cytokine of type IV reaction. It activates the resting macrophages into activated macrophages which are highly competent for microbial killing; mediated through several mechanisms such as:
 - ↑ Expression of MHC-II molecules so that they can act as efficient APCs
 - ↑ TNF receptors
 - ↑ Levels of oxygen radicals and nitric oxide.
- **Interleukin-2 (IL-2)**: It acts in autocrine manner; stimulates the proliferation of T_{DTH} cells
- **MCAF** (Monocyte chemotactic and activating factor) and **TNF β** —Help in migration of monocytes from blood to the site of DTH and transforming them into tissue macrophages
- **MIF** (migration inhibitory factor): It further inhibits migration of macrophages from the site of DTH
- **IL-3** and **GM-CSF** (granulocyte-monocyte colony stimulating factor)—help in local synthesis of monocytes.

Role of DTH: Protective vs Tissue Damage Response

Through type IV hypersensitivity reactions, host attempts to provide defense against many intracellular microorganisms such as *M. tuberculosis* as well as several chemicals and nickel salts (Table 16.6). Always, the attempts do not result in protection.

Protective Response

Under normal circumstances, the pathogens are usually cleared with little tissue damage; mediated by the enhanced microbicidal potency of activated macrophages.

Tissue Damage Response

However, in conditions, when the intracellular microbes escape the macrophage killing mechanisms; the enhanced phagocytic activity and release of various lytic enzymes by the activated macrophages in an attempt to kill the pathogen leads to nonspecific tissue destruction.

Pathology of DTH Reaction (Granuloma Formation)

Continuous DTH reaction for killing the intracellular microbes (especially persistent and/or nondegradable antigens) leads to formation of granuloma (e.g. tubercles in leprosy and tuberculosis).

- ❖ The initial T_H cell infiltrate is progressively replaced by macrophages in 2–3 weeks. Macrophages transform into two type of cells:
 1. They become large, flat, and eosinophilic; denoted as **epithelioid cells**
 2. The epithelioid cells occasionally fuse (induced by IFN- γ) to form **multinucleated giant cells**.

Granuloma consists of an inner zone of epithelioid cells, typically surrounded by a collar of lymphocytes

Table 16.6: Examples of delayed-type hypersensitivity (DTH).

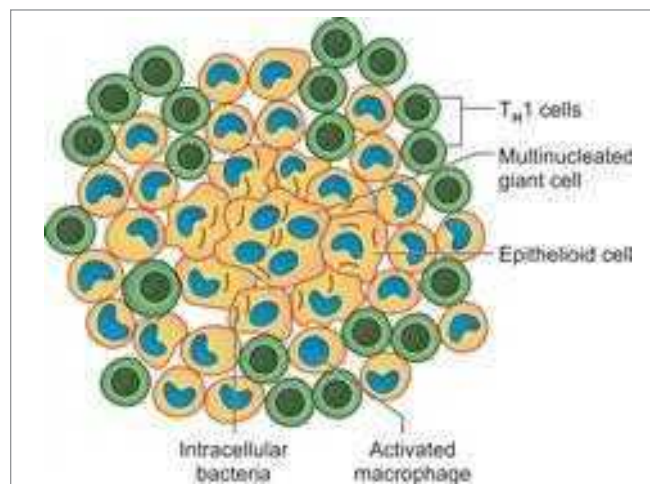
Intracellular pathogens inducing DTH	
Intracellular bacteria <ul style="list-style-type: none"> • <i>Mycobacterium leprae</i> • <i>M. tuberculosis</i> • <i>Listeria monocytogenes</i> • <i>Brucella abortus</i> 	Intracellular fungi <ul style="list-style-type: none"> • <i>Pneumocystis jirovecii</i> • <i>Candida albicans</i> • <i>Histoplasma capsulatum</i> • <i>Cryptococcus neoformans</i>
Intracellular viruses <ul style="list-style-type: none"> • Herpes simplex virus • Variola (smallpox) • Measles virus 	Skin test to demonstrate DTH <ul style="list-style-type: none"> • Tuberculin test (Mantoux test) • Lepromin test • Montenegro test (leishmaniasis) • Frei test—done in LGV
Contact dermatitis	
Following exposure to contact antigens: Nickel, poison ivy, poison oak, picryl chloride	
Other examples of DTH	
Noninfectious conditions <ul style="list-style-type: none"> • Diabetes mellitus type 1 • Multiple sclerosis • Peripheral neuropathies • Hashimoto's thyroiditis 	<ul style="list-style-type: none"> • Crohn's disease • Chronic transplant rejection • Graft-versus-host disease
Granuloma formation seen in Tuberculosis, sarcoidosis, schistosomiasis and other trematode infections	Other example Lepra reaction type I

Abbreviation: LGV, lymphogranuloma venereum.

and a peripheral rim of fibroblasts and connective tissue (Fig. 16.6).

Tuberculin Test

Tuberculin test is the prototype of delayed hypersensitivity. In sensitized individuals, (i.e. who possess sensitized T_{DTH} cells due to prior contact with *M. tuberculosis*); when a preparation of tuberculin antigen (glycerol extract of the tubercle bacillus) is injected intradermally, a local reaction develops after 48–72 hours consisting of induration surrounded by erythema.

**Fig. 16.6:** Structure of granuloma.

Contact Dermatitis

Many antigens such as nickel, poison oak, etc. (Table 16.6) act by producing DTH response:

- ❖ Most of these substances are haptens; they complex with skin proteins, which act as carrier to make the haptens immunogenic
- ❖ This hapten-skin protein complex is internalized by skin APCs (e.g. Langerhans cells), then presented to T_H cells to induce a TDH reaction
- ❖ Activated macrophages release lytic enzymes which result in skin lesions (e.g. redness and pustule seen following contact with poison oak).

EXPECTED QUESTIONS

I. Write essay on:

1. Define and classify hypersensitivity reactions. Write in detail about type IV hypersensitivity reaction.
2. Neha, a 17-year student who has recently joined MBBS, has come back to the hostel after the first vacation. After entering to her hostel room, she suddenly developed an episode of severe sneezing, and dyspnea. She had to be admitted to the casualty and when asked, she told that she has faced similar episodes since her childhood.
 - a. What type of immune reaction is this?
 - b. Describe the pathogenesis of this condition and management.

II. Write short notes on:

1. Type II hypersensitivity reaction.
2. Immune complex mediated hypersensitivity reaction.

III. Multiple Choice Questions (MCQs):

1. **Type I hypersensitivity is mediated by which of the following immunoglobulins?**
 - a. IgA
 - b. IgG
 - c. IgM
 - d. IgE
2. **The type of hypersensitivity reaction in myasthenia gravis is:**

- a. Type I
- b. Type II
- c. Type III
- d. Type IV

3. A positive tuberculin test is an example of:

- a. Type I hypersensitivity
- b. Type II hypersensitivity
- c. Type III hypersensitivity
- d. Type IV hypersensitivity

4. Example of antibody-dependent cellular dysfunction or ADCD:

- a. Graves' disease
- b. Hemolytic anemia
- c. Pemphigus vulgaris
- d. Transfusion reaction

5. Which of the following statement is wrong about type III hypersensitivity reaction?

- a. Antibody excess—produce a localized type III reaction
- b. Antigen excess—produce a systemic type III reaction
- c. Antigen-antibody equivalence—produce a localized type III reaction
- d. Soluble immunocomplexes—produce a localized type III reaction

Answers

1. d 2. b 3. d 4. a 5. d

CHAPTER PREVIEW

- Immunological Tolerance
- Mechanisms of Autoimmunity
- Autoimmune Diseases
- Single Organ or Cell Type Autoimmune Diseases
- Systemic Autoimmune Diseases
- Laboratory Diagnosis of Autoimmune Diseases

Autoimmunity is a condition in which the body's own immunologically competent cells or antibodies act against its self-antigens resulting in structural or functional damage. Paul Ehrlich had first introduced the concept of autoimmunity; he termed this condition as "horror autotoxicus".

- ❖ Normally immune system does not react to its own antigens due to a protective mechanism called tolerance. Any breach in tolerance mechanisms predisposes to several autoimmune diseases
- ❖ Therefore, before going into the details on mechanisms of autoimmunity; it is essential to know about the various tolerance mechanisms that the human immune system possesses.

IMMUNOLOGICAL TOLERANCE

Immunological tolerance is a state in which an individual is incapable of developing an immune response against his own tissue antigens. It is mediated by two broad mechanisms—central tolerance and peripheral tolerance.

Central Tolerance

This refers to the deletion of self-reactive T and B lymphocytes during their maturation in central lymphoid organs (i.e. in the thymus for T cells and in the bone marrow for B cells).

- ❖ **In thymus:** During the T cell development in thymus, if any self-antigens are encountered, they are processed and presented by thymic antigen presenting cells (APCs) in association with self-MHC. Any developing T cell that expresses a receptor for such self-antigen is negatively selected (i.e. deleted by apoptosis). Therefore, the resulting peripheral T cell pool is devoid of self-reactive cells
- ❖ **In bone marrow:** When developing immature B cells in the bone marrow encounter a self-antigen during their development, the tolerance is developed by:

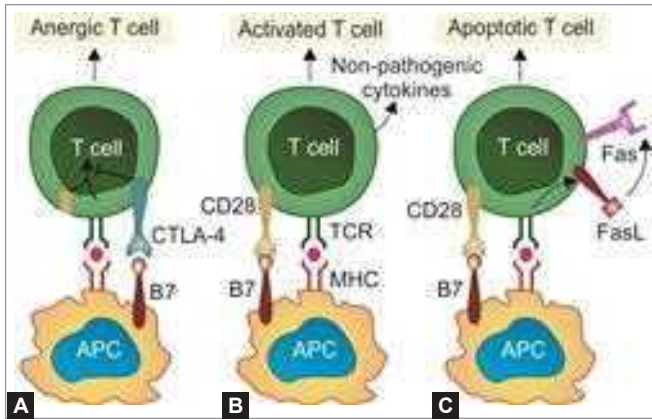
- **Receptor editing:** It is a process by which B cells reactivate the machinery of antigen receptor gene rearrangement (mainly genes coding for light chains), so that a different (edited) B cell receptor will be produced which no longer recognizes the self-antigen
- **Negative selection:** After receptor editing, if the B cells again recognize a self-antigen, then they are destroyed by subjecting them to apoptosis.

However, the process of central tolerance is not completely perfect. Many self-reactive T and B cells bearing receptors for self-antigens escape into the periphery. Hence, for counteracting those lymphocytes, peripheral tolerance takes a lead role.

Peripheral Tolerance

This refers to several back-up mechanisms that occur in the peripheral tissues to counteract the self-reactive T cells that escape central tolerance. It is provided by several mechanisms (Fig. 17.1).

- ❖ **Ignorance:** The self-reactive T cells might never encounter the self-antigen which they recognize and therefore remain in a state of ignorance
- ❖ **Anergy:** It can be defined as unresponsiveness to antigenic stimulus. The self-reactive T cells interact with the APCs presenting the self-antigen, but the co-stimulatory signal is blocked. The B7 molecules on APC bind to CTLA-4 molecules on T cells instead of CD28 molecules (Fig. 17.1A).
Note: Normally, T cell activation requires two signals—Main signal (provided by antigen MHC complex of APC interacts with TCR on T cell) and a co-stimulatory signal (B7 molecules on APCs bind to CD28 on T cells). If self-antigens are processed and presented by APCs, that do not bear the co-stimulators, a negative signal is delivered, and the cell becomes anergic
- ❖ **Phenotypic skewing:** Self-reactive T cells interacting with APCs presented with self-antigens, undergo full



Figs 17.1A to C: Mechanisms of peripheral tolerance: **A.** Anergy; **B.** Phenotypic skewing; **C.** Apoptosis.

activation, but might secrete nonpathogenic cytokines and chemokine receptors profile, hence although they are activated, fail to induce autoimmune response (Fig. 17.1B)

- ❖ **Apoptosis by AICD:** Self-reactive T cells are activated after interacting with APCs presented with self-antigens. But the activation of T cells induces upregulation of Fas ligand which subsequently interacts with the death receptor Fas leading to apoptosis. This mechanism is called as activation-induced cell death (AICD) (Fig. 17.1C)
- ❖ **Regulatory T cells (T_{reg} cells):** T_{reg} cells can down regulate the self-reactive T cells through secreting certain cytokines (e.g. IL-10 and transforming growth factor β [TGF- β]) or killing by direct cell-to-cell contact
- ❖ **Dendritic cells (DCs):** When certain dendritic cells such as immature DCs and tolerogenic DCs capture the self-antigen for processing, they down regulate the expression of molecules of costimulatory ligands such as CD40 and B7 molecules or act indirectly by induction of regulatory T cells
- ❖ **Sequestration of self-antigen:** Certain self-antigens can evade immune recognition by sequestration in immunologically privileged sites, e.g. corneal proteins, testicular antigens and antigens from brain.

B cells can also exhibit peripheral tolerance. The self-reacting B cells that have escaped (10%) the central tolerance at bone marrow are further destroyed at spleen by several mechanisms such as downregulation of a B cell growth factor called B cell activating factor (BAFF).

MECHANISMS OF AUTOIMMUNITY

Autoimmunity results due to breakdown of one or more of the mechanisms of immunological tolerance.

Breakdown of T Cell Anergy

Normal cells that do not usually express costimulatory molecules (B7) can be induced to do so. Such induction

may occur in presence of tissue necrosis and local inflammation. This mechanism has been postulated for—

- ❖ Multiple sclerosis
- ❖ Rheumatoid arthritis
- ❖ Psoriasis.

Failure of AICD

Failure of the autoreactive activated T cells to undergo activation-induced cell death (AICD), i.e. apoptosis via Fas-Fas ligand can lead to autoimmunity. It is observed in patients suffering from systemic lupus erythematosus (SLE).

Loss of T_{reg} Cells

Autoimmunity can result following the loss of regulatory T cell-mediated suppression of self-reactive lymphocytes.

Providing T Cell help to Stimulate Self-reacting B Cells

Antibody response to self-antigens occurs only when potentially self-reactive B cells receive help from T cells. For example, in autoimmune hemolytic anemia, administration of certain drugs may result in drug-induced alterations in the red cell surface that create antigens which can be recognized by helper T cells (Fig. 17.2).

Release of Sequestered Antigens

The sequestered antigens are usually viewed as foreign to the immune system as they are never been exposed to the tolerance mechanisms during development of immune system. Injury to the organs leads to release of such sequestered antigens which are very well capable of mounting an immune response. Spermatozoa and ocular antigens released after trauma or surgery can cause post-vasectomy orchitis and post-traumatic uveitis.

Infectious agents may participate in the pathogenesis of autoimmunity by the following mechanisms:

Molecular Mimicry

Some microorganisms share antigenic determinants (epitopes) with self-antigens, and an immune response

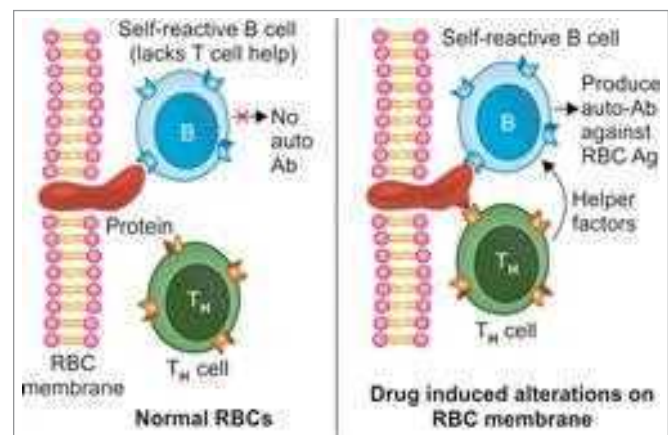


Fig. 17.2: T cells help to stimulate self-reacting B cells.

against such microbes would produce antibodies that can crossreact with self-antigen.

- ❖ For example, acute rheumatic fever results due to antibodies formed against streptococcal antigens (M protein), cross react with cardiac antigens (glycoproteins), due to antigenic cross reactivity
- ❖ Molecular mimicry involving T cell epitopes—Examples include multiple sclerosis, where T cell clones reacting to myelin basic protein probably would have been induced by reacting against peptides derived from many microbes including viruses.

Polyclonal Lymphocyte Activation

Several microorganisms and their products are capable of causing polyclonal (i.e. antigen-nonspecific) activation of T cells or B cells.

- ❖ **Polyclonal T cell activation:** Superantigens released from microbes (e.g. *Staphylococcus aureus*), polyclonally activate the T cells directly by binding to antigen non-specific V β region of T cell receptors
- ❖ **Polyclonal B cell activation:** It can be induced by products of various microbes such as Epstein-Barr virus, HIV, etc.

Exposure of Cryptic Self-epitopes

Research has proved that “molecular sequestration” of antigens is much more common than anatomic sequestration.

- ❖ During development of immune system, not all epitopes of an antigen are effectively processed and presented to T cells. There are some nondominant cryptic epitopes which remain sequestered. Hence, T cell clones reacting against such epitopes are not deleted

- ❖ Such cryptic self-epitopes can be released secondary to inflammation at a site of tissue injury, which can induce increased protease production and differential processing of released self-epitopes by APCs.

Epitope Spreading

The self-peptides released due to persistent inflammation induce tissue damage (as occurs in chronic microbial infection) and are processed and presented by APCs along with microbial peptides. It is possible that, there may occur a shift or spread of T cell recognition to self-epitopes presented on APCs rather than recognizing microbial epitope.

Bystander Activation

It is the nonspecific activation of bystander self-reactive T_H1 cells. Activation of microorganism-specific T_H1 cells leads to cytokine influx which causes an increased infiltration of various nonspecific T cells at the site of infection.

AUTOIMMUNE DISEASES

The immunological attack of self-reacting T lymphocytes or autoantibodies on tissues leads to the development of various autoimmune diseases. There are broad ranges of autoimmune diseases which can either be localized into single organ/cell type or may involve many organs and cause systemic manifestations (Table 17.1).

Laboratory Diagnosis of Autoimmune Diseases

Autoimmune diseases are diagnosed by detection of various autoantibodies in serum of the patients;

Single organ or cell type autoimmune diseases		
Disease	Self-antigen present on	Type of immune response and Important features
Autoimmune anemias		
Autoimmune hemolytic anemia	RBC membrane proteins	Autoantibodies to RBC antigens trigger complement mediated lysis or antibody-mediated opsonization of the RBCs
Drug-induced hemolytic anemia	Drugs alter the red cell membrane antigens	Drugs such as penicillin or methyl dopa interact with RBCs so that the cells become antigenic
Pernicious anemia	Intrinsic factor (a membrane-bound protein on gastric parietal cells)	Autoantibodies to intrinsic factor block the uptake of vitamin B12; leads to megaloblastic anemia
Idiopathic thrombocytopenic purpura	Platelet membrane proteins (glycoproteins IIb-IIIa or Ib-IX)	Auto-antibodies against platelet membrane antigens leads to ↓platelet count
Goodpasture syndrome	Renal and lung basement membranes	Auto-antibodies bind to basement-membrane antigens on kidney glomeruli and the alveoli of the lungs followed by complement mediated injury leading to progressive kidney damage and pulmonary hemorrhage
Myasthenia gravis	Acetylcholine receptors	Blocking type of autoantibody directed against Ach receptors present on motor nerve endings, leads to progressive weakening of the skeletal muscles
Graves' disease	Thyroid-stimulating hormone (TSH) receptor	Anti-TSH-autoantibody (stimulates thyroid follicles, leading to hyperthyroid state)

Contd...

Contd...

Hashimoto's thyroiditis	Thyroid proteins and cells	Autoantibodies and T _{DTH} cells targeted against thyroid antigen leads to suppression of thyroid gland <ul style="list-style-type: none"> • Seen in middle-aged females • Hypothyroid state is produced (↓ production of thyroid hormones)
Post-streptococcal glomerulonephritis	Kidney	Streptococcal antigen–antibody complexes are deposited in glomerular basement membrane
Systemic autoimmune diseases		
Disease	Self-antigen present on	Type of immune response and Important features
Systemic lupus erythematosus (SLE)	Autoantibodies are produced against various tissue antigens such as DNA, nuclear protein, RBC and platelet membranes	<ul style="list-style-type: none"> • Age and sex: Women (20–40 years of age) are commonly affected; female to male ratio is 10:1 • Immune complexes (self Ag–autoAb) are formed; which are deposited in various organs • Major symptoms: Fever, butterfly rash over the cheeks, arthritis, pleurisy, and kidney dysfunction
Rheumatoid arthritis (RA)	Here, a group of auto-antibodies against the host IgG antibodies are produced called RA factor . It is an IgM antibody directed against the Fc region of IgG Anticitrullinated peptide antibodies (ACPA) are also produced	<ul style="list-style-type: none"> • Age and sex: Women (40–60 years of age) affected • Autoantibodies bind to circulating IgG, forming IgM–IgG complexes that are deposited in the joints and can activate the complement cascade <p>Major symptoms:</p> <ul style="list-style-type: none"> • Main feature: Arthritis (chronic inflammation of the joints, begins at synovium; most common joints involved are–small joints of the hands, feet and cervical spine) • Other features: Hematologic, cardiovascular, and respiratory systems are also frequently affected
Sjögren syndrome	Ribonucleoprotein (RNP) antigens SS-A (Ro) and SS-B (La) present on salivary gland, lacrimal gland, liver, kidney, thyroid	Auto-antibodies to the RNP antigens SS-A (Ro) and SS-B (La), lead to immune-mediated destruction of the lacrimal and salivary glands resulting in dry eyes (<i>keratoconjunctivitis sicca</i>) and dry mouth (xerostomia)
Scleroderma (Systemic sclerosis)	Nuclear antigens such as DNA topoisomerase and centromere present in heart, lungs, GIT, kidney, etc.	Helper T cell (mainly) and auto-antibody mediated Excessive fibrosis of the skin, throughout the body Two types <ul style="list-style-type: none"> • Diffuse scleroderma: Autoantibodies against DNA topoisomerase I (anti-Scl 70) is elevated • Limited scleroderma: ↑Anticentromere antibody, characterized by CREST syndrome—calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia
Seronegative spondyloarthropathies	Sacroiliac joints and other vertebrae Several types: <ul style="list-style-type: none"> • Ankylosing spondylitis • Reiter syndrome • Psoriatic arthritis • Spondylitis with inflammatory Bowel disease • Reactive arthritis 	Common characteristics: They present as rheumatoid arthritis like features, but differ from it by: <ul style="list-style-type: none"> • Association with HLA-B27 • Pathologic changes begin in the ligamentous attachments to the bone rather than in the synovium • Involvement of the sacroiliac joints, and/or arthritis in other peripheral joints • Absence of RA (hence the name “seronegative”) • Auto-Ab and immune complex mediated
Multiple sclerosis	Brain (white matter)	Self-reactive T cells produce characteristic inflammatory lesions in brain that destroy the myelin sheath of nerve fibers; leading to numerous neurologic dysfunctions

- ❖ **Autoimmune hemolytic anemia:** Diagnosed by Coombs test, in which the red cells are incubated with an anti–human IgG antiserum. If IgG autoantibodies are present on the red cells, the cells are agglutinated by the antiserum
- ❖ **Goodpasture syndrome:** Biopsies from patients are stained with fluorescent-labeled anti-IgG and anti-C3b reveal linear deposits of IgG and C3b along the basement membranes
- ❖ **SLE** is diagnosed by:
 - Detection of autoantibodies against various nuclear antigens by indirect immunofluorescence assay (most widely used) and ELISA-based techniques
 - ♦ **Antinuclear antibody (ANA):** Positive in >90% of cases, used as screening method (Fig. 17.3)
 - ♦ Anti-double stranded DNA (dsDNA): Highly specific, used for confirmation of cases
 - ♦ Anti-Sm antibodies.

- **Lupus band test:** It is a direct immunofluorescence test, can detect deposits of immunoglobulins and complement proteins in the patient's skin

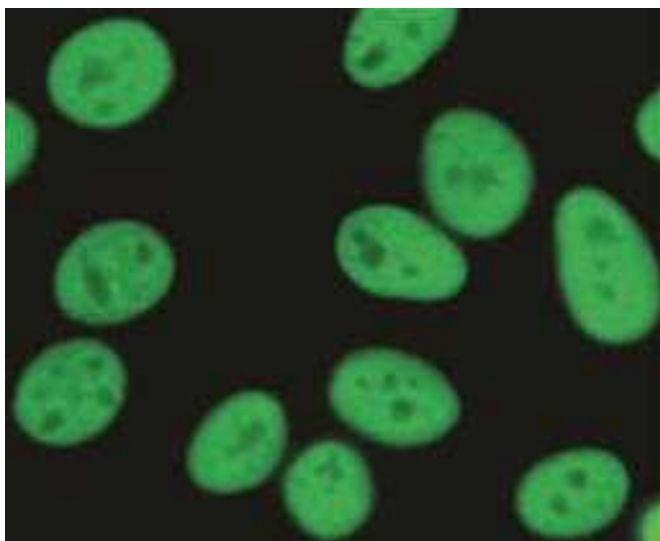


Fig. 17.3: Antinuclear antibody (homogeneous pattern) by indirect immunofluorescence staining.

Source: EUROIMMUN AG Pvt Ltd. (with permission).

- **LE cell test:** The lupus erythematosus (LE) cell test was commonly used for diagnosis, but it is no longer used because the LE cells are only found in 50–75% of SLE cases.
- ❖ **Scleroderma:** Anti-Scl 70 antibody is raised, detected by indirect immunofluorescence assay
- ❖ **Sjögren's syndrome:** Is diagnosed by detection of SS-A (or anti-Ro) and SS-B (or anti-La) antibodies by indirect immunofluorescence assay
- ❖ **Rheumatoid arthritis:** RA is diagnosed by detection of two important autoantibodies-RA factor and ACPA
 - **RA factor** (by latex agglutination test): RA factor is an IgM autoantibody directed against Fc portion of IgG
 - ◆ RA factor detection has good sensitivity (negative in only 15% of cases)
 - ◆ False positive detection of RA factor is seen in other autoimmune diseases.
 - **Anti-cyclic citrullinated peptide (anti-CCP):** It is an autoantibody to citrullin protein. It is positive in about 86% of patients with established RF-positive RA and in 25% of patients with RF-negative RA; but is highly specific (96%)
 - **Rose-Waaler test** to detect RA factor is of historical importance, no longer used now.

EXPECTED QUESTIONS

I. Write essay on:

1. Define autoimmunity. Classify various autoimmune diseases and briefly explain various mechanisms involved in the development of autoimmunity with suitable examples.

II. Multiple Choice Questions (MCQs):

1. Lens antigens of the eye are a type of:

- a. Sequestered antigens
- b. Neoantigens
- c. Cross reacting antigens
- d. None of the above

2. Autoimmunity can be caused due to all of the following, *except*:

- a. The pressure of forbidden clones
- b. Expression of cryptic antigens
- c. Negative selection of T cells in the thymus
- d. Release of sequestered antigens

3. All of the following are systemic autoimmune diseases, *except*:

- a. Hashimoto's thyroiditis
- b. Systemic lupus erythematosus

- c. Rheumatoid arthritis
- d. Scleroderma

4. Autoantibodies bind to basement-membrane antigens on kidney glomeruli and the alveoli. This is the hallmark of:

- a. Goodpasture syndrome
- b. Myasthenia gravis
- c. Graves' disease
- d. Hashimoto's thyroiditis

5. Autoantibodies to ribonucleoprotein (RNP) antigens SS-A (Ro) and SS-B (La) are produced in which disease?

- a. Sjögren syndrome
- b. Systemic sclerosis
- c. Multiple sclerosis
- d. Systemic lupus erythematosus (SLE)

6. Anti-citrullinated peptide antibodies (ACPA) are diagnostic for:

- a. Systemic lupus erythematosus (SLE)
- b. Rheumatoid arthritis
- c. Sjögren syndrome
- d. Scleroderma

Answers

1. a 2. c 3. a 4. a 5. a 6. b

Immunodeficiency Disorders

CHAPTER PREVIEW

- Definition and Classification
- Primary Immunodeficiency Diseases
 - Humoral Immunodeficiency (B Cell Defects)
 - Cellular Immunodeficiency (T Cell Defects)
- Combined Immunodeficiencies
- Disorders of Phagocytosis
- Secondary Immunodeficiencies

DEFINITION AND CLASSIFICATION

Immunodeficiency is a state where the defence mechanisms of the body are impaired, leading to enhanced susceptibility to microbial infections as well as to certain forms of cancer.

Immunodeficiency diseases are broadly classified as primary or secondary.

- ❖ Primary immunodeficiency diseases result from inherited defects affecting immune system development
- ❖ Secondary immunodeficiency diseases are secondary to some other disease process that interferes with the proper functioning of the immune system (e.g. infection, malnutrition, aging, immunosuppression, autoimmunity, or chemotherapy).

PRIMARY IMMUNODEFICIENCY DISEASES

Most primary immunodeficiency diseases are genetically determined and can be further classified into diseases resulting from deficiency of either specific immunity (i.e. humoral or cellular or both) or nonspecific host defense mechanisms (mediated by complement proteins and cells such as phagocytes or NK cells) (Table 18.1).

However, the distinction of diseases affecting specific immunity (humoral or cellular) components is not clear. In particular, T cell defects almost always lead to impaired antibody synthesis, and hence isolated deficiencies of T cells are usually indistinguishable from combined deficiencies of T and B cells.

The type of infections in a given patient depends largely on the component of the immune system that is affected (Table 18.2).

- ❖ Patients with defects in humoral immunity, complement, or phagocytosis typically suffer from recurrent infections with pyogenic bacteria
- ❖ On the other hand, those with defects in cell-mediated immunity are prone to infections caused by viruses, fungi, and intracellular bacteria.

Table 18.1: Classification of primary immunodeficiency diseases.

Humoral immunodeficiency (B cell defects)
<ul style="list-style-type: none"> ● Bruton disease (X-linked agammaglobulinemia) ● Common variable immunodeficiency ● Isolated IgA deficiency ● Hyper-IgM syndrome
Cellular immunodeficiencies (T cell defects)
<ul style="list-style-type: none"> ● DiGeorge syndrome (thymic hypoplasia) ● Chronic mucocutaneous candidiasis ● Purine nucleoside phosphorylase (PNP) deficiency
Combined immunodeficiencies (B and T cell defects)
<ul style="list-style-type: none"> ● Severe combined immunodeficiencies <ul style="list-style-type: none"> ➢ Cytokine receptor mutation ➢ Adenosine deaminase (ADA) deficiency ● Wiskott–Aldrich syndrome ● Ataxia telangiectasia ● Nezelof syndrome
Disorders of phagocytosis
<ul style="list-style-type: none"> ● Chronic granulomatous disease ● Myeloperoxidase deficiency ● Chediak–Higashi syndrome ● Leukocyte adhesion deficiency ● Lazy leukocyte syndrome ● Job's syndrome or Hyper-IgE syndrome
Disorders of complement*
<ul style="list-style-type: none"> ● Complement component deficiencies ● Complement regulatory protein deficiencies

*Described in detail in Chapter 13.

Most primary immunodeficiencies come to attention early in life (between 6 months and 2 years of life); usually because of the susceptibility of infants to recurrent infections.

HUMORAL IMMUNODEFICIENCY (B CELL DEFECTS)

Bruton Disease (X-linked Agammaglobulinemia)

Bruton disease is one of the more common forms of primary immunodeficiency. It is characterized by:

Pathogen type	T cell defect	B cell defect	Granulocyte defect	Complement defect
Bacteria	Bacterial sepsis	<i>Streptococcus pneumoniae</i> <i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	Staphylococci <i>Pseudomonas, Nocardia</i>	<i>Neisseria</i> Other pyogenic infections
Viruses	Cytomegalovirus Epstein–Barr virus Severe varicella Chronic infections with respiratory and intestinal viruses	Enterovirus encephalitis	—	
Fungi	<i>Candida, Pneumocystis jirovecii</i>	—	<i>Candida, Aspergillus</i>	
Parasites	—	Giardiasis	—	
Special features	Aggressive disease with opportunistic pathogens, failure to clear infections	Recurrent sinopulmonary infections, sepsis, chronic meningitis	—	

- ❖ Failure of pre-B cells to differentiate into immature B cells in the bone marrow—due to absence of an enzyme called **Bruton's tyrosine kinase** which is involved in transformation of pre-B cell into immature B cell
- ❖ As a result, there occurs total absence of B cells and plasma cells in the circulation, with depressed serum levels of all classes of immunoglobulins. However, Pre-B cells are found in normal numbers in bone marrow and the T cell-mediated responses are also normal
- ❖ The B cell maturation stops at pre-B cell stage; after the synthesis of heavy-chain without forming the light chains. Hence the cytoplasm of pre-B cell may have incomplete immunoglobulins
- ❖ Bruton's tyrosine kinase is X-linked; hence, this disease is seen primarily in males; nevertheless, sporadic cases have been described in females
- ❖ Secondary infections are seen after 6 months of age, (when maternal antibodies are depleted), such as:
 - Recurrent bacterial infections caused by pathogens that are usually cleared by antibody opsonization (e.g. *Haemophilus influenzae*, *Streptococcus pneumoniae*, or *Staphylococcus aureus*) leading to acute and chronic pharyngitis, sinusitis, otitis media, bronchitis, and pneumonia
 - Viruses that are cleared by neutralizing antibodies, e.g. enteroviruses
 - Parasites which are usually resisted by secretory IgA, e.g. *Giardia lamblia*.
- ❖ Autoimmune diseases (such as SLE and dermatomyositis) also occur in up to 20% of cases.

Common Variable Immunodeficiency

This is a heterogeneous group of both sporadic and inherited forms of the disease characterized by hypogammaglobulinemia, increased susceptibility to infection, autoimmune disorders (hemolytic anemia, pernicious anemia), as well as lymphoid tumors. The clinical manifestations are superficially similar to those of Bruton diseases; but differ in the following aspects:

- ❖ Both sexes are affected equally
- ❖ Onset of symptoms is much later, in the second or third decade of life
- ❖ It is also B cell development defect; B cells may be present in circulation in normal numbers, but they appear defective in their ability to differentiate into plasma cells and secrete immunoglobulins
- ❖ The diagnosis is usually one of exclusion (after other causes of immunodeficiency are ruled out); the basis of the immunoglobulin deficiency is variable (hence the name)
- ❖ The defect in the antibody production has been variably attributed to intrinsic B cell defects, deficient T cell helper, or excessive T cell suppressor activity.

Isolated IgA Deficiency

IgA deficiency is the most common of all the primary immunodeficiency diseases, affects about 1 in 700 white individuals.

- ❖ In healthy normal individuals, IgA is predominant in mucosal secretions and involves in providing immunity at mucosal sites of intestine and respiratory tract
- ❖ Therefore, the weakened mucosal defences due to IgA deficiency predispose patients to recurrent sinopulmonary infections and diarrhea. There is also a significant (but unexplained) association with autoimmune diseases
- ❖ **Pathogenesis:** IgA deficiency occurs due to a block in the terminal differentiation of IgA-secreting B cells to plasma cells, which in turn is due to altered T cell production of cytokines that drive IgA responses (e.g. TGF- β and IL-5) or due to intrinsic B cell defect. The levels of other immunoglobulins are usually normal or even excess.

Hyper-IgM Syndrome

Hyper-IgM syndrome is an X-linked disorder; results due to a defect in **isotype class switchover** of B cells.

- ❖ Underlying genetic defect is mutations in either CD40L or CD40 genes, leading to prevention of interaction between T and B cell; thus blocking the class switchover

- ❖ A block in class switchover results in lack of synthesis of other classes of antibodies such as IgG, IgA, and IgE with a normal or supernormal levels of IgM
 - Deficiency of IgG leads to defect in opsonization and complement activation (predisposes to recurrent pyogenic infections) and IgA deficiency leads to increased recurrent sinopulmonary infections and diarrhea
 - Excess IgM antibodies can react with blood cells, resulting in autoimmune hemolytic anemia, thrombocytopenia, or neutropenia.
- ❖ Because CD40L signals are involved in macrophage activation and thus producing delayed hypersensitivity response, hence patients with defect in CD40L are more susceptible to *Pneumocystis jirovecii* infection
- ❖ Hyper-IgM syndrome is X-linked in 70% of the cases affecting males; in the remaining patients, the precise mutations have not been fully characterized.
- ❖ Transfer factor therapy, along with amphotericin B has been reported to be effective.

Purine Nucleoside Phosphorylase Deficiency

It is a rare autosomal recessive disorder (chromosome 14), characterized by deficiency of an enzyme of purine metabolism called purine nucleoside phosphorylase (PNP).

- ❖ PNP is a key enzyme required for purine degradation; catalyzes the conversion of guanosine to hypoxanthine
- ❖ Its deficiency leads to elevated Deoxyguanosine triphosphate levels resulting in T cell toxicity. However, B cells are not affected
- ❖ T cell depletion predisposes to increased susceptibility to infection and autoimmune disorders.

COMBINED IMMUNODEFICIENCIES (B AND T CELLS DEFECTS)

Severe Combined Immunodeficiencies (SCID)

SCID represents groups of genetically distinct syndromes; all having in common, defects in both humoral and cell-mediated immune responses.

Types of Genetic Defect in SCID

CELLULAR IMMUNODEFICIENCY (T CELL DEFECTS)

DiGeorge Syndrome (Thymic Aplasia)

DiGeorge syndrome results from a congenital defect in thymic development leading to defect in T cell maturation.

- ❖ Infants are extremely vulnerable to viral, fungal, intracellular bacterial and protozoan infections
- ❖ **Genetic defect:** In 90% of cases, there occurs a deletion affecting chromosome 22q11 which leads to developmental malformation affecting the third and fourth pharyngeal pouches in embryonic life
- ❖ As a result, all the structures that develop from third and fourth pharyngeal pouches such as thymus, parathyroid glands, and portions of the face and aortic arch become defective
- ❖ Thus, in addition to the thymic defects, there may be associated:
 - Parathyroid gland hypoplasia resulting in neonatal tetany and hypocalcemia
 - Anomalies of the heart and the great vessels (Fallot's tetralogy)
 - Characteristic facial appearance.
- ❖ B cells and serum immunoglobulin levels are generally unaffected
- ❖ Treatment: Thymus transplantation has been found to be successful in restoration of immune function. In others (with partial defects), immunity may improve spontaneously with age.

Chronic Mucocutaneous Candidiasis

It represents an impaired cell-mediated immunity against *Candida albicans* leading to superficial infections of the skin, mucous membranes, and nails.

- ❖ They do not show increased susceptibility to other infections but often associated with endocrinopathies and autoimmune disorders

- ❖ **Mutation in cytokine receptor:** Approximately 50–60% of the cases of SCID are X-linked (seen in males), resulting from mutations in the gene encoding the common γ chain shared by the cytokine receptors for IL-7 and others (IL-2, IL-4, IL-9, and IL-15)

- IL-7 being lymphopoietic growth factor, defective IL-7 receptor signalling leads to defect in survival and expansion of immature B and T cell precursors in the bone marrow
- Defect in IL-15 receptor signaling leads to deficiency of NK cell.

- ❖ **The remaining cases of SCID** are inherited as autosomal recessive manner include:

- **Adenosine deaminase (ADA) deficiency:** It is the most common type of autosomal recessive SCID. ADA is an enzyme required for purine degradation; its deficiency leads to accumulation of deoxyadenosine which is toxic to rapidly dividing immature T lymphocytes. B cell deficiency is not profound
- **RAG Mutation:** Recombinase-activating genes (RAG) are essential for somatic gene rearrangements of T cell receptor and immunoglobulins. Thus, defect in RAG blocks the development of T and B cells
- **Jak3 mutation:** Jak3, an intracellular kinase, is essential for signal transduction through the common cytokine receptor γ chain. Hence Jak3 mutation is another way of blocking the cytokine receptor signalling
- **Class II MHC deficiency:** Mutations that impair the expression of class II MHC molecules prevent the development of CD4+ T cells. This condition is also called the **Bare lymphocyte syndrome**.

Infections

Irrespective of the underlying genetic defect, the affected infants are susceptible to severe recurrent infections by a wide array of pathogens, including *Candida*, *Pneumocystis*, cytomegalovirus and *Pseudomonas* (see Table 18.2).

Treatment

Bone marrow transplantation is the mainstay of treatment. Gene therapy replacing the mutated genes has been successful in X-linked cases.

Wiskott–Aldrich Syndrome (WAS)

It is an X-linked recessive disease, characterized by immunodeficiency with thrombocytopenia, eczema, etc. The severity of WAS increases with age.

- ❖ It first manifests itself by defective responses to bacterial polysaccharides and by lower IgM levels. IgG levels are usually normal. Paradoxically the levels of IgA and IgE are often elevated
- ❖ Other T and B cell responses are normal initially, but with increase of age, there are recurrent bacterial infections and a gradual loss of humoral and cellular responses
- ❖ Patients are also prone to develop non-Hodgkin B cell lymphomas
- ❖ Patients may present with bloody diarrhea secondary to thrombocytopenia.

Pathogenesis

The underlying genetic defect is due to a mutation in the gene encoding Wiskott–Aldrich syndrome protein (WASP) present in precursor lymphoid cells of bone marrow. It is a cytoskeletal glycoprotein (sialophorin or CD43), required for actin polymerization.

Ataxia Telangiectasia

The syndrome is characterized by:

- ❖ Difficulty in maintaining balance while walking (cerebellar ataxia)
- ❖ Appearance of broken capillaries (telangiectasia) in the eyes and choreoathetoid movements (usually noticed in infancy)
- ❖ Deficiency of IgA and sometimes IgE
- ❖ Profound sinopulmonary infections.

Genetic defect: The primary defect appears to be in a kinase involved in regulation of the cell cycle. The relationship between the immune deficiency and the other defects in ataxia telangiectasia remains obscure.

Nezelof Syndrome

It is an autosomal recessive condition characterized by cellular immunodeficiency resulting from thymus hypoplasia. Affected individuals suffer from chronic diarrhea, viral and fungal infections, and failure to thrive.

DISORDERS OF PHAGOCYTOSIS

Chronic Granulomatous Disease (CGD)

Pathogenesis

Pathogenesis of CGD involves inherited defects in the gene encoding components of oxidase system, e.g. Nicotinamide adenine dinucleotide phosphate (NADP) oxidase of phagocyte which breaks down hydrogen peroxide to generate free oxygen radicals (O_2^-) that are involved in microbial killing. As a result, there occurs decreased oxidative burst which predisposes to recurrent bacterial infections. CGD is a genetic disease that runs in family in two forms:

- ❖ In X-linked form (more common, 70%), membrane component of phagocyte oxidase is defective
- ❖ In autosomal recessive form, cytoplasmic component of phagocyte oxidase is defective.

Manifestations

- ❖ The bacteria involved in the recurrent infections are catalase positive; pyogenic pathogens such as staphylococci, *Pseudomonas* and coliforms. Catalase negative pathogens such as streptococci and pneumococci are handled well
- ❖ Patients also undergo excessive inflammatory reactions that result in gingivitis, swollen lymph nodes, and nonmalignant granulomas (lumpy subcutaneous cell masses)
- ❖ Nitroblue tetrazolium reduction test (NBT) is used as screening test to detect deficiency of NADPH oxidase activity.

Myeloperoxidase Deficiency

It is a common genetic disorder characterized by deficiency in either quantity or function, of myeloperoxidase, an enzyme produced by neutrophils. Patients present with immune deficiency and recurrent infections, especially with *Candida albicans*.

Chediak–Higashi Syndrome

It is an autosomal recessive disease, characterized by:

- ❖ Defective fusion of phagosomes and lysosomes in phagocytes which leads to increased susceptibility to recurrent and severe pyogenic infections
- ❖ Abnormalities in melanocytes leading to albinism (lack of skin and eye pigment)
- ❖ Abnormalities in cells of the nervous system (associated with nerve defects), and
- ❖ Platelets abnormalities, causing bleeding disorders
- ❖ Aggressive but non-malignant infiltration of organs by lymphoid cells.

Genetic defect: Pathogenesis of this syndrome is due to a mutation in a protein called LYST which is believed to regulate lysosomal trafficking.

- ❖ The mutation impairs the targeting of proteins to secretory lysosomes, which makes them unable to lyse bacteria
- ❖ Phagocytes from patients with this immune defect contain *giant granules* but do not have the ability to kill bacteria.

Leukocyte Adhesion Deficiency (LAD)

LAD is rare autosomal recessive disorder, characterized by a defect in the adhesion of leukocytes which results in poor leukocyte chemotaxis particularly of neutrophils. Thus it predisposes to various bacterial and fungal infections. LAD is due to mutations in $\beta 2$ integrin subunit (CD18) of the leukocyte cell adhesion molecule or fucosyltransferase enzyme.

Lazy Leukocyte Syndrome

It is an idiopathic condition due to defect in neutrophil chemotaxis which results in increased pyogenic infections such as gingivitis, abscess formation, pneumonia and neutropenia.

Job's Syndrome (Hyper-IgE Syndrome)

Hyper-IgE syndrome is a rare primary immunodeficiency disease characterized by eczema, recurrent staphylococcal skin abscesses, recurrent lung infections (pneumatocele), eosinophilia and high serum levels of IgE. Underlying mechanism of this immunodeficiency disease is defect in

neutrophil chemotaxis; due to mutations in either STAT3 or DOCK8 genes.

SECONDARY IMMUNODEFICIENCIES

Secondary immunodeficiencies, also known as acquired immunodeficiencies, are due to the secondary effects of other diseases, such as:

- ❖ Malnutrition (due to inadequate immunoglobulin synthesis)
- ❖ Aging (suppression of immune system with age)
- ❖ Patients with several infections that suppress immune system causing lymphocyte depletion, e.g. HIV (human immunodeficiency virus) infection
- ❖ Underlying cancers (particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma)
- ❖ Underlying proteinuric renal diseases—leads to loss of immunoglobulins
- ❖ Sarcoidosis
- ❖ Patients on immunosuppressive medications
- ❖ Patients receiving chemotherapy or radiation therapy for malignancy.

As a group, the secondary immunodeficiencies are more common than the primary immunodeficiency disorders. Acquired immunodeficiency syndrome (AIDS), the most widespread and important of the secondary immunodeficiency diseases, is discussed in detail in Chapter 48.

EXPECTED QUESTIONS

I. Write short notes on:

1. Severe combined immunodeficiencies.
2. Chronic granulomatous disease.
3. Wiskott–Aldrich syndrome.

II. Multiple Choice Questions (MCQs):

1. **Chronic granulomatous disease is due to deficiency of:**
 - a. Tyrosine kinase
 - b. NADPH oxidase
 - c. Adenosine deaminase
 - d. Myeloperoxidase
2. **The most common underlying mechanism of severe combined immunodeficiency disease (SCID) is:**

- a. Mutation in cytokine receptor
- b. Adenosine deaminase (ADA) deficiency
- c. Recombinase-activating genes
- d. Jak3 mutation

3. **Wiskott–Aldrich syndrome (WAS): All are true, except:**

- a. Thrombocytopenia
- b. Low IgA and IgE
- c. Defective response to bacterial polysaccharides
- d. Prone to develop non-Hodgkin B cell lymphomas

4. **Ataxia telangiectasia all are true, except:**

- a. Difficulty in maintaining balance while walking
- b. Telangiectasia
- c. Hyper IgA
- d. Profound sinopulmonary infections

Answers

1. b 2. a 3. b 4. c

CHAPTER PREVIEW

- Transplant Immunology
 - Classification of Transplants
 - Histocompatibility Antigens
 - Types of Graft Rejection
- Mechanism of Graft Rejection
- Prevention of Graft Rejection
- Graft-versus-host Reaction
- Cancer Immunology
 - Tumor Antigens
 - Cancer Immunotherapy

Organ transplantation and cancer are two situations in which the host immune system plays a deciding role in the survival of such transplants or tumors inside the host.

- ❖ In organ transplantation, immune response against the graft is a barrier to successful transplantation, and suppression of the immune system is the key for the transplant survival
- ❖ In cancer, the situation is precisely the reverse: suppressed immune system gives opportunity for many tumors to take birth and hence, enhancing the immunity against the tumor cells, is the principle used for treatment of cancers.

TRANSPLANT IMMUNOLOGY

Transplantation refers to transfer of a graft or transplant (cells, tissues, or organs) from one site to another. The individual from whom the transplant is taken is referred to as the **donor**; while the individual to whom it is transplanted, is called **recipient**.

CLASSIFICATION OF TRANSPLANTS

Transplants are classified in various ways.

- ❖ **Based on the organ or tissue transplanted:** Examples are kidney, heart and skin grafts, etc.
- ❖ **Based on the anatomical site of the graft:**
 - Orthotopic grafts: When the tissue or organ grafts are transplanted to their anatomically 'normal' sites in the recipient, then such grafts are known as orthotopic grafts, e.g. as in skin grafts
 - Heterotopic grafts: They are placed in anatomically 'abnormal' sites, as when thyroid tissue is transplanted in a subcutaneous pocket.
- ❖ **Vital and static transplants:**
 - Vital grafts are the live grafts, such as the kidney or heart, are expected to survive and function physiologically in the recipient

- Static grafts are nonliving structures, like bone or artery which merely provide a scaffolding on which new tissue is laid by the recipient.

❖ **Based on the genetic relationship** between the donor and the recipient:

- Autograft: It is a self-tissue transferred from one part of the body site to another in the same individual. Examples include transferring healthy skin to a burned area in burn patients and use of healthy blood vessels of the same person to replace blocked coronary arteries
- Isograft or syngeneic graft: It is a tissue transferred between genetically identical individuals (e.g. monozygotic twins)
- Allograft: It is a tissue transferred between genetically non-identical members of the same species (e.g. kidney or heart transplant)
- Xenograft: It is a tissue transferred between different species (e.g. the graft of a baboon heart into a man).

In humans, allografts are the most commonly used graft in transplant centres; hence our further discussion will be confined to allografts.

HISTOCOMPATIBILITY ANTIGENS

Histocompatibility

Histocompatibility between the graft and recipient would decide whether the graft is going to be accepted or rejected.

- ❖ If a graft and recipient tissues are histocompatible to each other (i.e. antigenically similar); then the graft is accepted. Usually, autografts and isografts are histocompatible
- ❖ On the contrary, histoincompatible (i.e. antigenically dissimilar) grafts are generally rejected by the recipient. Allografts and xenografts are usually histoincompatible.

Transplantation Antigens

Transplantation antigens are the antigens of allografts against which the recipient would mount an immune response.

- ❖ MHC molecules (major histocompatibility antigens) are the most important transplantation antigens
- ❖ Apart from that, ABO and Rh blood group systems also play a role in determining the histocompatibility
- ❖ **Minor histocompatibility antigens (MHA):** They are the peptides derived from normal cellular proteins of donated organs. Immune response against MHA molecules is weaker; hence they pose problems of rejection less frequently than MHC molecules. One exception is when a graft is transferred from a male donor to a female recipient
 - The graft tissues of a male donor (XY) would have some male-specific minor histocompatibility (H-Y) antigens determined by the Y chromosome which will be absent in the female (XX) recipient
 - Hence, it is observed that the rejection of the grafts when transferred from a male donor to female recipient is more as compared to female to male transplantation
 - This unilateral sex linked histoincompatibility is known as the **Eichwald-Silmser effect**.

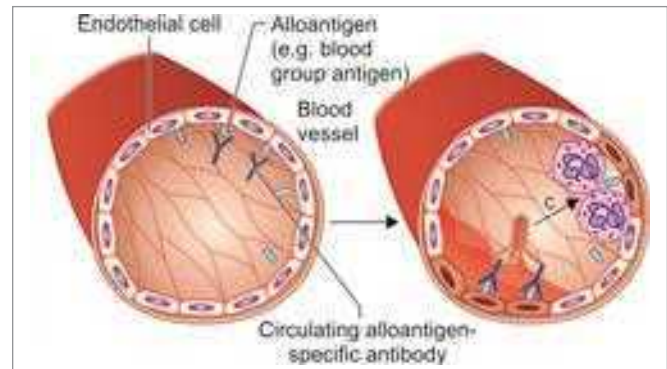
TYPES OF GRAFT REJECTION

Graft rejection is classified into hyperacute, acute, and chronic, on the basis of time taken for the rejection, types of immune response mounted against the graft and clinical and pathologic features (Table 19.1).

Hyperacute Rejection

This occurs within minutes to hours of transplantation and is characterized by thrombosis of graft vessels and ischemic necrosis of the graft.

- ❖ It is mediated by **circulating antibodies** that are specific for antigens on the graft endothelial cells and that are present before transplantation
- ❖ In an individual, exposure to foreign HLA antigens can occur as a consequence of previous blood transfusions, pregnancy, or organ transplantation. Following which, the individual develops antibodies against these antigens. These preformed antibodies may be anti-ABO or anti-



Preformed antibodies react with alloantigens on the vascular endothelium of the graft, activate complement (C) and trigger rapid intravascular thrombosis and necrosis of the vessel wall.

Fig. 19.1: Hyperacute graft rejection.

HLA specific for allogenic (i.e. graft's) MHC molecules. If an individual with these pre-existing antibodies to a foreign HLA antigen receives a graft (containing same foreign HLA antigen), then the graft will be rejected earlier and more vigorously (Fig. 19.1)

- ❖ Hyperacute rejection is not a common problem in clinical transplantation, because it can be avoided by matching the donor and the recipient. Potential recipients are tested for antibodies against the prospective donor's blood group antigens (by cross matching) and HLA antigens (by HLA typing).

Acute Graft Rejection

Acute graft rejection occurs within days or weeks after transplantation. It is due to an active immune response of the host stimulated by alloantigens in the graft.

- ❖ Acute graft rejection is mediated by T cells (mainly cytotoxic T cells, occasionally helper T cells) and antibodies specific for alloantigens in the graft
- ❖ Cytotoxic T cells directly destroy the graft cells, or cytokines secreted by the helper T cells induce inflammation, which destroys the graft
- ❖ Antibodies contribute especially to the vascular component of acute rejection. Antibody-mediated injury to graft vessels is caused mainly by complement activation by the classical pathway (Fig. 19.2)
- ❖ Current immunosuppressive therapy is designed mainly to prevent and reduce acute rejection by blocking the activation of alloreactive T cells.

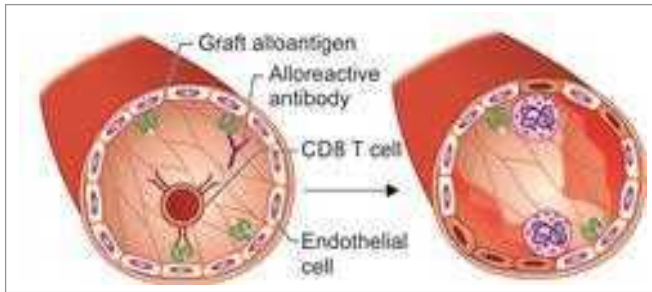
Chronic Graft Rejection

Chronic graft rejection is an indolent form of graft damage that occurs over months or years, leading to progressive loss of graft function.

- ❖ Chronic rejection may be manifested as fibrosis of the graft and by gradual narrowing of graft blood vessels, called graft arteriosclerosis
- ❖ T cells that react against graft alloantigens secrete cytokines, which stimulate the proliferation and

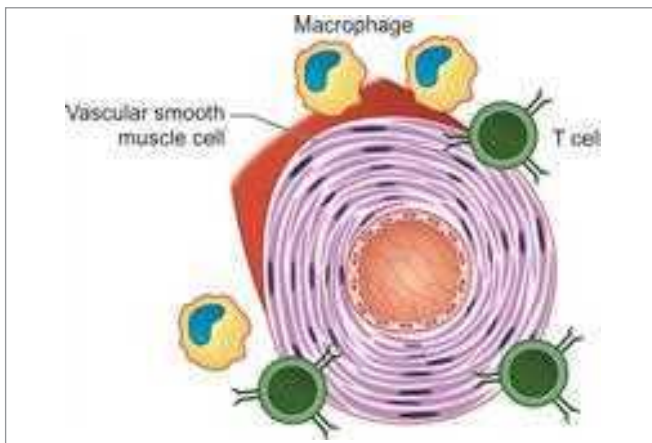
Table 19.1: Comparison of various types of graft rejection.

Graft rejection	Time taken for rejection	Immune mechanisms involved
Hyperacute	Minutes to hours	Preformed antibodies (anti-ABO and/or anti-HLA)
Acute	Weeks to months	<ul style="list-style-type: none"> • Cytotoxic T cell mediated • Antibody mediated
Chronic	Months to years	<ul style="list-style-type: none"> • Chronic DTH mediated • Antibody mediated



CD8 T cells react with graft alloantigens and destroy the endothelial and parenchymal cells or antibodies react with alloantigens of the graft's endothelium and causes endothelial cell damage via complement activation.

Fig. 19.2: Acute graft rejection.



T cells react with graft alloantigens may produce cytokines that induce inflammation and proliferation of intimal smooth muscle cells, leading to luminal occlusion and graft arteriosclerosis.

Fig. 19.3: Chronic graft rejection.

activities of fibroblasts and vascular smooth muscle cells in the graft. The smooth cell proliferation in the vascular intima may represent a specialized form of chronic delayed type hypersensitivity (DTH) reaction (Fig. 19.3)

- ❖ Alloantibodies also contribute to chronic rejection
- ❖ Chronic rejection is refractory to most of the therapeutic options available and is becoming the leading cause of graft failure.

FACTORS INFLUENCING ALLOGRAFT REJECTION

The rate of allograft rejection varies according to the—

- ❖ **Tissue involved**, e.g. skin grafts are rejected faster than other tissues such as kidney or heart
- ❖ **Genetic distance** between the donor and recipient—More the genetic distance; faster is the rejection. Autografts and isografts are well accepted
- ❖ **Immunological memory**: Rejection is faster when another graft is placed to a recipient from the same donor. This occurs due to the memory cells produced against the

first graft would differentiate quickly into effector cells; and that in turn reject the second graft faster.

An example is given below which describes the pathological sequences that take place when a skin graft is placed: (1) as an autograft to the same donor (leads to acceptance), (2) as an allograft to a recipient for the first time (leads to first set rejection), (3) as an allograft to the same recipient for the second time (leads to second set rejection).

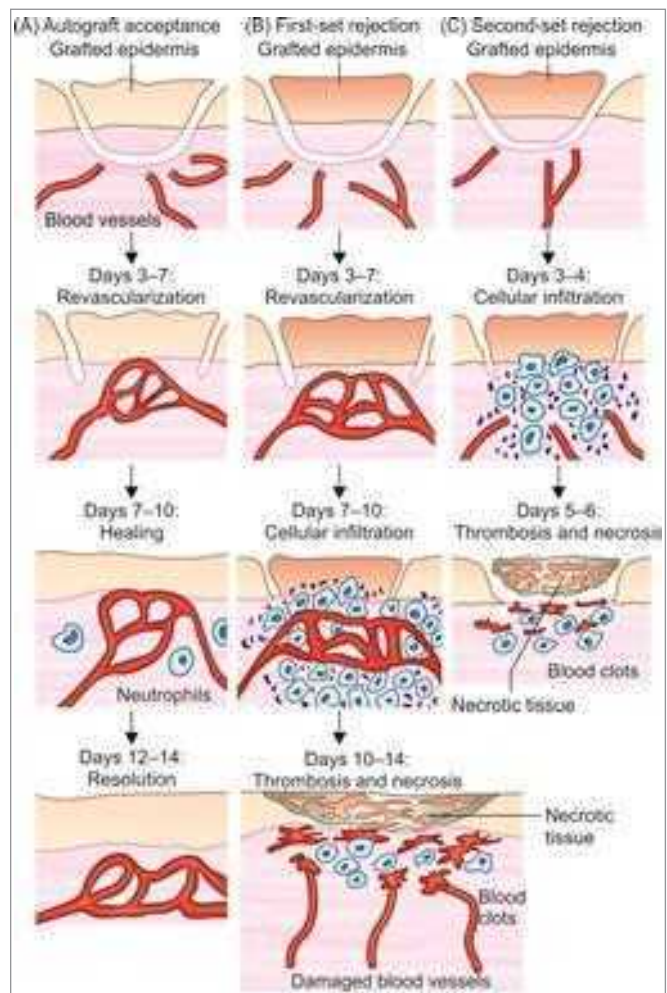
Autograft Acceptance

When a skin graft is transplanted to the same individual at a different site, revascularization takes place by day 3–7; followed by healing (within day 7–10) and then resolution and acceptance of the graft (by day 12–14) (Fig. 19.4A).

First-set Rejection

When an allograft is placed for the first time from a donor to a recipient, the type of primary graft rejection that develops is known as, **first-set rejection** (Fig. 19.4B).

- ❖ The skin first becomes revascularized between days 3 and 7; as the reaction develops, the vascularized transplant



Figs 19.4A to C: Graft acceptance and graft rejection.

becomes infiltrated with lymphocytes, monocytes, neutrophils, and other inflammatory cells

- ❖ There is decreased vascularization of the transplanted tissue by 7–10 days, visible necrosis by 10 days, and complete rejection by 12–14 days.

Second-set Rejection

If, in a recipient who has rejected a graft by the first set response, another graft from the same donor is transplanted, it will be rejected in an accelerated fashion.

- ❖ Though vascularization starts but is soon interrupted by the inflammatory response
- ❖ Necrosis sets in early and the graft sloughs off by the sixth day (Fig. 19.4C).

MECHANISM OF GRAFT REJECTION

Graft rejection is caused principally by a T cell-mediated immune response to alloantigens expressed on the graft cells, primarily the MHC molecules (Fig. 19.5).

The T cell response to MHC antigens involves recognition of both the donor MHC molecule as well as the associated peptide ligand present in the cleft of the MHC molecule.

- ❖ The peptides present in the groove of allogeneic (i.e. donor) class I MHC molecules are derived from proteins synthesized within the allogeneic cell
- ❖ The peptides present in the groove of allogeneic (i.e. donor) class II MHC molecules are generally proteins taken up and processed by the allogeneic APCs.

The process of graft rejection can be divided into two stages: (1) A sensitization phase—which involves alloantigen (mainly graft MHC molecules) presentation to recipient's T cells and (2) An effector stage, in which immune destruction of the graft takes place due to activation of recipient's T cells.

Sensitization Phase

T cells in the recipient may recognize donor alloantigens in the graft in two different ways: (1) direct pathway, and (2) indirect pathway; depending on what cells in the graft

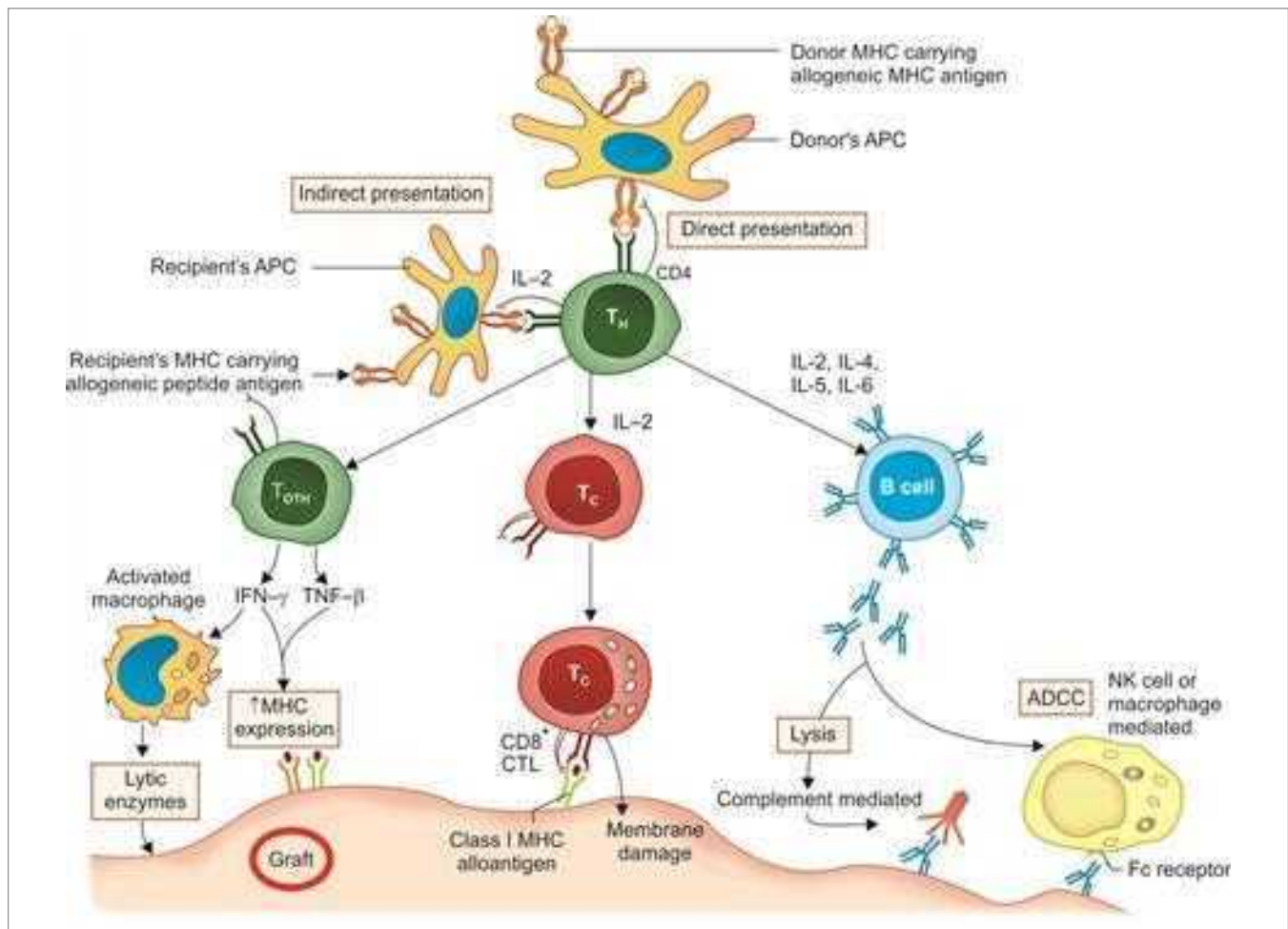


Fig. 19.5: Mechanisms involved in graft rejection.

Abbreviations: MHC, major histocompatibility complex; NK, natural killer; APC, antigen presenting cells; IL, interleukin; TNF, tumor necrosis factor; IFN, interferons; CTL, cytotoxic-T lymphocytes.

are displaying these alloantigens to the recipient T cells (Fig. 19.5).

Direct Pathway of Alloantigen Presentation

Many graft tissues contain antigen presenting cells (APCs, e.g. dendritic cells and macrophages) and when the tissues are transplanted, the APCs are also carried along with the graft to the recipients.

- ❖ The allogeneic MHC molecules on graft's APCs are directly presented to the recipient's helper T cells
- ❖ This pathway is responsible for most of the acute graft rejections mediated by cytotoxic T cells (described in effector phase).

Indirect Pathway of Alloantigen Presentation

This is similar to that for recognition of any foreign antigen by the host APCs.

- ❖ The graft cells are ingested by recipient APCs, donor alloantigens are processed and presented by the MHC molecules present on recipient APCs to recipient's helper T cells
- ❖ This pathway is responsible for most of the chronic rejection mediated by helper T cells via specialized form of chronic DTH reaction (described in effector phase).

Effector Phase

A variety of effector mechanisms participate in allograft rejection. The most common are cell-mediated reactions involving delayed-type hypersensitivity T cells and cytotoxic T cells.

- ❖ **Delayed-type hypersensitivity:** Activated helper T cells differentiate into T_{DTH} cells. Cytokines secreted from T_{DTH} (e.g. interferon- γ) activate macrophages which destroy the target graft cells by producing lytic enzymes
- ❖ **Cytotoxic T cells:** $CD8^+ T_C$ cells kill the graft cells by recognizing the allogeneic MHC-I molecules
- ❖ **Antibody-mediated mechanisms:** Cytokines produced by helper T cells activate B cells to produce antibodies. Antibodies are also important in mounting immune response against the graft. They take a lead role in mediating hyperacute graft rejections; however, in acute and chronic rejections, they play a minor role. Antibody-mediated destruction of the graft occurs by the following mechanisms:
 - Complement-mediated lysis
 - Antibody-dependent cell-mediated cytotoxicity (ADCC) via NK cell or macrophage mediated destruction.

PREVENTION OF GRAFT REJECTION

Laboratory Tests to Determine Histocompatibility

Prior to transplantation, various laboratory tests should be carried out to assess the histocompatibility between the donor and recipient.

- ❖ ABO blood group compatibility testing by blood grouping and cross matching
- ❖ HLA typing (see highlight box).

Immunosuppressive Therapy

- ❖ Hyperacute rejection manifests severely and within minutes, and so the treatment indicated is immediate removal of the tissue

HLA Typing

In this test, donor's antigens expressed on the surface of leukocytes or their gene to that of recipient are matched. The HLA compatibility is determined by:

- ❑ **Phenotypic method**, such as
 - Serology: Microcytotoxicity
 - Tissue typing: Mixed lymphocyte reaction
- ❑ **Genotypic methods**, such as
 - PCR detecting HLA genes
 - PCR-RFLP (restriction fragment length polymorphism)
 - PCR-SSOP (PCR sequence-specific oligonucleotide probing)
 - PCR-SSP (PCR-sequence-specific primer)
 - PCR- DNA sequencing
 - Conformational analysis.

The phenotypic methods were used widely in the past. But with the advent of molecular methods, they are not preferred now. PCR-SSOP, PCR-SSP and PCR-DNA sequencing are the most reliable methods currently in use; have shown high resolution matching.

- ❖ Chronic rejection is generally considered irreversible and poorly amenable to treatment—only retransplant generally indicated if feasible—though inhaled cyclosporine is being investigated to delay or prevent chronic rejection of lung transplants
- ❖ Acute rejection is treated with therapeutic regimens consisting of one or combination of various immunosuppressive therapies as given in Table 19.2.

Graft-versus-Host Reaction

Graft-versus-host (GVH) reaction is a condition, where graft mounts an immune response against the host (i.e. recipient) and rejects the host, in contrary to the usual situation of graft rejections, in which the recipient mounts an immune response against the graft antigens.

The GVH reaction occurs when the following three conditions are present:

1. The graft must contain immunocompetent T cells (e.g. stem cells or bone marrow or thymus transplants).
2. The recipient should possess transplantation antigens that are absent in the graft.
3. The recipient may be immunologically suppressed, therefore cannot mount immune response against the graft.

Types

GVH disease occurs in two forms:

1. Acute or fulminant GVH disease occurs within first 100 days of post-transplantation. It is a major challenge in case of bone marrow transplantation.

Contd...

Table 19.2: Immunosuppressive agents used to improve graft survival.

Immunosuppressive drugs	
Corticosteroids	Prednisolone, hydrocortisone
Calcineurin inhibitors	Cyclosporine, Tacrolimus
Mitotic inhibitors	Azathioprine Cyclophosphamide Methotrexate
Antiproliferatives	Mycophenolic acid
mTOR inhibitor (mammalian target of rapamycin)	Sirolimus (rapamycin) Everolimus
Monoclonal antibody based agents	
mAb to CD2 molecule present on T cell surface	OKT2
mAb to CD3 molecule present on T cell surface	OKT3
mAb to CD4 molecule present on T cell surface	OKT4
Monoclonal anti-IL-2R α receptor antibodies	Basiliximab Daclizumab
Monoclonal anti-CD20 antibodies	Rituximab
mAb to TNF α	Infliximab
Antithymocyte globulin (ATG)	
Antilymphocyte globulin (ALG)	

Contd...

- Chronic GVH disease is less severe form, occurs after 100 days of transplantation.

Clinical Manifestations

- The acute GVH disease is characterized by selective damage to the liver (hepatomegaly), skin (rash), mucosa, and the intestine (diarrhea) mediated by graft's immunocompetent T cells. Experimentally, GVH can be produced in mice, called **Runt disease**
- Chronic GVH disease also attacks the above organs, but in addition, it causes damage to the connective tissues and exocrine glands.

Treatment

Glucocorticoids (administered intravenously) are the standard treatment given for both acute and chronic GVH disease.

CANCER IMMUNOLOGY

Tumor immunology involves the study of antigens on tumor cells and the immune response to these antigens.

TUMOR ANTIGENS

Two types of tumor antigens have been identified on tumor cells:

- Tumor-specific transplantation antigens (TSTAs)
- Tumor-associated transplantation antigens (TATAs).

Tumor-specific Transplantation Antigen

Tumor-specific antigens are present only on tumor cells and are absent in normal cells of the body.

They may result from mutations in tumor cells that generate altered cellular proteins; cytosolic processing of these proteins would give rise to novel peptides that are presented with class I MHC molecules, inducing a cell-mediated immune response by tumor-specific cytotoxic T lymphocytes.

TSTAs are induced on tumor cells either by chemical or by physical carcinogens, and also by viral carcinogens.

- In chemically/physically induced tumors, the TSTA is tumor specific. Different tumors possess different TSTA, even though induced by the same carcinogen. Methylcholanthrene and ultraviolet light are the examples of chemical and physical carcinogens that are extensively studied
- In contrast, the TSTA of virus induced tumors is virus specific; all tumors produced by one virus would possess the same antigen. Examples include Epstein-Barr virus which causes nasopharyngeal carcinoma and several types of lymphoma.

Tumor-associated Transplantation Antigens

Tumor-associated antigens (TATAs) are not unique to tumor cells and may also be expressed by normal cells, but at a very low level. Their level gets exponentially high in tumor cells. Examples include (Table 19.3):

- Oncofetal antigens:** They are the proteins that are expressed on normal cells during fetal life, but not expressed in the adult normally
 - Reactivation of the embryonic genes that encode these proteins in tumor cells results in their expression on the fully differentiated tumor cells

Table 19.3: TATAs used as tumor markers for diagnosis of cancers.

Tumor markers	Tumor types
Oncofetal proteins	
Alpha-fetoprotein (AFP)	Hepatoma Testicular cancer
Carcinoembryonic antigen (CEA)	Gastrointestinal cancers Lung, ovarian cancers
Secreted tumor antigens	
CA 125	Ovarian cancers Other epithelial cancers
CA 19-9	Various carcinomas
Prostate-specific antigen	Prostate cancer
β 2-microglobulin	Multiple myeloma
Hormones	
β subunit of chorionic gonadotropin	Hydatidiform mole Choriocarcinoma Testicular cancers

- Examples include alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA).
- ❖ **Non-oncofetal TATAs:** Examples include carbohydrate antigens (CA 125, CA 19-9), prostate specific antigen and macroglobulin.

IMMUNE RESPONSE AGAINST TUMOR CELLS

Both humoral and cell-mediated immune responses are induced by tumor antigens that result in the destruction of the tumor cells. In general, the cell-mediated response appears to play the major role, especially cytotoxic T cell and NK cell.

Cytotoxic T Cells

A number of tumors have been shown to induce tumor-specific T_c cells that recognize tumor antigens presented by class I MHC on the tumor cells. However, as the expression of class I MHC molecules are decreased in a number of tumors, thereby limiting the role of specific T_c cells in their destruction.

Natural Killer (NK) Cells

The recognition of tumor cells by NK cells is not MHC restricted. The activity of NK cells is not compromised; but enhanced by the decreased MHC expression exhibited by some tumor cells.

- ❖ This is due to withdrawal of inhibitory receptors induced NK cells suppression. The inhibitory receptors of NK cells will be no longer functional in the absence of MHC I molecules on the target cells so that the activation receptors become active
- ❖ The activation receptors can be Fc receptors on NK cells which can bind to antibody-coated tumor cells, leading to ADCC
- ❖ The importance of NK cells in tumor immunity is suggested by the mutant mouse strain called **beige** and **Chediak-Higashi syndrome** in humans. In each case, a genetic defect causes marked impairment of NK cells and an associated increase in certain types of cancer.

CANCER IMMUNOTHERAPY

Cancer immunotherapy is the use of the immune system to treat cancer. Three main groups of immunotherapy are used to treat cancers: cell-based therapies, antibody therapies and cytokine therapies. They all provoke the immune system to attack the tumor cells by using these cancer antigens as targets.

Cell-based Therapies

Cell-based therapies, also known as **cancer vaccines**, usually involve the removal of immune cells from patients

with cancer, either from the blood or from a tumor → immune cells specific for the tumor will be activated, grown and returned to the person with cancer → where the immune cells provoke an immune response against the cancer.

- ❖ Cell types that can be used in cancer vaccines include NK cells, cytotoxic T cells and dendritic cells
- ❖ The only cell-based therapy currently approved for use is dendritic cells (Provenge) for the treatment of prostate cancer.

Monoclonal Antibodies

Monoclonal antibody (mAb) therapies are currently the most successful form of immunotherapy. Many mAbs are approved for treatments of a wide range of cancers (Table 19.4).

Cytokine Therapies

Administration of cytokines can regulate and coordinate the behavior of the immune system. Examples include:

- ❖ **Interferon α:** It is used in the treatment of hairy-cell leukemia, AIDS-related Kaposi's sarcoma, follicular lymphoma, chronic myeloid leukemia and malignant melanoma
- ❖ **Interleukin-2:** It is used in the treatment of malignant melanoma and renal cell carcinoma.

Cancer Vaccine

They are used for treatment of existing cancers or prevention of emergence of new cancers.

- ❖ **Preventive cancer vaccines:** Examples of HPV vaccine and hepatitis B vaccine will prevent the emergence cervical and liver cancers, respectively
- ❖ **Therapeutic cancer vaccines:** They are used to treat existing cancers. Research is ongoing for preparation of such vaccines. Vaccines against some oncogenic viruses have proven extremely effective.

Table 19.4: Monoclonal antibodies approved for treatment of cancers.

Monoclonal antibodies	Target	Approved for treatment of cancers
Alemtuzumab	CD52	Chronic lymphocytic leukemia (CLL)
Bevacizumab	Vascular endothelial growth factor	Colorectal, lung and renal cell cancer
Cetuximab	Epidermal growth factor receptor	Colorectal, head and neck cancer
Ipilimumab	CTLA4	Metastatic melanoma
Rituximab	CD20	CLL
Tositumomab	CD20	Non-Hodgkin lymphoma
Trastuzumab	ErbB2	Breast cancer

EXPECTED QUESTIONS

I. Write essay on:

1. A 55-year-old male patient with chronic kidney disease underwent a kidney transplantation donated by an unrelated donor. Patient developed rejection reaction within 3 weeks.
 - a. What is the immunological process of rejection?
 - b. Mention the pretransplantation investigations to know the suitability of transplant.
 - c. How can this be prevented?

II. Write short notes on:

1. Graft-versus-host reaction.
2. Tumor antigens.

III. Multiple Choice Questions (MCQs):

1. Application of skin graft for the second time from the same donor will result in:

- a. First set rejection
- b. Second set rejection
- c. Both
- d. None

2. Graft rejection due to preformed antibodies occurs in:

- a. Hyperacute rejection
- b. Acute rejection
- c. Subacute rejection
- d. Chronic rejection

3. Best example of syngeneic graft:

- a. Between dizygotic twins
- b. Between monozygotic twins
- c. Between two members of same or different species
- d. Between two sites of same person

4. Which is not correct about graft rejection:

- a. Hyperacute graft rejection—anti-ABO mediated
- b. Acute graft rejection—cytotoxic T cell mediated

- c. Chronic Graft rejection—delayed type hypersensitivity (DTH) mediated
- d. Acute graft rejection—manifested as fibrosis of the graft

5. Which is a typical example of second-set rejection?

- a. Revascularization (day 3–7) → healing (day 7–10) → resolution (day 10–12) → rejection (by day 12–14)
- b. Revascularization (day 3–7) → cellular infiltration (day 7–8) → decreased vascularization (day 7–10) → necrosis (day 10–12) → rejection (by day 12–14)
- c. In-complete revascularization (day 1–2) → cellular infiltration (day 3–4) → thrombosis and necrosis (day 5–6) → rejection (by day 6)
- d. Decreased vascularization (day 3–7) → Revascularization (day 7–10) → necrosis (day 10–12) → rejection (by day 12–14)

6. Which statement is not true about direct pathway of alloantigen presentation?

- a. The allogeneic MHC molecules on graft's APCs are presented to the recipient's helper T cells.
- b. Responsible for most of the acute graft rejections mediated by cytotoxic T cells
- c. Seen if graft tissues contain macrophages and dendritic cells
- d. The donor alloantigens are processed and presented by recipient APCs

7. Which of the following HLA typing gives high resolution matching?

- a. Microcytotoxicity
- b. Mixed lymphocyte reaction
- c. PCR-RFLP (restriction fragment length polymorphism)
- d. PCR – DNA sequence typing

Answers

1. b 2. a 3. b 4. d 5. c 6. d 7. d

CHAPTER PREVIEW

- Active Immunoprophylaxis
- Passive Immunoprophylaxis
- National Immunization Schedule

IMMUNOPROPHYLAXIS

Immunoprophylaxis against microbial pathogens can be classified into active immunoprophylaxis (or vaccination) and passive immunoprophylaxis (or immunoglobulin administration).

VACCINATION (ACTIVE IMMUNOPROPHYLAXIS)

Vaccine is an immunobiological preparation that provides specific protection against a given disease. Following vaccine administration, the immunogen (active ingredient of the vaccine) stimulates the immune system of the body to produce active immunity in the form of protective antibody and/or immunocompetent T cell response.

- ❖ **History:** The terms vaccine and vaccination are derived from '*Variolae vaccinae*' (smallpox of the cow), the term devised by Edward Jenner to denote cowpox. Later, Louis Pasteur proposed the term 'vaccine' to cover all the new protective preparations being developed, in the memory of Edward Jenner
- ❖ **Valency:** Vaccines may be **monovalent** that contains single antigen or single serotype of a microorganism) or **polyvalent** (contains two or more strains of the same microorganism), e.g. trivalent vaccines such as influenza vaccine and polio vaccine
- ❖ **Homologous and heterologous vaccine:** In most vaccines, the immunizing substance is derived from the same microorganism against which it is used (homologous vaccine). However, there are few exceptions, where the vaccine organism is different from the disease-causing organism. Such vaccines are called as heterologous or "Jennerian" vaccines. Examples include—
 - The classic example is Jenner's use of cowpox to protect against smallpox
 - Use of BCG vaccine made from *Mycobacterium bovis* to protect against human tuberculosis caused by *M. tuberculosis*.

- ❖ **Types:** Vaccine may be prepared by live modified organisms, inactivated or killed organisms, extracted or cellular fractions, toxoids or combinations of all these. Preparations that are more recent are subunit vaccines and recombinant vaccine. Vaccines of future prospects include DNA vaccine and edible vaccine.

Live Attenuated Vaccine

Live vaccines, such as BCG (Table 20.1) are prepared from live (usually attenuated) organisms.

- ❖ The live attenuated organisms lose their ability to induce full blown disease, but retain their immunogenicity
- ❖ Attenuation is achieved by passing the live organisms serially through a foreign host, such as chick embryo/tissue culture or live animals.

Note: Smallpox vaccine is a live vaccine which is not attenuated. The nonpathogenic cross reactive vaccinia virus or cowpox virus were used to vaccinate against smallpox virus (i.e. variola).

Advantages

Live vaccines in general, are more potent immunizing agents compared to killed vaccines, due to the following reasons:

- ❖ The live organisms **multiply** in the host and the resultant antigenic dose would be larger than what is administered
- ❖ Live vaccines retain all the immunogenic components (major and minor) of the organisms
- ❖ They are capable of inducing mucosal immunity by stimulating secretory IgA antibody production at the local mucosal sites.

Precautions While Using Live Attenuated Vaccines

- ❖ **Contraindications:** Live vaccines should not be administered in individuals with immunodeficiency diseases or any conditions that suppresses the immunity, such as leukemia, lymphoma, malignancies, on corticosteroid or any other immunosuppressive drug therapy

Table 20.1: Example of commonly used vaccines.	
Bacterial	Viral
Live attenuated vaccines	
BCG vaccine	Measles vaccine
Typhoral vaccine	Mumps vaccine
Epidemic typhus vaccine	Rubella vaccine
	Live attenuated influenza vaccine
	Chickenpox vaccine
	Oral polio vaccine (OPV, Sabin vaccine)
	Rotavirus vaccine
	Yellow fever 17D vaccine
	Hepatitis A vaccine
	Japanese B encephalitis vaccine (14-14-2 strain)
Killed/inactivated vaccine	
Typhoid vaccine	Injectable polio vaccine (IPV or Salk vaccine)
Cholera vaccine	Killed influenza vaccine
Pertussis vaccine	Rabies vaccine
Plague vaccine	Hepatitis A vaccine
	Japanese B encephalitis vaccine
Toxoid vaccine	
DT (Diphtheria toxoid)	–
TT (Tetanus toxoid)	–
Subunit vaccine	
–	Hepatitis B vaccine
–	HPV (Human papillomavirus) vaccine
Cellular fraction	
Meningococcal vaccine	–
Pneumococcal vaccine	–
<i>Haemophilus influenzae</i> type b (Hib) vaccine	–
Combined vaccine	
DPT vaccine (Diphtheria, pertussis and tetanus)	Mumps, measles, rubella (MMR) vaccine
Pentavalent vaccine (DPT + Hib + Hepatitis B)	

Note: Details about individual vaccine is discussed in the respective chapters.

- ❖ **Pregnancy** is another contraindication, unless the risk of infection exceeds the risk of harm to the fetus by giving the live vaccine
- ❖ When **two live vaccines** are required to be given; they should be administered with an interval of at least 4 weeks. Exception is yellow fever vaccine which can be given less than 4 weeks after MMR vaccine
- ❖ **Dosage:** Most live vaccines are given in single dose format as effective immunity is achieved with a single dose. Exception is oral polio vaccine (OPV) which is given as multiple doses at spaced intervals to achieve effective immunity

- ❖ **Risk of gaining the virulence:** The attenuation of the live vaccine has to be done in an effective way otherwise there is always a risk of gaining the virulence back
- ❖ **Storage:** Live vaccines must be stored cautiously to retain effectiveness, especially the OPV and measles vaccine.

Inactivated or Killed Vaccine

It consists of organisms, which are grown in culture under controlled conditions and then killed using methods, such as heat or formaldehyde.

- ❖ They are generally safer but less efficacious than live vaccines
- ❖ Compared to the live vaccines, killed vaccines require large doses, adjuvants, and multiple doses to confer immunity. In most cases, a booster dose is also needed
- ❖ Adjuvants increase the immunogenicity of the vaccine antigen (e.g. alum is used as adjuvant in DPT vaccine)
- ❖ Killed vaccines are usually administered in subcutaneous or intramuscular routes. The only absolute contraindication is a severe local or general reaction to the previous dose.

Various characteristics of killed and live vaccines are given in Table 20.2.

Toxoid Vaccine

The exotoxins produced by certain bacteria can be detoxicated to form toxoid by treating with acidic pH, formalin or by prolonged storage.

- ❖ Toxoid is a form of toxin that loses its virulence property but retains immunogenicity
- ❖ When a toxoid preparation is given as vaccine, it induces formation of neutralizing antibodies that are capable

Table 20.2: Characteristics of killed and live vaccines.

Characteristics	Killed vaccine	Live vaccine
Number of doses	Multiple	Single*
Need for adjuvant	Yes	No
Duration of immunity	Shorter	Longer
Effectiveness of protection	Lower	Greater
Mimics natural infection	Less closely	More closely
Immunoglobulins produced	IgG	IgA and IgG
Mucosal immunity	Absent	Induced
Cell-mediated immunity	Poor	Induced
Reverts back to virulent form	No	Possible
Excretion of vaccine virus and transmission to non-immune contacts	No	Possible
Interference by other microorganisms in host	No	Possible
Stability at room temperature	High	Low
Immunodeficiency and pregnancy	Safe	Unsafe

*Exception is oral polio vaccine (OPV), which is given as multiple doses at spaced intervals to achieve effective immunity.

of neutralizing the toxin moiety produced during an infection; rather than acting upon the organism

- ❖ Examples include diphtheria toxoid (from *Corynebacterium diphtheriae*) and tetanus toxoid (from *Clostridium tetani*).

Extracted or Cellular Fractions Vaccine

Vaccines, in certain instances, are prepared from extracted cellular fractions; examples include meningococcal vaccine, pneumococcal vaccine and *Haemophilus influenzae* type b vaccine—all are prepared from the capsular polysaccharide antigens of the respective organism.

Subunit Vaccines

For certain viruses, only a particular subunit of the virus is necessary to initiate the immunity, e.g. hepatitis B surface antigen (HBsAg) is the immunogenic component of hepatitis B virus. So, this viral component alone can be used as vaccine rather than the whole virus.

- ❖ Examples of subunit vaccines include hepatitis B vaccine and human papillomavirus (HPV) vaccine
- ❖ DNA recombinant technology is used for the preparation of such sub viral components. For example, in hepatitis B vaccine preparation, the gene coding for HBsAg is inserted into the chromosome of baker's yeast, so that, with the multiplication of the yeast, the gene of interest would also replicate resulting in production of large quantity of HBsAg which can be used as vaccine.

Combinations

If more than one immunizing agents are included in a vaccine preparation, it is called combined vaccine. The aim of the combined vaccine is to—

- ❖ Simplify administration and
- ❖ Augment the immunogenicity of the immunogen. For example, in DPT vaccine, the pertussis component acts as an adjuvant, which increases the immunogenicity of both diphtheria toxoid and tetanus toxoid.

Newer Vaccine Approaches

DNA Vaccine

DNA vaccines are experimental at present, have many advantages such as cost effectiveness and mounting a stronger and wider range of immune response.

The small pieces of DNA containing genes from the pathogenic microorganism are injected into the host. The gene of interest gets integrated with the host cell genome and starts transcribing the proteins against which the host mounts an immune response. Several vaccine trials are going on based on DNA vaccines.

Edible Vaccine

The edible vaccine is a new concept introduced recently.

- ❖ The gene encoding the orally active antigenic protein is isolated from the pathogen and is transferred to suitable

plant bacteria, which are then used to infect a transgenic plant (e.g. banana, potato, etc.)

- ❖ The plants infected by the bacteria then start producing the antigen of interest in large scale. The appropriate plant parts having the antigen may be fed raw to animals or humans to bring about immunization
- ❖ The advantages of the edible vaccines are—(1) low cost, (2) ability to produce in large scale, (3) administered orally, (4) induce local immunity, and (5) heat stable
- ❖ **Applications:** The edible vaccines are still under experimental stage; some formulations available include—
 - Transgenic potatoes and tomatoes against diarrheagenic organisms
 - Edible banana against Norwalk virus.

Cold Chain

“Cold chain” refers to a system of transport, storage, and handling of vaccines, starting at the manufacturer level and ending with the site of administration of the vaccine to the client. The optimum temperature for refrigerated vaccines is between +2°C and +8°C. For frozen vaccines the optimum temperature is -15°C or lower. In addition, protection from light is a necessary condition for some vaccines. Improper cold chain maintenance is one of the most common causes of vaccine failure; especially oral polio vaccine which is the most sensitive vaccine to heat; must be stored at -20°C.

- ❖ Vaccines which must be stored in the freezer compartment are polio and measles vaccines
- ❖ Vaccines which must be stored in the COLD part but never allowed to freeze are—DPT, TT, Td, BCG, hepatitis B, *H. influenzae* type b and diluents.

Vaccine Vial Monitor

Vaccine vial monitor is a tool to monitor the stability/potency of a vaccine and to check the efficiency of cold chain.

It is heat sensitive label lining the vaccine vial. It contains an outer blue circle and an inner white square. With time and exposure to higher temperature, the inner square changes its color gradually from white towards blue, whereas the outer circle is not heat sensitive; it remains blue throughout (Table 20.3 and Fig. 20.1).

National Immunization Schedule 2020 (NIS)

Immunization is one of the most logical and cost effective strategies of any country for the prevention of childhood sicknesses and disabilities and is thus a basic need for all children. The following is the national immunization

Table 20.3: Staging of vaccine vial monitor.

	Inner square	Outer circle	Vaccine
Stage 1	White	Blue	Can be used
Stage 2	Light blue	Blue	Can be used
Stage 3	Blue	Blue	Discard
Stage 4	Dark blue	Blue	Discard

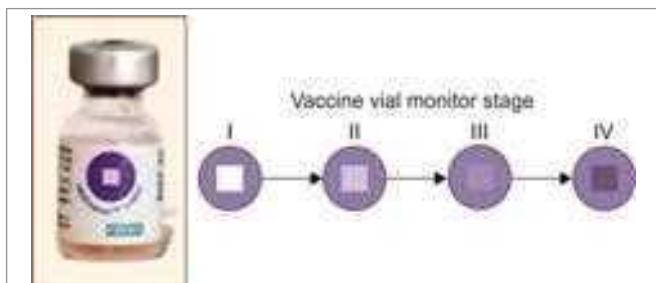


Fig. 20.1: Various stages of vaccine vial monitor (Vaccine is usable up to stages I and II and should be discarded for stages III and IV)

Source: Pondicherry Institute of Medical Sciences (with permission).

schedule recommended by the Ministry of Health, Government of India and it includes those vaccines that are given free of cost to all children of our country (Table 20.4).

PASSIVE IMMUNOPROPHYLAXIS (IMMUNOGLOBULINS)

Passive immunoprophylaxis is given in the form of commercially available ready made **immunoglobulins** prepared against the pathogenic microorganism. Unlike vaccines, immunoglobulins act faster, without involvement of host immune apparatus.

Table 20.4: National Immunization Schedule 2020 (NIS) for infants, children and pregnant women.

Vaccine	When to give	Maximum age	Dose	Dilution	Route	Site
For pregnant women						
TT/Td-1	Early in pregnancy		0.5 mL	No	IM	Upper arm
TT/Td-2	4 weeks after TT/Td-1*	<36 weeks of pregnancy (if missed, can be given later)	0.5 mL	No	IM	Upper arm
TT/Td- Booster	If received 2 TT/Td doses in a pregnancy within the last 3 years*		0.5 mL	No	IM	Upper arm
For infants						
BCG	At birth or as early as possible	Till 1 year	0.05 mL (0.1 mL for >1 month)	Saline	ID	Left upper arm
Hepatitis B - Birth dose	At birth or as early as possible	Within 24 hour	0.5 mL	No	IM	Anterolateral side of mid-thigh
OPV-0	At birth or as early as possible	Within first 15 days	2 drops	No	Oral	Oral
OPV 1, 2 and 3	At 6 weeks, 10 weeks and 14 weeks	5 years of age	2 drops	No	Oral	Oral
Pentavalent [†] 1, 2 and 3	At 6 weeks, 10 weeks and 14 weeks	1 year of age	0.5 mL	No	IM	Anterolateral side of mid-thigh
PCV [^] (3 doses)	At 6 weeks and 14 weeks, booster at 9-12 months	–	0.5 mL	–	IM	Anterolateral side of mid-thigh
Rotavirus ^{##}	At 6 weeks, 10 weeks and 14 weeks	1 year of age	5 drops	No	Oral	Oral
IPV	Two fractional doses at 6 and 14 weeks of age	1 year of age	0.1 mL	No	ID	Right upper arm
Measles /MR 1st Dose	9 completed months–12 months	5 years of age (only measles vaccine)	0.5 mL	Sterile water	SC	Right upper arm
JE - 1 ^{**}	9 completed months–12 months	15 years of age	0.5 mL	Phosphate buffer	SC	Left upper arm
Vitamin A (1st dose)	At 9 completed months, given along MR vaccine	5 years of age	1 mL (1 lakh IU)	No	Oral	Oral
For Children						
DPT booster-1	16–24 months	7 years of age	0.5 mL	No	IM	Anterolateral side of mid-thigh
MR 2nd dose [§]	16–24 months	5 years of age	0.5 mL	Sterile water	SC	Right upper arm
OPV Booster	16–24 months	5 years of age	2 drops	No	Oral	Oral
JE-2	16–24 months	–	0.5 mL	Phosphate buffer	SC	Left upper arm
Vitamin A ^{***} (2nd to 9th dose)	16–18 months. Then one dose every 6 months up to the age of 5 years	5 years of age	2 mL (2 lakh IU)	No	Oral	Oral

Contd...

Contd...

Vaccine	When to give	Maximum age	Dose	Dilution	Route	Site
DPT Booster-2	5–6 years	7 years of age	0.5 mL	No	IM	Upper arm
TT/Td	10 years and 16 years		0.5 mL	No	IM	Upper arm

***TT/Td:** Tetanus toxoid (TT) is given alone, or in combination with adult diphtheria toxoid (Td). The second or booster dose is ideally given before 36 weeks of pregnancy, but should be given even if presented late in pregnancy or during labor.

****JE Vaccine** is introduced in selected endemic districts after the campaign: UP, Bihar, Assam, West Bengal and Karnataka.

***The 2nd to 9th doses of Vitamin A can be administered to children 1–5 years old during biannual rounds, in collaboration with ICDS (Integrated Child Development Services).

***Pentavalent vaccine-** contains combination of DPT, hepatitis B and *H.influenzae* type b vaccines.

Interval between two doses of pentavalent vaccine or OPV should never be less than 1 month.

****Rotavirus vaccine:** Given in selected states such as Andhra Pradesh, Assam, Haryana, Himachal Pradesh, Jharkhand, Madhya Pradesh, Odisha, Rajasthan, Tamil Nadu, Tripura and Uttar Pradesh.

^**Pneumococcal conjugate vaccine (PCV):** Given in selected states such as Bihar, Himachal Pradesh, Madhya Pradesh, Uttar Pradesh (12 districts) & Rajasthan (9 districts).

Children who have not been received a single vaccine coming after 1 year: Will be given 3 doses of DPT at an interval of 4 weeks; Measles-1st dose, JE-1st dose (wherever applicable) up to 2 years of age.

Abbreviations: IM, intramuscular; SC, subcutaneous; ID, intradermal; TT/Td, Tetanus and adult diphtheria toxoid; BCG, Bacillus Calmette Guerin; PCV, Pneumococcal conjugate vaccine.

Passive immunization is useful in the following circumstances:

- ❖ For immunocompromised individuals who cannot synthesize antibodies
- ❖ For post-exposure prophylaxis to achieve an immediate effect.

For the treatment of toxin mediated diseases to ameliorate the effect of toxin. Antibiotics cannot neutralize the toxin; hence, they cannot be used for the treatment of toxin mediated diseases.

Passive immunoprophylaxis available against various microbial diseases is given in Table 20.5.

Immunoglobulin preparations	Source	Indications
Diphtheria antitoxin	Equine	Treatment of respiratory diphtheria
Tetanus immune globulin (TIG)	Equine, Human	Treatment of tetanus as PEP, for people not adequately immunized with tetanus toxoid
Botulinum antitoxin	Equine, Human	Treatment of botulism
Varicella-zoster immune globulin (VZIG)	Human	PEP for immunosuppressed contacts of acute cases or newborn contacts
Cytomegalovirus immune globulin (CMV-IG)	Human	PEP in hematopoietic stem cell and kidney transplant recipients
Rabies immunoglobulin (RIG)	Equine, Human	Treatment of rabies and PEP in people not previously immunized with rabies vaccine
Hepatitis B immunoglobulin (HBIG)	Human	PEP for percutaneous or mucosal or sexual exposure Newborn of mother with HBsAg +ve
Hepatitis A immunoglobulin (HAIG)	Human	Postexposure prophylaxis: For family contacts and travelers
Rubella	Human	Women exposed during early pregnancy
Measles	Human	Infants or immunosuppressed contacts of acute cases exposed <6 days previously
Rh-isoimmunization (RhIG)	Human	Treatment of Rh-ve mother following delivery of a Rh +ve baby

Abbreviation: PEP, post-exposure prophylaxis.

EXPECTED QUESTIONS

I. Write short notes on:

1. Live vaccines vs. killed vaccines.
2. National Immunization Schedule.
3. Passive immunoprophylaxis.

II. Multiple Choice Questions (MCQs):

1. All of the following are live attenuated vaccines, **except:**

- a. MMR
- b. Yellow fever 17D
- c. Salk polio
- d. Sabin polio

2. All the following vaccines are given at birth, **except:**

- a. BCG
- b. Hepatitis B
- c. DPT
- d. OPV

Answers

1. c 2. c

March 16

National Vaccination Day



Prevention is better than cure.

The day is observed to enhance awareness for the eradication of polio from planet earth.



***Serving the Community
with the Gift of Immunity.***

Hospital Infection Control

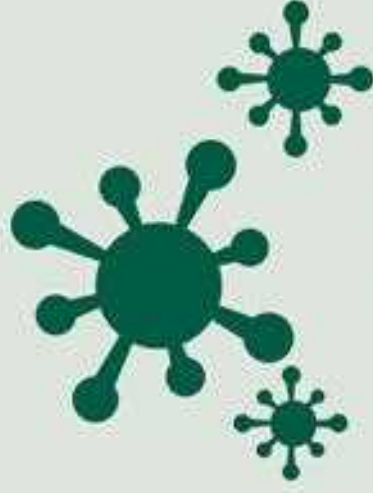
SECTION

3

SECTION OUTLINE

21. Healthcare-associated Infections
22. Major Healthcare-associated Infection Types
23. Sterilization and Disinfection
24. Biomedical Waste Management
25. Needle Stick Injury
26. Antimicrobial Stewardship
27. Environmental Surveillance
(Bacteriology of Water, Air and Surface)





INFECTION CONTROL

**The need of the hour,
Every healthcare personnel
must adhere to.**

CHAPTER PREVIEW

- Healthcare-associated Infections
- Major HAI Types
- Prevention of HAIs
 - Standard Precautions
- Transmission-based Precautions
- Hospital Infection Control Committee

INTRODUCTION

Definition

Healthcare-associated infections (HAIs) can be defined as—(i) the infections acquired in the hospital by a patient admitted for a reason other than the infection in context, (ii) the infection should not be present or incubating at the time of admission, and (iii) the symptoms should appear at least after 48 hours of admission. This also include:

- ❖ Infections that are acquired in the hospital but symptoms appear after discharge
- ❖ Occupational infections among staff of the healthcare facility (e.g. needle stick injury transmitted infections)
- ❖ Infection in a neonate that results while passage through the birth canal (in contrast to congenital infections due to transplacental transmission, which are not HAIs).

CDC (Centers for Disease Control and Prevention, Atlanta) has established the National Healthcare Safety Network (NHSN) to monitor the incidence of nosocomial infections.

As the site of healthcare facility has increasingly shifted from inpatient hospital care based service to the ambulatory setting, the relevance of traditional terminologies such as “hospital-associated or nosocomial” infections has diminished.

Burden of HAI

HAIs are one of the most common adverse events in the care delivery system. According to World Health Organization (WHO), on average at any given time 7% of patients in developed and 10% in developing countries acquire at least one HAI. Mortality from HAI occurs in about 10% of affected patients. Treatment of these HAIs adds a huge economic burden to the hospital.

Factors Affecting HAIs

The principal factors that determine the likelihood that a given patient would acquire a HAI are:

- ❖ **Immune status:** Most admitted patients have impaired immunity either as a part of their preexisting disease or in

some instances, due to the treatment they have received in the hospital

- ❖ **Hospital environment:** The hospital environment harbors a greater magnitude of microorganisms than that of the community. Transmission of these organisms to the patients can cause nosocomial outbreaks of infection
- ❖ **Hospital organisms:** Most of the organisms present in the hospital environment are multidrug-resistant. This is because of the increased antibiotic usage in the hospital. The minor population of resistant organisms present initially flourish in the presence of constant antibiotic pressure and slowly replace the susceptible strains in the hospital
- ❖ **Diagnostic or therapeutic interventions** such as insertion of a central line or urinary catheters, or endotracheal tube, may introduce infection in susceptible patients; most of which are due to the patient’s endogenous flora
- ❖ **Transfusion:** Blood, blood products and intravenous fluids used for transfusion, if not properly screened, can transmit many blood-borne infections (BBI) such as HIV, hepatitis B and C viruses
- ❖ **Poor hospital administration:** Strong administrative support is essential to control the HAIs; failing of which promote the spread of HAIs.

Sources of Infection

Endogenous Source

The majority of nosocomial infections are endogenous in origin, i.e. they involve patient’s own microbial flora which may invade the patient’s body during some surgical or instrumental manipulations.

Exogenous Source

Exogenous sources are from the hospital environment, healthcare workers (HCW), or patients.

- ❖ **Environmental sources** include inanimate objects, air, water and food in the hospital. Inanimate objects in the hospital are medical equipment (endoscopes, catheters, etc.), bedpans, surfaces contaminated by patients’ excretions, blood and body fluids

- ❖ **Healthcare workers** may be potential carriers, harboring many organisms; which may be multidrug-resistant, e.g. nasal carriers of Methicillin-resistant *Staphylococcus aureus* (MRSA)
- ❖ **Other patients** of the hospital may also be the source of infection.

Microorganisms Implicated in HAIs

HAIs can be caused by almost any microorganism, but those which survive in the hospital environment for long periods and develop resistance to antimicrobials and disinfectants are particularly important.

The ESKAPE pathogens: They are responsible for a substantial percentage of nosocomial infections in the modern era and represent the vast majority of multidrug resistant isolates present in a hospital.

- ❖ *Enterococcus faecium*
- ❖ *Staphylococcus aureus*
- ❖ *Klebsiella pneumoniae*
- ❖ *Acinetobacter baumannii*
- ❖ *Pseudomonas aeruginosa*
- ❖ *Enterobacter* species.

Other infections that can spread in hospitals include:

- ❖ *Escherichia coli*
- ❖ SARS-CoV-2 (COVID-19)
- ❖ Nosocomially-acquired *Mycobacterium tuberculosis*
- ❖ *Legionella pneumophila*
- ❖ *Candida albicans*
- ❖ *Clostridium difficile* diarrhea
- ❖ Blood-borne infections transmitted through needle prick injury or mucocutaneous exposure of blood includes HIV, hepatitis B and C viral infections.

Modes of Transmission

Microorganisms spread in the hospital through several modes such as contact, droplet and airborne transmissions. They are discussed subsequently in this chapter under transmission-based precautions.

MAJOR HAI TYPES

Though several types of HAIs exist, there are four most common types (listed below) which are often monitored to estimate the burden of HAI in a hospital. Out of these, the first three are together called as device associated infections (DAIs).

1. Catheter-associated urinary tract infection (CAUTI, 33%)
2. Central line-associated blood stream infection (CLABSI, 13%)
3. Ventilator-associated pneumonia (VAP, 15%)
4. Surgical site infection (SSI, 31%).

These major HAI types have been discussed in detail in Chapter 22.

PREVENTION OF HAIs

The preventive measures for HAIs can be broadly categorized into (i) standard precautions and (ii) transmission-based or specific precautions.

STANDARD PRECAUTIONS

Standard precautions are a set of infection control practices (see highlight box below) used to prevent transmission of diseases that can be acquired by contact with blood, body fluids, non-intact skin (including rashes), and mucous membranes. These measures should be followed when providing care to or handling:

- ❖ All individuals, whether they appear infectious/symptomatic or not
- ❖ All specimens (blood or body fluids) whether they appear infectious or not
- ❖ All needles and sharps whether they appear infectious or not.

Note: Universal precautions was a term used in the past to refer to the infection control practices to avoid contact with patients' body fluids, by means of wearing the nonporous articles such as medical gloves, goggles, and face shields. Now it is replaced by the word "standard precaution" which in addition include contact with all body fluids regardless of whether blood is present.

Standard Precautions

They are indicated while handling all patients, specimens and sharps. Components of standard precautions include:

- ❑ **Hand hygiene:** (details explained later)
 - Wash hands promptly after contact with infective material
 - Use no touch technique wherever possible.
- ❑ **Personal protective equipment (PPE):** described later
- ❑ **Biomedical waste:** All biomedical waste including sharp should be segregated and disposed appropriately (Chapter 24)
- ❑ **Spillage cleaning:** Clean up spills of infective material promptly
- ❑ **Disinfection of patient care items:** Ensure that all patient-care items such as instruments, devices and linens are disinfected before reuse
- ❑ **Environmental cleaning** of surface and floor (Chapter 23)
- ❑ **Sharp:** Safe use and disposal of sharp (Chapters 24 and 25)
- ❑ **Respiratory hygiene and cough etiquette.**

Hand Hygiene

Hands of the HCWs are the main source of transmission of infections in healthcare facilities. Hand hygiene is therefore the most important measure to avoid the transmission of harmful microbes and prevent healthcare-associated infections.

Types of Hand Hygiene Methods

Hand Rub

Alcohol based (70–80% ethyl alcohol) and chlorhexidine (0.5–4%) based hand rubs are available. The duration of contact has to be at least for 20–30 seconds.

- ❖ **Advantage:** After a period of contact, it gets evaporated of its own, hence drying of hands is not required separately
- ❖ **Indications:** Hand rub is indicated during routine patient care activities or taking rounds in the wards or ICUs—whenever opportunity for hand hygiene arises, except when the hands are visibly soiled with blood or other specimens.

Hand Wash

Antimicrobial soaps (liquid, gel or bars) are available containing 4% chlorhexidine. If facilities are not available, then even ordinary soap and water can also be used. The duration of contact has to be at least for 40–60 seconds. Hand washing is indicated in the following situations:

- ❖ When the hands are visibly soiled with blood, excreta, pus, etc.
- ❖ Before and after eating
- ❖ After going to toilet
- ❖ Before and after shift of the duty
- ❖ When giving care to a patient with diarrhea.

Surgical Hand Scrub (3–5 min): This is indicated prior to any surgical procedure and also in between the cases; using 4% chlorhexidine hand wash.

Indications (Five Moments for Hand Hygiene)

The WHO has published standard guidelines describing the situations or opportunities when hand hygiene is indicated in healthcare sectors (Fig. 21.1)—known as ‘My Five Moments for Hand Hygiene’; which include:

1. Before touching a patient
2. Before clean/aseptic procedures
3. After body fluid exposure/risk
4. After touching a patient
5. After touching patient’s surroundings.

Steps of Hand Rubbing and Hand Washing

WHO has also laid down the guidelines describing the appropriate steps involved for an effective hand rubbing and hand washing (Fig. 21.2).

Personal Protective Equipment (PPE)

Personal protective equipment are used to protect the skin and mucous membranes of HCWs from exposure to blood and/or body fluids and from the HCW to the patient during sterile and invasive procedures.

- ❖ The various PPE used in healthcare settings are gloves, mask/respirator, gown/plastic apron/coverall, goggles or face shield, shoe cover and head cover (Figs 21.3A to N)
- ❖ Selection of appropriate PPE is based on:



Fig. 21.1: My five moments for hand hygiene.

Source: World Health Organization (WHO) (with permission).

- The level of risk associated with contamination of skin, mucous membranes, and clothing by blood and body fluids during a specific patient care activity or intervention (as a part of standard precaution)
- Route of transmission of suspected organisms—contact, droplet and inhalation (as a part of transmission-based precaution).
- ❖ Though most PPE are used as a part of transmission-based precautions; there are few indications where some PPE are used as a part of standard precaution
- ❖ The PPE must be removed immediately following the indication for which it was used.

Gloves

Gloves can protect both patients and HCWs from exposure to microorganisms that have colonized their hands. It is used as part of standard, contact and droplet precautions.

Gloves should be worn only when there is an indication (Table 21.1). The use of gloves in situations when their use is not indicated represents a waste of resources and gives a false sense of security. Therefore gloves should not be used when not clinically indicated (Table 21.1).

Hand Hygiene and Glove Use

Glove is not a substitute for hand hygiene. In no way does the glove use modify hand hygiene indications or replace hand hygiene. The following measures should be adapted during gloves use.

- ❖ **Hand hygiene before gloves use:** This is to prevent possible cross-contamination of gloves with HCW’s flora
- ❖ **Hand wash after glove use:** To prevent cross-contamination, hands must be washed immediately after

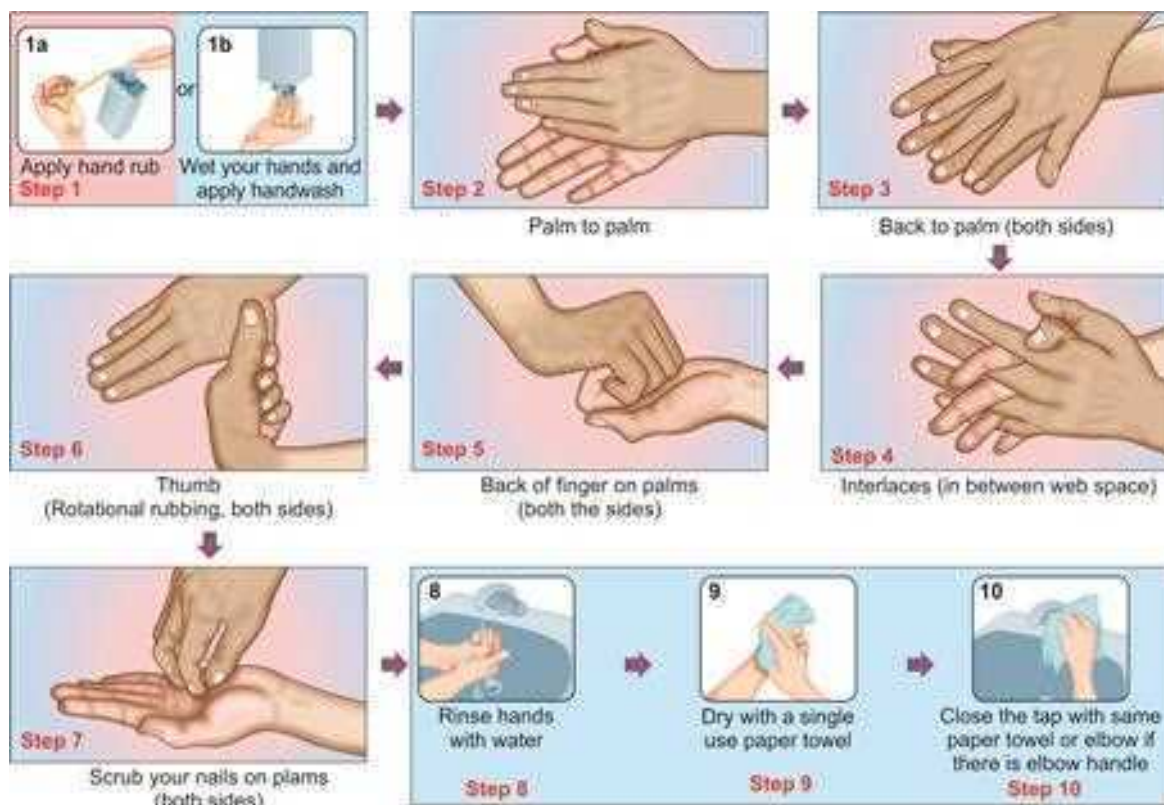


Fig. 21.2: Steps of hand rubbing and hand washing (WHO): Hand rub step 1 to 7 (20–40 seconds); Hand wash step 1 to 10 (40–60 seconds).

Table 21.1: Indications for appropriate use of glove use.
Indications for glove use
<ul style="list-style-type: none"> As a part of standard precautions <ul style="list-style-type: none"> Before a sterile procedure Anticipation of contact with blood or body fluid, regardless of the existence of sterile conditions and including contact with non-intact skin and mucous membrane As a part of contact precautions: Contact with a patient (and his/her immediate surroundings) Heavy duty gloves: To protect from sharp injuries, mainly used by biomedical waste handlers
Indications for glove removal
<ul style="list-style-type: none"> As soon as gloves are damaged Gloves are meant for single-use, must be changed in-between patients or patient care activities When there is an indication for hand hygiene
Clinical situations where use of gloves is not recommended
<ul style="list-style-type: none"> For routine patient care activities if there is no anticipated risk to blood/body fluid or no indication for contact precautions Examples: Measuring blood pressure, temperature, and pulse, while administering medications (oral or injections), during maintenance of IV cannula, during dressing and transporting patient, writing in the patient's case sheet, etc.

the removal of gloves as it creates a moist, warm, and occlusive environment between the skin and the glove which is 'safe haven' for microorganisms. Furthermore, microtears can occur in gloves which may lead to

transmission of organism if the HCW has had contact with blood or body fluid

- ❖ **Change:** Gloves should be worn for a single patient care activity and not beyond. Gloves must be changed between patient contacts and between separate procedures on the same patient
- ❖ **No hand hygiene over the gloved hand:** Gloved hands should neither be wiped with any form of handrub nor washed with soap and water.

The technique for donning and doffing of gloves has been depicted in Figures 21.4 and 21.5.

Surgical (3-ply) Mask and Respirators

Respiratory protection is essential when there is a risk of transmission of droplets and aerosols. There are two type of PPEs available for respiratory protection; surgical mask and respirators.

Surgical Mask (3-ply Mask)

Surgical masks (also called as medical mask or 3-ply mask) are loose fitting, single-use item that cover the nose and mouth.

- ❖ They are used as part of standard precautions to prevent splashes or sprays from reaching the mouth and nose of the person wearing them
- ❖ They also provide some protection from respiratory secretions and are worn when caring for patients on droplet precautions.



Figs 21.3A to N: Personal protective equipment (PPE): **A.** Gloves; **B.** heavy duty gloves; **C.** Surgical mask; **D.** N95 respirator; **E.** Plastic apron; **F.** Linen gown; **G.** Disposable gown; **H.** Coverall; **I.** Goggles; **J.** Face shield; **K.** Cap; **L.** Shoes; **M.** Gum boot; **N.** Shoe cover.

Composition

It has three layers (Fig. 21.3C):

- 1. Outer fluid repellent layer:** Hydrophobic layer that can repel water, blood and body fluids
- 2. Middle filter layer:** It is made up of melt-blown material; filters bacteria/viruses and also filters out the water droplets. In contrast to N95 respirator, the filter pore size of a surgical mask is not standardized



Fig. 21.4: Steps of gloves donning (wearing).

- 1.** Donning of the first glove: Wear by touching and pulling only the edge of the cuff.
- 2.** Donning of the second glove: Avoid touching the forearm skin by pulling external surface of second glove by the finger of gloved hand.

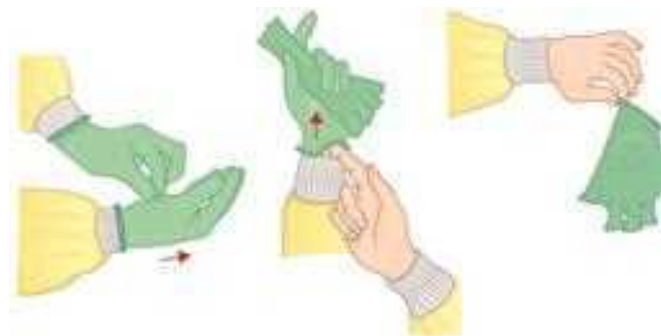


Fig. 21.5: Steps of gloves removal (doffing).

Do not touch the outside of the gloves (contaminated)

- Using a gloved hand, grasp the palm area of the other gloved hand peel off first glove.
- Hold removed glove in gloved hand slide fingers of ungloved hand under the other glove at wrist and peel off second glove over first glove.
- First glove will remain inside the pouch of the second glove.
- Perform hand hygiene after removal.

- 3. Inner hydrophilic layer:** Absorbs water, sweat and spit; made up of non-woven fabric.

Note: 2-ply masks may look similar to 3-ply mask in appearance. However, they have only two layers (outer and inner), but no middle filter layer. They should be used for hygienic and sanitation purposes—in restaurants, spa centers, food industry; but not in the hospitals.

Instructions

When using a surgical mask, the following measures should be considered:

- ❖ **Shelf-life:** Disposable (single-use); should be discarded or changed after 4-6 hours of use or earlier if it becomes soiled or wet
- ❖ **Donning:** Place the mask carefully, ensuring it covers the mouth and nose, adjust to the nose bridge, and tie it securely to minimize any gaps between the face and the mask
- ❖ **Hanging mask syndrome:** Masks should not be left dangling around the neck, a common practice observed



Fig. 21.6: Steps of mask donning (wearing).

- Pull the straps tight and pull the mask to below chin and then apply knots.
- Press on the nasal bridge part of the mask to seal tightly and for N95 respirator, perform fit check.



Fig. 21.7: Steps of mask removal (doffing).

- Do not touch front part of the mask.
- Untie the lower knot first, then the upper knot and remove the mask by holding its straps, without touching the front; hand wash after removal.

among doctors, doing so may contaminate the inner side of mask

- ❖ Touching the **front of the mask** while wearing should be avoided
- ❖ Mask should not be worn with beard or unshaven face
- ❖ Hand hygiene should be performed before donning the mask, upon touching or discarding a used mask.

The technique of donning and doffing of surgical mask has been depicted in Figures 21.6 and 21.7.

Respirator (N95 Respirator)

A respirator is a device designed to protect the wearer from airborne microorganisms (e.g. *M. tuberculosis*). There are many types of respirators. The most common respirator used in hospital settings is N95 respirator.

- ❖ N95 refers to 'not resistance to oil and ability to filter off 95% of airborne particles'
- ❖ **Composition:** The N95 respirator is comprised of four layers of material: an outer and inner layers of spun-bond polypropylene and middle two layers of cellulose/polyester, melt-blown polypropylene filter
- ❖ **Negative-pressure:** N95 respirators are described as "negative-pressure" because the pressure inside the facepiece is negative during inhalation compared to the pressure outside the respirator

- ❖ **Removal:** N95 respirator should be removed or changed once in 8 hours or earlier if it gets clogged, wet or dirty on the inside, or deformed, or torn
- ❖ **Single-use:** N95 respirator is for single-use only, should not be reused as it cannot be cleaned or disinfected
- ❖ **Fit checking:** After wearing the N95 respirator, the HCW must perform a fit check to ensure if it is properly fitted. No clinical activity should be undertaken until a satisfactory fit check has been achieved. It includes the following steps
 - **Sealing:** The respirator is compressed to ensure a seal across the face, cheeks and the nasal bridge
 - **The positive pressure seal** of the respirator is checked by gently exhaling. If air escapes, the respirator needs to be adjusted
 - **The negative pressure seal** of the respirator is checked by gently inhaling. If the respirator is not drawn in towards the face, or air leaks around the face seal, the respirator is readjusted.
- ❖ **Fit testing:** Fit testing is done to identify which size and style of N95 respirator is suitable for an individual and to train the HCW on how to don and doff N95 respirator. It should be done at the time of joining and thereafter annually.

Protective Body Clothing

Laboratory coats, plastic aprons, disposable gowns and coverall (full body cover) are examples of protective body wears used in hospitals. They are worn when there is a risk that clothing may become exposed to blood or body fluids

- ❖ **Laboratory coats:** They are used as a part of a standard precaution by all laboratory staff which protect their clothing and skin from the splash of blood or body fluid; however, they are not fluid resistant
- ❖ **Plastic aprons:** Worn when there is a low risk of contamination of blood/body fluid. They are fluid-resistant and for single-use only, i.e. used for one procedure or one patient care activity (Fig. 21.3E)
- ❖ **Disposable gowns:** They are long-sleeved, fluid resistant; indicated when there is a moderate risk of contamination with blood/body fluid (Fig. 21.3G)
- ❖ **Coverall:** It comprises of a gown with pant and hood, which covers the whole body including the head. Coverall should be used in the following situations
 - Anticipated risk of splashing with a large volume blood/body fluid (e.g. cardiac surgeries)
 - Anticipated risk of extensive skin to skin contact with a patient known to harbor organisms of contact transmission (e.g. lifting a patient with uncontrolled diarrhea)
 - Handling patients infected with pathogens of high mortality (e.g. Nipah or Ebola) or in the laboratory while handling their specimens (Fig. 21.3H).
- ❖ **Donning:** Gown should be fully covered, torso from neck to knees, arms to end of the wrist and then wrapped



Fig. 21.8: Steps of gown donning (wearing). Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back. Fasten it in the back of neck and waist.



Fig. 21.9: Steps of gown removal (doffing).

Do not touch front part of the gown

- Unfasten gown ties, taking care that sleeves don't touch the body when reaching for ties.
- Pull the gown away from neck and shoulders, touching inside of gown only.
- Turn gown inside out and roll into a bundle and discard.
- Perform hand hygiene after removal.

around the neck. It should be fastened in the back of neck and waist (Fig. 21.8)

- ❖ **Doffing:** Once the task is performed, the gown must be removed immediately after use by unfastening the gown ties taking care that sleeves should not contact the body while reaching for the ties (Fig. 21.9).

Protective Eye/Face Wear

Protective eyewear (goggles, or face-shields) are used to protect the mucous membranes of the eyes, nose, and mouth

- ❖ Prevents exposure to blood and/or body fluids that may be splashed, sprayed, or splattered into the face during clinical procedures
- ❖ Eyewear must be worn during procedures that are likely to generate droplets or aerosols of blood and/or high-risk body fluids (Figs 21.3I and J).

Head Cover and Shoe Cover

- ❖ **Head cover or cap** (Fig. 21.3K) is used when spillage of blood is suspected, e.g. during major cardiac surgeries, etc.

- ❖ **Shoe covers** include: (1) Surgical shoes (slippers) and shoe covers (Figs 21.3L and N): Used mainly in ICUs and operation theaters to protect HCWs from organisms present in floor and (2) Gumboots: Used for anticipated risk of sharp injuries (e.g. for biomedical waste handlers, laundry staff and housekeeping staff) (Fig. 21.3M).

Donning and Doffing

In order to minimize the risk of transmission of infection, donning (wearing) and doffing (removing) of PPE must be performed in a particular sequence.

Donning (wearing): Gown first → Mask or respirator → Goggles or face shield → Gloves

Doffing (removing): Gloves first → Face shield or goggles → Gown → Mask or respirator.

Doffing is extremely important as even a minor breach in the doffing procedure would subject the HCW to a huge risk of acquiring the infection. This could be a potential reason why many HCWs got infected during COVID-19 pandemic.

- ❖ All PPE should be removed just before exiting the patient room **except a respirator**, which should be removed after leaving the patient room and closing the door
- ❖ Discard into appropriate BMW bins:
 - Yellow bag: Gown/coverall, mask/respirator, shoe cover and cap
 - Red bag: Plastic apron, goggles/face shield, gloves.

Blood Spill Management

Spillage of blood and body fluid poses a substantial risk for the transmission of blood-borne viruses such as hepatitis B, C and HIV. Therefore, any spillage (small, few drops to large, few mL) should be considered infectious, and need to be cleaned at the earliest.

Steps of Spill Management (CDC)

The following steps need to be sequentially followed for the management of blood or body fluid spillage.

1. Any spillage, should be attended immediately
2. Mark the spill area, place the wet floor signage
3. Wear appropriate PPE (gloves and gown) as mentioned in the spill kit
4. Confine the spill and wipe immediately with an absorbent towel or cloth, which is spread over the spill to solidify the blood or body fluid. Then it is disposed as infectious waste
5. Clean with hypochlorite (freshly prepared)
 - For large spills (>10 cm size): Use 1:10 dilution of 5% hypochlorite (5000 ppm) i.e. 0.5%
 - For small spills (<10 cm size): Use 1:100 dilution of 5% hypochlorite (500 ppm), i.e. 0.05%
6. Allow the disinfectant to remain wet on the surface for at least a contact time of 10 min
7. Rinse the area with clean water to remove the disinfectant residue.

TRANSMISSION-BASED PRECAUTIONS

Definition

Transmission-based precautions (TBPs), also called as specific precautions are set of infection control practices which should be followed over and above the standard precautions.

- ❖ TBPs should be practiced when giving care for the patients who are infected with infectious agents having specific mode of transmissions such as contact, droplet and airborne
- ❖ Accordingly, there are three types of TBPs—contact precautions, droplet precautions and airborne precautions
- ❖ TBPs should be followed even when the specific infections are suspected and may be discontinued later when the diagnosis is ruled out.

Contact Precautions

Contact precaution should be followed when there is a definitive or suspected evidence of certain infectious agents that are transmitted by direct or indirect contact during patient care.

- ❖ **Direct transmission** occurs when infectious agents are transferred from one person to another person without a contaminated intermediate object or person. For example, direct contact through contaminated hands (most common mode of transmission of organism in healthcare settings) or direct contact with blood or body fluids from an infectious person
- ❖ **Indirect transmission** involves the transfer of an infectious agent through a contaminated intermediate object (clothes, patient-care devices, environmental surfaces, fomite) or person.

Agents Transmitted Through Contact

- ❑ MRSA (Methicillin resistant *S. aureus*)
- ❑ CRE (carbapenem resistant Enterobacteriaceae)
- ❑ VRE (vancomycin resistant enterococci)
- ❑ MDR nonfermenting gram-negative bacilli such as *Acinetobacter*, *Pseudomonas*, etc.
- ❑ Agents of conjunctivitis (e.g. adenovirus, gonococcus, *Chlamydia*)
- ❑ Any highly contagious skin lesions (abscess, impetigo, infected ulcers) infected with Group A *Streptococcus*, *Staphylococcus*, HSV lesions
- ❑ Skin infestations (e.g. scabies)
- ❑ Agents of diarrhea such as rotavirus, cholera, *C. difficile*
- ❑ Enterically transmitted hepatitis viruses (HAV and HEV).

Infection Control Measures

The following infection control measures should be applied in addition to other standard precaution measures.

- ❖ **Hand hygiene:** Strict adherence to hand hygiene is an absolute requirement of contact precaution as transmission via contaminated hands accounts for majority of contact transmission

- ❖ **PPEs:** Gloves and gown are the essential PPE that the HCW should wear upon entry to the patient-care area and must be removed before leaving the patient-care area. Surgical mask and protective eyewear are optional PPEs, needed if there is a risk of exposure to splashes or sprays of blood and body substances into the face and eyes
- ❖ **Equipment:** Single-use patient-dedicated equipment (e.g. blood pressure cuffs, stethoscopes, thermometers) must be used. If not possible, then the equipment should be cleaned and allowed to dry before use on another patient
- ❖ **Patient placement: Single isolation room** with a bathroom facility is preferred. If not available, then cohorting is recommended. **Cohorting** may be carried out in various ways
 - Patients with similar infections requiring contact precautions can be placed together either in the same isolation room, or in the same cubicle or corner of a ward or
 - Spatial separation of minimum of **3 feet distance** between the beds with privacy curtains.
- ❖ **Transfer of patients:** Patient movement should be limited only to medically-necessary purposes. When transport is necessary, the HCW must wear PPE before transport and the infected areas of the patient's body should be covered to contain the infection
- ❖ **Disinfection of the rooms:** Patient rooms must be frequently cleaned and disinfected adequately (e.g., at least daily and before use by another patient) focusing on frequently-touched surfaces and equipment in the immediate vicinity of the patient.

Droplet Precautions

Droplet precautions when used in addition to standard precautions are intended to prevent the spread of infectious agents that are transmitted through respiratory droplet via close respiratory or mucous membrane contact with respiratory secretions.

- ❖ Respiratory droplets are large-particles (>5 µm in size) that are generated by a patient who is coughing, sneezing or talking
- ❖ Transmission via large droplets requires close contact (<3 feet) as droplets do not remain suspended in the air and generally, only travel shorter distances
- ❖ Some infectious agents transmitted by droplet route can also be significantly transmitted by contact mode. This is because the larger droplets settle on the surfaces and inanimate objects within 1-meter distance, which subsequently spread to other individuals when they touch the contaminated surfaces and then touch their eyes, nose or mouth.

Agents Transmitted Through Droplets

- ❑ Diphtheria (pharyngeal)
- ❑ *Haemophilus influenzae* type b (pneumonia, meningitis)

Contd...

Contd...

- ❑ *Neisseria meningitidis*—meningitis, sepsis, pneumonia
- ❑ Pertussis (whooping cough)
- ❑ Pneumonic plague
- ❑ *Mycoplasma pneumoniae*
- ❑ Streptococcal (group A) pharyngitis
- ❑ Influenza viruses, seasonal
- ❑ SARS-CoV2 (COVID-19)
- ❑ Viral hemorrhagic fevers due to Lassa, Ebola, Marburg, Crimean Congo Fever viruses
- ❑ Other viruses: Mumps, Parvovirus B19, Rhinovirus, Rubella, Adenovirus

Infection Control Measures

The following infection control measures should be applied in addition to standard precautions.

1. Hand Hygiene

Droplet transmission is also associated with contact transmission (as discussed earlier). Therefore, hand hygiene is an important component of droplet precautions.

2. PPE

HCWs should wear a **surgical mask** when close contact (<3 feet) with the patient is anticipated and also upon room entry.

- ❖ Patients should wear a surgical mask (all the time)
- ❖ HCWs should wear protective eyewear if there is a risk of splashes or spray to eye/face. Gown and gloves should also be worn to prevent contact transmission
- ❖ The primary function of surgical mask is for '**source control**'; which prevents the transmission of droplets from the wearer to the environment. N95 respirator does not provide additional environmental protection and therefore, should not be used for this purpose
- ❖ Secondary function of the surgical mask is to protect the person wearing it from larger droplets in the environment
- ❖ **AGPs:** For certain diseases like seasonal influenza, viral hemorrhagic fever or COVID 19, the HCWs should wear N95 respirator during aerosol generating procedures (AGPs), described subsequently in this chapter.

3. Respiratory Hygiene/Cough Etiquette

The following measures are recommended for all individuals with respiratory symptoms (Fig. 21.10).

- ❖ Directly coughing or sneezing on hands or rubbing of the nose should be strictly avoided
- ❖ Mouth and nose should be covered with a tissue when coughing or sneezing. Tissues should be disposed into the yellow waste bins after use
- ❖ If no tissues are available, coughing or sneezing can be done into the inner elbow (sleeves), turning away from other patients
- ❖ Hand hygiene should be performed after having contact with respiratory secretions



Fig. 21.10: Respiratory hygiene/cough etiquette.

- ❖ Contaminated hands should be kept away from the mucous membranes of the eyes
- ❖ In outpatient settings, patients with respiratory symptoms should be segregated separately, provided with mask and attending the cases must be fast-tracked
- ❖ **Social distancing:** Individuals with respiratory symptoms should always maintain a distance of least 1 meter from others.

4. Patient Placement

A single room is preferred for patients who require droplet precautions. If is not available, alternative placement options can be looked for similar to contact precaution such as cohorting, spatial separation of >3 feet and drawing the curtain between patient beds.

5. Transfer of Patients

Transfer of patients on droplet precautions should be limited as there is a high-risk of transmission. If unavoidable, then the following precautions should be undertaken.

- ❖ The patient should wear a surgical mask while they are being transferred
- ❖ Patients should follow respiratory hygiene and cough etiquette
- ❖ HCW transporting the patient should wear surgical mask, gloves, gown and protective eyewear.

6. Disinfection of the Rooms

Patient-care items, bedside equipment, frequently touched surfaces area and environmental surfaces should be cleaned daily with appropriate disinfectants according to the hospital policy.

Airborne Precautions

Airborne precautions when used in addition to standard precautions are intended to prevent the spread of infectious agents that are transmitted through respiratory aerosols.

Aerosols are small-particles (<5µm) generated by an infectious person during coughing, sneezing, talking or performing certain aerosol-generating procedures (e.g. intubation). These smaller droplets remain suspended in air for long periods and may disperse to a distant place along with the air current.

Agents Transmitted Through Aerosols

- ❑ *Mycobacterium tuberculosis*
- ❑ Measles virus
- ❑ Varicella (chickenpox and zoster)
- ❑ Smallpox (variola) and monkeypox virus
- ❑ Aerosolizable spore-containing powders such as *Bacillus anthracis*
- ❑ *Aspergillus* (pulmonary aspergillosis)

Aerosol-generating Procedures (AGPs)

AGPs are procedures that can generate much higher concentrations of aerosols as compared to coughing, sneezing, or speaking and are associated with higher risk of pathogen transmission.

- ❖ Therefore, it is recommended to follow airborne precautions such as isolating the patient in negative pressure room and wearing appropriate PPEs like N95 respirator
- ❖ Examples of AGPs include: Endotracheal intubation, open respiratory and airway suctioning, tracheostomy care, cardiopulmonary resuscitation, sputum induction and bronchoscopy.

Infection Control Measures

A prudent approach is to implement infection control measures at the earliest based on clinical suspicion, and discontinue it later if the patient is subsequently diagnosed with a disease that does not require airborne precautions.

1. PPE

While giving care to a patient with airborne precaution, the HCWs must wear N95 or higher level respirator. The HCW must perform *fit checking* every time before donning the N95 respirator to ensure it is properly applied. Gloves, gown and protective eyewear may be needed if the exposure risk is likely to be present.

2. Patient Placement

Patients should be placed in an airborne infection isolation room (AIIR). The components of AIIR include: adequate ventilation, ultraviolet germicidal irradiation (UVGI) and filtration.

3. Ventilation

Ventilation can reduce the risk of infection through dilution and removal of room air containing infectious aerosols by

introduction of clean or fresh air into the room, either by natural or mechanical ventilation.

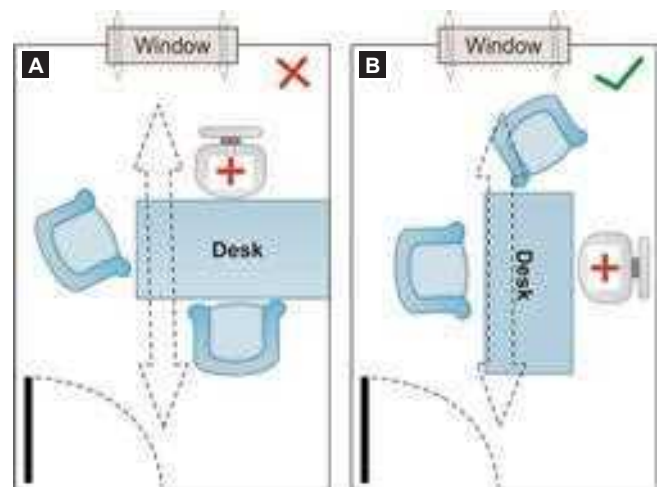
Natural Ventilation

Natural ventilation refers to the fresh air that enters and leaves a room through openings such as windows or doors.

- ❖ The effect of natural ventilation is maximized when the door and windows are placed at opposite to each other and are kept open to maintain airflow at all times
- ❖ In a consultation room with natural ventilation, the seating arrangement for patient and doctor should be made in such that doctor should sit away from the direction of natural airflow, thus has a lesser risk of exposure (Figs 21.11A and B)
- ❖ In ward set-up, the beds should be placed away from air flow (door-window direction) (Fig. 21.12).

Mechanical Ventilation (Negative-pressure Room)

Negative-pressure room includes a mechanical ventilation system which maintains the pressure of the room slightly lesser than the pressure of the entry area (i.e. creating a “negative pressure”), so that it allows air to flow into the isolation room but not escape from the room, as air naturally flows from areas with higher pressure to the areas



Figs 21.11A and B: Schematic diagram showing seating directions of patient and doctor in a consultation room.

- In room (A), the seating arrangement is along with direct of natural ventilation of air flow, so that the doctor has a higher risk of exposure to the potentially infected air.
- In room (B), doctor is sitting away from the direction of natural airflow, thus has a lesser risk of exposure.

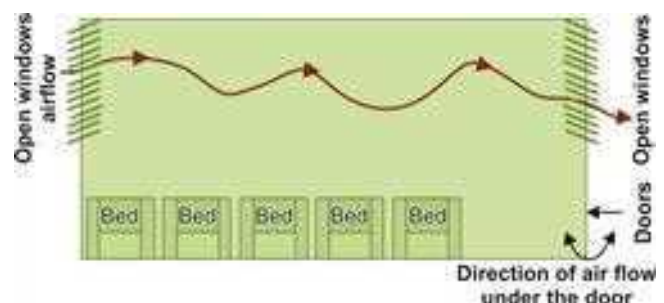


Fig. 21.12: Schematic diagram of a room with natural ventilation.

with lower pressure, thereby preventing contaminated air from escaping the room. The negative pressure room should have the following properties:

- ❖ **Air changes per hour (ACH):** Minimum 12 ACH should take place in the high-risk area, compared to 6 ACH per hour in a low-risk area for airborne transmission. High-risk areas include TB/chest department (outpatient and inpatient), bronchoscopy procedure rooms, MDR-TB wards and clinics, airborne precaution rooms
- ❖ **Negative pressure differential** between airflow from adjacent spaces to the patient room should be >2.5 Pascal
- ❖ **Door** should be kept closed at all times
- ❖ **Anteroom:** The negative pressure room should be preceded by an anteroom (a small outer waiting room which proceeds to the patient room).

4. Ultraviolet Germicidal Irradiation (UVGI)

If adequate ventilation is not possible, ultraviolet germicidal irradiation (UVGI) devices can be used as in addition to negative pressure ventilation. UVGI kills the organisms by irradiating. **UVGI** can be wall-mounted and should be installed at a higher level than (>8 feet from the floor) so that it will not directly irradiate on patients (Fig. 21.13).

5. Filtration

The room air directly exhausted to the outside through an exhaust fan or through HEPA (high efficiency particulate air) filtration. Exhaust fans must be properly installed closely fitting to the window (Figs 21.14A and B).

6. Transfer of Patients

The patient on airborne precaution should be transferred outside the negative pressure room only when it is absolutely necessary. In such a case, the following measures should be undertaken.

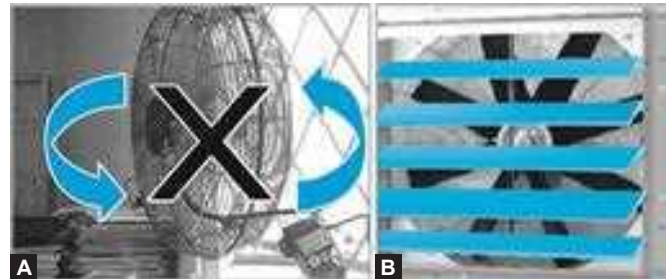
- ❖ The patient should wear a surgical mask and follow respiratory hygiene and cough etiquette
- ❖ Any skin lesions associated with the condition (e.g. chickenpox) should be covered
- ❖ The HCW must wear N95 respirator and other PPEs as indicated.

7. Respiratory Hygiene and Cough Etiquette

Patients must be explained in detail about cough hygiene as described under droplet precautions and Figure 21.10.



Fig. 21.13: Wall mounted ultraviolet germicidal irradiation.



Figs 21.14A and B: Exhaust fan: **A.** Poorly-installed in an open window space; **B.** well-installed, closely fitted.

8. Visitors and Staff

Entry of the visitors and staff should be absolutely restricted or they should wear PPE before entry into the room. The staff who are immune to the specific airborne disease (e.g. chickenpox) should preferably be posted for airborne precaution room whenever possible.

The infection control measures need to be taken for various standard and transmission-based precautions have been summarized in Table 21.2.

HOSPITAL INFECTION CONTROL COMMITTEE

The hospital infection control program is organized and run by the Medical Superintendent (MS), for which he/she constitutes the Hospital Infection Control Committee (HICC).

The HICC provides a forum for multidisciplinary input and cooperation, and information sharing, required for hospital infection control and prevention. The HICC is advisory to the MS and makes its recommendations to the MS.

HICC Constitution

The hospital infection control committee (HICC) should include wide representations from relevant departments/health sectors as follows:

- Chairperson, usually the Medical Superintendent
- Secretary, mostly the head of department of Microbiology
- Hospital Infection Control Officer (HICO), generally a representative from the department of Microbiology
- Hospital Infection Control Nurses (HICN)
- Head of all the clinical (all medical and surgical) departments
- Nursing Superintendent
- Head of the staff clinic
- Operation Room Supervisor
- In-charge of Central Sterile Supplies Department (CSSD)
- In-charge of biomedical waste management
- In-charge of pharmacy
- In-charge of hospital linen and laundry
- In-charge of hospital kitchen
- Epidemiologist
- In-charge of engineering department of hospital.

Functions of HICC

The HICC supervises the implementation of the hospital infection control program. The various functions of the committee include:

Table 21.2: Measures be followed during standard and transmission-based precautions.

Type	Isolation room or cohorting	Hand hygiene	Gloves	Apron or gown	Mask	Eye protection	Handling of equipment	Visitors
Standard	Not required	Yes	As required*	If soiling likely [#]	As required [§]	As required [§]	Single use or reprocessed	No additional precautions
Contact	Essential	Yes	Essential	Essential	As required [§]	As required [§]	Same as standard	Same precautions as for HCWs
Droplet	Essential	Yes	As required*	If soiling likely [#]	Surgical mask is essential	As required [§]	Same as standard	Restricted. Precautions, same as for HCWs
Airborne	Essential (negative pressure)	Yes	As required*	If soiling likely [#]	N95 respirator is essential	As required [§]	Same as contact	Same as for droplet

*Gloves are used when there is likely exposure to blood, body fluids and contaminated items.

[§]Mask or eye protection is required during procedures likely to generate droplets or anticipated splash of specimens on face respectively.

[#]Soiling is likely to occur during procedures that are expected to generate contamination from blood and body fluids.

Environmental cleaning with an appropriate disinfectant is required for all type of precautions.

- ❖ **HAI surveillance:** Maintains surveillance of hospital-acquired infections. The four key parameters used for HAI surveillance are as follows (refer chapter 22):
 1. CA-UTI (Catheter-associated urinary tract infection)
 2. CLABSI (Central line-associated bloodstream infection)
 3. VAP (Ventilator-associated pneumonia)
 4. SSI (Surgical site infection).
- ❖ **Develops a system** for identifying, reporting, analyzing, investigating and controlling healthcare-associated infections
- ❖ **Antimicrobial stewardship program (AMSP):** Develops antibiotic policies, monitors the antibiotic usage, advises the MS on matters related to the proper use of antibiotics, and also recommends remedial measures when antibiotic-resistant strains are detected (Refer Chapter 26 for detail)
- ❖ **Policies:** Reviews and updates on the hospital infection control policies and guidelines from time to time
- ❖ **Education:** Conducts teaching sessions for healthcare workers regarding matters related to HAIs
- ❖ **Staff health:** Monitors employee health activities regarding matters related to HAIs such as needle stick injury prevention, hepatitis B vaccination, etc.
- ❖ **Outbreak management:** Develops strategies to identify infectious outbreaks, their source and implements preventive and corrective measures
- ❖ **Other departments:** Communicates and cooperates with other departments of the hospital with common interests such as:
 - Pharmacy
 - Central Sterile Supplies Department (CSSD)
 - Linen and Laundry Department(s)
 - Antimicrobial Usage Committee
 - Biomedical Safety Committee
 - Blood Transfusion Committee.
- ❖ **Reviews** risks associated with new technologies, and monitor infectious risks of new devices and products, prior to their approval for use
- ❖ **HICC meetings:** HICC shall meet regularly not less than once a month and as often as required. However, in an emergency (such as an outbreak), this committee must be able to meet promptly as and when required.

EXPECTED QUESTIONS

I. Write short notes on:

1. Modes of transmission of healthcare-associated pathogens.
2. Prevention of healthcare-associated infections.
3. Hospital infection control committee (HICC).
4. Hand hygiene.
5. Standard precautions.

II. Multiple Choice Questions (MCQs):

1. **Hand rub should not be used in which condition?**
 - a. Before touching patient
 - b. After touching patient
 - c. After touching patient's surrounding

d. Hands are visibly soiled

2. **How many moments of hand hygiene have been laid down by WHO?**
 - a. 5
 - b. 6
 - c. 7
 - d. 8
3. **Hand rub should be performed for minimum of how much duration?**
 - a. 20 seconds
 - b. 40 seconds
 - c. 60 seconds
 - d. 2 minutes
4. **Hand wash should be performed for minimum of how much duration?**
 - a. 20 seconds
 - b. 40 seconds
 - c. 60 seconds
 - d. 2 minutes

Answers

1. d 2. a 3. a 4. b

Major Healthcare-associated Infection Types

CHAPTER 22

CHAPTER PREVIEW

- Catheter-associated Urinary Tract Infection
- Catheter-related Bloodstream Infection
- Ventilator-associated Pneumonia
- Surgical Site Infection
- Prevention of Device-associated Infections
- HAI Surveillance

INTRODUCTION

Theoretically, any infection developing in a patient after two days of hospitalization can be labelled as healthcare-associated infection (HAI). Among them, there are four major types which are commonly encountered and therefore need to be discussed in detail. These are also the HAIs for which surveillance is recommended.

1. Catheter-associated urinary tract infection (CAUTI)
2. Catheter-related bloodstream infection (CRBSI)
3. Ventilator-associated pneumonia (VAP)
4. Surgical site infection (SSI).

Out of these, the first three (CAUTI, CRBSI, VAP) are together called as device associated infections (DAIs).

CATHETER-ASSOCIATED URINARY TRACT INFECTION (CAUTI)

CAUTI is considered as the most common HAI worldwide, accounting for up to 40% of nosocomial infections. About 70–80% of healthcare-associated UTI are attributable to the presence of an indwelling urinary catheter.

Definitions

Catheter-associated bacteriuria (CA-bacteriuria) has been defined as presence of significant bacteriuria in a catheterized patient. It can be classified as:

- ❖ Catheter-associated UTI (CAUTI): CA-bacteriuria with symptoms or signs referable to the urinary tract
- ❖ Catheter-associated asymptomatic bacteriuria (CA-ASB): CA-bacteriuria without symptoms or signs referable to the urinary tract.

Epidemiology

Approximately 15 to 25% of hospitalized patients require urinary catheterization at some time during their hospital stay.

- ❖ The risk of developing CA-bacteriuria increases with time with an average risk of 3–10% per catheter day, which

may rise up to 25% at end of one week to nearly all cases (100%) in one month. However, only a fraction (less than a quarter) of these cases progress to CAUTI

- ❖ The CAUTI rate varies from 0 to 5 per 1000 catheterized depending upon the hospital location (ward vs ICU).

Microbiology

A broad range of bacteria can cause CAUTI, most of which are multidrug resistant.

In short-term catheterized patients: Most CAUTI are caused by the monomicrobial pathogens such as gram-negative bacilli or enterococci.

- ❖ *E. coli* is the predominant agent, although it is not as prevalent as in community-associated UTI
- ❖ Other gram-negative bacilli such as *Klebsiella*, *Pseudomonas* and *Acinetobacter* and gram-positive cocci such as *Enterococcus* account for most of the other infections.

In long-term catheterized patients, CAUTI is usually polymicrobial. In addition to the pathogens of short-term catheterization, other organisms such as *Proteus*, *Providencia* and *Morganella* are also encountered.

Pathogenesis

There are four main entry points through which the microorganism may reach the bladder in a catheterized patient (Fig. 22.1). The risk factors for the development of CAUTI is depicted in Table 22.1. Microorganisms may ascend to the urinary tract by either extraluminal or intraluminal surface of the catheter.

- ❖ **Extraluminal spread:** This accounts for two-third of cases
 - The source of the infection in catheterized patients may include patients' endogenous flora, hands of health care personnel, or inanimate objects
 - If asepsis is not maintained at the time of insertion or during maintenance of the catheter, there is always a risk of extraluminal migration of bacteria from one of these sources into the bladder

Table 22.1: Risk factors for CAUTI.

Device-related risk factors	Patient-related risk factors	Caregiver-related risk factors
<ul style="list-style-type: none"> • Duration: Long-term (≥ 30 days) catheterization has a higher risk than short-term (< 30 days) • Type of catheter material: Latex catheter has higher CAUTI risk (causes more urethritis, stricture formation, and obstruction) than silicone catheters 	<ul style="list-style-type: none"> • Female gender • Fatal underlying illness • Older age (> 50 years) • Diabetes mellitus • Poor personal hygiene • Incomplete emptying of bladder • Fecal incontinence 	<ul style="list-style-type: none"> • Failure in adherence to aseptic technique (and other care bundle components) both during insertion and maintenance of catheter • Emergency catheter insertion outside the operating room

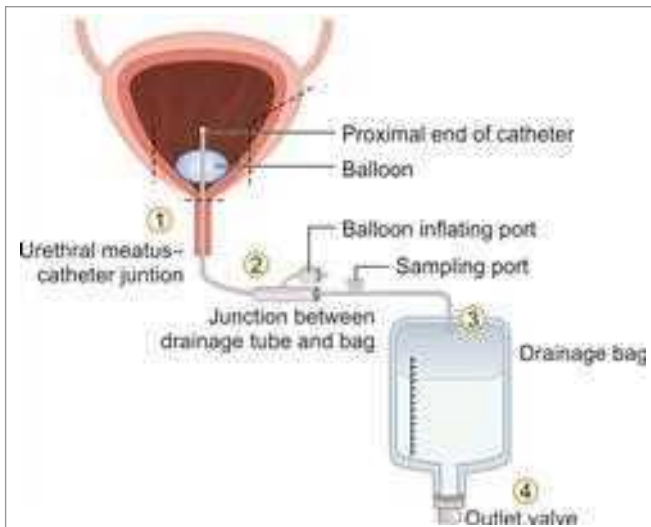


Fig. 22.1: Entry points through which the microorganism may reach the bladder in a catheterized patient.

- When a urinary catheter is inserted, the mechanism by which the urethral flora is constantly flushed out is interrupted. This helps in extraluminal migration of urethral and perineal flora into the bladder causing colonization and subsequent infection.
- ❖ **Intraluminal spread:** If the drainage bag is open type or when the closed drainage system is breached, there is a risk of reflux of contaminated urine from the drainage bag. This accounts for one-third of cases.

Why Indwelling Catheter is a Risk for UTI?

The presence of an urinary catheter is the single most important risk factor. The risk of developing CAUTI is directly proportional to the duration of catheterization.

- **Urethral pressure:** An indwelling catheter exerts lateral urethral pressure causing decreased mucosal blood flow, urothelial mucosal disruption and impaired mucin secretion; all together predispose to infection
- **Incomplete emptying:** In catheterized patients, bladder is often incompletely emptied because of pressure differentials created by patient movement or catheter manipulation. A small amount of urine always pools around the balloon which serves as a nidus for infection.

Laboratory Diagnosis

Urine should be collected through the catheter port using aseptic technique or, if a port is not present, by puncturing

the catheter tubing with a needle and syringe. Urine should never be collected from the urobag.

The clinical diagnosis of CAUTI is based on the following three criteria.

1. **Catheter criteria:** Catheterized or history of recent catheterization within 48 hours
2. **Clinical criteria:** Presence of at least one signs or symptoms of UTI such as fever, suprapubic tenderness, costovertebral angle pain, urinary urgency, frequency or dysuria (pain during micturition)
3. **Urine culture criteria:** Presence of significant bacteriuria, defined as colony count exceeding:
 - $\geq 10^3$ CFU/mL: in symptomatic patients
 - $\geq 10^5$ CFU/mL: in asymptomatic patients.

TREATMENT

CAUTI

Management of CAUTI includes removal of catheter and institution of appropriate antimicrobial therapy based on the susceptibility pattern of the organism isolated.

Treatment of asymptomatic bacteriuria (CA-ASB) is not recommended except when:

- Bacteriuria persists for > 48 hours after removal of the catheter
- In pregnancy, as there is 20–30 fold increased risk of developing pyelonephritis and risk of premature delivery with low birth weight
- Prior to traumatic urological procedures such as transurethral resection of prostate where mucosal bleeding is anticipated which may cause bacteremia.

Prevention of CAUTI is discussed later this chapter along with other major HAIs, under care bundle approach.

CATHETER-RELATED BLOODSTREAM INFECTION (CRBSI)

CRBSI refers to the development of bloodstream infections (BSI) in hospitalized patients which is attributed to the presence of a central line as a source of infection and is not associated with any other secondary cause of BSI. There is another related terminology called **CLABSI** (central line-associated bloodstream infection), which is strictly used only for surveillance purpose.

Central Line or Central Venous Catheter

A central line (CL) is an intravascular device that terminates in the great vessels. It is needed for various purposes such as central venous pressure monitoring and administration of

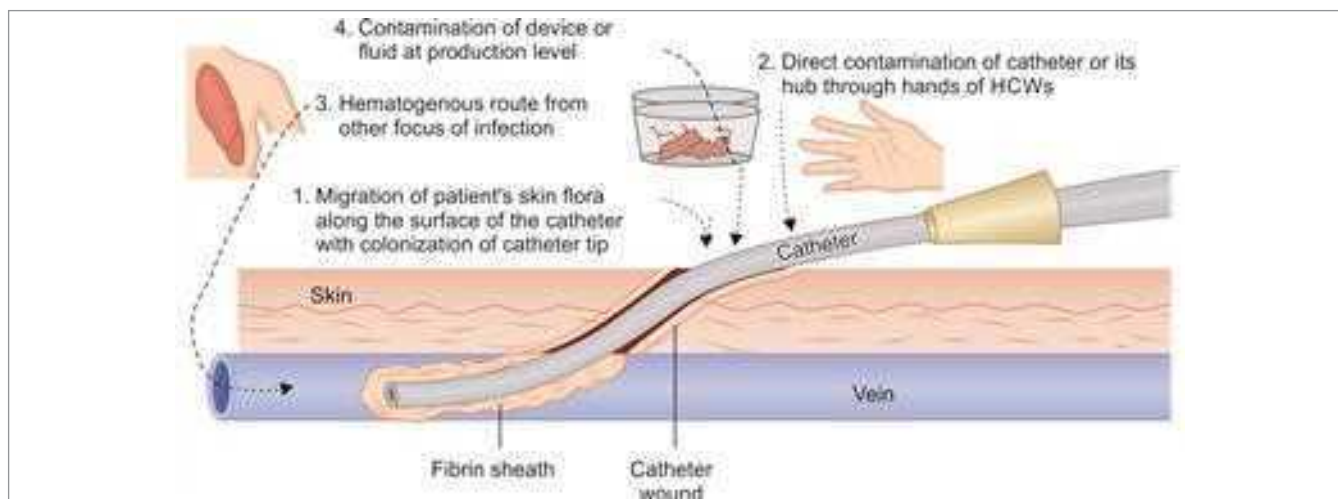


Fig. 22.2: Routes of access of organisms to central line.

Table 22.2: Risk factors for CRBSI.

Device-related	Patient-related	Caregiver-related
<ul style="list-style-type: none"> • Duration of central line (CL): Longer duration (≥ 72 hrs) has a higher risk than shorter duration (< 72 hrs) • Site: Femoral vein CL has higher risk than jugular vein and subclavian vein • Catheter type: Non-tunneled catheters have higher risk than tunneled catheters • Number of lumens: Multi-lumen CLs have a higher risk than single-lumen • Insertion circumstances: Emergency insertion has a higher risk than elective insertion 	<ul style="list-style-type: none"> • Immunodeficiency • Severe underlying illness • Hematologic malignancy • Loss of skin integrity (e.g. burns, psoriasis) • Presence of distant infection • Alteration in the patient's cutaneous microflora 	<ul style="list-style-type: none"> • Poor hand hygiene • Lack of infection control practices (e.g. care bundle) • Skin antisepsis: Use of alcohol has a higher risk than chlorhexidine

drugs, total parenteral nutrition, etc. and for hemodialysis access (hemodialysis catheters)

Central line can be classified in various ways depending upon:

- ❖ Its intended life span, e.g. temporary or short-term (< 72 hrs) versus permanent or long-term (≥ 72 hrs)
- ❖ Its site of insertion (e.g. subclavian, femoral, internal jugular and peripheral veins)
- ❖ Its pathway from skin to great vessel (e.g. tunneled versus non-tunneled).

Epidemiology

Approximately $< 3\%$ of hospitalized patients require central line at some time during their stay, out of which 3-8 % develop CLABSI. The CLABSI rate varies from 0% to 2.9 % depending upon the location (wards or ICUs).

Pathogenesis

There are several routes by which the organisms gain access to the extraluminal or intraluminal surface of the CVC as given below in the decreasing order of frequency (Fig. 22.2).

1. Migration of patient's skin flora along the surface of the catheter with colonization of catheter tip
2. Direct contamination of the catheter or its hub through the hands of healthcare workers (HCWs)

3. Hematogenous route from other focus of infection
4. Contamination of the device or fluid at the production level.

There are various risk factors associated with pathogenesis of CRBSI, described in Table 22.2. The source of infection may be intraluminal (contamination occurs during device or fluid production) or extraluminal (contamination at the time of insertion) (Table 22.3). Following events take place after the entry of the organism into the CL.

- ❖ Foreign body reaction, around the catheter insertion site
- ❖ **Colonization** of the organism by microbial adherence
- ❖ **Biofilm formation on catheter surface:** This is observed with many organisms such as coagulase-negative

Table 22.3: Intrinsic and extrinsic contamination of central line.

Intrinsic contamination (intraluminal source)	Extrinsic contamination (extraluminal source)
Contamination during device or fluid production	Contamination at the time of insertion
Due to defect during manufacture May cause outbreaks	Poor sterile precautions during drug or IV fluid admixture
Most common causative agents include <i>Klebsiella</i> , <i>Enterobacter</i> or <i>Pseudomonas</i>	Most common causative agents include skin commensals like CoNS and <i>S. aureus</i>

Abbreviation: CoNS, coagulase negative staphylococci.

staphylococci, *S. aureus*, *Pseudomonas aeruginosa*, and *Candida* species.

Diagnosis of CRBSI

The diagnosis of CRBSI is established when a patient on CL meets the clinical criteria and microbiological criteria; in the absence of evidence of other sources of BSI.

- ❖ **Clinical criteria:** Presence of fever, chills, rigor or hypotension after the insertion of CL and/or signs of catheter site infection such as erythema, tenderness, warmth, swelling at the catheter exit site
- ❖ **Microbiological criteria:** Simultaneous blood culture from CL and peripheral line (PL) is carried out and the CL blood culture bottle flags ≥ 2 hrs earlier to peripheral line blood culture (i.e. differential time to positivity ≥ 2 hrs).

TREATMENT

CRBSI

Treatment of CRBSI consists of institution of appropriate systematic antimicrobial therapy and removal of the central line.

- **Systematic antimicrobial therapy (SAT):** The empirical therapy should be started as soon as the clinical suspicion is made, which should be modified later based on susceptibility report
- **Antibiotic lock therapy (ALT):** In situations where salvage of the catheter is considered (e.g. infection with CoNS, those with limited venous access and a history of recurrent CLABSIs), ALT is given along with SAT. It involves instillation of a highly concentrated antibiotic solution into the CL lumen and is left to dwell within the lumen for a short period.

Prevention of CRBSI is discussed later in this chapter along with other major HAIs, under care bundle approach.

VENTILATOR-ASSOCIATED PNEUMONIA

Ventilator-associated pneumonia (VAP) is the second most common nosocomial infection (after CAUTI) and accounts for 15–20% of the total HAIs.

- ❖ It is the most common cause of death among HAIs, with a mortality rate of up to 40% and is the primary cause of death in ICUs
- ❖ The VAP rate varies from 1.0 to 46.0 per 1000 mechanical ventilation (MV) days, depending up on the ICU facility and the hospital.

Microbiology

VAP can be divided into early- and late-onset.

- ❖ **Early-onset VAP:** It occurs during the first 4 days of mechanical ventilation. It is caused by typical community organisms such as pneumococcus, *H. influenzae*, methicillin susceptible *S. aureus* (MSSA), etc.
- ❖ **Late-onset VAP:** It develops ≥ 5 days after mechanical ventilation and is commonly caused by typical multidrug resistant hospital pathogens—*P. aeruginosa*, *Acinetobacter baumannii*, *E.coli*, *Klebsiella* and methicillin resistant *S. aureus* (MRSA). It is associated with high attributable mortality. Here, the source of infection may be:
 - **Endogenous**, i.e. patient's own oropharyngeal microbial flora transmitted to lungs by aspiration
 - **Exogenous**, e.g. hospital environmental sources like air, water, reusable equipment, nebulized medication, etc. contaminated with environmental organisms.

Pathogenesis and Risk Factors

The pathogenesis of VAP involves a complex interplay between various risk factors (Table 22.4).

- ❖ **Colonization:** Following hospitalization of critically-ill patients, the normal oropharyngeal flora (e.g. viridans streptococci, *Haemophilus*, anaerobes) rapidly shifts toward “hospital-associated” pathogens such as *Pseudomonas*, *Acinetobacter* species, etc.
- ❖ **Endotracheal (ET) intubation** is the most important risk factor. It disrupts normal ciliary clearance of bronchial secretions, inhibits the cough reflex, damages the respiratory epithelium, and helps oropharyngeal bacteria to gain access directly into the lower respiratory tract
- ❖ **Biofilm:** The organism begins to form biofilm both inside and outside the endotracheal tube within a day of placement, which acts as a reservoir of infection, preventing the entry of antimicrobials and the host immune system
- ❖ **Subglottic secretions:** Secretions pool on and above the ET tube cuff and intermittently seep (*microaspiration*) to the lower respiratory tract, particularly if the cuff is underinflated or gets shifted during patient movement (Fig. 22.3). This can be prevented by:

Table 22.4: Risk factors for the development of VAP.

Device- or intervention-related	Patient-related	Healthcare personnel-related
Device-related <ul style="list-style-type: none"> • Duration of ventilation • Nasogastric tube • Frequent changes of ventilator circuit • Failed subglottic aspiration • Intra-cuff pressure < 20 cm of H₂O 	<ul style="list-style-type: none"> • Advanced age (> 60 years) • Prior hospitalization • Patient position: Supine position • Critically-ill with comorbidities • Underlying condition such as COPD, ARDS, head trauma • Patients with coma • Immobilization • Thermal injury (burns) 	<ul style="list-style-type: none"> • Improper adherence to aseptic techniques specially hand washing • Contaminated environmental sources
Intervention-related <ul style="list-style-type: none"> • Use of antibiotics or sedatives • Stress ulcer prophylaxis • Tracheostomy 		

Abbreviations: COPD, chronic obstructive pulmonary disease; ARDS, adult respiratory distress syndrome.

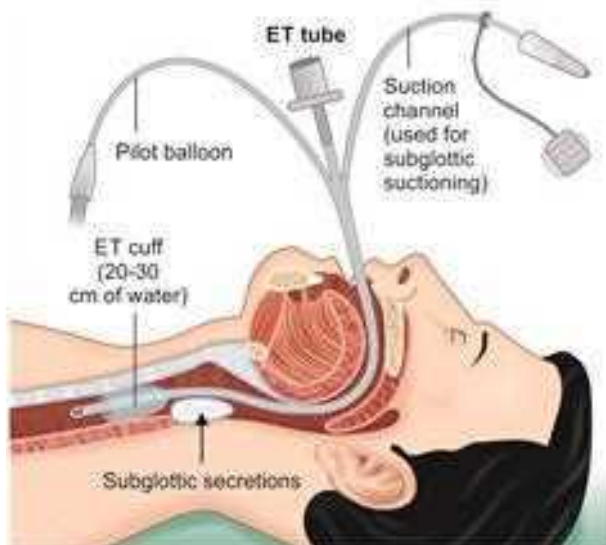


Fig. 22.3: Endotracheal (ET) tube with suction.

- Maintaining the cuff pressure at 20–30 cm of H₂O
- Subglottic suctioning should be done regularly to remove the pooling of secretion above the cuff.
- ❖ **Sedation:** Sedation, coma or unconsciousness inhibits the natural ability to clear secretions and thereby increases the risk of aspiration
- ❖ **Supine position** facilitates microaspiration. Therefore, patients should be put on a semi-recumbent position (30–45°)
- ❖ **Nasogastric tubes:** Ventilated patients are very often kept on nasogastric tubes, which disrupt the lower esophageal sphincter and increase the risk of aspiration of gastric contents
- ❖ **Critical illness with comorbidities,** poor nutrition and immobilization may increase patients' susceptibility to infection
- ❖ **Stress ulcer prophylaxis:** Intubated patients are at high-risk for stress ulcers, which may lead to upper gastrointestinal hemorrhage. Therefore, stress ulcer prophylaxis is a common practice in ventilated patients. However, this itself is a risk factor for aspiration

pneumonia. The only acceptable prophylaxis is by sucralfate, which is associated with lower risk of VAP.

Diagnosis

The diagnosis of VAP is based on a combination of clinical, radiological, and microbiological criteria.

Till date, there is no gold standard criteria available which can define VAP accurately. The most popular and widely used criteria is CPIS system.

Clinical pulmonary infection score (CPIS) system is a scoring system, based on six parameters (clinical, radiological and microbiological) with each parameter given a score scale ranging from 0 to 2 (Table 22.5).

- ❖ The maximum score that can be obtained is 12 and a score >6 is diagnostic of VAP
- ❖ CPIS score is prone to significant inter-observer variability, mainly in the interpretation of the tracheal secretions and the chest X-ray.

Microbiological Criteria

The specimens for VAP include endotracheal aspirate (most common), bronchoalveolar lavage (BAL), protected specimen brush (PSB) or lung biopsy. Specimens should be processed immediately. Delay of no more than 2 hours is permissible.

- ❖ **Gram staining:** Gram stain should be performed from the mucopurulent part. The diagnosis of VAP is likely if Gram staining demonstrates—higher numbers of bacteria, intracellular bacteria or presence of fibrin strands. A negative Gram stain result suggests that VAP is unlikely
- ❖ **Culture:** The specimens are subjected to either quantitative or semi-quantitative culture
 - Quantitative culture: Considered significant if the colony count exceeds $\geq 10^5$ CFU/mL for endotracheal aspirate, $\geq 10^4$ CFU/mL for BAL and $\geq 10^3$ /mL for PSB
 - Semi quantitative culture: Moderate to heavy growth is suggestive of colony count of $\geq 10^5$ CFU/mL.

Radiological Criteria

Radiological diagnosis of VAP is highly subjective as many other clinical conditions may show similar findings. In

Table 22.5: Modified Clinical Pulmonary Infection Score (CPIS) used for ventilator-associated pneumonia.

CPIS points	0	1	2
Temperature (°C)	$\geq 36.5^\circ\text{C}$ and $\leq 38.4^\circ\text{C}$	$\geq 38.5^\circ\text{C}$ and $\leq 38.9^\circ\text{C}$	$\geq 39^\circ\text{C}$ or $\leq 36^\circ\text{C}$
Leukocyte count (per mm ³)	4,000–11,000	<4,000 or >11,000	<4,000 or >11,000 + band forms $\geq 50\%$
Tracheal secretions	Rare	Nonpurulent	Abundant + purulent
Oxygenation PaO ₂ /FiO ₂ mm Hg	>240 with ARDS	-	≤ 240 and no ARDS
Chest radiograph	No infiltrate	Diffuse or patchy infiltrate	Localized infiltrate
Tracheal aspirate culture report	Light growth or no growth	Moderate or heavy growth of pathogenic bacteria	Moderate or heavy growth of bacteria and presence of bacteria with similar morphology on Gram stain

Abbreviations: ARDS, acute respiratory distress syndrome; FiO₂, fraction of inspired oxygen; PaO₂, arterial partial pressure of oxygen.

general, the most accepted radiological criteria is chest X-ray or CT scan showing one of the following—infiltrate, consolidation or cavitation, in the absence of underlying pulmonary or cardiac disease.

TREATMENT

VAP

Treatment of VAP consists of institution of empirical antimicrobial therapy once the clinical diagnosis of VAP is made, which can be modified subsequently based on antimicrobial susceptibility report.

- Empirical regimen should include a combination of antimicrobial agents active against *S. aureus*, *Pseudomonas* and other gram-negative bacilli
- The choice of empirical regimen should be based on local antimicrobial resistance pattern of the hospital.

Prevention of VAP is discussed later this chapter along with other major HAIs, under care bundle approach.

SURGICAL SITE INFECTION

Surgical site infections (SSI) are defined as infections that develop at the surgical site within 30 days of surgery (or within 90 days for some surgeries such as breast, cardiac and joint surgeries including implants).

- ❖ SSIs can cause significant morbidity and mortality as well as economic burden if left untreated
- ❖ SSI affects up to one third of patients who have undergone a surgical procedure, incidence is higher following abdominal operations
- ❖ In India, several studies reported SSI rate ranging from 4 to 11 per 100 surgeries.

Microbiology

The type of etiological agents implicated in SSI depends upon the site of surgical procedure and the source of infection from which they are acquired.

- ❖ **Endogenous source** such as the patient's own flora present on
 - **Skin:** *S. aureus* (the most common organism causing SSI), coagulase negative staphylococci (CoNS)
 - **Mucosa** (from opened viscus such as GIT, respiratory or genitourinary): Consists of predominantly aerobic gram-negative bacilli (*E.coli*, *Klebsiella*), gram-positive cocci (*Enterococcus*) and anaerobes such *Bacteroides*, and others.
- ❖ **Exogenous source** from contact with the operative room personnel or instruments or environment: *S. aureus* and gram-negative bacilli including nonfermenters such as *Pseudomonas* and *Acinetobacter*.

The inoculum load and the virulence of the microorganism can determine the risk of SSI

- ❖ **Inoculum of bacteria:** Surgical procedures involving the sites (e.g. bowel, vagina) which are heavily colonized with bacteria have a higher risk of developing SSI as large inoculum of bacteria lodge into the wound during surgery

- ❖ **Virulence of bacteria:** Higher is the virulence of infecting organism, more is the risk of development of SSI.

Pathogenesis and Risk Factors

In most patients, infection does not develop at surgical site due to the presence of strong host innate immunity eliminating microbial contaminants at the surgical site. However, when host defense mechanisms fail to eliminate the microbial contamination, compounded by the greater load and higher virulence of the invading microbes, all together pave path to the development of SSI.

The **risk factors** leading to the development of SSIs can be classified into patient-related, procedure-related, organism-related and environmental-related risk factors (Table 22.6); out of which the type of wound class is the most important, discussed below.

Wound Class Type

Depending up on the degree of microbial contamination, wounds are classified as clean, clean-contaminated, contaminated, or dirty/infected. The contaminated and dirty wound classes have a higher risk of developing SSI.

Class I, Clean Wound

It is an uninfected operative wound in which no inflammation is encountered and the hollow viscus such as respiratory, alimentary, genital, or urinary tract is not entered. SSI rate is usually less than 2% in clean operated wounds.

Class II, Clean-contaminated Wound

It is an operative wound in which the hollow viscus such as respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination.

Table 22.6: Risk factors for development of SSI.

Patient-related	Procedure-related
Age >60 years	Improper surgical scrub
Malnutrition, diabetes	Inadequate skin antisepsis
Immunosuppression	Prolonged operative time
Skin colonization at the time of surgery (e.g. MRSA carrier)	Inadequate antimicrobial prophylaxis
Duration of hospital stay	Poor perioperative glycemic control
Smoking, obesity	Emergency procedures
Higher wound class	Preoperative shaving
Organism-related	Environmental-related
Inoculum size (e.g. bowel surgery)	Presence of blood/ clot, suture material and foreign bodies at the surgical site
Bacterial virulence	Inadequate ventilation
Ability to form biofilm	Contaminated medications

- ❖ Surgeries included in this category involve the biliary tract, appendix, vagina, and oropharynx; provided that no evidence of infection or major breach in the technique is encountered
- ❖ SSI rate is about 3% to 11% in clean-contaminated wounds.

Class III, Contaminated Wound

Contaminated wound includes the following:

- ❖ Open, fresh, accidental wounds
- ❖ Operations with major breaks in the sterile technique (e.g. open cardiac massage) or
- ❖ Operations with gross spillage from the gastrointestinal tract (colonic surgeries)
- ❖ Entry into biliary or genitourinary tract in the presence of infected bile or urine
- ❖ Incisions in which acute, nonpurulent inflammation is encountered including necrotic tissue without evidence of purulent drainage (for example, dry gangrene).

SSI rate is >10% in contaminated wounds even with administration of surgical antimicrobial prophylaxis.

Class IV, Dirty/Infected Wound

Surgical procedures performed when active infection is already present are considered dirty wounds. Examples include:

- ❖ Abdominal exploration for acute bacterial peritonitis or perforated viscera
- ❖ Intra-abdominal abscess

- ❖ Old traumatic wounds with retained devitalized tissue. In dirty wound, the SSI rate can exceed 20–40%.

Classification and Diagnosis of SSI

SSIs are classified based on the level where infection developed into three types:

- ❖ **Superficial SSI**—develops at the level of superficial incisional site (skin and subcutaneous level) within 30 days regardless of the type of surgery
- ❖ **Deep SSI**—develops at the level of deep incisional site (muscle and fascial level) within 30 days for all surgeries except for breast, cardiac and implant surgeries (90 days)
- ❖ **Organ space SSI**—develops at the level of organ space site within 30 days for all surgeries except breast, cardiac and implant surgeries (90 days).

The criteria for diagnosis of above mentioned three types of SSIs have been discussed in detail under surveillance of SSI, subsequently in this chapter.

TREATMENT

SSI

Treatment of SSI includes suture removal plus incision and drainage with adjunctive systemic antimicrobial therapy.

Prevention of SSI

Preventive measures of SSI can be categorized into preoperative, perioperative and postoperative measures. Both WHO and CDC recently published the guidelines for prevention of SSI which has been summarized in Table 22.7.

Table 22.7: Prevention of surgical site infections (SSIs).

Preoperative measures

1. **Preoperative bathing:** It should be performed using a plain soap or an antimicrobial soap to reduce the bacterial load, especially at the site of incision
2. **For MRSA carriers:** Decolonization with mupirocin ointment must be done for patients undergoing surgery who are nasal carriers of MRSA
3. **Hair removal:** For patients undergoing any surgical procedure, hair removal should not be done or, if absolutely necessary, it should be removed only with a clipper. Shaving is strongly discouraged at all times

Intraoperative measures

1. **SAP:** Surgical antimicrobial prophylaxis (SAP) must be provided for all except for clean surgeries.
 - *Timing*—SAP must be administered within 60–120 minutes before incision, which usually coincides with the induction of anesthesia
 - *Choice*—It depends upon local antibiotic policy. Cefazolin or cefuroxime are usually preferred
 - *Frequency*—SAP is usually given as a single dose. Repeat dose may be required only for:
 - ◆ Duration of surgery exceeds 4 hours
 - ◆ Cardiac surgeries
 - ◆ Drugs with lower half-lives (redosing required if duration of surgery exceeds 2 half-lives)
 - ◆ Extensive blood loss during surgery
 - *For ESBL prevalent area*—SAP should not be modified. ESBL screening for patients is not routinely recommended.
2. **Surgical hand disinfection**—Scrubbing with either antimicrobial soap (chlorhexidine) or with alcohol-based hand rub must be performed before donning sterile gloves, before surgery and in between surgeries
3. **Surgical site preparation** should be performed with alcohol-based chlorhexidine antiseptic solution before the commencement of surgery
4. **Perioperative maintenance** of oxygenation (target FiO_2 80%), temperature (normothermia), blood glucose level (target level of <200 mg/dL), adequate circulating volume (normovolemia) and nutritional support are necessary during the surgery and immediate 4–6 hours postoperative period

Contd...

Contd...

Postoperative measures

1. **Wound dressing**—Daily dressing of surgical site and removal of any discharge present at the site must be performed. Perform hand hygiene and use gloves before dressing
2. **OT disinfection**—Thorough postoperative disinfection of operation theater must be performed with a high level disinfectant in between cases and after the last case (terminal disinfection)
3. **Periodic monitoring of the air quality of operation theater** for various parameters must be performed such as no. of air exchanges, temperature, humidity, pressure and microbial contamination
4. **SAP prolongation is not recommended** in any situations (e.g. presence of a wound drain) for the purpose of preventing SSI as it promotes development of antimicrobial resistance

Source: Adapted from WHO's Global guidelines on the prevention of surgical site infection, 2016 and CDC's Guideline for the prevention of surgical site infection, 2017.
Abbreviation: ESBL, extended spectrum beta lactamase.

PREVENTION OF DEVICE-ASSOCIATED INFECTIONS

The majority of device-associated infections (DAIs) encountered in hospital are CAUTI, CLABSI and VAP.

- ❖ Presence of device itself is a major risk factor for development of such infection. This is because of various reasons:
 - Risk of introduction of patient's own flora
 - Risk of introduction of HCW's hand flora due to improper handling during insertion or daily maintenance of the device
 - Ability of the invading organism to produce biofilm over the device.
- ❖ Strict aseptic techniques must be followed while insertion and daily maintenance of the devices

- ❖ The preventive measures for each of the DAIs are grouped as care bundle approach (described below).

Care Bundle Approach

Healthcare facilities must adhere to care bundle approach for the prevention of DAIs.

- ❖ Care bundle comprises of 3 to 5 evidence-based elements with strong clinician agreement; each of the components must be followed during the insertion or maintenance of the device
- ❖ Compliance to the care bundle is calculated as all-or-none way, i.e. failure of compliance to any of the components leads to non-compliance to the whole bundle
- ❖ The components of care bundle approach for prevention of DAIs have been described in Table 22.8.

Table 22.8: Care bundle approach for prevention of device-associated infections (DAIs).

Care bundle for urinary catheter	
Insertion bundle	Maintenance bundle
<ol style="list-style-type: none"> 1. Catheter should be inserted only when appropriate indication is present (e.g. acute urinary retention) 2. Only the sterile items are used for insertion of catheter 3. Catheter is inserted by non-touch technique with strict asepsis 4. Closed drainage system must be used 5. Catheter of appropriate size must be used 6. Catheter must be properly secured after placement (by plaster-tube-plaster technique) 	<ol style="list-style-type: none"> 1. Daily catheter care (vaginal or meatal care) must be given regularly and by strict aseptic measures such as hand hygiene and single use gloves 2. Catheter is properly secured all the time 3. Drainage bag must be always above the floor and below the bladder level 4. Closed drainage system is used all the time 5. While collection of urine from bag, the following steps must be followed — Hand hygiene, change of gloves between patients; use of separate jug for each bag, use of alcohol swabs for disinfection of outlet 6. Daily assessment of readiness for removal of catheter must be documented
Care bundle for central line	
Insertion bundle	Maintenance bundle
<ol style="list-style-type: none"> 1. Hand hygiene before and after insertion of central line 2. Use maximum sterile PPE: gloves, gown, drapes, cap and mask 3. Site of insertion—Subclavian preferred, avoid femoral 4. Skin preparation—by antiseptics such as chlorhexidine 5. Skin must be completely dry after use of antiseptics 6. Use semi-permeable dressing 7. Document data and time of insertion 	<ol style="list-style-type: none"> 1. Daily aseptic central line care during handling: <ul style="list-style-type: none"> ➢ Hand hygiene must be performed ➢ Hub decontamination by alcohol 2. Daily documentation of local signs of infection 3. Change of dressing with 2% chlorhexidine 4. Daily assessment of readiness for removal of central line must be documented
Maintenance care bundle for mechanical ventilator	
<ol style="list-style-type: none"> 1. Adherence to hand hygiene 2. Elevation of the head of the bed to 30–45°—this is to prevent oropharyngeal aspiration to respiratory tract 3. Daily oral care with chlorhexidine 2% solution 4. Need of PUD (peptic ulcer disease) prophylaxis should be assessed daily; if needed only sucralfate should be used 5. DVT (deep vein thrombosis) prophylaxis should be provided if needed 6. Daily assessment of readiness to remove mechanical ventilator must be documented 	

HAI SURVEILLANCE

Healthcare-associated infections (HAIs) surveillance is a system that monitors the HAIs in a hospital. Main objectives of HAI surveillance include:

- ❖ Provides endemic or baseline HAI rate and information on type of HAIs in the hospital
- ❖ Helps in comparing HAI rates within and between hospitals
- ❖ Identifies the problem area; based on which root cause analysis can be conducted to find out the breakdowns in infection control measures and then the appropriate corrective measures are implemented
- ❖ Provides timely feedback to the clinicians; thus, reinforces them to adopt best practices.

Targeted Surveillance

National Healthcare Safety Network (NHSN) division of CDC (centers for disease control and prevention) provides guidelines for the surveillance of HAIs.

- ❖ **Where to conduct:** HAI surveillance should be conducted only for high-risk locations such as intensive care units (ICUs)
- ❖ **What type of HAIs to be monitored:** As technically difficult, only the major types of HAIs can be monitored such as CAUTI, CLABSI, VAP and SSI
- ❖ **Who will conduct:** The infection control nurses (ICNs) under the supervision of the officer in-charge of HICC conduct HAI surveillance
- ❖ **HAI surveillance diagnostic criteria:** The NHSN has provided the diagnostic criteria for four major types of HAIs (Tables 22.9 to 22.12)
 - These criteria are made very objective so as to maintain the uniformity of data collection between hospitals which helps in accurate comparison of HAI rates between hospitals of same and different nations
 - The surveillance criteria are different from clinical and diagnostic criteria, and therefore these should strictly be used only for surveillance purpose, not for clinical diagnosis and treatment.

Method of Conducting HAI Surveillance

The HAI surveillance cycle consists of data collection → data analysis → data interpretation → data dissemination.

Device criteria	Presence of a urinary catheter for >2 days
Clinical criteria	Presence of any one symptom of UTI such as fever, suprapubic tenderness, urgency, frequency or dysuria
Culture criteria	Isolation of significant count ($\geq 10^5$ /mL) of a UTI pathogen from urine

- ❖ **Data Collection:** The infection control nurses (ICNs) visit daily to the high-risk areas (ICUs) and collect the clinical data of patients on devices (urinary catheter, central line, ventilator) and also patients admitted following surgeries. They also prospectively check the laboratory investigations to confirm a diagnosis

Table 22.10: NHSN Surveillance diagnostic criteria for CLABSI (Central line-associated bloodstream infection).

	Age	Blood culture criteria		Clinical criteria
		Organism isolated	No. of cultures positive	
LCBI-1	Any age	LCBI pathogen ¹	1	Symptoms not required
LCBI-2	<1 year	LCBI commensal ²	2	Any one symptom ³
LCBI-3	<1 year	LCBI commensal ²	2	Any one symptom ⁴

Device criteria = catheter present for > two calendar days
 LCBI *plus* catheter criteria met = called as CLABSI
 LCBI *without* catheter criteria met = called as non-CLABSI

LCBI- laboratory confirmed bloodstream infection.

¹LCBI pathogen, e.g. common healthcare-associated pathogens.

²LCBI commensal, e.g. coagulase negative staphylococci.

³LCBI-2 symptoms—fever, chills, hypotension.

⁴LCBI-3 symptoms—fever, hypothermia, bradycardia, apnea.

Table 22.11: NHSN surveillance diagnostic criteria for VAE (Ventilator associated events).

Stage-1: VAC (ventilator-associated condition)	
Device criteria	Presence of a mechanical ventilator at least for two calendar days
Worsening oxygenation criteria	<ul style="list-style-type: none"> • Baseline period during which the daily minimum FiO₂ (fraction of inspired oxygen) and PEEP (positive end-expiratory pressure) values are stable or decreasing for 2 days followed by • Period of worsening of oxygenation—increased FiO₂ (by $\geq 20\%$) or PEEP (≥ 3 cm water) for at least 2 consecutive days
Stage-2: IVAC (infection-related ventilator associated complications): VAC plus the following criteria	
Clinical criteria	Any one of the following: Fever or hypothermia or Leukocytosis or leukopenia
Antibiotic criteria	New antimicrobial agent started and continued for ≥ 4 days
Stage-3: PVAP (Possible ventilator-associated pneumonia): IVAC plus the culture criteria	
Culture criteria	Isolation of significant count of a pneumonia pathogen from respiratory specimens such as endotracheal aspirate, bronchoalveolar lavage, etc.

Table 22.12: NHSN surveillance diagnostic criteria for surgical site infection (SSI).**Definition and types of SSIs**

Definition: Surgical site infections (SSI) are defined as infections that develop at the surgical site within 30 days of surgery (within 90 days for breast, cardiac and joint surgeries)

SSIs are classified based on the level where infection is developed:

- *Superficial SSI:* Develops at the level of superficial incisional site (skin and subcutaneous level) within 30 days regardless of type of surgery
- *Deep SSI:* Develops at the level of deep incisional site (muscle and fascial level) within 30 days for all surgeries except breast, cardiac and implant surgeries (90 days)
- *Organ space SSI:* Develops at the level of organ space site within 30 days for all surgeries except breast, cardiac and implant surgeries (90 days)

One among the following must be met:

Clinical criteria	i. Presence of purulent pus from the corresponding level of surgical site or ii. Presence of local signs of infections (pain/tenderness, swelling, erythema, heat, etc.)
Culture criteria	Positive culture from the discharge collected at the corresponding level of surgical site
Other evidence	i. For superficial SSI—Surgeon's diagnosis is taken as diagnostic criteria ii. For deep or organ space SSI—histopathological, imaging or gross anatomical evidence of abscess should be present

❖ **Data Analysis:** The four types of HAIs are diagnosed according to HAI surveillance criteria of NHSN/CDC (Tables 22.9 to 22.12)

- Then the HAI rates are calculated as per the formulae given in Table 22.13
- Then the monthly report of location wise HAI rates of the hospital is generated.

❖ **Data Interpretation:** HAI rates are compared:

- For the same location across different time frames
- Between different locations of same or different hospital during same time frame.

❖ **Data Dissemination:** The monthly HAI surveillance report should be shared with all clinical departments and administrators. It is also presented during HICC meetings. Accordingly, the appropriate corrective actions are planned in the problem areas.

Table 22.13: Formulae of HAI infection rates.

HAI infection rates	Formulae
CAUTI Rate	No. of CAUTI cases/ total no. of urinary catheter days × 1000
CLABSI Rate	No. of CLABSI cases/ total no. of central line days × 1000
VAE Rate	No. of VAE cases/ total no. of ventilator days × 1000
SSI Rate	No. of SSI/No. of surgeries done × 100

EXPECTED QUESTIONS

I. Write short notes on:

1. HAI surveillance.
2. Care bundle approach for prevention of device-associated infections.
3. Prevention of surgical site infection.

II. Multiple Choice Questions (MCQs):

1. **Which parameter is not included in HAI surveillance?**
 - a. CAUTI (catheter-associated urinary tract infection)
 - b. CLABSI (central line-associated bloodstream infection)
 - c. VAP (ventilator-associated pneumonia)
 - d. Open wound infections
2. **For device-associated infection, the device should be present in place at least for how many calendar days?**
 - a. 1
 - b. 2
 - c. 3
 - d. 4
3. **Among the following ventilator associated events, which requires to meet the worsening oxygenation criteria?**

- a. VAC (ventilator-associated condition)
- b. IVAC (infection-related ventilator-associated complication)
- c. PVAP (possible ventilator-associated pneumonia)
- d. All of the above

4. Among the following ventilator-associated events, which requires culture to be positive?

- a. VAC (ventilator-associated condition)
- b. IVAC (infection-related ventilator associated complications)
- c. PVAP (possible ventilator-associated pneumonia)
- d. All of the above

5. A patient is admitted since 5 days. He develops fever after placement of a urinary catheter for >2 days. Urine culture revealed *Escherichia coli* ($\geq 10^5$ /mL). What is the surveillance diagnosis?

- a. Healthcare-associated CAUTI
- b. Healthcare-associated non-CAUTI
- c. Community-acquired CAUTI
- d. Community-acquired non-CAUTI

Answers

1. d 2. b 3. d 4. c 5. a

CHAPTER PREVIEW

- Definitions
- Sterilants
- High-level Disinfectants
- Intermediate-level Disinfectants
- Low-level Disinfectants
- Cleaning Agents
- Environmental Cleaning
- Methods to Test Efficacy of Sterilant/Disinfectant

INTRODUCTION

The sterilization and disinfection practices in a hospital is of paramount importance in preventing transmission of healthcare-associated infections.

Definitions

Sterilization, disinfection and cleaning are three separate but interrelated terminologies, all aiming at removing or destroying the microorganisms from materials or from body surfaces. However they vary in their efficacy of destroying the microorganisms (Table 23.1).

Sterilization

Sterilization is a process by which all living microorganisms including viable spores, are either destroyed or removed from an article, surface or medium

- ❖ Results in reduction of $\geq 10^6$ log colony forming units (CFU) of microorganisms and their spores
- ❖ The agents which achieve sterilization are called as sterilants (Table 23.2).

Disinfection

It refers to a process that destroys or removes most if not all pathogenic organisms but may or may not destroy bacterial spores.

- ❖ Results in reduction of $\geq 10^3$ log CFU of most microorganism but not spores
- ❖ Achieved by a physical agent or a chemical agent and are normally used only on inanimate objects, not on body surfaces
- ❖ The agents which achieve disinfection are called as disinfectants (Table 23.2).

Type of Disinfectants

Depending upon their efficacy, the disinfectants are further classified into three categories.

- ❖ **High-level disinfectants (HLD)** are capable of killing bacterial spores when used in sufficient concentration under suitable conditions. They can kill all other microorganisms
- ❖ **Intermediate-level disinfectants (ILD)** destroy all microorganisms, but not bacterial spores
- ❖ **Low-level disinfectants (LLD)** destroy vegetative bacteria and enveloped viruses; variable action on nonenveloped viruses, and fungi, but no action on tubercle bacilli and spores.

Note: **Antiseptics** are a type of disinfectants which are safe to apply on body surfaces (skin and mucosa) resulting in the destruction of organisms present on the body surfaces. This type of disinfection is termed as **asepsis**.

Cleaning (Decontamination)

Cleaning refers to the reduction in the pathogenic microbial population to a level at which items are considered as safe without protective attire

- ❖ Results in reduction of at least ≥ 1 log CFU of most of the microorganism but not spores
- ❖ Achieved by manual or mechanical cleaning by soap and detergents to eliminate debris or organic matter from the medical devices or surfaces (Table 23.2).

In a healthcare facility, most of the sterilization practices for surgical instrument and other critical care items are carried out in Central Sterile Supply Department (CSSD).

Table 23.1: Level of sterilant/ disinfectants according to their microbicidal action.

Level of disinfectant/sterilant	Bacterial spores	Tubercle bacilli	Non-enveloped viruses	Fungi	Vegetative bacteria	Enveloped viruses
Sterilant	Yes	Yes	Yes	Yes	Yes	Yes
Disinfectant						
High level	+/-	Yes	Yes	Yes	Yes	Yes
Intermediate level	No	Yes	Yes	Yes	Yes	Yes
Low level	No	No	+/-	+/-	Yes	Yes

Table 23.2: Agents used in the hospital for achieving sterilization, disinfection and cleaning.		
Agents	Physical methods	Chemical methods
Sterilants		
Agents of sterilization	<ul style="list-style-type: none"> • Steam sterilizer (autoclave) • Dry heat sterilizer (hot air oven) • Filtration • Radiation: Ionizing and non-ionizing (infrared) • Others: Incineration, microwave 	<ul style="list-style-type: none"> • Ethylene oxide (ETO) sterilizer • Plasma sterilizer
Disinfectants		
High-level disinfectants	No physical methods in this category	<ul style="list-style-type: none"> • Aldehydes- glutaraldehyde, orthophthaldehyde, formaldehyde • Peracetic acid • Hydrogen peroxide
Intermediate-level disinfectants	<ul style="list-style-type: none"> • Heat-based methods: Pasteurization, boiling and steaming • Ultraviolet (non-ionizing) radiation 	<ul style="list-style-type: none"> • Alcohols-ethyl alcohol and isopropyl alcohol • Phenolics-phenol, cresol, lysol • Halogens-iodine and chlorine
Low-level disinfectants	No physical methods in this category	<ul style="list-style-type: none"> • Quaternary ammonium compound (QAC) • Chlorhexidine
Cleaning		
Agents of cleaning	Automated washers such as ultrasonic washers, washer-disinfector and automated cart washers	<ul style="list-style-type: none"> • Enzymatic solution • Detergent • Soap (antimicrobial or plain soap)

Therefore, it is important to understand the workflow of CSSD.

Central Sterile Supply Department (CSSD)

CSSD is an integrated place in hospitals that performs sterilization of medical devices, equipment and consumables; that are used in the operating theater (OT) of the hospital and also for other aseptic procedures.

The processing area of CSSD consists of four unidirectional zones starting from an unsterile area to a sterile area separated by a physical barrier (Fig. 23.1).

Decontamination area → Packaging area → Sterilization area → Sterile storage area

1. **Decontamination area:** The items are collected and then decontaminated/cleaned by either manual wash or by automated machines (ultrasonic washer and washer-disinfector)
2. **Packaging area:** Here, the items (medical devices) are enclosed in materials or a container designed to allow the penetration and removal of the sterilant during sterilization and then to protect the device from contamination and other damage following sterilization and until the time of use
3. **Sterilization area:** The packed medical devices received from the packaging area are subjected to sterilization process by steam sterilizer, ethylene oxide sterilizer (ETO) or plasma sterilizer
4. **Sterile storage area:** After sterilization the sterilized items are stored in this area. It has an issue counter to supply the items to OTs and various other areas of the hospital.

Factors Influencing Efficacy of Sterilant/Disinfectant

The efficiency of a sterilant/disinfectant is affected by various factors.

- ❖ **Organism load:** As the bioburden increases, the contact time of the disinfectant also needs to be increased



Fig. 23.1: Central Sterile Services Department (CSSD).

- ❖ **Nature of organisms:** Organisms vary greatly in their resistance to disinfectants and sterilants

The decreasing order of resistance of microorganisms to various agents used for sterilization or disinfection is as follows. Prions > bacterial spores > coccidian oocyst > mycobacteria > non-enveloped viruses > fungi > vegetative bacteria > enveloped viruses

- ❖ **Concentration:** The agents should be used at their optimal concentration to produce the desired antimicrobial action. Higher concentrations may corrode the material and lower concentrations may be less effective
- ❖ **Contact time:** It is the most crucial factor for a disinfectant to be effective. It refers to the time period, a disinfectant is in direct contact with the surface or item to be disinfected. For surface disinfection, this period is framed by the application to the surface until complete drying has occurred. Lower exposure time doesn't achieve effective killing

- ❖ **Temperature:** The activity of most agents increases as the temperature increases. However, inappropriate higher temperatures may degrade the agent
- ❖ **Stability:** Some agents are unstable at in-use concentration, e.g. hypochlorite, and should be freshly prepared each day
- ❖ **Local pH:** The pH influences the antimicrobial activity. An increase in pH improves the antimicrobial activity of some disinfectants (e.g. glutaraldehyde, quaternary ammonium compound or QAC) but decreases the antimicrobial activity of others (e.g. phenols, hypochlorite, and iodine)
- ❖ **Relative humidity** is an important factor influencing the activity of gaseous disinfectant such as ethylene oxide (ETO)
- ❖ **Organic matter** such as pus, serum, blood, and stool can interfere with the antimicrobial activity of some disinfectants (e.g. hypochlorites and QAC)
 - This can be overcome by—(i) mechanically cleaning the instrument or surface/floor before it is subjected for disinfection/sterilization and (ii) increase in exposure time or concentration of the agent
 - However, few other disinfectants such as phenolics or glutaraldehyde retain their efficacy in the presence of organic matter.
- ❖ **Biofilm:** Formation of biofilm is another mechanism which prevents the entry of disinfectant/sterilant to act on the microorganisms which are embedded inside the biofilm.

Property of an Ideal Sterilant/Disinfectant

An ideal disinfectant/sterilant should have various properties—(i) broader microbicidal activity, (ii) fast acting, (iii) not affected by environmental factors such as organic matter, (iv) nontoxic, (v) compatible with surfaces/materials to which it is used, (vi) odorless or pleasant odor, (vii) economical and (viii) environmental friendly.

Spaulding's Classification of Medical Devices

Earle H. Spaulding in 1971 devised a rational approach to classify the medical devices into three categories according to the degree of risk for infection involved in use of the items (Table 23.3).

- ❖ This classification is used to categorize medical devices according to their intended use and the subsequent level of reprocessing required to render the devices safe for reuse
- ❖ Spaulding's classification scheme is so clear and logical that it has been retained, refined, and successfully used by infection control professionals in planning methods for disinfection or sterilization.

The various agents used in the hospital for achieving sterilization, disinfection and cleaning are enlisted in Table 23.2 and are discussed below.

STERILANTS

Steam Sterilizer (Autoclave)

Principle

Steam sterilizer functions similar to a pressure cooker and follows the general laws of gas.

- ❖ Water boils when its vapor pressure equals that of the surrounding atmosphere
- ❖ When the atmospheric pressure is raised, the boiling temperature is also raised
- ❖ At normal pressure, water boils at 100°C but when the pressure inside a closed vessel increases, the temperature at which water boils also increases.

Mechanism of action: Moist heat destroys microorganisms by irreversible coagulation, denaturation of enzymes and structural proteins.

Components of Steam Sterilizer (Autoclave)

Steam sterilizer is a pressure chamber; consists of a cylinder, a lid and an electrical heater.

- ❖ **Pressure chamber:** It consists of:
 - A large **cylinder** (vertical or horizontal) made up of gunmetal or stainless steel, in which the materials to be sterilized are placed
 - A **steam jacket** (water compartment).
- ❖ **Lid:** It bears the following:
 - A discharge tap for the passage of air and steam
 - A pressure gauge (sets the pressure at a particular level)
 - A safety valve (to remove the excess steam).
- ❖ **Electrical heater:** It is attached to the jacket; that heats the water to produce steam.

Risk category	Definition	Recommended method	Medical equipment or surfaces
Critical device (high risk)	Items that enter a normally sterile site	Sterilization	Surgical instruments, implants/prosthesis, rigid endoscopes, syringes, needles
Semi-critical device (intermediate risk)	Items in contact with mucous membranes or body fluids	Disinfection (HLD)	Respiratory equipment, non-invasive flexible endoscopes, bedpans, urine bottles
Non-critical (low-risk)	Items in contact with intact skin	Disinfection (ILD or LLD)	Non-critical patient items ¹ Non-critical environmental surfaces ²

¹**Non-critical patient items**—examples include blood pressure cuffs, ECG electrodes, thermometer and stethoscopes

²**Non-critical environmental surfaces**—e.g. medical equipment, computers, bedrails, food utensils, bedside tables, patient furniture and floor

Abbreviations: HLD, high-level disinfectant; ILD, intermediate-level disinfectant; LLD, low-level disinfectant.

Procedure

The materials to be sterilized are placed inside the cylinder. The steam jacket is filled with sufficient water, lid is closed and the electrical heater is put on. The sterilization process can be divided into three phases.

- ❖ **Conditioning phase:** After the water boils, the air in the chamber is completely displaced by the steam produced. The steam pressure rises inside and when it reaches the desired set level (15 pounds per square inch), the safety valve opens and excess steam escapes out
- ❖ **Exposure phase:** The holding period is counted from this point of time, which is about 15 minutes in most cases
- ❖ **Exhaust phase:** After the holding period, the electrical heater is switched off and the steam sterilizer is allowed to cool till it reaches atmospheric pressure.

Sterilization Conditions

The steam sterilizer can be set to provide higher temperatures by adjusting the pressure provided to the vessel.

- ❑ Cycle duration varies (3 to 18 min) depending on the sterilization temperature (121°C–135°C)
- ❑ The most commonly used sterilization condition is 121°C for 15 min at a pressure of 15 pounds (lbs) per square inch (psi).

Uses of Steam Sterilizer (Autoclave)

Steam sterilizer is the most commonly used sterilization method in the hospital. It is used for:

- ❖ All critical and semi-critical items that are heat and moisture resistant: surgical instruments, anesthetic equipment, dental instruments, implanted medical devices and surgical drapes and linens
- ❖ Culture media preparation
- ❖ Biomedical waste treatment of waste and sharps.

Precautions

- ❖ It should not be used for sterilizing waterproof materials such as oil and grease or dry materials such as glove powder
- ❖ The chamber should not be overfilled and the material should not touch the sides or top of the chamber
- ❖ Separate steam sterilizers should be used for treatment of biomedical waste.

Types of Steam Sterilizer

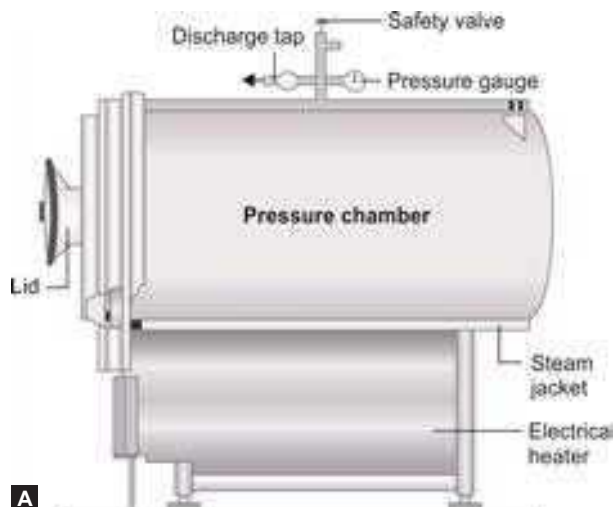
Steam sterilizers are available in various sizes and dimensions.

- ❖ **Horizontal type** (large volume capacity) (Figs 23.2A and B): It is used in CSSD, large-size laboratories and for biomedical waste treatment
- ❖ **Vertical type** (small volume capacity) (Fig. 23.2C): It is used for small-size laboratories.

Advantages

Steam sterilizer has the following advantages:

- ❖ It is low cost than ETO and plasma sterilizers
- ❖ Sterilization cycles are fast compared to ETO sterilizers



A



B

C

Figs 23.2A to C: Steam sterilizer (autoclave): **A.** Schematic diagram; **B.** Horizontal autoclave; **C.** Vertical autoclave.

- ❖ It is nontoxic and leaves no by-product behind (unlike ETO).

Disadvantages

Disadvantages of steam sterilizer include:

- ❖ Heat can damage acrylics and styrene, PVC material and corrode some metals
- ❖ Higher temperature for a prolonged time can harm or shorten the life of instruments
- ❖ Moisture also can adversely affect electronics and can cloud the sensitive materials or leave watermark stains on them.

Sterilization Control

The effectiveness of the sterilization achieved by steam sterilizer can be monitored by:

- ❖ **Biological indicator:** Spores of *Geobacillus stearothermophilus* are the best indicator. Their spores are killed in 12 minutes at 121°C
- ❖ **Chemical indicators**
 - External pack control, e.g. autoclave tape
 - Bowie-Dick test
 - Internal pack control.

- ❖ **Physical indicators:** For example, digital displays on the equipment displaying temperature, time and pressure.

Flash Sterilization

Flash sterilization is a modification of conventional steam sterilization, designed to be used at emergency or during unplanned procedures.

- ❖ It involves fast sterilization (134°C for 3-10 minutes) of surgical instruments in an unwrapped condition in steam sterilizers located close to the operation theatre
- ❖ This practice should only be restricted for emergency situation, e.g. instrument has been contaminated and needs to be replaced in the surgical field immediately
- ❖ It is not suitable for porous and cannulated instruments, implants and suction tubing
- ❖ As the instruments are not packed, they remain wet following sterilization; therefore, there is a high-risk of recontamination.

Ethylene Oxide (ETO) Sterilizer

Ethylene oxide (ETO) is one of the widely used gaseous chemical sterilants in CSSD.

- ❖ **Mechanism of action:** ETO has broad microbicidal action including spores; causes alkylation of cell components such as cell proteins, DNA and RNA
- ❖ **Sterilization cycle:** It is carried out in a special equipment called ethylene oxide sterilizer (Fig. 23.3). The process comprises of three stages
 - **Preconditioning:** At first, air is removed from the chamber and vacuum is created. Then the physical conditions (temperature, pressure and humidity) for sterilization are set in the chamber
 - **Sterilization:** ETO is allowed to enter the chamber. The four essential parameters that influence the effectiveness of ETO sterilization are—gas concentration, temperature, relative humidity and exposure time.

At ETO concentration of 700 mg/liter and 40–80% relative humidity, sterilization is achieved in 4–5 hours at 38°C or 1 hour at 55°C

- **Aeration (Degassing):** The ETO residues left on surgical instruments and tubing may be toxic to the patients and staff. Therefore, extensive aeration of the sterilized materials for 8–12 hours is necessary to remove residual ETO.
- ❖ **Uses:** ETO is used by CSSD to sterilize critical items (and sometimes semicritical items) that are moisture or heat sensitive and cannot be sterilized by steam sterilization. Examples include:
 - Heart-lung machine components
 - Sutures, catheters and stents
 - Respirators and dental equipment
 - Devices with electronic components
 - Assembled complex devices
 - Multi-lumen tubings, etc.
- ❖ **Advantages of ETO:** (i) Large chamber capacity than plasma sterilization, (ii) suitable for heat sensitive items, (iii) high penetration power- ETO is highly diffusible, penetrates areas that cannot be reached by steam, (iv) non-corrosive to plastic, metal and rubber materials
- ❖ **Disadvantages:** (i) ETO is highly inflammable, irritant, explosive and carcinogenic, (ii) ETO is usually supplied in a 10–20% concentration; mixed with inert gases like either CO₂, (iii) long duration of cycle (12–14 hours), (iv) high cost of instrument and consumables
- ❖ **Sterilization control:** Spores of *Bacillus atrophaeus* is used as biological indicator to check the effectiveness of sterilization. Physical and chemical indicators are same as discussed for autoclave.

Plasma Sterilization

Plasma refers to a gaseous state consisting of ions, photons, free electrons and free radicals (such as O and OH). Plasma sterilizer is a special device used to create the plasma state



Fig. 23.3: Ethylene oxide sterilizer.

Source: 3M India Pvt. Ltd.



Fig. 23.4: Plasma sterilizer (Sterrad).

Source: Johnson & Johnson Pvt. Ltd.



Fig. 23.5: Dry heat sterilizer (hot air oven).

(commercial brands, such as *Sterrad*). It has the following steps (Fig. 23.4).

- ❖ **Vacuum:** First, the chamber is evacuated to create a vacuum
- ❖ **Chemical sterilant:** Next step is injection of chemical sterilant hydrogen peroxide (H_2O_2) solution from a cassette, which gets vaporized in the sterilization chamber to a concentration of 6 mg/L
 - The H_2O_2 vapor diffuses through the chamber (50 minutes), exposes all surfaces of the load to the sterilant
 - Low temperature is maintained 37-44°C throughout the cycle.
- ❖ **Gas plasma:** In the next step, an **electrical field** is applied to the chamber to create a gas plasma. H_2O_2 breaks into free radicals such as hydroxyl (OH^-) and hydroperoxyl (HO_2) which initiate microbicidal action, which subsequently interact with essential cell components (e.g. enzymes, nucleic acids)
- ❖ **Finally**, the excess gas is removed. The by-products of the cycle (e.g. water vapor, oxygen) are nontoxic and therefore, there is no need of an additional aeration step
- ❖ **Cycle duration:** It has a cycle time of 75 min. The newer versions have shorter cycles of 52 min and 24 min
- ❖ **Sterilization control:** Spores of *Bacillus stearothermophilus* is used as a biological indicator to check the effectiveness of sterilization. Physical and chemical indicators are the same as discussed for the autoclave.

Uses of Plasma Sterilizer

It is used by CSSD for sterilization of materials and devices that cannot tolerate high temperature and humidity of steam sterilizer, such as some plastics, electrical devices, and corrosion-susceptible metals such as arthroscope, micro and vascular instruments, spine sets and laparoscope.

Precautions/Disadvantages

The following precautions should be followed while using plasma sterilizer.

- ❖ Items should be dried before loading
- ❖ Linen or paper or cellulose or liquid cannot be processed
- ❖ It may not penetrate well, especially in channels or devices designed with long lumens
- ❖ It has a small chamber, therefore cannot be used for bulk items
- ❖ High cost of equipment and packing materials.

Dry Heat Sterilizer (Hot Air Oven)

This method is used for materials that might be damaged by moist heat or that are impenetrable to the moist heat (e.g. glass wares, powders, petroleum products, sharp instruments).

- ❖ **Procedure:** It has a sterilization chamber, which is electrically heated and has a fan or a motor to ensure adequate and even distribution of hot air in the chamber (Fig. 23.5)
 - Dry heat acts by oxidation of cell constituents

- The most common cycles used are 170°C for 60 minutes, 160°C for 120 minutes, and 150°C for 150 minutes.

- ❖ **Advantages:** (i) It is non-toxic and does not harm environment, (ii) low operating costs, (iii) penetrates well into materials, (iv) noncorrosive for metals
- ❖ **Disadvantages:** The high temperatures are not suitable for most materials
- ❖ **Sterilization control:** Spores of *Bacillus atrophaeus* is used as biological indicator as they are more resistant to dry heat than are *G. stearothermophilus* spores.

Filtration

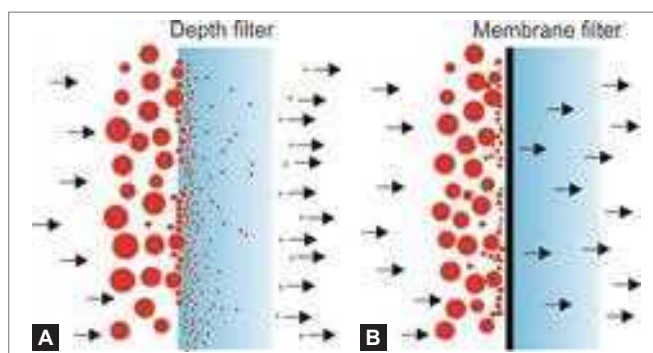
Filtration acts by removing microorganisms, not by killing. CDC considers filtration as a sterilization method, although some authors arguably disregard this as it does not kill the microorganisms, rather only filters them out.

There are two types of filters.

- ❖ **Depth filters:** They retain particles throughout the depth of the filter, rather than just on the surface (Fig. 23.6 A). They are used as drinking water purifiers. They do not achieve sterilization and are not suitable for hospital use
- ❖ **Membrane filters:** They are the most widely used filters in hospitals. They retain all the particles on the surface that are larger than their pore size (Figs 23.6B and 23.7)
 - Bacterial filters have 0.22 μm pore size which removes most of the bacteria; allowing the viruses to pass-through
 - Viral filters have even smaller pore size.
- ❖ Membrane filtration has two wider applications in hospital settings—filtration of air and water.

Filtration of Air

- ❖ **Surgical (3-ply) mask and respirators:** They are simplest examples of filters being used for purification of air. They remove microorganisms based on their pore size. These filters are made up of flat, non-woven fibers
- ❖ **HEPA filters** (High-efficiency particulate air filters):
 - HEPA filter removes 99.97% of particles that have a size of 0.3 μm or more



Figs 23.6A and B: Filtration methods: **A.** Depth filters; **B.** Membrane filters.



Fig. 23.7: Filter apparatus with membrane filter.

Source: Department of Microbiology, JIPMER, Puducherry.

- HEPA filters in hospitals are used in biological safety cabinets, airflow system, operation theatre, and isolation rooms.
- ❖ **ULPA filters** (Ultra-low particulate/penetration air): An ULPA filter can remove from the air at least 99.999% of dust, pollen, mold, bacteria and any airborne particles with a size of 0.12 μm or larger.

Filtration of Liquid

- ❖ Used for bacteriological examination of water in hospital settings, especially dialysis water
- ❖ Also used to remove bacteria from pharmaceutical fluids that are heat labile and cannot be purified by any other means—e.g. sera, sugar, toxin, vaccine and antibiotic solutions.

The sterilization control of membrane filters includes *Brevundimonas diminuta* and *Serratia marcescens*.

Radiation

Ionizing Radiation

Ionizing radiations include cobalt 60 gamma rays or electron accelerators.

- ❖ **Use:** It is a low-temperature sterilization method that has been used for a number of medical products (e.g. tissue for transplantation, pharmaceuticals, medical devices)
- ❖ **Mechanism:** It causes ionization of the molecules in organisms leading to breakage of DNA
- ❖ **Advantages** of ionizing radiation—(1) high penetrating power, (2) rapidity of action, and (3) temperature is not raised (hence this method is also called as **cold sterilization**)
- ❖ **Disadvantages:** High sterilization costs and may have deleterious effects on the equipment made up of polyethylene
- ❖ **Sterilization control:** Efficacy of ionizing radiation is tested by using *Bacillus pumilus*.

Non-ionizing Radiation

Examples include infrared and ultraviolet radiations.

- ❖ **Infrared radiation** technology can be used as an alternate method of sterilization for selected heat-resistant instrument
- ❖ **Ultraviolet radiation** does not achieve sterilization, described under intermediate-level disinfectant.

Incineration

Incineration is used for the treatment of biomedical waste materials (for non-plastic infectious waste). It burns (sterilizes) the waste by providing a very high temperature 870–1,200°C and thereby converting the waste into ash, flue gas and heat (Chapter 24).

Microwave

Microwaves are used in hospitals for disinfection of soft contact lenses, dental instruments, dentures, and urinary catheters (for intermittent self-catheterization). It is available in various size from home-type microwave ovens to large-size.

- ❖ Large size microwaves are used for disposal of biomedical waste (for plastic infectious waste)
- ❖ However, microwaves must only be used with products that are compatible
- ❖ **Mechanism of action:** Microwaves are radio-frequency waves, which are usually used at a frequency of 2450 MHz. They produce friction of water molecules which generates heat.

Other sterilization methods which are less commonly used include low temperature steam formaldehyde, beta-propiolactone vapor, gaseous chlorine dioxide, vaporized peracetic acid, vaporized hydrogen peroxide and ozone.

HIGH-LEVEL DISINFECTANTS (HLD)

HLD agents are capable of killing bacterial spores when used in sufficient concentration under suitable conditions. They can kill all the other microorganisms.

Aldehyde

Formaldehyde, glutaraldehyde and ortho-phthalaldehyde are the commonly used disinfectants. They combine with nucleic acids, proteins and inactivate them, probably by cross-linking and alkylating the molecules.

Glutaraldehyde

- ❖ **Semicritical items:** It remains active in the presence of organic matter and is non-corrosive to equipment. Therefore, glutaraldehyde is the most common HLD used for semicritical equipment, such as endoscopes and cystoscopes
 - It is used at 2% or 2.4% concentration (e.g. Cidex). It disinfects objects within 20 minutes but may require longer time to kill spores (10–14 hours)

- It is available in inactive form; has to be activated by alkalization before use. Once activated, it remains active only for 14 days.
- ❖ **Aerial disinfection and cleaning:** It is also used for fogging and cleaning of floor and surfaces of critical areas such as operation theatre (e.g. Bacillocid Extra)
- ❖ **Advantages:** It remains active in the presence of organic matter, has excellent material compatibility
- ❖ **Disadvantages:** It has a pungent odour, can produce eye irritation, occupational asthma and contact dermatitis.

Ortho-phthalaldehyde (0.55%)

This can also be used for disinfection of semicritical items, has many advantages over glutaraldehyde—(1) it does not require activation, (2) better odor, (3) less eye irritation, (4) acts faster (5–10 min). However, it does not kill spores effectively and stains skin gray.

Formaldehyde

Although it is an excellent HLD, the health-care uses of formaldehyde are limited because it produces irritating fumes and pungent odor. It is also a potential carcinogen, corrosive to the metals and causes skin irritation and asthma (when inhaled).

- ❖ Therefore, formaldehyde is largely restricted to non-patient care area only; used for—preservation of anatomical specimen and stool specimen and as an embalming agent
- ❖ It was used for fumigation of closed areas, such as operation theatre, but now this is an obsolete practice
- ❖ Formaldehyde tablets and gaseous formaldehyde were used for sterilization of surgical instruments, but unreliable.

Peracetic Acid

Peracetic acid is used in automated machines. It is also available for manual immersion; 0.1–0.2%, used for 5–15 min.

- ❖ **Use:** It can be used to sterilize medical (e.g. endoscopes, arthroscopes), surgical, and dental instruments. Peracetic acid in combination with hydrogen peroxide has been used for disinfecting hemodialyzers
- ❖ **Disadvantages:** Expensive, has material compatibility issues, causes chemical irritation and eye damage.

Hydrogen Peroxide (H₂O₂)

H₂O₂ works by producing destructive hydroxyl free radicals that can attack various cell components. H₂O₂ can be degraded by catalase-producing organisms to water and oxygen, which can be overcome by using higher concentrations of H₂O₂

- ❖ **Uses:** H₂O₂ has several usages at various concentrations. It is sporicidal only at >4–5%
 - 3% H₂O₂ is used for environmental surface disinfection, fogging and for wound cleaning

- 3–6% H₂O₂ is used to disinfect soft contact lens, tonometer biprisms, ventilators, fabrics, and endoscopes, etc.
- 6–7.5% H₂O₂ is used as chemical sterilant in plasma sterilization
- Vaporized H₂O₂ is used for industrial sterilization of medical devices and for decontamination of large and small area.
- ❖ **Advantages:** It is rapid in action, nontoxic, has detergent properties with good cleaning ability, and is active in the presence of organic material
- ❖ **Disadvantages** include—expensive, has material compatibility issue (contraindicated for use on copper, brass, zinc, aluminium), can produce chemical irritation and corneal damage. It should be properly stored in dark containers.

INTERMEDIATE-LEVEL DISINFECTANTS

Alcohol

Ethyl alcohol and isopropyl alcohol are the most popular alcohols used in hospitals.

- ❖ **Action:** They are rapidly bactericidal to most organisms except spores. The cidal activity drops sharply when diluted below 50% concentration. They act by denaturation of proteins
- ❖ **Uses:** Alcohol (60–80%) is used for various purposes
 - **Alcohol based handrub** (ABHR), e.g. Sterillium, a popular commercial product
 - Disinfecting **smaller non-critical instruments** such as thermometers, which are immersed in alcohol for 10–15 minutes
 - Disinfection of **small medical items/surfaces** such as rubber stoppers of multiple-dose medication vials or vaccine bottles and hubs of the central line
 - Disinfection of **external surfaces of equipment** such as stethoscopes, ventilators, manual ventilation bags, ultrasound machines, etc.
 - Disinfection of **non-critical surfaces** such as laboratory bench, medication preparation areas.
 - **Spirit** (70% alcohol): Used a skin antiseptic
- ❖ **Disadvantages:** (i) Flammable and must be stored in a cool, well-ventilated area, (ii) Evaporate rapidly, making exposure time difficult to achieve unless the items are immersed, (iii) May damage tonometer tips and lenses, (iv) Inactivated by organic matter.

Phenolics

Phenol (carbolic acid) was the first widely used antiseptic and disinfectant; was introduced for surgery in 1867 by Joseph Lister (the father of antiseptic surgery). The phenol and its derivatives (called phenolics) are produced by distillation of coal tar.

- ❖ **Mechanisms:** Phenolics act as protoplasmic poison, disrupt the cell wall and precipitate the cell proteins

- ❖ **Used as disinfectants:** Cresol, and lysol are the common phenolics used for disinfecting environmental surfaces (e.g. bedside tables, bedrails, and laboratory surfaces) and noncritical medical devices. They are toxic to skin, hence not used as antiseptics. **5% phenol** is mycobactericidal, used for disinfection of sputum specimen
- ❖ **Used as antiseptics:** Certain phenolics are compatible with skin and are widely used as antiseptics. The classical example is chloroxylenol (the active ingredient of the commercial brand, Dettol)
- ❖ **Advantages:** Phenolics are the only ILD that retain activity in the presence of organic materials
- ❖ **Disadvantages:** They can cause hyperbilirubinemia in infants and therefore should not be used in nurseries.
- ❖ **Mechanisms:** All preparations yield hypochlorous acid (HClO), which causes oxidation of cellular materials and destruction of vegetative bacteria and fungi
- ❖ **Uses (free chlorine):** Chlorine is used for disinfection of municipal water supplies and swimming pool water. It is also employed in the dairy and food industries
- ❖ **Uses (sodium hypochlorite):** It is available at 5.25–6.15%, which is equivalent to 50,000 ppm of available chlorine. It should be used in appropriate dilutions (by adding with water) for disinfection of various hospital supplies. The contact time is about 10–20 minutes
 - Large blood spill: 0.5% (1:10 dilution or 5,000 ppm) is used
 - Small blood spill: 0.05% (1: 100 dilution, or 500 ppm) is used
 - Pre-treatment of liquid waste before disposal : 1% (1:5 dilution, 10,000 ppm) is used
 - Laundry items : 0.1% (1 in 50 dilution 1,000 ppm) is used
 - Surface disinfectant: 0.5% (1:10 dilution or 5,000 ppm) is used
 - *C. difficile* (diarrheal stool): Hypochlorite is sporicidal only >0.5% (5000 ppm).

Halogens

Among the halogens, iodine and chlorine have antimicrobial activity. They exist in free state, and form salt with sodium and other metals.

Iodine

Iodine acts by disruption of protein and nucleic acid. Two preparations are available.

- ❖ **Tincture of iodine:** It is a preparation of iodine (2%) in potassium iodide. It used as antiseptic for wound cleaning, but can cause staining and skin allergy
- ❖ **Iodophor (e.g. povidone iodine):** It is prepared by complexing iodine with carrier (povidone) which helps in sustained-release of iodine. It is nonstaining and free of skin toxicity. Some popular brands available are Wescodyne and Betadine.
- Uses:** Iodophors are used both as antiseptics and disinfectant at different concentrations. Therefore, antiseptic iodophors are not suitable for use as disinfectants and vice-versa
- ❖ **Advantages:** Hypochlorites are broad spectrum, rapid in its action, non-flammable, low cost and are widely available
- ❖ **Disadvantages:** (1) Inactivated by organic matter, which can be overcome by adding excess chlorine, (2) Toxic to skin and mucosa, and carcinogenic, (3) Daily preparation—hypochlorite is unstable, evaporates on exposure to sunlight or air. Hence, it has to be prepared daily and stored in opaque container, (4) Corrosive, damages fabrics, carpets, (5) Leaves residue, requires rinsing or neutralization, (6) Offensive odors, (7) Bleaches the fabrics and carpets.

- **Used as antiseptics**

- ◆ 5% topical solution and ointment is used for wound cleaning
- ◆ 7.5% is used for hand scrub
- ◆ 10% is used for surgical skin preparation
- ◆ 1% is used as an oral antiseptic, for mouth wash.

- **Used as disinfectant** for medical equipment, such as hydrotherapy tanks and thermometers.

Chlorine and Hypochlorite

Chlorine is one of the most commonly available disinfectant in hospital.

- ❖ **Preparations:** Chlorine occurs as—(1) free chlorine, (2) hypochlorite—it is available in two preparations
 - Liquid form (sodium hypochlorite or household bleach), or
 - Powder form (calcium hypochlorite or bleaching powder)
 - Other forms: include sodium dichloroisocyanurate (NaDCC) available as tablets and chlorine dioxide.
- ❖ **Boiling at 100°C:** Boiling of the items in water for 15 minutes may kill most of the vegetative forms but not the spores, hence not suitable for sterilization of surgical instruments
- ❖ **Steaming at 100°C:** When the autoclave is used without closing the pressure valve, the temperature does not rise beyond 100°C. It may be useful for disinfecting those items which cannot withstand the high temperature of autoclave
- ❖ **Insipissation:** In microbiology laboratory, the egg-based culture media such as Lowenstein-Jensen medium are

Heat-based Methods

The following heat-based methods can act as ILD, kill all organisms except the spores.

sterilized by heating at 80–85°C for 30 minutes on 3 successive days so that the spores can be killed.

Ultraviolet (UV) Radiation

Ultraviolet (UV) radiation is a form of non-ionizing radiation that is emitted by the sun and artificial sources such as mercury vapor bulbs.

- ❖ **Mechanism of action:** Causes destruction of nucleic acid through induction of thymine dimers. Mercury vapor lamps emit UV rays at 253.7 nm wavelength. Bacteria and viruses are more easily killed by UV light but not spores
- ❖ **Uses:** UV radiation has been employed for:
 - Disinfection of drinking water, titanium implants and contact lenses
 - Disinfection of air and/or surfaces as in operating rooms, isolation rooms, and biologic safety cabinets
 - Sun-rays also contain UV rays, which may disinfect organisms present on environmental surfaces.
- ❖ **Disadvantages:** The effectiveness is influenced by organic matter. In isolation rooms, it may cause skin erythema and keratoconjunctivitis in patients and visitors. Therefore, UV lamps should be placed at least above 2-meters height from the floor level.

LOW-LEVEL DISINFECTANTS

Low-level disinfectants (LLD) destroy vegetative bacteria and enveloped viruses, variable action on nonenveloped viruses, and fungi, but no action on tubercle bacilli and spores.

Quaternary Ammonium Compound (QAC)

QAC are commonly used in ordinary environmental sanitation of noncritical surfaces, such as floors, furniture, and walls. Some products are also used for disinfecting non-critical medical equipment that contacts intact skin (e.g. blood pressure cuffs). QAC are also good cleaning agents as they have surfactant like action.

- ❖ **Mechanism:** They act by inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane
- ❖ **QAC formulations:** Benzyl ammonium chloride is the most popular QAC used in the healthcare. It does not act in the presence of hard water. The newer generation of QACs (e.g. didecyl dimethyl ammonium bromide) remain active in hard water and are better compatible.

Chlorhexidine Gluconate (CHG)

CHG is a biguanide disinfectant, acts by disruption of cytoplasmic membrane.

- ❖ **Uses:** CHG is widely used in antiseptic products, at various concentrations
 - **Hand hygiene product:** Hand rub (0.5%), hand wash (4%) (e.g. Microshield, a commercial product)
 - **Mouthwash** (0.1-0.2%)

- **Body wash** solutions (used before surgery)
- **Skin disinfectant** before surgery (2 %)
- **Antiseptic** for wound cleaning: Commercially available as **Savlon** which is a combination of CHG 0.3%, cetrimide and isopropyl alcohol.
- ❖ **Advantages:** The wide use of CHG is due to its residual activity (prolonged action than alcohol hand rub) and is less irritant
- ❖ **Disadvantages:** It is slower in action, activity is pH dependent and is greatly reduced in the presence of organic matter. It produces dermatitis on prolonged use as handrub.

CLEANING AGENTS

Most disinfectants act well only when the instrument or the surface is free from organic matter such as dirt, blood, or other specimens.

- ❖ Therefore, cleaning is a very important step which needs to be performed before the disinfectants are applied
- ❖ An ideal cleaning agent should have the following properties: easily emulsifiable, saponifiable, water softening, non-toxic and have surfactant like action.

Cleaning Products

Broadly two types of cleaning agents are available.

- ❖ **Enzymatic (proteolytic) cleaners:** They contain enzymes such as amylase, lipase, cellulase, protease which break down proteinaceous matter present on equipment. Enzymatic cleaners are not disinfectants; they only remove protein from surfaces
- ❖ **Cleaning chemicals (detergents):** These agents act by reducing surface tension and dissolving fat and organic matter. Detergents used for surface and floor cleaning are different than that used for instrument cleaning. Mild alkaline detergents (pH 8.0–10.8) are more efficient cleaning agents for surgical instruments.

Cleaning Methods

The cleaning methods are grouped into:

- ❖ **Manual cleaning** by immersion of instruments into the cleaning solution, or by wiping the surfaces with a cloth soaked with the cleaning solution
- ❖ **Automatic or mechanical cleaning:** They clean faster with a higher standard of cleaning than manual cleaning
 - It is very useful for cleaning the hard-to-reach parts of surgical instruments
 - Several equipment are available for mechanical cleaning such as ultrasonic washers, washer-disinfector and automated cart washers.

ENVIRONMENTAL CLEANING

Environmental cleaning of the floor and surface of hospitals play a vital role in controlling the spread of infections. The general principles of environmental cleaning are as follows.

- ❖ **Cleaning followed by disinfections:**
 - **Cleaning:** Always cleaning with a detergent is performed first, before applying disinfectant
 - **Disinfection:** CDC recommends to use low- to intermediate-level disinfectants for environmental cleaning such as QAC, hypochlorite and improved hydrogen peroxide.
- ❖ **Cleaning sequence:** Cleaning should be performed in correct sequence to prevent recontamination
 - **Cleaner to dirtier:** The cleaner areas are cleaned first, followed by the dirtier areas; for e.g. low-touch surfaces should be cleaned first followed by high-touch surfaces
 - **High to low:** Top area should be cleaned first, then proceed towards bottom (e.g. bedrails → bed legs and table surfaces → floors)
 - **Inward to outwards:** Clean the farthest point from the door first and then proceed towards the door.
- ❖ **Frequency of cleaning depends upon:**
 - **Probability of contamination:** e.g. heavily contaminated vs low-contaminated surfaces or instrument
 - **Vulnerability of population to infection:** e.g. immunocompromised vs healthy adults
 - **Frequency of hand contact:** e.g. high-touch vs low-touch surfaces.
- ❖ **Frequency of cleaning for common situations:**
 - Non-critical surfaces and floors can be cleaned 2-3 times a day
 - Mattress used for patients should be cleaned weekly and after discharge
 - Doors, windows, walls and ceiling should be cleaned once a month and spot-cleaning when soiled
 - **High touch areas** such as doorknobs, elevator buttons, telephones, bedrails, light switches, computer keyboards, monitoring equipment should be cleaned more frequently, every 3-4 hours.

Disinfection of Operation Theatre

Environmental cleaning in operation theatre (OT) minimizes patients' and HCWs' exposure to potentially infectious microorganisms.

- ❖ **Surface disinfection:** Cleaning should be performed first with a cleansing agent, followed by disinfection by using an aldehyde-based disinfectant. Disinfection of OT is carried out in the following situations
 1. First cleaning of the day (before cases begin)
 2. In between cases (cleaning 3 to 4 feet perimeter around the OT table)
 3. Terminal cleaning of OT after the last case
 4. Detailed wash-down of the OT complex once a week
 5. During renovation or construction of OT or nearby places.
- ❖ **Fogging:** Also called aerial disinfection, involves spraying of a disinfectant (e.g. glutaraldehyde, H₂O₂ or QAC based product) with the help of a fogger machine (Fig. 23.8)



Fig. 23.8: Fogging of dental unit following construction.

Source: Sanitary Department, JIPMER, Puducherry.

- The procedure takes around 1-2 hours, during which OT should be closed down and personnel need to be vacated
- Indication: Routine periodic fogging is not recommended, but is indicated only when any outbreak of infection is suspected or any change in infection control practice implemented or during renovation or construction of OT or nearby places.

METHODS TO TEST EFFICACY OF STERILANT/DISINFECTANT

Tests for Chemical Disinfectants

Chemical disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy. Various methods are available.

- ❖ **Rideal and Walker test or Phenol coefficient test:** It tests the efficacy of a phenolic disinfectant to kill *Salmonella* Typhi, when compared with that of phenol
- ❖ **Chick Martin test:** It is a modification of Rideal and Walker test, in which the disinfectant acts in the presence of organic matter (e.g. dried yeast, feces, etc.) to simulate the natural conditions
- ❖ **Capacity (Kelsey-Sykes) test:** It tests the capacity of a disinfectant to retain its activity when the microbiological load keeps increasing. It is used to test new disinfectants procured in hospitals to know which dilutions are suitable for use
- ❖ **In-use (Kelsey-Maurer) test:** It simulates real-time situation. It is used to determine whether an in-use solution of disinfectant in hospital is microbiologically contaminated.

Tests for Sterilizers (Indicators)

The efficacy of sterilizers can be assessed by using physical, chemical and biological indicators.

Physical Indicator

These are the digital displays of the sterilizer equipment showing parameters such as temperature, time and pressure, etc.

Chemical Indicator

They use heat or chemical sensitive materials which undergo a color change if the sterilization parameter (e.g. time, steam quality and temperature) for which it is issued is achieved. Common types used are:

- ❖ **Class I:** Also called as exposure indicator or external pack control. They are used on the external surface of each pack, to indicate that the pack has been directly exposed to the sterilant. However, it does not assure sterility (Fig. 23.9A)
- ❖ **Class II:** It is called as Bowie- Dick test or as equipment control; i.e. it checks the efficacy of air removal, air leaks and steam penetration and ensures that the steam sterilizer is functioning well
- ❖ **Class IV and V:** Also called as internal pack control indicator. It is placed inside the packs and therefore it verifies whether the critical parameters such as time, steam quality and temperature are attained inside the pack or not (Fig. 23.9B).



Figs 23.9A and B: Chemical indicator: **A.** Type-I (autoclave tape); **B.** Type V (internal pack control indicator).

Source: Department of CSSD, JIPMER, Puducherry.

Biological Indicator (BI)

It is the most reliable indicator as it uses bacterial spores to check the effectiveness of sterilization. The spores are highly resistant and will be destroyed only when the effective condition is achieved.

- ❖ *Geobacillus stearothermophilus* for steam sterilizer and gas plasma (hydrogen peroxide) and liquid acetic acid sterilizer
- ❖ *Bacillus atrophaeus* for ethylene oxide sterilizer and dry heat sterilizer (hot air oven)
- ❖ Spore containing vials are incubated. Depending upon the incubators used, the result is obtained in 24 min to 48 hrs time (Figs 23.10A and B).



Figs 23.10A and B: Biological indicator: **A.** Vial; **B.** Incubator.

Source: Department of CSSD, JIPMER, Puducherry.

EXPECTED QUESTIONS

I. Write essay on:

1. Define sterilization and disinfection. Describe principle and uses of steam sterilizers.
2. What are chemical sterilants. Discuss their application in healthcare settings.

II. Write short notes on:

1. Membrane filters.
2. Application of glutaraldehyde in healthcare setting.
3. Central Sterile Services Department (CSSD).

III. Multiple Choice Questions (MCQs):

1. Which of the following disinfectant is used in plasma sterilization?
 - a. Formaldehyde
 - b. Glutaraldehyde
 - c. Hydrogen peroxide
 - d. Ethylene oxide
2. *Geobacillus stearothermophilus* is used as indicator for efficacy of:
 - a. Hot air oven
 - b. Autoclave
 - c. Filtration
 - d. Ultraviolet rays
3. Which of the following is most resistant to sterilization?
 - a. Tubercle bacilli
 - b. Viruses

- c. Spores
- d. Prions

4. Endoscope is sterilized by:

- a. Glutaraldehyde
- b. Formaldehyde
- c. Autoclaving
- d. Hot air oven

5. Which is a form of cold sterilization?

- a. Infrared rays
- b. Steam sterilization
- c. Gamma rays
- d. UV rays

6. Which of the following disinfectant is used for hand wash?

- a. Ethylene oxide
- b. Formaldehyde
- c. Chlorhexidine
- d. Povidone iodine

7. Which of the following is used for disinfection of blood spillage area?

- a. Phenol
- b. Hypochlorite
- c. Lysol
- d. Formaldehyde

8. Which of the following disinfectant is currently NOT used for fogging of operation theaters?

- a. Formaldehyde
- b. Glutaraldehyde based
- c. Hydrogen peroxide based
- d. Quaternary ammonium compound

Answers

1. c 2. b 3. d 4. a 5. c 6. c 7. b 8. a

CHAPTER PREVIEW

■ Biomedical Waste Rule

■ Waste Segregation in Hospitals

■ Treatment and Disposal Methods

INTRODUCTION

The waste generated from the hospital carries a higher potential for infections and injuries. Therefore, it is essential to have safe and reliable methods of segregation and disposal of hospital waste.

Definition

Biomedical wastes (BMW) are defined as wastes that are generated during the laboratory diagnosis, treatment or immunization of human beings or animals, or in research activities pertaining thereto, or in the production of biologicals.

Waste Generated in Hospitals

It is estimated that the quantity of solid waste generated in hospitals varies from 1/2 to 2 kg/bed. However, BMW accounts for a minor proportion of the total waste generated in hospitals (~250 gram per bed per day). In developing countries, the waste generated in hospitals falls into two categories:

1. **General (non-hazardous solid waste, 80%):** A large amount of waste falls in the general waste category, which may be disposed of with the usual domestic and urban waste management system. They do not cause any harm to humans. They are not considered as BMW. They should not be mixed with BMW
2. **Biomedical waste:** This includes infectious waste (10%) and chemical/radioactive waste (5%)

Hazards Associated with BMW

Inappropriate and inefficient disposal of BMW can lead to infectious hazards, malignancies, malformations, and environmental (air, land and water) pollution not only to the current generation but also for future generations. The various hazards are:

- ❖ **Hazards from infectious wastes:** This is the component of hospital waste that produces maximum hazards
 - **Pathogens in the infectious waste** may infect HCWs by entering through ingestion, inhalation or direct skin-to-skin contact and can cause various type of

infections such as gastrointestinal, respiratory, skin infections, etc.

- **Hazards from infectious sharps**—leads to transmission of blood borne viruses (hepatitis B, C and HIV).
- ❖ **Hazards from chemical wastes:** They include laboratory reagents, disinfectants, and waste with high content of heavy metals, e.g. mercury from broken thermometers. Most chemicals are toxic, corrosive, explosive and flammable; can cause various physical injuries including chemical burns
- ❖ **Pharmaceutical waste:** It includes expired, unused and contaminated drugs, vaccines, sera, etc. Exposure to these agents may cause several adverse effects depending upon the nature of the pharmaceutical waste
- ❖ **Hazards from cytotoxic waste:** Cytotoxic drugs used in the treatment of cancers and autoimmune disorders are extremely hazardous to the environment and human health owing to their mutagenic, teratogenic, or carcinogenic properties
- ❖ **Hazards from radioactive waste:** They include materials contaminated with radionuclides
 - They are produced as a result of procedures performed by radiology and nuclear medicine departments
 - They are genotoxic, in higher doses can cause severe injuries, including tissue destruction, necessitating the amputation of body parts.

Situation in India

According to the Ministry of Environment and Forests (MoEF), the gross generation of BMW in India is about 484 TPD (tons per day). Unfortunately, only 447 TPD is treated, and 37 TPD (8%) is left untreated. Karnataka tops the chart among all the states in generation of BMW followed by Maharashtra.

Waste Management Hierarchy

The waste management hierarchy is largely based on the concept of the “3Rs”, namely reduce, recycle and recover. If none of these methods is available, then the last method opted is disposal.

- ❖ **Prevent and reduce:** The most preferable approach, is to prevent or reduce the production of waste as far as possible
- ❖ **Reuse and recycle:** The next best option is to reuse the waste as such (if feasible) or after recycling
- ❖ **Recover:** Where practicable, recovering waste items for secondary use can be done. It is of two types
 - *Energy recovery*, whereby waste is converted to fuel for generating electricity or for direct heating
 - *Waste recovery* is a term used for composting of organic waste matter to produce compost or soil conditioner which can be used in agriculture.
- ❖ **Treatment:** Wastes that cannot be recycled or recovered, can be subjected to treatment by various methods such as incineration.

Note: Treatment is also necessary for the biomedical waste before sending for recycling or recovery as these wastes are potentially infectious. This is usually carried out by the autoclave or microwave (explained later)
- ❖ **Disposal:** It involves disposal of the waste in landfill or dump yard. This is the least preferable option among all waste management strategies.

BIOMEDICAL WASTE RULE, INDIA

The Ministry of Environment and Forests (MoEF) has formulated biomedical waste rule in 1998; had classified the waste into 10 categories, which used to be segregated into five color-coded containers. There was considerable overlapping between categories which created ambiguity and confusion.

The new BMW guideline was published in 2016 with an amendment added in 2018 and 2019 (Table 24.1).

- ❖ It was implemented with a vision of simplifying categorization of BMWs, while improving the ease of segregation, transportation and disposal methods to decrease environmental pollution
- ❖ According to this new rule, there are four categories of BMWs, each is segregated by a single color-coded container.

Steps of BMW Management

The management of BMW can overall be summarized into six simple steps.

1. Waste segregation (at the point of generation) into color-coded containers
2. Pre-treatment for laboratory liquid waste
3. Transport of waste from generation site to central storage area of the hospital
4. Transport of waste from central storage area to common bio-medical waste treatment facility (CBMWTF)
5. Treatment and/or disposal (within 48 hours of generation).

Waste Segregation in Hospitals

Waste segregation refers to the basic separation of different categories of waste generated at source in the hospital and

thereby reducing the risks as well as the cost of handling and disposal.

According to BMW Rule (2016), segregation of waste should be done by using containers of four different colors—each is designated for segregation of a particular waste category (see Table 24.1).

- ❖ Yellow bag—for infectious non-plastic waste
- ❖ Red bag—for infectious plastic waste
- ❖ White or translucent sharp container (puncture-proof box)—for metal sharps
- ❖ Blue container (puncture-proof box)—for broken glass items and metal implants.

The following general principles need to be followed during segregation, transport and storage of BMW.

- ❖ **Waste receptacles:** The waste receptacles should have the following properties
 - **Plastic bags** must be labelled with biohazard logos (Fig. 24.1) and should be non-inflammable, autoclave stable and non-chlorinated with a thickness of $\geq 50 \mu\text{m}$
 - **Containers** should have well-fitting lids, either removable by hand or preferably operated by a foot pedal
 - **Sharp box** should be puncture-proof, leak-proof and tamper-proof impermeable container.
- ❖ **Importance of segregation:** Segregation is the most crucial step in BMW management. Wrong segregation may lead to serious consequences such as:
 - Needle stick injury transmitting hepatitis B or HIV (if sharp items are segregated in to yellow or red bags)
 - Production of carcinogens (if plastic items are wrongly segregated into yellow bag and subjected to incineration, leads to production of carcinogenic furans).
- ❖ **Securement:** All the bags used for waste collection need to be sealed once they are filled to 3/4th of their capacity
- ❖ **Labelling:** Bags and containers should be labelled properly with the date and place
- ❖ **Pre-treatment:** The laboratory liquid waste should always be pre-treated either with chemical (1-2% hypochlorite) or autoclave before segregating into appropriate containers

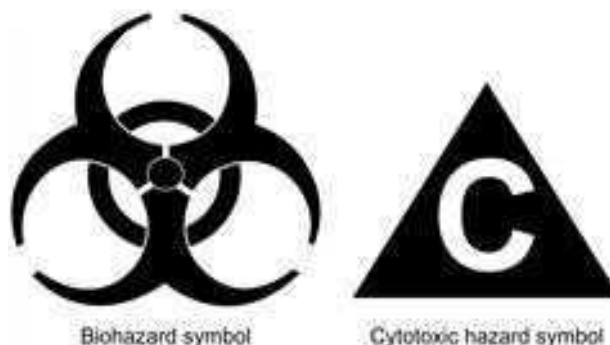


Fig. 24.1: Logos used for segregation of biomedical waste.

Table 24.1: Biomedical Waste Management Rule, India, 2016 (including the amendment added in 2018 and 2019).			
Category	Type of waste	Type of Bag/container	Treatment/disposal options
Yellow 	A. Human anatomical waste	Yellow colored non-chlorinated plastic bags	Incineration/plasma pyrolysis/deep burial
	B. Animal anatomical waste		Incineration/plasma pyrolysis/ deep burial/autoclaving or hydroclaving + shredding/mutilation
	C. Soiled waste		
	D. Expired/discarded medicines— pharmaceutical waste, cytotoxic drugs	Yellow colored containers/non-chlorinated plastic bags with cytotoxic label	Sent back to manufacturer/ CBMWTF for incineration (cytotoxic drugs at temperature >1200°C)
	E. Chemical solid waste	Yellow colored containers/ nonchlorinated plastic bags	Incineration or plasma pyrolysis or encapsulation
	F. Chemical liquid waste such as discarded disinfectants, infected body fluids and secretions, liquid from house-keeping related activities	To be discharged into separate collection system, which leads to effluent treatment system Not to be discarded into yellow bag	Pre-treated ¹ before mixing with other wastewater
	G. Discarded linen waste contaminated with blood/body fluids, mask, cap, gown and shoe cover	Non-chlorinated yellow plastic bags/suitable packing material	Non-chlorinated chemical disinfection ² followed by incineration/plasma pyrolysis
	H. Microbiology, other clinical laboratory waste, blood bags, live attenuated vaccines	Autoclave safe plastic bag/ container	Pre-treat to sterilize with non-chlorinated chemicals ² on-site as per NACO/ WHO guidelines (Blue book 2014) + incineration
Red 	Infectious plastic waste Disposable items such as tubing, bottles, intravenous tubes and sets, catheters, urine bags, syringes (without needles and fixed needle syringes) and vacutainer with their needles cut), gloves, plastic apron and goggles	Red colored non-chlorinated plastic bags or containers	<ul style="list-style-type: none"> • Autoclaving/microwaving/ hydroclaving + shredding • Mutilation/sterilization+ shredding Treated waste sent to authorized recyclers or for energy recovery
White (Translucent) 	Waste sharps including metal sharps Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp (used or discarded)	Puncture-proof, leak-proof, tamper-proof containers	Autoclaving/dry heat sterilization followed by: <ul style="list-style-type: none"> • Shredding or mutilation or encapsulation in metal container or cement concrete or • Sanitary landfill or • Designated concrete waste sharp pit
Blue 	a. Glasswares: Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes, microscope slides b. Metallic body implants Dental implants, other body implants and plates	Puncture proof and leak-proof container	Disinfection can be carried out by: <ul style="list-style-type: none"> • Soaking the washed glass waste after cleaning with detergent and sodium hypochlorite treatment (1–2%) or • Autoclaving/microwaving/ hydroclaving and then it is sent for recycling

Note:

- Biomedical waste rule does not specify any specific color coded bag for general waste segregation in hospital. Depending upon the local policy, hospitals choose any color coded bag for general waste (for e.g. JIPMER uses black bag for general waste).
- ¹**Chemical treatment:** Hypochlorite should be used at 1–2% concentration having 30% residual chlorine with contact time of 20 minutes.
- ²**Non-chlorinated chemicals** include 5% phenol, 5% cresol or 5% lysol.
- The chlorinated plastic bags (except blood bags) and gloves should be phased out and replaced by non-chlorinated bags and gloves.
- Every health care facility should have their own STP (sewage treatment plant).
- Barcoding system should be introduced to monitor the segregation compliance.

Abbreviations: NACO, National AIDS Control Organization; WHO, World Health Organization; CBMWTF, common bio-medical waste treatment facility.

- ❖ **Transport:** The waste should be transported within 24 hours by **dedicated trolley** to the central BMW storage facility of the hospital. Separate routes should be used for transport to prevent exposure to staff and patients and to minimize the passage of loaded carts through patient care and other clean areas. Interim storage of the waste at ward is strongly discouraged
- ❖ **Central storage area:** This is a temporary storage facility present within a hospital where different types of waste should be brought for safe retention until it is treated or collected for transport to CBMWTF
- ❖ **PPE:** HCWs handling BMW during transport or in the storage area should wear appropriate personal protective equipment (PPE) such as heavy duty gloves, 3-ply mask, gowns and gumboots.

Treatment and Disposal Methods

As per the mandate of the BMWM rules, 2016, the final disposal and recycling must be performed at common bio-medical waste treatment facility (CBMWTF). Only when there is no CBMWTF within 75 km, the hospital can create its own the disposal facility. The following are the methods used for treatment/disposal of BMW.

Incineration

It has been the method of choice for the disposal of BMW.

- ❖ Incineration is a high temperature (800-1200°C) dry oxidation process that reduces organic and combustible waste into nonorganic incombustible matter, resulting in a very significant reduction of waste volume and weight
- ❖ Incineration is usually done for those wastes that cannot be reused, recycled or disposed of in a landfill site, for example human and animal anatomical waste, microbiological waste, and solid non-plastic infectious waste
- ❖ Halogenated plastics such as PVC should never be incinerated as it generates furans which are carcinogenic.

Autoclave

Autoclaving is a thermal process where steam is brought into direct contact with waste in a controlled manner and for sufficient duration to sterilize the wastes. It is mainly used for the treatment of infectious plastic and sharp waste.

Chemical Disinfection

A chemical such as hypochlorite 1-2% is mixed to waste which results in disinfection. It is more suitable for liquid waste such as discarded blood and body fluid and also for hospital sewage.

Effluent Treatment Plant

The liquid waste (effluent) generated in the hospital if mixes directly with groundwater it can create significant health risks.

- ❖ Therefore, it is first subjected to chemical treatment and then is drained into effluent treatment plant (ETP)
- ❖ ETP removes the suspended solids and organic matter in wastewater and then disinfects the wastewater (with hypochlorite) and finally drain the water to municipal drainage.

Microwaving

Microwaves are radio-frequency waves, used at a frequency of 2450 MHz. They produce friction of water molecules which generates heat. Large size microwaves are used for disposal of BMW—mainly infectious plastics and sharp wastes.

Hydroclaving

Hydroclaving is a low-temperature steam sterilizer, involving steam treatment with fragmentation and drying of waste. It breaks up the waste into small pieces of fragmented material; thus obviates postcycle shredding (unlike autoclave).

Shredder

Shredding is a process by which wastes are de-shaped or cut into smaller pieces so as to make the wastes unrecognizable. It helps in prevention of reuse of BMW and also helps to reduce the waste volume.

Deep Burial

Deep burial is a pit dug about two meters deep. It needs to be half-filled with waste, and then covered with lime within 50 cm of the surface, before filling the rest of the pit with soil. The groundwater level should be a minimum of six meters below the lower level of a deep burial pit.

Sharp Pit

A sharp pit constructed within the hospital premises provides an alternative method for disposal of the sharp wastes generated from the facility (Fig. 24.2).

Encapsulation

Encapsulation method involves filling the containers with waste, adding immobilizing material and sealing



Fig. 24.2: Sharp pit.

Source: Department of Biomedical Waste Management, JIPMER, Puducherry (with permission).

the containers, to prevent the access to unscrupulous activities. The process uses cubic boxes made up of metallic drums which are three quarters filled with sharps or chemicals or pharmaceutical wastes and then filled with a medium such as plastic foam, cement mortar or clay material.

Inertization

The process of inertization involves mixing waste with cement and other substances before disposal to minimize the risk of toxic substances contained in the waste migrating to surface or groundwater. It is especially suitable for pharmaceuticals and for incinerated ashes with a high metal content.

Plasma Pyrolysis

Plasma pyrolysis uses ionized gas in the plasma state to convert electrical energy to temperatures of several thousand degrees using plasma arc torches or electrodes. The system provides high temperatures combined with high UV radiation flux which destroys pathogens completely.

Disposal of Cytotoxic Drug Waste

Expired cytotoxic drugs to be returned back to the manufacturer or supplier or CBMWTF for incineration at $>1,200^{\circ}\text{C}$ or encapsulation or plasma pyrolysis at $>1,200^{\circ}\text{C}$

Disposal of General Waste (Solid Waste)

They constitute the large component of hospital waste (80%). They are not biomedical waste; their disposal can be carried out by several strategies.

- ❖ Composting: It is the decomposition of organic matter by microorganism in warm moist environment
- ❖ Waste-to-energy: By various methods such as incineration, pelletisation, biomethanation, etc.
- ❖ Recycling of the waste
- ❖ Landfilling in dump yard (least preferred method).

MONITORING OF BMW MANAGEMENT

Monitoring is an essential component of managing biomedical waste in the hospital. BMW management committee should be formed in a healthcare facility, which serves several functions—(1) to oversee the implementation of BMW practices, (2) to educate HCWs about BMW practices, and (3) to monitor BMW management in a hospital. Monitoring of BMW management practices can be carried out through the following ways:

- ❖ Biomedical waste segregation audit by direct observation
- ❖ Biomedical waste segregation audit by CCTV camera
- ❖ Onsite inspection of BMW segregation at common storage area
- ❖ Conducting surveys through structured questionnaires
- ❖ Barcoding-based tracking of BMW, starting from segregation to disposal.

EXPECTED QUESTIONS

I. Write short notes on:

1. Categories of biomedical waste.
2. Disposal methods available for biomedical waste.
3. Type of containers used for disposal of biomedical waste.

II. Multiple Choice Questions (MCQs):

1. Anatomical waste should be segregated in which color bags?
 - a. Yellow
 - b. Red
 - c. Blue
 - d. Black
2. Microbiological waste should be segregated in which color bags?
 - a. Yellow
 - b. Red
 - c. Blue
 - d. Black
3. Sharps should be segregated in which color box?

- a. Yellow
- b. Red
- c. Blue
- d. White

4. Solid waste (items contaminated with blood and body fluids including cotton, dressings) belong to which category of biomedical waste?

- a. Yellow
- b. Red
- c. Blue
- d. White

5. Plastic infectious items should be segregated in which color bag?

- a. Yellow
- b. Red
- c. Blue
- d. White

6. Before segregation of microbiological wastes, pre-treatment with what concentration of hypochlorite is recommended?

- a. 1–2%
- b. 5%
- c. 10%
- d. 15%

Answers

1. a 2. a 3. d 4. a 5. b 6. a

CHAPTER PREVIEW

■ Prevention of Needle Stick Injury

■ Post-exposure Management

INTRODUCTION

An occupational exposure is defined as:

- ❖ Percutaneous injury, e.g. needle stick injury (NSI) or other sharp injury
- ❖ Splash injury:
 - Contact with the mucous membrane (e.g. eye or mouth)
 - Contact with non-intact skin (abraded skin or afflicted with dermatitis)
 - Contact with the intact skin when the duration is prolonged (e.g. several minutes or more).

An occupational injury is often loosely termed as needle stick injury though it includes injury through needle or other sharps and splashes.

Agents transmitted:

Hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV are three major blood-borne viruses (BBVs) that are transmitted through NSI. The risk of transmission is highest for HBV (30%) followed by HCV (3%) and HIV (0.3%).

Infectious specimens for NSI:

- ❖ *Potentially infectious body fluids* include blood, genital secretions (semen, vaginal secretions) and all body fluids (CSF, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid)
- ❖ *The following are not considered potentially infectious*, unless visibly contaminated with blood: Feces, nasal secretions, saliva, sputum, sweat, tears, urine and vomitus.

Factors that influence the risk of contracting infection following NSI:

The risk of infection following exposure depends on the following factors:

- ❖ Type of needle (hollow bore needle has a higher risk than solid needle)
- ❖ Device visibly contaminated with blood
- ❖ Depth of injury (higher is the depth, more is the risk)
- ❖ Volume of blood involved in the exposure
- ❖ Viral load present in the blood at the time of exposure
- ❖ Timely performing first aid

- ❖ Timely start of appropriate post-exposure prophylaxis (PEP) for HBV and HIV.

PREVENTION OF NEEDLE STICK INJURY

Precautions During Handling Needles

The following measures should be taken during handling needles to prevent occupational exposures:

- ❖ **Standard precautions** must be followed such as hand hygiene and appropriate use of personal protective equipment (PPE) (e.g. gloves, gowns, masks, and goggles) while handling blood or body fluids
- ❖ **Work surfaces** must be disinfected with 0.5% sodium hypochlorite solution
- ❖ **HBV vaccination:** Health care workers (HCWs) must be immunized against HBV and protective titer must be documented
- ❖ **Spill management:** Spillage of blood and other body fluids must be promptly cleaned and surface disinfected with 0.5% sodium hypochlorite solution
- ❖ **Disposable needles** should be used. Needles should never be reused
- ❖ **Never recap needles:** If unavoidable, single hand-scoop technique may be followed (Figs 25.1A and B)
- ❖ **Disposal after use:** Needles must be disposed into the sharp box immediately after use. Needles/sharps should not be left on trolleys and bedside tables



Figs 25.1A and B: Recapping of needle: **A.** Wrong method; **B.** Correct method (single hand 'scoop' technique).

- ❖ **Engineering control measures:** Various devices are specially designed with safety features to prevent NSI such as retractable lancets, safety lock syringe with a protective sheath and needleless IV systems.

Precautions During Surgical Procedures

Confine and contain approach should be implemented for every surgical procedure.

- ❖ *Passing of sharp instruments* during surgery must be according to the plan decided by the surgeon and his assistant nurse. Sharp instruments should always be passed by non-touch approach, not directly by hands
- ❖ *Suturing:* Needles must never be picked up with the fingers while suturing. Forceps or a needle holder is ideal for holding a needle. Where practical, blunt needles should be used to close the abdomen
- ❖ Preoperative testing of a patient for BBVs is not mandatory; should be performed only if a clinical indication is present
- ❖ **Patient known to have BBV infections** may require the following additional precautions for surgical operation:
 - The lead surgeon should ensure that all members of the team know about infection hazards and appropriate measures should be followed, such as use of *double gloves*
 - The surgical team must be limited to essential members of *trained staff* only
 - It may help theater decontamination if such cases *posted last in the list*, but this is not mandatory.

POST-EXPOSURE MANAGEMENT

Steps of Post-exposure Management

The following are the sequential steps to be followed following an occupational exposure (Table 25.1):

1. **First aid:** First aid has to be started as early as possible (Table 25.2)
2. **Report to the designated nodal center:** Every hospital must have a nodal center for the management of NSI. In most hospitals, HICC office acts as a nodal center, other hospitals may designate staff clinic or casualty for the purpose. Nodal centers perform the following functions as mentioned below (steps 3–9)

Table 25.1: Steps of post-exposure management.

1. First aid
2. Report to designated nodal center
3. Take first dose of PEP for HIV
4. Testing for BBVs
5. Decision on PEP for HIV and HBV
6. Documentation and recording of exposure
7. Informed consent and counseling
8. Follow-up testing of HCWs
9. Precautions during the follow-up period

Abbreviations: PEP, post-exposure prophylaxis; HCW, health-care worker; BBV, blood-borne virus.

Table 25.2: First Aid: Management of exposed site.

Do's	Don'ts
Earlier the first aid, lesser is the chance of transmission of BBVs <ul style="list-style-type: none"> • For splash injury: Irrigate thoroughly the site (e.g. eyes or mouth or other exposed area) vigorously with water at least for 5 minutes • Spit fluid out immediately if gone into mouth and rinse the mouth several times • If wearing contact lenses, leave them in place while irrigating. Once the eye is cleaned, remove the contact lens and clean them in a normal manner 	<ul style="list-style-type: none"> • Do not panic • Do not place the pricked finger into the mouth reflexively • Do not squeeze blood from wound • Do not use antiseptics and detergents

3. Take first dose of PEP for HIV:

- The first dose of PEP for HIV should be taken as early as possible. Effect is maximum if taken <2 hours and effect is nil if taken after 72 hours of exposure
- **NACO recommendation:** The first dose regimen comprises of a fixed-dose combination of five tablets; given on the first day of exposure
 - ♦ Tenofovir 300 mg + Lamivudine 300 mg, one tablet once daily and
 - ♦ Lopinavir (200 mg) + Ritonavir (50 mg) two tablets twice daily.
- If the HIV negative status of the source is documented in patient's case record or in the hospital information system, then the first dose of PEP is not required
- If test report is not available, then administer the first dose regimen immediately without waiting for the laboratory result.

4. Testing for BBVs:

The following tests are done for both source and HCW. The test format should be a rapid method (immuno-chromatographic test or flow through assay) and result should be available within 1–2 hours

- Anti-HIV antibody detection
- HBsAg detection
- Anti-HCV antibody detection
- Anti-HBs antibody (done for HCW if previously vaccinated for HBV and titer not tested).

HCW's baseline status is determined because later it maybe difficult to attribute whether the infection was acquired due to this occupational exposure or any other prior exposure. This may guide while taking a decision, when the HCW claims for compensation from the health authorities.

5. Decision on post-exposure prophylaxis (PEP) for HIV and HBV is taken based on standard guidelines (NACO for HIV and CDC for HBV) as described in Tables 25.3 and 25.4 respectively

- ### 6. Informed consent and counseling:
- Almost every person feels anxious after exposure. They should be counseled and provided with psychological support
- They should be informed about the risks and benefits of PEP medications

- PEP is not mandatory. If the exposed person refuses to take the PEP, it should be documented. However, he should be made to understand about the risk of acquiring infection if PEP is not taken.
7. **Documentation and recording of exposure:**
- A **structured proforma** should be used to collect the detail information related to exposure such as date, time, and place of exposure, type of procedure done, type of exposure, duration of exposure, source status, volume and type of specimen involved
 - **Consent form:** For prophylactic treatment, the exposed person must sign a consent form. If the individual refuses to initiate PEP, it should be

Table 25.3: Revised NACO Guidelines for post-exposure prophylaxis (PEP), 2018.

Exposure code (EC)	Source HIV status code (SC)	PEP Recommendation
1, 2 or 3	Negative	Not warranted
1	1	Not warranted
1	2	PEP is recommended
2	1	Duration of PEP: 28 days
2	2	Primary TL+LR regimen*: Regimen comprises of fixed dose combination of five tablets to be taken every day.
3	1 or 2	<ul style="list-style-type: none"> • Tenofovir (300 mg) + Lamivudine (300 mg), one tablet once daily and • Lopinavir (200 mg) + Ritonavir (50 mg) two tablets twice daily
2 or 3	Unknown (in area with high prevalence)	Alternative TLE regimen: Fixed dose combination of tenofovir (300 mg)+ Lamivudine (300 mg) + Efavirenz (600 mg), one tablet once daily

Source material: Blood, body fluids or other potentially infectious material (CSF, synovial, pleural, pericardial and amniotic fluid, and pus) or an instrument contaminated with any of these substances

Exposure code:

1. **EC-1 (Mild exposure):** Mucous membrane/non-intact skin exposure with small volumes, or less duration

2. **EC-2 (Moderate exposure):**

- Mucous membrane/non-intact skin with large volumes/splashes for several minutes or more duration OR
- Percutaneous superficial exposure with solid needle or superficial scratch

3. **EC-3 (Severe exposure):** Percutaneous exposure with:

- Large volume transfer
- By hollow needle, wide bore needle or deep puncture
- Visible blood on device
- Needle used in patient's artery or vein

Source HIV Status Code (SC):

1. **SC-1:** HIV positive, asymptomatic or low viral load (<400 copies/mL)
2. **SC-2:** HIV positive, symptomatic (advanced AIDS or primary HIV infection), high viral load
3. **SC Unknown:** Status of the patient is unknown and neither the patient nor his/her blood is available for testing
4. **HIV negative:** Tested negative according to NACO strategy

The first dose of PEP

Should be started within 2 hours (for greater impact) and definitely within 72 hours. No need to provide PEP if exposure occurred >72 hours

PEP not required in the following situations:

1. If exposed person is HIV positive: Exposed individuals who are known or discovered to be HIV positive should not receive PEP. They should be referred to ART clinic for counseling and initiation of ART
2. If the exposure is on an intact skin
3. If source is HIV negative
4. Exposure with low-risk specimens like tear, saliva, urine, stool, vomitus, nasal secretion, sweat, etc.
5. For exposures with EC-1 and SC-1
6. Source unknown if HIV prevalence is low
7. In case of delay in reporting the exposure by > 72 hours, PEP initiation becomes optional

Side effects and compliance to PEP:

- Common side effects are:
 - At the initial phase of the course: Nausea, diarrhea, muscular pain, headache or fatigue
 - Later during the course: Anemia, leukopenia or thrombocytopenia
- For most side effects except jaundice or liver tenderness, **PEP should never be discontinued.**
- Compliance of >95% to the PEP schedule is required to maximize the efficacy of PEP. Hence, the person should be counseled to continue the PEP and to take medication to minimize the side effects of PEP.

*Regimen for exposure in pregnant women is essentially same as that of non-pregnant persons.

Abbreviations: NACO, National AIDS Control Organization; ART, antiretroviral therapy.

Table 25.4: Post-exposure prophylaxis (PEP) for hepatitis B.

HCW status	If source is positive or unknown for HBsAg	If source is negative for HBsAg
If the exposed person is completely vaccinated and the antibody titer is protective (≥ 10 mIU/mL)	No further treatment is required: <ul style="list-style-type: none"> • Regardless of the HBV status of the source* • Regardless if the titer falls down later* 	
If the exposed person is completely vaccinated and the titer is not protective (< 10 mIU/mL)	HBIG-1 dose should be started immediately; maximum within 7 days Vaccine: Start the second series (3 doses)	Vaccine: Start the second series (3 doses)
If the exposed person is not vaccinated or partially vaccinated	HBIG-1 dose should be started immediately; maximum within 7 days Vaccine: Complete the vaccine series from the last dose given (do not restart)	Vaccine: Complete the vaccine series from the last dose given (do not restart)
Nonresponders (If the exposed person is vaccinated for 2 series, i.e. 6 doses and the titer is not protective)	HBIG-2 doses at 1 month apart (0.06 mL/kg or 10–12 IU/kg)	Nothing is required

Note:

- HCW is said to be protected when titer rises (anti-HBs ≥ 10 mIU/mL), after three or more doses of vaccination. Rise of titer after one or two doses of vaccine should not be considered as protective.
- HCWs who are not protected must be checked for their HBsAg status at baseline and follow-up testing 6 months later, regardless of their vaccination status.
- Anti-HBs antibody titer should be checked only after 2 months of last dose of vaccine and 6 months after HBIG administration; otherwise, it will give erratic results.
- HBIG and HBV vaccine can be administered simultaneously but at different sites.
- HBIG provides a temporary protection for 3–6 months.
- Previous report of Anti-HBs titer is acceptable only if it is documented. Verbal reports should not be considered.

* In a previously protected person, the memory B cells will start producing antibodies soon after the antigenic challenge, hence revaccination by booster doses is not recommended even if the titer falls down later.

Adapted from CDC guideline, 2013.

Abbreviations: HBIG, hepatitis B immunoglobulin; HCW, health-care worker; HBsAg, hepatitis B surface antigen.

documented. The designated officer for PEP should keep this document.

8. Follow-up testing of HCWs for BBVs should be done if the source status is positive/unknown

- **HIV testing follow-up is done:** At 6 weeks, 3 months and 6 months after exposure
- HBV and HCV follow-up testing is done at 6 months after exposure.

9. Precautions during the follow-up period: If the source status is positive/unknown, then the following

precautions should be adopted by the HCW during the follow-up period, especially the first 6–12 weeks

- Refraining from blood, semen, organ donation
- Abstinence from sexual intercourse or use of latex condom till both baseline and 3 months HIV tests are found negative
- Women should not breastfeed their infants
- The exposed person is advised to seek medical evaluation for any febrile illness that occurs within 12 weeks of exposure.

EXPECTED QUESTIONS

I. Write short notes on:

1. Sequential steps to be followed after a needle stick injury.
2. Post-exposure prophylaxis for HIV.
3. Post-exposure prophylaxis for hepatitis B.

II. Multiple Choice Questions (MCQs):

1. The decreasing order of risk of transmission following occupational exposure:

- a. HIV>HBV>HCV
- b. HBV>HIV>HCV
- c. HBV>HCV>HIV
- d. HCV>HBV>HIV

2. All are potentially highly infectious specimen for occupational injury; except:

- a. Blood
- b. Semen
- c. CSF
- d. Saliva

3. The sequence of steps to be followed after accidental exposure to blood/fluid:

- a. First aid → Reach to nodal center and try to get report of source status → Take first dose of PEP for HIV → Testing of source and HCW status for BBVs → Prophylactic treatment for HIV and HBV
- b. Reach to nodal center and try to get report of source status → First aid → Take first dose of PEP for HIV → Testing of source and HCW status for BBVs → Prophylactic treatment for HIV and HBV
- c. Take first dose of PEP for HIV → Testing of source and HCW status for BBVs → First aid → Reach to nodal center and try to get report of source status → Prophylactic treatment for HIV and HBV
- d. Prophylactic treatment for HIV and HBV → Take first dose of PEP for HIV → Testing of source and HCW status for BBVs → First aid → Reach to nodal center and try to get report of source status

Answers

1. c 2. d 3. a

CHAPTER PREVIEW

- Implementation of Antimicrobial Stewardship Program
- Monitoring the Compliance to Antimicrobial Stewardship Program
- Rational Use of Antimicrobial Agents
- Hospital Antibigram

INTRODUCTION

Antimicrobial stewardship program (AMSP) provides strategies for rationalizing the use of antimicrobials in the hospital.

Definition

Centers for disease control and prevention (CDC) has defined antimicrobial stewardship as use of the right antimicrobial agent, for the right patient, at the right time, with the right dose, route and frequency, causing the least harm to the patient and future patients.

Why AMSP is Needed?

Antimicrobial stewardship program in a hospital is required for the following reasons.

Antimicrobial Resistance (AMR)

AMR is a rising threat across the globe. The multidrug resistant organisms (MDROs) are prevalent in every country though the extent and the severity of the problem varies. Extensive use of antimicrobials is the single most important factor for the bacteria to undergo mutation, which leads bacteria to become resistant to antimicrobials and then the resistant strain flourish exponentially in the presence of selective pressure of antimicrobials.

Misuse and Over-use of Antimicrobials

The last eight decades since the discovery of penicillin witnessed the saving of millions of lives due to use of antimicrobials in treating infections. However, at the same time, this has also led to their misuse through various ways—(1) use without a prescription, (2) overuse for self-limiting infections, non-bacterial infections and (3) treatment of colonizer/contaminant.

Widespread Use of Antimicrobials in Other Sectors

World's largest antimicrobial use occurs for animal non-therapeutic purpose (70%), followed by animal therapeutic purpose (15%). Human use accounts only 15%

of total antimicrobial consumption, out of which only 9% is being used for human therapeutic purpose. This data explains that just bringing in stewardship program in health care facility would not bring down antimicrobial use dramatically. A robust plan should also be in place for control of antimicrobial use in animals.

Poor Antimicrobial Research

Research in the development of new antimicrobial is a huge investment for the pharmaceutical industry. More so, soon after the discovery of an antimicrobial, the bacteria develop resistance mechanisms to tackle the antimicrobial. As a result, investment goes waste. It is also hypothesized that there could be a return to the pre-antibiotic era, where many people could suffer or die from untreatable bacterial infections.

IMPLEMENTATION OF ANTIMICROBIAL STEWARDSHIP PROGRAM

The key steps of implementation of AMSP in a hospital is as follows.

Administrative Support (Leadership)

The most important prerequisite for implementing AMSP is a strong administrative support. They should be publicly committed to the program and provide necessary funding and infrastructure support.

Formulating AMS Team

Antimicrobial stewardship team (AMS team) is a multidisciplinary committee which is responsible for framing, implementing and monitoring the compliance to antimicrobial policy of the hospital.

- ❖ AMS team is led by the antimicrobial steward who may be an infectious disease physician or infection control officer or clinical microbiologist
- ❖ **Antimicrobial steward** is the central driving force behind this program. A larger hospital may require more than one antimicrobial steward

- ❖ Other members of AMS team include stewardship nurses, clinical pharmacists and officer in-charge of pharmacy.

Infrastructure Support

Infrastructure support is essential to initiate appropriate pathogen-directed antimicrobial agent at the earliest.

- ❖ **Support from the microbiology laboratory**
 - **Automations:** Facility for automated culture (e.g. BACTEC, BacT/ALERT or Virtuo), identification (MALDI-TOF) and sensitivity (e.g. VITEK) should be available. This reduces the turn-around time to 24–48 hours; compared to conventional cultures which takes 2–5 days
 - **Biomarkers:** Facility for testing biomarkers such as procalcitonin and C-reactive protein (CRP) must be available (discussed subsequently)
 - **Molecular tests:** Facility to perform rapid molecular tests must be available; e.g. Biofire FilmArray multiplex PCR
 - **Emergency laboratory:** Emergency lab functioning round the clock is a marker of a quality microbiology laboratory.
- ❖ **Hospital information system (HIS):** Fully functional HIS including laboratory information system will augment the stewardship program by many folds
- ❖ Supporting manpower availability.

Framing Antimicrobial Policy

Every hospital should frame their own hospital antimicrobial policy which is usually a pocket handbook, comprising of system/syndrome wise indications for antimicrobial choice and their dosage.

- ❖ It should be prepared by AMS team after discussing with all the clinicians, microbiologists and administrators
- ❖ The policy must be compliant to the standard national and international antimicrobial guidelines and local antibiogram pattern
- ❖ Common consensus between all clinicians must be arrived, before framing the policy; which facilitates better adherence to policy.

Implementing AMS Strategies

Two types of strategies are available for implementing AMSP.

- ❖ Front end strategies (formulary restriction)
- ❖ Back end strategies (prospective audit and feedback).

Front End Strategy (Formulary Restriction)

This involves classifying antimicrobial agents into restricted, semi-restricted and non-restricted antimicrobials with indications for their use combined with an approval system regulated by the AMS team (Table 26.1).

- ❖ This strategy sounds more attractive, impact is immediate and appears to be the most ideal way to

Table 26.1: Proposed formulary restriction.

Restricted antimicrobials	Semi-restricted antimicrobials	Unrestricted antimicrobials
Pharmacy supply of >1 days requires prior approval by AMS team	Pharmacy supply of >3 days requires prior approval by AMS team	Pharmacy supply does not require AMS team approval
Colistin Carbapenem Tigecycline	Teicoplanin, Vancomycin, Daptomycin, Linezolid, Third and fourth generation cephalosporins	First and second generation cephalosporins, Cotrimoxazole, Azithromycin, Clarithromycin, Fluoroquinolones

achieve antimicrobial stewardship, but practically implementing formulary restrictions is challenging and a difficult task

- ❖ It creates a lot of confusion as it directly compromises the clinician's freedom to choose antimicrobials
- ❖ More so availability of the AMSP consultants to give approval all the time further complicates the problem, especially in emergency situations.

Back End Strategy

This is carried out by **prospective audit and feedback**. Though difficult to perform, but it is the most effective strategy to implement AMSP.

- ❖ The AMS team goes for stewardship round during which they discuss with the clinical team in detail about the compliance to the antimicrobial policy in terms of appropriateness of the antimicrobials used, dosage with renal adjustment, compliance to susceptibility report, etc. The clinical team gives justification about the non-compliance occurred, if any
- ❖ The prospective audit and feedback is a mutually agreed upon constructive discussion between AMS team and the clinical team on the cases with daily follow up
- ❖ Although the back end strategy is more labor-intensive, it has several advantages:
 - It is more widely practiced
 - It is more easily accepted by clinicians
 - It provides a higher opportunity for educating and training health care professionals
 - Impact is delayed but sustainable improving the overall quality of antimicrobial prescribing practice.

Education and Training

Similar to any other health care program, AMSP also needs continuous education, training, motivation and assessment of the health care providers. Developing antimicrobial stewardship is a behavioral change within the person. Hence, adequate motivational education is a must to bring in such change.

MONITORING THE COMPLIANCE TO ANTIMICROBIAL STEWARDSHIP PROGRAM

It is said that “If you cannot measure it, you cannot improve it”. Measurement of the compliance to AMSP is achieved by looking at both process and outcome indicators.

1. **Policy adherence indicator** (process indicator): This is achieved by conducting antimicrobial stewardship audit as described under backward strategy. Both prescription and administrative compliance can be calculated

Indicators of Prescription Compliance

Examples of prescription compliance indicators include:

- ❑ Percentage of time the empirical antibiotic given, is according to the infective syndrome suspected
- ❑ Percentage of time the empirical antibiotic is modified according to antimicrobial susceptibility report.
- ❑ Percentage of time cultures are taken before the start of antibiotics
- ❑ Percentage of time the choice of surgical antimicrobial prophylaxis given is according to the policy

Indicators of administrative compliance

Examples of administrative compliance indicators include:

- ❑ Percentage of time the antibiotic is administered in correct dose, correct frequency, correct route (IV, oral or infusion)
- ❑ Percentage of time the surgical antimicrobial prophylaxis is administered in correct dose, correct time and correct frequency

2. **Antimicrobial usage outcome indicators** such as defined daily dosage (DDD) and days of therapy (DOT). These indicators are used to estimate the antibiotic consumption. They are discussed below
3. **AMR outcome indicator:** The change in AMR pattern is analyzed by conducting periodic AMR surveillance
4. **Clinical outcome indicators** such as morbidity (e.g. length of stay) and mortality (e.g. infection-related deaths) indicators
5. **Financial outcome indicators** such as antimicrobial cost per patient day or per year or per admission.

DDD (Defined Daily Dosage)

Defined Daily Dose (DDD) is the average maintenance dose per day for a drug used for its main indication in adults.

- ❖ Therapeutic dose should not be used for calculating antibiotic usage because it varies between the persons depending upon the weight, disease type, associated factors such as renal adjustment, etc. Therefore, DDD is a better indicator to calculate the antimicrobial consumption
- ❖ WHO assigned DDD are available in website, which should be used while calculating the number of DDDs consumed
- ❖ DDD cannot be used for estimating antibiotic consumption in patients with renal failure and

pediatric patients, because the daily dose actually prescribed is typically lower than the average dose defining the DDD.

Calculation of DDDs

$$\text{No. of DDDs} = \frac{\text{Therapeutic dose (No. of tablets/vials used} \times \text{gm per tablet/vial)}}{\text{WHO defined DDD of the antimicrobial agent}}$$

Example

Levofloxacin is administered as 750 mg PO daily for 7 days. The WHO assigned DDD for levofloxacin is 0.5 g.

Therefore the number of DDD is calculated as:
 $= (0.75 \text{ g dose} \times 7 \text{ days} / 0.5 \text{ g DDD}) = 10.5 \text{ DDDs}$

Days of Therapy (DOT)

DOT of an antibiotic is the number of days that patient receives at least one dose of that antibiotic. It can be used for estimating antibiotic consumption in patients with renal failure, pediatric patients and therefore is preferred over DDDs.

Examples include:

- ❑ A patient has received meropenem 1 g, twice daily for 3 days; the DOT is 3
- ❑ A patient has received meropenem 0.5 g, thrice daily for 3 days; the DOT is 3
- ❑ A patient has received meropenem 1 g, twice daily and vancomycin 1g thrice daily for 3 days; the DOT is 3+3=6

RATIONAL USE OF ANTIMICROBIAL AGENTS

When prescribing antimicrobial agents, the clinicians should consider the following advice.

Prescribe Only when Indicated

Prescribe antibiotics only when it is indicated. There are various conditions where antibiotics are not required.

- ❖ **Diarrhea:** Oral rehydration solution is the mainstay of treatment, not antibiotics. More so, the most common cause of diarrhea is of viral etiology
- ❖ **Upper respiratory tract infections** such as common cold and sore throat, where the primary cause is viral infections (except when bacterial infections such as streptococcal sore throat or diphtheria are strongly suspected)
- ❖ When an **alternative diagnosis** is suspected/confirmed such as dengue, chikungunya, malaria, etc.
- ❖ **Prophylaxis:** Routine antibiotic prophylaxis should not be given to prevent infection, except for particular situations such as cotrimoxazole prophylaxis in HIV-infected individuals.

Culture of Cultures

Antibiotics should always be started only after site-specific specimens are collected for culture. If specimens are

collected after antibiotic start, then cultures become false-negative and thus it will not help in targeted therapy.

Empirical vs Targeted Therapy

Empirical therapy: Empirical antibiotic should not be given randomly, but based on three important elements.

- ❖ The infective syndrome likely to be present
- ❖ The common etiological bacterial agents for that infective syndrome
- ❖ The local antibiogram for those organisms, indicating the antimicrobial resistance pattern.

Targeted or pathogen-directed therapy: The empirical therapy should be modified subsequently, based on antimicrobial susceptibility test (AST) report. The modifications may be of two types—escalation or de-escalation.

Escalation vs De-escalation Approach

There are two approaches by which antimicrobial agents are prescribed—escalation and de-escalation.

- ❖ The approach needs to be chosen based on local antimicrobial resistance pattern and the spectrum of activity of the antibiotic
- ❖ Antibiotics prescribed for an organism can be ranked based on their spectrum of activity and local antimicrobial resistance pattern
- ❖ For example; In hospital X, the antibiotics given for gram-negative organisms such as *E. coli* are ranked according to decreasing order of susceptibility: colistin (rank-1) → tigecycline → carbapenems → piperacillin-tazobactam → cefoperazone sulbactam → amikacin → cefepime → ceftazidime → cotrimoxazole → ceftazidime → ciprofloxacin → ceftriaxone (lowest rank).

Escalation Approach

This approach is chosen if local antimicrobial resistance pattern is unlikely and/or the patient is clinically stable. The empirical therapy is started with a narrow spectrum antibiotics (e.g. ceftriaxone for *E.coli*). If AST report shows resistance, then can be escalated to a higher rank antibiotic subsequently (e.g. meropenem for *E.coli*).

De-escalation Approach

This approach is chosen if local antimicrobial resistance pattern is expected to be high and/or patient is critically ill. Empirical therapy is started with broad spectrum antibiotics (e.g. meropenem for *E.coli*). If AST report shows susceptible, it can be de-escalated to a narrow spectrum antibiotic subsequently (e.g. ceftriaxone for *E.coli*).

However, the reserved/restricted antimicrobials such as colistin and tigecycline should not be given as empirical therapy even under de-escalation strategy. They should be prescribed only when AST report is available and shows resistant results to all other antimicrobials tested.

Site-specific Antimicrobials

Only those antimicrobials should be prescribed which are active at the infection site. The following antimicrobials are not active at the respective sites and therefore should be excluded from treatment.

- ❖ **Lungs:** Daptomycin is not active at respiratory site as it gets inactivated by pulmonary surfactants
- ❖ **CSF:** Any oral antibiotic, 1st and second generation cephalosporins, tetracyclines, macrolides, quinolones and clindamycin are not active in CSF
- ❖ **Urine:** Antibiotics such as chloramphenicol, macrolide and clindamycin should be avoided in UTI; as they do not achieve adequate urinary concentrations.

Avoid Administration Errors

Antimicrobials must be administered at the correct dose (as per the age/body weight), and frequency and duration of therapy.

- ❖ **Loading dose:** Certain concentration dependent antimicrobials such as aminoglycoside, vancomycin and colistin should be administered with a loading dose
- ❖ **Infusion:** The efficacy of certain antimicrobials such as vancomycin is better when mixed with saline and given as an IV infusion over 2–3 hours
- ❖ **Renal adjustment:** The dosage of the nephrotoxic drugs (such as aminoglycoside, vancomycin, and colistin) should be adjusted according to the creatinine clearance.

MIC-guided Therapy

The AST can be performed by disk diffusion or by MIC (minimum inhibitory concentration)–based method; the latter being more accurate and reliable. There are certain situations, where the antibiotic treatment is MIC-guided.

- ❖ Clinical conditions such as endocarditis, pneumococcal meningitis/pneumonia, etc.
- ❖ Vancomycin for *S. aureus*: Vancomycin should be avoided if MIC is >1 µg/mL.

MIC helps to select the most appropriate antibiotic: Lower is the MIC, better is the therapeutic efficacy. If >1 antimicrobial agents are found susceptible, then the antibiotic having the lowest MIC (when compared with the susceptibility breakpoint) should be chosen for therapy. This is better guided by calculating the **therapeutic index**.

Therapeutic Index

It is the ratio of susceptibility breakpoint divided by MIC of the test isolate.

Therapeutic index = Susceptible breakpoint/Test MIC

Higher the therapeutic index, better is the efficacy of the antimicrobial agent.

For example, if a clinical isolate of *E.coli* is susceptible to both meropenem (MIC 1 µg/ml) and amikacin (MIC 8 µg/ml), then meropenem falsely appears to be more efficacious as its absolute MIC value is lower than amikacin. However, the MIC

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value compared with the standard susceptibility breakpoint (and not the absolute MIC value) determines therapeutic efficacy.

Susceptible breakpoints of meropenem and amikacin for *E.coli* are 1 µg/mL and 16 µg/mL respectively, according to CLSI guideline 2020.

The therapeutic index is calculated as:

□ Therapeutic index of meropenem = 1/1 = 1

□ Therapeutic index of amikacin = 16/8 = 2

Therefore, in this case amikacin is superior to meropenem.

Therapeutic Drug Monitoring

Therapeutic efficacy is not only relies on in vitro susceptibility result (MIC), but also dependent on in vivo activity which is in turn dependent on pharmacokinetic and pharmacodynamics (PK/PD) parameters of the antimicrobial agent.

- ❖ Therefore, therapeutic drug monitoring is necessary to find out how the drug behaves in vivo
- ❖ It is particularly important for antibiotic such as vancomycin, amikacin and colistin.

Depending up on PK/PD parameters, antibiotics are classified as:

- ❖ **Concentration dependent antibiotics**, e.g. aminoglycosides: They work better if the drug concentration in serum is much higher than the MIC of the drug (determined in vitro). They are usually given as a loading dose so that the drug concentration reaches its peak immediately
- ❖ **Time-dependent antibiotics**, e.g. beta-lactams: Here, the efficacy of the drug is dependent upon how much time the drug concentration remains higher than the MIC. Therefore, these drugs are given in frequent intervals (e.g. thrice daily) so that the drug level in serum would remain always higher than the MIC.

Timely Stoppage of Antimicrobial

Antimicrobial agent must be stopped at appropriate time, which may be determined by clinical improvement or after obtaining negative culture or by use of biomarkers.

Biomarkers-guided Therapy

Biomarkers such as procalcitonin (PCT) or C-reactive protein (CRP) may be used for predicting bacterial infection. PCT is more reliable marker than CRP.

Procalcitonin (PCT)

It is a peptide precursor of the hormone calcitonin, secreted at very low level (<0.05 ng/mL) normally by the body, but the level goes up by manifolds in bacterial infection. The various diagnostic application of PCT include:

- To differentiate bacterial vs viral infection

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- For predicting bacterial infections such as local or systemic infection (e.g. bacterial sepsis, or bacterial pneumonia), depending upon PCT level and associated clinical presentation
 - 0.05–2.0 ng/mL indicates possible local infection
 - 2–10 ng/mL indicates systemic infection
 - >10 ng/mL indicates septic shock
- Helps to decide whether to continue antibiotics in culture-negative cases
- Helps to decide whether to stop antibiotics
- Severity: PCT levels parallel with the severity of the bacterial infections; higher the PCT level, severe is the infection. Therefore PCT can be used as prognostic indicator. However, PCT should not be used alone, but should always be used in adjunct with cultures.

Misuse of Antimicrobials

The following are the common examples of misuse of antimicrobials which should be avoided.

- ❖ **Avoid overlapping spectra:** Meropenem and piperacillin–tazobactam combination therapy for double gram-negative coverage should be avoided as both these drugs belong to beta-lactam group of antimicrobials and therefore share similar antibacterial spectra
- ❖ **Redundant antibiotic:** Meropenem and metronidazole combination therapy for suspected gram-negative/anaerobic sepsis should be avoided as meropenem is active against anaerobes in addition to gram-negative bacteria. Therefore, metronidazole can be withdrawn from therapy
- ❖ **Ineffective antibiotic:** Cloxacillin in MRSA (methicillin resistant *S. aureus*) infection is ineffective and therefore should be avoided. Vancomycin is the drug of choice for MRSA
- ❖ **Inferior antibiotic:** Vancomycin is an inferior cell wall acting agent compared to cloxacillin for MSSA (methicillin susceptible *S. aureus*) infection.

HOSPITAL ANTIBIOGRAM

An **antibiogram** is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial agents (Table 26.2). It is

Table 26.2: Hospital antibiogram of gram-negative bacteria for the year 2020, expressed in terms of susceptibility rate.

	Ciprofloxacin	Amikacin	Ceftazidime	Piperacillin-tazobactam	Meropenem	Colistin
<i>E. coli</i>	25	85	55	75	82	99
<i>Klebsiella</i>	15	75	35	65	70	85
<i>Pseudomonas</i>	32	80	60	80	80	99
<i>Acinetobacter</i>	29	70	10	18	20	99

the responsibility of the department of Microbiology to construct a hospital antibiogram and share it with clinicians. It has the following uses.

- ❖ Antibiogram guides the clinicians in selecting the best empirical antimicrobial treatment in the event of pending culture and susceptibility results
- ❖ It is also a useful tool for detecting and monitoring trends in antimicrobial resistance within the hospital
- ❖ Antibiogram can also be used to compare susceptibility rates across institutions and track resistance trends and thereby contributing to national AMR surveillance database.

EXPECTED QUESTIONS

I. Write short notes on:

1. Strategies of antimicrobial stewardship program.
2. Monitoring of antimicrobial stewardship program.

II. Multiple Choice Questions (MCQs):

1. Antimicrobial stewardship program in a hospital is required for the following reasons, *except*:

- a. Rapid development of antimicrobial resistance
- b. Misuse and over-use of antimicrobials
- c. Widespread use of antimicrobials in humans compared to animal industry
- d. Poor antimicrobial research

2. Who can act as antimicrobial steward?

- a. Infectious disease physician
- b. Clinical microbiologists
- c. Medicine consultant
- d. Any of the above

3. Which is not a Back End Strategy of Antimicrobial stewardship program?

- a. Prospective audit and feedback is an example
- b. Formulary restriction is an example

- c. It is labor intensive than front-end strategy
- d. Sustainable than front-end strategy

4. Which is the correct method of framing antimicrobial policy by Antimicrobial stewardship (AMS) team?

- a. AMS team discuss with each other and frame the policy based on the standard guideline
- b. AMS team discuss with each other and frame the policy based on the standard guideline as well as local AMR pattern
- c. AMS team discuss with each other and frame the policy based on the standard guideline as well as local AMR pattern, then discuss with each clinicians for their suggestions
- d. AMS team just copy the guideline from any other renowned institute of India

5. Maximum consumption of antibiotics occurs for:

- a. Human therapeutic use
- b. Human non-therapeutic use
- c. Animal therapeutic use
- d. Animal non-therapeutic use

Answer

1. c 2. d 3. b 4. c 5. d

CHAPTER PREVIEW

■ Water Surveillance

■ Air Surveillance

■ Surface Surveillance

The environment in the hospital plays an important role in the occurrence of healthcare-associated infections. The various environmental sources from which microorganisms can be transmitted to patients and healthcare workers include water, air and environmental surfaces. Therefore, monitoring of microbiological quality of water, air and surfaces are of paramount importance for safe hospital environment.

INDICATIONS

Microbiological sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is an expensive and time-consuming process. Therefore, random, or periodic sampling is not recommended. CDC recommends to perform targeted microbiological sampling of air, water and surfaces for defined indications, as given below.

- 1. Outbreak investigation:** To determine whether environmental microorganisms are the source of the outbreak
- 2. To evaluate the change in infection control practice:** For example,
 - Assessing a new method introduced for equipment sterilization
 - Assessing the change in housekeeping practices.
- 3. Construction:** For example,
 - During construction or renovation work in the hospital premises, it is necessary to assure that the environment is clean
 - Commissioning newly constructed space in special care areas (OTs and transplant units).
- 4. Research purpose** such as studying the environmental microbial contamination that compared HAI rates in an old hospital and a new facility before and shortly after occupancy.

Routine environmental microbiological sampling is **not recommended** as part of a quality assurance program except for the following situation where routine sampling is indicated.

- ❖ Biological monitoring of sterilization processes by using bacterial spores
- ❖ Monthly conducting water surveillance in hemodialysis units for the final dialysate use
- ❖ Use of **air particle counter** for regular maintenance of the air handling system, biological safety cabinets.

WATER SURVEILLANCE

Waterborne Pathogens

Hospital water and water-containing devices may serve as a reservoir of healthcare associated waterborne pathogens. Microbial contamination of water in healthcare settings is broadly of two types.

Category 1 (Enteric Pathogens)

This results from fecal contamination of drinking water supplies. This group of waterborne pathogens are common in community settings than in hospitals. These agents are transmitted by ingestion of contaminated water. Most of them cause diarrheal outbreaks, while few agents cause extraintestinal illness. Examples include:

- ❖ **Bacteria:** Gram-negative bacilli such as diarrheagenic *E.coli* such as O157:H7, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Campylobacter*
- ❖ **Viruses:** Rotavirus, norovirus, hepatitis A and E viruses, polioviruses
- ❖ **Parasites:** *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, and *Schistosoma* spp.

Category 2 (Common Hospital Pathogens)

These include multidrug resistant gram-negative bacilli (MDR-GNB), nontuberculous mycobacteria (NTM), legionellae, etc.

- ❖ This group of waterborne pathogens are more important in hospital-setting, than community
- ❖ They are commonly present in hospital environment and can contaminate various hospital water reservoirs

Bacteriological examination	Indications
Multiple-tube method	<ul style="list-style-type: none"> Extensively used for drinking water analysis For highly turbid samples
Membrane filtration method	<ul style="list-style-type: none"> For testing dialysis water For testing clean water, where the bacterial count in water is expected to be low For testing large volume of water
Presence/absence method	To monitor quality of drinking water where contamination is likely to be rare

such as potable water, sinks, dialysis water, ice and ice machines, etc.

- ❖ They are transmitted by various routes such as ingestion, contact, aspiration, etc.
- ❖ Waterborne outbreaks caused by these pathogens and their reservoirs in healthcare settings have been a serious threat to high-risk patients who are critically ill or immunocompromised.

The methods employed for detection of microbial contamination of water must have capability to detect both the category of pathogens (Table 27.1).

Test of Drinking Water Contaminated with Enteric Pathogens

Hospital drinking water should be free of enteric pathogens and safe for drinking. Therefore, water supplies should be regularly tested to confirm that they are free from such contamination.

- ❖ However, it is impracticable to attempt directly to detect the presence of all types of enteric pathogen in water because they are usually present in minute quantity; such as *Shigella*, *Salmonella*, etc.
- ❖ Instead, it is wise to test the water supplies for those microorganisms which indicate that fecal contamination has taken place. These organisms are called as indicator organisms (Table 27.2).

Indicator Organisms

Indicator organisms are usually the commensal bacteria of intestine which satisfy two properties:

1. They should be present in excess number than any pathogen so that they can be detected easily; at the same time, they should not be able to proliferate in water to any extent
2. They should be more resistant than the pathogens to the stresses of aquatic environment and disinfection processes.

Indicator organisms themselves are not pathogens, but their presence in water supplies indicates that there is a contamination of sewage and the water supplies needs to be disinfected. However, it is also to be kept in mind

Indicator organisms	Interpretation
Presence in water indicates:	
Coliform (other than <i>Escherichia coli</i>)	Remote contamination—either by fecal (presumptive) or soil and vegetation
Fecal (thermotolerant) <i>Escherichia coli</i>	Confirms recent fecal contamination of water; most sensitive indicator
Fecal streptococci	Confirms remote fecal pollution
<i>Clostridium perfringens</i>	Remote contamination
<i>Pseudomonas aeruginosa</i>	Least reliable indicator Useful in hospitals and food establishments
Bacteriophages	Phage specific for <i>E.coli</i> —indicate fecal pollution of water Indirectly indicates viral pollution

that mere presence of these indicator organisms does not assure the presence of water borne pathogens.

There are a number of intestinal commensals, used as indicator organisms (Table 27.2), described below.

Coliform (Presumptive Coliform)

They are the members of Enterobacteriaceae that are found in human and animal intestine and also present in large numbers in sewage. Therefore, detection of coliforms in higher concentrations than pathogenic bacteria is used as an index of the potential presence of enteric pathogens in water.

- ❖ Among them, only *Escherichia coli* is a reliable indicator which is not found in other sources (described below)
- ❖ However other coliforms such as *Klebsiella*, *Enterobacter*, etc. are much less abundant in feces than *E.coli*, and survive for longer time than *E.coli*
- ❖ They are also found in the environment as saprophytes
- ❖ Therefore, their presence in water may indicate either remote fecal pollution of water (long enough to have allowed *E.coli* to die out) or contamination from soil and vegetations.

Thermotolerant or Fecal *Escherichia coli*

It is regarded as the most reliable indicator of fecal pollution of water.

- ❖ Fecal *E.coli* is the most abundant coliform in human and animal intestine and is derived almost exclusively from these sources
- ❖ It does not survive in water for long time, and therefore is the *best indicator of recent human or animal fecal pollution of water*
- ❖ Its presence in water indicates a potentially dangerous fecal pollution of water
- ❖ They can ferment lactose at 44–45°C with production of acid and gas.

Other indicator organisms are less reliable of fecal pollution of water, which include fecal streptococci, sulphite reducing clostridia (*C. perfringens*), *Pseudomonas aeruginosa*, and bacteriophages (Table 27.2).

Collection and Transport of Water Sample

Water specimen should be collected in a screw-capped wide sterile container.

- ❖ **Volume:** At least 150–200 mL of water should be collected
- ❖ **Neutralizer:** Sodium thiosulfate is added to neutralize the bactericidal effect of residual chlorine present in water if any
- ❖ **Sampling points** in hospitals must represent different sources from which water is obtained such as portable water from pipelines, endoscopy rinse water, dialysis water, dental chair unit waterline, etc.
- ❖ **Sampling method from the tap:** Care should be taken while collecting water to minimize extraneous contamination. Hand washing should be performed and gloves should be worn before collection (Fig. 27.1)
- ❖ **Water from streams or lake or swimming pool:** The bottle should be opened only after immersed at a depth of 30 cm with its mouth facing the current
- ❖ **Tap swabs:** Sterile swab is inserted into the nozzle of the tap carefully, without touching the outer tap surface. The swab is then rubbed around— that is, moved backwards and forwards and up and down, as much as possible, on the inside surface of the tap outlet or flow straightener (Fig. 27.1).

Multiple-tube Method

It is the most common method used for water surveillance. It is so named as it involves mixing of specific volume of water samples to multiple tubes containing a special culture medium—MacConkey purple broth.

- ❖ **Procedure:** Most of the hospital water supplies are non-turbid and unpolluted. As per WHO guideline, for testing of unpolluted water samples the following method should be followed (Fig. 27.2)
 - 50 mL of water is added to one tube having 50 mL of culture medium
 - 10 mL of water is added to each of five tubes containing 10 mL of culture medium.



Fig. 27.1: Water sampling methods.



Fig. 27.2: MacConkey purple broth for multiple tube method (one 50 mL and five 10 mL tubes are needed for testing unpolluted water); if negative, media appears purple; if positive, media turn yellow.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ **Positive result:** After incubated for 24–48 hours, the medium turns to yellow from purple (due to lactose fermentation), along with turbidity of the medium and gas collected in the Durham's tube
- ❖ **Determination of MPN:** The number of tubes giving positive reaction is compared with McCrady statistical table (Table 27.3) to determine the most probable number (MPN) of coliform count present per 100 mL of water. This is called as presumptive coliform count.

For example: As shown in Figure 27.2, multiple tube method performed on an unpolluted water sample gives positive result for one 50 mL tube and two numbers of 10 mL tubes. When matched with Table 27.3, the presumptive coliform count (MPN) is estimated as 6 coliforms per 100 mL water sample

Table 27.3: MPN values per 100 mL of sample for non-polluted/treated water samples (McCrady statistical table).

No. of tubes giving a positive reaction out of		MPN per 100 mL of water
1 of 50 mL	5 of 10 mL	
0	0	<1
0	1	1
0	2	2
0	3	4
0	4	5
0	5	7
1	0	2
1	1	3
1	2	6
1	3	9
1	4	16
1	5	>18

Table 27.4: Classification of quality of drinking water supply.

Quality of drinking water supply	Most probable number (MPN)/100 mL of water	
	Coliform count/100 mL	Thermotolerant <i>E. coli</i> count/100 mL
Excellent	0	0
Satisfactory	1–3	0
Intermediate	4–9	0
Unsatisfactory	≥10	≥1

- ❖ **Quality of water supply:** Depending upon the MPN/100 mL, the quality of the water specimen can be interpreted as excellent, satisfactory, intermediate or unsatisfactory (Table 27.4).

Differential Coliform Count (Eijkman Test)

Detection of coliform bacteria does not always indicate fecal contamination as some of them may be found in environment. Hence, it is further tested by differential coliform count to detect the fecal *E. coli*. This is done by sub-culturing the positive tubes (from the multiple tube method) on lactose containing medium such as brilliant green bile broth for:

- ❖ Detection of lactose fermentation with the production of acid and gas at 44°C and
- ❖ Demonstrating a positive indole test at 44°C.

Membrane Filtration Method

This method is based on the filtration of a known volume (e.g. 100 mL) of water through a cellulose membrane of pore size 0.2 or 0.45 µm.

- ❖ **Procedure:** The bacteria retained on the surface of the membrane filter are transferred into a Petri dish containing a suitable medium (e.g. membrane lauryl sulphate broth) and incubated
- ❖ **Result:** Characteristic yellow colonies of coliforms are produced, which can be counted directly to obtain CFU/100 mL of water
- ❖ **Confirmation:** The colonies are further processed for detection of lactose fermentation with the production of acid and gas at 44°C to check for thermotolerant *E. coli*
- ❖ **Advantages:** Membrane filtration is the recommended method for—(1) testing dialysis water, (2) for testing clean water, where the bacterial count in water is expected to be low and (3) for testing a large volume of water
- ❖ **Disadvantages:** It is not suitable for turbid water. Expensive than multiple tube method.

Presence Absence Method

This is a qualitative method, detects just the presence or absence of the organism in water.

- ❖ **Method:** Various commercial kits are available such as Manjas method where H₂S coated strips used for detection of *Salmonella* in water

- ❖ **Advantages:** This test is useful for monitoring good-quality drinking-water where positive results are known to be rare and in outbreak situation where urgent report is needed.

Test of Water Contaminated with Healthcare Associated Pathogens

Most of the healthcare associated pathogens are recovered by membrane filtration method, followed by plating on to a suitable culture medium; for e.g. *Legionella*, on to buffer charcoal yeast extract (BCYE) medium.

Endotoxin Detection

Endotoxins are the component of the cell wall of gram-negative bacteria. Dialysis water, devices, etc. contaminated with endotoxin can elicit a variety of inflammatory responses in our body and thereby cause serious toxic effects. Therefore, apart from bacteriological testing, the dialysis water used for hemodialysis is also tested for presence of endotoxin.

- ❖ **Methods:** Three methods are available—gel clot assay (Limulus amoebocyte lysate assay) and turbidimetric method and chromogenic technique
- ❖ **Permissive level:** Water used to prepare dialysate and to reprocess hemodialyzers should contain endotoxin unit <0.25 EU/mL
- ❖ Endotoxin testing is also performed for testing injectable **pharmaceuticals** (drugs, solutions), which may get contaminated during manufacture of medical devices (e.g. endoscopes) which may get contaminated while in use.

AIR SURVEILLANCE

Air is an important vehicle of transmission of many pathogenic organisms. Therefore, the examination of air to detect the number of bacteria carrying particles is important particularly in critical areas such as operation theatres (OTs), bone marrow transplant units, etc.

Indication (CDC Recommendations)

Routine air sampling (i.e. random or periodic sampling) is not recommended because (i) HAI rates are not related with levels of general microbial contamination of air or environmental surfaces, (ii) there is no standard guideline mentioning for permissible levels of microbial contamination of environmental surfaces or air.

CDC recommends targeted air surveillance, which should be carried out for the following indications:

- ❖ Investigation of an outbreak
- ❖ For research purpose
- ❖ After reconstruction or newly constructed buildings
- ❖ After fogging (to monitor the quality)
- ❖ For short-term evaluation of a change in infection control practice.

Evaluation of the Quality of Air in OT

Evaluation of the quality of air includes both microbiological and non-microbiological (physical) parameters.

Microbiological Parameters

There are two principle means of monitoring the microbiological parameters present in the air, passive monitoring and active sampling.

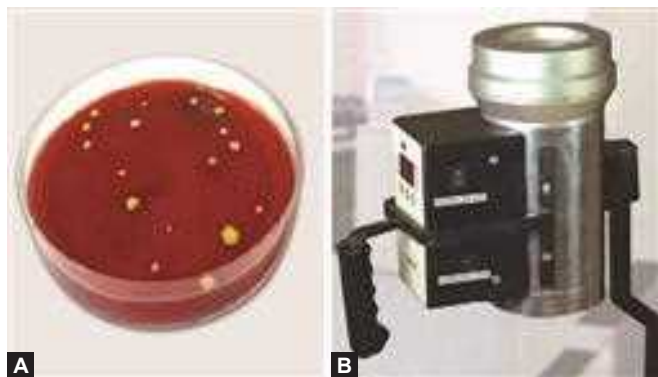
Passive Monitoring (Settle Plate) Method

Standard Petri dishes containing culture media (e.g. blood agar) are exposed to the air for a given time and then the plates are incubated at 37°C for 24 hours aerobically.

- ❖ **1,1,1 method:** The ideal recommendation is the 1,1,1 method where the plates are placed at different locations in the OT one meter away from the side walls, one meter above the floor and for a duration of one hour
- ❖ **Interpretation:** Bacteria sediment/settle on the plates which subsequently form colonies on incubation. Maximum acceptable levels in operating theatres with turbulent air flow is:
 - ≤ 786.4 CFU/m²/h (≤ 5 CFU/9 cm diameter plate/h) at rest, and
 - ≤ 3932.1 CFU/m²/h (≤ 25 CFU/9 cm diameter plate/h) when operational.
- ❖ **Disadvantages:** (1) It cannot detect smaller particles or droplets suspended in the air, (2) This method cannot quantify the volume of air sampled, (3) This method is not recommended when sampling air for fungal spores, because single spores can remain suspended in air indefinitely.

Active Monitoring (Slit Sampler Method)

In active monitoring, a microbiological air sampler (e.g. sieve impactor) is used. It has a vacuum pump, and a perforated lid (Fig. 27.3B), in which an agar plate can be placed.



Figs 27.3A and B: A. Air sampler method showing blood agar with bacterial colonies; B. Air sampler (HiMedia).

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ The vacuum pump physically draws a known volume of air through the perforated lid and allows it to impact on the agar plate (e.g. blood agar)
- ❖ Following incubation, the quantity of microorganisms present in the culture plate is measured in terms of CFU/m³ of air (Fig. 27.3A)
- ❖ Active monitoring is applicable when the concentration of microorganisms is not very high, such as in an operating theatre, bone marrow transplant unit, etc.

Air Particle Counters

Air particle counters have been developed recently, that are capable of detecting airborne particles containing microorganisms in real time.

- ❖ These employ **laser technology** to induce fluorescence in any viable air particles in air drawn through the instrument, which can be detected and quantified in real time
- ❖ The particle count of an OT is considered acceptable only when it falls in the acceptable range of clean room standard, according to the international standards system ISO 14644-1
 - Most of the HEPA filtered OT should satisfy ISO 6 level of clean room standard, which refers that the room should maintain <35,200 particles of 0.5 μ m in size per mm³
 - Ultra-clean OT (e.g. orthopedics) should have clean room standard ISO 5; which refers that the room should maintain <3,520 particles of 0.5 μ m size per mm³.

Non-microbiological Parameters

The number of bacteria in air at any given point of time depends upon various non-microbiological parameters such as air changes per hour, air velocity, positive pressure environment, temperature and relative humidity inside OT, etc. (Table 27.5). Therefore, there should be periodic monitoring of these parameters.

SURFACE SURVEILLANCE

Environmental surface sampling has been used to determine (a) reservoirs of potential environmental pathogens, and (b) the sources of the environmental contamination.

- ❖ **Locations:** It is required for high-risk locations such as operation theatres and ICU settings
- ❖ **Sites for sampling (high touch areas):** Surface sampling is taken from sites where there is high-risk of contaminations
- ❖ **Indications (CDC recommendation):**
 - Surface sampling is currently indicated for research, as a part of an epidemiologic investigation, or during an outbreak investigation
 - Routine periodic surface surveillance is not recommended.

Table 27.5: Quality of air in OT (non-microbiological parameters).	
Properties	Recommendations
Air changes per hour	Minimum 20 numbers of air changes per hour; out of which four should be fresh air
Air velocity	25–35 FPM (feet per minute) It is checked by anemometer Airflow direction should be unidirectional and downward from OT table
Positive pressure (PP)	Difference in positive pressure between OT and adjoining areas is required to prevent outside air entry into OT <ul style="list-style-type: none"> • PP should be maintained in OT at all times (operational and non-operational hours) • Minimum PP of 2.5 Pascal is recommended
Temperature	21 +/- 3°C (general OT) (orthopedic theaters, 18°C +/- 2°C)
Relative humidity	20 to 60% (ideal 55%)
Air handling in the OT	There should be dedicated AHU (air handling unit) for each OT and should not be linked to air conditioning of any other area/OT <ul style="list-style-type: none"> • Window and split AC should never be used in OT as they are pure recirculating units and have pockets for microbial growth • Air is supplied through terminal HEPA filters in the ceiling • The minimum size of the filtration area should extend one feet on each side of the OT table to cover the entire OT table and surgical team • Validation of HEPA filters is done biannually by DOP test. • Air quality at the supply should be <ul style="list-style-type: none"> ➢ ISO Class 5 (at rest condition) for super specialty OT ➢ ISO Class 6 (at rest condition) for general OT
Wall, floor and ceiling	<ul style="list-style-type: none"> • Should be anti-static and made up of non-porous, smooth, seamless materials • Paints used should have antibacterial, anti-fungal property
Occupancy	Maximum occupancy of 5–8 persons at any time inside the OT is allowed

Abbreviations: HEPA, high efficiency particulate air; DOP, dispersed oil particulate test.

- ❖ **Method:** Moistened sterile swabs (soaked in sterile saline) are used to collect the samples from high-risk areas and then inoculated on to blood agar for the recovery of aerobic bacteria
- ❖ **Reporting:** Only pathogenic organisms isolated are reported. A semi-quantitative report (as heavy, moderate or light growth) should be provided. Contaminants such as aerobic spore bearers are not reported
- ❖ **Newer techniques** such as luminometer (expresses bacterial contamination as CFU/mL) and glow gel techniques are available which are easy to perform though expensive.

EXPECTED QUESTIONS

I. Write short notes on:

1. Indicator organisms for fecal contamination of water.
2. Eijkman test.
3. Settle plate method.

II. Multiple Choice Questions (MCQs):

1. Which is not used as indicator organism of fecal contamination of water?

- a. Fecal *E. coli*
- b. Fecal streptococci
- c. *Pseudomonas*
- d. *Vibrio cholerae*

2. Evaluation of the quality of air in OTs can be performed by all the following methods, except:

- a. Settle plate
- b. Slit Sampler
- c. Particle count
- d. Presence- absence

Answer

1. d
2. d

SAY NO TO HANGING MASK SYNDROME

DO NOT BRING DOWN YOUR MASK TO THE CHIN



When you want to eat, drink or do any activity where you have to remove your mask, just remove it completely



The contaminated mask will cross-contaminate your scrub when mask is worn hanging around the neck.



Don't touch front part of mask



Dispose of the mask into the waste bin



Handwash after removal

Mask is meant to protect you from infection,
Do not make it as a mean to acquire infection

PART II

Systemic Microbiology (Infectious Diseases)

Section 4: Bloodstream and Cardiovascular System Infections

Section 5: Gastrointestinal Infections

Section 6: Hepatobiliary System Infections

Section 7: Skin, Soft Tissue and Musculoskeletal System Infections

Section 8: Respiratory Tract Infections

Section 9: Central Nervous System Infections

Section 10: Urogenital Tract Infections

Section 11: Miscellaneous Infective Syndromes

Bloodstream and Cardiovascular System Infections

SECTION

4

SECTION OUTLINE

28. Cardiovascular System Infections
29. Bloodstream Infections
30. Enteric Fever (*Salmonella Typhi* and *Salmonella Paratyphi*)
31. Rickettsial Infections
32. Miscellaneous Bacterial Bloodstream Infections: Brucellosis, Leptospirosis and Borreliosis
33. HIV/AIDS
34. Viral Hemorrhagic Fever
Arboviral VHF (Dengue, Chikungunya and Others), Filoviral VHF (Ebola and Marburg Virus), Hantaviral and Other Agents of VHF
35. Malaria and Babesiosis
36. Visceral Leishmaniasis and Trypanosomiasis
37. Lymphatic Filariasis
38. Systemic Candidiasis and Systemic Mycoses





ANTIBIOTIC GUARDIAN

*If we use antibiotics when not needed,
we may not have them when they are most needed*



CHAPTER PREVIEW

- Infective Endocarditis
- Other Infections of CVS
- Acute Rheumatic Fever

INTRODUCTION

Cardiovascular system infections include infections of heart and blood vessels.

- ❖ **Infections of heart:** This includes infection of the three layers of the heart wall
 - **Endocardium:** It is the innermost layer of the cardiac wall that lines the cavities and valves of the heart. Infection of the endocardium is known as *infective endocarditis*
 - **Myocardium:** It is the middle layer, composed of cardiac muscle; an involuntary striated muscle. It is responsible for the contractions of the heart. Infection of the myocardium is known as *myocarditis*
 - **Pericardium:** It is the outermost layer of the heart, composed of connective tissue and fat. The connective tissue secretes a small amount of lubricating fluid into the pericardial cavity. Infection of the pericardium is known as pericarditis. Collection of excess fluid in pericardial cavity can lead to pericardial effusion.
- ❖ **Infections of blood vessels:** Infections of blood vessels include mycotic aneurysm, and infective endarteritis
- ❖ **Device-related infections:** These include CRBSI (catheter-related bloodstream infection) and suppurative thrombophlebitis
- ❖ **Autoimmune-mediated:** Acute rheumatic fever.

INFECTIVE ENDOCARDITIS

Infective endocarditis (IE) refers to microbial invasion of the heart valves or mural endocardium—characteristically results in formation of bulky friable *vegetations*, composed of mass of platelets, fibrin, microcolonies of organisms, and scanty inflammatory cells.

Vegetations are most commonly present on the heart valves, followed by the low-pressure side of a ventricular septal defect, and on the mural endocardium.

Classification

Infective endocarditis can be classified into acute and subacute forms based on rapidity of evolution, severity

of infection and virulence of the implicated organism (Table 28.1).

Acute Endocarditis

It rapidly damages the cardiac structures, and spreads to extracardiac site, frequently involving a highly virulent organism (e.g. *S. aureus*) attacking a previously normal valve, and capable of causing substantial morbidity and mortality even with appropriate antibiotic therapy and/or surgery.

Subacute Endocarditis

It usually occurs in a previously damaged heart (e.g. scarred or deformed valves) and the implicating organisms are of low virulence (e.g. viridans streptococci). The disease typically has an insidious onset, metastasizes slowly and is gradually progressive over weeks to months; most patients recover after appropriate antibiotic therapy.

Pathogenesis of IE

The pathogenesis of infective endocarditis involves the following sequential steps.

- ❖ **Underlying risk factors:** IE is usually predisposed by various risk factors
 - Underlying cardiac defect: The cardiac conditions most commonly associated with infective endocar-

Table 28.1: Differences between acute and subacute endocarditis.

Acute endocarditis	Subacute endocarditis
Evolution is rapid	Evolution is slow
Involves normal cardiac valve	Involves previously damaged heart (scarred or deformed valve)
Implicated organism is of high virulence, e.g. <i>S. aureus</i>	Implicated organism is of low virulence, e.g. viridans streptococci
Causes substantial morbidity and mortality even with the appropriate antibiotic therapy and/or surgery	Follows a gradually progressive course of weeks to months; most patients recover after antibiotic therapy
Less common type, accounts for 10–20% of all cases	More common type, accounts for 50–60% of all cases

ditis are congenital valvular diseases such as mitral regurgitation, aortic stenosis, aortic regurgitation, ventricular septal defects

- Use of intravenous catheter
 - Prosthetic valve replacement surgery.
- ❖ **Endothelial injury:** The endothelium, unless damaged, is resistant to infection by most bacteria and to thrombus formation
- Therefore, though IE can develop on previously normal valves, but it is more common to develop on a defective valve
 - This is because predisposing cardiac abnormalities produces turbulence in blood flow to the heart which can damage cardiac endothelium. Use of IV catheter induces direct trauma that can damage cardiac endothelium
 - This damage to the endothelial surface results in the deposition of platelets and fibrin—a condition called *nonbacterial thrombotic endocarditis (NBTE)*.
- ❖ **Colonization:** The thrombus subsequently serves as a site of bacterial attachment during transient bacteremia. When bacteria transiently gain access to the bloodstream (e.g. after brushing the teeth), the organisms may stick to and then colonize the damaged cardiac endothelial surface
- ❖ **Formation of vegetations:** After colonization, the endothelial surface gets rapidly covered with a protective layer of fibrin and platelets. This protective environment is favorable to further bacterial multiplication. This web of platelets, fibrin, inflammatory cells, and entrapped organisms is called as **vegetation** (Fig. 28.1)
- ❖ **Metastasis:** The resulting vegetations ultimately seed bacteria into the blood at a slow but constant rate, which can metastasize to distant sites.

Etiological Agents of IE

The causative organisms of IE differ depending on the underlying risk factors such as native or prosthetic valve IE, acute or subacute IE, other risk factors such as IV drug abuser (Table 28.2).

The organisms differ from each other in their primary portal through which they enter into the bloodstream and reach to the heart; for example,

- ❖ Oral cavity- for viridans streptococci
- ❖ Skin for staphylococci
- ❖ Upper respiratory tract for HACEK organisms
- ❖ Gastrointestinal tract for *Streptococcus gallolyticus* and enterococci.

Clinical Manifestations

The clinical spectrum of IE includes both cardiac and noncardiac manifestations.

- ❖ **Cardiac manifestations** include the appearance of a new/worsened regurgitant murmur, which is more useful for the diagnosis of IE involving a normal valve



Fig. 28.1: Subacute bacterial endocarditis involving mitral valve showing large vegetations on valve leaflets.

Source: Public Health Image Library, ID # 851 (Dr Edwin)/ Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Noncardiac manifestations** include fever, chills and sweats, anorexia, weight loss, myalgia, arthralgia, arterial emboli, splenomegaly, clubbing, petechiae, neurologic manifestations and peripheral manifestations (Osler's nodes, subungual hemorrhages, Janeway lesions)
- ❖ **Laboratory manifestations** such as anemia, leucocytosis, microscopic hematuria, elevated ESR, CRP, or rheumatoid factor.

Diagnosis (Modified Duke Criteria)

The diagnosis of IE is established with the help of a highly sensitive and specific diagnostic schema—known as the **modified Duke criteria**; which is based on clinical, laboratory, and echocardiographic findings (Table 28.3).

Blood Cultures

Isolation of the causative microorganism from blood cultures is critical for diagnosis, determination of antimicrobial susceptibility, and planning of treatment. Blood cultures should be collected before antibiotic therapy.

- ❖ Two blood culture sets should be collected at an interval of >12hr between 1st and 2nd set
- ❖ Alternatively, three blood culture sets can be collected over one hour (e.g. 30 min gap between 1st and 2nd set and 30 min gap between 2nd and 3rd set).

Note: Blood culture set refers to 'pair of bottles'; collected from different venipuncture sites.

A major criterion can be fulfilled (Table 28.3), if:

- ❖ A typical IE organism is isolated from two separate blood cultures, or
- ❖ Agent other than typical IE organisms is isolated persistently from blood cultures (see Table 28.3) in the absence of an extra-cardiac focus of infection.

Table 28.2: Agents of infective endocarditis.

Etiological agents of infective endocarditis
<ul style="list-style-type: none"> • <i>Staphylococcus aureus</i> • Coagulase-negative staphylococci (e.g. <i>Staphylococcus epidermidis</i>) • Streptococci (Viridans streptococci and others) • Enterococci • Pneumococci • Fastidious gram-negative coccobacilli (HACEK group) • Enterobacteriaceae • <i>Pseudomonas</i> spp. (usually in drug users) • <i>Candida</i> species • Diphtheroids
Most common agent in specific types of endocarditis
<p>Native valve endocarditis: <i>Staphylococcus aureus</i></p> <ul style="list-style-type: none"> • Community acquired—Viridans streptococci • Healthcare associated—<i>S. aureus</i> • Overall—<i>S. aureus</i> <p>Prosthetic valve endocarditis: It occurs following cardiac valve replacement</p> <ul style="list-style-type: none"> • Early prosthetic valve endocarditis (occurs within 12 months of valve replacement)—It is generally nosocomial, results from intra-operative contamination of the prosthesis or a bacteremic postoperative complication. CoNS (e.g. <i>S. epidermidis</i>) and <i>S. aureus</i> are the most common agents • Late prosthetic valve endocarditis (occurs after 12 months of valve replacement)—Usually community-acquired; viridans streptococci are the most common agents • Overall—Regardless of the time of onset after the surgery, CoNS are the most common agents (at least 68–85%); and the majority are methicillin resistant CoNS <p>Endocarditis in IV drug abusers: Young males are the most common victims. The skin is the commonest source of infection.</p> <ul style="list-style-type: none"> • Right-sided (tricuspid valve) endocarditis—<i>Staphylococcus aureus</i> is the most common agent, majority are MRSA • Left-sided (mitral valve) endocarditis—has more varied etiology. <i>Enterococcus</i>, followed by <i>S. aureus</i> are the most common agents. However, <i>Pseudomonas aeruginosa</i>, <i>Candida</i> species and sporadically by unusual organisms such as <i>Bacillus</i>, <i>Lactobacillus</i>, and <i>Corynebacterium</i> species can also be implicated • Overall—Most common agent is <i>Staphylococcus aureus</i> <p>Subacute endocarditis: Viridians streptococci</p> <p>Culture-negative endocarditis: From 5–10% of IE have negative blood cultures; majority (one-third to one-half) of which are because of prior antibiotic exposure. The remainder of these patients are infected by fastidious organisms, such as:</p> <ul style="list-style-type: none"> • Nutritionally variant streptococci (<i>Granulicatella</i> and <i>Abiotrophia</i> species) • HACEK organisms—HACEK organisms • <i>Coxiella burnetii</i> • <i>Bartonella</i> species • <i>Brucella</i> species • <i>Tropheryma whipplei</i>—causes an indolent, culture-negative, afebrile form of endocarditis <p>Some fastidious organisms occur in characteristic geographic settings (e.g. <i>C. burnetii</i> and <i>Bartonella</i> species in Europe, <i>Brucella</i> species in Middle East).</p>

Note: Majority of *S. aureus* causing IE are methicillin resistant *S. aureus* (MRSA). Abbreviations: HACEK, *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*; CoNS, coagulase-negative staphylococci.

Table 28.3: Modified Duke criteria for the clinical diagnosis of infective endocarditis.

Major Criteria
<p>1. Positive blood culture: Any one of the following:</p> <ol style="list-style-type: none"> A. Typical IE organism isolated from two separate blood cultures (Viridans streptococci, <i>Streptococcus gallolyticus</i>, HACEK group, <i>S. aureus</i> or enterococci) or B. Persistently positive blood culture with agents other than typical IE organisms: <ul style="list-style-type: none"> ➢ Blood culture sets drawn >12 h apart; or ➢ All of 3 sets or a majority of ≥4 separate blood cultures, with first and last drawn at least 1 h apart C. Single positive blood culture for <i>Coxiella burnetii</i> or phase I IgG antibody titer of >1:800 <p>2. Evidence of endocardial involvement: Any one</p> <ol style="list-style-type: none"> A. Positive echocardiogram <ul style="list-style-type: none"> ➢ Oscillating intracardiac mass on valve or ➢ Abscess, or ➢ New partial dehiscence of prosthetic valve B. New valvular regurgitation
Minor Criteria
<ol style="list-style-type: none"> 1. Predisposition: Predisposing heart conditions or IV drug use 2. Fever ≥ 38.0°C (≥100.4°F) 3. Vascular phenomena: Major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages or Janeway lesions 4. Immunologic phenomena: Glomerulonephritis, Osler's nodes, Roth's spots or rheumatoid factor 5. Microbiologic evidence: Positive blood culture but not meeting major criterion as noted previously* or serologic evidence of active infection with organism consistent with infective endocarditis <p>Definite endocarditis if the followings are present:</p> <ul style="list-style-type: none"> • Two major criteria or • One major criterion and three minor criteria or • Five minor criteria

*Excluding single positive blood cultures for coagulase-negative staphylococci and diphtheroids, which are common culture contaminants, and organisms that do not cause endocarditis frequently, such as gram-negative bacilli. Abbreviation: IE, infective endocarditis.

A **minor criterion** is considered to be fulfilled (Table 28.3) if blood cultures show positive but not meeting major criterion.

Blood culture collection technique and processing is discussed in detail in Chapter 29.

Non-blood-culture Tests

Various non-blood-culture tests that can be used for the diagnosis of IE include:

- ❖ Serologic tests can be used to implicate some organisms that are difficult to recover by blood culture: *Brucella*, *Bartonella*, *Legionella*, *Chlamydomphila psittaci*, and *Coxiella burnetii*
- ❖ Isolation of the pathogens in vegetations by culture
- ❖ Microscopic examination with special stains (e.g. periodic acid-Schiff stain for *Tropheryma whipplei*)
- ❖ Direct fluorescence antibody techniques
- ❖ PCR to recover unique microbial DNA or 16S rRNA that, when sequenced, allows identification of the etiologic agent.

Echocardiography

Echocardiography allows anatomic confirmation of infective endocarditis, sizing of vegetations, detection of intracardiac complications, and assessment of cardiac function.

TREATMENT

Infective endocarditis

1. Regimen for *S. aureus* IE

For native valve IE:

- For MSSA (methicillin susceptible *S. aureus*): Cloxacillin or nafcillin is given for 6 weeks
- For MRSA (methicillin resistant *S. aureus*): Vancomycin is given for 6 weeks

For prosthetic valve IE:

In addition to above regimen, rifampin (for 6 weeks) and gentamicin (for 2 weeks) are added.

2. Regimen for viridans streptococci and *S. gallolyticus* IE

- For native valve IE: Penicillin or ceftriaxone is given for 4 weeks
- For prosthetic valve IE: Gentamicin is added to the above regimen for 6 weeks

3. For HACEK endocarditis

Ceftriaxone or ciprofloxacin is given for 4 weeks. Treatment may be extended for 6 weeks in case of prosthetic valve IE.

Therapeutic drug monitoring

Optimum dosage is key behind a successful clinical outcome. Therapeutic drug monitoring should be performed to maintain optimum antibiotic level in the serum.

- Vancomycin dosage is 15–20 mg/kg, q8–12h. Target trough concentration of 15–20 µg/mL need to be maintained. Vancomycin should be avoided if the MIC is >1 µg/mL
- Gentamicin dosage is 1 mg/kg, q8h IV. It is used for synergy; peak levels need not exceed 4 µg/mL and troughs should be <1 µg/mL.

Agents Causing IE

Infective endocarditis due to staphylococci, Viridans streptococci, nutritionally variant streptococci, and HACEK group of pathogens are discussed in this chapter. The other etiological agents of IE are discussed under different systems they principally infect.

Staphylococcal Endocarditis

S. aureus is the most common cause of IE; usually runs an acute course.

- ❖ *S. aureus* IE presents with larger vegetations (>10 mm in diameter), and therefore is more frequently associated with features of septic embolization (due to breaking of vegetations leading to formation of emboli) such as subungual hemorrhage, Osler's nodes, etc.
 - Cerebrovascular emboli can cause strokes or occasionally encephalopathy
 - Embolization risk is higher for mitral valve IE.
- ❖ *S. aureus* appears gram-positive cocci arranged in cluster, produces golden yellow hemolytic colonies on blood agar and gives a positive coagulase test (Chapter 51)

- ❖ Coagulase-negative staphylococci (e.g. *S. epidermidis*) are increasingly associated with prosthetic valve endocarditis (at least 68–85% of cases) and majority of them are methicillin resistant

Staphylococci can cause infections of various other systems such as skin and soft tissue (Chapter 51).

Viridans Streptococci

Viridans streptococci are commensals of mouth and upper respiratory tract. Usually, they are nonpathogenic, however occasionally cause diseases such as:

- ❖ **Subacute bacterial endocarditis (SABE):** Viridans streptococci are the most common cause of SABE. The commensal viridans streptococci (*S. sanguinis*) in the oral cavity can enter blood to cause transient bacteremia while chewing, tooth brushing and dental procedures that can account for the predilection of these organisms to cause endocarditis
- ❖ **Dental caries:** It is mainly caused by *S. mutans* which breaks down dietary sucrose to acid and dextrans with the help of an enzyme glucosyl transferase. Acid damages the dentine, while adhesive dextran binds together with food debris, mucus, epithelial cells and bacteria to produce **dental plaques**
- ❖ **In cancer patients:** Viridans streptococci can cause prolonged bacteremia among neutropenic patients undergoing cancer chemotherapy
- ❖ ***S. milleri* group** (includes *S. intermedius*, *S. anginosus*, and *S. constellatus*): Produce suppurative infections, particularly brain abscess and empyema.

Laboratory Diagnosis

- ❖ On Gram stain, they appear as gram-positive cocci arranged in long chains (Fig. 28.2A)
- ❖ They produce minute α -hemolytic green-colored (rarely non-hemolytic) colonies on blood agar ("*viridis*" means green, Fig. 28.2B)
- ❖ They can be differentiated from *Streptococcus pneumoniae* (which is also α -hemolytic) by a number of tests such as resistant to optochin and insoluble in bile (Chapter 61)
- ❖ Accurate species identification is made by automated methods such as MALDI-TOF.

TREATMENT

Viridans streptococci

They are usually sensitive to penicillin except in neutropenic patients with bacteremia, where vancomycin is the treatment of choice.

Nutritionally Variant Streptococci

Abiotrophia and *Granulicatella* species are known as nutritionally variant streptococci as they require vitamin B (pyridoxal) in the culture medium for their growth. Earlier, they were grouped along with viridans streptococci.



Figs 28.2A and B: Viridans streptococci: **A.** Gram-positive cocci in long chains; **B.** α -hemolytic colonies on blood agar.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ **Manifestation:** They are normal inhabitants of the oral cavity and similar to other oral commensals, they can also cause endocarditis
- ❖ **Diagnosis:** They can be recovered in automated blood cultures such as BacT/ALERT. Multiple blood cultures and prolonged incubation may be necessary
 - They fail to grow when subcultured on solid media. However, they sometimes produce satellite colonies near the colonies of “helper” bacteria (e.g. near *Staphylococcus aureus* streak line). Therefore, they are also called as *Satelliting streptococci*
 - They are catalase negative, gram-positive cocci arranged in short chains
 - Species identification is made by automated systems such as MALDI-TOF.
- ❖ **Treatment:** Combination therapy with *penicillin plus gentamicin* is recommended for IE cases.

S. gallolyticus Endocarditis

S. gallolyticus (formerly *S. bovis*) is a group D *Streptococcus*, found as a commensal in intestine of animals. In humans, it occasionally causes bacteremia, subacute endocarditis, and also associated with colorectal cancer or polyps. Penicillin is the drug of choice (Chapter 52).

HACEK Endocarditis

HACEK is an abbreviation used to represent a group of highly fastidious, slow-growing, capnophilic, gram-negative bacteria, that normally reside in the oral cavity as commensal, but occasionally have been associated with local infections of the mouth and systemic infections such as bacterial endocarditis. Species belonging to this group include:

- ❖ *Haemophilus parainfluenzae*
- ❖ *Aggregatibacter* species: *A. actinomycetemcomitans*, *A. aphrophilus* and *A. paraphrophilus*
- ❖ *Cardiobacterium hominis*
- ❖ *Eikenella corrodens*
- ❖ *Kingella kingae*.

HACEK endocarditis accounts for 3% of total endocarditis cases.

- ❖ Typically has a subacute course
- ❖ Occurs in patients with preexisting valvular defects or those undergoing dental procedures
- ❖ The aortic and mitral valves are most commonly affected.

Clinical Manifestations

- ❖ ***Haemophilus parainfluenzae*:** It is a commensal in mouth and throat
 - Occasionally, it can be an opportunistic pathogen causing endocarditis, conjunctivitis, abscesses, genital tract infections and bronchopulmonary infections in patients with cystic fibrosis
 - It can be differentiated from *H. influenzae* either by its growth requirement (requires only factor X, but not V), or by automated identification systems such as MALDI-TOF or VITEK.
- ❖ ***Aggregatibacter actinomycetemcomitans*:** Formerly called as *Actinobacillus actinomycetemcomitans*
 - It is the most common member of HACEK to cause endocarditis
 - It can also be isolated from soft tissue infections and abscesses associated with *Actinomyces israelii*
 - Rarely, it can cause periodontitis, brain abscess, meningitis and endophthalmitis.
- ❖ ***Aggregatibacter aphrophilus* and *A. paraphrophilus*:** Earlier members of *Haemophilus*, now are renamed under genus *Aggregatibacter*
 - They are commensals of mouth and occasionally cause endocarditis, head and neck infections, invasive bone and joint infections
 - *A. aphrophilus* requires only factor X, whereas *A. paraphrophilus* requires only factor V.
- ❖ ***Cardiobacterium hominis*:** It frequently affects the aortic valve. It is also associated with arterial embolization, immune complex glomerulonephritis or arthritis
- ❖ ***Eikenella corrodens*:** Apart from endocarditis, it can also occasionally cause skin and soft tissue infections. The name ‘corrodens’ refers to the characteristic **pitting or corroded colonies** on blood agar
- ❖ ***Kingella kingae*:** In addition to endocarditis, it can also cause infections of bones, joints and tendons.

Laboratory Diagnosis

The laboratory diagnosis of HACEK endocarditis is as follows:

- ❖ **Culture:** Blood cultures are performed on automated systems such as BacT/ALERT
 - As they are highly fastidious, require multiple blood cultures, and prolonged incubation up to 1 week
 - They are capnophilic, growth is optimum in presence of 5–10% of CO₂
 - Identification is made by automated systems such as MALDI-TOF.
- ❖ **Molecular methods:** Simultaneous detection of HACEK members from clinical specimen is possible by

performing (i) broad-range bacterial PCR targeting 16S rRNA gene followed by sequencing; (ii) multiplex PCR or multiplex real-time PCR.

TREATMENT

HACEK endocarditis

The prognosis of HACEK endocarditis is good.

- ❑ Ceftriaxone (2 g/day) is the drug of choice for most of the HACEK organisms except *Eikenella corrodens* where ampicillin is indicated
- ❑ Quinolones are given if the strain is a β -lactamase producer
- ❑ Duration of treatment: Antibiotics are given for 4 weeks for native valve endocarditis and 6 weeks for prosthetic-valve endocarditis.

OTHER INFECTIONS OF CVS

Myocarditis

Myocarditis refers to inflammation of the myocardium, which is clinically manifested by chest pain, arrhythmias, or congestive heart failure. It is rapidly progressive and often fatal. It can be caused by both infectious and non-infectious etiology. The infectious etiological agents include:

- ❖ *Viruses* are the most common agents; most common being Coxsackievirus B, followed by adenoviruses, parvovirus B19, human herpesvirus 6, and dengue viruses
- ❖ *Parasitic agent* such as *Trypanosoma cruzi*, the agent of Chagas' disease
- ❖ *Bacterial agent*: It is rarely caused by bacteria, as a result of bacteremia, direct extension from a contiguous focus, or a bacterial toxin.

Laboratory Diagnosis

Endomyocardial biopsy can provide a definitive diagnosis. Evidence of viral infection by detection in peripheral samples or by serology provides only circumstantial evidence of possible etiology.

Pericarditis

Inflammation of the pericardium is clinically presented by one or more of the following manifestations- chest pain, pericardial friction rub, and pericardial effusion. It can be caused by both infectious and non-infectious etiology. The infectious etiologic agents include:

- ❖ *Viruses* are the most common agents; such as Coxsackievirus B (most common cause), Echovirus, Adenovirus, HIV and others
- ❖ *Bacteria* rarely may cause purulent pericarditis, usually as a complication of pneumonia due to *S. aureus*, *H. influenzae*, meningococcus and pneumococcus
- ❖ *M. tuberculosis* can cause pericarditis, usually as a complication of pulmonary tuberculosis.

Laboratory Diagnosis

Percutaneous pericardial biopsy or pericardiotomy with biopsy and drainage provide a definite diagnosis. Evidence

of coincident viral infection, either by culture or serology, is circumstantial.

Pericardial Effusion

It refers to excess fluid production in pericardial sac, usually secondary to pericarditis or other causes such as malignant, or autoimmune processes within the pericardium.

Infections of Blood Vessels

Mycotic Aneurysm

Aneurysm refers to inflammatory damage and weakening of an arterial wall; leading to a bulging of the arterial wall, that can eventually rupture. Though the word 'mycotic' is used to denote fungi; 'mycotic aneurysm' is used to describe aneurysms of any infectious etiology except syphilitic aortitis. The etiologic agents are similar to those that cause endocarditis such as streptococci and staphylococci.

Infective Endarteritis

Infective endarteritis refers to inflammation of the arterial wall, which may occur with or without coexistent aneurysmal dilation.

Device-related Infections

This includes infections of various devices inserted in the blood vessels such as central line (central venous catheters) and peripheral IV cannula

CRBSI (Catheter-related Bloodstream Infection)

Severely-ill patients in ICUs are often put on central line for administration of medications and parenteral nutrition. Central lines may get infected due to mishandling during insertion or during daily maintenance which leads to development of CRBSI. CRBSI is a major healthcare associated infection, discussed in Chapter 22.

Suppurative Thrombophlebitis

Suppurative thrombophlebitis (STP) is an inflammation of a vein wall. It occurs secondary to either dermal infection or use of indwelling intravenous catheters; the latter being the most common cause.

- ❖ **IV cannulation:** STP occurs frequently in hospitalized patients after 3-4 days of IV cannulation (e.g. veinflam), which gets colonized by the organisms present on patient's skin or hands (as normal skin flora) of the healthcare workers
- ❖ **Etiology:** Common agents of STP include *S. aureus*, members of Enterobacteriaceae, and yeasts (*Candida* and *Malassezia*)
- ❖ **Lemierre's syndrome:** It is a condition characterized by thrombophlebitis of the internal jugular vein and bacteremia—caused primarily by anaerobic organism *Fusobacterium necrophorum*, following a recent oropharyngeal infection.

ACUTE RHEUMATIC FEVER

Acute rheumatic fever (ARF) is a multisystem disease that occurs in people previously affected with streptococcal (group A) sore throat, as a result of an autoimmune reaction. Although ARF may involve many parts of the body, almost all the manifestations resolve completely; except the cardiac valvular damage, which is called as rheumatic heart disease (RHD).

Group A *Streptococcus* (*S. pyogenes*) principally causes infections of skin and soft tissues and is discussed in Chapter 52.

Pathogenesis

Primary ARF is mainly a disease of children age 5–14 years, and it is rare in persons aged more than 30 years. However, recurrent episodes of ARF is more common in adolescents and young adults. There is no clear gender association for ARF, but RHD more commonly affects females.

ARF results following upper respiratory tract infection with group A streptococci (usually by M-serotypes 1, 3, 5, 6, 14, 18, 19, 24, 27, and 29). Genetic predisposition may play a role; people with HLA-DR7 and HLA-DR4 appear to be more susceptible as compared to others.

Pathogenesis is unclear. It may be due to:

- ❖ **Autoimmune theory:** Pathogenesis is based on theory of **molecular mimicry**—the antibodies targeted against streptococcal antigens (M protein) cross react with human tissue antigens (e.g. heart and joint). These cross reactive antibodies bind to valvular endothelium, leading to damage of the heart valves
- ❖ **Cytotoxic theory:** Streptococcal toxins (e.g. streptococcal pyrogenic toxin) and enzymes (streptolysin O) are directly toxic to human heart.

Clinical Manifestations

The clinical manifestations usually appear after period of ~3 weeks following precipitating group A streptococcal infection. The prior streptococcal infection may be either subclinical (more common) or presents as sore throat.

Acute rheumatic fever affects heart, joints, skin and brain. The common manifestations in the order of frequency include:

- ❖ **Migrating polyarthritis:** It is the most common manifestation, characterized by migratory polyarthritis (hot, swollen, red, and/or tender joints), which moves from one joint to another over a period of hours. It is asymmetric and affects the large joints—most commonly the knees, ankles, hips, and elbows
- ❖ **Pancarditis**, affecting endocardium, pericardium, or myocardium
 - Valvular damage is the hallmark; leading to mitral regurgitation (most common) and aortic regurgitation

Table 28.4: Diagnostic criteria for rheumatic fever—modified Jones criteria (2015).

Major criteria	
Low-risk population	High-risk population
Carditis (clinical or subclinical)	Carditis (clinical or subclinical)
Arthritis—only polyarthritis	Arthritis —monoarthritis or polyarthritis
	Polyarthralgia
Chorea	Chorea
Erythema marginatum	Erythema marginatum
Subcutaneous nodules	Subcutaneous nodules
Minor criteria	
Low-risk population	High-risk population
Polyarthralgia	Monoarthralgia
Hyperpyrexia ($\geq 38.5^{\circ}\text{C}$)	Hyperpyrexia ($\geq 38.0^{\circ}\text{C}$)
ESR ≥ 60 mm/h and/or CRP ≥ 3.0 mg/dL	ESR ≥ 30 mm/h and/or CRP ≥ 3.0 mg/dL
Prolonged PR interval	Prolonged PR interval
Diagnostic criteria	
Initial ARF	Two major or One major + two minor
Recurrent ARF (with a reliable past history of ARF/RHD)	Two major or One major + two minor or Three minor criteria

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ARF, acute rheumatic fever; RHD, rheumatic heart disease.

Note: The supporting evidence (of previous streptococcal infection), which was a part of previous version of Jones criteria (1992), has been removed from the new modified Jones criteria, 2015.

- Myocardial inflammation may affect electrical conduction pathways, leading to P-R interval prolongation.
- ❖ **Subcutaneous nodules:** Occur as painless, small, mobile lumps beneath the skin overlying bony prominences, particularly of the hands, feet, and elbows
- ❖ **Chorea (Sydenham's):** It is an abnormal involuntary movement disorder, mainly affecting head and limbs
- ❖ **Erythema marginatum:** They are pink macular rashes that appear and disappear before the examiner's eyes.

Diagnosis of ARF (Jones Criteria)

The diagnosis of ARF is made based on diagnostic criteria known as revised Jones criteria (2015). It is based on the presence of a combination of typical clinical features together with ECG and laboratory (ESR, CRP) findings (Table 28.4).

Supportive evidence (of previous group A streptococcal infection within the last 45 days) was a part of the previous version of Jones criteria (1992); which was diagnosed by detection of one of the following:

- ❖ Elevated ASO titre—which will be much higher in patients with ARF than that seen in patients with GAS infections without ARF

- ❖ A positive throat culture
- ❖ Rapid antigen test for GAS
- ❖ Recent scarlet fever.

TREATMENT

Rheumatic fever

Penicillin is the drug of choice; can be given orally (as penicillin V or amoxicillin for 10 days) or intramuscularly as single dose of 1.2 million units of benzathine penicillin G. Supportive treatment (e.g. aspirin) should be given for arthritis, arthralgia, and fever.

Prevention

Primary Prevention

It includes timely and complete treatment of group A streptococcal sore throat with antibiotics (penicillin) within 9 days of sore throat onset, which will prevent almost all cases of ARF.

Secondary Prevention

The mainstay of controlling ARF and RHD is secondary prevention. Patients with ARF are at much higher risk of developing recurrent ARF. Therefore, long-term penicillin prophylaxis is indicated to prevent recurrences. The drug of choice for secondary prophylaxis is intramuscular benzathine penicillin G given every 4 weeks. In case of penicillin allergy, erythromycin (250 mg, twice a day) can be given as an alternative. The duration depends upon underlying carditis.

- ❖ **ARF without carditis:** For 5 years after the last attack or 21 years of age (whichever is longer)
- ❖ **ARF with carditis but no residual valvular disease:** For 10 years after the last attack, or 21 years of age (whichever is longer)
- ❖ **ARF with persistent valvular disease:** For 10 years after the last attack, or 40 years of age (whichever is longer) or sometimes lifelong prophylaxis.

EXPECTED QUESTIONS

I. Write essay on:

1. A 75-year-old man was hospitalized with fever (101°F), severe back-pain and weakness in lower limbs. On examination, few non-tender, small erythematous nodular lesions on soles were seen. Echocardiogram showed valvular vegetations on mitral valve. He was diagnosed to have a cardiac valve vegetations 3 years back. Laboratory tests showed CRP 2.5 mg/dL, ESR 66 mm/h, leukocytes $15.6 \times 10^9/L$ and creatinine 4.6 mg/dL. Two pairs of blood cultures were sent which subsequently were positive for viridans streptococci. The patient was immediately started on benzyl penicillin.
 - a. What is the probable clinical diagnosis?
 - b. What are the typical etiological agents?
 - c. Describe the diagnostic criteria used for this condition.
 - d. How will you collect specimen for this clinical condition?
2. A 7-year-old female child presented to the cardiology OPD with swollen, red, and/or tender joints, which migrates from one joint to another (knees, ankles, hips, and elbows) over a period of hours. The child was having an abnormal gait. She also complained of painless, small, mobile lumps beneath the skin overlying bony prominences, particularly of the hands, feet, and elbows. On auscultation, murmur was heard over the mitral valve area. ECG showed prolongation of P-R interval. On inquiry, it was found that the child had an episode of sore throat 3 weeks back.
 - a. What is the probable clinical diagnosis and its etiological agent?
 - b. Describe the diagnostic criteria used for this condition.
 - c. How will you prevent recurrence of such episodes?

CHAPTER PREVIEW

- Etiological Agents of BSI
- Clinical Manifestations
- Fever of Unknown Origin (FUO)
- Types of Bloodstream Infections
- Laboratory Diagnosis
- Infections Causing Anemia

INTRODUCTION

Bloodstream infections (BSI) refer to the presence of microorganisms in blood, which constitute one of the most serious situations among infectious diseases; as they are a threat to every organ in the body.

- ❖ Microbial invasion of bloodstream can have serious immediate consequences such as shock, multiple organ failure, and DIC (disseminated intravascular coagulopathies)
- ❖ Therefore, timely detection of the causative agent is one of the most important goals of the microbiology laboratory.

Terminologies

The suffix 'emia' is derived from the Greek word meaning "blood" and refers to the presence of a substance in the blood.

- ❖ Bacteremia refers to the presence of bacteria in blood without any multiplication
- ❖ Septicemia is a condition in which bacteria circulate and actively multiply in the bloodstream and may produce their products (e.g. toxins) that cause harm to the host
- ❖ Similarly, the presence of viruses, parasites and fungi in blood can be described as 'viremia', 'parasitemia' and 'fungemia' respectively.

Types of Bacteremia

Bacteremia may be transient, continuous, or intermittent.

1. **Transient bacteremia:** It may occur spontaneously or with minor events such as brushing teeth or chewing food, instrumentation of contaminated mucosal site and surgery involving a non-sterile site. These circumstances may also lead to septicemia; although normally the bacteria are cleared from blood by the host immune mechanisms
2. **Continuous bacteremia:** Here, the organisms are released into the bloodstream at a fairly constant rate. It occurs in conditions such as:
 - Septic shock, endocarditis and other endovascular infections

- During the early stage of certain infections including enteric fever, brucellosis, and leptospirosis.
3. **Intermittent bacteremia:** In most other infections, bacteria are released into blood intermittently
 - Sequestered focus of infection such as an undrained abscess (bacteria are released approximately 45 minutes before a febrile episode)
 - Early course of meningitis, pneumonia, septic arthritis and osteomyelitis.

ETIOLOGICAL AGENTS OF BSI

Pathogens of all four major groups of microbes—bacteria, viruses, fungi, and parasites can cause bloodstream infections.

Bacterial Etiology

Bacterial agents account for the majority of bloodstream infections. The common agents causing primary bloodstream infection include typhoidal salmonellae, brucellae or spirochetes (*Leptospira*, *Borrelia*), HACEK group of pathogens, viridans streptococci and rickettsiae (infect vascular endothelium). These agents are discussed in the subsequent chapters of this section.

However, there are various other bacterial agents which can primarily infect other sites and subsequently spill over to the bloodstream to cause secondary BSI. These include:

- ❖ Gram-positive cocci—staphylococci, beta-hemolytic streptococci, enterococci and pneumococci
- ❖ Gram-negative cocci—meningococci
- ❖ Gram-positive bacilli—*Bacillus anthracis* and *Listeria*
- ❖ Gram-negative bacilli—*E. coli*, *Klebsiella*, *Enterobacter*, non-fermenters (e.g. *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Stenotrophomonas*), *Haemophilus*, *Aeromonas*, etc.
- ❖ Anaerobes—*Bacteroides*.

Above agents are discussed under the primary systems they infect; for e.g. *Escherichia coli* is discussed under UTI (Chapter 76) and diarrhea (Chapter 41).

Viral Etiology

Although many viruses do circulate in the peripheral blood at some stage of the disease and have a viremic phase, the primary infection usually occurs in the target organs. There are a few viruses that preferentially infect blood cells, which can be considered as viral agents of bloodstream infections.

- ❖ Human immunodeficiency virus (HIV) and other human retroviruses—attack CD4 T lymphocytes and macrophages (discussed in Chapter 33)
- ❖ Agents of hemorrhagic fever such as dengue, chikungunya, Ebola, Marburg, Lassa, yellow fever, and other viruses—they infect endothelial cells (VHF is discussed in Chapter 34 and Yellow fever virus in Chapter 48)
- ❖ Epstein-Barr virus: Invades lymphocytes. It causes infectious mononucleosis and various malignancies (Chapters 68 and 80)
- ❖ Cytomegalovirus: Invades monocytes, polymorphonuclear cells, and lymphocytes. It causes hepatitis and congenital infections (Chapter 79).

Parasitic Etiology

The parasites causing bloodstream infections may be categorized into:

- ❖ Parasites that directly infect blood cells such as *Plasmodium* and *Babesia* infecting RBCs
- ❖ Parasites that may be found in the bloodstream before they migrate to other tissues or organs; e.g. include tachyzoites of *Toxoplasma gondii*, amastigote forms of *Leishmania*, and trypomastigote forms of *Trypanosoma*
- ❖ Parasites that maybe present in the lymphatics and come to bloodstream transiently; e.g. microfilariae of filarial parasites.

Note: Schistosoma (or blood flukes) reside in the venous plexus of the bladder and the intestine. However, the clinical manifestations are principally confined to the bladder and the intestine and therefore have been discussed in Chapters 46 and 76. Tachyzoites of *Toxoplasma gondii* also appear in blood. However, it principally causes CNS and congenital infections and therefore discussed under Chapters 75 and 79.

Fungal Etiology

Fungemia (the presence of fungi in the blood) is usually a serious condition, occurring primarily in immunosuppressed patients, patients with malignancies, patients on chemotherapy and in those with serious or terminal illness.

- ❖ *Candida* species are the most common etiological agents of fungemia; account for 8-10% of all nosocomial bloodstream infections. Both *Candida albicans* and non-albicans *Candida* species such as *C. tropicalis*, *C. parapsilosis* and *C. auris* can cause bloodstream infections
- ❖ Agents of systemic mycoses (*Histoplasma*, *Blastomyces*, *Coccidioides*, and *Paracoccidioides*) can also invade

bloodstream. These infections usually begin as pneumonia, and then the fungi may disseminate from the lungs to the bloodstream, from where they seed into other organs

- ❖ *Cryptococcus* predominantly causes meningitis (although fungemia is also observed) and therefore is discussed in Chapter 75.

As bacterial agents are the most common group to cause bloodstream infections, therefore the rest of the discussion in this chapter is largely limited to bacterial agents. The viral, parasitic and fungal agents of bloodstream infections have been described in detail in the subsequent chapters of this section.

TYPES OF BLOODSTREAM INFECTIONS

There are two major categories of bloodstream infections (BSIs): Intravascular and extravascular.

Factors that contribute to the initiation of BSI are:

- ❖ Immunosuppression
- ❖ Use of broad spectrum antimicrobial agents can suppress the normal flora; thus allowing the emergence of resistant strains of bacteria
- ❖ Invasive procedures or extensive surgeries that allow the bacteria to access the blood
- ❖ Prolonged survival of debilitated patients.

Intravascular Bloodstream Infections

They originate within the cardiovascular system which includes infection of the heart (endocarditis, myocarditis and pericarditis) and infection of blood vessels. These infections are discussed in Chapter 28.

Extravascular Bloodstream Infections

Most cases of clinically significant bacteremia are of extravascular origin.

- ❖ The organisms multiply at the primary site such as lungs and are drained by lymphatics and reach the bloodstream
- ❖ The organisms are either removed by the cells of the reticuloendothelial system or they multiply more widely and thereby causing septicemia
- ❖ **Portal of entry:** The most common portals of entry for bacteremia are the genitourinary tract (25%), followed by respiratory tract (20%), abscesses (10%), surgical site wound infections (5%), and biliary tract (5%). In up to 25% of cases, the portal of entry remains uncertain
- ❖ **Agents:** The bacteria invading the bloodstream depend upon the portal of entry and have been listed in Table 29.1.

CLINICAL MANIFESTATIONS

Bloodstream infections have a bacteremia stage followed by a septicemic stage. The clinical manifestations are evident only in the septicemic stage. In this stage, the bacteria multiply and release their products (e.g. toxins) which

Table 29.1: Bacteria causing extravascular bloodstream infections (BSIs) and their common sources.

Bacteria	Portal of entry/sources
<i>E. coli</i> and other gram-negative bacteria such as <i>Klebsiella</i> , <i>Proteus</i> , <i>Enterobacter</i> , <i>Pseudomonas</i>	Urinary tract (most common), Intestine (rarely)
<i>Haemophilus influenzae</i> type b	Meninges, epiglottis, lungs
Pneumococcus	Meninges, lungs
<i>Brucella</i>	Reticuloendothelial system
<i>Salmonella</i> Typhi	Small intestine, lymph nodes and reticuloendothelial system
<i>Listeria</i>	Intestine, meninges
<i>Staphylococcus aureus</i> and coagulase negative staphylococci	Surgical site infections

travel to various organs affecting their functions. Based on the severity and the extent of organ failure; bloodstream infection can be divided into two stages: sepsis and septic shock (Table 29.2).

- ❖ **Sepsis:** The common signs and symptoms include:
 - Fever or hypothermia with/without chills and rigors
 - Hyperventilation leads to excess loss of CO₂ and subsequent respiratory alkalosis
 - Skin lesions, change of mental status and diarrhea.
- ❖ **Septic shock:** This is the gravest late stage complication of septicemia and is manifested as—hypotension, DIC and multi-organ failure (e.g. acute respiratory distress, renal failure, tissue destruction, etc.). The endotoxins of gram-negative bacteria have a direct effect on the pathogenesis of septic (or endotoxic) shock.

In sepsis, the severity and degree of organ failure can be determined by an assessment score called as SOFA (Sepsis related Organ Failure Assessment) score Quick SOFA criteria (Table 29.2).

LABORATORY DIAGNOSIS

Diagnosis of bloodstream infection depends on the isolation of the causative agent from blood by performing blood culture.

Specimen Collection for Blood Culture

Extreme care should be taken while collection of blood for culture, as there is a high-risk of contamination with skin flora.

- ❖ **Site:** Blood for culture should always be collected in **pairs**; from two separate venipuncture and 2 separate skin decontamination process. If a central line is present, then one sample from the central line and one from the venipuncture should be collected
- ❖ **Preparation of the site:** To avoid contamination with skin flora, blood should be collected under strict aseptic conditions using sterile disposable syringe (Fig. 29.1)

Table 29.2: Definition of sepsis and the assessment of severity and organ failure.

Sepsis Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection
SOFA score Sepsis is diagnosed by SOFA (Sepsis-related organ failure assessment) score which in turn depends on six parameters. 1. Respiratory system—PaO ₂ /FiO ₂ 2. Coagulation system—Platelet count 3. Liver—Serum bilirubin 4. Cardiovascular—Mean arterial pressure (MAP) 5. Central nervous system—Glasgow coma scale score 6. Renal—Serum creatinine and urine output Organ dysfunction can be identified as an acute change in the total SOFA score ≥2 points following the infection
qSOFA (Quick SOFA) Criteria Determination of SOFA score takes considerable time as it depends upon a number of laboratory parameters. However, before the result of SOFA score is available, sepsis can promptly be identified at the bedside with qSOFA score • Respiratory rate ≥22/min • Altered mentation • Systolic blood pressure ≤100 mm Hg
Septic shock It is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound Patients with septic shock can be identified with a clinical construct of sepsis with: • Persisting hypotension requiring vasopressors to maintain MAP (mean arterial pressure) ≥65 mm Hg and • Serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation Patients with septic shock have a mortality of >40% in contrast to 10%, for sepsis cases

Note: PaO₂/FiO₂ is the ratio of arterial oxygen partial pressure to fractional inspired oxygen.

- ❖ **Skin decontamination:** Skin should be disinfected by two-step procedure—first, treated with 70% isopropyl alcohol and then a second antiseptic solution such as povidone iodine or chlorhexidine should be applied
 - The disinfectants should be applied in a circular motion (5 cm in diameter), starting from the centre to the periphery
 - The area should be allowed to air dry before venipuncture.
- ❖ **Timing of collection:** Blood should be collected before starting antimicrobial therapy. If the antimicrobial agent is already started, then the best time of collection is just before the next dose of the antimicrobial agent
- ❖ **Blood volume:** Blood specimen is drawn using a sterile syringe and needle. Higher the volume of blood, greater is the chance of isolation (yield increases by 3.2% per mL of blood cultured). At least **8–10 mL** of blood per bottle for an adult and **1–3 mL** per pediatric bottle is recommended
- ❖ **Number of blood cultures:** At least 2–3 blood culture sets (each set consists of two bottles: 1 aerobic and

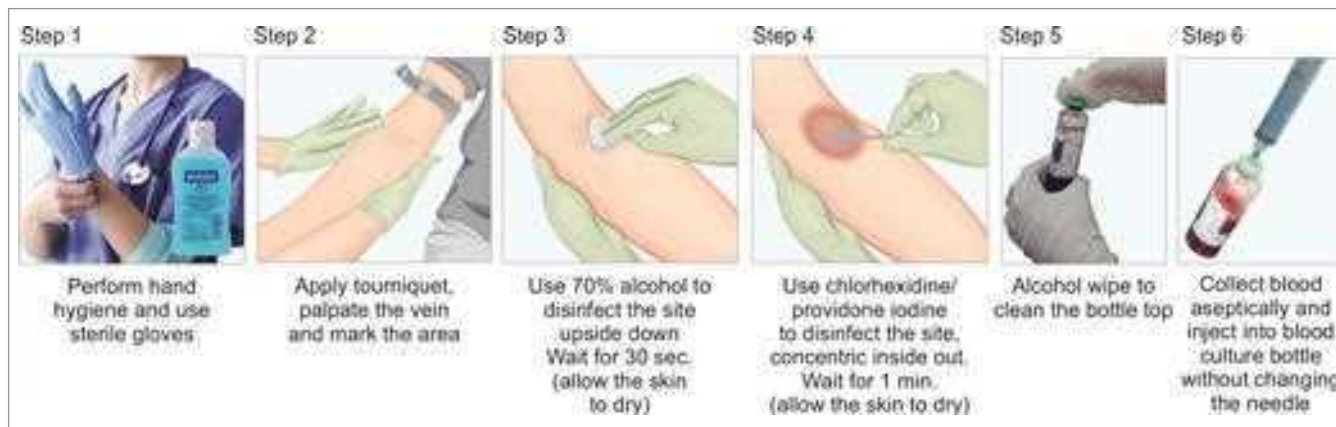


Fig. 29.1: Steps of collection of blood for culture.

1 anaerobic) are required to have good isolation rate (around 65%, 80% and 95% with one, two and three sets respectively). Multiple blood cultures should be collected for endocarditis cases

- ❖ **Dispensing:** Collected blood is then directly dispensed into either blood culture bottle at the bedside; either a conventional or automated blood culture. Change of needle between collection and dispensing, an old practice is no longer recommended
- ❖ **Transport of blood specimen:** The collected blood is gently mixed with the broth and then transported immediately to the Microbiology laboratory. In case of delay, blood culture bottle **should never be refrigerated**. It can be kept at 35°C in an incubator (if available) or left at room temperature.

Conventional Culture Medium

The method used for the conventional blood culture is as follows (refer Chapter 3.3)

- ❖ **Types of media:** There are two types of conventional blood culture media (Figs 29.2A and B)
 - Monophasic medium: It contains 50–100 mL of brain heart infusion (BHI) broth
 - Castaneda's biphasic medium: It consists of BHI agar slope and BHI broth (50 mL).
- ❖ **Dilution:** The blood is inoculated in the medium at a dilution of 1:5 so that the antibacterial components in the blood, if any, will get diluted
- ❖ **SPS** (sodium polyanethol sulfonate) is added to the medium as an anticoagulant. It also counteracts the bactericidal action of blood
- ❖ **Incubation:** Upon receipt, the bottles should be directly incubated in the upright position at 37° C for up to 7 days
- ❖ **Repeat subcultures** are made from the BHI broth onto blood agar and MacConkey agar
 - **From monophasic medium:** Subcultures are made when the broth becomes turbid or periodically (blind subcultures) for one week. There is a risk of contamination due to opening of the cap of the bottle every time when subcultures are done



Figs 29.2A to C: Blood culture bottles: **A.** Monophasic medium (BHI broth); **B.** Biphasic medium (Castaneda's), containing BHI broth and BHI agar slant; **C.** Bact/ALERT bottle.

Source: A to C. Department of Microbiology, JIPMER, Puducherry (with permission).

- **In biphasic medium,** the subcultures can be made just by tilting the bottles so that the broth runs over the agar slope. There is lower risk of contamination as it obviates the opening of the cap of the bottle. The colonies appear over the agar slant, which is further used for identification.

Automated Culture Media

BACTEC and Bact/ALERT are the automated blood culture systems. The most advanced system is Bact/ALERT Virtuo. In these systems, the growth is continuously monitored and reading is recorded every 15–20 min (Fig. 29.2C).

When the growth is detected, the system gives a positive signal. Then the bottle is removed and processed similarly as done for conventional bottles. Automated systems are much superior to conventional media in terms of faster isolation and increased sensitivity. More so, they also help in diagnosing catheter related

bloodstream infection (CRBSI) by determining the differential time to positivity.

Identification

The isolated organism is identified by colony morphology, Gram staining, followed by either conventional biochemical reactions or automated identification system such as MALDI-TOF or VITEK.

Antimicrobial Susceptibility Test (AST)

Antimicrobial susceptibility test is carried out for guiding the institution of appropriate therapy. Minimum inhibitory concentration (MIC) based method (e.g. VITEK) is preferred over disk diffusion method when testing for blood isolates. It is ideal for endocarditis isolates, especially while reporting AST result of penicillin.

TREATMENT

Sepsis/Bloodstream infection

Due to higher prevalence of multi-drug resistant bacteria (MDROs) and higher mortality in sepsis, antibiotics should be instituted at the earliest, as soon as sepsis is clinically suspected. De-escalation approach is usually followed which means:

- ❑ Empirical treatment consists of higher class of antimicrobials with both gram-negative and gram-positive coverage; e.g. carbapenem such as meropenem plus vancomycin
- ❑ Definitive treatment can be tailored according to the culture sensitivity report.

FEVER OF UNKNOWN ORIGIN (FUO)

Fever of unknown origin (FUO) is a very common term used by clinicians to refer to any febrile illness without an initial obvious etiology.

- ❖ Most febrile illnesses either resolve before a diagnosis can be made or eventually show typical clinical features or positive for specific investigations that lead to arrive at a correct diagnosis. These group of febrile illnesses are not called as FUO
- ❖ The term FUO is reserved only for prolonged febrile illnesses without an established etiology despite of intensive evaluation and diagnostic testing.

Definitions

With the advent of modern diagnostic tools, the definition of FUO has changed over time.

Petersdorf and Beeson had defined fever of unknown origin (FUO) in 1961 as patients having:

- ❖ Temperatures of more than 38.3°C (more than 101°F) at least on two occasions
- ❖ For a duration of more than 3 weeks
- ❖ Failure to reach a diagnosis despite 1 week of inpatient investigation.

This traditional definition has been modified from time to time.

- ❖ In recent days, patients with FUO undergo various investigations in outpatient department and are hospitalized only if their clinical condition warrants.

Thus the in-patient evaluation requirement has been eliminated from the definition

- ❖ As in immunocompromised patients, the diagnostic workup requires an entirely different and extensive list of investigations, they were excluded from the FUO definition
- ❖ The diagnostic workup (investigation) criteria has been changed from quantitative criterion (diagnosis uncertain after 1 week of evaluation) to a qualitative criterion which requires the performance of a specific list of investigations before labeling a case as FUO. This will help in optimal comparison of patients with FUO from different geographic areas.

The Current Definition of FUO

Accordingly, FUO is now defined as follows:

1. Fever $\geq 38.3^{\circ}\text{C}$ ($\geq 101^{\circ}\text{F}$) on at least two occasions
2. Duration of illness of ≥ 3 weeks
3. No known immunocompromised state
4. Diagnosis that remains uncertain after a thorough history-taking, physical examination, and the following obligatory investigations:
 - ESR and CRP (C-reactive protein) level
 - Platelet count, leukocyte count (total and differential), and hemoglobin
 - Electrolytes, creatinine, total protein, ferritin and protein electrophoresis
 - Enzymes such as alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase
 - Antinuclear antibodies, and rheumatoid factor
 - Urinalysis
 - Culture: blood cultures (3 negative cultures) and urine culture
 - Radiology: Chest X-ray, abdominal USG
 - Tuberculin skin test or interferon γ release assay.

Etiology of FUO

FUO has both infectious and non-infectious etiology.

- ❖ **Infections (36%):** This accounts for majority of FUO cases. All groups of microbial infections (both localized and systemic) can cause FUO (Table 29.3)
- ❖ **Neoplasms (19%):** For example, lymphoma, leukemia, myeloma, renal, colon and liver cancers, etc.
- ❖ **Non-infectious Inflammatory Diseases (19%):** For example, connective tissue disorders like rheumatoid arthritis, SLE (systemic lupus erythematosus), etc.
- ❖ **Miscellaneous Causes (19%):**
 - Granulomatous diseases
 - Inherited and metabolic diseases
 - Thermoregulatory disorders.
- ❖ Undiagnosed cases (7%).

Laboratory Diagnosis

Specimen Collection

Prior to specimen collection, a complete clinical history (including details of travel, immunization, exposure to

Bacterial causes		Non-bacterial causes		
Localized pyogenic infections	Systemic bacterial infections	Viral infections	Parasitic infections	Fungal infections
<ul style="list-style-type: none"> • Appendicitis • Cholangitis • Cholecystitis • Localized abscess • Mesenteric lymphadenitis • Osteomyelitis • Pelvic inflammatory disease • Sinusitis • Suppurative thrombophlebitis • Intravascular infections 	<ul style="list-style-type: none"> • Mycobacterial infections • Typhoid fever • Rickettsial infections • <i>Mycoplasma</i> infections • Chlamydial infections • Brucellosis • Melioidosis • Listeriosis • Bartonellosis • Actinomycosis and Nocardiosis • Spirochete infections: <ul style="list-style-type: none"> ➢ Syphilis ➢ Lyme disease ➢ Relapsing fever ➢ Leptospirosis 	<ul style="list-style-type: none"> • Cytomegalovirus and EBV infections • Coxsackievirus group B infection • Viral hepatitis • HIV infection • HSV infection • Dengue • West Nile virus infection • Colorado tick fever • Hantavirus infection • Parvovirus infection 	<ul style="list-style-type: none"> • Malaria • Amoebiasis • Leishmaniasis • Chagas' disease • Toxoplasmosis • Strongyloidiasis • Echinococcosis • Schistosomiasis • Toxocariasis 	<ul style="list-style-type: none"> • Aspergillosis • Mucormycosis • Blastomycosis • Histoplasmosis • Coccidioidomycosis • Paracoccidioidomycosis • Candidiasis • Cryptococcosis • <i>Pneumocystis</i> infection • Sporotrichosis

Abbreviations: EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus.

any other patients) and physical examination should be carried out that may be helpful in choosing the appropriate specimen such as blood, urine, bone marrow aspirate, pus from abscesses, etc.

Microscopy

- ❖ **Blood microscopy:** Useful for detection of malaria parasites (ring forms and gametocytes), filarial nematodes (microfilariae), *Leishmania donovani* (LD bodies or amastigote forms), *Toxoplasma* (tachyzoites) and trypanosomes (trypomastigote forms)
- ❖ **Stool wet mount:** For the detection of cyst, trophozoite or ova of a parasitic agent causing FUO (e.g. *Entamoeba histolytica*)
- ❖ **Gram stain** of pus, sputum and other specimens can be carried out for detection of the causative agent
- ❖ Ziehl-Neelsen stain for *M. tuberculosis*.
- ❖ Periodic acid-schiff (PAS) or Gomori methenamine silver (GMS) stain for the detection of fungal morphology.

Culture

- ❖ Blood culture is done for typhoid fever, brucellosis
- ❖ Culture on Lowenstein Jensen medium is done for *M. tuberculosis*
- ❖ Culture of pus and exudate specimen from the abscesses: for the detection of the causative agent
- ❖ **Sabouraud dextrose agar (SDA) culture:** For fungal isolation
- ❖ **Cell line culture:** Culture in appropriate cell lines is useful for the isolation of virus, e.g. human diploid cell line for cytomegalovirus (CMV).

Serological Tests

- ❖ ELISA and rapid tests for viral diseases such as hepatitis B and C, HIV, CMV, EBV infections, etc.
- ❖ **Standard agglutination test:** For brucellosis

- ❖ **Microscopic agglutination test:** For leptospirosis
- ❖ **Cold agglutination test:** For *Mycoplasma*
- ❖ **Weil Felix test:** For rickettsial diseases
- ❖ **Paul-Bunnell test:** For infectious mononucleosis
- ❖ **Widal test:** For typhoid fever
- ❖ Microimmunofluorescence test for chlamydial infections
- ❖ **Rheumatoid arthritis (RA) factor:** For rheumatoid arthritis
- ❖ Antinuclear antibody detection by immunofluorescence or ELISA for diagnosis of SLE.

Molecular Tests

If the infective organism load is very low, PCR can be very useful as it amplifies the specific gene. **Multiplex PCR** can be carried out which can simultaneously detect the common etiological agents of bloodstream infections.

Other Tests

- ❖ **Complete blood count:** Increased neutrophil count indicates pyogenic infections
- ❖ **Raised ESR** (erythrocyte sedimentation rate)
- ❖ Histopathological examinations of the biopsies obtained from tumors may suggest the underlying etiology
- ❖ **Imaging methods:** Chest X-ray (for diagnosis of tuberculosis) and CT or MRI scan to identify the malignant tumors and their extension
- ❖ ECG and echocardiography for rheumatic fever and infective endocarditis.

INFECTIONS CAUSING ANEMIA

Anemia refers to decrease in the total amount of red blood cells (RBCs) or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen. It may occur due to several etiologies. The infective causes are listed in Table 29.4.

Table 29.4: List of microorganisms causing anemia.**Iron deficiency anemia**

It occurs due to blood loss, which occurs as a result of infection with various agents, leading to microcytic hypochromic anemia.

- Hookworm (*Necator americanus* and *Ancylostoma duodenale*)
- *Trichuris trichiura*
- *Schistosoma* species

Hemolytic anemia

It occurs due to destruction of RBCs. The various infectious agents include:

- Malaria (*Plasmodium falciparum*)
- *Babesia microti*
- *Bartonella bacilliformis*
- Clostridial sepsis (*Clostridium perfringens*)
- *Mycoplasma pneumoniae*
- Infectious mononucleosis (Epstein-Barr virus)
- Hepatitis A virus

Megaloblastic anemia

It is caused by a parasite, *Diphyllobothrium latum*

It causes dissociation of vitamin B₁₂-intrinsic factor complex within the gut lumen, which leads to decreased absorption of vitamin B₁₂ in ileum.

Aplastic anemia

Aplastic anemia occurs as a result of bone marrow dysfunction (or failure) leading to a normocytic normochromic type of anemia. This can occur due to infection with various agents such as:

- *M. tuberculosis*
- Rickettsial infections
- *Leishmania donovani*
- Cytomegalovirus (CMV)
- Epstein-Barr virus
- Varicella-zoster virus
- Parvovirus B19 (aplastic crisis in patients with chronic hemolytic anemia)
- Human immunodeficiency virus (HIV)
- Human herpesvirus 6 (HHV-6)
- Hepatitis C virus
- Dengue virus (rarely)

EXPECTED QUESTIONS**I. Write essay on:**

1. A 42-year-old female presented with fever, chills and rigors, confusion, anxiety, difficulty in breathing, malaise and vomiting. On examination, the following signs were noticed: body temperature 102°F, heart rate 106 per minute and respiratory rate 22 per minute. Urine output was significantly decreased.
 - a. What is the probable clinical diagnosis?
 - b. What scoring system is used to assess the severity of infection and extent of organ failure?
 - c. How will you collect the specimen?
 - d. Describe the laboratory diagnosis in detail
2. A 28-year-old male is presented with elevated temperature of (102°F) for >3 weeks. The patient is hospitalized for the past five days without elucidation of a cause.
 - a. What is your probable clinical diagnosis?
 - b. List the various etiological agents
 - c. How will you approach for laboratory diagnosis in such case?

CHAPTER PREVIEW

- Classification and Nomenclature
- Antigenic Structure
- Typhoidal *Salmonella*

Enteric fever is a potentially fatal multisystem illness caused by *Salmonella* Typhi (typhoid fever) and, *S. Paratyphi* A, B and C (paratyphoid fever).

CLASSIFICATION AND NOMENCLATURE

Salmonella is a gram-negative bacterium, belongs to the family Enterobacteriaceae.

- ❖ The credit of discovery of '*Salmonella*' goes to Salmon and Smith (1885)
- ❖ The most important serotype of *Salmonella*, *S. Typhi*, was first observed by Eberth (1880) and Gaffky (1884) and hence was formerly called Eberth-Gaffky bacillus or *Eberthella* Typhi.

Salmonella is antigenically complex. The classification and nomenclature of salmonellae have undergone several modifications over the past years. There are several classifications proposed so far.

Clinical Classification

It is the oldest, user friendly classification which is still widely used. It divides salmonellae into two groups:

1. **Typhoidal *Salmonella*:** It includes serotypes *S. Typhi* and *S. Paratyphi*. They are restricted to human hosts, in whom they cause enteric fever (typhoid/paratyphoid fever)
2. **Non-typhoidal salmonellae or NTS:** The remaining serotypes can colonize the intestine of a broad range of animals, including mammals, reptiles, birds and insects. They also infect humans causing food-borne gastroenteritis and septicemia.

Antigenic Classification (Kauffmann–White Scheme)

The classification within the genus is based on the presence of different somatic (O) and flagellar (H) antigens which can be detected by agglutination with the respective antisera (Table 30.1).

- ❖ **Serogroups:** Based on O antigen, salmonellae are initially classified into serogroups:
 - Earlier, serogroups were named as letters, e.g. A, B, C and so on

Table 30.1: Kauffmann–White antigenic classification for *Salmonella*.

Serogroup	Serotype		O Ag*	Vi Ag	H Ag*	
	New	Old			Phase 1	Phase 2
2	A	<i>S. Paratyphi</i> A	1, 2, 12	–	a	[1,5]
4	B	<i>S. Paratyphi</i> B	1, 4, [5], 12	–	b	1,2
		<i>S. Typhimurium</i>	1, 4, [5], 12	–	i	1,2
7	C1	<i>S. Paratyphi</i> C	6, 7	+	c	1,5
		<i>S. Choleraesuis</i>	6, 7	–	c	1,5
9	D1	<i>S. Typhi</i>	9, 12	+	d	–
		<i>S. Enteritidis</i>	1, 9, 12	–	g,m	[1,7]

Abbreviation: Ag, antigen.

Note: *Antigen in brackets are not always present. Only some representative serotypes are given in the table.

There are >2,500 serotypes of salmonellae. Only the important human pathogens are enumerated in this table.

- However, as the numbers increased, the serogroups are re-designated as numbers, e.g. 1, 2, 3 and so on
- Currently, there are up to 67 serogroups, each containing group-specific O antigen, for example:
 - ◆ Serogroup-2 (formerly, serogroup A)—contains group specific O antigen type 2
 - ◆ Serogroup-4 (formerly, serogroup B)—contains group-specific O antigen type 4
 - ◆ Serogroup-9 (formerly, serogroup D)—contains group specific O antigen type 9.
- ❖ **Serotypes:** Each serogroup is further differentiated into serotypes, based on the type of flagellar antigens present. Currently, there are more than 2,500 serotypes of salmonellae.

Molecular Classification

Based on DNA hybridization studies, the genus *Salmonella* consists of two species—(1) *Salmonella enterica*, and (2) *S. bongori*.

- ❖ Within the species *S. enterica*, there are six subspecies; namely *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*
- ❖ Each subspecies is further differentiated into serotypes (based on O and H antigens as described in the Kauffmann–White scheme) (Table 30.1)

- ❖ Most of the pathogenic typhoidal and non-typhoidal *Salmonella* serotypes are placed under species *enterica* and subspecies *enterica*.

Nomenclature

Taxonomically, the correct nomenclature of the members of salmonellae is very much complicated, e.g. *Salmonella species enterica subspecies enterica serotype Typhi*.

However, for routine use a simplified format is followed where only the genus and serotype names are included, for example *Salmonella* serotype Typhi or in short, *S. Typhi*.

ANTIGENIC STRUCTURE

Salmonellae possess three important antigens on their cell wall, based on which they are classified. The antigens are:

1. Somatic antigen (O)
 2. Flagellar antigen (H)
 3. Surface envelope antigen (Vi)—found in some species.
- The O and H antigens are described in Table 30.2.

Vi Antigen

Vi antigen is a surface polysaccharide envelope or capsular antigen covering the O antigen. The naming is due to the belief that Vi antigen is related to virulence.

- ❖ It is expressed in only few serotypes, such as *S. Typhi*, *S. Paratyphi C*, *S. Dublin* and some stains of *Citrobacter freundii* (the Ballerup-Bethesda group)
- ❖ As Vi antigen is **poorly immunogenic** and antibody titers are low, it is not helpful in the diagnosis of cases. Hence, the Vi antigen is not employed in the Widal test
- ❖ However, it is believed that the complete absence of the Vi antibody in a proven case of typhoid fever indicates poor prognosis

Table 30.2: Differences between somatic (O) and flagellar (H) antigen.	
Somatic (O) antigen	Flagellar (H) antigen
It is a part of cell wall lipopolysaccharide (LPS)	Made up of protein flagellin It confers motility to the bacteria
In Widal test, O antigen of <i>S. Typhi</i> is used	In Widal test, H antigens of <i>S. Typhi</i> , <i>S. Paratyphi A</i> and <i>B</i> are used
It is less immunogenic	It is more immunogenic
O antibody appears early, disappears early: indicates recent infection	H antibody appears late, disappears late: indicates convalescent stage
When O antigen reacts with O antibody forms compact, granular, chalky clumps <ul style="list-style-type: none"> • Agglutination takes place slowly • Optimum temperature for agglutination is 55°C 	When H antigen reacts with H antibody forms large, loose, fluffy clumps <ul style="list-style-type: none"> • Agglutination takes place rapidly • Optimum temperature for agglutination is 37°C
Serogrouping of salmonellae is based on the O antigen	Serogroups are differentiated into serotypes based on H antigen

- ❖ The Vi antibody usually disappears early in convalescence, but if persists, indicates the development of the carrier state
- ❖ Phage typing of *S. Typhi* can be done by using Vi specific bacteriophages
- ❖ Vi antigens can also be used for vaccination.

TYPHOIDAL SALMONELLA

Typhoidal salmonellae include *S. Typhi* and *S. Paratyphi A*, *B* and *C* which cause enteric fever. Non-typhoidal salmonellae cause mainly gastrointestinal manifestations and have been discussed in Chapter 41.

Pathogenesis

Salmonellae are transmitted by oral route, through ingestion of contaminated food or water.

- ❖ **Infective dose of *Salmonella*** is higher than that of *Shigella*. Minimum 10^3 – 10^6 bacilli are needed to initiate the infection
- ❖ **Risk factors** that promote transmission include the conditions that decrease:
 - Stomach acidity (<1 year age, antacid ingestion, or achlorhydria or prior *Helicobacter pylori* infection)
 - Intestinal integrity (inflammatory bowel disease, prior GIT surgery or suppression of the intestinal flora by antibiotics).
- ❖ **Entry through epithelial cells (M cells)** lining the intestinal mucosa—Salmonellae can trigger the formation of membrane ruffles on the cell membrane of M cells. These ruffles reach out and enclose the adherent bacteria within the large vesicles. This process of uptake is called **bacteria-mediated endocytosis (BME)**; which is mediated by a specialized type III secretion system. Following entry, the bacilli remain inside the vacuoles in the cytoplasm
- ❖ **Entry into macrophages:** Salmonellae containing vacuoles cross the epithelial layer to reach submucosa, where they are phagocytosed by the macrophages
- ❖ **Survival inside the macrophages:** *S. Typhi* induces certain alterations on its surface (in LPS), so that it is no longer susceptible to the lysosomal enzymes of macrophages
- ❖ **Primary bacteremia:** Salmonellae contained inside the macrophages spread via the lymphatics to enter the bloodstream (transient primary bacteremia)
- ❖ **Spread:** Then, the bacilli disseminate throughout the reticuloendothelial tissues (liver, spleen, lymph nodes and bone marrow) and other organs, such as gallbladder, kidneys and lungs where further multiplication takes place
- ❖ **Secondary bacteremia** occurs from the seeded organs, which leads to the onset of clinical disease.

Clinical Manifestations of Enteric Fever

Incubation period is about 10–14 days. Enteric fever is named after the mode of transmission (enteric route) of

its causative agent. However, the manifestations seen are largely extraintestinal.

- ❖ **Fever (step ladder pattern of remittent fever):** Fever rises gradually to a higher level with every spike; then falls down, but does not touch normal
- ❖ **Other symptoms:** Headache, chills, cough, sweating, myalgia and arthralgia
- ❖ **Rashes (called rose spots):** Faint, salmon-colored, blanching, maculopapular rash on the trunk and chest seen in 30% of patients at the end of the first week
- ❖ **Early intestinal manifestations** such as abdominal pain, nausea, vomiting and anorexia
- ❖ **Important signs** include hepatosplenomegaly, epistaxis and relative bradycardia
- ❖ **Complications:** Gastrointestinal bleeding and intestinal perforation can occur mostly in the third and fourth weeks of illness
- ❖ **Neurologic manifestations** occur rarely which include meningitis, cerebellar ataxia and neuropsychiatric symptoms (described as “muttering delirium” or “coma vigil”) such as paranoid psychosis, hysteria, delirium and aggressive behavior.

Epidemiology

- ❖ **Host:** Humans are the only natural hosts for typhoidal salmonellae
- ❖ **Transmission:** It is transmitted by ingestion of contaminated water and food
- ❖ **Prevalence:** As per WHO, an estimated 11–21 million cases of typhoid fever with 1.2–1.6 lakh deaths occur annually worldwide; compared to 6 million cases of paratyphoid fever with 54,000 deaths annually. India bears the major brunt of the disease, with estimated >6 million cases annually
- ❖ **Incidence:** Varies between the countries—
 - Highest (>100 cases per 100,000 population per year) in South central and Southeast Asia
 - Medium (10–100 cases per 100,000) in the rest of Asia, Africa, Latin America
 - Low (<10 cases per 100,000) in other parts of the world.
- ❖ **Locality and age:** Enteric fever is—
 - More common in urban than rural areas
 - More common among young children and adolescents than in adults.
- ❖ **Factors** that favor transmission include:
 - Poor sanitation and improper cleaning of drinking water
 - Contaminated water, food and drinks.
- ❖ Lack of hand washing and toilet access
- ❖ Evidence of prior *Helicobacter pylori* infection
- ❖ **Typhi vs Paratyphi:** *S. Typhi* infection is more common than *S. Paratyphi A* (ratio is 4:1). However, *S. Paratyphi A* appears to be increasing, especially in India; may be due to increased vaccination for *S. Typhi*
- ❖ **Carriage:** Untreated patients become carriers and excrete *S. Typhi* in feces or urine
 - **Carriers are of two types:**
 1. Fecal carriers: Typhoid bacilli multiply in the gallbladder and are excreted in the feces. Fecal carriers are more common
 2. Urinary carriers: Multiplication takes place in kidneys and bacilli are excreted in urine. Urinary carries are rare.
 - **Duration of shedding:** Carriers continue to shed the bacilli in feces and urine for:
 - ♦ Temporary carriers: Shed *S. Typhi* in the feces for up to 3 months; up to 10% of untreated patients excrete *S. Typhi*
 - ♦ Chronic carriers: They shed *S. Typhi* in either urine or stool for >1 year; seen up to 2–5% of patients.

Chronic Carriers

It occurs in about 1–4% of infected people. Chronic carriage is more common in:

- ❑ Women, infants and old age
- ❑ Biliary tract abnormalities which leads to increased fecal excretion—Salmonellae form biofilms on gallstones and chronic carriage is associated with an increased risk of gallbladder cancer.
- ❑ Abnormalities of the urinary tract and associated *Schistosoma haematobium* infection of the bladder—Leads to increased urinary excretion.

- **Food handlers or cooks** who become chronic carriers are particularly dangerous, can excrete the bacilli for many years. The best known example of such typhoid carrier was Mary Mallon (“**Typhoid Mary**”), a New York cook who gave rise to more than 1,300 cases during her lifetime causing several outbreaks.

LABORATORY DIAGNOSIS

Enteric fever

- ❑ **Culture isolation**
 - Blood and bone marrow culture (in first week of illness):
 - ♦ Conventional: BHI broth/agar
 - ♦ Automated blood culture systems—BACTEC or BacT/ALERT).
 - Stool culture (in 3–4 weeks of illness):
 - ♦ Enrichment broth such as Selenite F broth, tetrathionate broth and gram-negative broth
 - ♦ Low selective medium: MacConkey agar (translucent NLF colonies)
 - ♦ Highly selective media: DCA, XLD agar and Wilson Blair’s Bismuth sulphite medium.
 - Urine culture (in 3–4 weeks of illness)—on MacConkey agar.
- ❑ **Culture smear and motility:** Motile, gram-negative bacilli
- ❑ **Biochemical identification**
 - Catalase positive and oxidase negative
 - ICUT: Indole(–), Citrate(+/-), Urease(–)
TSI: K/A, gas(+) except in *S. Typhi*, H_2S (*S. Typhi*- small speck, *S. Paratyphi A*-absent, *S. Paratyphi B*-abundant).
- ❑ **Slide agglutination test:** To confirm the serotype

Contd...

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LABORATORY DIAGNOSIS**Enteric fever**

- ❑ **Serum antibody detection (Widal test):** 2–3 weeks of illness. Antibodies are detected against TO, TH, AH, BH antigens
 - In *S. Typhi* infection: ↑TO and TH antibodies
 - In *S. Paratyphi A* infection: ↑TO and AH antibodies
 - In *S. Paratyphi B* infection: ↑TO and BH antibodies.
- Result and interpretation**
 - O antibodies: Produce granular chalky clumps when react with O Ag
 - H antibodies: Produce cottony woolly clumps when react with H Ag.
- ❑ **Antigen detection (serum and urine):** By ELISA
- ❑ **Molecular methods:** PCR detecting *flagellin* gene, *iro B* and *fliC* gene
- ❑ **Nonspecific findings:** For example, neutropenia
- ❑ **Antimicrobial susceptibility testing.**

Laboratory Diagnosis

Type of specimen to be collected depends on the duration of illness. The preferred specimen(s) to be collected are:

- ❖ **First week of illness:** Blood culture, bone marrow or duodenal aspirate culture
- ❖ **Second/third week of illness:** Serum specimen for serology (e.g. Widal test)
- ❖ **Third/fourth week of illness:** Urine and stool culture.

Culture and Identification**Blood Culture**

Blood culture is the ideal method for diagnosis in the first week of fever, which becomes positive in about 90% of cases. There after the positivity declines to 75% in the second week and 60% in the third week and 25% till the fever subsides. The specimen collection and procedure of blood culture is discussed in Figure 29.1.

- ❖ **Blood culture bottles:** 8–10 mL of blood may be collected in blood culture bottle; either conventional bottle (brain heart infusion medium—monophasic or biphasic) or automated bottle (e.g. BacT/ALERT) (Figs 29.2A to C)
- ❖ **Incubation:** Blood culture bottles are incubated at 37°C. Salmonellae are nonfastidious, growth occurs within 24 hours. From positively flagged blood culture broth, subcultures are made on to blood agar and MacConkey agar
- ❖ **Colony appearance:**
 - Blood agar: Nonhemolytic moist colonies
 - MacConkey agar: Colonies are round, translucent, pale and non-lactose fermenting.

Stool and Urine Culture

It is useful for the isolation of *Salmonella* in the third and fourth weeks of illness. They remain positive even after antibiotic treatment. Stool and urine culture are also done for the detection of carriers.

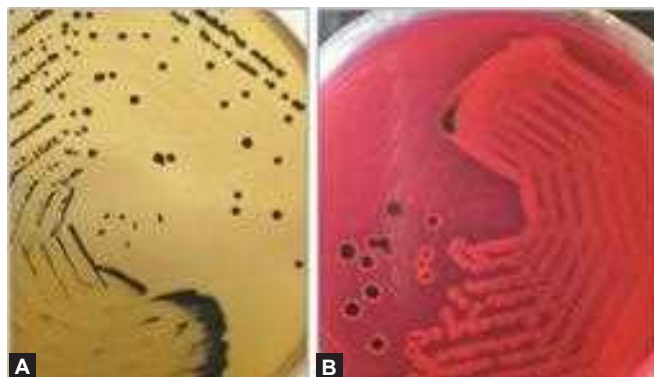
Urine culture seldom becomes positive as salmonellae are shed in urine infrequently. Urine is centrifuged and the deposit is inoculated onto MacConkey agar.

Stool culture is done similar to the method followed for *Shigella* (described in Chapter 41). Appropriate media should be used to inhibit the commensals in the stool.

- ❖ **Enrichment broth** such as Selenite F broth, tetrathionate broth and gram-negative broth are used
- ❖ **Selective media** such as:
 - **Low selective media** such as MacConkey agar
 - **Highly selective media:** Growth of *S. Typhi* occurs as follows (Figs 30.1A and B):
 - ◆ **DCA** (deoxycholate citrate agar): Produces non-lactose fermenting pale colonies with black center
 - ◆ **XLD agar** (xylose lysine deoxycholate): Produces red colonies with black center
 - ◆ **Wilson Blair's** brilliant green bismuth sulfite medium is particularly useful for the isolation of *S. Typhi* from heavily contaminated specimens. *S. Typhi* produces characteristic **jet black** colored colonies with a **metallic sheen** due to production of H₂S. *S. Paratyphi A* and others that do not form H₂S produce green colored colonies.

Other Specimens

- ❖ **Bone marrow** culture is employed during the first week of illness (55–90% sensitive) when blood culture is negative, especially when patient is on antibiotics
- ❖ **Duodenal aspirate** culture is recommended during first week of illness if both blood and bone marrow cultures turn negative
- ❖ **Combination** of blood, bone marrow, and intestinal secretions culture is the best method in the first week, which shows a sensitivity of more than 90%
- ❖ **Other specimens** from which salmonellae can be isolated are rose spots, pus from suppurative lesions, cerebrospinal fluid (CSF), sputum and autopsy specimens such as gallbladder, liver and spleen.



Figs 30.1A and B: Colonies of *S. Typhi*: **A.** DCA (Deoxycholate citrate agar) showing pale colonies with black center; **B.** XLD agar (Xylose lysine deoxycholate) showing red colonies with black center.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Culture Smear and Motility Testing

Gram-stained smear made from colonies reveals gram-negative, non-sporing and non-capsulated bacilli. They are motile with peritrichous flagella.

Identification

Identification of *Salmonella* from the colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as described below.

- ❖ It is catalase positive and oxidase negative
- ❖ Indole test (negative), citrate test (negative), urease test (negative)
- ❖ TSI (triple sugar iron test) shows:
 - Alkaline/acid
 - Gas present (except for *S. Typhi*, which is anaerogenic)
 - Abundant H₂S present except for:
 - ♦ *S. Paratyphi A*: H₂S not produced
 - ♦ *S. Typhi*: Speck of H₂S present at the junction of slant and butt.
- ❖ MALDI-TOF can identify *Salmonella* up to genus level. However they poorly differentiate between serotypes as they share the same ribosomal proteins.

Slide Agglutination Test

Identification of *Salmonella* at genus level can be confirmed by slide agglutination using polyvalent O antisera. Then, the serotypes can be identified by using type specific O antisera.

- ❖ *S. Typhi*: Agglutinates with O9 antisera
- ❖ *S. Paratyphi A*: Agglutinates with O2 antisera
- ❖ *S. Paratyphi B*: Agglutinates with O4 antisera.

Flagellar antigens can also be determined by using type-specific H antisera.

Antimicrobial Susceptibility Testing (AST)

AST is performed by disk diffusion method (on Mueller-Hinton agar) or by automated MIC detection method by microbroth dilution (e.g. by VITEK).

Demonstration of Serum Antibodies

Widal Test

Widal test is one of the oldest and most widely used serological tests for diagnosis of enteric fever. It was discovered by Fernand Widal in 1896.

- ❖ **Principle:** It is an agglutination test where H and O antibodies against *S. Typhi* and *S. Paratyphi A* and B are detected and measured in the patient's sera by using O and H antigens
- ❖ **Antigens used:** Four antigens are used
 1. O antigens of *S. Typhi* (TO)
 2. H antigens of *S. Typhi* (TH)
 3. H antigens of *S. Paratyphi A* (AH)
 4. H antigens of *S. Paratyphi B* (BH).

Note: The paratyphoid O antigens cross-react with the typhoid O antigen due to their sharing of factor 12, hence they are not used in the test.

- ❖ **Procedure:** Patient's serum is serially diluted in normal saline in test tubes from 1 in 10 to 1 in 640 dilutions. Four such sets are made
 - To each set of diluted sera, respective four antigen suspensions (TO, TH, AH, BH) are added
 - Test tubes are incubated in water bath at 37°C overnight.
- ❖ **Results** (Fig. 30.2):
 - O agglutination appears as compact granular chalky clumps (disk-like pattern), with clear supernatant fluid
 - H agglutination appears as large loose fluffy cotton-woolly clumps, with clear supernatant fluid
 - If agglutination does not occur, *button* formation occurs due to deposition of antigens and the supernatant fluid remains hazy
 - **Titer:** The highest dilution of sera, at which agglutination occurs, is taken as the antibody titer.
- ❖ **Interpretation** (Table 30.3 and Fig. 30.3):
 - **Significant titer:** Any titer is not significant. In endemic countries like India, due to prior exposure, people will always have some baseline antibodies. Higher titers are only significant. The cut-off varies from place to place depending on endemicity of the disease

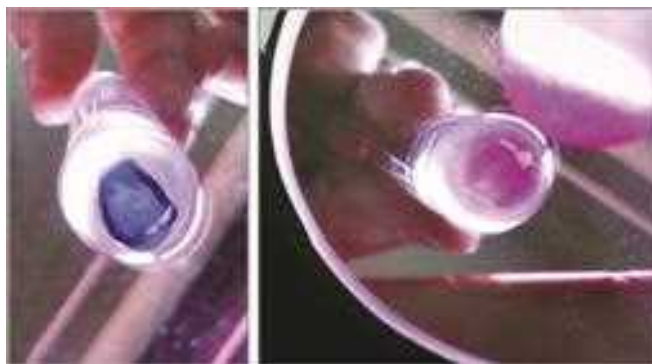


Fig. 30.2: O and H agglutination in Widal test (reading taken in a mirror).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences.

Table 30.3: Interpretation of Widal test.

Widal test result	Suggestive of
Rise of TO and TH antibody	Enteric fever due to <i>S. Typhi</i>
Rise of TO and AH antibody	Enteric fever due to <i>S. Paratyphi A</i>
Rise of TO and BH antibody	Enteric fever due to <i>S. Paratyphi B</i>
Rise of only TO antibody	Recent infection: Due to <i>S. Typhi</i> or <i>S. Paratyphi A</i> or B
Rise of only TH antibody	? Convalescent stage/anamnestic response
Rise of all TH, AH, BH antibodies	Post TAB vaccination

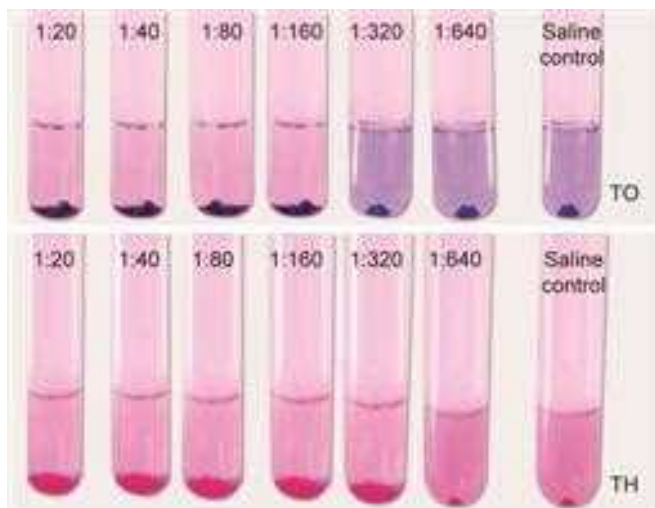


Fig. 30.3: Widal test showing titre of TO 1:160 and TH 1:320.

Abbreviation: TO and TH, antibody titre to *S. Typhi* O and H antigens in patient's serum.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences.

- ♦ Significant titer in most of the places in India is taken as:
 - H agglutinin titer more than 200 and
 - O agglutinin titer more than 100.
- ♦ Low titers should be ignored and considered as baseline titers in endemic areas.
- **False-positive:** Widal test may occur due to:
 - ♦ **Anamnestic response:** It refers to a transient rise of titer due to unrelated infections (malaria, dengue) in persons who have had prior enteric fever
 - ♦ If bacterial antigen suspensions are not free from fimbriae
 - ♦ Persons with inapparent infection or
 - ♦ Persons with prior immunization (with TAB vaccine).
- **A fourfold rise** in antibody titer demonstrated by testing paired sera at 1-week interval is more meaningful than a single high titer. Rise in titers in anamnestic responses are transient that usually falls after 1 week whereas, in true infection, the titer increases by fourfold after 1 week
- **False-negative:** Widal test may occur in:
 - ♦ Early-stage (1st week of illness)
 - ♦ Late-stage (after fourth week)
 - ♦ Carriers
 - ♦ Patients on antibiotics
 - ♦ Due to prozone phenomena (antibody excess)—this can be obviated by serial dilution of sera.
- O agglutinins appear early and disappear early and indicate recent infection. H agglutinins appear late and disappear late
- O antibodies are serotype nonspecific. They are raised in all infections, i.e. *S. Typhi*, *S. Paratyphi* A and B
- H antibodies are specific. TH, AH and BH antibodies are raised in *S. Typhi*, *S. Paratyphi* A and B infections respectively.

Other Antibody Detection Tests

Various commercial methods available are:

- ❖ **Typhidot test:** 50 kDa OMP (outer membrane protein) antigen is used; it uses a dot ELISA format to detect both IgM and IgG separately after 2-3 days of infection
- ❖ **IDL Tubex test:** O9 antigen is used, detects only IgM antibodies against *S. Typhi* by a semiquantitative colorimetric method
- ❖ **IgM dip stick test** and **ELISA** detect anti-LPS IgM antibodies
- ❖ **Dot blot assay:** Flagellar antigen is used, detects only IgG antibodies.

Demonstration of Serum Antigens

Antigens of typhoidal salmonellae are consistently present in the blood in the early course of the disease, and also in the urine of patients during the late phase. Several methods such as ELISA are available for antigen detection.

Molecular Methods

Several polymerase chain reaction (PCR) based methods (e.g. nested PCR) are available to detect and differentiate typhoidal salmonellae by targeting various genes, such as *flagellin* gene, *Iro B* and *fliC* gene.

Other Nonspecific Methods

- ❖ WBC count: Neutropenia is seen in 15–25% of cases. Leukocytosis is more common among children, during early phase and in cases complicated by intestinal perforation or secondary infection
- ❖ Liver function tests moderately deranged
- ❖ Muscle enzyme levels moderately elevated.

Antimicrobial Susceptibility Testing

It is done by disk diffusion method on Mueller Hinton agar or MIC based method (e.g. VITEK).

Detection of Carriers

- ❖ **Culture:** By stool and bile culture (detects fecal carriers) and urine culture (detects urinary carriers)
- ❖ **Detection of Vi antibodies:** It is done by tube agglutination test by using *S. Typhi* suspension carrying Vi antigen (Bhatnagar strains). Even a titer of 1:10 is also considered as significant. However, the diagnosis should always be confirmed by culture
- ❖ **Isolation of salmonellae from sewage:** It is carried out to trace the carriers in the communities. It can be done by:
 - Sewer-swab technique: Gauze pads left in sewers are cultured on highly selective media, such as Wilson and Blair media
 - Filtration: Sewage can be filtered through millipore membranes and the membranes are cultured on highly selective media.

T TREATMENT

Enteric fever

Prompt administration of appropriate antibiotics prevents severe complications and reduces the mortality to <1%.

Treatment of cases depends on the susceptibility of the strains.

The currently recommended drugs are as follows:

- ❑ Third generation cephalosporins: Ceftriaxone is the drug of choice for empirical treatment; given 1–2 g/day, IV, for 10–14 days
- ❑ Azithromycin: Alternative drug for empirical therapy. Advantage is, it is given orally, 1g/day for 5 days
- ❑ Fluoroquinolones, e.g. ciprofloxacin: Because of increased drug resistance, it should not be given empirically. It can be given only if, found susceptible. It is given as 500 mg twice a day oral for 5 days.

Drugs that were used in the past were: Chloramphenicol, amoxicillin and cotrimoxazole.

Treatment of carriers include: Ampicillin or amoxicillin plus probenecid; given for 6 weeks.

Drug Resistance in Typhoidal Salmonellae

Antimicrobial resistance in typhoidal salmonellae is a global problem.

- ❖ **Multidrug-resistant (MDR) S. Typhi:** It is defined as resistant to chloramphenicol, ampicillin and cotrimoxazole. These antibiotics were in use to treat enteric fever in the past. MDR strains emerged in 1989 in China and Southeast Asia including India and since then they have disseminated widely
- ❖ **Fluoroquinolone (FQ) resistance:** After the emergence of MDR strains, ciprofloxacin became the drug of choice since the year 1990 and was extensively used for three decades. This led to the emergence of drug resistance to FQs; mainly seen in India and other regions of southern Asia
 - In India, ciprofloxacin resistance in *Salmonella* is very high (>70% in 2019)
 - The key mechanisms responsible for FQ resistance mutations in *gyrA* and *parC* genes.
- ❖ **Resistance to ceftriaxone:** It is very rare (<1%), has been reported recently. Both extended spectrum β -lactamases (ESBLs) and AmpC β -lactamase producing *S. Typhi* have been detected
- ❖ **Old is gold:** Interestingly, it is noticed that many strains reverted susceptible to the olden days drugs (amoxicillin, chloramphenicol, cotrimoxazole) as they were not in use for long time.

Prophylaxis

Theoretically, it is possible to control or eliminate enteric fever since the agents survive only in the human hosts and are spread by contaminated food and water. Many developed countries have proven this. However, in developing countries, this goal is currently unrealistic due to lack of adequate sewage disposal and water treatment. There are three lines of prophylactic measures.

Control of Reservoir

Control of Cases

- ❖ By early diagnosis and prompt effective treatment
- ❖ Disinfection of stool or urine soiled clothes with 5% cresol, 2% chlorine or by steam sterilizer
- ❖ Follow-up examination of stool and urine culture to detect carriers (twice, at 3–4 months and at 12 months).

Control of Carriers

- ❖ Early detection of carriers by stool/urine culture or by detection of Vi antibodies
- ❖ Effective treatment of carriers by:
 - Ampicillin or amoxicillin (4–6 g/day) plus probenecid (2 g/day) for 6 weeks. These drugs get concentrated in bile and may eliminate 70% of carrier state
 - Surgery: Cholecystectomy plus ampicillin is regarded as the most effective approach for carrier state elimination (80% cure rate).

Sanitation Measures

Sanitation measures include the following:

- ❖ Protection and purification of drinking water supplies
- ❖ Hand washing and improvement of basic sanitation
- ❖ Promotion of food hygiene
- ❖ Health education.

Vaccine

Immunization provides short time protection. It is indicated in following situations:

- ❖ Travelers going to endemic areas
- ❖ People attending *melas* and *yatras*
- ❖ Household contacts
- ❖ People at increased risk (school children)
- ❖ People living in endemic area (optional).

There are three types of vaccines available (see below).

Vaccines for Typhoid Fever

There are two types of typhoid vaccines available currently.

Vi antigen capsular polysaccharide (Vi-CPS) vaccine:

It is composed of purified Vi capsular polysaccharide antigen derived from *S. Typhi* strain Ty2.

- ❑ Dosage: Single dose containing 25 μ g of Vi antigen is given IM or subcutaneously
- ❑ Vaccine confers protection for 2 years; booster is given every 2 years
- ❑ Age: It is given only after 2 years of age. Capsular antigen being T independent antigen, is poorly immunogenic to children < 2 years
- ❑ **Vi-rEPA:** Vi antigen is conjugated with recombinant *Pseudomonas aeruginosa* Exotoxin A. Conjugation increases immunogenicity of the Vi antigen; therefore this vaccine can be given to children less than two years.

Typhoral (oral live attenuated S. Typhi Ty21a vaccine):

Typhoral is a stable live attenuated mutant of *S. Typhi* strain Ty21a, which lacks the enzyme UDP-galactose-4-epimerase (Gal E mutant)

Contd...

Contd...

- ❑ On ingestion, it multiplies for some time, initiates the immune response but self-destructs (dies of its own after 4–5 cell divisions, due to lack of Gal E enzyme) and therefore cannot induce any pathogenesis
- ❑ It is indicated only after 6 years of age
- ❑ The vaccine is available in lyophilized form as **enteric coated capsules**
- ❑ It is given orally before food, on alternate days- 1, 3, 5, 7. No antibiotics should be given during this period
- ❑ **Revaccination** is recommended every 5 years with a full 4-dose series
- ❑ **Protective immunity** starts after 7 days of the last dose and lasts for 4 years.

Parenteral TAB vaccine

It is a heat-killed whole cell *S. Typhi*/*S. Paratyphi A* and *B* vaccine. It is no longer in use because of significant side effects.

NON-TYPHOIDAL SALMONELLAE

Non-typhoidal salmonellae cause mainly gastrointestinal manifestations, and have been described in Chapter 41.

However, up to 8% of patients with NTS gastroenteritis develop into bacteremia; which can lead to either endovascular infection or seedling to various organs leading to metastatic infections.

Risk factors for bacteremia include:

- ❖ **NTS serotype:** Most common being *S. Choleraesuis* (source-pig) and *S. Dublin* (source—cattle)
- ❖ **Age:** Infants and elderly people are at higher risk
- ❖ **Immunity:** HIV and other conditions with low immunity
- ❖ **People with pre-existing valvular heart disease:** NTS can cause endovascular infection such as endocarditis and arteritis.

EXPECTED QUESTIONS**I. Write essay on:**

1. Meena, a young adult female was admitted to the hospital with intense headache, abdominal discomfort for the past 5 days. She had also developed fever which is of remittent type with gradual rise in a step ladder fashion. On examination, she was toxic with temperature of 101° F, tongue was coated and mild splenomegaly was present.
 - a. What is the most probable etiological diagnosis?
 - b. Describe the pathogenesis of this condition.
 - c. Mention sample collection and laboratory diagnosis in detail.
 - d. Add a note on treatment and vaccination available for this clinical condition.

II. Write short notes on:

1. Typhoid carriers.
2. Drug resistance in salmonellae.

III. Multiple Choice Questions (MCQs):

1. ***S. Typhi* is the causative agent of typhoid fever. The infective does of *S. Typhi*:**
 - a. One bacillus
 - b. 10^3 – 10^6 bacilli
 - c. 10^8 – 10^{10} bacilli
 - d. 1–10 bacilli

2. In a patient with typhoid, diagnosis after 15 days of onset of fever is best done by:

- a. Blood culture
- b. Stool culture
- c. Urine culture
- d. Widal test

3. Antibodies against which of the following antigen appear early following infection with *S. Typhi*?

- a. Vi antigen
- b. O antigen
- c. H antigen
- d. Capsular antigen

4. In Widal test, rise of TO and AH indicates infection with:

- a. *S. Typhi*
- b. *S. Paratyphi A*
- c. *S. Paratyphi B*
- d. *S. Paratyphi C*

5. Antibodies against which of the following antigen appear in typhoid carrier?

- a. Vi antigen
- b. O antigen
- c. H antigen
- d. Capsular antigen

6. Bacteria mediated endocytosis (BME) is observed in:

- a. *Shigella*
- b. *Listeria*
- c. *Salmonella*
- d. *Campylobacter*

7. Currently the drug of choice for enteric fever is:

- a. Ciprofloxacin
- b. Azithromycin
- c. Ceftriaxone
- d. Chloramphenicol

Answers

1. b 2. d 3. b 4. b 5. a 6. c 7. c

CHAPTER PREVIEW

- General Properties
- *Rickettsia* Infections
- Scrub Typhus (*Orientia*)
- Ehrlichiosis
- Q Fever (*Coxiella burnetii*)
- Bartonellosis

GENERAL PROPERTIES

Rickettsiae comprise of group of small non-motile gram-negative coccobacilli that possess the following common characteristics:

- ❖ They are obligate intracellular organisms
- ❖ They are not cultivable in artificial media, although they can grow in cell lines, or by animal and egg inoculation
- ❖ They are transmitted by arthropod vectors, such as tick, mite, flea or louse
- ❖ They infect the vascular endothelial cells (final target site).

Classification

The order Rickettsiales has two families:

1. Family Rickettsiaceae comprises of two pathogenic genera—*Rickettsia* and *Orientia*
2. Family Anaplasmataceae includes four genera—(1) *Ehrlichia*, (2) *Wolbachia*, (3) *Anaplasma*, and (4) *Neorickettsia*.

Former members such as *Coxiella* and *Bartonella* are now excluded from the family. This is because:

- ❖ *Coxiella* is not arthropod borne; infection is transmitted by inhalational mode
- ❖ *Bartonella* is not an obligate intracellular parasite; capable of growing in cell-free media. It also differs in genetic properties.

Rickettsiae Versus Viruses

Because of the small size and obligate intracellular properties, Rickettsiae were once thought to be viruses, however now they are confirmed to be bacteria due to having following characters:

- ❖ They possess gram-negative cell wall (however, they are poorly gram-stained, better stained with Giemsa stains)
- ❖ They contain both DNA and RNA
- ❖ Rickettsiae multiply by binary fission
- ❖ They are susceptible to antibacterial agents
- ❖ Rickettsiae are large enough to be seen under the light microscope
- ❖ They are held back by bacterial filters.

History

Rickettsia is named after Howard Taylor Ricketts (1911), who discovered that Rocky Mountain spotted fever is transmitted by tick

- ❖ *Rickettsia prowazekii* is named by Da Rocha Lima in honor of von Prowazek
- ❖ Both von Prowazek and H Ricketts died of typhus which they contracted during their study.

FAMILY RICKETTSIACEAE INFECTIONS

Family Rickettsiaceae comprises of two pathogenic genera *Rickettsia* and *Orientia* (Table 31.1).

RICKETTSIA INFECTIONS

Rickettsia can be categorized into two groups based on the clinical manifestations (Table 31.1):

1. Typhus group
2. Spotted fever group.

Antigenic Structure

The cell wall of rickettsiae is similar to that of gram-negative bacteria, composed of peptidoglycan, lipopolysaccharide (LPS), and an outer membrane containing few outer membrane proteins (OMP).

- ❖ **OMP antigens** are species-specific; highly immunogenic, thus can be used for vaccine as well as for serodiagnosis
- ❖ **LPS antigens** are group-specific; found in some rickettsiae and is shared by certain strains of *Proteus*. This antigenic cross reactivity serves as the basis of Weil-Felix reaction; the serological test used for the diagnosis of rickettsial infections.

Pathogenesis

- ❖ **Transmission:** All rickettsiae are transmitted to humans by arthropod vectors (Table 31.1)
 - Transmission occurs either by their bite (e.g. tick and mite) or following rubbing or scratching of the vector on abraded skin or mucosa (louse or flea)

Broad group	Species	Disease	Vector	Distribution	Rash	Eschar	LN	Weil-Felix test
Typhus group	<i>R. prowazekii</i>	Epidemic typhus	Louse	Worldwide (Africa and South America)	80% (All over the body except palm and sole)	–	–	OX19 ++++ OX2 +/-
		Brill–Zinsser disease						Negative or weakly positive
	<i>R. typhi</i>	Endemic typhus	Flea	Worldwide	80% (trunk)	–	–	OX19 ++++ OX2 +/-
Spotted fever group	<i>R. rickettsii</i>	Rocky Mountain spotted fever (RMSF)	Tick	America	90% (extremities and trunk, more hemorrhagic)	<1%	+	OX19 ++ OX2 ++
	<i>R. conorii</i>	Indian tick typhus (ITT)	Tick	Europe, Asia	97%	50%	+	
	<i>R. africae</i>	African tick bite fever	Tick	Sub-Saharan Africa	50% (vesicular)	90%	++++	
	<i>R. akari</i>	Rickettsialpox	Mite (gamasid)	USA, Ukraine, Turkey, Mexico	100% (vesicular)	90%	+++	All negative
Scrub typhus	<i>Orientia tsutsugamushi</i>	Scrub typhus	Mite (trombiculid)	Asia, Australia	50%	35%	+++	OXK +++

- Tick and mite can act as reservoirs, maintain the organism by **transovarial transmission** to offspring.
 - ❖ **Spread:** Rickettsiae spread through the lymphatics from the portal of entry, multiply in the regional lymph nodes and then spread via bloodstream
 - ❖ **Target sites:** For all rickettsiae, the final target site is the endothelial cells (in addition, *R. akari* and *O. tsutsugamushi*, attack the monocytes)
 - ❖ **Phagocytosis:** Adhesion to the endothelial cells is mediated by outer membrane proteins; OmpA and OmpB present on rickettsial surface. Following adhesion, the organisms are phagocytosed
 - ❖ **Intracellular survival:** Being obligate intracellular; following phagocytosis they survive inside the host cells by exhibiting various mechanisms such as resisting lysosomal killing or inhibiting phagolysosomal fusion, etc.
 - ❖ **Multiplication:** Inside the host cells, they multiply slowly by binary fission (generation time is about 9–12 hours)
 - ❖ **Cell-to-cell spread:** Spotted fever rickettsiae can spread from cell-to-cell by actin polymerization. In contrast, other rickettsiae accumulate in the cell until the lysis of the cell takes place.
- Epidemic Typhus (Louse-borne)**
- Epidemic typhus is caused by *R. prowazekii*.
- ❖ **Vector:** Human body louse, *Pediculus humanus corporis* acquires the organism while taking the blood meal from an infected patient
 - ❖ **Mode of transmission:** (1) By rubbing or scratching of abraded skin or mucosa contaminated by louse feces, (2) rarely, by inhalation of louse feces, during laboratory work exposure or during bioterrorism
 - ❖ **Clinical manifestations:** Epidemic typhus is an acute febrile disease; accompanied by headache, myalgia, eye discharge and rashes occurring after an incubation period of 1–2 weeks
 - **Rash** begins on the upper trunk, usually on the fifth day, and then becomes generalized, involving the entire body except for the face, palms and soles
 - **Myalgia** is usually severe, was referred to as sutama (“crouching”) in Burundi outbreak, a designation reflecting the posture of the patients attempting to alleviate the pain
 - **Complications** include interstitial pneumonitis, CNS involvement manifests as mental confusion and coma (*‘typhus’* name comes from the Greek word *typhos* meaning smoky or hazy, describing the state of mind of the affected patients). If not treated promptly can be fatal in 7–40% of cases.
 - ❖ **Risk factors:** Outbreaks occur when louse population increases; especially in unhygienic conditions. Typical settings include refugee camps, prisons and overcrowded communities
 - ❖ **Zoonotic cycle:** Eastern flying squirrels and their lice and fleas maintain *R. prowazekii* in the environment
 - ❖ **Geographical distribution:** It is endemic in Africa (notably Burundi, Rwanda and Ethiopia) and South America (Peru, Bolivia and Ecuador). Burundi outbreak in 1997 had involved nearly 1 Lakh people in refugee camps
 - ❖ **Brill–Zinsser disease:** It is a recrudescent illness occurring years after acute epidemic typhus. *R. prowazekii* remains latent for years; its reactivation occurs due to waning immunity, which leads to sporadic infection or outbreaks.

Endemic Typhus (Flea-borne)

Endemic (murine) typhus is caused by *R. typhi*.

- ❖ **Vectors:** The vector for endemic typhus is rat flea (*Xenopsylla cheopis*) or rarely by cat flea (*Ctenocephalides felis*)
- ❖ **Mode of transmission:** It is transmitted by rubbing or scratching on skin or inhalation of flea's dried feces, less frequently by the flea bite
- ❖ **Reservoir:** Rodents such as *Rattus rattus* and *R. norvegicus* species are the natural reservoirs; whereas the opossum/cat flea (*C. felis*) cycle is prominent in southern Texas and southern California
- ❖ **Clinical manifestations:** Incubation period is 1–2 weeks (average 11 days)
 - Symptoms are similar to epidemic typhus but milder and rarely fatal
 - Common symptoms include fever, headache, myalgia, anorexia and rash (involving the trunk more often than the extremities).
- ❖ **Geographical distribution:** It is endemic worldwide, especially in warm (often coastal) areas throughout the tropics having high rat infestations
 - Recent days, it is increasingly reported from Southeast Asia and Western Pacific
 - **India:** It has been reported from many places, such as Shimla, Kashmir, Mumbai, Lucknow and Pune.

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever (RMSF) is caused by *Rickettsia rickettsii*.

- ❖ **Transmission:** By the bite of an infected tick such as—Dermacentor (in USA) and Amblyomma (in South America)
- ❖ **Reservoir:** Ticks serve as vector as well as reservoir. Other mammals like dogs, small rodents can also act as reservoir
- ❖ **Clinical manifestations:** Incubation period ranges from 4 to 14 days
 - RMS fever is an acute potentially fatal disease characterized by fever, headache, rash, myalgia and anorexia
 - Rashes appear typically on extremities (wrist and ankles) and trunk. Initially they are maculopapular, later become hemorrhagic
 - **Complications:** They appear late, include: vascular damage, increased permeability, edema, hemorrhage, disseminated intravascular coagulation, interstitial pneumonitis, and CNS involvement
 - It is the most fatal rickettsial disease and is associated with higher mortality rate.
- ❖ **Geographical distribution:**
 - RMS fever is endemic in high tick population areas of USA, Central and South America
 - It is more common during tick season (summer in tropics) and among children and males.

Indian Tick Typhus

Indian tick typhus (ITT) is caused by *Rickettsia conorii*.

- ❖ **Vector:** Transmitted by tick bite (*Rhipicephalus sanguineus*)
- ❖ **Clinical manifestations:** They are similar to that of RMS fever. In addition, an eschar is observed at the site of the tick bite in 50% of cases. Disease is more severe in patients with diabetes, alcoholism, or heart failure
- ❖ **Geographical distribution:** *R. conorii* is prevalent in Southern Europe, Africa and Southern Asia. In India, ITT is widespread. Cases have been reported from Nagpur, Jabalpur, Sagar, Pune, Lucknow, Bengaluru and Secunderabad.

Rickettsialpox

Rickettsialpox is caused by *Rickettsia akari*.

- ❖ **Vector:** Transmitted by bite of infected mites (*Liponyssoides sanguineus*)
- ❖ **Reservoir:** Mice are the principal reservoir of *R. akari*
- ❖ **Clinical manifestations** of rickettsialpox are similar to any other rickettsial diseases, but differ from the later by the presence of:
 - Vesicular rashes (often confused for the rashes of chickenpox, hence so named)
 - Eschar (painless black crusted lesions surrounded by an erythematous halo), is present at the site of mite bite
 - Regional lymphadenopathy.
- ❖ **Geographical distribution:** *R. akari* is endemic in USA, Ukraine, Turkey and Mexico.

LABORATORY DIAGNOSIS

Rickettsial infections

Serology (antibody detection)

- Non-specific test (**Weil-Felix test**)—Rickettsial antibodies detected against *Proteus* OX 19, OX 2 and OX K antigens
 - In epidemic and endemic typhus—↑OX 19 antibody
 - In tick-borne spotted fever—↑OX 19 and ↑OX 2 antibodies
 - In scrub typhus—↑OX K antibody.
- Specific antibody detection—by Indirect IF, ELISA and Indirect immunoperoxidase assay

Histological examination of a cutaneous biopsy sample (from rash)

Isolation—by inoculating into cell lines (Vero, WI-38, HeLa), embryonated egg (yolk sac), or animal (guinea pig)

PCR—detecting gene encoding 16S rRNA or *Omp* genes.

Laboratory Diagnosis of Rickettsiosis

Serology

Serology (antibody detection) is the mainstay of diagnosis of rickettsial diseases. They can be categorized into non-specific test (Weil-Felix test) and specific tests.

Weil-Felix Test

It is heterophile agglutination test works on the principle of antigenic cross reactivity. Though this test lacks high

sensitivity and specificity but still serves as a useful and inexpensive diagnostic tool.

- ❖ **Principle:** The alkali-stable LPS antigens of some rickettsiae is also shared by certain strains of *Proteus* (OX19, OX2 and OXK strains). Hence, rickettsial antibodies are detected by using *Proteus* antigens
- ❖ **Procedure:** It is a tube agglutination test; serial dilutions of patient's serum are treated with non-motile strains of *P. vulgaris* OX19 and OX2 and *P. mirabilis* OXK
- ❖ **Results:** Test should be carried out only after 5–7 days of onset of fever. Titre of 1:80 is to be considered as possible infection
 - In epidemic and endemic typhus—sera agglutinate mainly with OX19 and sometimes with OX2
 - In tick-borne spotted fever—antibodies to both OX19 and OX2 are elevated
 - In scrub typhus—antibodies to OXK are raised
 - The test is negative in rickettsialpox, Q fever, ehrlichiosis and bartonellosis.
- ❖ **False-positive titer** may be seen in presence of underlying *Proteus* infection. Hence, fourfold rise of antibody titer in paired sera is more meaningful than a single high titer
- ❖ **False-negative result** may occur due to excess antibodies in patient's sera (prozone phenomenon). This can be obviated by testing with serial dilutions of patient's sera
- ❖ Weil–Felix test being a nonspecific test should always be confirmed by specific tests.

Specific Antibody Detection Tests

- ❖ **Indirect immunofluorescence assay (IFA):** It is the gold standard and reference serologic test used for confirmation of the rickettsial diagnosis
 - Antibodies appear only after 7–10 days of infection
 - Titer of $\geq 1:64$ is considered as significant.
- ❖ **ELISA (IgM capture ELISA):** It is useful in early diagnosis (<1 week) with excellent sensitivity and specificity.

Other Methods of Diagnosis

- ❖ **Histological examination** of a cutaneous biopsy sample from a rash lesion can be done even during acute illness
- ❖ **Isolation:** Rickettsiae cannot be cultivated in cell free media
 - However, isolation can be done by cell lines (Vero and HeLa), egg (yolk sac inoculation), or animal inoculation (guinea pig)
 - As rickettsiae are highly infectious, isolation should be attempted cautiously only in laboratories equipped with biosafety level III facilities.
- ❖ **Neil-Mooser reaction:** It is an animal pathogenicity testing performed using guinea pigs to speciate various rickettsial species; now not in use
- ❖ **Molecular tests:** Polymerase chain reaction (PCR) and real time PCR formats are available

- They are rapid and specific; can detect specific rickettsial DNA (e.g. gene encoding the major surface antigens, 16S rRNA or *Omp* genes)
- Useful specimens are: Whole blood, buffy coat fraction, skin rash biopsies, lymph node biopsies or tissue specimen
- The results are best within the first week for blood samples as rickettsemia is present usually in first 7–10 days.

TREATMENT

Rickettsiosis

Doxycycline (for 1–5 days) is the drug of choice for treatment of most rickettsial illnesses. Chloramphenicol is used as alternative.

Prevention of Rickettsiosis

Preventive measures include:

- ❖ Vector control strategies such as use of insecticides
- ❖ Control of rodents and other animals
- ❖ Improvement of personal hygiene.

No vaccine is available at present against rickettsial infections.

SCRUB TYPHUS (ORIENTIA)

Scrub typhus is caused by *Orientia tsutsugamushi* (formerly classified under *Rickettsia*). It differs from *Rickettsia* both genetically and by its cell wall composition (i.e. it lacks lipopolysaccharide layer).

- ❖ **Naming:** Scrub typhus is so named because as it can occur in areas where scrub vegetations consisting of low lying trees and bushes are encountered. However sandy, semi-arid and mountain desert areas can also be endemic harboring the vector
- ❖ **Vector:** It is transmitted by the bite of infected trombiculid mites of genus *Leptotrombidium* (*L. akamushi* in Japan and *L. deliensis* in India)
 - Chiggerosis: Among all stages of mite, the larvae (called **chiggers**) are the only stage that feed on humans. Hence, scrub typhus is also called *chiggerosis*
 - Mites can maintain the organisms through transovarian transmission.
- ❖ **Clinical manifestations:** The classic presentation of scrub typhus consists of triad of an eschar (at the site of bite), regional lymphadenopathy and maculopapular rash. However, it is seen only in 40–50% of cases
 - Non-specific manifestations may appear early, such as fever, headache, myalgia, cough, and gastrointestinal symptoms
 - Complications such as encephalitis and interstitial pneumonia may occur rarely in the late stage (due to vascular injury).
- ❖ **Epidemiology:** Among the rickettsial diseases, scrub typhus is most widespread
 - **Zoonotic tetrad:** Four elements are essential to maintain *O. tsutsugamushi* in nature:

1. Trombiculid mites
2. Small mammals (e.g. field mice, rats, shrews)
3. Secondary scrub vegetations or forests (hence named as scrub typhus)
4. Wet season (when mites lay eggs).

- **World scenario:** Scrub typhus is endemic to a part of the world known as the “**tsutsugamushi triangle**”, which extends from Japan-Russia in the north, to Australia in the south, and to Pakistan in the west. Various countries included in this triangle are Japan, China, Philippines, and Southeast Asia, including India
- **Indian scenario:** Scrub typhus is a re-emerging infectious disease in India. It is the most common rickettsial disease in India; prevalent in many parts of the country such as sub-Himalayan belt, Bihar, Rajasthan, Maharashtra, Karnataka, Tamil Nadu, Pondicherry and Kerala.
- ❖ **Rural to urban shift:** Though it is mainly reported from rural areas, however recently cases have been increasingly reported from urban areas also
- ❖ **Treatment:** Doxycycline is the drug choice. Chloramphenicol or azithromycin is given alternatively.

LABORATORY DIAGNOSIS

Scrub typhus

Serology (antibody detection)

Serology remains the mainstay of diagnosis. In primary infection IgM antibodies appear by the end of the 1st week, and IgG by end of the 2nd week, whereas in re-infection IgG antibodies are detectable by day 6, with IgM antibody titers being variable.

- **Weil-Felix test:** Nonspecific, detects high titers of heterophile antibodies to *Proteus* OXK antigens
- **Indirect immunofluorescence antibody (IFA):** It is specific, considered as the gold standard serological test
- **ELISA:** It is performed using 56-kDa recombinant major surface protein antigens. It is cost-effective and considered as alternative to IFA for diagnosis of acute infection and for seroprevalence.

Molecular test

Various formats of PCR have been developed, targeting specific genes such as major 56-kDa gene, 47-kDa gene and 16S rRNA gene. For more details, refer to 'laboratory diagnosis of rickettsial diseases, described earlier in this chapter.

EHRlichiosis

Family Anaplasmataceae comprises of four obligatory intracellular organisms named *Ehrlichia*, *Wolbachia*, *Anaplasma* and *Neorickettsia*.

- ❖ They reside in vertebrate reservoirs and target vacuoles of hematopoietic cells
- ❖ **Pathogenic species:** Few of them are pathogenic, such as—
 - *Ehrlichia chaffeensis*: It is the agent of human monocytic ehrlichiosis, infects predominantly mononuclear phagocytes
 - *Ehrlichia ewingii*: It infects neutrophils and causes human granulocytic ehrlichiosis
 - *Anaplasma phagocytophilum*: It infects neutrophils; causes human granulocytic anaplasmosis
 - *Neorickettsia sennetsu* infects the lymphocytes and causes human lymphocytic ehrlichiosis.
- ❖ **Transmission:** They are vector-borne, transmitted by ticks; except *Neorickettsia sennetsu* which is transmitted by ingestion of fish carrying infected flukes
- ❖ **Epidemiology:** Ehrlichiosis is prevalent in USA, except infections caused by *Neorickettsia sennetsu* is seen in Japan and Malaysia. They have many animal reservoirs such as white-tailed deer and dogs
- ❖ **Clinical manifestations:** They produce an acute febrile disease; characterized by headache, myalgia, arthralgia, cough, pharyngitis, lymphadenopathy, diarrhea, nausea, vomiting, abdominal pain and changes in mental status
- ❖ **Inclusions:** They reside inside the phagosome, multiply to produce the following three stages of growth—elementary body, initial body, and mulberry like inclusions called Morula
- ❖ **Morulae** in neutrophil can be detected in 20–75% of cases by Giemsa-stained peripheral blood film examination
- ❖ **Treatment:** Drug of choice for ehrlichiosis is doxycycline.

Q FEVER (COXIELLA BURNETII)

Coxiella burnetii is an obligate intracellular organism that causes ‘Q fever’.

- ❖ **History:** For long time, the causative agent of the disease was unknown, hence was referred to as ‘Query’ or Q fever. Later, the agent was identified and named after their discoverers Cox and Burnet as *Coxiella burnetii*
- ❖ **Source of infection:** Q fever is a zoonotic; human infection occurs from cattle, sheep and goats
- ❖ **Mode of transmission:** (1) Inhalation of infected dust from soil, previously contaminated by urine and feces of diseased animals; (2) rarely by ingestion of contaminated milk
- ❖ **Geographical distribution:** Q fever is endemic in most parts of the world except in New Zealand and Antarctica. In India, it is reported from Rajasthan, Punjab, Haryana and Delhi
- ❖ **Clinical manifestations:** Incubation period ranges from 3–30 days
 - **Acute Q fever:** Presents as patients present with hepatitis, interstitial pneumonia, fever, CNS involvement and pericarditis or myocarditis
 - **Chronic Q fever** develops months to 2-3 years later, in 5% of cases; presents as endocarditis.
- ❖ **Laboratory diagnosis:** *C. burnetii* is small pleomorphic gram-negative coccobacillus
 - **Isolation:** It is extremely fastidious. It can be isolated from buffy-coat blood samples or tissue specimens by a shell-vial technique; but requires a biosafety level 3 laboratory
 - **Antibody detection:** Indirect immunofluorescence assay (IFA) is sensitive, specific and is the method of choice. It detects antibodies against Phase I and

II antigens of LPS. In acute Q fever; the phase II antibody titer is higher; whereas in chronic Q fever phase I antibody titer is elevated

- **Molecular methods:** PCR detects *C. burnetii* DNA in tissue specimens.

❖ **Treatment:**

- **Acute Q fever:** Doxycycline is the drug of choice; given for 14 days. Quinolones are also effective
- **Chronic Q fever:** Hydroxychloroquine is added to alkalinize the phagolysosome and to render doxycycline bactericidal against the organism.

❖ **Prevention:** Control measures include—

- **Vaccine:** Inactivated whole-cell vaccine (*Q-Vax*) is licensed in Australia. It is recommended for occupationally exposed workers
- Good animal husbandry practices should be followed such as proper disposal of animal excreta
- Pasteurization of milk should be done by Flash method (72°C for 20 sec followed by rapid cooling to 13°C or lower).

BARTONELLOSIS

Bartonella species are fastidious, facultative intracellular, gram-negative bacteria that have ability to invade mammalian cells and RBCs. Out of several species, only three are important human pathogens: *B. henselae*, *B. quintana* and *B. bacilliformis*.

- ❖ ***B. henselae*:** It is transmitted by exposure to cats (by scratch or bite). It is the agent of—
 - **Cat-scratch disease:** Characterized by typical features such as regional lymphadenopathy and painless papule at the site of cat scratch. Rarely, atypical features such as hepatitis, splenitis, and retinitis may be seen
 - **Bacillary angiomatosis:** An angioproliferative disorder, characterized by neovascular lesions involving skin and other organs

- **Bacillary peliosis:** Angioproliferative disorder involving liver and spleen.
- ❖ ***B. quintana*:** It is transmitted by louse. It is the agent of—
 - **Trench fever:** It occurred as epidemics in the trenches during World War I. It is a mild febrile illness which lasts for five days, subsequent episodes occurring every 5-day (hence also called as *5 days fever*). Thereafter, it was silent for decades. However, the disease has re-emerged in USA recently
 - **Others:** Bacillary angiomatosis, chronic bacteremia, and endocarditis.
- ❖ ***B. bacilliformis*:** It is transmitted by vector sandfly (*Lutzomyia*). It produces a biphasic disease—
 - **Oroya fever or Carrion's disease:** It is the initial, bacteremic, systemic illness presenting with or without anemia
 - **Verruga peruana:** It is a late manifestation, characterized by cutaneous vascular lesions
 - **Others:** Bacteremia and endocarditis.

Laboratory Diagnosis of Bartonella Infections

- ❖ **Specimens** collected are blood, lymph node or skin biopsies
- ❖ **Microscopy:** Warthin-Starry silver nitrate staining and immunofluorescence staining can be used to detect *B. henselae* from lymph node smears
- ❖ **Culture:** *Bartonella* can be grown on blood agar at 37°C and incubated for 12–15 days
- ❖ **Antibody detection:** Both indirect immunofluorescence assay (IFA) and enzyme immunoassay (EIA) based methods are available, which detects antibodies against *B. henselae* and *B. quintana* separately
- ❖ **PCR:** It can be done to amplify the genes such as 16S rRNA gene.

TREATMENT

Bartonellosis

- ❑ **Cat-scratch disease:** Treatment is not routinely indicated; For extensive lymphadenopathy, azithromycin is given for 5 days.
- ❑ **Bacillary angiomatosis:** Erythromycin or doxycycline is given for 3 months.

EXPECTED QUESTIONS

I. Write essay on:

- Mr Sarvanan, a 29-year-old military Jawan was brought to the hospital in a state of altered sensorium, which he had developed a few hours ago. There was history of high grade fever and headache associated with vomiting for the past 2 days. On examination he was febrile (102°F), his blood pressure was 90/60 mm Hg. There were petechial rashes noted throughout his body except palm and sole. On enquiry, he was found to have exposed to body lice. Similar symptoms were also reported from a few members of his battalion.
 - What is the most probable diagnosis?
 - List the other agents of the family to which the causative agent belongs to with their modes of transmission and the diseases they cause.
 - How this disease is diagnosed in the laboratory?

II. Write short notes on:

- A 39-year-old farmer presented to the emergency department with a 4-day history of fever, headache, and non-pruritic rashes on his face, extremities with an eschar on left lower leg. Identify the etiological diagnosis and discuss briefly pathogenesis of the same.
- Q fever.

III. Multiple Choice Questions (MCQs):

- All of the following rickettsiae belong to spotted fever group, except:**
 - R. rickettsii*
 - R. conorii*
 - R. typhi*
 - R. akari*
- Tick is the vector for following rickettsial infections, except:**
 - R. rickettsii*
 - R. akari*
 - R. africae*
 - R. conorii*

Answers

1. c 2. b

Miscellaneous Bacterial Bloodstream Infections

CHAPTER 32

CHAPTER PREVIEW

■ Brucellosis

■ Leptospirosis

■ Borreliosis

This chapter covers bloodstream infections due to bacterial diseases such as brucellosis, leptospirosis, and borreliosis.

BRUCELLOSIS

Brucella is an obligate aerobic, fastidious small gram-negative coccobacillus, responsible for a highly contagious febrile illness called **brucellosis**.

- ❖ Brucellosis (also called **undulant fever**) is primarily a zoonotic disease affecting various domestic animals, such as sheep, goat or cattle
- ❖ Human infection is usually associated with occupational or domestic exposure to infected animals or their products.

History

Brucella was named after British army physician Sir David Bruce (1886), who isolated the first recognized species, *Brucella melitensis* (*melita* is Roman name for Malta) from Malta Island (Europe); hence the disease was called Malta fever.

Nomen System of Classification

Brucella belongs to the family Brucellaceae.

- ❖ DNA hybridization studies reveal that the members of the genus *Brucella* are very closely related and probably represent variants of a single species
- ❖ However, for the sake of convenience, these have been classified into **nomen species**, based on various properties, (discussed under laboratory diagnosis)
- ❖ **Nomen species:** Important nomen species associated with human infections are as follows
 1. *B. melitensis*: It is usually pathogenic to sheep, goat and camel. Man is also a susceptible host
 2. *B. abortus*: The infection is acquired from cattle and buffalo
 3. *B. suis*: It infects most often pigs
 4. *B. canis*: It causes abortion in dogs. Occasional cases of human infection have been reported.

Antigenic Structure

Brucellae have two major types of lipopolysaccharide (LPS) antigens designated as **M** and **A**. They are present in varying proportion in the three major species of *Brucella*; however, one of them is predominant in each species

- ❖ In most biovars of *B. melitensis*, M antigen is predominant
- ❖ In most *B. abortus* biovars, A antigen is predominant
- ❖ *B. suis* biovars contain either M or A antigens.

Pathogenesis

B. melitensis is the most pathogenic species, followed by *B. abortus* and *B. suis*. Human infection with other species is extremely rare.

- ❖ **Transmission:** Brucellosis is zoonotic, transmitted from infected animals to man by various modes:
 - **Direct contact:** The most common route is direct contact of abraded skin or mucosa with the infected animal tissue, blood, urine, vaginal discharge or placenta
 - **Food-borne:** By ingestion of unpasteurized milk or dairy products or undercooked meat from infected animals or rarely vegetables or water contaminated with animal excreta
 - **Air-borne:** By inhalation of dust or aerosols in the infected cowshed or slaughter houses
 - **Person-to-person spread** is extremely rare; through breast milk or by tissue transplantation or blood transfusions.
- ❖ **Spread:** From the initial site of infection, the organisms spread to bloodstream resulting in bacteremia and then disseminate to involve various organs
- ❖ **Organs involved:** Brucellae primarily infect organs of the reticuloendothelial system, such as lymph nodes, spleen, liver and bone marrow. Other organs such as placenta, musculoskeletal tissues and genitourinary systems are also involved
- ❖ **Local tissue response:** Initially, neutrophilic infiltration occurs; which is later on replaced by chronic inflammatory cells leading to granuloma formation

- ❖ **Intracellular survival:** Brucellae are facultative intracellular pathogen; infect macrophages and monocytes. The cell-wall LPS appears to be the major virulence factor; plays a key role in their intracellular survival
- ❖ **Host immune response:** Cell-mediated immunity (CMI) is the key to control the infection. TH1 cells produce interferon- γ , which cause macrophage activation leading to killing of intracellular organisms. Antibodies play only a minor role as they are active only in extracellular milieu.

Clinical Manifestations

The incubation period varies from 1 week to several months and the onset is either abrupt or more often insidious.

- ❖ **Classic triad:** Though the manifestations vary, the classic triad of fever with profuse night sweats, arthralgia/arthritides and hepatosplenomegaly are present in most patients. Foul-smelling perspiration is considered as a classical sign, but uncommon
- ❖ **Typhoid-like illness:** Overall brucellosis resembles typhoid-like illness except that it is less acute, less severe with undulating pattern of fever and more musculoskeletal symptoms
- ❖ **Undulating fever:** Fever has a typical remittent course, i.e. in between febrile periods (which last for weeks), there will be afebrile periods. It is also called Malta fever or Mediterranean fever
- ❖ **Musculoskeletal symptoms** are present in about one-half of all patients, which may mimic skeletal tuberculosis
 - Vertebral osteomyelitis involves lumbar and low thoracic vertebrae commonly
 - Septic arthritis: Most commonly affected joints are knee, hip, sacroiliac and shoulder joints.
- ❖ **Other nonspecific symptoms:** These include abdominal pain, headache, diarrhea, rash, weakness/fatigue, weight loss, vomiting, cough, pharyngitis, and refusal to eat (children)
- ❖ **CNS:** Depression and lethargy with meningitis or lymphocytic meningoencephalitis are the most common neurological manifestations
- ❖ **CVS:** Rarely causes endocarditis, affecting the aortic valve
- ❖ **Genitourinary manifestations:** Include acute epididymo-orchitis, prostatitis, salpingitis and pyelonephritis.

Epidemiology

Brucellosis is a worldwide zoonotic disease. The occurrence in humans is closely related to their prevalence in various domestic animals.

- ❖ **Endemic area:** Human brucellosis is endemic in areas where animals are raised in large numbers, such as countries of Mediterranean zone, Eastern Europe, Central Asia, Mexico and South America

- ❖ The disease is rare in most European countries, Australia and North America
- ❖ **Prevalence:** The true prevalence of human brucellosis is difficult to estimate. Many cases are under-reported either because they are inapparent or due to difficulty in diagnosis
- ❖ **Sources of infection** are:
 - Infected animals excreting the organisms in urine, milk, placenta or vaginal discharge
 - Contaminated animal food products, such as dairy products, especially soft cheeses, milk, icecream and rarely raw meats and bone marrows.
- ❖ **Occupational risk:** Farmers, shepherds, goatherds, butchers, abattoir workers, veterinarians, laboratory workers in endemic areas are at higher risks of occupational exposure to brucellosis.

LABORATORY DIAGNOSIS

Brucellosis

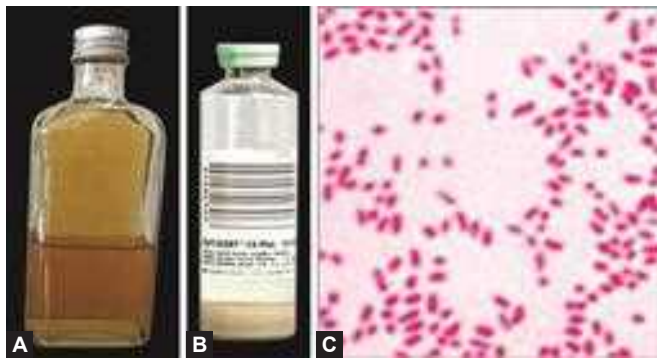
- ❑ **Specimens:** Blood, bone marrow, etc.
- ❑ **Blood culture by:**
 - Castaneda's biphasic media (BHI broth/agar)
 - Automated techniques such as BACTEC or BacT/ALERT.
- ❑ **Culture smear and motility testing:** Reveals non-motile gram-negative coccobacilli
- ❑ **Identification:** By automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests
- ❑ **Serological tests (antibody detection)**
 - Standard agglutination test (SAT)—detects IgM
 - Tests to detect IgG antibody—2ME test, ELISA.
- ❑ **Molecular method:** PCR detecting *rrs-rrl* gene, *Omp2* gene, protein *BCSP31* and IS711 insertion sequence.

Laboratory Diagnosis

Culture

Brucellae are recovered from blood, bone marrow, CSF, joint fluid or other tissues.

- ❖ Blood should be collected aseptically during the febrile period before starting antibiotics. Multiple blood cultures (8–10 mL per bottle, 2–3 times a day) over 3 consecutive days yield better result
- ❖ Bone marrow culture remains positive even after starting antibiotics and gives a higher yield than blood culture
- ❖ **Biosafety precautions:** As *Brucella* is an highly infectious pathogen, all laboratory procedures should be carried out with adequate biosafety precautions
- ❖ **Culture conditions:** They are obligate aerobes, but growth is promoted in the presence of 5–10% CO₂. Primary isolation requires prolonged (several weeks) incubation at 37°C
- ❖ **Blood culture:** Automated blood culture such as BacT/ALERT takes lesser time for isolation (7–10 days) and have better recovery rates (Fig. 32.1B). Therefore they are preferred over conventional blood culture media [Castaneda's biphasic media with brain heart infusion (BHI) broth/agar] (Fig. 32.1A)



Figs 32.1A to C: A. Blood culture bottles (Biphasic medium); B. BacT/ALERT; C. Gram-stained smear of *Brucella* species showing small gram-negative coccobacilli.

Source: A and B. Department of Microbiology, JIPMER, Puducherry; C. Public Health Image Library, ID# /15243, Dr WA Clark/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Subcultures** are made from positively flagged blood culture bottles onto blood agar and then incubated in candle jar at 37°C overnight
- ❖ **On blood agar**, colonies are small, smooth, transparent and nonhemolytic.

Culture Smear and Motility Testing

- ❖ Gram-staining of colonies reveals non-capsulated, small, gram-negative coccobacilli (Fig. 32.1C)
- ❖ *Brucellae* are nonmotile by hanging drop test.

Identification

Identification of brucellae from colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as given below.

- ❖ *Brucella* is catalase and oxidase positive
- ❖ Urease test is rapidly positive for *B. suis* and *B. canis*
- ❖ **Nomen species of identification:** The nomen species are identified based on the following tests:
 - Preference of animal host
 - CO₂ requirement and H₂S production
 - Tolerance to bacteriostatic dyes, such as basic fuchsin and thionine
 - Agglutination with specific antisera
 - Lysis by bacteriophages: A standard reference strain of Tbilisi phage is used for phage typing.

Serological Tests (Antibody Detection)

As isolation is difficult, serological tests are of paramount importance in diagnosis of brucellosis. Several tests have been developed, such as standard agglutination test, and ELISA.

Standard Agglutination Test (SAT)

SAT is considered as the gold standard serological test.

- ❖ **Procedure:** It is a tube agglutination test. It detects antibodies against LPS antigen of *B. abortus*. Serial dilutions

of patient's sera are mixed with killed suspension of *B. abortus* and incubated at 37°C for 48 hours

- ❖ **Positive test:** The diagnostic cut-off titer depends on disease endemicity
 - In nonendemic areas: Titer of $\geq 1:160$ along with compatible clinical manifestations is considered significant
 - In endemic area or following occupational exposure: Titer of $\geq 1:320$ or rising titer by repeating the test after 2-4 weeks is considered diagnostic.
- ❖ **Interpretation:** A positive SAT result indicates either acute or chronic brucellosis. As SAT detects total antibodies (IgM + IgG); it cannot differentiate between acute and chronic infection
- ❖ **2-mercaptoethanol (2ME) SAT test:** 2ME destroys IgM antibodies. Therefore, SAT performed with 2ME treated serum detects only IgG and confirms chronic brucellosis
 - SAT positive and 2ME SAT negative—indicates acute brucellosis (IgM)
 - SAT positive and 2ME SAT positive—indicates chronic brucellosis (IgG).
- ❖ **False-negative SAT** may occur due to:
 - Prozone phenomenon (due to excess of antibodies in patient's sera)
 - Presence of blocking or non-agglutinating antibodies.
- ❖ **False-positive:** SAT may become false-positive in infection with other gram-negative bacteria having similar LPS antigen.

Other Antibody Detection Tests

- ❖ **ELISA** is a highly sensitive test; uses either cytoplasmic proteins or LPS antigens to detect IgM, IgG, and IgA antibodies individually. Therefore, it is useful for diagnosis chronic brucellosis. However, the result has to be confirmed by SAT. ELISA is also useful in diagnosis neurobrucellosis
- ❖ **Dipstick assays** for anti-*Brucella* IgM are available for diagnosis of acute infection, but is less sensitive.

Molecular Methods

- ❖ **PCR assay** using primers for 16S- 23S rRNA operon (*rrs-rrl* gene), *Omp2* gene (outer-membrane protein), insertion sequence *IS711*, and protein *BCSP31* are available
 - It is rapid, sensitive and specific and can also differentiate between the species and biovars
 - Blood and tissues are ideal samples for PCR assays.
- ❖ **FilmArray BioThreat panel** is an automated multiplex PCR, useful for simultaneous detection 17 human pathogens causing bioterrorism including *Brucella*.

Diagnosis of Brucellosis in Animals

Brucellosis is diagnosed in animals by various methods such as: Milk ring test and Rose Bengal card test; detecting antibodies in infected milk.

TREATMENT

Brucellosis

Various regimens are recommended for the treatment of brucellosis.

- ❑ **Standard regimen** in adults: Gentamicin for 7 days plus doxycycline for 6 weeks. Streptomycin can be given alternative to gentamicin
- ❑ **WHO regimen** in adults: Rifampin for 6 weeks plus doxycycline for 6 weeks
- ❑ Relapse or treatment failure occurs in 5–10% of cases
- ❑ **For CNS involvement:** Ceftriaxone is added to the regimen and treatment is prolonged for 3–6 months.

Prevention

Prevention in Animals

The most rational approach to control human brucellosis is to control and eradicate infection from its animal reservoirs.

- ❖ **Test and slaughter:** Active case finding is done and infected animals are slaughtered
- ❖ **Vaccine:** Live attenuated vaccine using *B. abortus* 19 strain for cattle and *B. melitensis* rev-1 strain for sheep and goat are available.

Prevention in Humans

General precautions such as:

- ❖ Use of pasteurized milk or properly cooked food
- ❖ Use of protective measures to prevent direct contact with animals.

Vaccine: Live attenuated *B. abortus* 19-BA is available for human use but provides short term protection and had shown high reactogenicity.

SPIROCHETAL INFECTIONS

CLASSIFICATION AND MORPHOLOGY

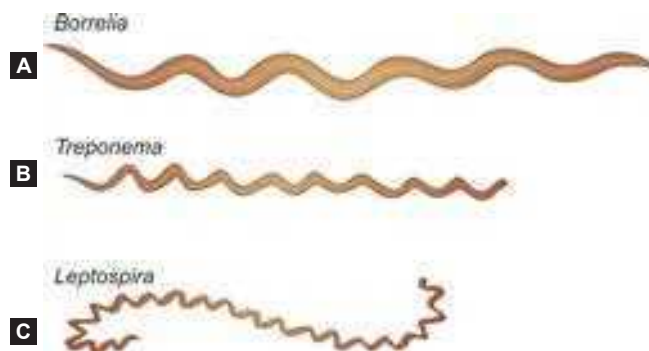
Spirochetes are thin, flexible, elongated spirally coiled helical bacilli (*speira*, meaning coil; and *chaite*, meaning hair).

- ❖ **Pathogens:** Most of the spirochetes are saprophytes. Only three of them are major human pathogens—*Treponema*, *Borrelia* and *Leptospira*
- ❖ Spirochetes are gram-negative bacteria, however their cell wall differs from other gram-negative bacilli by presence of endoflagella
- ❖ **Endoflagella** do not protrude outside, but present in the periplasmic space between peptidoglycan layer and outer membrane
- ❖ **Motility:** Endoflagella are responsible for various motility of spirochetes such as: flexion-extension type, corkscrew type rotatory movement and translatory type motility

The pathogenic spirochetes differ in their mode of transmission and spectrum of diseases produced (Table 32.1). They also differ in their morphological properties such as length and spirals (Figs 32.2A to C)

Table 32.1: Pathogenic spirochetes.

Spirochetes	Disease	Transmission
<i>Treponema</i>		
<i>T. pallidum</i>	Syphilis	Sexual
<i>T. pertenue</i>	Yaws	Direct contact
<i>T. endemicum</i>	Endemic syphilis	
<i>T. carateum</i>	Pinta	
<i>Borrelia</i>		
<i>B. recurrentis</i>	Relapsing fever (epidemic)	Louse borne
<i>B. duttonii</i> , <i>B. hermsii</i>	Relapsing fever (endemic)	Tick borne
<i>B. burgdorferi</i>	Lyme disease	Tick borne
<i>B. vincentii</i>	Vincent's angina	Direct contact
<i>Leptospira</i>		
<i>L. interrogans</i>	Leptospirosis • Milder form • Severe form (Weil's disease)	Contact with rodent urine



Figs 32.2A to C: Morphology of spirochetes.

- ❖ *Treponema* measure 6–14 μm in length; possess 6–12 number of spirals
 - *T. pallidum* causes a sexually transmitted disease, called syphilis (Chapter 77)
 - Nonvenereal treponematoses: Other *Treponema* species such as *T. pertenue*, *T. endemicum*, *T. carateum* are transmitted by nonsexual mode (direct contact) and cause mainly cutaneous lesions (Chapter 55)
- ❖ *Borrelia* are larger in size (10–30 μm in length), possess 3–10 number of spirals. They cause various diseases such as relapsing fever, Lyme disease and Vincent's angina (discussed in this chapter)
- ❖ *Leptospira* measure 6–20 μm in length. They possess numerous, tightly coiled spirals and with hooked ends. They cause Weil's disease (discussed here).

LEPTOSPIROSIS

Classification (Leptospira)

Leptospira is antigenically complex; comprises of two species: *L. interrogans* and *L. biflexa*; both can further be classified into many serovars and serogroups based on LPS (lipopolysaccharide) antigens

Table 32.2: *Leptospira* serogroups.

Serogroups of <i>Leptospira interrogans</i>		
Australis	Grippotyphosa	Sarmin
Autumnalis	Hebdomadis	Sejroe
Ballum	Icterohaemorrhagiae	Semarang
Bataviae	Javanica	Tarassovi
Canicola	Leptonema	Hurstbridge
Celledoni	Lyme	Ranarum
Cynopteri	Mini	Turneria
Djasiman	Pomona	Manhao
	Pyogenes	

- ❖ *L. interrogans* comprises of 26 serogroups (Table 32.2); which further consist of over 300 serovars. They differ in their geographical distribution and in severity of infection
- ❖ *L. biflexa* has 65 serovars arranged in 38 serogroups. It is a saprophyte and not pathogenic to man.

Epidemiology

Leptospira interrogans causes leptospirosis or Weil's disease; a zoonotic disease that can cause liver and kidney damage, meningitis, respiratory distress and even death, if not treated promptly.

- ❖ **Mode of transmission:** Leptospirosis is zoonotic. Direct human-to-human transmission does not occur. It is transmitted by:
 - Indirect contact with water, moist soil and wet surfaces contaminated with animal urine or
 - Direct contact with urine and products of parturition, placenta of infected animals.
- ❖ **Source:** Although more than 100 animals can be infected; important sources of infection are rats, dogs, cattle and pigs. Even asymptomatic animals can transmit the infection via urine
- ❖ **Seasonality:** Leptospirosis is more common in rainy and post monsoon period
- ❖ **Risk factors** that promote transmission include:
 - Lower socioeconomic status
 - Urban and rural slum areas
 - Rainfall and floods
 - Occupational exposure: Agricultural workers (e.g. rice field planters), fishermen, sewer workers and all those persons who are liable to work in rodent infested environment are at increased risk.
- ❖ **3R's:** The three important epidemiological determinants for leptospirosis include exposure to rodents, rainfall and rice field
- ❖ **Incidence:** The incidence rate ranges from 0.1–1/100,000 per year in temperate climates to 10–100/100,000 in tropical countries. During outbreak the incidence may reach over 100/100,000
- ❖ **Global distribution:** Leptospirosis is worldwide in distribution. About one million severe cases of human leptospirosis occur every year, with a case-fatality rate

of 10%. Highest burden of the disease has been reported from area with high population density such as urban slums of Brazil, India and Thailand

- ❖ **In India:** Leptospirosis has been reported more commonly from coastal districts of Andaman and Nicobar (hence called as **Andaman hemorrhagic fever**)
 - **Other states** affected include Gujarat, Kerala, Maharashtra and Tamil Nadu followed by Andhra Pradesh, Karnataka, Dadar and Nagar Haveli, Daman and Diu, Puducherry, Goa and Odisha
 - The serovars predominantly present in India are *L. Andamana*, *L. Pomona*, *L. Grippotyphosa*, *L. Hebdomadis*, *L. Semoranga*, *L. Javanica*, *L. Autumnalis*, *L. Canicola*.

Pathogenesis

There are two distinct phases of pathogenesis following leptospiral infection:

1. **First phase (septicemic phase):** After entering through the mucosa (conjunctival or oral) or abraded skin, *L. interrogans* spill over to the bloodstream and then disseminate hematogenously to various organs including brain, liver, lung, heart and kidney
 - **Vascular damage:** Spirochetes can be found in the walls of capillaries, medium and large-sized vessels. The exact mechanism of vascular damage is not clear
 - Penetration and invasion of tissues is due to active motility and release of hyaluronidase.
2. **Second phase (immune phase):**
 - As antibodies develop, spirochetes disappear from the blood. Antigen antibody complexes are deposited in various organs
 - Renal colonization: Bacilli become adherent to the proximal renal tubular brush border and are excreted in urine.

Clinical Manifestations

The incubation period is around 10 (1 to 30) days. In general, the manifestations can be divided into two distinct clinical syndromes:

1. **Mild anicteric febrile illness:** It occurs in 90% of patients. It is biphasic; a septicemic phase occurs first, followed by immune phase. It presents as flu-like illness with fever, chills, headache, conjunctival suffusion, nausea, vomiting, abdominal pain, and myalgia
2. **Weil's disease** (Hepato-renal-hemorrhagic syndrome): It is a severe form of icteric illness and occurs in 10% patients. Typical biphasic course may not be present. It often progresses rapidly with a case-fatality rate of 5 to 15% (Table 32.3).

LABORATORY DIAGNOSIS

Leptospirosis

Specimens: CSF, blood and urine

Microscopy

Dark ground or phase contrast microscope or silver impregnation staining: Reveals spirally coiled bacilli (tightly and

Contd...

	Mild anicteric febrile illness		Weil's disease	
	First stage 3–10 days (septicemic)	Second stage 10–30 days (immune)	First stage 3–10 days (septicemic)	Second stage 10–30 days (immune)
Clinical findings	<ul style="list-style-type: none"> • Fever • Myalgia • Headache • Conjunctival suffusion • Abdominal pain • Pharyngeal erythema without exudates • Vomiting 	<ul style="list-style-type: none"> • Meningitis • Uveitis, optic neuritis, chorioretinitis • Rash • Fever • Peripheral neuropathy 	<ul style="list-style-type: none"> • High-grade fever • Liver-jaundice (5–10%) and raised liver enzymes • Hemorrhages: <ul style="list-style-type: none"> ➢ Pulmonary hemorrhage ➢ Petechiae and purpura ➢ Conjunctival hemorrhage ➢ Gastrointestinal hemorrhage • Kidney—Raised serum urea and creatinine and renal failure 	
Isolation	From blood and CSF	From Urine	Blood and CSF	Urine
Serum IgM	Absent	Present	Absent	Present
Antibiotics	Susceptible to antibiotics	Refractory to treatment	Susceptible to antibiotics	Refractory to treatment

Contd...

L ABORATORY DIAGNOSIS

Leptospirosis

regularly coiled), with characteristic hooked ends like umbrella handle.

Isolation

- Culture condition: 30°C for 4–6 weeks
- Medium: EMJH medium, Korthof's and Fletcher's media.

Animal inoculation: Samples are inoculated into hamsters and young guinea pigs

Serology for antibody detection

- Genus specific tests: Macroscopic slide agglutination test, latex agglutination test, ELISA, ICT
- Serovar specific test: Microscopic agglutination test

Molecular methods:

- PCR detecting I6S or 23S rRNA or IS1533 genes
- PCR-RFLP and PFGE: to detect genomospecies

Nonspecific findings: altered renal and liver function tests

Laboratory Diagnosis

Useful specimens are CSF and blood (in first 10 days of infection) and urine (between 10 and 30 days of infection).

Microscopy

Leptospira are extremely thin; hence, cannot be seen under light microscope (leptos, meaning fine or thin)

- ❖ **Wet films:** They may be observed under dark ground or phase contrast microscope (Fig. 32.3B). They are highly motile; exhibit spinning and translational movements
- ❖ **Staining:** They do not stain by ordinary stain, but can be stained by *sliver impregnation stains* such as Fontana stain and modified Steiner technique
- ❖ ***L. interrogans*** is 6–12 μm long; tightly and regularly coiled, with characteristic hooked ends like umbrella handle (hence the species name *interrogans*—resembling interrogation or question mark)
- ❖ **Disadvantages:** Microscopy is less sensitive and requires technical expertise. Serum proteins and fibrin strands in blood may resemble leptospire.

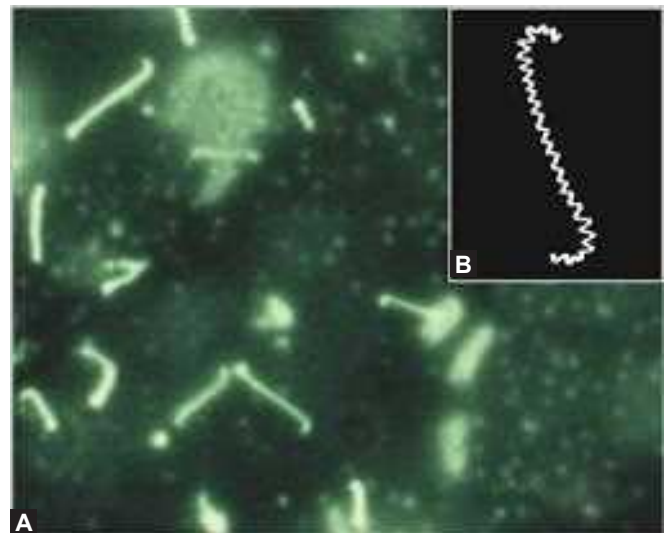
Culture Isolation

Culture technique is laborious, technically demanding and time-consuming; therefore not routinely used for diagnosis.

- ❖ **Culture condition:** *Leptospira* is obligate aerobe and slow growing. Cultures should be incubated at 30°C for 4–6 weeks. Culture fluid should be examined under dark ground microscope for the presence of leptospire
- ❖ **Culture media:** As *Leptospira* is highly fastidious, requires enriched media such as—(1) **EMJH liquid medium** (Ellinghausen, McCullough, Johnson, Harris), (2) **Korthof's** medium, and (3) **Fletcher's** semisolid medium.

Serology for Antibody Detection

- ❖ IgM antibodies appear early within one week of illness, reach peak levels in third or fourth week and then



Figs 32.3A and B: *Leptospira interrogans* (spirally coiled bacilli with hooked ends): **A.** Dark ground microscopy of the mount following microscopic agglutination test; **B.** Schematic diagram (viewed under dark-ground microscope).

Source: A. Public Health Image Library/ID#: 2888/ Mrs M Gatton, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

decline slowly and become undetectable within six months

- ❖ IgG antibodies appear later than IgM; reach peak level after few weeks of illness and may persist at low level for years.

Antibody detection tests can be broadly classified into:

- ❖ **Genus-specific tests** uses broadly reactive genus-specific antigen prepared from nonpathogenic *L. biflexa* Patoc 1 strain. They cannot detect the infecting serovar. Various tests available are:
 - ELISA: It detects IgM and IgG separately
 - Lepto dipstick assay: It detects IgM antibodies
 - Immunochromatographic test (ICT): It detects IgM and IgG antibodies separately (Fig. 32.4).
- ❖ **Serovar-specific test (Microscopic agglutination test, MAT):** It detects antibodies against specific serovars of *L. interrogans*
 - It is the gold standard method and the reference test for the diagnosis of leptospirosis
 - Patient's serum is mixed in a microtitre plate with live antigen suspensions of various leptospiral serovars endemic in the locality, incubated for 2–4 hours at 30°C and then examined under dark ground microscopy for the presence of agglutination (Fig. 32.3A).
- ❖ **Cross agglutination and absorption test (CAAT):** It is done to detect the relatedness between the strains.

Molecular Methods

PCR has been found particularly useful in severe disease, before seroconversion occurs

- ❖ Various genes such as 32-kDa lipoprotein (*lipL32*) gene, 16S or 23S rRNA or IS1533 insertion sequence are targeted
- ❖ However, PCR is not serovar-specific.

Non-specific Findings

- ❖ **Altered renal function:** Elevated levels of blood urea nitrogen and serum creatinine
- ❖ **Altered liver function:** Elevated bilirubin and liver enzymes in serum.

TREATMENT

Leptospirosis

- ❑ **Mild leptospirosis** should be treated with oral doxycycline (100 mg twice a day for 7 days). Amoxicillin can be given alternatively
- ❑ **Severe leptospirosis:** Penicillin is the drug of choice (1.5 million units IV, four times a day for 7 days), alternatives being ceftriaxone or cefotaxime.

Prevention

Vaccine

Whole cell vaccines (mono- or polyvalent) containing specific serovars of *Leptospira* are available in many countries. SPIROLEPT manufactured by Sanofi-Pasteur is



Fig. 32.4: ICT for *Leptospira* antibody detection (showing positive result for *Leptospira* IgM antibody).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

available for subcutaneous injection as two doses at 15-days interval, with the third dose 4–6 months after the first dose, followed by biannual revaccination. The efficacy rate is around 60–100%.

General Measures

- ❖ Chemoprophylaxis with doxycycline is recommended for anticipated short-term exposures, such as military training or travelling or fresh-water swimming
- ❖ General sanitation approaches including proper waste disposal
- ❖ Rodent control
- ❖ Avoidance of swimming in contaminated places
- ❖ Health education.

BORRELIOSIS

Borrelia is a larger spirochete, 10–30 µm in length; consists of lesser number (3–10) of spirals. Most of the species of *Borrelia* occur as commensals on the buccal and genital mucosa. Few are pathogenic to men, such as:

- ❖ *B. recurrentis* causes epidemic relapsing fever
- ❖ *B. burgdorferi* is the agent of Lyme disease
- ❖ *B. vincentii* causes an ulcerative gingivostomatitis called **Vincent's angina**, in association with fusiform bacilli (Chapter 59).

Relapsing Fever

Relapsing fever (RF) is characterized by recurrent episodes of fever and nonspecific symptoms following exposure to insect vector carrying *Borrelia* species. Relapsing fever is of two types:

1. **Epidemic RF:** It is caused by *B. recurrentis* and transmitted by louse
2. **Endemic RF** is caused by *Borrelia* species other than *B. recurrentis* such as *B. duttonii*, *B. hermsii* and *B. turicatae*. It is transmitted by tick.

Pathogenesis

- ❖ **Mode of transmission:** Relapsing fever is vector-borne
 - **Epidemic RF:** It is transmitted by human body louse (*Pediculus humanus*). Borreliae are introduced by crushing of the louse (e.g. by scratching) leading to

deposition of numerous spirochetes on the abraded skin and mucous membranes

- **Endemic RF:** It is transmitted by bite of an infected tick (*Ornithodoros* species).
- ❖ From the inoculated site, *Borrelia* spreads rapidly leading to bacteremia and fever. Host's immune system tries to eliminate the bacilli from the body
- ❖ However, the borrelial surface antigens frequently undergo **antigenic variation**. Each time, new antigens are produced which can evade host's immune system leading to repeated bacteremia and recurrent febrile episodes.

Clinical Manifestations

Both epidemic and endemic RF have similar manifestations although not identical. Incubation period is about 7–8 days.

- ❖ **Recurrent febrile episodes** lasting for 3–5 days occur intervening with afebrile periods of 4–14 days. Relapses can occur up to 10 times; subsequent episodes are shorter
- ❖ **Hemorrhages:** Petechiae, epistaxis and blood-tinged sputum are more likely in epidemic RF
- ❖ **Neurologic features** such as meningitis, seizure, focal deficits, paraplegia and psychosis may occur in 10–30% of cases and are more common in epidemic RF.

Laboratory Diagnosis

- ❖ **Microscopy:** Microscopic features of *Borrelia* have been described earlier. Various methods are available to detect *Borrelia* from blood
 - Peripheral thick or thin smear-stained by Wright- or Giemsa-stain (Fig. 32.5)
 - Direct fluorescent antibody test using monoclonal antibody is employed to identify the species

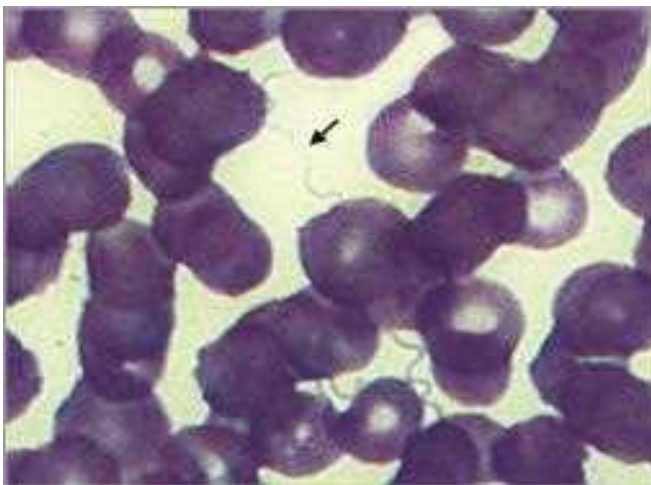


Fig. 32.5: Peripheral blood smear showing *Borrelia* species (Giemsa-staining) (arrow showing).

Source: Public Health Image Library, ID# 14495/Fort Collins/Centers for Diseases Control and Prevention (CDC), Atlanta (with permission).

- Dark ground or phase-contrast microscopy to demonstrate motile spirochetes (but low sensitivity)
- It is poorly gram-negative.
- ❖ **Culture:** During afebrile period, microscopy fails to detect *Borrelia*; hence, the confirmation is made by isolation of *Borrelia* from blood
- ❖ **Serology:** Done for detection of antibodies
 - **ELISA** and **IFA** (indirect fluorescence assay) are available to detect serum antibodies
 - **GlpQ assay:** It is the most reliable serological method. It is an immunoblot assay detecting antibody against the recombinant GlpQ antigen (Glycerophosphodiester phosphodiesterase).
- ❖ **Molecular methods:** Multiplex Real-time PCR has been developed targeting *16S rRNA* and *GlpQ* genes to identify the various species of *Borrelia* causing RF.

TREATMENT

Relapsing fever

Antibiotics such as doxycycline or erythromycin are the drug of choice for relapsing fever. Recommended schedule is single dose for epidemic RF, and 7–10 days course for endemic RF.

Lyme Disease

Agent Lyme disease or Lyme borreliosis is caused by *Borrelia burgdorferi*.

- ❖ It is widespread in USA, but also reported from other parts of the world like Europe and Asia
- ❖ Rodents and deer are main reservoirs
- ❖ It is transmitted by **tick bite** (*Ixodes ricinus* complex).

Clinical Manifestations

Lyme disease occurs through the following stages:

- ❖ **Stage 1: Early localized infection:** After an incubation period of 3–32 days, an annular maculopapular lesion develops at the site of the tick bite called **erythema migrans**, commonly involving thigh and groin (Chapter 55)
- ❖ **Stage 2: Early disseminated infection:** *B. burgdorferi* spreads hematogenously to many sites resulting in secondary annular skin lesions, arthralgia, malaise and neurological abnormalities
- ❖ **Stage 3: Late persistent infection (Lyme arthritis):** About 60% patients develop arthritis of large joints (e.g. knees), lasting for months; which may be refractory to the treatment.

Laboratory Diagnosis

- ❖ **Isolation** of *B. burgdorferi* can be done by culturing specimens like skin lesions, blood or CSF in special medium called BSK medium (Barbour-Stoenner-Kelly)
- ❖ **Molecular methods:** PCR detecting specific DNA is more sensitive in joint fluid. But its sensitivity is poor for CSF, blood or urine samples. Common gene targets include *16S rRNA*, *flaB*, and *ospA* (outer surface lipoprotein)
- ❖ **Serology (antibody detection):** The most common method of diagnosis of Lyme disease is by characteristic

clinical picture with a positive serological test. CDC recommends two-test approach. First ELISA should be performed → if found positive, it has to be confirmed by western blot

- ❖ ↑**Leukocyte count:** Joint fluid examination reveals elevated polymorphonuclear cells whereas CSF shows lymphocytosis.

TREATMENT

Lyme disease

- ❑ For all stages of Lyme disease except CNS and CVS infection: Oral doxycycline is the drug of choice, except for children where amoxicillin is given. Duration of treatment is 14 days (for skin lesions) and 30-60 days (for arthritis)
- ❑ For CNS or CVS infection: Ceftriaxone is given for 14–28 days.

EXPECTED QUESTIONS

I. Write essay on:

1. Kishan, a young farmer was complaining of fever, headache, and myalgia. Gradually, he developed yellow discoloration of skin and sclera. On examination, he had conjunctival suffusion and hepatosplenomegaly. His blood count showed neutrophilia with a thrombocytopenia. Liver function tests showed an elevated conjugated bilirubin with mild elevation of transaminases. He was also found to be oliguric and uremic.
 - a. What is the etiological agent and how is this disease transmitted?
 - b. What is the typical clinical presentation and pathogenesis of this condition?
 - c. How will you confirm the diagnosis?
 - d. How will you manage this clinical condition?

II. Write short notes on:

1. Laboratory diagnosis of brucellosis
2. Relapsing fever.
3. Lyme disease.

III. Multiple Choice Questions (MCQs):

1. *Brucella melitensis* is commonly found in which animal?
 - a. Pig
 - b. Dog
 - c. Cattle
 - d. Goat
2. Malta fever is also called as:
 - a. Undulant fever
 - b. Relapsing fever
 - c. Rat bite fever
 - d. Hemorrhagic fever
3. The following drugs are indicated in brucellosis, except:
 - a. Streptomycin
 - b. Doxycycline
 - c. Meropenem
 - d. Rifampin

4. Fever in *Brucella* infection is described as:

- a. Breakbone fever
- b. Step ladder fever
- c. Undulating fever
- d. Pontiac fever

5. The most common mode of transmission of *Brucella* is:

- a. Direct contact
- b. Ingestion of raw milk
- c. Air-borne
- d. Man to man

6. The classic triad of brucellosis includes all, except:

- a. Fever with profuse night sweats
- b. Meningoencephalitis
- c. Arthralgia/arthritis
- d. Hepatosplenomegaly

7. Which of the following is the gold standard test for the diagnosis of leptospirosis?

- a. Culture of urine on EMJH media
- b. Testing serum by dark field examination for the presence of leptospire
- c. Testing acute and convalescent phase sera for anti-leptospiral antibodies by microscopic agglutination test
- d. Culture of CSF on blood and chocolate agar

8. Which of the following statements about relapsing fever is correct?

- a. Each relapse is associated with an antigenically distinct variant
- b. Blood smears should be made when the patient is afebrile
- c. Transmitted by flea bite
- d. Caused by *Borrelia burgdorferi*

9. Weil's disease is caused by:

- a. *Leptospira interrogans*
- b. *Borrelia recurrentis*
- c. *Treponema carateum*
- d. *Treponema pallidum*

Answers

1. d 2. a 3. c 4. c 5. a 6. b 7. c 8. a 9. a

CHAPTER PREVIEW

- Morphology
- Pathogenesis
- Clinical Diagnosis
- Epidemiology
- Laboratory Diagnosis
- Post-exposure Prophylaxis

INTRODUCTION AND MORPHOLOGY

Retroviruses are group of RNA viruses that possess a unique enzyme called **reverse transcriptase** which directs the synthesis of DNA from the viral RNA after they infect a host cell.

They belong to family Retroviridae, which comprises of several genera; out of which two genera contain viruses that are pathogenic to humans.

- ❖ Genus *Lentivirus*: Contains human immunodeficiency virus (HIV)-1 and 2
- ❖ Genus *Deltaretrovirus*: Contains human T cell lymphotropic virus-1 (HTLV-1).

Human immunodeficiency virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS)—the biggest threat to mankind in last three decades.

History/Origin of AIDS

The first case of AIDS was described from New York (USA) in 1981; which was soon followed by the discovery (isolation) of HIV-1 from Pasteur Institute, Paris in 1983.

- ❖ HIV in humans was believed to be acquired from chimpanzee by the cross species infections of simian counterpart of HIV (simian immunodeficiency virus or *SIVcpz*) in rural Africa
- ❖ It has been postulated that though such zoonotic transmission to humans was going on repeatedly over many years in the past, only by the late 20th century the virus underwent changes which enabled it to adapt to human environment and to reach the epidemic level.

Structure

HIV and other lentiviruses have a unique structure (Fig. 33.1): They are spherical and 80–110 nm in size.

- ❖ **Envelope:** HIV is an enveloped virus. The envelope is made up of:
 - Lipid part: It is host cell membrane derived
 - Protein part: It has two components:
 1. Glycoprotein: 120 (gp 120)—They are projected as knob like spikes on the surface and

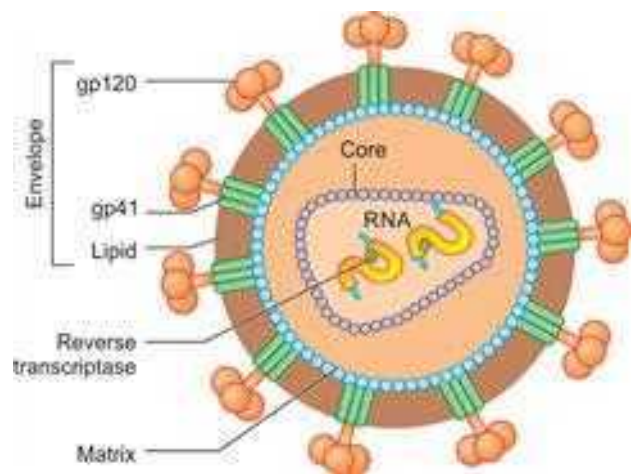


Fig. 33.1: Structure of HIV.

2. Glycoprotein: 41 (gp 41)—They form anchoring transmembrane pedicles.

- ❖ **Nucleocapsid:** Capsid is icosahedral in symmetry, made up of core protein. Inside, there is a dense cylindrical inner core which encloses:
 - **RNA:** Two identical copies of single-stranded positive sense linear RNA
 - **Viral enzymes** such as reverse transcriptase, integrase and proteases which are closely associated with HIV RNA.

HIV Genes and Antigens

HIV contains three structural genes—*gag*, *pol*, and *env* and six non-structural or regulatory genes.

Structural Genes

Structural genes code for various components of the virus.

- ❖ The **gag gene** codes for the core and shell of the virus. It is expressed as a precursor protein, p55* which is cleaved into three proteins:
 - p18—constitutes the matrix or shell antigen
 - p24 and p15—constitute the core antigens.

*In HIV, the proteins and glycoproteins are indicated by their mass which is expressed in kilodaltons (e.g. p55- means protein with molecular weight 55kDa.)

- ❖ The **pol gene** codes for viral enzymes such as reverse transcriptase, protease and integrase. It is expressed as a precursor protein, which is cleaved into proteins p31 (integrase), p51 (reverse transcriptase) and p66
- ❖ The **env gene** codes for the envelope glycoprotein (gp 160), which is cleaved into two components:
 1. gp120: It is the main receptor of HIV that binds to CD4 molecules on host cell to initiate the infection
 2. gp41: It is the fusion protein.

Non-structural Genes

Non-structural genes regulate viral replication and are important in disease pathogenesis *in vivo*. They can be grouped into:

- ❖ Essential regulatory genes: *tat* (transcriptional transactivator), *nef* (negative factor) and *rev* genes
- ❖ Accessory regulatory genes: *vif* (viral infectivity factor), *vpr* and *vpu* genes.

Antigenic Variation and Diversity

HIV shows extensive antigenic diversity as it undergoes high rates of mutation.

- ❖ This is believed to be due to the error-prone nature of reverse transcriptase enzyme
- ❖ Different mutants will be selected under different conditions (host factors, immune responses and tissue types)
- ❖ Although mutations may occur in any genes, most notably it is observed in *env* gene
- ❖ Unfortunately, envelope proteins happen to be the major target against which antibodies are produced. Hence mutations in *env* gene are the main reason which explains why:
 - HIV evades the host's immune response
 - Vaccination against HIV is extremely difficult.

HIV Serotyping

Based on sequence differences in *env* gene, HIV comprises of two serotypes HIV-1 and 2.

HIV-1

It is divided into three distinct groups (M, N, and O). Recently, a HIV strain related to gorilla SIV was identified in a Cameroonian woman in 2009 and has been proposed as *group P*.

- 'M' is the dominant group worldwide. It comprises of eleven **subtypes** or "clades" (A-K)
- Subtypes are sometimes further split into **sub-subtypes** such as A1 and A2 or F1 and F2
- There are also "**circulating recombinant forms**" or CRFs derived from recombination between different subtypes. For example, CRF01_AE is a recombination between subtypes A and E
- The same infected host may have a group of closely related viral subtypes and/or CRF at a given time which are collectively called as **quasispecies**
- HIV-1 subtypes or clades do not vary in pathogenesis or biology; but they differ in geographical distribution and transmission

Contd...

Geographical distribution

- Subtype A is common in West Africa
- Subtype B is predominant in Europe, America, Japan, and Australia
- Subtype C is the most common form worldwide (47%). It is also the dominant form in Southern and Eastern Africa, India, and China
- Greatest diversity: In Cameroon (West Africa), all known HIV groups and subtypes are found. It is probably, considered as the place of origin of the virus.

- **Transmission:** Asian and African subtypes (C and E) are more readily transmitted heterosexually, whereas American strains (subtype B) preferentially spread through blood and homosexual contact.

HIV-2

It comprises of eight subtypes (A–H); they are mainly confined to Africa and few other places including India. Group A is the most common form.

PATHOGENESIS

Mode of Transmission

HIV is transmitted through the following modes (Table 33.1):

- ❖ **Sexual mode** is by far the most common mode of transmission, accounts for 75% of total cases in the world
 - Heterosexual route (male to female via vaginal coitus) is the commonest mode
 - However, the risk of transmission through sexual route is minimal (0.1–1% per coitus)
 - Anal intercourse (among homosexual males or even male to female) has higher risk of transmission than vaginal intercourse.
- ❖ **Blood transfusion** is the least common mode of transmission (5%) but the risk of transmission is maximum (90–95%)

Table 33.1: Transmission of HIV.

Route of transmission	Risk of transmission (Worldwide, %)	% of total transmission	
		World	India*
Blood transfusion	>90	5	1**
Parent to child [#]	13–40	10	5.4
Sexual intercourse	0.1–1	75	88.9
Vaginal	0.05–0.1	60	87.4 (heterosexual)
Anal	0.065–0.5	15	1.5 (homosexual)
Oral	0.005–0.1	Rare	Not reported
Injection drug abuse	0.5–1.0	10	1.6%
Needle stick exposure	0.3	0.1	1**
Unknown	–	–	3

*Courtesy: NACO (National AIDS Control Organization, India).

**1% for Needle stick exposure plus blood transfusion together.

*Risk of transmission of HIV infection by breastfeeding is 30–45%.

Contd...

- ❖ **Percutaneous/mucosal** transmission modes such as needle stick injury, injection drug abuse and sharing razors or tattooing or splashes of infected blood on eyes, etc. are among the less effective modes of transmission
- ❖ **Perinatal mode:** In the absence of any intervention, the risk of transmission from mother to fetus is about 20–40%
 - Transmission may occur at any time during pregnancy and breastfeeding but the risk is maximum during delivery
 - Risk is maximum if mother is recently infected or has already developed AIDS.
- ❖ There is no evidence of HIV transmission by casual contact or kissing or insect bite
- ❖ **Viral load** is maximum in blood, genital secretions, and CSF; variable in breast milk and saliva; zero to minimal in other body fluids or urine
- ❖ Saliva may contain inhibitory substances like fibronectin and glycoproteins, which prevent transmission of the virus.

Receptor Attachment

The following receptor interaction is essential for HIV entry into the host cell.

- ❖ **Main receptor:** HIV enters into the target cells by binding its gp120 to the CD4 receptor on host cell surface. CD4 molecules are mainly expressed on helper T cells; and also on the surface of various other cells like monocytes, macrophages, Langerhans cells, astrocytes, keratinocytes and glial cells
- ❖ **A second co-receptor** in addition to CD4 is necessary for fusion of HIV to gain entry into the host cell. Usually, the chemokine receptors act as co-receptors for HIV and act by binding to gp120. Examples include:
 - CXCR4 molecules present on T lymphocytes
 - CCR5 molecules present on cells of macrophage lineage.
- ❖ **DC-SIGN**, a dendritic cell-specific lectin receptor present in skin and mucosal surfaces, can also bind to HIV-1 but does not mediate cell entry. Rather, it may facilitate transport of HIV by dendritic cells to lymphoid organs where HIV replicates further in T cells.

Mutation in CCR5 (Delta 32 Mutation)

This mutation results in blockade of HIV entry into the cells. It is observed principally in some lucky people of Europe and Western Asia who are either:

- ❑ **Completely resistant to HIV infection:** If they are homozygous for delta 32 mutation genes (seen in 1% of Northern Europeans, particularly in Sweden) or
- ❑ **Susceptible, but progression to AIDS is delayed:** If they are heterozygous for the same gene, seen in 10–15% of Europeans).

Replication

The replication of HIV (Refer Fig 4.5, Chapter 4) occurs through the following steps:

- ❖ **Fusion:** Following attachment of receptor and co-receptor to gp120, fusion of HIV to host cell takes place; mediated by the fusion protein gp41
- ❖ **Penetration and uncoating:** After fusion, HIV nucleocapsid enters into the host cell cytoplasm, which is followed by uncoating and release of two copies of ssRNA and viral enzymes
- ❖ **Reverse transcription:** Viral reverse transcriptase mediates transcription of its ssRNA into ssDNA so that DNA-RNA hybrid is formed. The RNA is degraded by viral endonuclease and ssDNA replicates to form dsDNA.
- ❖ Transcription of the DNA occurs to form some of the components of viral proteins
- ❖ **Pre-integration complex:** The nucleoprotein complex formed, comprises of linear dsDNA, gag matrix protein, accessory vpr protein and viral integrase. This is called **pre-integration complex**, which is transported into the host cell nucleus
- ❖ **Integration:** The viral dsDNA gets integrated into the host cell chromosome; mediated by viral integrase. The integrated virus is called as **provirus**
- ❖ **Latency:** In the integrated state, HIV establishes a latent infection for variable period. However, HIV is different from other latent viruses as it is able to replicate even in latent state and is infectious to other neighboring cells.

Disease Progression

Patients infected with HIV undergo various types of disease progression (Table 33.2); which in-turn depends upon various factors such as host, viral and environmental factors.

Natural Course (Typical Progressors)

About 80–90% of HIV, infected individuals are “typical progressors,” with a median survival time of approximately 10 years. The typical course of HIV infection includes the following five stages (Fig. 33.2A).

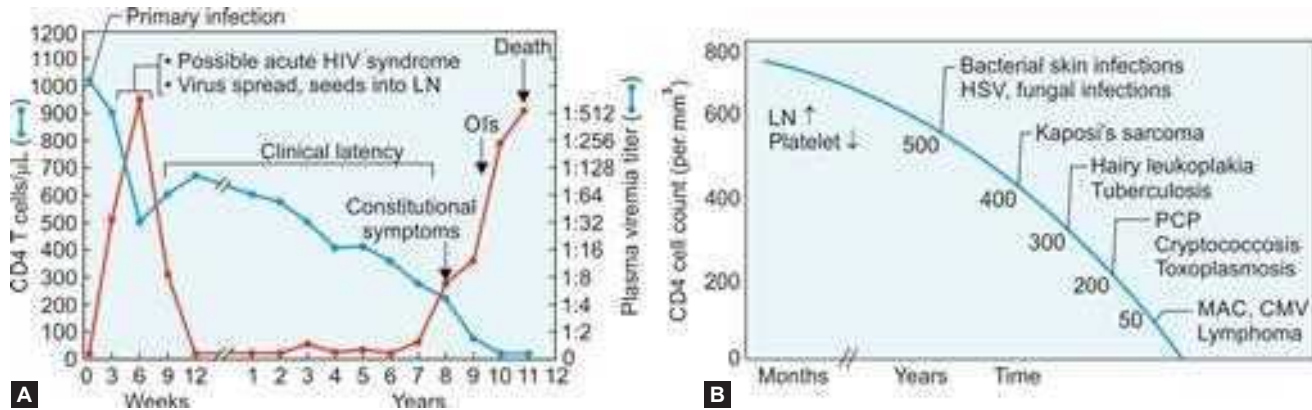
1. Acute HIV Disease or Acute Retroviral Syndrome

Following infection, HIV is carried to the lymph nodes and other lymphoid tissues where further multiplication occurs inside the T cells.

Table 33.2: Type of disease progressions in people living with HIV/AIDS (PLHA).

Progressors	Develops into AIDS	% of PLHA
Typical progressor	Within 10 years (described in the text)	80–90%
Rapid progressor	Within 2–3 years	5–10%
Long-term Non-progressor (LTNP)	After long time (10–30 years) without ART. They show <5000 HIV RNA copies/mL. Usually associated with CCR5 mutation	5%
Elite controller (subset of LTNP)	After long time (10–30 years) without ART. They show <50 RNA copies/mL (below detectable level)	<1%

Abbreviations: PLHA, people living with HIV/AIDS; ART, antiretroviral therapy.



Figs 33.2A and B: A. Natural course of HIV infection; B. Opportunistic infections associated with HIV infection and correlation with CD4 T cell counts.

Abbreviations: PCP, *Pneumocystis pneumonia*; MAC, *Mycobacterium avium* complex; CMV, cytomegalovirus; HSV, herpes simplex virus; LN, lymph node; OIs, opportunistic infections.

- Initially, HIV destroys the infected T cells and spills over into bloodstream to cause primary viremia (or acute mononucleosis-like syndrome) which coincides with an initial flu-like illness that occurs in many patients (50–75%) 3–6 weeks after the primary infection
- There is a significant drop in the number of circulating CD4 T cells at this stage.

2. Asymptomatic Stage (Clinical Latency)

Adequate immune response develops within 1 month in most of the patients.

- Both effective cell-mediated immune response (HIV specific CD8 T cells) and humoral immune response (HIV specific neutralizing antibodies) come into play
- As a result, viremia drops down and CD4 T cell count becomes normal
- It is important to note that this is a state of clinical latency, but **not microbiological latency**
- The immune response cannot clear the infection completely, HIV-infected cells persist in the lymph nodes, and there is a high level of ongoing viral replication
- This period of clinical latency is variable, may last for 10 years but ranges from few months to 30 years
- However, once the latency is broken, the disease progresses rapidly and death usually occurs within 2 years if left untreated.

3. Persistent Generalized Lymphadenopathy (PGL)

As a result of HIV replication in lymph nodes, 25–30% of infected people who are otherwise asymptomatic, develop lymphadenopathy.

- PGL is defined as enlarged lymph nodes of more than 1 cm size in two or more non-contiguous sites that persist for at least 3 months
- PGL must be distinguished from other causes of lymphadenopathies such as lymphoma.

4. Symptomatic HIV Infection (AIDS-related Complex)

After variable period of clinical latency, the CD4 T cell level starts falling. Eventually patients develop constitutional symptoms such as:

- Unexplained diarrhea, lasting for more than 1 month
- Weight loss (>10%), fatigue, malaise and night sweat
- Mild opportunistic infections such as oral thrush.

5. AIDS

Gradually, the patient moves towards the advanced end stage of HIV infection called AIDS; characterized by:

- Rapid fall in CD4 T cell count (usually <200 cells/μL)
- High viral load
- Lymphoid tissue is totally destroyed and replaced by fibrous tissue
- Opportunistic infections** set in secondary to profound immune suppression. Depending on the CD4 T cell count, various infections occur (Fig. 33.2B)
- Development of neoplasia (e.g. CNS lymphoma)
- Development of direct HIV induced manifestations such as HIV encephalopathy.

CLINICAL DIAGNOSIS

Classification systems for HIV disease have been developed which are useful for tracking and monitoring the HIV epidemic, for providing clinicians and patients with important information about HIV disease stage and clinical management. Two such systems are currently in use worldwide:

- CDC classification system** (Centers for Disease Control and Prevention, revised 1993): This system classifies HIV infection into nine stages based on associated clinical conditions and CD4 T cell count of the patient (refer CDC website)
- WHO clinical staging of HIV/AIDS for adults** (World Health Organization, revised 2007) is based only on

the clinical conditions associated with the patient. For, resource poor countries like India, where facilities for testing CD4 T cell count are not available widely, WHO clinical staging is more useful. It classifies HIV infection in adults and adolescents (>15 years) into four stages (Table 33.3).

EPIDEMIOLOGY

Global Situation (Till End of 2018)

Since the discovery of the AIDS epidemic (till end 2018), almost 74.9 million people have become infected worldwide with HIV with 32.0 million deaths.

- ❖ **Prevalence:** At the end of 2018, about 37.9 million people were living with HIV with a **global prevalence of 0.8%** in adults
- ❖ **Incidence:** In 2018, 1.7 million new HIV infections occurred globally with 7.7 lakh deaths
- ❖ **Sub-Saharan Africa** remains the most severely affected region, with nearly one in every 25 adults (3.9% prevalence) living with HIV and accounting for nearly two-thirds of the people living with HIV worldwide. South Africa has the largest HIV epidemic in the world, with 19% of the global PLHA
- ❖ **World AIDS Day:** Globally, 1st December is observed as World AIDS Day every year.

Situation in India (Till End of 2017)

- ❖ **HIV prevalence in India** was reported as 0.22% in adults (0.25% in males, 0.19% in females). It continues to have a steady decline since 2001 (0.38%)
- ❖ **Northeast states** such as Mizoram (2%), Manipur and Nagaland have highest prevalence
- ❖ **PLHIV:** Number of **People Living with HIV** were 21.4 lakh
- ❖ **Maharashtra** was the worst affected state followed by Andhra Pradesh, Karnataka and Telangana in terms of PLHIV
- ❖ **Incidence:** Around 87 thousand new HIV infections and 69 thousand AIDS-related deaths occurred in 2017. Incidence rate was highest from Mizoram, Manipur and Nagaland.

Reservoir

Infected people (both symptomatic as well as asymptomatic) are the only reservoir host. Once infected, they harbor the virus for life.

High-risk Groups

High-risk groups which commonly acquire infection are:

- ❖ **Extremely high-risk group:** Include female sex workers, men who have sex with men, Hijra/transgenders and IV drug abuser group
- ❖ **Moderately high-risk group:**
 - Health care workers (via needle pricks or splashes injury)

Table 33.3: WHO clinical staging of HIV/AIDS for adults (2007).

Clinical Stage 1
<ul style="list-style-type: none"> • Asymptomatic HIV infection • Persistent generalized lymphadenopathy
Clinical Stage 2
<ul style="list-style-type: none"> • Unexplained moderate weight loss (<10%) • Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis) • Herpes zoster • Angular cheilitis • Recurrent oral ulcers • Papular pruritic eruptions • Seborrheic dermatitis • Fungal nail infection
Clinical Stage 3
<ul style="list-style-type: none"> • Unexplained severe weight loss (>10%) • Unexplained chronic diarrhea: >1 month • Unexplained persistent fever: 1 month • Oral candidiasis • Oral hairy leukoplakia • Pulmonary tuberculosis • Severe bacterial infections (pneumonia, empyema, etc.) • Acute necrotizing ulcerative stomatitis, gingivitis, and periodontitis • Unexplained anemia, neutropenia or chronic thrombocytopenia
Clinical Stage 4
<ul style="list-style-type: none"> • HIV wasting syndrome (Slim disease): Characterized by profound weight loss (>10%), chronic diarrhea (>1 month), prolonged unexplained fever (1 month) • Bacterial opportunistic infections: <ul style="list-style-type: none"> ➢ Recurrent severe bacterial infections ➢ Extrapulmonary tuberculosis ➢ Disseminated non-tubercular mycobacterial infection ➢ Recurrent septicemia (including non-typhoidal salmonellosis) • Viral opportunistic infections: <ul style="list-style-type: none"> ➢ Chronic HSV infection ➢ Progressive multifocal leukoencephalopathy ➢ CMV (retinitis, or infection of other organs) • Fungal opportunistic infections: <ul style="list-style-type: none"> ➢ <i>Pneumocystis jirovecii</i> pneumonia ➢ Esophageal candidiasis ➢ Extrapulmonary cryptococcosis (meningitis) ➢ Disseminated mycoses (histoplasmosis and coccidioidomycoses) • Parasitic opportunistic infections: <ul style="list-style-type: none"> ➢ <i>Toxoplasma</i> encephalitis ➢ Chronic intestinal cystoisosporiasis (>1 month) ➢ Atypical disseminated leishmaniasis • Neoplasia (Chapter 80): <ul style="list-style-type: none"> ➢ Kaposi's sarcoma ➢ Invasive cervical cancer ➢ Lymphoma (cerebral, B cell and non-Hodgkin) • Other conditions (direct HIV induced): <ul style="list-style-type: none"> ➢ HIV encephalopathy ➢ Symptomatic HIV-associated nephropathy or cardiomyopathy

- Hemophiliacs and other recipients of blood products
- People with other STIs (sexually-transmitted infections).

Opportunistic Infections

Globally including India, tuberculosis is the most common opportunistic infection that occurs in HIV-infected people (see Fig. 33.2B).

- ❖ Common fungal infections are candidiasis (oral thrush) and *Pneumocystis jirovecii*
- ❖ Frequent viral infections are herpes simplex mucosal lesions and CMV retinitis
- ❖ Common parasitic infections are *Cryptosporidium parvum* diarrhea, *Toxoplasma* encephalitis and *Strongyloides stercoralis* hyperinfection syndrome.

AIDS Control Organizations

- ❖ **UNAIDS:** The Joint United Nations Program on HIV and AIDS (UNAIDS) is the main advocate for global action on the HIV/AIDS. It has initiated the 'Fast-Track strategy to end the AIDS epidemic by 2030' (Table 33.4)
- ❖ **NACO:** National AIDS Control Organization (NACO) has been constituted to implement the HIV/AIDS control program in India. It provides single national plan within one monitoring system
- ❖ **SACS:** State AIDS Prevention and Control Societies (SACS) are present in every state/union territory (35 numbers). They implement NACO program at state level
- ❖ **National Strategic Plan for HIV/AIDS and STI (2017–2024):** NACO has launched the national strategic plan 'Paving Way for an AIDS Free India'; going in line with UNAIDS for ending the AIDS epidemic by 2030.

KINETICS OF IMMUNE RESPONSE

An understanding of the kinetics of host immune response following infection is needed before we discuss the laboratory diagnosis; so as to understand the optimal usage of various tests during different stages of HIV disease.

- ❖ **Viremia:** Soon following the entry of the virus into the body, there occurs a transient period of high level viremia and p24 antigenemia. However, the levels of

these components fall down with concomitant immune response

- ❖ **Humoral response** is evidenced by formation of antibodies of different classes (IgM, IgA, IgG) against different **structural proteins** (*gag*: p15, p17, p24, p55; *env*: gp 41, gp 120, gp 160; and *pol*: p31, p51 and p66), **regulatory proteins** (*nef*, *rev*, *tat*) and **accessory proteins** (*vif*, *vpr* and *vpr*)
- ❖ All **structural components are strongly immunogenic** and induce formation of antibodies; whereas, immunogenicity of regulatory and accessory proteins is variable
- ❖ **Window period:** Following infection, antibodies appear in serum only after a period of interval, which is called window period. This is about 3 to 12 weeks
- ❖ **The antibodies to gag protein** (p24 and p55) usually appear first, though antibodies to *env* proteins and *pol* proteins may also be produced simultaneously
- ❖ As infection progresses to AIDS, antibody to p24 usually declines as p24 antigen levels rise concomitant with progression of disease to AIDS. However, antibodies to *env* proteins persist throughout the infection
- ❖ **Anti-HIV antibodies:** Among the antibodies (IgA, IgM and IgG) that appear, only IgG response is consistent and long lasting. Most currently available assays detect IgG antibodies. However detection of IgA is useful in specimens such as serum, mucous secretions and in newborn.

LABORATORY DIAGNOSIS

HIV infection

Specific Tests for HIV Infection

- ❑ **Screening tests** (antibody detection):
 - ELISA (takes 2–3 hours)
 - Rapid/Simple test (takes <30 minutes)
- ❑ **Supplemental tests** (antibody detection):
 - Western blot assay
 - Line immunoassay (LIA)
- ❑ **Confirmatory tests**
 - p24 antigen detection (after 12–26 days of infection)
 - Viral culture—by Co-cultivation technique
 - HIV RNA (best confirmatory method)—can be detected 10–14 days after infection
 - ◆ Reverse transcriptase PCR (RT-PCR)
 - ◆ Branched DNA assay
 - ◆ NASBA (nucleic acid sequence-based amplification)
 - ◆ Real time RT-PCR for estimating viral load
 - HIV DNA detection: Useful for diagnosis of pediatric HIV

Non-specific Immunological Methods

- ❑ Low CD4 T cell count
- ❑ Hypergammaglobulinemia:
 - Neopterin
 - β2-macroglobulin
- ❑ Altered CD4: CD8 T cell ratio

LABORATORY DIAGNOSIS

Diagnosis of HIV/AIDS is not like other infectious diseases. A number of moral, ethical, legal and psychosocial issues are associated with a positive HIV status. The disease is life long,

Table 33.4: Fast-track strategy ending the AIDS epidemic by 2030 by UNAIDS (United Nations Programme on HIV/AIDS).

Target indicators	Present status (2016)	By 2020	By 2030
% of infected people should know about their disease status	70%	90%	95%
% of infected people receiving antiretroviral treatment (ART)	56%	90%	95%
% of people receiving ART have viral suppression	60–70%	90 %	95 %
New infection among adults	1.8 million	5 Lakh	2 Lakh
To eliminate HIV-related stigma and discrimination	>50%*	Zero	Zero

*Findings from 50 countries indicate that roughly one in every eight people living with HIV is being denied of health services because of stigma and discrimination.

outcome is invariably fatal, no cure or vaccine is available so far, and in the majority, the transmission is through sexual contact. Hence, individuals known to be HIV infected are stigmatized and develop a fear of being discriminated and socially outcasted. Therefore, the following care should be taken (**3Cs**) while performing the test for HIV.

- ❖ **Consent** in written format should be taken before the test is done. The patient should be explained about the nature of the test being performed
- ❖ **Confidentiality** of a positive test result is a must. Patient name or the word “HIV positive” should not be written on the report form
- ❖ **Counseling** should be provided to motivate the individual to tell the spouse/family and induce behavioral change.

Antibody Detection

Detection of anti-HIV antibodies is the mainstay of diagnosis of HIV. Tests to detect specific HIV antibodies can be classified into:

Screening Assays

Screening assays usually take less time (2–3 hours for ELISA, less than 30 minutes for rapid/simple tests):

- ❖ **High sensitivity and specificity:** NACO recommends the use of ELISA and rapid kits which have a sensitivity of $\geq 99.5\%$ and a specificity of $\geq 98\%$
- ❖ **Should be confirmed:** Results of a single screening test should never be used as the final interpretation of HIV status as false positive results or technical errors can occur. It is always subjected to confirmatory tests
- ❖ Antigens used in most of the screening tests are:
 - HIV-1 specific (p24, gp 120, gp160, gp41)
 - HIV-2 specific gp36.
- ❖ They detect HIV-1 and 2 either separately or together.

ELISA (Enzyme-linked Immunosorbant Assay)

ELISA is the most commonly performed screening test at blood banks and tertiary care sites. It is easy to perform, adaptable to large number of samples. It is sensitive, specific, and cost effective.

ELISA kits: Most of the currently available ELISA kits are of two types:

- ❖ *3rd generation ELISA* that uses recombinant and/or synthetic peptides as antigen to detect HIV antibodies
- ❖ *4th generation ELISA* that detects both HIV antibodies and p24 antigen by using combination of recombinant/synthetic peptides as well as monoclonal antibodies respectively. It reduces the window period considerably.

Note: Older ELISA kits based on crude HIV antigen (first generation) and recombinant and/synthetic antigen (second generation) are obsolete now.

Types of ELISA: Various ELISA formats are in use depending on different principles such as: (i) indirect ELISA, (ii) competitive ELISA, (iii) sandwich ELISA.

Rapid/Simple Test

These assays have been developed for ease of performance and quick results. They generally require less than 30 minutes to perform and do not require special equipment. They are the most commonly used tests in India. They work on various principles such as:

- ❖ Dot blot assays (or Immunoconcentration or flow through method, e.g. Tridot test, see Fig. 12.13)
- ❖ Immunochromatography (or ICT, lateral flow assay)
- ❖ Particle agglutination assays (using latex, gelatin, RBCs)
- ❖ Dip stick/Comb tests (Enzyme immune assay-based tests).

Supplemental Tests

These assays are highly specific antibody detection methods; hence used for validation of positive results of screening tests. They are expensive, labor intensive, need expertise to interpret, and may also give equivocal/indeterminate results.

Western Blot

It is the most commonly used supplemental test available and is also recommended by NACO.

- ❖ It works on the principle of immunoblot technique (described in Chapter 12)
- ❖ It detects individual antibodies in serum separately against various antigenic fragments of HIV (Fig. 33.3)
 - Antibody to *gag* gene products (p55, p40, p24, p18)
 - Antibody to *pol* gene products (p65/66, p55/51, p31)
 - Antibody to *env* gene products (gp 120, gp160, gp41).
- ❖ The antigen antibody complexes appear as distinct bands on nitrocellulose strip
- ❖ Reactive results are interpreted as per:
 - *WHO criteria:* presence of at least two envelope bands (out of gp120, gp160 or gp41) with or without *gag* or *pol* bands
 - *CDC criteria:* presence of any two; out of p24, gp120, gp160 and gp41 bands.

Detection of p24 Core Antigen

The p24 antigen becomes detectable after 12–26 days of infection and lasts for 3–4 weeks thereafter. Again, it is elevated during the late advanced stage of AIDS. p24 Ag is detected by 4th generation ELISA (described earlier).

- ❖ **It is less sensitive** (~30%) because once the antibody is formed, it binds to the p24 protein and the antigen-antibody complex gets eliminated from the blood
- ❖ Recently, **antigen dissociation assay** has been developed that involves pretreatment of serum to an agent, that

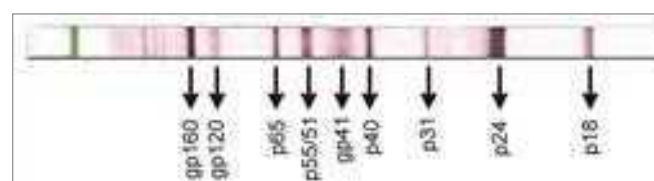


Fig. 33.3: HIV Western blot test strip.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

liberates p24 antigen from the immunocomplexes. This has shown better sensitivity

❖ **Uses of p24 antigen detection test:**

- For confirmation of diagnosis of HIV/AIDS
- Diagnosis of HIV during the window period
- To diagnose the late stage of HIV/AIDS (immune collapse) or CNS disease
- Diagnosis of HIV in infants (not reliable)
- Monitoring the progress of HIV infection
- To resolve equivocal western blot results.

Viral RNA Detection

Detection of viral RNA is the “**gold standard**” method for confirmation of HIV diagnosis. Various formats are available targeting pol and *env* genes.

- ❖ Reverse transcriptase polymerase chain reaction (RT-PCR)
- ❖ Branched DNA assay
- ❖ NASBA: Nucleic acid sequence-based amplification
- ❖ Real time RT-PCR: For estimating viral load.

Apart from the routine diagnosis of HIV, RNA detection has several other uses such as:

- ❖ It is the **most sensitive** and **specific** method, detects even few copies of viral RNA and is the best method for **confirmation** of HIV
- ❖ It is the best tool for diagnosis of HIV during **window period**, detects HIV earlier than all available methods (10–14 days postexposure)
- ❖ **Viral load monitoring:** Real time RT-PCR can quantify the viral load and is the most appropriate tool for monitoring the response to antiretroviral therapy
- ❖ **Typing:** RT-PCR can successfully differentiate between HIV-1 and HIV-2 infections and can detect the specific genotype or subtype
- ❖ Detection of **drug resistance** genes.

DNA PCR

PCR detecting proviral DNA is extremely useful for diagnosis of pediatric HIV and to differentiate latent HIV infection from active viral transcription. It is also useful during the window period, viral load estimation (real time PCR) and detection of genotypes.

Isolation of the Virus from Blood or Tissues

Isolation is time consuming, expensive, takes longer time (6 weeks or more) and not sensitive. It is used only for research and not for routine diagnostics.

Co-cultivation is the method used for virus isolation. Here, the peripheral blood mononuclear cells (PBMCs) obtained from the patient are co-cultured along with the PBMCs from healthy donor; followed by detection of viral RNA or antigen in culture suspension.

Non-specific/Immunological Tests

- ❖ **CD4 T cell count:** Measurement of CD4 T cell count is carried out by flow cytometry method. It is useful for:

- Assessing the risk of opportunistic infections (see Fig. 33.2B)
- Initiation of antiretroviral therapy—previously used. Current guideline says treatment should be started in all patients regardless of CD4 T cell count
- Monitoring the response to antiretroviral therapy.
- ❖ **Abnormal proteins** such as neopterin, beta 2-microglobulin and soluble IL-2 receptor are produced by peripheral blood mononuclear cells; stimulated by interferon-gamma or IL-2 which in turn are produced by HIV activated T_H1 cells.

NACO Strategy for HIV Diagnosis

For the resource poor countries, it is impracticable to confirm the result of HIV screening tests by PCR or western blot as these assays are expensive and available only at limited centers.

NACO (National AIDS Control Organization, India) has formulated a strategic plan for HIV diagnosis. The guidelines are as follows:

- ❖ Depending on the situation/condition, for which the test is done, the positive result of the first screening test should be either considered as such or confirmed by another one or two screening tests
- ❖ The first screening test should be highly sensitive, whereas the second and third screening tests should have high specificity
- ❖ The three screening tests should use different principles or different antigens. The same kit should not be used again
- ❖ Supplemental or confirmatory tests should be used only when the screening test(s) results are equivocal/intermediate.

There are four NACO Strategic Algorithms (Fig. 33.4):

1. **Strategy I:**
 - **Purpose:** It is done for transfusion and transplantation safety; i.e. for the screening of the blood donors in blood banks
 - Only one test should be done. If found reactive, then the unit of blood is destroyed.
2. **Strategy IIa:**
 - **Purpose:** It is done for sentinel surveillance of HIV infection to estimate the prevalence of infection
 - **UAT:** The method followed here is called as **unlinked anonymous testing (UAT)**, which involves screening of blood specimens taken for purposes other than HIV testing. Then the samples are permanently decoded of personal identifiers. This process occurs without informed consent
 - **Two tests format:** Positive results of the first test should be confirmed by a second test. If the second test is negative, then it is reported as negative.
3. **Strategy IIb:**
 - **Purpose:** It is followed for the diagnosis of HIV/AIDS in symptomatic patients
 - Positive result of the first test should be confirmed by a second test. If the second test is negative, then a third test is done for confirmation.

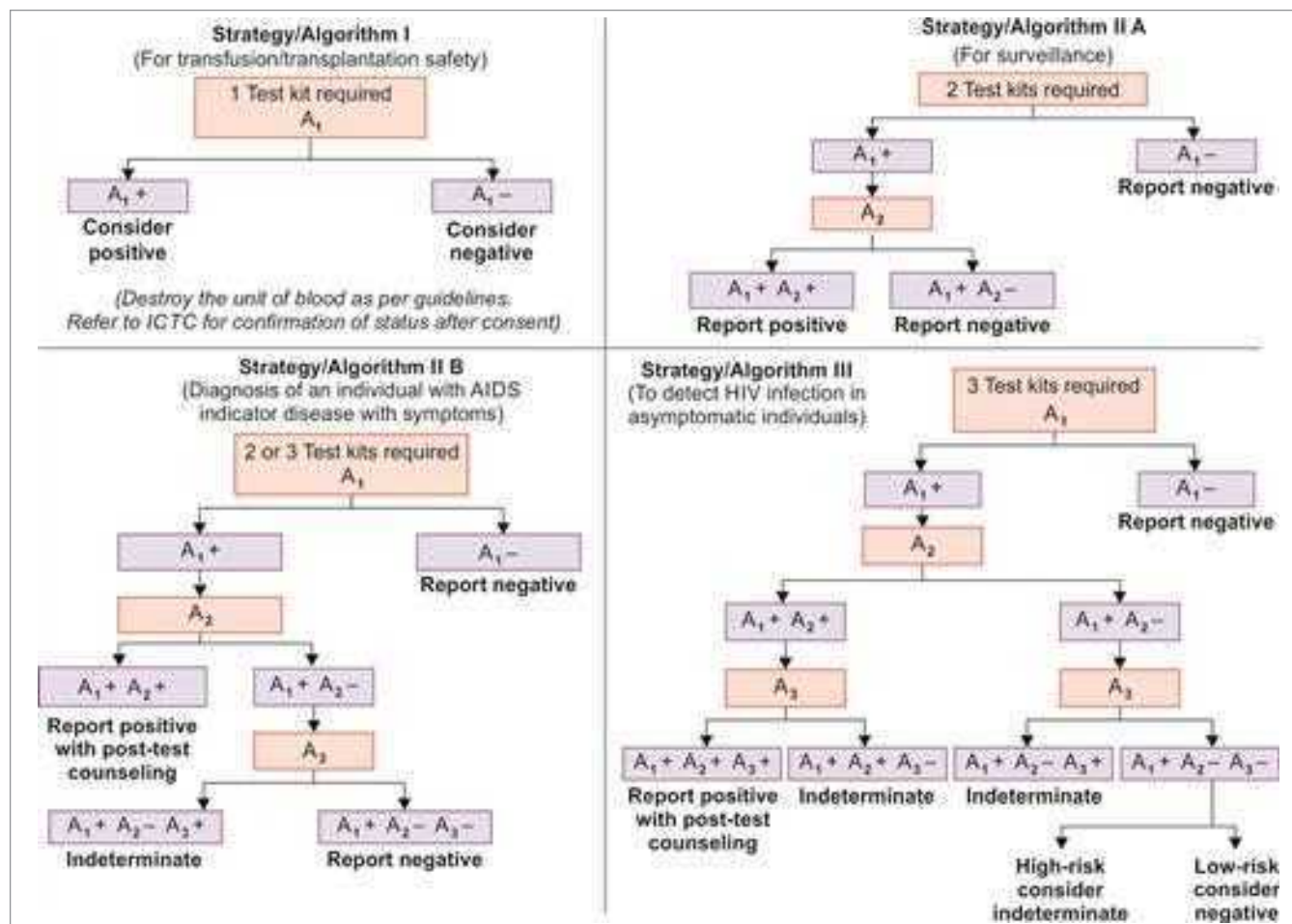


Fig. 33.4: NACO strategies/algorithms for diagnosing HIV infection.

Abbreviations: NACO, National AIDS Control Organization; ICTC, Integrated Counselling and Testing Centre.

4. Strategy III:

- **Purpose:** It is done for the diagnosis of asymptomatic HIV patients, antenatal screening and screening of patients awaiting surgeries
- **Three tests format:** All positive results in the first test should be confirmed by the second and third test. Positive report is sent only if all three test results are found reactive
- For indeterminate results of strategy IIB and III, (i.e. first test positive but second or third test negative), a repeat test is done after 14–28 days and the sample should be sent to the reference center for confirmation by western blot or RT-PCR.

Prognosis/Monitoring of HIV

Various tools available for monitoring the response to antiretroviral therapy include:

- **CD4 T cell count:** Most commonly used
- **HIV RNA load:** Most consistent and best tool at present
- p24 antigen detection
- Neopterin and β_2 macroglobulin level.

Note: Viral antibody levels are inconsistent and variable during late stage due to immune collapse; hence not reliable for prognosis.

Diagnosis of Pediatric HIV Infection

The routine screening methods (ELISA or rapid/simple tests) detect IgG antibodies.

- They cannot differentiate between baby's IgG or maternally transferred IgG, hence cannot be used for the diagnosis of pediatric HIV
- As all maternal antibodies would disappear by 18 months; therefore IgG assays can be performed after 18 months of birth.

Various methods used for diagnosis of pediatric HIV include:

- **HIV DNA PCR:** This is the most recommended method for diagnosis of pediatric HIV. Baby is tested for HIV DNA PCR at 6 weeks by DBS (Dry Blood Spot) collection. If found positive, then is reconfirmed by a repeat HIV DNA PCR. Then it is reported as positive and the baby is then initiated on lifelong ART
- HIV RNA detection
- p24 antigen detection
- IgG ELISA only after 18 months of age.

Diagnosis of HIV in Window Period

Window period refers to the initial time interval between the exposure and appearance of detectable levels of antibodies in the serum.

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- ❑ The antibodies appear in blood within 2–8 weeks after infection, but usually become detectable after 3 weeks to 12 weeks with the assays available presently. It can be as low as 22 days; when third generation antibody detection kits with high sensitivity are used
- ❑ p24 antigen detection (30% sensitive; by 4th generation ELISA): It can be detected by 12–26 days after infection
- ❑ HIV RNA detection (by RT-PCR) is the best method—it detects HIV RNA around 10–14 days after infection.

T TREATMENT**HIV infection****Antiretroviral Therapy (ART)**

Antiretroviral therapy (ART) denotes the approved drugs used for the treatment of HIV/AIDS (Table 33.5). The drugs do not kill all the viruses or cure the disease. However, they have the following goals:

- ❑ **Clinical goals:** Prolongation of life, improvement in quality of life
- ❑ **Virological goals:** Greatest possible sustained reduction in the viral load
- ❑ **Immunological goals:** Immune reconstitution; both quantitative and qualitative improvement
- ❑ **Transmission goals:** Reduction of HIV transmission from infected person to others.

ART Guideline, NACO 2018

Indication to start ART: TREAT ALL; i.e. ART has to be started in all patients irrespective of CD4 count, clinical stage, age, population or associated OIs.

Highly active antiretroviral therapy (HAART)

HAART is referred to the use of combination of at least three antiretroviral drugs to maximally suppress the HIV and stop the progression of the disease. Monotherapy with single drug is contraindicated due to inefficacy and chance of development of resistance.

Contd...

Table 33.5: Antiretroviral drugs.

NRTI	NNRTI	PI	Others
Zidovudine	Nevirapine	Saquinavir	Fusion inhibitor e.g. Enfuvirtide
Stavudine	Efavirenz	Ritonavir	
Lamivudine	Delavirdine	Nelfinavir	Integrase inhibitor e.g. Raltegravir, Dolutegravir, Elvitegravir
Didanosine	Rilpivirine	Amprenavir	
Zalcitabine	Etravirine	Indinavir	
Abacavir		Lopinavir	CCR5 receptor inhibitor e.g. Maraviroc
Emtricitabine		Fosamprenavir	
NtRTI		Atazanavir	Pharmacokinetic enhancer e.g. Cobicistat
Tenofovir		Tipranavir	
		Darunavir	

Abbreviations: NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; NtRTI, nucleotide reverse transcriptase inhibitors.

Contd...

T TREATMENT**HIV infection****NACO recommended first line HAART regimen**

- ❑ **Principle:** NACO recommends to include three drugs (2NRTIs/ NtRTI + 1NNRTI) as first line regimen. Protease inhibitor is added in place of NNRTI for HIV-2 infection
- ❑ NACO recommended first-line ART regimens for various situations; given in Table 33.6
- ❑ **Monitoring:** CD4 count and viral load have to be monitored every 3–6 months to monitor the response to treatment.

Opportunistic Infections (OIs)

OIs should be adequately treated with appropriate antimicrobial agents before starting ART.

Prophylaxis is indicated for the following OIs.

(1) Prophylaxis for *Pneumocystis pneumonia* (PCP)

Cotrimoxazole preventive therapy (CPT), at a dose of 800 mg/160 mg PO once daily may be initiated in the following scenarios:

- ❑ **Primary prophylaxis:** It is initiated in HIV patient in any of the condition
 - If CD4 count < 350 cells /cmm or
 - WHO clinical stage 3 and 4.
- ❑ **Secondary prophylaxis:** Given to HIV patients with PCP who have completed the treatment and continued until CD4 cell count remains at >350 cells/cmm for a period of 6 months.

(2) Prophylaxis for *Cryptococcal meningitis*

Secondary prophylaxis with fluconazole (200 mg daily) should be started for HIV patients with cryptococcal meningitis who have completed treatment and continued until the CD4 cell count remains at >200 cells/cmm for a period of 6 months. There is no role for primary prophylaxis against cryptococcal meningitis.

(3) Isoniazid Preventive Therapy (IPT)

- ❑ About 50% of the adults in the community have latent TB Infection
- ❑ Isoniazid protects against progression of latent TB infection to active disease
- ❑ It also prevents TB reinfection after exposure to an open case of TB.

Problems Pertaining to use of ART

Although early start of ART can reduce the risk of disease progression, there are many other factors that pose deleterious effects on quality of life.

- ❖ Toxicity and adverse effects of ARTs, especially lipid abnormalities and drug interactions
- ❖ High cost of the regimen
- ❖ Risk of development of drug resistance and dissemination of resistant virus
- ❖ Limited therapeutic options
- ❖ **IRIS (Immune reconstitution inflammatory syndrome):** It can occur in some cases of AIDS during the recovery phase following the start of ART. As the viral load decreases, the immune system begins to recover, which results in an exaggerated immune response to a previously acquired opportunistic infection causing an overwhelming inflammatory response, that paradoxically makes the symptoms of infection worse.

Table 33.6: First-line ART regimen in various situations (ART guideline, NACO, 2018).

Code	First-line ART regimen	Indication
TLE*	Tenofovir + Lamivudine + Efavirenz	HIV-1 infection in adults (body weight > 30 kg)
ALE	Abacavir + Lamivudine + Efavirenz	HIV-1 patients with abnormal serum creatinine values HIV-1 infection in children (body weight < 30 kg)
TL + LR	Tenofovir-Lamivudine + Lopinavir-Ritonavir	HIV-2 or HIV- 1 and HIV-2 co-infection Women received with single dose nevirapine in past pregnancy Post-exposure prophylaxis for healthcare workers
ZLN	Zidovudine + Lamivudine + Nevirapine	These old regimens if already initiated earlier, need to be continued on the same regimen unless failing
ZLE	Zidovudine + Lamivudine + Efavirenz	

* TLE regimen is available as Fixed Dose Combination (FDC), as a single pill to be taken once a day.

POST-EXPOSURE PROPHYLAXIS

Post-exposure prophylaxis (PEP) is required to reduce the risk of transmission after occupational exposures such as needle stick or sharp injury or mucocutaneous exposure.

- ❖ Every hospital should have a nodal center for PEP management and must provide PEP free of cost to the employee
- ❖ **TL+LR regimen:** Consists of Tenofovir-Lamivudine plus Lopinavir-Ritonavir
- ❖ **Indication:** If source is unknown/positive for HIV
- ❖ **Duration:** Should be started within 2 hours of exposure and continued for 28 days.

The revised NACO guideline for post-exposure prophylaxis (PEP), 2018 has been discussed in detail in Chapter 25.

Tenofovir 300 mg + Lamivudine 300 mg, one tablet, once daily and Lopinavir (200 mg) + Ritonavir (50 mg) two tablets, twice daily

NACO Guidelines to Prevent Neonatal HIV

Pregnant women who are found to be HIV reactive are initiated on lifelong ART (TLE regimen); their newborn (HIV exposed) babies are initiated on 6 weeks of Syrup Nevirapine immediately after birth; which may be extended up to 12 weeks, if the duration of the ART of mother is less than 24 weeks. The baby is also initiated on cotrimoxazole prophylaxis at 6 weeks till 18 months.

HIV Vaccine Strategies

Hurdles to Jump

After 40 years of discovery of HIV/AIDS, still medical research failed to invent an effective approved vaccine. This attributes to various factors:

- High mutability of the virus is the most important factor
- Long latency between exposure and appearance of symptoms
- Lack of ideal small animal models for studying HIV infection
- Ethical issue: Difficulty to get human volunteers.

Approaches and Trials

The researchers have explored a number of strategies and based on which more than 40 vaccine trials have been conducted in several countries so far.

- **SAV001:** It is a killed whole-virus vaccine trial, going on in Canada (2012–till now). The vaccine candidate is a genetically modified killed virus (by deleting the *nef* and *vpu* genes). Efficacy is good (antibody produced till 52-weeks); without side effect
- Other previously done unsuccessful trials were RV 144 trial, Vax Gen trial, Step trial, HVTM 505 trial
- HIV vaccine trials in India are tgAAC09 trial and Modified Vaccinia Ankara (MVA) trial.

In spite of intense research, effort and finance involved, none of the trials have been approved for human use till now.

EXPECTED QUESTIONS

I. Write essay on:

1. A 25-year-old male with history of multiple sex partners is admitted with complaints of unexplained fever, progressive loss of weight, persistent diarrhea and generalized lymphadenopathy for the past 6 months.
 - a. What is the most probable diagnosis?
 - b. Discuss the pathogenesis and laboratory diagnosis of the above condition.

II. Write short notes on:

1. Structure of HIV.
2. Diagnosis of pediatric HIV.

III. Multiple Choice Questions (MCQs):

1. The gene coding for core of HIV is:

- a. *gag*
- b. *env*
- c. *pol*
- d. *tat*

2. During the window period of patient with AIDS, best diagnostic test is:

- a. ELISA
- b. Western Blot
- c. Rapid test
- d. RT-PCR

3. Best indicator of HIV prognosis:

- a. CD4 T cell count
- b. CD8 T cell count
- c. HIV RNA
- d. ELISA

4. Highest risk of transmission of HIV:

- a. Sexual
- b. Blood product
- c. Needle/syringe
- d. Mother to fetus

Answers

1. a 2. d 3. c 4. b

CHAPTER PREVIEW

- Viral Hemorrhagic Fever
- Arboviruses
 - Dengue
- Chikungunya
- Kyasanur Forest Disease
- Other Viral Hemorrhagic Fevers
- Filoviruses
- Rodent Borne Viruses

VIRAL HEMORRHAGIC FEVER

Viral hemorrhagic fevers (VHF) are defined as a group of illnesses caused by different families of viruses that cause vascular damage that results in symptomatic bleeding (hemorrhage). VHFs are caused by viruses of three distinct groups:

1. **Arboviruses:** Transmitted by arthropod vectors. Examples include dengue, yellow fever viruses
2. **Filoviruses** such as Ebola and Marburg viruses
3. **Rodent borne** viruses such as Hantaviruses and Arenaviruses.

Note: Rarely, bacterial infections such as scrub typhus and leptospirosis can also cause hemorrhagic fever.

General Properties

Hemorrhagic fever viruses share a number of features.

- ❖ They are all enveloped RNA viruses
- ❖ **Distribution:** They are geographically restricted to the areas where their host species live (e.g. Ebola viruses in Africa)
- ❖ **Severity:** Some types of VHF (e.g. dengue) are relatively mild, whereas many of these diseases (e.g. Ebola) are severe and life-threatening
- ❖ **Reservoir:** Their survival is dependent on an animal or insect vector, which serves as natural reservoir. Humans are not the natural reservoir
 - They can be occasionally transmitted to humans when come in contact with the excretions of infected animals or arthropods
 - In some instances, once the virus infects humans, person-to-person transmission can occur when an uninfected person comes in contact with the bodily fluids (e.g. Ebola) or by the bite of an arthropod vector (e.g. dengue).
- ❖ **Symptoms:** VHF presents with fatigue, fever, weakness, dizziness, and muscle aches
 - Patients with more severe infections show bleeding under the skin, internal organs or even from external body orifices such as the mouth, eyes, or ears

- Some patients develop severe diarrhea that may also be bloody, and severely ill patients present with shock, delirium, seizures, kidney failure, and coma that often ends in death.

- ❖ **Epidemiological pattern:** VHF cases occur either sporadically or as seasonal outbreaks (dengue in India) or sometimes as explosive epidemics (e.g. Ebola in Africa, 2014). The occurrence of outbreaks cannot be easily predicted
- ❖ **Treatment:** With a few noteworthy exceptions (ribavirin for Lassa fever) there is no definitive treatment available for VHFs. Cases can only be managed by symptomatic treatment
- ❖ **Vaccine:** No vaccine is available for VHF, with the exceptions of yellow fever and dengue.

ARBOVIRAL INFECTIONS

Arboviruses (arthropod-borne viruses) are diverse group of RNA viruses that are transmitted by blood sucking arthropods (insect vectors) from one vertebrate host to another.

Viruses must multiply inside the insects and establish a lifelong harmless infection in them. Thus, the viruses which are just mechanically transmitted by insects are not included in this group.

INTRODUCTION

Arboviruses are taxonomically diverse, belong to five different families (Table 34.1). Still, the name 'arbovirus' is internationally accepted as the members under this group are comparable in many ecological and epidemiological factors—such as geographical distribution, mode of transmission (type of insect vector), clinical features and their control measures.

Classification

Members of arboviruses are RNA viruses, belonging to five different families: Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae and Rhabdoviridae (Table 34.1).

Table 34.1: General features of arbovirus.				
Virus	Manifestation	Distribution	Vector	Reservoir
Family: Togaviridae				
Chikungunya virus	Fever and arthritis (rarely hemorrhagic fever)	Asia, Africa	<i>Aedes aegypti</i>	Monkeys *
O'Nyong-nyong virus	Fever and arthritis	Africa	<i>Anopheles</i>	*
Mayaro virus	Fever and arthritis	South America	<i>Aedes aegypti</i>	Monkeys
Ross River virus	Epidemic polyarthrititis	Australia	<i>Aedes</i>	Small animals
Sindbis virus	Arthralgia, and rash	Africa, Europe, Australia	<i>Culex</i>	Birds, mammals
Semliki Forest virus	Fever and arthralgia	Africa	<i>Aedes</i>	Birds, rodents
Eastern equine encephalitis virus	Encephalitis	Eastern part of North America	<i>Aedes, Culex</i>	Birds
Western equine encephalitis virus	Encephalitis	Western part of North America	<i>Culex tarsalis, Aedes</i>	Birds
Venezuelan equine encephalitis virus	Encephalitis	South and Central America	<i>Aedes, Culex</i>	Horses
Family: Flaviviridae				
Japanese B encephalitis virus	Encephalitis	South East Asia	<i>Culex tritaeniorhynchus</i>	Pigs, Birds
St Louis encephalitis virus	Encephalitis	United States	<i>Culex</i>	Wild birds
West Nile encephalitis virus	Encephalitis	East Africa (Uganda), Algeria, Romania	<i>Culex, Aedes, Anopheles</i>	Birds
Murray Valley encephalitis virus	Encephalitis	America	<i>Culex annulirostris</i>	Birds
Rocio virus	Encephalitis	São Paulo, Brazil	<i>Culex</i>	*
Russian spring-summer encephalitis virus	Encephalitis	Central Europe, Russia	Tick	Rodents, other mammals, birds
Powassan virus	Encephalitis	America	Tick	Rodents
Louping-ill virus	Encephalitis	Europe	Tick	Sheep
Dengue virus	Hemorrhagic fever	India	<i>Aedes aegypti</i>	*
Yellow fever virus	Hemorrhagic fever	West Africa, Central South America	<i>Aedes aegypti</i>	Monkeys
Kyasanur Forest disease virus	Hemorrhagic fever	India (Karnataka)	Tick	Monkeys and rats
Omsk hemorrhagic fever virus	Hemorrhagic fever	Russia	Tick	Small mammals
Zika virus	Fever and arthritis	First occurred in Brazil, then spread to other countries	<i>Aedes aegypti</i>	Monkeys
Family: Bunyaviridae				
California encephalitis virus	Encephalitis	USA	<i>Aedes triseriatus</i>	Rodents
Oropouche virus	Rash and aseptic meningitis	Central and South America	<i>Culicoides paraensis</i>	Not known
Sandfly fever virus	Fever and myalgia	Southern Europe, North Africa, India	Sandfly	Small mammals
Rift Valley fever virus	Fever and myalgia	Africa	<i>Aedes</i>	Sheep, cattle
Crimean Congo hemorrhagic fever virus	Hemorrhagic fever	Africa	Tick	Small mammals
Ganjam virus	Fever	India	Tick	Small mammals
Severe fever with thrombocytopenia syndrome virus	Fever, thrombocytopenia	China, Korea	Tick	Sheep, goat, chicken
Family: Reoviridae				
Colorado tick fever virus	Fever, rarely encephalitis	America (mountains)	Tick	Rodents
Orungo virus	Fever	Sub-Saharan Africa	<i>Aedes</i>	*
Kemerovo virus	Fever, meningism	Russia	Tick	*
Family: Rhabdoviridae				
Vesicular stomatitis virus	Oral mucosal vesicles	Indiana	Sandfly	*
Chandipura virus	Encephalitis	India	Sandfly	*

* Not yet identified. For viruses like dengue, some studies have shown domestic dogs can be infected with dengue virus. Arboviruses causing encephalitis (e.g. Japanese encephalitis and West Nile encephalitis viruses) are discussed in Chapter 74.

Individual viruses under each family are named after various features such as:

- ❖ **Clinical features:** For example, yellow fever is named after its main clinical feature—jaundice
- ❖ **Place of discovery:** For example, Kyasanur Forest disease virus
- ❖ **Vector needed for transmission:** For example, sandfly fever virus
- ❖ **Peak season:** For example, Russian spring-summer encephalitis virus
- ❖ **Multiple features:** Japanese encephalitis virus is named after the place of discovery and clinical feature.

Clinical Manifestations

Arboviruses may also be arbitrarily divided based on the pattern of clinical syndromes they produce (Table 34.1).

- ❖ Fever and/or rash, and/or arthralgia group
- ❖ Encephalitis group
- ❖ Hemorrhagic fever group.

However, some of them may be associated with more than one clinical syndromes, e.g. dengue virus.

Epidemiology

- ❖ **Zoonotic:** Several hundred arboviruses exist in the world and all are believed to be endemic in animals. However, only about 100 are human pathogens
- ❖ **Transmission cycle:** Arboviruses are maintained in the nature between animals and their insect vectors
- ❖ **Humans are the accidental hosts** and do not play any role in the maintenance or transmission cycle of the virus, except for urban yellow fever and dengue
- ❖ **Arthropod vector:** Most arboviruses are transmitted by mosquitoes (*Aedes*, *Culex* or *Anopheles*) followed by ticks, and rarely sandfly and other insects (Table 34.1)
- ❖ **Climatic variation:** Arboviruses are more prevalent in the **tropics** than temperate climate, due to abundance of appropriate animals and arthropods in the former
- ❖ **Geographical distribution:** Arboviruses vary greatly in their geographical distribution which in turn depends on the various factors such as climatic condition and presence of vector. Viruses that are highly endemic in one place, may not be found in other areas (Table 34.1)
 - **Yellow fever** is highly endemic in West Africa, but not found at all in India in spite of its vector *Aedes aegypti* being widely distributed in India
 - **Encephalitic arboviruses:** Eastern, Western and Venezuelan equine encephalitis viruses are prevalent in North America whereas in India, Japanese encephalitis virus is the most common arbovirus causing encephalitis.
- ❖ **Arboviruses found in India:** Over 40 arboviruses have been detected in India, of which three are highly endemic and produce several outbreaks every year
 - **Common arboviruses** prevalent in India include:

- ♦ **Hemorrhagic fever group** (dengue and Kyasanur forest disease viruses) and fever with arthralgia group (chikungunya virus)

- ♦ **Encephalitis group:** and Japanese B encephalitis and West Nile encephalitis viruses (Chapter 74).

- **Rare:** Sindbis, Crimean Congo hemorrhagic fever, Ganjam, Vellore, Chandipura, Bhanja, Umbre, Sathuperi, Chittoor, Minnal, Venkatapuram, Dhori, Kaisodi and sandfly fever viruses are among the rare arboviruses found in India, with limited geographical distribution.

Arboviruses which are prevalent in other parts of the World, but not in India are not discussed, but only enlisted in Table 34.1, except for Zika virus and yellow fever virus.

- ❖ **Zika virus:** It causes fever, arthralgia and congenital infection; has recently caused an explosive epidemic in Brazil in 2015. It is discussed in this Chapter 79

- ❖ **Yellow fever virus:** It is endemic in West Africa and Central South America. It infects liver, causes hepatitis and jaundice and also hemorrhagic fever. Because of its vaccination importance for international travelers, it has been discussed in this book (Chapter 48).

LABORATORY DIAGNOSIS

Arboviral infections

- ❑ **Antibody detection:** ELISA (IgM and IgG specific) and immunochromatographic test (ICT)
- ❑ **Virus Isolation:**
 - Mosquito inoculation [adult or larval stage of *Toxorhynchites* (best), *A. aegypti* and *A. albopictus*]
 - Mosquito cell lines, such as C6/36 and AP61
 - Mammalian cell lines (such as Vero and LLC-MK2 cell lines)—least sensitive
 - Suckling mice (intracerebral inoculation).
- ❑ **Detection of antigen:**
 - In blood, e.g. dengue virus specific NS1 (nonstructural antigen 1) by ELISA and ICT
 - In fixed tissues by immunohistochemistry or direct-IF.
- ❑ **Molecular methods:** RT-PCR and Real time RT-PCR.

DENGUE

Dengue virus (DENV) is the most common arbovirus found in India. It belongs to family Flaviviridae. It is an enveloped virus, containing ssRNA.

- ❖ It is named after the Swahili word “dinga” meaning fastidious or careful, which would describe the gait of a person suffering from the bone pain of dengue fever
- ❖ It has four serotypes (DEN-1 to DEN-4). Recently, the fifth serotype (DEN-5) was discovered in 2013 from Bangkok.

Vector

Aedes aegypti is the principal vector followed by *Aedes albopictus*. They bite during the day time.

- ❖ *A. aegypti* is a nervous feeder (so, it bites repeatedly to more than one person to complete a blood meal) and resides in domestic places, hence is the most efficient vector

- ❖ *Aedes albopictus* is found in peripheral urban areas. It is an aggressive and concordant feeder, i.e. can complete its blood meal in one go; hence is less efficient in transmission
- ❖ *Aedes* becomes infective only when it feeds on viremic patients (generally from a day before to the end of the febrile period, i.e. 5 days)
- ❖ Extrinsic incubation period of 8–10 days is needed before *Aedes* to become infective. However, once infected, it remains infective for life
- ❖ *Aedes* can pass the dengue virus to its offsprings by transovarial transmission
- ❖ **Transmission cycle:** Man and *Aedes* are the principal reservoirs. Transmission cycle of four serotypes do not involve other animals; in contrast the fifth serotype follows the sylvatic cycle.

Pathogenesis

Primary dengue infection occurs when a person is infected with dengue virus for the first time with any one serotype. Months to years later, a more severe form of dengue illness may appear (called **secondary dengue infection**) due to infection with another second serotype which is different from the first serotype causing primary infection.

Antibody Response Against Dengue Virus

Infection with dengue virus induces the production of both neutralizing and non-neutralizing antibodies.

- The **neutralizing antibodies** are protective in nature. Such antibodies are produced against the infective serotype (which last lifelong) as well as against other serotypes (which last for some time). Hence, protection to infective serotype stays lifelong but cross protection to other serotypes diminishes over few months
- The **non-neutralizing antibodies** last lifelong and are heterotypic in nature; i.e. they are produced against other serotypes but not against the infective serotype
 - Such antibodies produced following the first serotype infection, can bind to a second serotype during secondary dengue infection; but instead of neutralizing the second serotype, it protects it from host immune system by inhibiting the bystander B cell activation against the second serotype
 - **ADE:** The above phenomena is called **antibody dependent enhancement (ADE)**. Non-neutralizing antibodies promote recruitment of mononuclear cells followed by release of cytokines which explains the reason behind the severity of secondary dengue infection
 - Among all the serotypes combinations, ADE is remarkably observed when serotype 1 infection is followed by serotype 2, which also claims to be the most severe form of dengue infection.

Clinical Classifications

The Traditional (1997) WHO Classification

This classification divides dengue into three clinical stages:

1. **Dengue fever (DF):** It is characterized by:
 - Abrupt onset of high fever (also called biphasic fever, break bone fever or saddle back fever)

- Maculopapular rashes over the chest and upper limbs
 - Severe frontal headache
 - Muscle and joint pains
 - Lymphadenopathy
 - Retro-orbital pain
 - Loss of appetite, nausea and vomiting.
2. **Dengue hemorrhagic fever (DHF):** It is characterized by:
 - High-grade continuous fever
 - Hepatomegaly
 - Thrombocytopenia (platelet count < 1 Lakh/mm³)
 - Raised hematocrit (packed cell volume) by 20%
 - Evidence of hemorrhages which can be detected by:
 - ◆ Positive tourniquet test (>20 petechial spots per square inch area in cubital fossa)
 - ◆ Spontaneous bleeding from skin, nose, mouth and gums.
 3. **Dengue shock syndrome (DSS):** Here, all the above criteria of DHF are present, and in addition manifestations of shock are present, such as:
 - Rapid and weak pulse
 - Narrow pulse pressure (<20 mm Hg) or hypotension
 - Presence of cold and clammy skin
 - Restlessness.

2009 WHO Classification

This is the most recently described classification by WHO which grades dengue into two stages based on the severity of infection (Fig. 34.1):

1. Dengue with or without warning signs
2. Severe dengue.

Factors Determining the Outcome

- ❖ **Infecting serotype:** Type 2 is apparently more dangerous than other serotypes
- ❖ **Sequence of infection:** Serotype 1 followed by serotype 2 seems to be more dangerous and can develop into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) more often than others
- ❖ **Age:** Though all age groups are affected equally, children less than 12 years are more prone to develop DHF and DSS.

Dengue during Pregnancy

Perinatal transmission of dengue infection can occur. Peripartum maternal infection may lead to symptomatic infection in the newborn, who may present with fever, thrombocytopenia, ascites or pleural effusions; typically during the first week of life.

Geographical Distribution

Global Scenario

Dengue is endemic in more than 100 countries with 2.5 billion people at risk.

- ❖ Tropical countries of Southeast Asia and Western Pacific are at highest risk

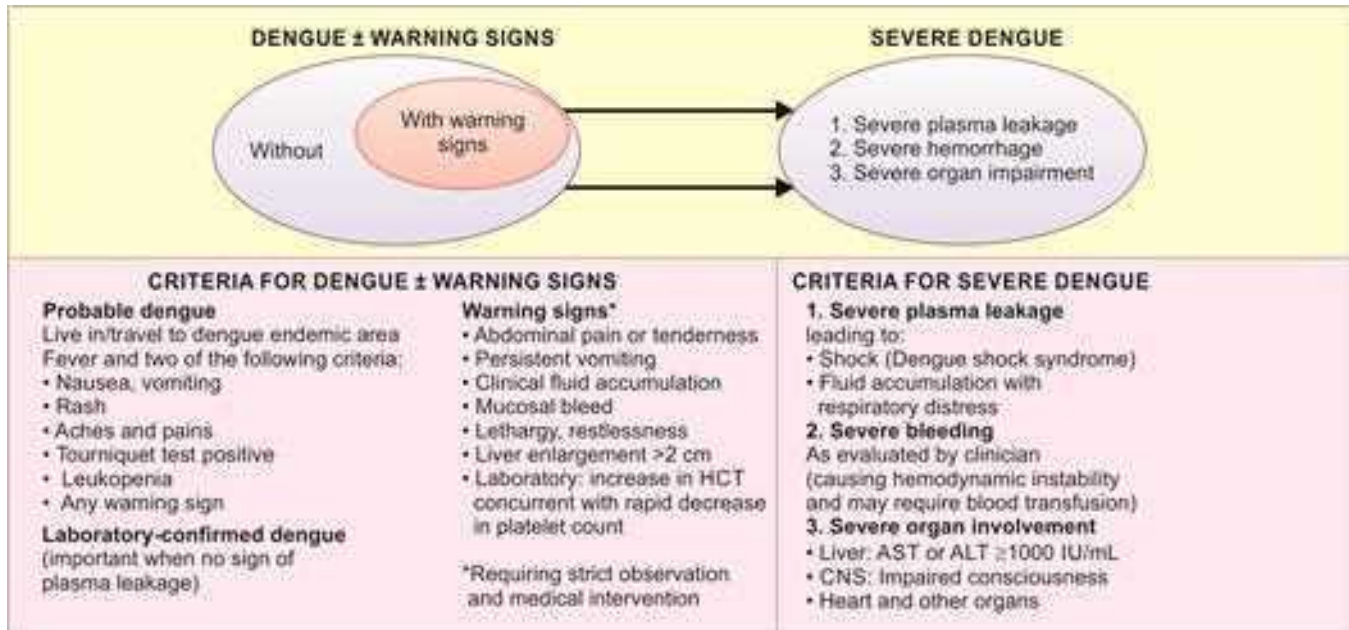


Fig. 34.1: Dengue case classification based on the severity (WHO 2009).

Abbreviations: HCT, hematocrit; DSS, dengue shock syndrome; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CNS, central nervous system.

- ❖ About 50 million of dengue cases occur every year worldwide, out of which 5 lakh cases (mostly children) proceed to DHF.

Situation in India

Disease is prevalent throughout India in most of the **urban cities/towns** affecting almost 31 states/Union territories.

- ❖ **Last decade:** Every year >1 Lakh cases of dengue with >200 deaths occur in India. Maximum cases have been reported (in descending order) from West Bengal, Tamil Nadu, Punjab, Kerala, Delhi, Karnataka, and Maharashtra
- ❖ **In 2019:** >1.37 lakh cases were reported with >130 deaths; maximum were from Karnataka and Gujarat
- ❖ All four dengue serotypes have been isolated from India. Serotype prevalence varies between seasons and places, but DEN-1 and DEN-2 are widespread. DEN-5 has not been reported yet.

Laboratory Diagnosis

The outline of laboratory diagnosis of dengue is similar to that of other arboviruses as described earlier.

NS1 Antigen Detection

ELISA and ICT formats are available for detecting NS1 antigen in serum. They gained recent popularity because of the early detection of the infection.

- ❖ NS1 antigen becomes detectable from day 1 of fever and remains positive up to 18 days
- ❖ **Highly specific:** It differentiates between flaviviruses. It can also be specific to different dengue serotypes.

Antibody Detection

- ❖ **In primary infection:** Antibody response is slow and of low titer. IgM appears first after 5 days of fever and dis-

appears within 90 days. IgG is detectable at low titer in 14–21 days of illness, and then it slowly increases

- ❖ **In secondary infection:** IgG antibody titers rise rapidly. IgG is often cross reactive with many flaviviruses and may give false positive result after recent infection or vaccination with yellow fever virus or JE. In contrast, IgM titer is significantly low and may be undetectable
- ❖ **In past infection:** Low levels of IgG remain detectable for over 60 years and in the absence of symptoms, is a useful indicator of past infection
- ❖ **MAC-ELISA (IgM antibody capture ELISA):** This is the recommended serological testing in India. Kits are supplied by NIV, Pune
 - Principle (Fig. 12.7B, Chapter 12): It is a double sandwich ELISA; which captures human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus four serotypes specific envelope protein antigens (this step makes the test specific) . There is a signal enhancement due to use of avidin-biotin complex (ABC) which makes the test more sensitive
 - Cross-reactivity with other flaviviruses is a limitation of this test.
- ❖ **Neutralization tests** such as plaque reduction test, and microneutralization tests are available. They are cumbersome; but are most specific serologic tests.

Rapid Diagnostic Tests (RDT) for Dengue

Rapid diagnostic tests (e.g. ICT) for dengue IgM antibodies or NS1 antigen are available, but have poor sensitivity and specificity. Government of India had passed an order in 2016, that a positive RDT for dengue NS1 or IgM should be considered as probable diagnosis; must be confirmed by ELISA.

Virus Isolation

Dengue virus can be detected in blood from -1 to +5 days of onset of symptoms. Virus isolation can be done by inoculation into mosquito cell line (such as C6/36 and AP61) or in mouse. It is available for research purpose and in reference centers.

Molecular Method

❖ **Detection of specific genes of viral RNA (3'-UTR region)** by real time RT-PCR:

- It is the most sensitive (80–90%) and specific assay (95%), can be used for detection of serotypes and quantification of viral load in blood
- Viral RNA can be detected in blood from -1 to +5 days of onset of symptoms
- A negative PCR result is interpreted as “indeterminate”; which has to be sent for serological confirmation after the 5th day of illness.

❖ **Genotype detection:** Each serotypes of dengue virus comprises of several genotypes which can be detected by molecular typing. A total of 13 genotypes have been detected so far; three for DENV-1, two for DENV-2, four each for DENV-3 and 4 serotypes respectively.

TREATMENT

Dengue

There is no specific antiviral therapy. Treatment is symptomatic and supportive such as:

- ❑ Replacement of plasma losses
- ❑ Correction of electrolyte and metabolic disturbances
- ❑ Platelet transfusion if needed.

Prevention

- ❖ **Vaccine:** Vaccine development for dengue has been a challenge as it should be effective against all four serotypes
 - After so many trials, recently a vaccine has been licensed for human use since 2015 (see the highlight box below)
 - Several more vaccine trials for dengue are on-going including two Indian trials—a live-attenuated vaccine (TetraVax-DV) and a tetravalent dengue subunit vaccine (DSV4).
- ❖ Mosquito control measures (as discussed for malaria, Chapter 35).

Dengue Vaccine (CYD-TDV)

This vaccine has been licensed for human use since 2015.

- ❑ It is a Chimeric Yellow Fever-Dengue, Live-Attenuated, Tetravalent Dengue Vaccine (CYD-TDV); commercially available as dengvaxia (developed by Sanofi Pasteur)
- ❑ It uses live attenuated yellow fever 17D virus as vaccine vector in which the target genes of all four dengue serotypes are integrated by recombinant technique
- ❑ **Age:** It is indicated for 9–45 years of age
- ❑ **Schedule:** 3 injections of 0.5 mL administered subcutaneously at 6 month intervals

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- ❑ It is available as lyophilized form; reconstituted with normal saline
- ❑ **Contraindications:** (i) Allergic reactions to vaccine, (ii) Immunodeficient individuals (e.g. HIV), (iii) Pregnant and breastfeeding women
- ❑ **Efficacy** against hospitalized dengue illness was found around 80%
- ❑ WHO recommends to start this vaccine in high burden countries (seroprevalence > 70%)
- ❑ WHO also recommends Dengvaxia be used **only in people previously infected with dengue**. If this vaccine is given to people who have not been previously infected with dengue, then they may be at a higher risk of developing severe dengue following subsequent DENV infection
- ❑ Currently, the vaccine is approved in Mexico, Philippines, Brazil, Indonesia, Thailand and Singapore. In India, it is not available yet because of its safety issues.

CHIKUNGUNYA

Chikungunya fever is a re-emerging disease characterized by acute fever with severe arthralgia. It belongs to family Togaviridae, of genus Alphavirus. It is an enveloped virus, containing ssRNA.

History

The name is derived from the *Makonde* word “kungunyala” meaning “that which bends up or gets folded” in reference to the stooped posture which develops as a result of the severe joint pain that occurs during the course of illness.

Transmission

- ❖ **Human transmission occurs by:**
 - *Aedes* mosquito, primarily *Aedes aegypti* which bites during day time
 - Rarely, by vertical transmission from mother to fetus or by blood transfusion or organ transplantation.
- ❖ **Transmission cycle:** Chikungunya virus is maintained in the environment through—**urban cycle** (between human and *Aedes aegypti*) and **syllan/jungle cycle** (between monkeys and forest species of *Aedes*).

Clinical Manifestations

- ❖ Incubation period is about 5 days (3–7 days)
- ❖ **Acute stage:** Most common symptoms are fever and severe joint pain (due to arthritis), worsened at morning
 - **Arthritis** is polyarticular, migratory and edematous (joint swelling), predominantly affecting the small joints of wrists and ankles
 - Other symptoms include headache, muscle pain, tenosynovitis or morbilliform skin rashes
 - Symptoms are often confusing with that of dengue. In general, Chikungunya is less severe, less acute and hemorrhagic manifestations are rare compared to dengue (Table 34.2)

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Table 34.2: Manifestations of chikungunya and dengue.

Features	Chikungunya	Dengue
Fever (onset, duration)	Acute, 2–4 days	Gradual, 5–7 days
Polyarthrititis	Frequent, may last longer (>1 month)	Less common Short duration
Tenosynovitis	Common	None
Rashes appear on	Day 1–4, maculopapular	Day 3–7, Petechiae or maculopapular
Myalgia	Possible	Common
Leukopenia	Common	Infrequent
Thrombocytopenia	Infrequent	Common
Retro-orbital pain	Rare	Common
Hypotension and shock	Possible	Common
Minor bleeding	Rare	Common
Hematocrit	Normal	Increased

- **Chik sign** (also called brownie nose appearance): Rare presentation; characterized by hyperpigmentation over centofacial area; occurs due to increased intraepidermal melanin retention triggered by the chikungunya virus. Most patients recover within a week, except for the joint pain (lasts for months).
- ❖ **Chronic stage** (10–15%): Most patients recover within a week, except for the joint pain (lasts for months; rarely up to a year)
- ❖ **High-risk group**: This group includes newborns, older adults (≥65 years), and persons with underlying hypertension, diabetes, or heart disease.

Epidemiology

Chikungunya virus was first reported in Africa (Tanzania, 1952), was subsequently introduced into Asia and had caused several outbreaks in various African and Southeast Asian countries (Bangkok and India).

- ❖ **India (past)**: Several outbreaks were reported during 1963–1973; e.g. Kolkata in 1963 and South India in 1964 (Puducherry, Chennai-Vellore region) and Barsi in Maharashtra in 1973
- ❖ Since then, it was **clinically quiescent** and no outbreaks were reported between 1973–2005 from most parts of the world, except for the few sporadic cases, which occurred in various places of the world including India (Maharashtra)
- ❖ **Re-emergence** (Reunion Outbreak): In 2005, Chikungunya re-emerged in Reunion Island of Indian Ocean and affected 2,58,000 people (almost one-third of country's population)

Reasons for Re-emergence

Re-emergence in 2005 was believed to be due to a novel mutation in the virus and a change in vector.

- ❑ **New mutation (E1-A226V)**: Chikungunya virus underwent an important mutation. Alanine in the 226 position of E1 glycoprotein gene is replaced by valine

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- ❑ **New vector (*Aedes albopictus*)**: This mutation led to a shift of vector preference. Mutated virus was found to be 100 times more infective to *A. albopictus* than to *A. aegypti*.

- ❖ **Spread**: Following the re-emergence, it has been associated with several outbreaks in India, other Southeast Asian and African countries and has also spread to some areas of America and Europe
- ❖ The most recent epidemic had occurred in Colombia during 2014–15; which witnessed 82,977 clinically confirmed cases by end of 2014
- ❖ **India (at present)**: Chikungunya is endemic in several states
 - **States**: Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal have reported higher number of cases
 - In 2019, nearly 65,217 suspected and 9,477 confirmed cases were reported
 - Karnataka accounted for the maximum number of cases followed by Maharashtra.

Laboratory Diagnosis

Laboratory diagnosis of chikungunya is similar to that of other arboviruses as described before.

- ❖ **Viral isolation** in mosquito cell lines (takes 1–2 weeks) is useful for early diagnosis (0–7 days), but available only in reference centers
- ❖ **Serum antibody detection**: IgM appears after 4 days of infection and lasts for 3 months; IgG appears late (after 2 weeks) and lasts for years. So, detection of IgM or a fourfold rise in IgG titer is more significant
 - **MAC (IgM Antibody Capture) ELISA** (using virus lysate) is the best format available showing excellent sensitivity (95%) and specificity (98%) with only little cross reactivity with other alphaviruses and dengue. In India, MAC ELISA kits are supplied by National Institute of Virology (NIV), Pune
 - Several other rapid tests (e.g. ICT using envelope antigens) are also available.
- ❖ **Molecular method**: Reverse-transcriptase PCR has been developed to detect specific gene (e.g. nsP1, nsP4) in blood
- ❖ **Hematological finding**: Such as leukopenia with lymphocyte predominance, thrombocytopenia (rare), elevated ESR and C-reactive protein.

TREATMENT

Chikungunya

Treatment of chikungunya is only by supportive measures; no specific antiviral drugs are available. Vaccine is also not available, although vaccine trials are on going.

KYASANUR FOREST DISEASE

Kyasanur Forest disease virus was identified in 1957 from monkeys from the Kyasanur Forest in Shimoga district of

Karnataka, India. It belongs to the family Flaviviridae. It is an enveloped virus, containing ssRNA.

Epidemiology

- ❖ **Vector:** Hard ticks (*Haemaphysalis spinigera*) are the vectors of KFD virus
- ❖ **Hosts:** Monkeys, rodents and squirrels are common hosts which maintain the virus through animal-tick cycles. Monkeys are the amplifier hosts, where the virus multiplies exponentially
- ❖ **Seasonality:** KFD is increasingly reported in dry months (November to June) which coincides with human activity in forest
- ❖ **Situation in India:** KFD is currently endemic in five districts of Karnataka—Shimoga, North Kannada, South Kannada, Chikkamagaluru and Udipi
 - **Largest outbreak** had occurred in 1983–84, which has witnessed 2,167 cases with 69 deaths. Currently only focal cases occur at a rate of 100–500 cases per year
 - There is a **declining trend** of incidence after the initiation of vaccine in 1999, except for the outbreak that occurred in 2013, which witnessed 215 suspects with 61 confirmed cases.

Clinical Manifestation in Humans

- ❖ Incubation period varies from 3–8 days
- ❖ **First stage (hemorrhagic fever):** It starts as acute high fever with malaise and frontal headaches, followed by hemorrhagic symptoms, such as bleeding from the nasal cavity, throat, and gums, as well as gastrointestinal bleeding
- ❖ **Second stage** in the form of meningoencephalitis may occur 7–21 days after the first stage.

Laboratory Diagnosis

Diagnosis is made by virus isolation from blood or by IgM antibody detection by ELISA.

- ❖ Recently, nested RT-PCR and real time RT-PCR have been developed detecting viral RNA (NS-5 non-coding region) in serum samples and can provide early, rapid and accurate diagnosis of the infection
- ❖ Non-specific findings such as leukopenia, thrombocytopenia and decreased hematocrit, albuminuria and abnormal CSF are found in second stage.

TREATMENT

KFD

Treatment of KFD is only by supportive measures; no specific antiviral drugs are available.

Killed KFD Vaccine

A formalin-inactivated chick embryo vaccine has been developed for KFD in the Haffkine institute, Mumbai.

- ❖ **Schedule:** Two-doses at interval of 2 months, followed by booster doses at 6–9 months and then every 5 years

- ❖ **Target area:** KFD vaccine is recommended in endemic areas of Karnataka (villages within 5 km of endemic foci).

OTHER VIRAL HEMORRHAGIC FEVERS

Viral hemorrhagic fevers can also be caused by Filoviruses (e.g. Ebola and Marburg viruses) and rodent-borne viruses such as Hantaviruses and Arenaviruses.

FILOVIRUS INFECTIONS

Family Filoviridae contains two antigenically distinct genera—*Ebola virus* and *Marburg virus*; both cause **African hemorrhagic fever**. A third genus has recently been described, *Cuevavirus*.

- ❖ **Morphology:** They are pleomorphic, mostly appear as long **filamentous threads**, ranging from 80–1000 nm, the average size being 665 nm (Marburg) to 805 nm (Ebola)
- ❖ **Highly fatal:** A great matter of concern is, of all the viral hemorrhagic fevers, Marburg and Ebola viruses have the highest mortality rates (25–90%).

Ebola Virus Disease

Ebola virus has become a global threat, because of its explosive outbreak in 2014; which was declared by WHO, as a public health emergency of international concern.

History

Ebola virus disease in humans appeared first in 1976 in two simultaneous African outbreaks occurring in Sudan, and Democratic Republic of Congo. The latter outbreak occurred in a village near the **Ebola River**, from which the virus takes its name.

Species

Ebola virus has six stable subtypes or species (*Zaire*, *Bundibugyo*, *Sudan*, *Tai Forest*, *Reston* and *Bombali*); all differ from each other by up to 40% of their nucleotide sequences.

- ❖ Species of epidemiological importance are *Ebola*, *Sudan*, *Tai Forest*, and *Bundibugyo* viruses can cause disease in humans, but *Reston* virus causes disease in nonhuman primates, but not in people. *Bombali* virus was recently identified in bats
- ❖ The virus that had caused an explosive outbreak in Democratic Republic of Congo and the 2014 West African outbreak belongs to the *Zaire* species.

Geographical Distribution

Since its discovery, Ebola virus has caused several outbreaks in various African countries.

- ❖ **DRC outbreak:** Democratic Republic of the Congo (DRC) continues to report Ebola outbreaks in recent years—11 outbreaks reported so far; the most recent being in June 2020

- ❖ **India:** There is no confirmed case documented yet.

West African Epidemic (2014–16)

The largest outbreak occurred in 2014–16; reported 28,616 cases with 11,310 deaths (40% mortality).

- ❑ Three primary countries affected were—Guinea, Liberia and Sierra Leone
- ❑ However few cases have also been reported from several other countries.

Reservoir

The reservoir hosts for Ebola viruses are unknown, but are suspected to be infected animals, such as a **fruit bat** or **primates** (apes and monkeys).

Transmission

In every outbreak, Ebola virus is introduced to human population through **close contact** with the blood, secretions, organs or other body fluids of infected animals such as chimpanzees, gorillas, fruit bats or monkeys.

- ❖ **Human-to-human transmission:** Once introduced to humans, Ebola virus spreads among people via **direct contact** (through broken skin or mucous membranes of eyes, nose, or mouth) with:
 - Blood, secretions, organs or other bodily fluids of infected people
 - Infected surfaces and materials (e.g. bedding, clothing, syringes, etc.).
- ❖ Health-care workers and close contacts/family members of infected individuals are at greater risk of contracting the infection
- ❖ Ebola virus can stay in semen for up to 3 months, although sexual transmission has not been reported yet.

Clinical Manifestations

- ❖ Incubation period is about 2–12 days
- ❖ Common symptoms include fever, headache, muscle pain and sore throat, followed by:
 - Abdominal pain, vomiting and severe watery diarrhea
 - Diffuse erythematous maculopapular rash, petechiae, ecchymosis/bruising, often leading to shock and death.
- ❖ **Mortality:** The average case fatality rate is around 50%; vary from 25 to 90% between outbreaks.

Laboratory Diagnosis

- ❖ **Serum antibody** detection:
 - ELISA detects both IgM and IgG separately by using recombinant nucleoprotein (NP) and glycoprotein (GP) antigens
 - IgM appears after seven days of symptoms and lasts for 3–6 months. IgG appears after 2 weeks and persists for 3–5 years or more

- Other antibody detection assays include immunofluorescence test and antibody-phage indicator assay.

- ❖ **Serum antigen** is detected by capture ELISA. The target proteins are NP, VP40, and GP. Immunohistochemical staining and histopathology can also be used to localize Ebola viral antigen in tissue
- ❖ **Molecular methods** such as RT-PCR and real time RT-PCR assays are useful to detect specific RNA such as NP and GP gene. Virus is detectable after 3 days of onset of fever and remains positive for 2–3 weeks
- ❖ **Electron microscopy** of the specimen shows typical filamentous viruses (Fig. 34.2)
- ❖ **Virus isolation in Vero cell line:** Processing the specimen should be carried out in biosafety level-4 cabinets as there is a great risk of laboratory spread of the virus.

TREATMENT

Ebola virus disease

Supportive care such as rehydration and symptomatic treatment improves survival. No proven treatment or vaccine is available yet.

Prevention (General Measures)

People who may be exposed to patients suspected with Ebola should follow the following steps:

- ❖ Practice proper infection control and sterilization measures such as strict hand hygiene and personal protective equipment (PPE such as coverall and N95 mask)
- ❖ Isolate patients with Ebola from other patients
- ❖ Avoid direct or indirect contact (clothes, bedding, needles) with blood or body fluids or other secretions of suspected Ebola cases
- ❖ Avoid attending funeral or wear PPE while attending funeral
- ❖ If traveling to Ebola outbreak area, should be monitored for 21 days after returning.

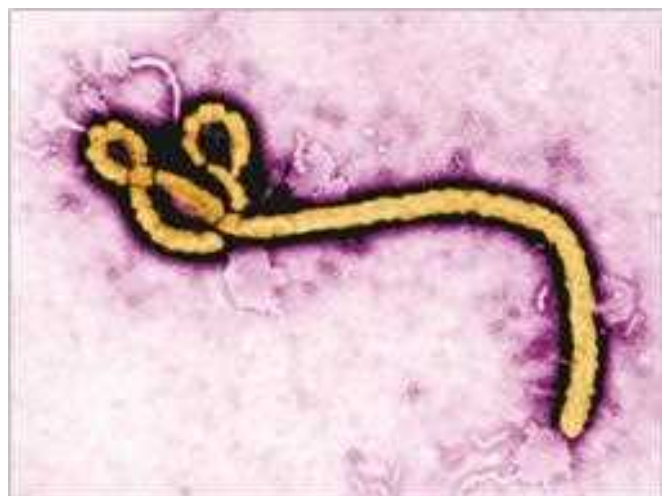


Fig. 34.2: Ebola virus, filamentous shaped (Electron micrograph).

Source: ID# 10815, Public Health Image Library, /Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Vaccine

- ❖ No Ebola vaccine is approved for human use yet. However, several vaccine trials are going on
- ❖ **STRIVE trial** (Sierra Leone Trial to Introduce a Vaccine against Ebola): The vaccine candidate used here is 'rVSV-ZEBOV', which is recombinant vesicular stomatitis virus—Zaire Ebola virus vaccine. This vaccine is being used in the ongoing 2018-2019 Ebola outbreak in DRC.

Marburg Virus Disease

Marburg virus disease was first reported in Germany and Yugoslavia (1967) among laboratory workers exposed to tissues of African green monkeys imported from Africa.

- ❖ Since then, over 450 cases have been reported in various African countries such as Kenya, South Africa, Democratic Republic of Congo, Uganda and Angola
- ❖ The most recent outbreak was in Angola (2005), affecting 252 people with 227 deaths (with mortality rate of 90%).

RODENT BORNE VIRUS INFECTIONS

Rodent-borne viruses or roboviruses are transmitted from rodents to man by contact with infected body fluids or excretions. They are maintained in nature by transmission from rodent to rodent without participation of arthropod vectors. Major rodent-borne viruses include Hantaviruses and Arenaviruses.

Hantavirus Disease

Genus *Hantavirus* belongs to the family Bunyviridae.

- ❖ **Morphology:** They are spherical, enveloped viruses; contain triple-segmented, negative-sense ssRNA
- ❖ **Clinical manifestations:** They cause two categories of manifestations

- *Hemorrhagic fever with renal syndrome (HFRS)*—is caused by several members of hantaviruses such as Hantaan virus, Dobrava virus, Puumala virus and Seoul virus
- *Hantavirus pulmonary syndrome (HPS)*—is caused by another member, Sin Nombre virus.
- ❖ **Epidemiology:** Worldwide, about 1-2 Lakh cases of hantavirus infections occur annually
 - HFRS due to Hantaan and Dobrava viruses occur in Asia, particularly in China, Russia, and Korea
 - Puumala virus causes a mild form of nephritis called nephropathia epidemica, prevalent in Scandinavia
 - Sin Nombre virus (causing HPS) is prevalent in America.
- ❖ **Diagnosis** is made by detection of viral RNA by RT-PCR
- ❖ **Treatment:** There is no specific antiviral therapy for hantaviral diseases. Only supportive symptomatic treatment is given.

Arenavirus Infections

Arenaviruses are pleomorphic, 50-300 nm in size, enveloped with large, **club-shaped peplomers** and contain a segmented ssRNA (**two segments**).

Based on geographical distribution, they are grouped into:

- ❖ **New world viruses:** Examples include Junin, Machupo, Guanarito and Sabia viruses. They cause South American hemorrhagic fever
- ❖ **Old world viruses:** Examples include—
 - Lassa viruses—cause hemorrhagic fever in Africa
 - Lymphocytic choriomeningitis (LCM) viruses—They primarily infect mouse; rarely cause meningitis in humans.

EXPECTED QUESTIONS

I. Write essay on:

1. Sunita, a 29-year-old female came to casualty with complaints of high-grade fever, severe joint pain, back pain and myalgia. Gradually, she developed petechial rashes over the body. On examination, she was found to have jaundice, hepatomegaly and a low platelet count (30,000/cmm). A tourniquet test done over the cubital fossa demonstrated 25 petechial spots/square inch area. On inquiry, she told that she has been bitten by the mosquitoes.
 - a. What is the clinical diagnosis and how is this disease transmitted?
 - b. What are the typical clinical presentation and pathogenesis of this condition?
 - c. How will you confirm the diagnosis?

II. Write short notes on:

1. Chikungunya.

2. Kyasanur Forest disease.
3. Ebola virus disease.

III. Multiple Choice Questions (MCQs):

1. **Kyasanur Forest disease is transmitted by:**
 - a. Mite
 - b. Louse
 - c. Tick
 - d. Mosquito
2. **In dengue infection, earliest detectable number of petechial spots per square inch in cubital fossa should be:**
 - a. >5
 - b. >10
 - c. >15
 - d. >20
3. **Antibody dependent enhancement (ADE) is observed with:**
 - a. Dengue hemorrhagic fever
 - b. Japanese encephalitis
 - c. Yellow fever
 - d. Chikungunya fever

Answers

1. c 2. d 3. a

CHAPTER PREVIEW

■ Malaria

■ Babesiosis

MALARIA

History

Malaria is one of the oldest documented diseases of mankind. The name “Malaria” (“Mal” means bad and “aria” means air) was derived from the ancient false belief that “disease is spread by air pollution through stagnant water and marshy lands”

- ❖ Sir Alphonse Laveran in 1902 and Sir Ronald Ross in 1907 won the Nobel Prize for their contributions in malaria
 - Alphonse Laveran (1880) was the first to discover the causative agent *Plasmodium*, in the red blood cell (RBC) of a patient in Algeria
 - Sir Ronald Ross, in 1897 had described the sexual cycle of the parasite in female *Anopheles* mosquito in Secunderabad, India.
- ❖ Ms Tu Youyou, a chemist was awarded Nobel prize (2015), for the discovery of artemisinin, which is used for the treatment of falciparum malaria.

Agent

Although several species of *Plasmodium* exist infecting wide range of birds, reptiles and mammals, human infection is mainly caused by five species.

1. *P. vivax* causes benign tertian malaria (periodicity of fever is once in 48 hours, i.e. recurs every third day)
2. *P. falciparum* causes malignant tertian malaria (severe malaria, periodicity of fever is once in 48 hours, recurs every third day)
3. *P. malariae* causes benign quartan malaria (periodicity of fever is once in 72 hours, i.e. recurs every fourth day)
4. *P. ovale* causes ovale tertian malaria (periodicity of fever is once in 48 hours, i.e. recurs every third day)
5. *P. knowlesi* causes quotidian or simian malaria (fever periodicity is once in 24 hours, i.e. recurs every day). It is a parasite of monkey but can also infect humans and many cases affecting man were recently reported from Asia.

Life Cycle (Fig. 35.1)

Host: *Plasmodium* completes its life cycle in two hosts: definitive host—female *Anopheles* mosquito (sexual cycle

or sporogony takes place) and intermediate host—man (asexual cycle or schizogony takes place)

- ❖ Male *Anopheles* does not feed on man and feeds exclusively on fruit juices, i.e. why male *Anopheles* mosquito does not transmit the disease. Whereas female *Anopheles* needs at least two blood meals before laying eggs
- ❖ Although several species of *Anopheles* can transmit, the vectors of primary importance include: *Anopheles culicifacies* in rural areas, *A. stephensi* in urban areas and *A. fluviatilis* in hilly areas.

Human Cycle

Mode of Transmission and Infective Form

- ❖ Man acquires infection by the bite of female *Anopheles* mosquito. Sporozoites (infective form) from the salivary gland of the mosquito are directly introduced into the cutaneous venules and enter the blood circulation
- ❖ Rarely, it can also be transmitted by blood transfusion or transplacental transmission—here, trophozoites (or merozoites) act as the infective form.

In humans, the asexual cycle takes place through three stages: (1) pre-erythrocytic schizogony (2) erythrocytic schizogony, and (3) gametogony.

Pre-erythrocytic (Hepatic) Stage

This stage occurs in the liver and it is so named because it occurs before the invasion of RBC. It is also called exoerythrocytic stage or intrahepatic or tissue stage.

- ❖ The motile sporozoites leave the circulation within 30 minutes and enter the liver
- ❖ **Attachment:** The circumsporozoite proteins present on the surface of sporozoites bind to the receptors present on the surface of hepatocytes facilitating the entry of sporozoites
- ❖ **Trophozoites:** After entering into hepatocytes, the spindle shaped sporozoites become rounded and transform into trophozoites
- ❖ **Schizogony:** Trophozoite is the feeding stage of the parasite which later on undergoes several nuclear divisions and transforms into pre-erythrocytic schizont

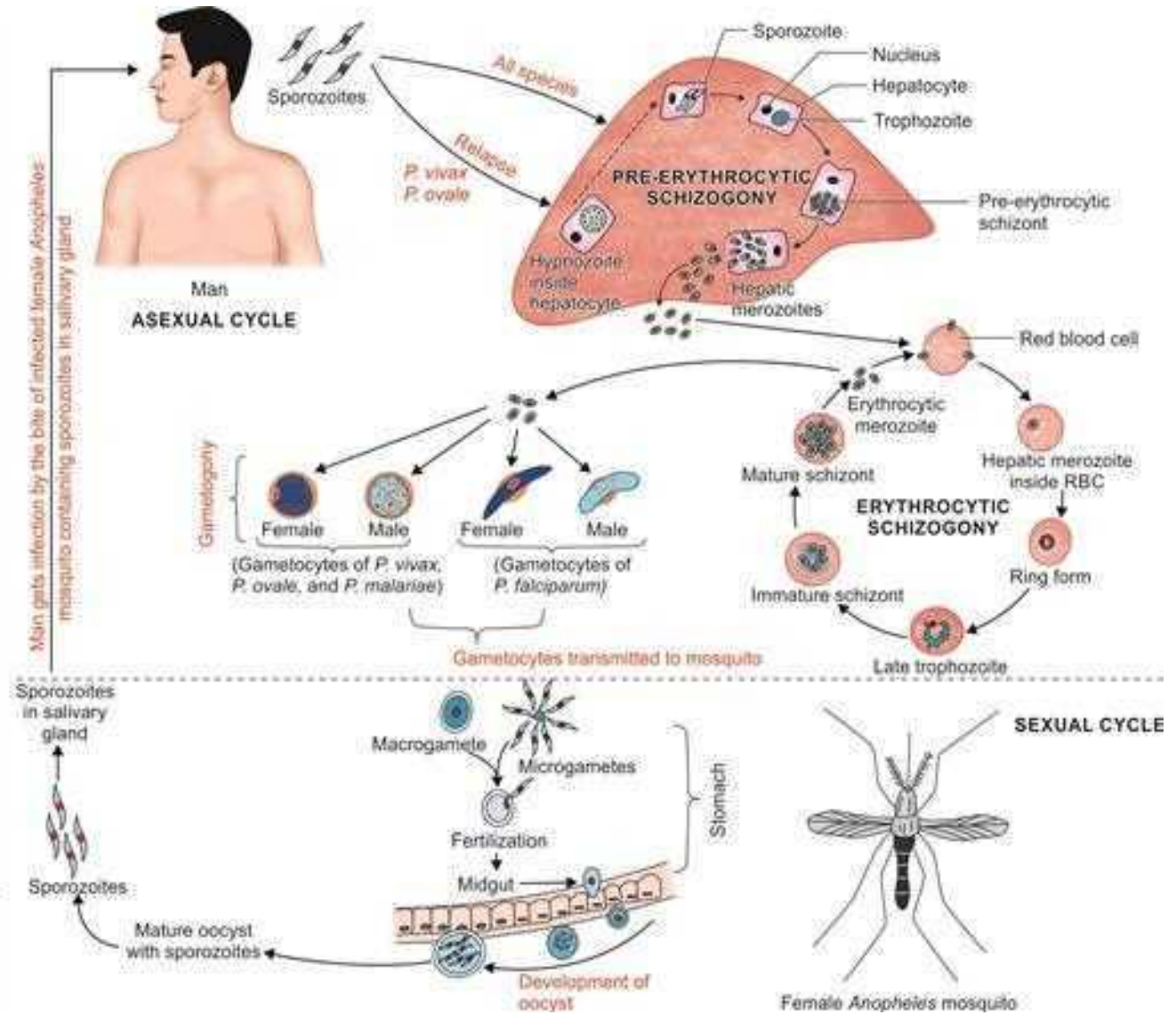


Fig. 35.1: Life cycle of malaria parasite.

- ❖ **Pre-erythrocytic schizont** contains several merozoites; released outside on rupture of hepatocyte. Merozoites then attack RBCs to initiate erythrocytic stage
- ❖ **No liver injury:** As only few hepatocytes are infected by *Plasmodium*, so hepatic damage does not occur in malaria
- ❖ **Duration** of pre-erythrocytic schizogony varies from 5 days to 15 days depending on the species
- ❖ **Hypnozoites:** Some sporozoites of *P. vivax* and *P. ovale* do not develop further and may remain in liver as hypnozoites and cause relapse of malaria after many years
- ❖ **Relapse** should be differentiated from another phenomena seen in *P. falciparum* and *P. malariae* called as **recrudescence** (Table 35.1).
- ❖ Merozoites bind to the **glycophorin receptors** on RBC surface, enter by endocytosis and are contained within a parasitophorous vacuole inside the RBCs
- ❖ **Trophozoite:** Soon the hepatic merozoites transform into trophozoites
- ❖ **Early trophozoites** are called as ring forms, which are annular or signet ring in appearance containing a central vacuole with a peripheral thin rim of cytoplasm and a nucleus
- ❖ **Late trophozoite:** Ring form enlarges and becomes more irregular and transforms into late trophozoite or amoeboid form
- ❖ **Malarial pigment:** *Plasmodium* feeds on hemoglobin, releasing the undigested products (hematin and iron porphyrin), which combine to form malarial pigment (hemozoin pigment)
- ❖ **Schizogony:** Late trophozoite undergoes schizogony to produce 6–30 daughter merozoites arranged in the form of rosette. This form is known as **erythrocytic schizont**

Erythrocytic schizogony

The hepatic merozoites after released from pre-erythrocytic schizont, attack RBCs.

Table 35.1: Relapse and recrudescence in malaria.

Relapse	Recrudescence
<ul style="list-style-type: none"> Seen in <i>Plasmodium vivax</i> and <i>P. ovale</i> infections 	<ul style="list-style-type: none"> Although seen in all species, more common in <i>P. falciparum</i> followed by <i>P. malariae</i>
<ul style="list-style-type: none"> Few sporozoites do not develop into pre-erythrocytic schizont, but remain dormant (known as <i>hypnozoites</i>) for 3 weeks to one year 	<ul style="list-style-type: none"> In <i>falciparum</i> malaria—recrudescence is due to persistence of drug resistant parasites, even after the completion of treatment
<ul style="list-style-type: none"> Reactivation of hypnozoites leads to initiation of erythrocytic cycle and relapse of malaria 	<ul style="list-style-type: none"> In <i>P. malariae</i> infection, long-term recrudescences are seen for as long as 60 years This is due to long-term survival of erythrocytic stages at a low undetectable level in blood
<p>Concept of secondary exo or pre-erythrocytic stage:</p> <ul style="list-style-type: none"> Formerly, it was postulated that relapse occurs due to secondary exo or pre-erythrocytic stage where a proportion of hepatic merozoites released from pre-erythrocytic schizont, again attack the liver cells But now, it is believed that secondary pre-erythrocytic stage does not occur and relapse occurs due to sporozoites undergoing dormancy during the primary pre-erythrocytic stage 	

- ❖ RBCs then rupture to release the daughter merozoites, malarial pigments and toxins into the circulation which result in malarial paroxysm of fever at the end of each erythrocytic cycle
- ❖ Each merozoite is potentially capable of invading a new RBC and repeating the cycle. Intra-erythrocytic life cycle takes 48–72 hours depending upon species (Table 35.2)
- ❖ In *P. falciparum* infection, the later stages of erythrocytic cycle occur in the capillaries of brain and internal organs. Hence, only the ring forms are found in peripheral blood examination, but not late trophozoites and schizonts.

Gametogony

After a series of erythrocytic cycles, some merozoites after entering into RBCs, instead of developing into trophozoites, they transform into sexual forms called as **gametocytes**.

- ❖ The gametocytes are usually round in shape, except in *P. falciparum* in which they are crescent or banana-shaped
- ❖ They are of two types—(1) male gametocyte (or microgametocyte) and (2) female gametocyte (or macrogametocyte)
- ❖ Gametocytes neither cause any clinical illness nor they divide, but play an important role in the transmission of the disease

- ❖ Gametocytes are the infective form to mosquito. They are capable of transmission only when they are **mature**, **viable**, and present in **sufficient density** (12 gametocytes per cubic mm of blood) to infect mosquitoes.

Mosquito Cycle

A female *Anopheles* mosquito during the blood meal, takes both asexual and the sexual forms. The asexual forms get digested whereas the sexual forms, i.e. the gametocytes undergo further development.

- ❖ Each male gametocyte undergoes **exflagellation** and divides into eight flagellated actively motile bodies called as male gamete or microgametes
- ❖ Female gametocyte does not undergo exflagellation but directly develop into one female gamete or macrogamete
- ❖ **Zygote:** The male gamete fertilizes with the female gamete to form zygote
- ❖ **Ookinete:** Zygote transforms into a motile elongated form called ookinete in the midgut
- ❖ **Oocyst:** The ookinete penetrates the stomach wall of the mosquito and becomes rounded, covered by a thin elastic membrane to form oocyst
- ❖ **Sporozoites:** Each oocyst undergoes sporogony (meiosis) to produce four spindle-shaped sporozoites. On rupture

Table 35.2: Differences between the four malaria parasites.

Properties	<i>Plasmodium vivax</i>	<i>Plasmodium falciparum</i>	<i>Plasmodium malariae</i>	<i>Plasmodium ovale</i>
Relapse (Hypnozoites)	Seen	Not seen	Not seen	Seen
Recrudescence	Not seen	Seen	Seen (Up to 60 years)	Not seen
Erythrocytic cycle	48 hours	36–48 hours	72 hours	48 hours
Prepatent period	8 days	5 days	13 days	9 days
Incubation period	14 days	12 days	28 days	17 days
R-G interval ^a	4–5 days	10–12 days	11–14 days	5–6 days
Extrinsic IP ^b	8–10 days	9–10 days	25–28 days	14–16 days

Abbreviations: ^aR-G interval, interval between appearance of ring form and gametocyte; ^bIP, incubation period.

Prepatent period: It is the time between the entry of the parasite into man and demonstration of the parasite in the peripheral blood. It varies between the species.

Incubation period: Time interval between the entry of the parasite into man and appearance of first clinical feature. It varies between species.

Duration of erythrocytic cycle corresponds to the frequency of febrile paroxysms seen in malaria; e.g. every 48h for *P. vivax*.

of the mature oocyst, the sporozoites are released and migrate to salivary gland and the cycle is repeated

- ❖ **Extrinsic incubation period:** Time required to complete the life cycle in mosquito varies from 1 to 4 weeks, depending up on the species.

The differences in the life cycle between the four malaria parasites have been depicted in Table 35.2. *Plasmodium knowlesi* has been discovered recently and therefore discussed separately.

Plasmodium knowlesi

It is a malaria parasite of monkeys, but can also rarely affect humans. *Anopheles leucosphyrus* is the main vector.

Epidemiology: The first human case was documented in 1965. However, cases in humans increasingly being reported from Asia since 2008.

- ❑ **World:** Maximum cases have been reported from Malaysia (highest), Thailand and Myanmar. The largest foci are located at Malaysian Borneo; 3,122 cases have been reported between 2004–2015
- ❑ **India:** The only report of *P. knowlesi* infection has documented from Andamans. However, India has all the potential of getting cases as the vector is found in the coastal region of Kerala and Maharashtra.

Clinical features: *P. knowlesi* produces an acute illness and relatively high parasitemia

- ❑ Paroxysms of fever occur daily (quotidian malaria) because of short RBC cycle (24 hours)
- ❑ Clinically it resembles *P. vivax*, but severe malaria is seen more frequently (7–10%), compared to 3% of *P. vivax*. However, it infects RBCs of all ages.
- ❑ Common complications seen are respiratory distress (most frequent) and renal failure. No cerebral malaria has been reported so far.

Laboratory diagnosis:

- ❑ On blood smear examination, early trophozoite of *P. knowlesi* is indistinguishable from *P. falciparum*, sometimes shows multiple ring forms, accolé forms and double dot ring forms
- ❑ The late trophozoites (with band forms), and round gametocytes are morphologically similar to that of *P. malariae*
- ❑ Currently, no specific rapid diagnostic tests (RDTs) are available to detect *P. knowlesi*
- ❑ *P. knowlesi* specific nested PCR assays are available using the primers Pmk8 and Pmk9 targeting small subunit rRNA.

Treatment: It responds well to chloroquine or primaquine. As the disease rapidly progresses, treatment should be promptly started.

Pathogenesis and Clinical Feature

Benign Malaria

Benign malaria is milder in nature, can be caused by all four species. It is characterized by a triad of febrile paroxysm, anemia and splenomegaly.

Febrile Paroxysm

Fever comes intermittently depending on the species. It occurs every fourth day (72 hour cycle for *P. malariae*) and every third day (48 hour cycle for other three species).

- ❖ Paroxysm corresponds to the release of the successive broods of merozoites into the bloodstream, at the end of RBC cycle
- ❖ Each paroxysm of fever is comprised of three stages
 1. **Cold stage:** Lasts for 15 minutes to 1 hour. The patient feels lassitude, headache, nausea, intense cold, chills and rigor
 2. **Hot stage:** Patient develops a high-grade fever of 39–41°C and dry burning skin. Headache persists but nausea diminishes
 3. **Sweating stage:** Fever comes down with profuse sweating. The skin becomes cold and moist. Patient feels relieved and often asleep. This stage lasts for 2–4 hours.
- ❖ The classical paroxysm may not be present always due to maturation of generations of parasites at different times
- ❖ In *P. falciparum*, the fever is more irregular or even continuous with marked prostration, headache and nausea.

Anemia

Patient develops normocytic normochromic anemia which may be attributed to various factors.

- ❖ Parasite induced RBC destruction—Lysis of RBC due to release of merozoites
- ❖ Splenic removal of both infected RBC and uninfected RBC coated with the immune complexes
- ❖ Bone marrow suppression leading to decreased RBC production.

Splenomegaly

After a few weeks of febrile paroxysms, spleen gets enlarged and becomes palpable. Splenomegaly is due to massive proliferation of macrophages that engulf parasitized and nonparasitized coated RBCs.

Falciparum Malaria (Malignant Tertian Malaria)

The pathogenesis of *P. falciparum* is different from other species.

Sequestration of the Parasites

An important feature of the pathogenesis of *P. falciparum* is its ability to sequester (holding back) the parasites in the blood vessels of deep visceral organs like brain, kidney, etc. This leads to blockage of vessels, congestion and hypoxia of internal organs. Sequestration is mediated by:

- ❖ **Cytoadherence:** It refers to the binding of infected erythrocytes to endothelial cells. It is mediated by a specialized antigen called as *P. falciparum* **erythrocyte membrane protein-1 (PfEMP-1)**, which binds to specific receptors present on the vascular endothelium of deep organs
- ❖ PfEMP-1 also helps in binding of infected RBCs to uninfected RBCs by a process called **rosetting**
- ❖ Since the parasites are sequestered back in deep vessels, they can avoid frequent spleen passage, hence can escape splenic clearance

- ❖ PfEMP undergoes frequent antigenic variation, thus helps the parasite in evading the host immune response.

Complications

Complications of *Falciparum* Malaria

P. falciparum infection is more acute and severe in nature with more complications than the benign malaria.

- ❖ **Cerebral malaria:** this is the most serious complication seen in falciparum malaria. It results due to plugging of brain capillaries by the sequestered parasitized RBCs leading to vascular occlusion and cerebral anoxia (discussed in detail in Chapter 75)
- ❖ **Pernicious malaria:** It is characterized by blackwater fever, algid malaria and septicemic malaria
- ❖ **Black water fever:** This syndrome is characterized by sudden intravascular hemolysis followed by fever, hemoglobinuria and dark urine
 - It occurs following quinine treatment to subjects previously infected with *P. falciparum*
 - Autoimmune mechanism: Antibodies develop against parasitized and quinized RBCs. With subsequent infection and quinine treatment, there is immunocomplex formation followed by complement mediated massive destruction of both parasitized and nonparasitized RBCs.
- ❖ **Algid malaria:** Characterized by cold clammy skin, hypotension, peripheral circulatory failure and profound shock
- ❖ **Septicemic malaria:** Characterized by high-grade fever with the dissemination of the parasite to various organs leading to multiorgan failure
- ❖ **Pulmonary edema and adult respiratory distress syndrome:** Severe falciparum malaria in adults may lead to noncardiogenic pulmonary edema, often aggravated by over hydration. It does not usually respond to antimalarial therapy; mortality rate is more than 80%
- ❖ **Hypoglycemia:** It is associated with a poor prognosis and is particularly problematic in children and pregnant women and following quinine therapy
- ❖ **Renal failure:** It occurs due to erythrocyte sequestration in renal microvasculature leading to acute tubular necrosis. It is common among adults than children
- ❖ **Bleeding/disseminated intravascular coagulation:** Patient presents with significant bleeding and hemorrhages from the gums, nose and intestine with or without evidence of disseminated intravascular coagulation
- ❖ **Severe jaundice:** More common among adults than children; it results from hemolysis, hepatocyte injury and cholestasis
- ❖ **Severe normochromic, normocytic anemia:** Characterized by hematocrit of less than 15% or hemoglobin level of less than 5 g/dL with parasitemia level $>10^5/\mu\text{L}$ ($>2\%$)
- ❖ **Acidosis:** Results from the accumulation of organic acids like lactic acid.

Chronic Complications of Malaria

1. **Tropical splenomegaly syndrome:** It is also called as hyper-active malarial splenomegaly; occurs in malaria-endemic areas in tropical Africa and Asia (including India)
 - It results from an abnormal immunologic response to repeated malaria infections and is characterized by—elevated IgM (due to polyclonal Bt cell activation) and massive splenomegaly
 - Patients respond well to antimalarial chemoprophylaxis (proguanil).
2. **Quartan malarial nephropathy:** It is a chronic complication seen with *P. malariae* (rarely *P. knowlesi* and other species). It occurs due to injury to the renal glomeruli by the immune complexes, resulting in nephrotic syndrome
3. **Promotes Burkitt's lymphoma:** Malaria induced severe immunosuppression in African children provoke Epstein-Barr virus infection to develop Burkitt's lymphoma.

Malaria in Special Situations

Transfusion Malaria

Malaria can be transmitted by blood transfusion, needle stick injury, or organ transplantation. The clinical features and management of these cases are same as for naturally acquired infections (mosquito-borne) but differs in many other ways:

- ❖ The infective form can be intraerythrocytic forms such as merozoites, trophozoites or schizonts but not gametocytes
- ❖ There is no pre-erythrocytic stage of development and no relapse
- ❖ The incubation period is often short
- ❖ Radical chemotherapy with primaquine is unnecessary as there is no relapse.

Malaria in Pregnancy

Malaria during pregnancy increases the risk of fetal distress and can result in premature labor low birth weight and still birth. In areas with high malaria transmission, pregnant women are particularly vulnerable to severe anemia, hypoglycemia and acute pulmonary edema.

Malaria in Children

Nearly one million children die of falciparum malaria each year in endemic countries.

Certain complications are relatively common among children like convulsions, coma, hypoglycemia, metabolic acidosis and severe anemia; whereas other complications like jaundice, acute renal failure, and acute pulmonary edema are unusual in children.

Immunity against Malaria

Both innate and acquired immunity contribute to the resistance against malaria.

Innate Immunity

This refers to the inherent mechanisms of host resistance against malaria parasite. This depends upon various factors.

- ❖ **Age of RBCs:** *P. falciparum* attacks RBCs of any age, *P. vivax* and *P. ovale* attack the young RBCs and reticulocytes; whereas *P. malariae* attacks older RBCs
- ❖ **Nature of hemoglobin:** Sick cell disease, hemoglobin C and E, fetal hemoglobin and thalassemia hemoglobin are resistant to falciparum malaria
- ❖ **Hereditary ovalocytosis:** In this condition, the rigid RBCs are resistant to falciparum malaria
- ❖ **G6PD deficiency:** RBCs with glucose-6-phosphate dehydrogenase deficiency are resistant to falciparum malaria
- ❖ **Duffy negative red blood cells:** Duffy blood group antigens present on RBC membrane act as receptors for *P. vivax*. So, people with Duffy negative RBCs (e.g. West Africans) are resistant to vivax malaria
- ❖ **Age:** Children are more prone to infection and complications. However, newborn are protected from falciparum malaria because of the high concentration of fetal hemoglobin in first few months of life
- ❖ **Nutritional status:** It has a paradoxical effect. Severe malaria is rare in children suffering from malnutrition.

Acquired Immunity

Both cellular and humoral immunity contribute to the resistance against malaria.

- ❖ **Humoral immunity:** Circulating antibodies against asexual forms give protection by inhibiting the RBC invasion and sequestration, whereas antibodies against sexual forms help in reducing the transmission of malaria
- ❖ **Cell-mediated immunity:** It also plays role in providing protection against malaria. Cytokines released from T cells stimulate the macrophages and also stimulate the B cells to produce antibodies
- ❖ **Premunition:** Immunity against *Plasmodium* lasts till the original infection remains active, which prevents further infection. This is called as premunition or infection immunity or concomitant or incomplete immunity.

Epidemiology of Malaria

Malaria is the most lethal parasitic disease of humans, transmitted in 108 countries containing 3 billion people.

Predisposing Factors

The transmission of malaria is directly proportional to:

- ❖ Density of the vector and number of human bites per day per mosquito
- ❖ Time of mosquito bite (more after the dusk)
- ❖ Mosquito longevity (as sporogony lasts for 7–30 days, thus, to transmit malaria, the mosquito must survive for >7 days)

- ❖ Optimum temperature (20–30°C), humidity (60%) and rainfall.

Situation in World (WHO Malaria Report, 2018)

In 2018, 228 million cases of malaria with about 4 lakh deaths occurred worldwide; Africa affected the worst (93%), followed by South-East Asia (3.4%).

- ❖ **Incidence rate:** It is about 57 cases per 1000 population at risk. The South-East Asia recorded the largest decline (70%) in incidence rate compared to 2010
- ❖ ***P. falciparum*** is the most common species worldwide; accounting for 99.7% of malaria cases in African region, 50% in South-East Asia
- ❖ Globally, 53% of the ***P. vivax*** burden is in South-East region, with 47% of the vivax cases being reported from India. *P. vivax* is the predominant species in America (75%).

Malaria Situation in India (NVBDCP Report)

According to National Vector Borne Disease Control Programme (NVBDCP), 3.3 lakh malaria cases were reported from India in 2019, with 73 deaths.

- ❖ Over the last decade, **Eastern Indian states** such as Odisha, Chhattisgarh and Jharkhand have accounted for maximum malaria cases; *P. falciparum* being the predominant species
- ❖ However, in 2018 and 2019, **Uttar Pradesh** accounted for highest malaria burden in India, where majority of cases were due to *P. vivax*. As a result, *P. vivax* became the most common species in India (>50%), followed by *P. falciparum* (46%)
- ❖ *P. malariae* infections are <1% and are reported time to time from various places such as Karnataka (Tumkur and Hassan districts, largest foci), Chhattisgarh (Bastar area), Odisha, West Bengal, Madhya Pradesh, Tamil Nadu, Kerala and Assam
- ❖ *P. ovale* is mainly confined to tropical Africa. Only few cases are reported from India such as Odisha (Koraput district, the 1st case of India), Chhattisgarh (Bastar area), Delhi, Assam, Gujarat and Kolkata.

Malaria Elimination in India

The malaria control in India has been operated through NVBDCP since 2006.

- ❖ WHO has initiated The Global Technical Strategy for Malaria (2016–2030), which aims at elimination of malaria by year 2030. Till date 38 countries have already achieved the elimination status from WHO
- ❖ In line with WHO, NVBDCP India has launched **National Framework for Malaria Elimination (NFME)** in 2016 with vision of malaria elimination by 2030. All states/UTs of India are stratified into four categories based on annual parasite incidence (API) (Table 35.3).

Table 35.3: Stratification of States/UTs in India based on annual parasite incidence (API).

Categories	Definition
Category 0 (Prevention of re-establishment phase)	States/UTs with zero indigenous cases of malaria
Category 1 (Elimination phase)	States/UTs including their districts with API of < 1/1,000 population at risk
Category 2 (Pre-elimination phase)	States/UTs with API < 1/1,000 population, but some of their districts are reporting API of ≥ 1/1,000 population at risk
Category 3 (Intensified control phase)	States/UTs with API of ≥ 1/1,000 population at risk

The objectives set for malaria elimination in India include:

- ❖ Eliminate malaria from all Category 1 and 2 transmission states and union territories (UTs) by 2022
- ❖ Interrupt indigenous transmission of malaria throughout the entire country, including states/UTs in Category 3 by 2027
- ❖ Prevent re-introduction of local transmission of malaria in areas where it has been eliminated and maintain national malaria free-status by 2030 and beyond.

Observations

- ❖ World Malaria Day: Every year, 25th April is being celebrated as “World Malaria Day”
- ❖ Antimalarial month is celebrated every June.

LABORATORY DIAGNOSIS

Malaria

Microscopic tests:

- ❑ **Peripheral blood smear**—gold standard
 - **Thick smear**—more sensitive
 - **Thin smear**—speciation can be done based on the following features:
 - ◆ *P. vivax*—amoeboid ring form and schizont
 - ◆ *P. falciparum*—ring forms (multiple ring form, accolé form, headphone shaped ring forms), banana shaped gametocytes
 - ◆ *P. malariae*—band forms
 - ◆ *P. ovale*—enlarged fimbriated oval RBC with ring forms
- ❑ **Fluorescence microscopy** (Kawamoto's technique)
- ❑ **Quantitative buffy coat examination**—parasitized RBCs appear as brilliant green dots.

Nonmicroscopic tests:

- ❑ **Antigen detection tests** (RDTs) or ICTs—detect pan malarial Ag (LDH, aldolase), falciparum specific Ag (HRP-II)
- ❑ **Culture**—RPMI 1640 medium
- ❑ **Molecular diagnosis**—PCR targeting 18S rDNA.

Laboratory Diagnosis

The diagnostic tests for malaria can be divided into microscopic and nonmicroscopic tests (see the laboratory diagnosis box).

Peripheral Blood Smear

Peripheral smear study still remains the simple and gold standard confirmatory test for detection of malarial parasites.

Specimen

Peripheral blood is the specimen of choice, collected from ear lobe or by finger prick in older children and adults and from the great toe in infants.

- ❖ Blood films should be prepared directly from the capillary blood. In case of anticoagulated blood, smears should be made within an hour of collection of blood
- ❖ **Time for taking blood:** Blood should be collected few hours after the height of the paroxysm of fever and before taking antimalarial drugs. Parasite density is maximum during this period
- ❖ **Frequency:** Smears should be examined at least twice daily until parasites are detected.

Types of Peripheral Blood Smear

It is of two types—(1) thin, and (2) thick smears. Both the smears are made at the same time from capillary blood either on the same or different slides (Fig. 35.2). At least two thick and two thin smears should be made.

- ❖ **For thick smear**, a big drop of blood is spread over 1–2 cm square area on a clean glass slide. The thickness of the film should be such that it allows newsprint to be read
- ❖ **For thin smear**, a small drop of blood is taken on a corner of a slide. It is spread by another spreader slide at an angle of 45° and then is lowered to an angle of 30° and is pushed gently to the left, till the blood is exhausted
- ❖ The **surface of a good thin film** is: (i) even and uniform, (ii) consists of a single layer of RBCs, (iii) forms a “feathery tail end” near the center of the slide, and (iv) margins of the film do not touch the sides of the slide
- ❖ **Stains:** They are stained with one of the Romanowsky's stains such as Leishman's, Giemsa and Field's, Wright's or JSB (Jaswant Singh and Bhattacharya) stain

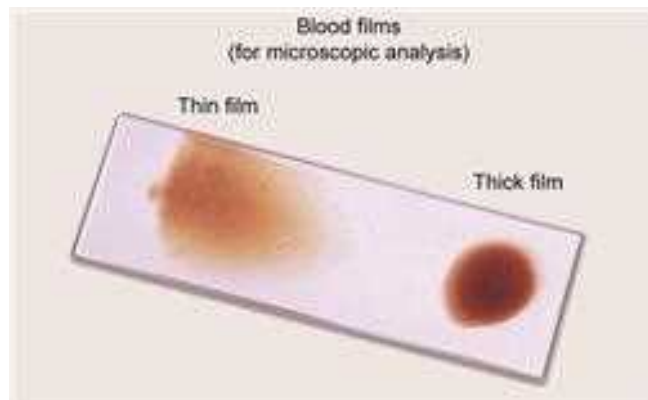


Fig. 35.2: Glass slide showing thin and thick blood smear.

- ❖ **Examination:** Both the smears are examined. The thin smear is screened near the feathery tail end. At least 200–300 oil immersion fields should be examined before the smears are considered as negative
- ❖ **Advantages:** Peripheral smear is simple, rapid and cheap
 - Thick smear is useful in—(1) **Detecting the parasites:** It is 40 times more sensitive than thin smear, can detect as low as 5–10 parasites per μL of blood; (2) Quantification of parasitemia; (3) Demonstrating the malaria pigments
 - Thin smear is useful in speciation of malaria parasites (see the highlight box)
- ❖ **Disadvantages:** (1) It is labor intensive and requires experienced microscopist; (2) Low sensitivity—the detection limit of thin smear is >200 parasites per μL of blood.

Speciation of Malaria Parasites

The speciation by thin smear is based on the detection of the ring forms, schizonts, gametocytes, type of pigments produced and RBC size (Fig. 35.3).

- **RBC size:** Parasitized RBC is normal in size and shape for *P. falciparum* and *P. malariae*; enlarged in size for *P. vivax*,

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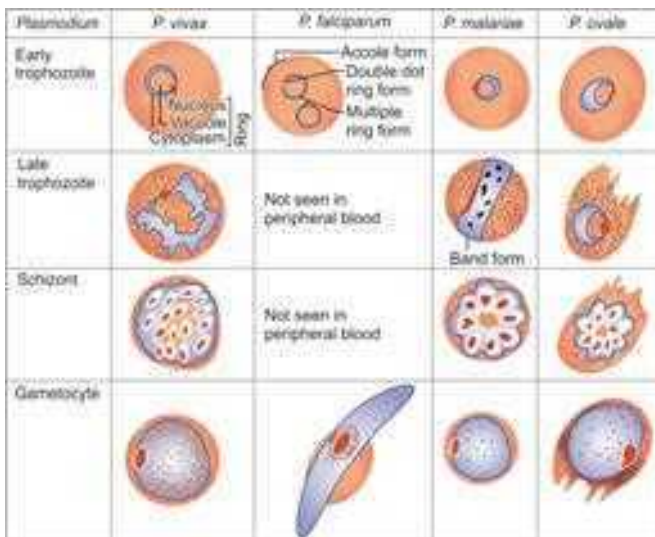


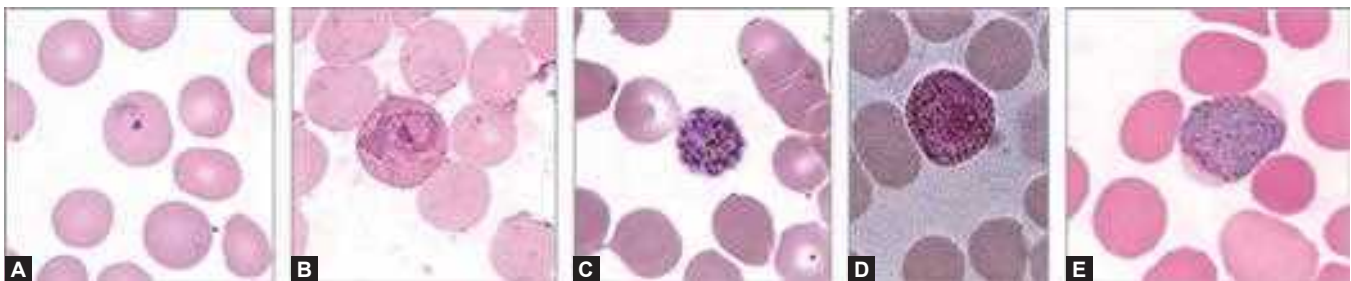
Fig. 35.3: Morphological forms of malaria parasites seen in the peripheral smear.

Contd...

whereas for *P. ovale* RBCs are enlarged with fimbriated margin.

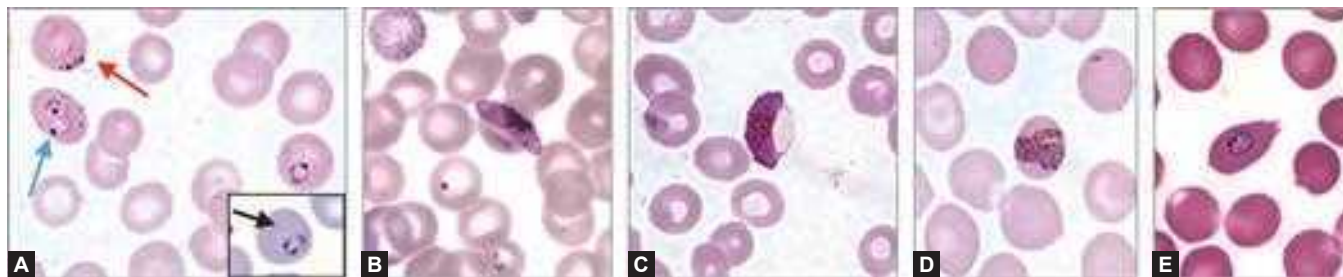
- **Pigments:** Malaria pigments in most species are dark-brown in color, except for *P. vivax*, where the pigments are yellowish-brown. Level of pigments correlates with severity of disease.
- **Schizont:** Speciation is made based on number of merozoites present per schizont.
 - *P. vivax*: Schizont is large (9–10 μm), completely fills the enlarged RBC and contains 12–24 merozoites /schizont (Fig. 35.4C).
 - *P. malariae* and *P. ovale*: Schizont is small (6 μm), almost completely fills or fills 3/4th of RBC and contains 8 merozoites /schizont.
 - *P. falciparum*: Schizonts are not seen in the peripheral smear
- **Gametocytes:** In *P. falciparum*, the gametocyte is crescentic or banana-shaped and larger than RBCs, whereas for other species, it is spherical and almost occupies the RBC (Figs 35.4D, E and 35.5B and C).
- **Ring forms:** It is the most important form that helps in accurate speciation. It comprises of a vacuole in the center, peripheral thin rim of blue cytoplasm, surrounding the red nucleus.
 - *P. vivax*: Rings are 2.5 μm size, occupies 1/3rd of the RBC size. Cytoplasm opposite to the nucleus is thick. Late trophozoites are amoeboid-shaped (Figs 35.4A and B).
 - *P. falciparum*: Rings are 1.5 μm size, smaller than in *P. vivax*, occupying 1/6th of RBC. Three variants of ring forms may be seen such as (Fig. 35.5A):
 - ◆ Multiple ring forms (inside the same RBC)
 - ◆ Accole (appliqué) form: Ring form attached to RBC membrane
 - ◆ Double dot/head phone shaped ring form: Ring form with fragmented nucleus
 - *P. malariae*: Early trophozoite is similar to that of *P. vivax*, but late trophozoite is band-shaped (called band forms) (Fig. 35.5D)
 - *P. ovale*: Ring forms are similar to that of *P. vivax*, but present inside oval-shaped RBC (Fig. 35.5E)

Note: In *falciparum* malaria, only the gametocytes and ring forms are demonstrated in peripheral blood but not schizonts and late trophozoites (as the later stages of erythrocytic cycle occurs in deep vessels, not in peripheral blood).



Figs 35.4A to E: Thin blood smear showing different forms of *Plasmodium vivax*: **A.** Ring form; **B.** Amoeboid form; **C.** Schizont; **D.** Male gametocyte; **E.** Female gametocyte.

Source: DPDx Image Library, Centre for Disease Control and Prevention (CDC), Atlanta (with permission).



Figs 35.5A to E: Thin blood smear showing: **A.** *P. falciparum* ring forms such as multiple rings (blue arrow), accolé form (red arrow) and head phone-shaped ring form (black arrow); **B.** Female gametocyte of *P. falciparum*; **C.** Male gametocyte of *P. falciparum*; **D.** Band form of *P. malariae*; **E.** Ring form of *P. ovale*.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Quantification of Parasites

Thick smear is preferred to thin smear for quantification of parasitemia.

- ❖ Previously, the “plus system” was used, which is simple but far less accurate for establishing parasite density in thick blood films. Now this is obsolete
- ❖ Currently, the quantification is done by calculating the number of parasites counted compared to number of white blood cells (WBCs) in the thick smear or number of RBCs counted in the thin smear

Quantification by thick smear =

$$\frac{\text{No. of parasites counted}}{\text{No. of WBC counted}} \times \text{Total WBC count}$$

- ❖ Quantification is helpful for:
 - Assessing the severity of infection
 - Monitoring the response to the treatment
 - Detecting drug resistance of *P. falciparum*.

Fluorescence Microscopy

Kawamoto technique is a fluorescent staining method for demonstrating malaria parasites. Blood smears are prepared on a slide and are stained with acridine-orange and examined under a fluorescence microscope. Nuclear DNA is stained green.

Quantitative Buffy Coat Examination

The quantitative buffy coat (QBC) is an advanced microscopic technique for malaria diagnosis. It consists of three basic steps:

1. Blood (60 μL) is collected in a capillary tube coated internally with acridine orange (Fig. 35.6A)
2. Capillary tube is centrifuged, which causes separation of components of blood according to their densities, forming discrete layers as RBCs, WBCs, lymphocytes and platelets (Fig. 35.6B)
3. Examination of capillary tube at the buffy coat region under ultraviolet (UV) light source (Figs 35.6C and D).

Interpretation

Acridine orange has a property of staining the nuclear DNA fluorescent brilliant green. Normal RBCs don't take up the stain (as they are a nucleated). However, parasitized RBCs appear as brilliant green dots. WBCs also take up the stain (Figs 35.6C and D).

Advantages

QBC is faster (the entire tube can be screened within minutes), more sensitive (at least as good as a thick film), and quantification is possible.



Figs 35.6A to D: **A.** QBC capillary tube; **B.** Magnified view of QBC capillary tube after centrifugation; **C.** Crescent shaped gametocyte of *Plasmodium falciparum*; **D.** Ring forms of *Plasmodium falciparum* seen as fluorescent dots.

Source: C and D. Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka (with permission).

Disadvantages

It is expensive, less specific and speciation is difficult.

Antigen Detection by Rapid Diagnostic Tests

Rapid diagnostic tests (RDTs) have revolutionized the diagnosis of malaria.

- ❖ **Antigens:** Several malarial antigens can be detected:
 - **Parasite lactate dehydrogenase (pLDH):** It is produced by all *Plasmodium* species. Currently available test kits can differentiate pan-LDH common to all species and Pf-LDH specific to *P. falciparum*
 - **Parasite aldolase:** Produced by all *Plasmodium* species
 - **Histidine rich protein-2 (HRP-II):** It is produced only by *P. falciparum*.
- ❖ **Principle:** Test kits currently available use a nitrocellulose membrane (NCM) strip with two parasite detection lines and a control line (Fig. 35.7A):
 - Test line-1: Coated with capture antibodies specific for *P. falciparum* (e.g. HRP-II or Pf-LDH)
 - Test line-2: Coated with capture antibodies common to all *Plasmodium* spp. (e.g. pan-LDH or aldolase).
- ❖ **Procedure:** Drop of blood specimen is added along with a buffer solution to sample window. Malarial antigens combine with polyclonal malarial antibody labeled with colloidal gold present in buffer and move

along with NCM to meet its corresponding antibodies at different test lines (Fig. 35.7)

- ❖ **Interpretation** is based on immobilization of malarial antigens at test lines 1 and/or 2 forming colored bands (Figs 35.7A and B)
 - If band is formed at only test line 1: indicates *P. falciparum* infection
 - If band is formed at only test line 2: indicates *Plasmodium* species other than *P. falciparum* infection
 - If bands are formed at both test lines 1 and 2: indicates *P. falciparum* or mixed infection.

Note: The band at control line must come to validate the test; if it does not come, test is considered invalid. Control line is coated with antibody against polyclonal malarial antibody present in buffer.

Advantages of Rapid Diagnostic Tests

Rapid diagnostic tests are simple to perform, do not need extra equipment or trained microscopist.

- ❖ **Sensitivity:** Rapid diagnostic tests are more than 90% sensitive at >100 parasites/μL. But the sensitivity is markedly reduced at <100 parasites/μL
- ❖ **Prognosis:** pLDH is produced by the viable parasites, hence it is used to monitor the response for treatment (microscopy is the best to assess prognosis)
- ❖ **Pregnancy:** HRP-II is a reliable marker to diagnose malaria in pregnancy
- ❖ **Severity:** Intensity of the band is directly proportional to the parasitemia and severity of the disease.

Disadvantages of Rapid Diagnostic Tests

RDTs have several disadvantages.

- ❖ It cannot differentiate between the non-falciparum malaria species
- ❖ Expensive than peripheral smear
- ❖ Gametocytes cannot be detected
- ❖ Low sensitivity: The lower limit to detect HRP-II is 40 parasites/μL and pLDH is 100 parasites/μL
- ❖ RDT has not been developed for *P. knowlesi* yet.

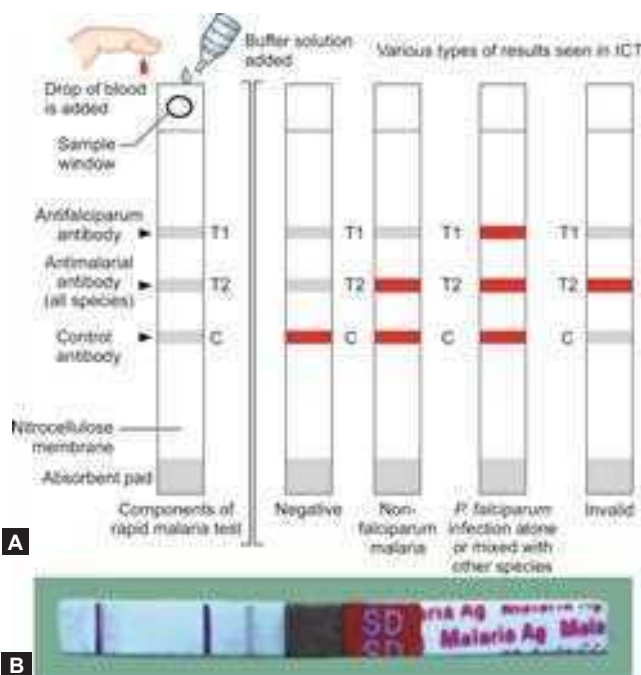
Comparison of peripheral smear, QBC and RDTs are described in Table 35.4.

Antibody Detection

Antibodies persist even after the clinical cure. Serology does not detect current infection, but only measures past exposure. Therefore, Government of India has banned the use of antibody detection tests for malaria diagnosis.

Culture

Culture techniques are mainly used for preparation of malaria antigens, not for diagnosis. **Trager and Jensen** method using RPMI 1640 medium (Roswell Park Memorial Institute) has been the most widely used technique for culture of malaria parasite.



Figs 35.7A and B: A. Schematic diagram of rapid diagnostic test kit showing negative, non-falciparum, pure or mixed infection with *Plasmodium falciparum* and invalid result of malaria; B. Real image of rapid diagnostic test kit.

Source: B. Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka (with permission).

Table 35.4: Comparison of peripheral smear, quantitative buffy coat and rapid diagnostic tests.

Features	Peripheral smear	Quantitative buffy coat	Rapid diagnostic tests
Method	Cumbersome	Easy	Easy
Time	Longer, 60–120 minutes	Faster, 15–30 minutes	Faster, 15–30 minutes
Sensitivity	Detection limit: • 5 parasites/μL in thick film • 200 parasites/μL in thin film	Claimed to be more sensitive, at least as good as a thick film	<ul style="list-style-type: none"> • >100 parasites/μL, sensitivity >90% • <100 parasites/μL, sensitivity falls
Specificity	Gold standard	False positives—artifacts may be reported as positive by nontrained technicians	False positive in RA factor (rheumatoid arthritis) positive cases
Speciation	Accurate, gold standard	Difficult	Detect <i>Plasmodium falciparum</i> but cannot differentiate non-falciparum species
Cost	Inexpensive	Costly equipment and consumables	Kits are costly but no extra equipment required. Good for field study
Experienced Microscopist	Required	Not required, minimal training is sufficient	Not required, minimal training is sufficient

Molecular Methods

Various molecular tests have recently gained attention for malaria diagnosis.

- ❖ Nested multiplex PCR targeting 18S rDNA has been developed for detection of all five human malaria parasites including *P. knowlesi*
- ❖ PCR can also be used to detect drug resistant genes
- ❖ Real time PCR is useful for quantification and speciation of parasites.

Automated Systems

Automated systems include Parasite F digital cytometry and automated microscopy methods have been recently developed for rapid detection of malaria parasites.

Other Nonspecific Tests

Other nonspecific tests include normocytic hemolytic anemia, leukopenia, metabolic acidosis, raised ESR, and hypoglycemia.

TREATMENT

Malaria

Various antimalarial drugs are depicted in Table 35.5. NVB-DCP, India has given guideline for the treatment of vivax malaria and falciparum malaria, as described in Tables 35.6 and 35.7.

Severe malaria is a medical emergency, develops quickly over 1–2 days and may lead to death. Its diagnostic criteria is given in Table 35.8. Hence, the treatment should be given promptly. The drug of choice include IV artemisinin derivatives or IV quinine (Table 35.9).

Antimalarial Drug Resistance

Antimalarial drug resistance has emerged as one of the greatest challenge facing malaria control today.

Drug Resistance in Falciparum Malaria

Drug resistance in *P. falciparum* is widespread and is described against several antimalarial drugs, however, there are geographical variations.

Table 35.5: Antimalarial drugs and their activity.

Class	Drugs	Active against parasitic stages
Quinolines and related compounds	Chloroquine	Asexual RBC stages
	Quinine	Gametocytes (except <i>Plasmodium falciparum</i>)
	Mefloquine	Asexual RBC stages
	Primaquine	Liver stages and hypnozoites, gametocytes
Artemisinin and its derivatives	Artemisinin, artemether and arte-ether	Asexual RBC stages and gametocytes
Hydroxynaphthoquinones	Atovaquone	Asexual RBC stages,
Biguanide derivative	Proguanil	Liver stages (only for <i>P. falciparum</i>)
Diaminopyrimidines	Pyrimethamine	Asexual RBC stages,
Sulfonamides	Sulfadiazine and sulfadoxine	Liver stages (+/-)
Tetracyclines	Tetracycline and doxycycline	Asexual RBC stages (+/-)

Abbreviations: (+/-) indicates doubtful activity; RBC, red blood cell.

Note: **Newer antimalarial drugs:** Arterolane, tetraoxanes, N-tert-Butyl isoquine, cycloguanil-pyrimethamine, azithromycin-chloroquine combination, spiroindolones, albitiazolium and AQ-13 (a next generation 4-aminoquinoline) are some of the newer antimalarial drugs in the pipeline.

Table 35.6: Treatment of Vivax Malaria (NVBDCP guideline, India).

Chloroquine	25 mg/kg, divided over three days, i.e. 10 mg/kg on day 1 and 2 and 5 mg/kg on day 3
Primaquine	0.25 mg/kg body weight; daily for 14 days; should be given under supervision, aiming to kill hypnozoites of <i>P. vivax</i> (to prevent relapse) Contraindicated in infants, pregnant women* and individuals with G6PD deficiency

* *P. vivax* infection in pregnancy, chloroquine is the treatment of choice.
Abbreviation: NVBDCP, National Vector Borne Disease Control Programme.

Table 35.7: Treatment of Falciparum Malaria (NVBDCP guideline, India).

North-Eastern States	ACT-AL , i.e. co-formulated tablet of <i>artemether and lumefantrine</i> ; total dose of 80 mg/480 mg (in adults), given over twice daily for 3 days <i>plus</i> Primaquine (0.75 mg/kg) single dose on second day aiming to kill gametocytes of <i>P. falciparum</i> . (ACT-AL is contraindicated in 1 st trimester pregnancy and children <5 kg weight)
Other states	ACT-SP , i.e. artesunate (4 mg/kg) for 3 days <i>plus</i> sulfadoxine (25 mg/kg)/ pyrimethamine (1.25 mg/kg), 1 tablet given on first day <i>plus</i> Primaquine (0.75 mg/kg) single dose on second day

In uncomplicated *P. falciparum* cases in pregnancy:

First trimester: Quinine salt 10 mg/kg 3 times daily for 7 days
Second/third trimester: Area-specific ACT; as given above

In mixed infection (*P. vivax* plus *P. falciparum*)

ACT-AL and ACT-SP as per geographical area as given above plus Primaquine (0.25 mg/kg) for 14 days

Abbreviations: ACT-AL, artemisinin combination therapy-artemether-lumefantrine; ACT-SP, artemisinin combination therapy-artesunate/sulfadoxine-pyrimethamine; NVBDCP, National Vector Borne Disease Control Programme

Northeastern states includes Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura.

World's Highest Burden

Border areas of Thailand, Cambodia, and Myanmar possess the highest risk for resistance to chloroquine and other classes of antimalarial drugs.

- ❖ Many of the strains of *P. falciparum* are multiple-drug resistant (MDR), defined as resistance to at least ≥3 classes of antimalarial drugs
- ❖ Resistance to artemisinin has not been reported from World; although slow clearance phenotypes have been documented.

India: Chloroquine resistance in *P. falciparum* has been reported since 1973 (first case from Assam). Northeast states show a higher proportion of chloroquine resistance in *P. falciparum*.

The factors that contribute to emergence of resistance are:

- ❖ Longer half-life of drug
- ❖ Mutation in the parasite gene (described below)
- ❖ Inadequate and irregular usage of drug

Table 35.8: Severe Malaria.**Characterized by ≥1 of the following features:**

- Impaired consciousness/coma
- Repeated generalized convulsions
- Renal failure (Serum creatinine >3 mg/dL)
- Jaundice (Serum bilirubin >3 mg/dL)
- Severe anemia (Hb <5 g/dL)
- Pulmonary edema/acute respiratory distress syndrome
- Hypoglycemia (Plasma glucose <40 mg/dL)
- Metabolic acidosis
- Circulatory collapse/shock (Systolic BP <80 mm Hg, <50 mm Hg in children)
- Abnormal bleeding and disseminated intravascular coagulation (DIC)
- Hemoglobinuria
- Hyperpyrexia (Temperature >106°F or >42°C)
- Hyperparasitemia (>5% parasitized RBCs)

Table 35.9: Treatment options available for severe malaria.

Drugs	Dosage
Artesunate	2.4 mg/kg IV, given on admission, then at 12 and 24 hours, then once a day
Quinine	Loading dose of 20 mg/kg at admission (IV infusion in 5% dextrose over a period of 4 hours) followed by maintenance dose of 10 mg/kg 8 hourly
Artemether	3.2 mg/kg IM, given on admission then 1.6 mg/kg body weight per day
Arteether	150 mg daily IM, for 3 days in adults only (not recommended for children)

Note:

- In pregnancy, quinine is preferred in first trimester.
- In severe *P. vivax* malaria, treatment is same as of *P. falciparum*, except primaquine is given for 14 days.
- For artemisinin derivatives, switch over to oral therapy when the patient becomes stable or after 24 hours of parenteral therapy whichever is later.

❖ **Host immunity.**

The development of resistance can be *delayed by combination of drugs*, i.e. combining one drug that rapidly reduces parasite biomass with a partner drug that can remove any residual parasites, e.g. artemisinin combination therapy (ACT).

Drug Resistance in Vivax Malaria

Only sporadic cases of resistance to chloroquine and/or primaquine have been reported from some areas of India, Burma, Indonesia and few others.

Mechanism of Drug Resistance

- ❖ **Chloroquine resistance in *P. falciparum*:** Occurs due to mutations in the genes encoding the transporter proteins such as PfCRT (*P. falciparum* chloroquine resistance transporter) and PfMDR1 (*P. falciparum* multidrug resistance gene 1). Such mutation results in impaired transport of chloroquine

- ❖ **Resistance to antifolates** such as pyrimethamine is due to point mutation in DHFR (dihydrofolate reductase) gene
- ❖ **Resistance to artemisinins** has not been reported yet however it is observed in experimental animals. Monotherapy with artemisinins is banned in India as it promotes resistance.

WHO Guideline for Assessing Degree of Resistance

Antimalarial drug resistance is detected by in vivo and in vitro methods.

In vivo method (2002): The degree of resistance is assessed based on two factors—persistence of clinical manifestations and level of parasitemia following administration of the antimalarial drug.

In vitro tests: Various in vitro methods are also available for antimalarial drug susceptibility testing such as:

- ❖ The WHO in vitro micro test using RPMI 1640 medium
- ❖ ELISA for measurement of HRP-II or pLDH
- ❖ PCR to detect the *P. falciparum* specific drug resistance genes.

Prophylaxis against Malaria

Prophylaxis against malaria includes chemoprophylaxis, vector control strategies and vaccine prophylaxis.

Chemoprophylaxis

Chemoprophylaxis is recommended for travelers, migrant laborers and military personnel exposed to malaria in highly endemic areas.

- ❖ **For short-term chemoprophylaxis (<6 weeks):** Doxycycline is recommended, at a dose of 100 mg daily. The drug should be started 2 days before travel and continued for 4 weeks after leaving the malaria endemic area
- ❖ **Long-term chemoprophylaxis (>6 weeks):** Mefloquine is recommended at a dose of 5 mg/kg weekly and administered two weeks before, during and four weeks after leaving the area.

Vector Control Strategies

Vector control is still one of the prime weapons to control malaria in endemic areas.

Anti-adult Measures

- ❖ **Residual spraying:** Spraying the houses with residual insecticides such as dichlorodiphenyltrichloroethane (DDT), malathion and fenitrothion is highly effective against adult mosquito
- ❖ **Space application** of pesticide in the form of fog or mist by ultra-low volume method of pesticide dispersion
- ❖ **Individual protection:** Done by reduction of human mosquito contact by using insecticide-treated bed nets, repellents and protective clothing.

Anti-larval Measures

- ❖ **Larvicide:** Use of mineral oil or Paris green has been extensively used to kill mosquito larvae and pupae
- ❖ **Source reduction** (to reduce the mosquito breeding sites): Includes environmental sanitation, water management and improvement of the drainage system
- ❖ **Biological larvicide:** *Gambusia affinis* (fish) and *Bacillus thuringiensis* (bacteria) can be used to kill the mosquito larva.

Vaccination for Malaria

Despite of intense research, till date, there is no vaccine licensed for human use. Currently, there are several vaccine trials going on globally in Africa, Asia and America. Approaches are made targeting the various stages of malaria cycle.

- ❖ **Pre-erythrocytic vaccine** targeting sporozoites and liver schizonts: Aims at preventing infection, disease and transmission
- ❖ **Erythrocytic vaccine** targeting merozoites, blood schizonts: These vaccines help in preventing the disease thus, are useful for people living in hyperendemic areas of malaria
- ❖ **Sexual stage vaccine** targeting gametocytes: They are *transmission-blocking*; do not prevent malaria in the individual taking the vaccine, but antibodies are passed to the mosquito during blood meal, block the further transmission of the parasite.

The main problems in malaria vaccine include:

- ❖ The vaccine candidates are poor inducers of cell-mediated immune response
- ❖ Antigenic variation in malarial antigens such as PfEMP
- ❖ Different immune mechanisms occur in different stages of malaria life cycle.

RTS, S/AS01

It is the only vaccine candidate that has successfully completed phase III trial. It is pre-erythrocytic vaccine containing Pf-CSP (circumsporozoite protein of *P. falciparum*) fused with hepatitis B surface antigen and a chemical adjuvant (AS01).

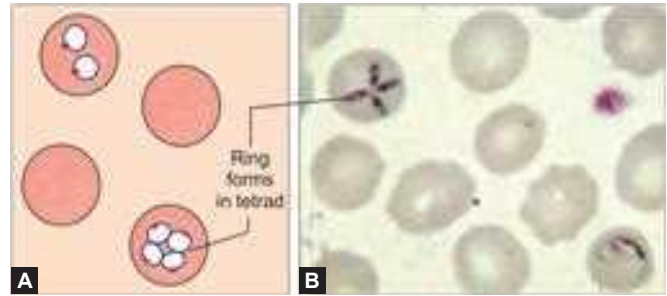
BABESIOSIS

Babesiosis is a malaria like illness seen in cattle and sheep. *B. microti* is the most common species, others being *B. bovis* and *B. divergens*. It is similar to malaria parasite in its life cycle and pathogenesis except for the following differences:

- ❖ **Hard tick** (*Ixodes scapularis*) is the primary vector (definitive host) of the parasite
- ❖ There is no liver-stage, sporozoites enter directly into RBCs
- ❖ **Clinical feature:** It differs from falciparum malaria being less severe, low parasitemia, no cerebral involvement,

less severe anemia and no periodicity seen in fever cycle. The disease is more severe in splenectomized and immunocompromised patients

- ❖ **Epidemiology:** Babesiosis is endemic in temperate area of USA and Europe; not reported from India yet
- ❖ **Diagnosis:** Peripheral blood smear examination reveals ring forms inside RBC arranged in pair or tetrads (called as *Maltese cross forms*) (Fig. 35.8). It is often confused with the multiple ring forms of *P. falciparum*, but can be differentiated by lack of pigments and lack of crescentic gametocytes
- ❖ **Treatment:** Atovaquone plus azithromycin is given for treatment.



Figs 35.8A and B: Giemsa stained blood smear showing Maltese cross form: **A.** Schematic diagram; **B.** Peripheral blood smear.

Source: B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

EXPECTED QUESTIONS

I. Write essay on:

1. A 54-year-old male from Chhattisgarh presented with fever, chills and rigor for a duration of four days. The patient developed convulsions prior to admission. He was started on ceftriaxone by a private medical practitioner, but did not improve. On physical examination, muscle tone and tendon reflexes were reduced. Anemia and splenomegaly were present. The blood sample was collected for peripheral blood smear examination which showed acrole form, multiple ring forms and crescent shaped gametocytes inside RBCs.
 - a. What is the etiological agent based on history?
 - b. Write briefly about the life cycle of the etiological agent.
 - c. Describe the pathogenesis, clinical manifestations and complications produced.
 - d. What are the various diagnostic modalities?
 - e. How will you treat this condition?
2. An 18-year-old female from Udupi, Karnataka, presented with high-grade fever which rises every third day with associated chills and rigor. Her blood sample was subjected to a rapid diagnostic test which revealed bands near pLDH line and control line, but no band near the HRP-II antigen line.
 - a. What is the probable etiological agent based on history?
 - b. Describe a note on epidemiology of this clinical condition.
 - c. What are the various diagnostic modalities?
 - d. How will you treat this condition?

II. Write short notes on:

1. Cerebral malaria.
2. *Plasmodium knowlesi*.

III. Multiple Choice Questions (MCQs):

1. Which is the infective form of the malaria parasite to man?

- a. Merozoite
- b. Sporozoite
- c. Trophozoite
- d. Gametocyte

2. Which is the infective form of the malaria parasite to mosquito?

- a. Merozoite
- b. Sporozoite
- c. Trophozoite
- d. Gametocyte

3. Which stage of the malaria parasite causes relapse?

- a. Sporozoite
- b. Trophozoite
- c. Merozoite
- d. Hypnozoites

4. Which is true about *Plasmodium falciparum*?

- a. High level of parasitemia
- b. It invades erythrocytes of all ages
- c. Its erythrocytic schizogony takes place in the capillaries of internal organs
- d. All of the above

5. Crescent-shaped or banana-shaped gametocytes are seen in infection with:

- a. *Plasmodium vivax*
- b. *Plasmodium falciparum*
- c. *Plasmodium ovale*
- d. *Plasmodium malariae*

6. Appearance of fever paroxysm every 72 hours (Quartan periodicity of malaria) is seen in infection with:

- a. *Plasmodium vivax*
- b. *Plasmodium falciparum*
- c. *Plasmodium malariae*
- d. *Plasmodium ovale*

7. Babesiosis is transmitted by bite of:

- a. *Anopheles*
- b. Sandfly
- c. Mite
- d. Tick

8. Maltese cross form is seen in:

- a. Babesiosis
- b. *Plasmodium ovale*
- c. *Plasmodium vivax*
- d. *Plasmodium malariae*

Answers

1. b 2. d 3. d 4. d 5. b 6. c 7. d 8. a

Visceral Leishmaniasis and Trypanosomiasis

CHAPTER 36

CHAPTER PREVIEW

■ Visceral Leishmaniasis

■ Chagas' Disease

■ African Sleeping Sickness

INTRODUCTION

Hemoflagellates are the flagellated protozoa that are found in peripheral blood circulation. Examples include *Leishmania* and *Trypanosoma*; both are transmitted by the bite of the insect vector.

- ❖ They have an oval to elongated body, nucleus, and a single flagellum arising from kinetoplast. **Kinetoplast** represents multiple copies of mitochondrial DNA. The intracellular portion (root) of the flagellum is called as the **axoneme**
- ❖ Based upon the arrangement of the flagellum, they exist in four morphological stages (Figs 36.1A to D)
 1. **Amastigote form:** Round to oval, lacks flagellum
 2. **Promastigote form:** Lanceolate shaped; kinetoplast is anterior to nucleus and flagellum arises from the anterior end
 3. **Epimastigote form:** Elongated, kinetoplast is placed close to the nucleus. Flagellum arises from the lateral side and traverses the body as a short undulating membrane and comes out from the anterior end
 4. **Trypomastigote form:** Elongated, kinetoplast lies near the posterior end. Flagellum arises posteriorly and runs as a long undulating membrane.

In *Leishmania* amastigote and promastigote forms are seen.

- ❖ Amastigotes are diagnostic form, found in man
- ❖ Promastigotes are infective stage to man, found in insect vector.

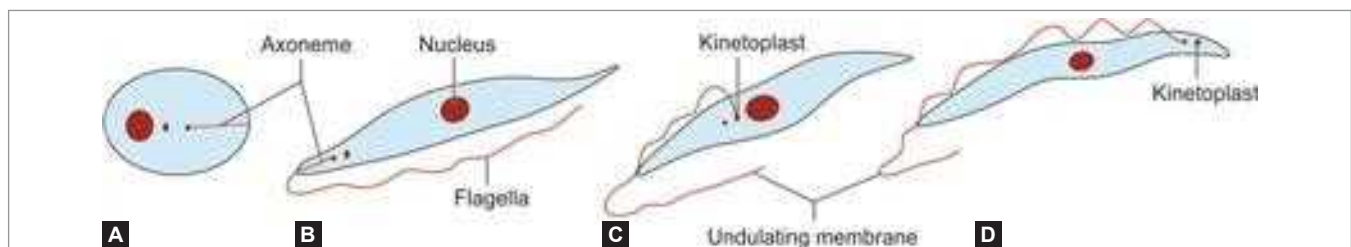
In *Trypanosoma*, epimastigote and trypomastigote forms are seen.

- ❖ In man, trypomastigotes are diagnostic form
- ❖ In insect vector, both epimastigotes and trypomastigotes are found, the latter being the infective stage to man.

LEISHMANIASIS

Leishmaniasis is caused by an obligatory intracellular protozoa of the genus *Leishmania*, which primarily affects the reticulo-endothelial system of the host.

- ❖ **Vector:** Leishmaniasis is transmitted by the bite of the female sandfly vector
- ❖ **Clinical forms:** Leishmaniasis presents in various clinical forms
 - **Visceral leishmaniasis (VL)**
 - **Post-kala-azar dermal leishmaniasis (PKDL):** It occurs few months to years following VL
 - **Cutaneous forms:** Occurs in various forms such as cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), leishmaniasis recidivans (LR) and mucocutaneous leishmaniasis (MCL) (Chapter 57).
- ❖ **Subgenera:** *Leishmania* has two subgenera, *L. Leishmania* (*L.L.*) and *L. Viannia* (*L.V.*). Both of the subgenera comprise of nearly 20 species
- ❖ **Old and New World:** Depending up on the geographical distribution, leishmaniasis is classified into two groups



Figs 36.1A to D: Various morphological forms of flagellates (schematic diagrams):
A. Amastigote; B. Promastigote; C. Epimastigote; D. Trypomastigote.

Old World Leishmaniasis

Seen in Asia, Africa and less frequently in Europe; transmitted by sandfly of genus *Phlebotomus*.

- *L.L. donovani*: Causes VL and PKDL
- *L.L. infantum*: Causes VL and PKDL
- *L.L. tropica* complex: Causes CL (Chapter 57)

New World Leishmaniasis (Chapter 57)

Seen in Central and South America; transmitted by sandfly of genus *Lutzomyia*.

- *L.L. chagasi*: Causes VL and CL
- *L.L. mexicana* complex: Causes CL
- *L. Viannia braziliensis* complex: Causes MCL and CL

- ❖ In Indian subcontinent, leishmaniasis is anthroponophilic affecting only humans; whereas in other parts of the world, it is a zoonotic disease, transmitted from various animals.

Visceral leishmaniasis (VL) and PKDL are discussed here. The various cutaneous and mucocutaneous forms are discussed in Chapter 57.

VISCERAL LEISHMANIASIS

Visceral leishmaniasis (VL) is mainly caused by the old world species *L. donovani* and *L. infantum*, together known as *L. donovani* complex and also by new world species, *L. chagasi*.

L. donovani is the most common species causing VL. It is found in India and Africa. It was named after Sir William Leishman and Sir Donovan, who simultaneously contributed to the discovery of this parasite in a patient from Kolkata and Chennai respectively in the same year 1903.

The subsequent discussion in this chapter is confined to VL due to *L. donovani*. The differences from VL due to *L. infantum* and *L. chagasi* is described in Table 36.1.

Epidemiology of VL

Leishmaniasis is endemic in 97 countries; most of them are developing countries of tropical and temperate regions.

- ❖ **World:** About 50-90 thousand new cases of VL occur worldwide each year, out of which >90% of cases are from three regions—(i) South-East Asia: India, Bangladesh, and Nepal; (ii) East Africa: Ethiopia, Sudan, and Kenya; and (iii) Brazil

- ❖ **India:** India is one of the worst affected country. In 2019, about 3,139 cases of VL and 821 cases of PKDL have been reported from India with nil death. Maximum cases were reported from Bihar (76% of VL, and 52% of PKDL) and followed by Jharkhand.

Life Cycle (*Leishmania donovani*, Fig. 36.2)

L. donovani completes its life cycle in two hosts —(i) Vertebrate host (man, dog, rodents, etc.) (ii) Invertebrate host (female sandfly, of genus *Phlebotomus argentipes*).

- ❖ **Infective form:** Promastigote forms found in alimentary canal of female sandfly serve as the infective form
- ❖ **Mode of transmission:** By the bite of an infected female sandfly, discharging promastigotes (infective forms) into skin of man

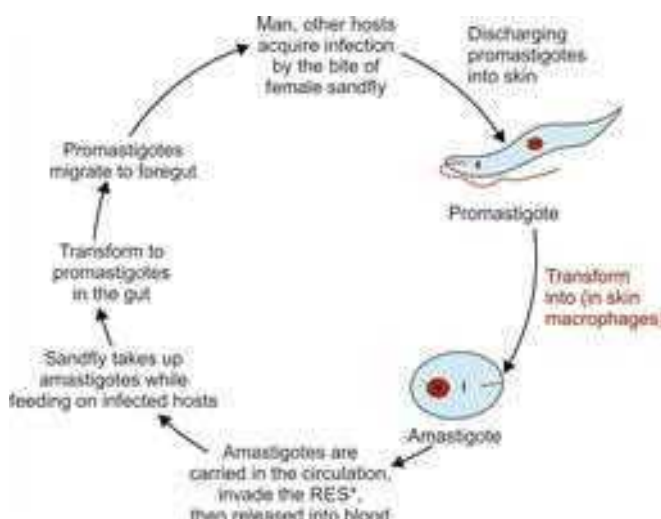


Fig. 36.2: Life cycle of *Leishmania donovani*.

*RES-Reticuloendothelial system.

Characters	Old World VL			New World VL
	Indian VL (kala-azar)	Infantile VL	African VL	Mediterranean VL
Agent	<i>Leishmania donovani</i>	<i>L. infantum</i>	<i>L. donovani</i>	<i>L. chagasi</i>
Vector	<i>Phlebotomus argentipes</i>	<i>P. perniciosus</i>	<i>P. orientalis, P. martini</i>	<i>Lutzomyia longipalpis</i>
Epidemiology	India	Middle East, Central Asia, China and Mediterranean basin	Sudan, Ethiopia, Kenya and Uganda	Central and South America
Age affected	Young adults	Infants and children <5 years of age	Adults	Children
Reservoir	Anthroponotic (human)	Zoonotic (canine)	Anthroponotic, Rarely-Zoonotic (rodents)	Zoonotic (canine)
PKDL	Common	Less common	Common	Less common
Lymph node involvement	Less common	More common, Aggravated by poor nutrition	Less common	Less common

Abbreviations: VL, visceral leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis.

- ❖ **In humans:** Promastigotes are phagocytosed by the skin macrophages, where they transform into amastigote forms
 - The amastigote forms multiply inside the macrophages, causing cell rupture and are released into the circulation
 - Amastigotes are carried out in the circulation to various organs like liver, spleen and bone marrow and invade the reticuloendothelial cells like macrophages, endothelial cells, etc.
- ❖ **In sandfly:** During the blood meal, the amastigotes are ingested and transformed into promastigote forms in the insect gut, which multiply and then migrate to their foregut. The cycle continues when this sandfly bites a new host.

Pathogenicity

Various factors contribute to the pathogenesis of VL.

- ❖ The phagocytosis of the promastigotes is facilitated by binding of its surface antigens such as 63 kDa glycoprotein (gp-63) and lipophosphoglycan (LPG) to specific receptors on macrophages
- ❖ LPG is the principle virulence factor, exhibits variety of functions. It prevents phagosome maturation and protects the parasite against hydrolytic enzymes secreted from the phagolysosome
- ❖ GPI (glycosyl-phosphatidyl-inositol) is a major surface protein on amastigotes, helps in protecting from phagolysosomal attack inside the macrophage.

Host Immune Response

Depending on the host immune response (T_H1 or T_H2) the amastigotes are either killed or allowed to multiply inside the macrophages.

T helper-1 Response

If T_H1 cells are activated, leads to a cell-mediated immune response which contain the disease spread by secreting interferon γ (IFN- γ) to activate macrophages, which in turn kill the amastigotes.

- ❖ T_H1 response is observed in cutaneous leishmaniasis and patients after recovery/treated for VL
- ❖ These individuals exhibit a delayed-type hypersensitivity (DTH) to leishmanial antigens (positive leishmanin skin test).

T helper-2 Response

If T_H2 cells are activated, leads to a humoral immune response due to increased production of IL-10 and IL-4; which in turn causes polyclonal B cell activation leading to hypergammaglobulinemia.

- ❖ It is observed in patients developing active VL and in diffuse CL
- ❖ Patients do not show positive leishmanin skin test
- ❖ The parasites use the macrophage much like a Trojan horse. Amastigotes are released periodically by rupture of the macrophages

- ❖ They disseminate through the regional lymphatics and the vascular system to infect the reticuloendothelial cells of various organs
- ❖ This results in remarkable enlargement of the spleen, liver and bone marrow dysfunction.

Clinical Features (VL)

VL is also called as kala-azar (a hindi term meaning “black fever”). Incubation period ranges from 2–6 months. The hallmark of VL is a **pentad** of fever, progressive weight loss, hepatosplenomegaly, pancytopenia and hypergammaglobulinemia.

- ❖ **Fever:** The most common symptom of VL; abrupt in onset, moderate to high-grade and associated with chills and rigors. Typically, it is described as double or triple rise of fever in 24 hours
- ❖ **Splenomegaly:** It is the most consistent sign. The spleen may become hugely enlarged (soft nontender and friable) and palpable below the umbilicus (Fig. 36.3A)
- ❖ **Hepatomegaly** (non-tender, moderate degree) soon follows splenomegaly
- ❖ **Lymphadenopathy:** Common in most of the African endemic regions (rare in Indian subcontinent). Femoral and inguinal nodes are affected commonly
- ❖ **Hyperpigmentation** is observed on face, hands, feet, and abdomen; hence the name kala-azar or black fever. This is a characteristic feature of Indian VL
- ❖ **Pedal edema and ascites:** Occur due to hypoalbuminemia, may be seen in advanced illness
- ❖ **Mucosal lesions** in mouth and nasopharynx—seen in Sudan, rare in India
- ❖ **Hematological abnormalities:** Occurs due to bone marrow dysfunction
 - Pancytopenia: Anemia, leukopenia and thrombocytopenia
 - Hypergammaglobulinemia (due to polyclonal B cell activation).
- ❖ **Leishmanoma:** Nodular skin lesions seen in African cases only
- ❖ **Secondary infections:** Such as measles, pneumonia, tuberculosis, bacillary or amoebic dysentery and gastroenteritis are common
- ❖ **Other findings** include weight loss and hair changes (thinning, hypopigmentation, etc.)
- ❖ Death occurs due to superimposed infection, severe anemia and hemorrhages.

Post-kala-azar Dermal Leishmaniasis (PKDL)

PKDL is a nonulcerative lesion of skin occurs in 2–50% of patients of VL following treatment with antimonials. It is aggravated by exposure to sunlight.

- ❖ Mainly seen in India and East African countries
- ❖ It develops as hypopigmented macule near mouth which spreads to face, arms and trunk and finally becomes nodules resembling leprosy (Figs 36.3B to D)



Figs 36.3A to D: Clinical features of leishmaniasis: **A.** Splenomegaly seen in visceral leishmaniasis; **B.** Hypopigmented skin changes in early PKDL; **C and D.** Extensive facial nodular lesions in late PKDL.

Source: World Health Organization "Manual on visceral leishmaniasis control" Slide 1/Desjeux; Slide 4 and 5/ El Hassan; Slide 6/ Bryceson (with permission).

- ❖ Ocular lesions like conjunctivitis and uveitis are associated in some patients
- ❖ Sometimes, PKDL may directly occur in subclinical patients without a history of VL
- ❖ **Diagnosis:** By detection of amastigotes in the nodular lesions and by serological tests such as direct agglutination test (DAT) and antibodies to rK39 antigen; positive in most of the cases
- ❖ **PKDL in Indian subcontinent:** Occurs in 2–20% of VL cases, usually after 2–10 years and persists for long periods (20 years). It can affect any age group. Amphotericin B is the drug of choice, given for 4 months.

Leishmaniasis with HIV Co-infection

Co-infection of HIV with VL has been reported from many countries.

- ❖ Southern Europe (France, Italy, Spain and Portugal) accounts for majority of co-infections; where 50–75% of VL are HIV positive and 7–17% of HIV-infected people with fever have amastigotes
- ❖ It is also reported from other places like Africa (Ethiopia, Sudan), Brazil and India
- ❖ In India, it is reported from Bihar, sub-Himalayan region and other North Indian states
- ❖ Both HIV and *Leishmania* affect each other's pathogenesis
 - **Effect on HIV:** *Leishmania* appears to cause activation of latent HIV. It expresses high level of chemokine receptors (CCR5) on macrophages
 - **Effect on *Leishmania*:** HIV causes activation of T_H2 cells response leading to disease progression.
- ❖ **Clinical feature:** In HIV co-infected patients, apart from the classical features of VL, other forms such as CL, MCL, PKDL, and atypical presentation such as chronic diarrhea and pleural effusion may be observed

- ❖ **Diagnosis:** Antibody detection tests are usually negative. Amastigotes are demonstrated from unusual sites such as bronchoalveolar lavage fluid and buffy coat region of blood.

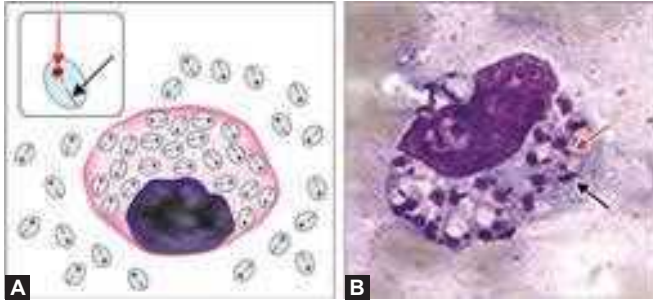
LABORATORY DIAGNOSIS

Visceral leishmaniasis

- ❑ **Microscopy**—Giemsa staining, detects LD bodies (i.e. macrophage filled with amastigote forms)
 - Splenic aspiration: Most sensitive
 - Bone marrow aspiration: Most common specimen
 - Lymph node aspiration
 - Liver biopsy
 - Peripheral blood smear (in HIV-infected people)
 - Biopsy of various organs (in HIV-infected people)
- ❑ **Culture** (detects promastigotes)—useful for species identification and drug sensitivity testing
 - NNN medium
 - Schneider's liquid medium
- ❑ **Antibody detection** in serum
 - ELISA, IFA and direct agglutination test
 - ICT using rk39 or rKE16 antigens
- ❑ **Antigen detection**—carbohydrate antigen in the urine (latex agglutination test)
- ❑ **Molecular method**—PCR, real-time PCR detecting specific kinetoplast (mitochondrial) DNA
- ❑ **Leishmanin test** (montenegro test)—indicates good CMI (DTH reaction); positive in all stages, except active VL and diffuse CL
- ❑ **Animal inoculation**—golden hamster
- ❑ **Nonspecific tests to detect:**
 - Hypergammaglobulinemia—by Napier's aldehyde test and Chopra's antimony test
 - Pancytopenia—by complete blood count

Laboratory Diagnosis

In an endemic area, any case presented with fever >2 weeks, splenomegaly and/or weight loss is suspected of having VL and should be subjected to laboratory confirmation.



Figs 36.4A and B: *L. donovani* amastigotes: showing a macrophage containing multiple *Leishmania* amastigotes: **A.** Schematic; **B.** In bone marrow smear stained with Giemsa. Note that each amastigote has a nucleus (red arrow) and a rod-shaped kinetoplast (black arrow).

Source: B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Microscopy

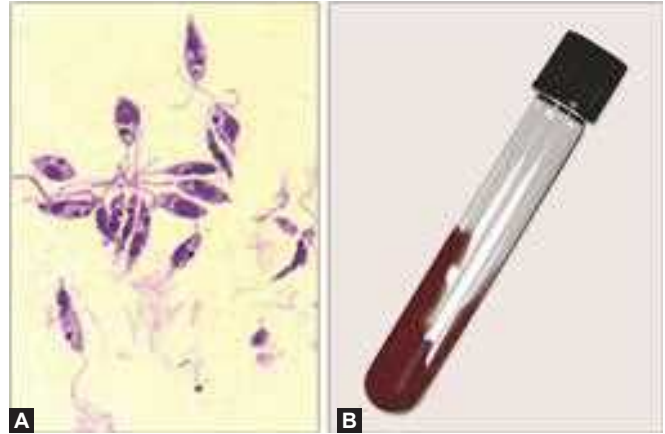
Demonstration of amastigotes inside the macrophages (also known as **Leishman-Donovan bodies** or **LD bodies**) is the gold standard method for the diagnosis of VL (Figs 36.4A and B). Smears should be stained with Leishman, Giemsa or Wright stains. The various samples include:

- ❖ **Splenic aspiration:** The sensitivity of splenic smear examination is excellent (>98%) but less preferred because of the risk of splenic puncture. Grading of LD bodies from splenic smear is useful in determining the parasitic load and monitoring the response to treatment
- ❖ **Bone marrow aspiration:** It is the most common specimen. The sensitivity is around 80–85%
- ❖ **Lymph node aspiration:** It is useful only in African cases of VL and has a low sensitivity (53–65%)
- ❖ **Liver biopsy:** Less sensitive and carries the risk of hemorrhage
- ❖ **Peripheral blood:** Smears made from buffy coat area (after blood is centrifuged) are particularly useful in HIV patients. Amastigotes are found within mononuclear cells and neutrophils
- ❖ **Biopsy specimens of various organs:** Like oropharynx, stomach, or intestine; useful in patients with AIDS.

Culture

Useful specimens are aspirations from spleen, bone marrow and buffy coat.

- ❖ **NNN medium:** Novy-MacNeal-Nicolle (NNN) medium is the culture medium of choice (Fig. 36.5B)
- ❖ **Schneider's *Drosophila* insect medium** can also be used alternatively
- ❖ Inoculated specimens are incubated at ambient temperature (24–26°C) and examined weekly for 4 weeks before declared as negative
- ❖ Amastigotes transform into **promastigotes** in the culture fluid which are detected by staining with Giemsa stain (Fig. 36.5A)



Figs 36.5A and B: **A.** Smear made from culture fluid shows promastigote forms (Giemsa stain); **B.** NNN medium.

Source: A. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission); B. World Health Organization, "Manual on visceral leishmaniasis control" (Slide22/Alvar) (with permission).

- ❖ Culture is found to be positive in 75% of cases
- ❖ Culture is useful for species identification and drug sensitivity testing.

Antibody Detection in Serum

In general, the serological tests are sensitive, but less specific. False-positive results may occur due to cross-reacting antibodies in patients with leprosy, Chagas' disease, CL, and other infections. Antibodies cannot differentiate current and past infection. More so, antibodies may be absent or present in low titer in patients with AIDS

- ❖ **Direct agglutination test (DAT):** Serial dilution of patient serum is added with stained extract of *L. donovani* amastigote on microtiter plate and incubated for 18 hours
 - If specific antibodies are present, agglutination (matt formation) is visible by naked eyes. Button formation indicates absence of antibodies (Fig. 36.6)

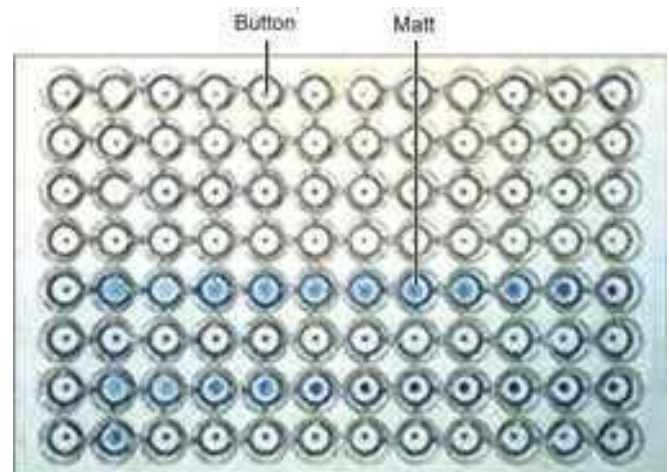


Fig. 36.6: Direct agglutination test.

Source: "Manual on visceral leishmaniasis control", World Health Organization (Slide32/Alvar) (with permission).

- It is found to be 100% sensitive and specific. It is simple, rapid and does not need any instrument
- Disadvantage: However, antibodies persist up to 5 years after the treatment.
- ❖ **Immunochromatographic test (ICT):** ICT detects leishmanial antibody by using *L. chagasi* recombinant kinesin antigen (rK39)
 - It claims 100% sensitivity and 98% specificity, however, the sensitivity is low in East Africa and in HIV patients
 - Like DAT, it is also simple, rapid and does not need any instrument (useful in field studies)
 - Recently, ICT based on another novel antigen rKE16 (from *L. donovani*) has been developed.
- ❖ Other tests available are **ELISA** and indirect fluorescent antibody (**IFA**) test.

Antigen Detection

Recently, latex agglutination test has been available detecting a heat-stable, low-molecular-weight carbohydrate antigen in the urine of VL patients.

- ❖ It has good specificity but variable sensitivity (40–80%)
- ❖ Antigen detection is more useful (i) in HIV-VL co-infection, (ii) as a prognostic marker, (iii) indicating active infection.

Molecular Methods

Various formats such as PCR, nested PCR and quantitative detection by real-time PCR are available targeting *Leishmania* specific kinetoplast (mitochondrial) DNA. It is mostly confined to the reference laboratories with sensitivity varying from 70% to 93%.

Leishmanin Test (Montenegro Test)

It is a skin test to detect delayed hypersensitivity to a suspension of killed *L. donovani* promastigote injected intradermally.

- ❖ Positive test (induration of >5 mm in 72 hours) indicates prior exposure to *Leishmania* antigens. Therefore, it is useful for epidemiological survey to estimate the burden of the disease
- ❖ It is positive in people with good CMI: Asymptomatic individuals, cutaneous leishmaniasis, after recovery from VL and leishmaniasis recidivans
- ❖ However, this test is negative when CMI is low: Such as in case of active VL and diffuse CL.

Animal Inoculation

Intranasal inoculation of specimens to golden hamsters yields amastigotes after several months. It is not in use nowadays.

Nonspecific Tests

- ❖ Complete blood count—to detect pancytopenia
- ❖ Elevated liver enzymes

- ❖ Reversal of albumin globulin ratio (reflects hypergammaglobulinemia)
- ❖ Other Non-specific tests such as Napier's Aldehyde test and Chopra's antimony test were in use in the past to detect hypergammaglobulinemia; now not in use.

TREATMENT

Visceral leishmaniasis

The various drugs used in the treatment of VL are: (i) pentavalent antimonials, (ii) liposomal amphotericin B, (iii) miltefosine, (iv) paromomycin. The WHO recommended regimens used for treatment of VL is given in Table 36.2.

Pentavalent antimonials

It has been the drug of choice and widely used in the past for several decades. However as the resistance has been emerged, currently, its use is restricted to regions where resistance has not been developed.

- ❑ **Dosage:** It is given as 20 mg/kg per day IM or IV for 30 days
- ❑ **Mechanism of action:** It is converted to its active trivalent form in body, induces oxidative stress in the parasite
- ❑ **Resistance:** Resistance to antimonials has been reported mostly from Bihar, India.

Liposomal amphotericin B

It has been the current drug of choice of leishmaniasis, especially in areas where resistance to antimonials have been reported.

- ❑ **Dosage:** It is given as 3–5 mg/kg per daily dose by IV infusion for 3–5 days up to a total dose of 15 mg/kg
- ❑ **Mechanism of action:** It acts by disrupting the cell membrane by forming pores.

Miltefosine

- ❑ **Dosage:** It is given as 150 mg/day; orally for 28 days
- ❑ **Mechanism of action:** by interacting with lipids, inhibiting cytochrome C oxidase
- ❑ **Resistance:** Resistance to miltefosine is rare, mainly reported from India and Nepal.

Paromomycin

It is an aminoglycoside antibiotic with anti-leishmanial activity. It is given IM at a dose of 15 mg per kg per day for 21 days.

Immunotherapy

Interferon- γ has been used in antimonial resistant and in selected relapse cases from Kenya, Brazil, and India.

Prevention of VL

National Vector-borne Disease Control Programme

National Vector-borne Disease Control Programme (NVBDCP) is a national program in India which works for the control of six common vector-borne diseases in India. It has launched the **accelerated plan for kala-azar elimination** in 2017.

- ❖ The target for elimination is to reduce the annual incidence of kala-azar to less than one per 10,000 populations at block PHC level
- ❖ The blocks in endemic area of India are classified into four categories based on annual incidence of kala-azar; each category has a specific action plan aiming towards kala-azar elimination, as described in Table 36.3.

Table 36.2: WHO recommendation for treatment of VL in different regions of the world.

Region	Drug regimen(s)
Indian subcontinent (ranked by preference)	<ul style="list-style-type: none"> • Liposomal amphotericin B • Combinations <ul style="list-style-type: none"> ➢ Liposomal amphotericin B plus miltefosine ➢ Liposomal amphotericin B plus paromomycin ➢ Miltefosine plus paromomycin • Amphotericin B deoxycholate • Miltefosine • Paromomycin • Pentavalent antimonials
East Africa and Yemen	Pentavalent antimonial plus paromomycin
Mediterranean Basin, Middle East, Central Asia and in America (due to <i>L. infantum</i>)	Liposomal amphotericin B

Abbreviation: WHO, World Health Organization.

Table 36.3: Categorization of blocks in endemic area* of India based on annual incidence of kala-azar, 2014.

Categories	Definition	Measures to be taken
Category I (94 blocks)	Blocks above elimination threshold (high transmission areas) Annual incidence >1 case per, 10,000 population	Intensive case detection Vector control activities Case based surveillance
Category II (23 blocks)	Blocks with borderline endemicity with Annual incidence 0.8 to <1 per 10,000 population (low transmission area)	Requires enhanced surveillance
Category III (290 blocks)	Fluctuation blocks with increasing or decreasing trend either year to year or with a gap of few years	Operation factors to be looked in
Category IV (207 blocks)	Silent blocks, i.e. areas reporting nil cases annually for at least two consecutive years	Robust surveillance for validation of nil status Preparedness for detection if cases occur

*Endemic region include Bihar, Jharkhand, Uttar Pradesh and West Bengal.

Vaccine Trials

Currently, no vaccine is available for the prevention of leishmaniasis. However, several trials are on-going.

Control Measures

Vector control measures should be followed such as:

- ❖ Personal prophylaxis by using insect repellents or bed nets (as discussed in Chapter 35)
- ❖ Control of canine or rodent reservoir
- ❖ *Phlebotomus* does not fly high above the ground level and it is nocturnal in habitat. So, sleeping at top floors also can prevent transmission
- ❖ Early treatment of all cases (mainly anthroponotic VL and PKDL cases).

TRYPANOSOMIASIS

Trypanosomes are hemoflagellates that reside in peripheral blood and tissues of their host. Based on their geographical distribution, they can be classified into two types:

- ❖ **African trypanosomes:** *Trypanosoma brucei* complex. They are transmitted by the vector tsetse fly. They cause African sleeping sickness
- ❖ **American trypanosomes:** *Trypanosoma cruzi*; which is the causative agent of Chagas' disease and is transmitted by the insect vector reduviid bug (also called triatomine bug).

CHAGAS' DISEASE (TRYPANOSOMA CRUZI)

Trypanosoma cruzi is the causative agent of South American trypanosomiasis or Chagas' disease, named after its discoverer Carlos Chagas, in 1909.

Epidemiology

Chagas' disease is mainly restricted to South and Central American countries like Brazil, Argentina, Venezuela, etc.

- ❖ Currently, it is estimated that 16–18 million people are infected with *T. cruzi*. Annual incidence is around 2 Lakh new cases with 50,000 deaths
- ❖ It is a zoonotic disease, have many animal reservoirs like dogs, and cats.

Life Cycle

T. cruzi is transmitted to man by rubbing of its vector reduviid bug's feces on abraded skin.

- ❖ **Human cycle:** The trypomastigotes from the bite wound are carried to various tissues where they transform into amastigote forms
 - **Amastigotes** multiply actively. They are the intracellular form found in tissues such as reticuloendothelial cells (spleen, liver, lymph node, bone marrow), muscles (cardiac, skeletal and GIT) and nervous tissue
 - **Trypomastigotes:** Amastigotes transform back to trypomastigote forms, which are motile C-shaped non-multiplying, extracellular forms found in peripheral blood (as long slender or invasive forms and short stubby forms). Invasive forms migrate to many organs and short stubby forms are ingested by reduviid bugs during a blood meal.
- ❖ **Vector cycle:** In the gut of reduviid bugs, the trypomastigotes transform into epimastigotes, which further develop into metacyclic trypomastigotes in the hindgut, and are excreted in the bug's feces.

Pathogenesis and Clinical Feature

Chagas' disease occurs in four stages.

1. **Early Stage Disease:** It is characterized by cutaneous and ocular manifestations
 - **Chagoma:** It forms a painful subcutaneous nodule at the site of deposition of bug's feces, commonly seen on face (Chapter 57)

- **Romana's sign:** It causes unilateral painless edema of the eye lid and conjunctivitis (Chapter 78).
- 2. **Acute Chagas' disease:** About 1% of patients, progress to acute stage disease, characterized by fever, hepatosplenomegaly and lymphadenopathy
- 3. **Indeterminate stage:** It is the asymptomatic phase; lasting for years to decades before progressing into symptomatic chronic stage
- 4. **Chronic Chagas' disease:** The parasite multiplies in the muscles (cardiac and GIT) and nervous tissue; producing various types of manifestations
 - **Cardiac form:** Occurs in 30% of the patients. Patient develops dilated cardiomyopathy, rhythm disturbances and thromboembolism
 - **Gastrointestinal form:** For example, megaesophagus and megacolon
 - **Autoimmune hypothesis:** *T. cruzi* antigens cross react with mammalian antigens (molecular mimicry) producing autoantibodies which may be responsible for tissue injury seen in chronic Chagas' disease
 - **Immune response:** CMI, to be particular antibody-dependent cell cytotoxicity (ADCC) is mainly involved in tissue destruction in the chronic stage such as cardiomyopathy and megacolon
 - **HIV and HTLV-II:** HIV and human T-lymphotropic virus (HTLV)-II infected people are at a greater risk of reactivation of underlying *T. cruzi* infection.

Congenital Trypanosomiasis

Rarely, *T. cruzi* can be transmitted transplacentally both in acute and chronic stages of the disease. It manifests as low birth weight, stillbirth, rarely myocarditis and neurological alterations (Chapter 79).

Laboratory Diagnosis

Peripheral Blood Microscopy

In acute Chagas' disease, the trypomastigotes are frequently found in peripheral blood which can be detected by: (1) wet mount preparation or (2) thick and thin smear examination.

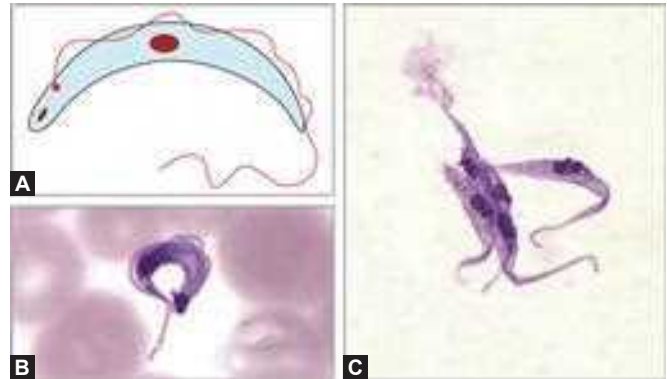
- ❖ Blood concentration techniques like microhematocrit method may be employed if the parasite count is low
- ❖ Thick smear is more sensitive in detecting the trypomastigotes, whereas the thin smear helps in detailed study of its morphology (described in highlight box).

Trypomastigote forms of *T. cruzi*

Trypomastigote forms of *T. cruzi* measure 20 µm in size, 'C' shaped with a large, terminal kinetoplast, with short blunt posterior end (Figs 36.7A and B). On wet mount examination, the motility can be demonstrated.

Other Diagnostic Methods

- ❖ **Culture:** Blood is inoculated onto NNN medium or Yager's liver infusion tryptose medium, incubated at 25°C



Figs 36.7A to C: *Trypanosoma cruzi*: **A.** Trypomastigote form (schematic); **B.** Trypomastigote form (in thin blood smear stained with Giemsa); **C.** Epimastigotes from NNN medium culture.

Source: B and C. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

and observed for the presence of epimastigote forms (Fig. 36.7C)

- ❖ **Antibody detection:** Chronic Chagas' disease is diagnosed by the detection of specific IgG antibodies by methods such as ELISA, Western blot and Chagas' RIPA (radioimmunoprecipitation assay)
- ❖ **Antigen detection:** *T. cruzi* specific antigens from serum and urine of the infected patients are detected by ELISA which are very useful for diagnosing acute and congenital infection
- ❖ **Molecular methods:** PCR is available that detects *T. cruzi* specific kinetoplast or nuclear DNA in blood. It is more sensitive than microscopy and serology for the diagnosis of chronic disease. It is also useful in monitoring the response to treatment and for the diagnosis of congenital infection
- ❖ **Animal inoculation:** By intraperitoneal inoculation of blood or CSF into mice
- ❖ **Xenodiagnosis:** The infected patients are exposed to laboratory maintained uninfected reduviid bugs and then after 1-3 months, the feces of the insects are examined by microscopy for the presence of the epimastigote forms.

TREATMENT

Chagas' disease

Therapy for Chagas' disease is still unsatisfactory.

In acute disease: Benznidazole is considered as the drug of choice in Latin America. Nifurtimox or allopurinol is given alternatively.

In chronic disease: These drugs lack efficacy and may cause many side effects. Supportive treatment such as pacemakers to manage arrhythmias and surgery for correction of megaesophagus and megacolon may be useful.

Prophylaxis

Prevention of the disease in endemic countries depends on the control of vector. This includes residual insecticides,

health education, measures to reduce insect exposure and housing improvement.

AFRICAN SLEEPING SICKNESS

Trypanosoma brucei was named after Sir Bruce in 1909, proved that the disease is transmitted by tsetse fly. *T. brucei* complex consists of three subspecies, out of which only two cause human disease:

1. *T. brucei gambiense*: Agent of West African sleeping sickness
2. *T. brucei rhodesiense*: Agent of East African sleeping sickness
3. *T. brucei brucei*: Causes “nagana”, a disease affecting cattle in Africa. It does not infect humans.

Epidemiology

African trypanosomiasis is endemic in 36 countries of Africa with 60 million people at risk. Approximately 50,000 new cases occur annually.

Life Cycle

T. brucei is transmitted to man by the bite of the vector **tsetse fly** (genus *Glossina*), inoculating the infective form—metacyclic trypomastigote forms into the skin.

- ❖ **Human cycle:** At the site of inoculation, they transform into long slender trypomastigote forms, which multiply by binary fission. Subsequently they transform into non-dividing short stumpy trypomastigote forms which either:
 - Invade the bloodstream and migrate to various organs or
 - Ingested by the tsetse fly during a blood meal.
- ❖ **Vector cycle:** The short stumpy trypomastigote forms become long slender forms, then to epimastigote forms and finally develop into metacyclic trypomastigote forms which are the infective forms to man.

Antigenic Variation

Trypomastigotes undergo periodic antigenic variation leading to frequent change of antigenic nature of variable surface glycoprotein (VSG) antigens present on their surface. This serves as the key mechanism of evading host immune (humoral) response.

Pathogenesis and Clinical Feature

In general, *T. brucei gambiense* develops a chronic course with slow progression; whereas *T. brucei rhodesiense* runs an acute course with rapid progression and early death.

Stage I Disease (Hemolymphatic Stage)

- ❖ **Trypanosomal chancre:** It is a self-limited lesion that appear a week after the bite of an infected tsetse fly
- ❖ **Asymptomatic period:** Following this, the clinical course runs through an asymptomatic phase which lasts for few months
- ❖ **Systemic febrile illness:** Months to years later, a systemic febrile illness develops; due to dissemination of the

parasite through the lymphatics and bloodstream. It is characterized by:

- Remittent irregular fever with night sweats
- Lymphadenopathy: The posterior cervical nodes are commonly involved, called as **Winterbottom’s sign**. It is a consistent feature of West African trypanosomiasis.

Stage II Disease (CNS Invasion)

It involves invasion of the CNS which leads to progressive chronic meningoencephalitis. Patients develop characteristic daytime somnolence (hence called as “sleeping sickness”), with restlessness and insomnia at night. Detail is discussed in Chapter 75.

T. brucei gambiense differs from *T. brucei rhodesiense* in several ways (Table 36.4).

Laboratory Diagnosis

Direct Microscopy

Useful samples are multiple blood samples collected during the febrile period, chancre fluid, CSF, lymph node and bone marrow aspirate.

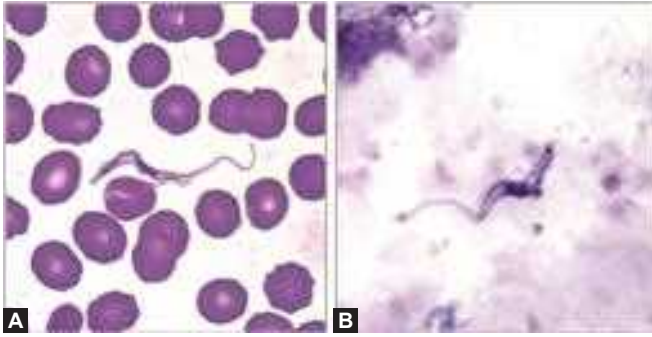
- ❖ **Blood examination:** Trypomastigote forms are seen by serial blood sample examination, which is performed either by (i) wet mount examination, (ii) peripheral blood smear examination by thin and thick smear (Figs 36.8A and B), (iii) concentration methods such as microhematocrit centrifugation, if parasitemia is low

Trypomastigotes of *T. brucei*

Trypomastigotes are elongated 14–33 µm long, having a flagellum with undulating membrane. Cytoplasm stains pale blue containing dark blue granules with nucleus stained red.

Table 36.4: Comparison between *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.

	<i>Trypanosoma brucei gambiense</i>	<i>Trypanosoma brucei rhodesiense</i>
Disease	West African sleeping sickness	East African sleeping sickness
Vectors	Tsetse flies (<i>Glossina palpalis</i> group)	Tsetse flies (<i>Glossina morsitans</i> group)
Primary reservoir	Humans	Animals (Antelope and cattle)
Human illness	Chronic central nervous system (CNS) disease	Acute (early CNS disease) up to 9 months
Duration of illness	Months to years	<9 months (before that the death occurs)
Lymphadenopathy	Frequent, cervical lymphadenopathy (winter bottom sign)	Minimal (Axially and inguinal)
Virulence	Less, as the parasitemia is low	More, as the parasitemia is high
Rodent inoculation	Not useful	Diagnostic
Drug of choice	Stage I: Pentamidine Stage II: Eflornithine	Stage I: Suramin Stage II: Melarsoprol



Figs 36.8A and B: Trypomastigote form of *T. brucei* in peripheral blood smear examination (Giemsa stain): **A.** Thin smear; **B.** Thick smear.

Source: A and B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **CSF examination:** This is necessary to confirm the CNS invasion; either by—(i) detection of trypomastigotes in CSF or by (ii) WBC count of >20 cells/ μL of CSF with parasite detected in blood or lymph node aspirate
- ❖ **Lymph node aspirate:** It is useful for *T. brucei gambiense* and shows variable sensitivity of 40–80%.

Antigens from Serum and CSF

Antigen detection by ELISA in serum or CSF is useful for clinical staging of disease to determine CNS infection and for monitoring the response to treatment (antigens are rapidly cleared following improvement).

Antibodies from Serum and CSF

- ❖ **Card agglutination test** for trypanosomes (CATT): It detects antibodies to VSG antigen
- ❖ **Semi-quantitative ELISA** using VSG antigen of *T. brucei gambiense* is available to detect various antibodies in serum and CSF.

Molecular Methods

- ❖ PCR assays have been developed which show a high sensitivity with a detection limit of 5 parasites/mL
- ❖ FISH (fluorescence in situ hybridization) using peptide nucleic acid probe appears to be an excellent diagnostic tool with detection limit same as PCR.

Culture

Samples can be inoculated into KIVI (kit for in vitro isolation) and trypomastigotes are recovered in 7–10 days. However, culture is not routinely performed.

Animal Inoculation in Mice

It is highly sensitive for the isolation of *T. brucei rhodesiense* but not useful for *T. brucei gambiense*.

Other non-specific Findings

Sustained high levels of IgM may be seen; due to antigenic switching in the parasite. Absence of elevated IgM levels in serum in an immunocompetent host rules out trypanosomiasis.

TREATMENT

African sleeping sickness

The drugs used for the treatment of African sleeping sickness are pentamidine and suramin. Alternative drugs are eflornithine, and the organic arsenical melarsoprol. Treatment is based on the type of disease (West or East African) and presence or absence of CNS invasion.

Prophylaxis

Vector control strategies like destruction of the insect's habitats, elimination of reservoir sources, etc. can be done to control African trypanosomiasis. Vaccine is not available as the parasite undergoes frequent antigenic variations.

EXPECTED QUESTIONS

I. Write essay on:

1. A 31-year-old man from Bihar presented with splenomegaly, anemia, and fever. The bone marrow aspirate collected was subjected to Giemsa staining which revealed amastigotes filled within a macrophage.
 - a. Identify the etiological agent and the clinical diagnosis.
 - b. Write briefly about the life cycle of the etiological agent.
 - c. Describe the pathogenesis and clinical manifestations produced.
 - d. What are the various diagnostic modalities?
 - e. How will you treat this condition?

II. Write short notes on:

1. Post-kala-azar dermal leishmaniasis.
2. African sleeping sickness.
3. Chagas' disease.

III. Multiple Choice Questions (MCQs):

1. Vector for leishmaniasis:

- a. Sandfly
- b. Reduviid bugs
- c. Tsetse fly
- d. *Anopheles* mosquito

2. Amastigote form of *Leishmania donovani* resides in the:

- a. Gastrointestinal tract of insect vector
- b. Salivary gland of mosquito
- c. Cells of reticuloendothelial system
- d. NNN culture media

3. *Leishmania donovani* can be cultivated in:

- a. Blood agar
- b. NNN medium
- c. Diamond's medium
- d. RPMI 1640 medium

4. American trypanosomiasis (Chagas' disease) is caused by:

- a. *T. rangeli*
- b. *T. brucei gambiense*
- c. *T. cruzi*
- d. *T. brucei rhodesiense*

Answers

1. a 2. c 3. b 4. c

CHAPTER PREVIEW

■ Filarial Nematode

■ *Wuchereria bancrofti*

■ *Brugia malayi*

FILARIAL NEMATODE

Filarial nematodes are vector-borne parasites that reside in the lymphatic system, skin, subcutaneous tissue and rarely in body cavities and accordingly they cause various types of clinical manifestations in man.

- ❖ **Lymphatic filariasis:** Filarial worms reside in the lymphatics; produce chronic obstruction and fibrosis of lymphatics. Agents include *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. They differ from each other in various properties (Table 37.1)
- ❖ **Cutaneous and ocular filariasis:** Include *Loa loa*, *Onchocerca volvulus* and *Mansonella* species, which reside in skin, subcutaneous tissues and some in eyes and serous cavity. They produce various cutaneous and ocular manifestations; discussed in Chapters 57 and 78 respectively.

Morphology and Habitat

Filarial nematodes are viviparous; exist in two morphological forms: adult worm and larvae. There is no egg stage.

- ❖ **Adult worm:** The adult worms are slender, round measuring 2–10 cm in length. Some adult filarial worms can survive for many years in humans, causing chronic obstructive and inflammatory conditions such as elephantiasis and hydrocele—a condition known as lymphatic filariasis
- ❖ **Larvae:** Like other nematodes, there are four larval stages. The first stage larva is called as **microfilaria**. The

third stage larva is called as **filariform larva**; which is the infective form to man

- ❖ **Microfilariae:** They are usually nonpathogenic, but sometimes hypersensitivity reactions can occur against the microfilarial antigen, resulting in tropical pulmonary eosinophilia (TPE).

Microfilarial Periodicity

It is defined as the time when most of the microfilariae are found in the peripheral blood

- ❑ Microfilariae usually reside in pulmonary blood vessels and occasionally come to the peripheral blood at different time (e.g. day time or night time), depending upon the species (Table 37.2)
- ❑ Periodicity occurs due to biological and evolutionary co-adaptation of the microfilariae to the feeding habit of the mosquito (*Culex* bites in the night, *Aedes* bites in daytime)
- ❑ However, other factors like the sleeping pattern of the individual, temperature and other climatic conditions also contribute.

LYMPHATIC FILARIAL NEMATODES

Lymphatic filariasis is caused by *Wuchereria bancrofti* (90%), *Brugia malayi* (10%) and *B. timori* (rare). It is characterized by chronic obstruction and fibrosis of the lymphatics leading to lymphedema, hydrocele and elephantiasis.

The existence of lymphatic filariasis has been recorded in ancient Indian, Chinese, and Egyptian

Table 37.1: Differences between various filarial nematodes causing lymphatic filariasis.

Parasite/ Disease	Location of adult	Location of microfilaria	Microfilaria periodicity	Vector	Epidemiology
<i>Wuchereria bancrofti</i> (bancroftian filariasis)	Lymphatic tissue	Blood, rarely hydrocele fluid and chylous urine	Nocturnal (mostly)	<i>Culex</i> —Worldwide <i>Anopheles</i> in rural Africa	Cosmopolitan, (South America, Africa, South Asia)
			Subperiodic (rare)	<i>Aedes</i>	Pacific islands Andaman and Nicobar
<i>Brugia malayi</i> (brugian filariasis)	Lymphatic tissue	Blood	Nocturnal (mostly)	<i>Mansonia</i> <i>Anopheles</i>	Southeast Asia, Indonesia and India
			Subperiodic (rare)	<i>Coquillettidia</i> and <i>Mansonia</i>	Southeast Asia
<i>Brugia timori</i>	Lymphatic tissue	Blood	Nocturnal	<i>Anopheles barbirostris</i>	Indonesia

Periodicity	Microfilaria in blood	Examples
Nocturnal	Peak at night (9 pm to 4 am)	<i>Wuchereria</i> , <i>Brugia</i>
Diurnal	Present throughout the day and night, peaks at mid-day (12 noon–2.00 pm)	<i>Loa loa</i>
Subperiodic	Present throughout; with (i) slight increase in the afternoon (3–5 pm, diurnal sub-periodic) or (ii) slight increase in the night (7–9 pm, nocturnal sub-periodic)	<i>Wuchereria</i> transmitted through <i>Aedes</i>
Nonperiodic	No periodicity is noticed	<i>Onchocerca</i> <i>Mansonella</i>

writings. Indian physician Sushruta was the first to describe elephantiasis in his famous book **Sushruta Samhita**, in 6th century BC. Madhavakara in 7th century AD, described the disease in his treatise ‘Madhava Nidhana’. In 1709, Clarke called elephantoid legs in Cochin as Malabar legs.

Epidemiology

About 893 million people in 49 countries worldwide are at risk of developing lymphatic filariasis. In 2000, over 120 million people were infected, with about 40 million disfigured and debilitated by the disease.

- ❖ **Southeast Asia** accounts for the highest burden; comprises of 50% of globally infected lymphatic filariasis (LF) cases; followed by Sub-Saharan Africa, Pacific Island, some areas of South America and the Caribbeans
- ❖ Globally, 90% of lymphatic filariasis are caused by *Wuchereria bancrofti* and the remainder by *Brugia* species and in India, the ratio is 99.4 and 0.6% respectively.

India

It is estimated that about 650 million people are at risk, residing in 256 districts of 21 states in India; accounting for 40% of global burden.

- ❖ Highly endemic states are Uttar Pradesh, Jharkhand, Bihar and West Bengal, which account for two-thirds of the lymphatic filariasis burden in India
- ❖ Prevalence is low in North-eastern states, Jammu and Kashmir and Punjab.

BANCROFTIAN FILARIASIS

W. bancrofti, is the most widely distributed filarial parasite of humans; accounts for >90% of cases of lymphatic filariasis. It is named after the scientists Wucherer and Bancroft who contributed to its discovery. In general, *W. bancrofti* is nocturnally periodic, except in Pacific Islands; where it is subperiodic.

Life Cycle (Fig. 37.1)

Host and vector: *W. bancrofti* completes its life cycle in two hosts—(i) man (definitive host), and (ii) intermediate host (mosquito).

- ❖ *Culex quinquefasciatus* is the principle vector worldwide
- ❖ Rarely, *Anopheles* (in rural Africa) or *Aedes* (in Pacific Island) can serve as a vector. Subperiodic *W. bancrofti* is transmitted by *Aedes* mosquito.

Infective form: Third stage (L₃) filariform larvae are the infective form, found in the proboscis of the mosquito.

Human Cycle

L₃ filariform larvae get deposited in skin by the mosquito bite.

- ❖ **Develop into adults:** Larvae penetrate the skin, enter into lymphatic vessels and migrate to the local lymph nodes where they molt twice to develop into adult worms
- ❖ **Adults lay L₁ larvae:** Adult worms reside in the lymphatics or lymph nodes where undergo fertilization to produce L₁ larvae (**microfilariae**). Male worms die after mating, whereas the female worms live up to 20 years. A gravid female worm can discharge 50,000 microfilariae/day. Microfilariae have a life span of up to 1 year
- ❖ **Prepatent period:** It is the time period between the infection (entry of L₃ larvae) and diagnosis (detection of microfilariae in blood). This is variable ranging from 82–142 days.

Mosquito Cycle

When the mosquito bites an infected man, the microfilariae are ingested. *Culex* bites at night, whereas *Aedes* bites in daytime.

- ❖ Microfilariae come out of the sheath, penetrate the stomach wall of the mosquito and migrate to the thoracic muscle, where they molt twice to develop into L₃ larvae
- ❖ The L₃ larvae migrate to the proboscis of the mosquito and serve as the infective stage to man

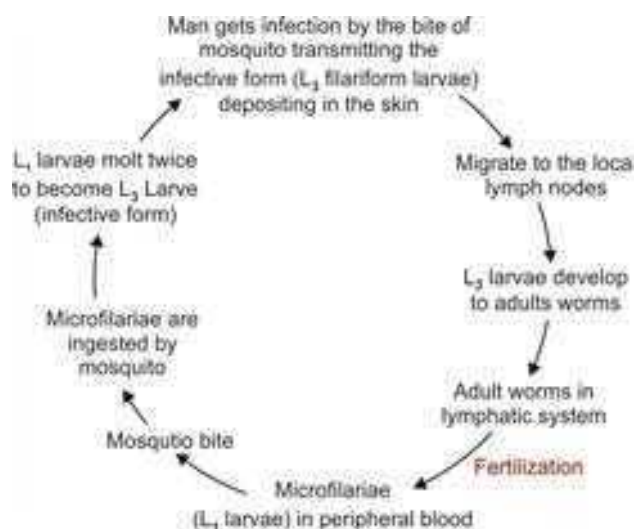


Fig. 37.1: Life cycle of *Wuchereria bancrofti*.

- ❖ **Extrinsic incubation period:** Under optimum conditions, the mosquito cycle takes around 7–21 days.

Pathogenesis and Pathology

The pathologic changes occur as a result of inflammatory damage to the lymphatics which in turn is due to summation of many effects such as:

- ❖ Tissue alterations related to migration of live adult worms such as lymphatic dilatation and thickening of the vessel walls
- ❖ Tissue alterations related to antigen and toxic metabolites released from dead adult worm
- ❖ Secondary bacterial and fungal infections
- ❖ Host's inflammatory response to both live and dead parasites
 - Infiltration of plasma cells, eosinophils, and macrophages in the infected vessels
 - This leads to tortuosity of the lymphatics and damage to lymph valves resulting in lymph edema of limbs.
- ❖ As long as the worm remains viable, the lymphatic vessels though damaged, still remains patent. However, the death of the worm leads to enhanced granulomatous reaction, and fibrosis of the lymph vessels
- ❖ This results in severe lymphatic obstruction. The lymphatic function is severely compromised
- ❖ **Endosymbiosis:** Pathogenic *W. bancrofti* is found to be infected with a *Rickettsia* group of bacteria called *Wolbachia* and maintain an endosymbiotic relationship. It is proved that this symbiosis is essential for the parasite survival, and pathogenesis
- ❖ **Age and gender:** Microfilaremia increases with age; starts at 5 years and peaks at >30 years. Males are more commonly affected than females. Hormonal factors in females may be attributed to their higher resistance to infection.

Host Immune Response

Both cellular and humoral immune responses are altered. Antigens of both adult worms and microfilariae are processed by the antigen-presenting cells (macrophages) and presented to T helper cells (T_H cells). T_H cells are stimulated and differentiated into T_{H1} cells and or T_{H2} cells.

- ❖ **In early infection** (amicrofilaremic individuals): Both T_{H1} cells and T_{H2} cells are stimulated
- ❖ **In microfilaremic individuals** (asymptomatic and acute stage): Here, T_{H1} response is down regulated and T_{H2} cells response is activated; which leads to:
 - Elevation of IL-4, IL-5, IL-13 and IL-10
 - Profound eosinophilia
 - Increased parasite specific IgG-4 antibodies is observed; level of which decreases with successful therapy
 - Hyper IgE levels.
- ❖ **In chronic filariasis:** There is increased production of T_{H2} cells induced cytokines like IL-4, IL-5 and IL-13. There occurs elevation of parasite specific IgG-1, IgG-2 and IgG-3.

Clinical Features

Incubation period is about 8–16 months. Clinical manifestations can be categorized into:

- ❖ Lymphatic filariasis
- ❖ Tropical pulmonary eosinophilia (TPE) or occult filariasis
- ❖ Immune complex mediated manifestations.

Lymphatic Filariasis

Lymphatic filariasis passes through four stages.

Endemic Normal

These are the normal people residing in endemic area. Their prevalence ranges from 0 to 50%. They are not infected by the parasite. This might be due to various reasons—insufficient exposure, immunological resistance or in the prepatent period at the time of study.

Asymptomatic Microfilaremia

In endemic area, many infected individuals do not exhibit any symptoms of filarial infection, but microfilariae can be demonstrated in their peripheral blood. They may show the following features when examined.

- ❖ Microscopic hematuria and/or proteinuria
- ❖ Dilated and tortuous lymphatics (visualized by imaging)
- ❖ Filarial dance sign (ultrasound showing motile adult worm in scrotal lymphatics).

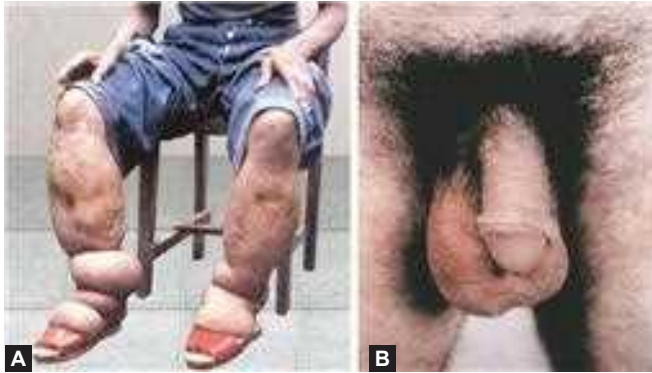
Acute Filariasis (Acute Adenolymphangitis)

It is characterized by recurrent episodes of:

- ❖ Filarial fever (high-grade fever)
- ❖ Lymphatic inflammation (lymphangitis and lymphadenitis):
 - Lower extremities are more commonly affected than the upper limbs
 - Lymph nodes most often affected are the epitrochlear, axillary, femoral or inguinal
 - The lymph nodes are firm, discrete, tender and enlarged, while the lymph vessels are inflamed and indurated
 - In addition, lymphatics of the male genital organs are frequently involved that leads to funiculitis, epididymitis and orchitis (not seen in brugian filariasis).
- ❖ **Transient local edema:** Early pitting edema; reversible on limb elevation
- ❖ **Dermatolymphangitis:** Plaque like lesion is formed over the affected skin with fever, chill and lymphatic inflammation
- ❖ In brugian filariasis, the episodes are more frequent and abrupt in onset.

Chronic Filariasis

It develops 10–15 years after infection. Chronic host immune response against the dead worms leads to granuloma formation and fibrosis of the lymph vessels causing severe lymphatic obstruction and pedal edema.



Figs 37.2A and B: Clinical features of filariasis: **A.** Elephantiasis; **B.** Hydrocele of scrotum.

Source: A. ID#-373; B. ID# 354, Public Health Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

The manifestations in descending order of occurrence are:

- ❖ **Hydrocele** (most common manifestation): Accumulation of clear straw colored fluid in the cavity of tunica vaginalis of testes (Fig. 37.2B)
- ❖ **Elephantiasis** (swelling of lower limb or less commonly arm, vulva or breast) (Fig. 37.2A)

Lymphedema is initially pitting type (stage-1) which becomes nonpitting and irreversible on limb elevation (stage-2) followed by brawny lymphedema with thickening of the skin (grade-3), finally lead to fibrosis and fissuring (grade-4)

- ❖ Chronic funiculitis and epididymitis
- ❖ **Chyluria:** Excretion of chyle, a milky white fluid in urine may occur rarely. This is due to rupture of lymph vessels into the urinary system.

Tropical Pulmonary Eosinophilia (TPE)

TPE is also called as occult filariasis, represents a hypersensitivity reaction to microfilaria antigen. Microfilariae are rapidly cleared from the bloodstream and filtered, lodged and destroyed in the lungs initiating an allergic response. Hence, microfilariae are not detected in the peripheral blood. It is discussed in detail in Chapter 69.

Immune Complex Mediated Manifestations

Circulating immune complexes containing microfilarial antigens are found to be deposited in various organs such as:

- ❖ Kidney (causes nephrotic syndrome, hematuria and proteinuria)
- ❖ Joints (causes filarial arthritis of knee or ankle).

LABORATORY DIAGNOSIS

Bancroftian filariasis

- ❑ **Demonstration of microfilariae** by thin or thick smear stained with Giemsa or by QBC examination—blood collected during night hours (or day time after DEC provocation test)

Contd...

Contd...

LABORATORY DIAGNOSIS

Bancroftian filariasis

- *W. bancrofti*—tail tip pointed, free of nuclei
- *B. malayi*—tail tip blunt, nuclei extended upto tail tip
- ❑ **Antigen detection** (ELISA, ICT)— detects Ag by using mAb to Og4C3 Ag and AD12 Ag
- ❑ **Antibody detection**
 - Flow-through assay using WbSXP-1 Ag
 - Luciferase immunoprecipitation system using Wb123 Ag
- ❑ **Imaging methods**—filarial dance sign in USG, indicates serpentine movement of adult worms in lymphatics
- ❑ **Molecular methods**—real-time PCR detecting genes such as *Sspl* repeat, *pWb12* repeat, *pWb-35*, etc.
- ❑ **Other methods**—eosinophilia, elevated IgE.

Laboratory Diagnosis

Microscopy (To Detect Microfilariae)

Microfilariae can be found in blood, and occasionally in hydrocele fluid, urine or other body fluids.

- ❖ **Direct wet mount:** Demonstrates serpentine movement of microfilariae
- ❖ **Thick and thin smears:** Leishman's or Giemsa staining can be performed to observe the sheath and nuclei of microfilaria (Fig. 37.4 and the highlight box below). Microfitemia ranges from 1–1,000 or some time up to 10,000 microfilariae/mL of blood
- ❖ **Concentration techniques:** Blood can be examined after concentration techniques to increase sensitivity
 - Membrane filtration technique
 - Knott's centrifugation technique.
- ❖ **Collection time:** It is critical and should be based on the periodicity of the microfilariae. For nocturnal periodicity, blood should be collected between 9 pm and 4 am
- ❖ **DEC provocation test:** This test is done to collect the blood in the day time
 - Patient takes a DEC tablet orally (2 mg/kg) so that the nocturnal microfilariae are stimulated and come to peripheral blood within 15 minutes to 1 hour
 - However, in case of subperiodic *W. bancrofti*, the microfilariae level falls rather rise after DEC provocation
 - This test is contraindicated in *Onchocerca* and *Loa loa* infection.
- ❖ **QBC (Quantitative buffy coat examination):** It involves centrifugation of blood, staining with acridine orange stain and examination under fluorescence microscope. This technique is more sensitive than smear microscopy
- ❖ **False negative:** Microfilariae may not be found in blood because of many reasons such as:
 - Occult filariasis
 - Chronic filariasis and endemic normal people
 - Wrong time of blood collection.

Microfilaria (*W. bancrofti*)

Detection of microfilariae in peripheral blood is diagnostic of filariasis.

- It measures 260 $\mu\text{m} \times 7.5\text{--}10\ \mu\text{m}$ covered by a long hyaline sheath
- The head end is blunt while the tail end is pointed
- In cephalic space, length to width ratio is 1:1
- The nuclei are large, coarse, well-separated present throughout the body except near the tail end
- Nuclei are also absent in few places where various primordial organs are present like nerve ring, excretory pore, anal pore and genital cells
- It differs from the microfilaria of *Brugia malayi* in head and tail region (Figs 37.3 and 37.4).

Antigen Detection

Circulating antigens of *W. bancrofti* can be detected by using monoclonal antibodies against Og4C3 and AD12 antigens.

- ❖ Both ELISA and rapid ICT are commercially available; shows 95–100% sensitivity and specificity
- ❖ No antigen detection methods are available for *Brugia* infection
- ❖ **Advantages of antigen detection:**
 - More sensitive than microscopy

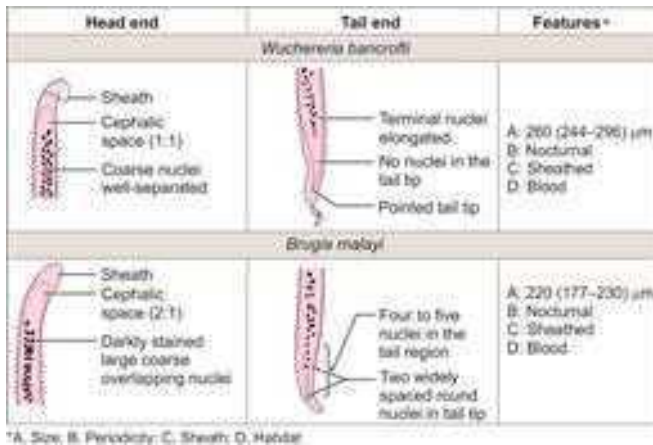


Fig. 37.3: Differences between the microfilaria of *Wuchereria bancrofti* and *Brugia malayi*.



Figs 37.4A to C: A. Microfilaria of *Wuchereria bancrofti* (schematic); B and C. Thick blood smears stained with Giemsa stain showing microfilariae of (B) *Wuchereria bancrofti*; (C) *Brugia* species.

Source: B. ID# 3009; C. ID# 3003; Dr Mae Melvin, Public Health Image Library, Centers for Disease Control and Prevention (CDC), Atlanta.

- Can be detected in day time
- Antigens can differentiate the current from past infection. Antigen disappears after clinical cure
- Can be detected in urine.

Antibody Detection

In endemic area, most people have a raised antibodies (IgG) due to prior exposure to non-human filarial worms and cross-reaction to other helminths. Therefore, antibody detection tests are useful only for epidemiologic purposes (seroprevalence studies). In non-endemic area, it may be of diagnostic value.

In contrast, detection of parasite-specific antibodies (e.g. IgG4) has a better diagnostic value, as described below:

- ❖ Parasite-specific IgG4 is increased in active filariasis and is less cross-reactive
- ❖ IgG2 level appears to be elevated in patients with elephantiasis
- ❖ The young people who are resistant to filarial infection in endemic areas, often express protective anti-sheath antibodies.

Though most tests lack both sensitivity and specificity, some newer methods as described below, have showed promising results such as:

- ❖ **Flow-through assay:** Detects total IgG antibodies to recombinant filarial WbSXP-1 antigen
- ❖ **Luciferase immunoprecipitation system:** It is another rapid test using *W. bancrofti* Wb123 antigen.

Imaging Methods

Imaging methods are useful for the following purposes.

- ❖ **Ultrasound:** It can demonstrate dilated and tortuous lymphatic vessels. Serpentine movement of adult worms within the lymphatic vessels of the scrotum, called **filarial dance sign**—is positive in 80% of cases
- ❖ **Lymphoscintigraphy** of the limbs reliably demonstrates the functional abnormalities of lymphatics; even in asymptomatic microfilaremic persons
- ❖ **X-ray** can detect dead and calcified worms.

Molecular Methods

Molecular methods such PCR and real-time PCR are available which provide several advantages.

- ❖ Can detect low level of parasitemia
- ❖ Can differentiate past from present infection
- ❖ Can distinguish between filarial parasites
- ❖ Useful for monitoring treatment response
- ❖ Detects parasites in carriers.

Other Methods

- ❖ Eosinophilia (absolute eosinophil count >3000/ μ L)
- ❖ Elevated serum concentrations of IgE (>1000 ng/mL)
- ❖ **Cellular assays:** Filarial skin test and lymphocyte response to filarial antigen; both are less specific
- ❖ Biopsy of enlarged lymph node to demonstrate adult worm
- ❖ Urine examination reveals microscopic hematuria and proteinuria.

TREATMENT

Bancroftian filariasis

Diethylcarbamazine (DEC)

- ❑ It is the drug of choice for the treatment of filariasis
- ❑ It is given 6 mg/kg daily for 12 days
- ❑ It can kill both adult worms and microfilariae. However, adult worms are cleared slowly

Albendazole: It is given as 400 mg twice daily for 21 days. It has also demonstrated efficacy against adult worms and microfilariae

Ivermectin: 0.4 mg/kg single dose, can kill microfilariae but has no effect on adult worms. High rate of recurrence occurs, hence not used in India (used only in Africa)

Doxycycline: It is given to target the intracellular *Wolbachia*. It also shows significant microfilaricidal activity like DEC

Penicillin: Secondary infections due to bacteria such as streptococci can be treated with systemic antibiotics like penicillin till the infection subsides.

Prevention

Vector Control

- ❖ **Anti-larval measures:** They are highly expensive hence mainly restricted to urban areas. Chemicals used are—mosquito larvicidal oil, pyrethrum-based oil (pyrosene oil-E), organo-phosphorus larvicides like fenthion, temephos
- ❖ **Anti-adult measures** like pyrethrum spray can be used. However, DDT and hexachlorocyclohexane (HCH) are not effective.

National Vector Borne Disease Control Programme

The National Filariasis Control Programme in India is active since 1955; which was integrated with National Vector Borne Disease Control Programme (NVBDCP) in 2006.

Hathipaon Mukh Bharat: India (under NVBDCP) has launched a massive campaign in 2015 for achieving filaria free India named “Hathipaon Mukh Bharat”. It aims at assisting the filariasis elimination program of India.

Elimination of Lymphatic Filariasis (ELF)

Global program to eliminate lymphatic filariasis (ELF) was launched by WHO in 2000 aiming at global elimination by the year 2020. In India, ELF is in operation since 2004 in parallel with global strategy.

- ❖ **MDA strategy:** WHO recommends mass drug administration (MDA) once in a year to all people at-risk in the endemic areas for a period of five years
- ❖ **MDA regimen:** Single dose of DEC (6mg/kg) + albendazole (400 mg) is the MDA regimen of choice in all endemic areas of the World including India, except:
 - In onchocerciasis endemic areas where ivermectin + albendazole is given
 - In *Loa loa* endemic area: Albendazole is given twice per year.
- ❖ **Triple drug regimen:** WHO recently recommended IDA regimen (ivermectin + DEC+ albendazole). It is implemented in selected districts of India
- ❖ **Anti-Filaria Day** is observed on 5th June globally. In addition, the **National Filariasis Day** in India is celebrated on 11th November every year
- ❖ **Assessment:** The impact of MDA is assessed by conducting:
 - *Microfilaria survey* (by peripheral blood smear examination) once in a year; one month prior to the next MDA, followed by:
 - *Transmission assessment survey* (by antigen/antibody detection) during post-MDA period.
- ❖ **Global LF elimination status:** Till date, 16 countries achieved the LF elimination status. Seven additional countries are in the verge of elimination. Preventive chemotherapy is still required in 49 countries
- ❖ **India LF elimination status:** In India, five states (Assam, Tamil Nadu, Goa, Puducherry, Daman and Diu) stopped MDA after achieving elimination status.

BRUGIAN FILARIASIS

Brugia malayi

Brugia malayi accounts for 10% of lymphatic filariasis, named after its discoverer Brug (1927).

Epidemiology

There is considerable overlapping in the geographical distribution of brugian filariasis and bancroftian filariasis.

- ❖ *B. malayi* occurs primarily in Eastern India, Indonesia, Malaysia, Thailand and Philippines
- ❖ It shows two types of periodicity of microfilaremia. The nocturnal form is more common, transmitted in areas of coastal rice fields, while the subperiodic form is rare, found in the forests of Malaysia and Indonesia
- ❖ In India, the major states involved are Kerala, Odisha, Assam and West Bengal.

Life Cycle

The life cycle of *B. malayi* is similar to *W. bancrofti* except:

- ❖ **Vector:** *Mansonia* is the main vector for the nocturnal strains, *Anopheles* and *Aedes* can also transmit the infection. The subperiodic strains are transmitted by *Coquillettidia* and *Mansonia*
- ❖ **Reservoir:** Humans are the main reservoir; except for the subperiodic strains of *B. malayi* where monkeys, cats and dogs are the animal reservoirs.

Clinical Features

Both lymphatic filariasis and tropical pulmonary eosinophilia syndrome are observed in brugian filariasis. Clinical features are similar to bancroftian filariasis except:

- ❖ More frequent episodes of acute adenolymphangitis, adenitis (femoral nodes), and filarial abscesses
- ❖ Chronic manifestations (lymphedema and elephantiasis) occur less frequently
- ❖ The genital involvement is not seen
- ❖ Elephantiasis: Swelling is limited to leg below the knee
- ❖ Chyluria does not occur.

Laboratory Diagnosis

As in bancroftian filariasis, the diagnosis of brugian filariasis depends on:

- ❖ **Microscopy:** Microfilaria in blood can be detected by peripheral blood smear examination

Microfilaria (*Brugia malayi*)

Detection of microfilariae in peripheral blood is diagnostic of filariasis (Figs 37.3 and 37.4C).

- ❑ Measures 220 × 5–6 μm in size, covered by hyaline sheath
- ❑ Cephalic space is longer
- ❑ Nuclei are large, coarse, darkly stained, overlapping and present throughout the body, extending till the tail region
- ❑ Tail tip is blunt, contains two-widely spaced nuclei

- ❖ **Antibody detection methods:** Two methods are available, both are currently used under filariasis elimination program—ICT for screening and ELISA for confirmation
 - ***Brugia rapid:*** It is an ICT detecting parasite-specific IgG-4 antibodies against recombinant BmR1 antigen of *B. malayi*
 - **Bm14 ELISA** employing recombinant *B. malayi* antigen (Bm-14) is available.
- ❖ However, there is no antigen detection method is available
- ❖ Imaging methods like ultrasound can be employed
- ❖ **Molecular methods:** As described earlier, PCR and other molecular methods can differentiate between *B. malayi* and *W. bancrofti*.

Treatment

Treatment for brugian filariasis is same as for bancroftian filariasis; except that frequency of adverse effects following DEC medication is more; therapy should be started with lower dose.

Prevention

Same as for bancroftian filariasis (i.e. both chemoprophylaxis and vector control).

Removal of pistia plants: In South India and Sri Lanka where the *Mansonia* is the main vector, breeding is best controlled by removing/destroying the supporting plant *Pistia stratiotes* from all water collections by herbicidal agents like phenoxylyene 30 and shell weed killer-D.

Brugia timori

B. timori infection is limited to the Timor islands of Southeastern Indonesia.

- ❖ Transmitted by *Anopheles barbirostris*
- ❖ Morphologically microfilariae are similar to that of *B. malayi* except that it is longer (310 μm), and more nuclei at tail region
- ❖ Clinical feature, laboratory diagnosis and treatment are similar to that of *B. malayi*.

EXPECTED QUESTIONS

I. Write essay on:

1. A 35-year-old female from a village of Bihar came to the hospital with history of fever on and off for the past one year and recently developed unilateral swelling of the left lower limb. Her blood sample was sent for peripheral blood smear examination which revealed microfilariae, 240 μm in length, tail tip pointed free of nuclei.
 - a. What is the etiological diagnosis?
 - b. Write briefly about the life cycle, laboratory diagnosis and treatment of this clinical condition.

II. Write short note on:

1. Brugian filariasis.

III. Multiple Choice Questions (MCQs):

1. **Microfilaria of *Brugia malayi* differs from that of *Wuchereria bancrofti* by all, except:**
 - a. Coarse, overlapping and darkly-stained nuclei
 - b. Tail-tip free from nuclei
 - c. Cephalic space longer
 - d. Possesses secondary kinks
2. **Which of the following microfilaria comes to peripheral blood in the day time?**
 - a. *Brugia malayi*
 - b. *Wuchereria bancrofti*
 - c. *Loa loa*
 - d. *Brugia timori*

Answers

1. b 2. c

Systemic Candidiasis and Systemic Mycoses

CHAPTER 38

CHAPTER PREVIEW

- Systemic Candidiasis
- Systemic Mycoses
- Histoplasmosis
- Blastomycosis
- Coccidioidomycosis
- Paracoccidioidomycosis

This chapter covers various systemic fungal diseases; which include:

- ❖ **Systemic candidiasis:** The most common fungal infection affecting the bloodstream
- ❖ **Systemic mycoses:** Also known as deep or endemic mycoses
- ❖ *Cryptococcus* and *Aspergillus* can occasionally cause bloodstream infection, but principally cause infection of CNS and respiratory system respectively. Hence they have been described in Chapters 75 and 69 respectively.

SYSTEMIC CANDIDIASIS

Candidiasis is the most common fungal disease in humans, affecting the skin, mucosa, and various internal organs; caused by *Candida*, a yeast like fungus that produces pseudohyphae. Various species of *Candida* include:

- ❖ *Candida albicans*: It is the most pathogenic species of *Candida* infecting humans
- ❖ Other *Candida* species which can also cause infection are *C. tropicalis* (most common species), *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. kefyr*, *C. guilliermondii*, *C. viswanathii* and *C. auris*.

Pathogenesis

Candidiasis is worldwide in distribution, accounts for the most common fungal infection in humans, both in HIV and non-HIV infected people.

Predisposing Factors

Predisposing factors that are associated with increased risk of infection with *Candida* include:

- ❖ **Physiological state:** Extremes of age (infancy, old age), pregnancy
- ❖ **Low immunity:** Patients on steroid or immunosuppressive drugs, post-transplantation, malignancy, HIV-infected people
- ❖ Patients on **broad spectrum antibiotics**—suppress the normal flora
- ❖ **Others:** Diabetes mellitus, febrile neutropenia and zinc or iron deficiency.

Virulence Factors

Candida albicans possesses the following virulence factors that contribute to the pathogenesis:

- ❖ **Adhesins:** Help in adhesion to the skin and mucosa
- ❖ **Enzymes** such as aspartyl proteinases and serine proteinases—help in tissue invasion
- ❖ **Toxins:** Glycoprotein extracts of *Candida* cell wall are pyrogenic similar to bacterial endotoxins
- ❖ **Pseudohyphae:** Presence of pseudohyphae indicates active infection; phospholipase released from the hyphal tip may help in invasion, though not proved
 - *C. albicans* has a unique ability to transform frequently between three phenotypic forms in the tissue—yeast (blastospores), pseudohyphae, and true hyphae. This property is known as **phenotypic switching**
 - This enables adaptation to changing conditions in host and thereby assists the fungus in evading host defense system (Fig. 38.1).

Clinical Manifestations

Candida species produce a spectrum of infections ranging from skin and mucosal infection to invasive and allergic infections.

- ❖ **Invasive candidiasis:** It results from hematogenous or local spread of the fungi. Various forms are:
 - Urinary tract infection
 - Pulmonary candidiasis

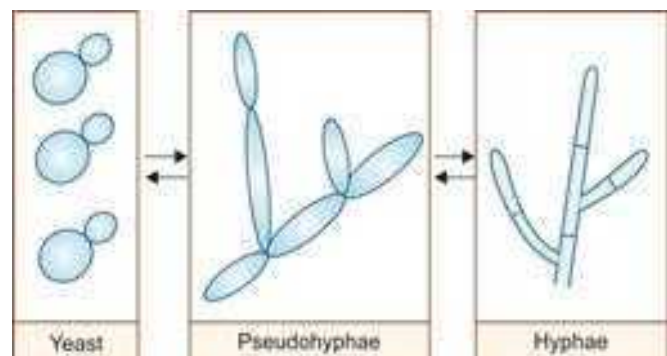


Fig. 38.1: Phenotypic switching of *Candida*.

- Septicemia (mainly by *C. albicans* and *C. glabrata*)
 - Arthritis and osteomyelitis
 - Meningitis
 - Ocular—keratoconjunctivitis and endophthalmitis
 - Hepatosplenic candidiasis
 - Disseminated candidiasis
 - Nosocomial candidiasis (mainly by *C. glabrata*).
- ❖ **Mucosal candidiasis:** The various mucosal manifestations include oropharyngeal candidiasis (oral thrush), vulvovaginitis, etc. (Chapter 58)
 - ❖ **Cutaneous candidiasis:** The cutaneous manifestations seen in candidiasis are intertrigo (pustules in the skin folds) and nail infections such as paronychia and onychomycosis (Chapter 58)
 - ❖ **Allergic candidiasis** such as candidid reaction.

LABORATORY DIAGNOSIS

Candidiasis

- **Direct microscopy:** Gram-positive oval budding yeast cells with pseudohyphae
- **Culture on SDA:** Produces creamy white and pasty colonies
- **Tests for species identification:**
 - Germ tube test (positive for *C. albicans*)
 - Dalmau plate culture for chlamyospore production
 - Growth on CHROMagar
 - Growth at 45°C (positive for *C. albicans*)
 - Carbohydrate assimilation test and carbohydrate fermentation test
 - Automated systems such as MALDI-TOF and VITEK
 - Molecular methods such as PCR.
- **Immunodiagnosis:**
 - Antibody detection against cell wall mannan antigen
 - Antigen detection such as cell wall mannan and cytoplasmic antigens.
- Enzyme detection, e.g. enolase
- Detection of metabolites, e.g. mannitol and arabinitol
- **β-d-Glucan assay:** marker of invasive fungal infection.

Laboratory Diagnosis

Specimen Collection

Depending on the site of infection, various specimens can be collected such as urine or blood.

Table 38.1: Differences between pseudohyphae and true hyphae.

Features	Pseudohyphae	True hyphae
Morphology	Elongated chains of budding yeast cells	Elongated branching filaments
Grows by	Budding	Apical elongation
Septa	Constricted	No constriction

Direct Microscopy

Gram staining reveals gram-positive oval budding yeast cells (4–6 μm size) with pseudohyphae (Fig. 38.2A). It has to be differentiated from true hyphae (Table 38.1).

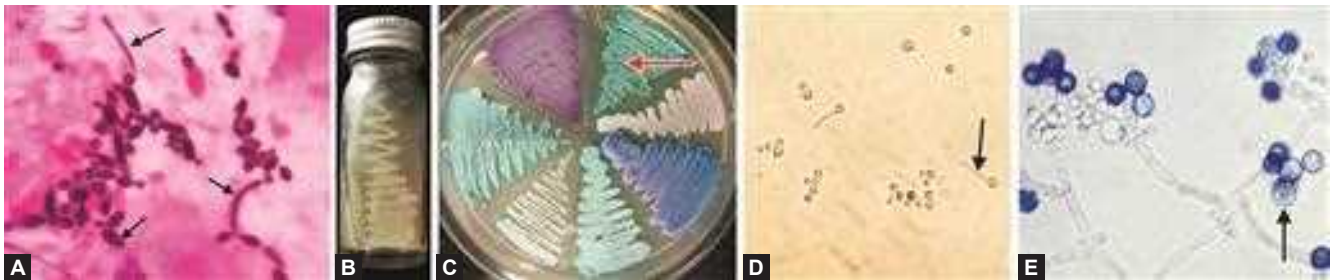
Culture

Specimens can be inoculated onto SDA with antibiotic supplements and then incubated at 37°C. *Candida* can also grow in bacteriological culture media such as blood agar. Blood for culture can be inoculated in to blood culture bottles (conventional or automated blood culture bottles such as BacT/ALERT).

- ❖ Colonies appear in 1–2 days and described as creamy white, smooth, and pasty with typical yeasty odor (Fig. 38.2B)
- ❖ Gram staining of the colonies shows gram-positive budding yeast cells with pseudohyphae except for *C. glabrata* which does not show pseudohyphae.

Tests for Species Identification

- ❖ **Germ tube test:** It is a specific test for *C. albicans*; also called Reynolds Braude phenomenon
 - Colonies are mixed with human or sheep serum and incubated for 2 hours. Wet mount preparation is examined under microscope
 - Germ tubes are formed, described as long tube like projections extending from the yeast cells
 - It is differentiated from pseudohyphae as there is no constriction at the origin (Fig. 38.2D, Table 38.1)
 - Though the test is specific for *C. albicans*, it may also be positive for *C. dubliniensis*.
- ❖ **Dalmau plate culture:** Culture on cornmeal agar can provide clue for species identification. *C. albicans* produces thick walled chlamyospores (Fig. 38.2E)



Figs 38.2A to E: **A.** *Candida albicans*—gram-positive oval budding yeast cells with pseudohyphae; **B.** *Candida albicans* on SDA shows creamy white colonies; **C.** CHROMagar showing colonies of various *Candida* species producing different colors (e.g. light-green color by *C. albicans*, red arrow); **D.** *Candida albicans* shows positive germ tube test (arrow showing); **E.** *Candida albicans* shows thick walled chlamyospores (arrow showing).

Source: A to D. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; E. ID#:2917/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **CHROMagar:** Different *Candida* species produce different colored colonies on CHROMagar (Fig. 38.2C)
- ❖ **Growth at 45°C:** It differentiates *C. albicans* (grows) from *C. dubliniensis* (does not grow at 45°C)
- ❖ **Carbohydrate fermentation test and carbohydrate assimilation test** can differentiate between various *Candida* species
- ❖ **Automated systems** such as MALDI-TOF and VITEK can also be used for speciation
- ❖ **Molecular methods** such as PCR using species specific primers are useful for species identification
 - Yeast specific universal primer and species specific primers are available targeting different genes from internal transcribed spacer region such as ITS-1 and ITS-2
 - Assays have been developed for the direct detection of *Candida* species from clinical specimens such as blood.

Immunodiagnosis

- ❖ **Antibody detection:** Various formats like ELISA, latex agglutination tests are available detecting serum antibodies against cell wall mannan antigen
- ❖ **Antigen detection:** *Candida* specific antigen such as cell wall mannan and cytoplasmic antigens can be detected by ELISA
- ❖ **Enzyme detection:** Assays are available to detect enzymes specific for *Candida* such as enolase, aspartate proteinase, etc.
- ❖ **Test for metabolites:** Specific metabolites of *Candida* such as mannitol, arabinitol can be detected.
- ❖ **β-d-Glucan assay:** See the highlight box below.

β-d-Glucan Assay

β-d-Glucan antigen can be detected in blood by colorimetric enzyme immunoassay.

- ❑ It is a marker of invasive fungal infections, raised in most invasive fungal infections (including invasive candidiasis); except for zygomycosis, blastomycosis and cryptococcosis.
- ❑ It can also be used for monitoring response to treatment.

TREATMENT

Candidiasis

The antifungal drugs recommended depends upon the type of candidiasis.

- ❑ Cutaneous candidiasis or oral thrush: the drug of choice is topical azole
- ❑ Esophageal and vulvovaginal candidiasis: the drug of choice is oral fluconazole or caspofungin
- ❑ Disseminated candidiasis: the drug of choice is liposomal amphotericin B or caspofungin or voriconazole.

C. glabrata and *C. krusei* exhibit intrinsic resistance to azoles and are refractory to treatment with azoles.

Note: Detection of *Candida* in urine and sputum may represent colonization; therefore, does not by itself warrant antifungal treatment.

Candida auris

It is an emerging species of *Candida*, increasingly reported in the recent past.

- ❑ **Manifestations:** It can cause outbreaks in healthcare settings; produces a wide range of clinical disease from asymptomatic colonization, to candidemia, abscesses, ear infections, etc.
- ❑ **Diagnosis:** It is difficult to identify with standard laboratory methods, and it can be misidentified easily. Hence, specific technologies such as molecular method or MALDI-TOF are useful for correct identification.
- ❑ **Treatment:** It is multidrug resistant and often shows resistance to commonly used antifungal drugs to treat *Candida* infections. Capsosfungins or lipid-based amphotericin B are the antifungals given for treatment.
- ❑ **Infection control:** Appropriate infection control measures (contact precautions, Chapter 21) need to be taken to prevent its spread in the hospital.

SYSTEMIC MYCOSES

Systemic mycoses include the four important fungal diseases that involve multiple organs.

1. Histoplasmosis, caused by *Histoplasma capsulatum*
2. Blastomycosis, caused by *Blastomyces dermatitidis*
3. Coccidioidomycosis, caused by *Coccidioides immitis*
4. Paracoccidioidomycosis, caused by *Paracoccidioides brasiliensis*.

They are saprophytic fungi, which spread by inhalation of spores leading to pulmonary infection. From lungs, they disseminate to cause various systemic manifestations.

All the four agents are **dimorphic fungi**, which exist as yeast at 37°C (inside human body) and mold at 25°C (in environment).

HISTOPLASMOSIS

Histoplasmosis is a systemic granulomatous disease caused by a dimorphic fungus, *Histoplasma capsulatum*. The species name is a misnomer as it is not capsulated. It was first reported by Samuel Darling (1905), hence is also known as **Darling's disease**. It has three varieties:

1. *H. capsulatum* var. *capsulatum*: It causes classical histoplasmosis (most common type)
2. *H. capsulatum* var. *duboisii*: It causes African histoplasmosis
3. *H. capsulatum* var. *farciminosum*: It causes epizootic histoplasmosis.

The description below is confined to *H. capsulatum* var. *capsulatum*. The other two varieties are described thereafter.

Epidemiology

Histoplasmosis occurs worldwide, but is endemic in USA, particularly in states bordering the Ohio River valley and the lower Mississippi River. In India, it is reported

frequently from the region of West Bengal along the Ganga River. The fungus inhabits in the humid and acidic soil that contains large amount of bird or bat droppings.

Pathogenesis

H. capsulatum is transmitted by inhalation of spores (i.e. microconidia) which usually circulate in the air after the contaminated soil is disturbed.

- ❖ After the spores enter into the lungs, they are engulfed inside the alveolar macrophages and then transform into yeast forms
- ❖ The yeasts survive within the phagolysosome of the macrophage by producing alkaline substances, such as bicarbonate and ammonia
- ❖ Then, the intracellular yeasts travel to the lymph nodes and spread to the other parts of the body through bloodstream
- ❖ Majority of the infected people show strong cell-mediated immune response (CMI) within 2 weeks. Granulomas are formed which later get healed with fibrosis and calcification. Unlike latent tuberculosis, histoplasmosis once healed, rarely reactivates
- ❖ However, in patients with impaired CMI, the disseminated infection sets in.

Clinical Manifestations

Clinically, the classical histoplasmosis ranges from asymptomatic infection (in immunocompetent people) to life-threatening illness seen in people with low CMI. The various clinical types include:

- ❖ **Pulmonary histoplasmosis:** It is the most common form
 - Acute form presents with mild flu like illness, pulmonary infiltrates in the chest X-ray with hilar or mediastinal lymphadenopathy
 - Chronic cavitary histoplasmosis may be seen in smokers with underlying structural lung disease.
- ❖ **Mucocutaneous histoplasmosis:** Skin and oral mucosal lesions may develop secondary to pulmonary infection. Oral lesions are particularly seen in Indian patients (Fig. 38.3)



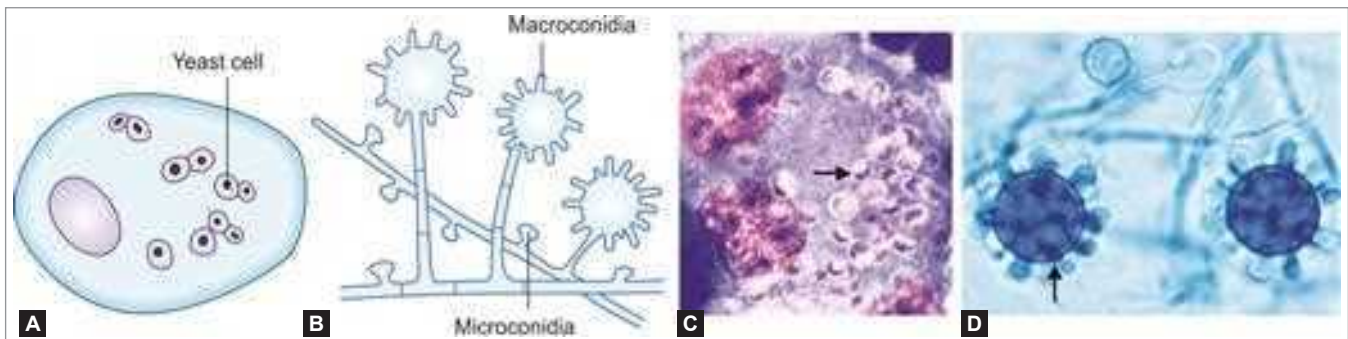
Fig. 38.3: Oral lesions of histoplasmosis (arrow showing).

Source: Public Health Image Library/Dr Lucille K Georg/ID#:15363/Centers for Disease Control and Prevention (CDC), Atlanta. (with permission).

- ❖ **Disseminated histoplasmosis:** It develops if CMI is very low (e.g. untreated HIV-infected people or following organ transplantation). The common sites affected are bone marrow, spleen, liver, eyes and adrenal glands.

Laboratory Diagnosis

- ❖ **Specimens:** Useful specimens include sputum, aspirate from bone marrow and lymph node, blood and biopsies from skin and mucosa
- ❖ **Direct microscopy:** Histopathological staining (such as PAS, Giemsa or GMS stain) of the specimens reveals tiny oval yeast cells (2–4 μm size) with narrow-based budding within the macrophages with an underlying granulomatous response (Figs 38.4A and C)
- ❖ **Culture:** It is the gold standard method of diagnosis. Specimens should be inoculated onto media such as SDA, blood agar and BHI agar in duplicate and incubated simultaneously at 25°C and 37°C. *Histoplasma* is a dimorphic fungus, hence:



Figs 38.4A to D: *Histoplasma capsulatum*: **A.** Yeast form (schematic); **B.** Mycelial form (schematic); **C.** 2–4 μm yeast cells with narrow-based budding (Giemsa stain) (arrow showing); **D.** Mold form, septate thin hyphae with tuberculate macroconidia (arrow showing).

Source: Public Health Image Library/C. Dr Lucille K. Georg/ID#:15365, D. Dr Libero Ajello/ID#:15364/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- **At 25°C:** It forms mycelial phase; produces white to buff brown colonies that consist of two types of conidia or spores:
 1. Tuberculate macroconidia, with typical thick walls and finger-like projections which is a characteristic feature of this fungus (Figs 38.4B and D)
 2. Microconidia are smaller, thin, and smooth-walled.
- **At 37°C:** It gets converted into yeast form (creamy white colonies), which is best developed in special media like Kelley's media.
- ❖ **Serology:** Antibodies in serum can be detected by CFT and immunodiffusion test
 - Antibodies appear after 1 month of infection; hence are more useful in chronic stage; but are often negative in early course and in disseminated stage
 - False positive result may occur due to past infection or cross infection with *Blastomyces*.
- ❖ **Skin test:** It may be done to demonstrate delayed type hypersensitivity response to histoplasmin antigen, which indicates prior exposure
- ❖ **Molecular test:** PCR targeting specific ITS D1/D2 gene (variable region of 28S rRNA) is available.

TREATMENT

Histoplasmosis

Liposomal amphotericin B is the antifungal agent of choice in acute pulmonary and disseminated histoplasmosis. Itraconazole is recommended for chronic cavitary pulmonary histoplasmosis.

H. capsulatum var. *duboisii*

It causes African histoplasmosis which is clinically distinct; characterized by frequent skin and bone involvement. Its yeast form exists as large thick walled oval yeast cells (7–15 μm) with prominent narrow based budding.

H. capsulatum var. *farciminosum*

It causes epizootic histoplasmosis. It is a form of lymphangitis in horses and mules.

BLASTOMYCOSIS

Blastomycosis (also known as North American blastomycosis or Gilchrist's disease or Chicago disease) is a fungal infection of humans and other animals, notably dogs and cats, caused by the dimorphic fungus, *Blastomyces dermatitidis*.

Pathogenesis

Blastomycosis is transmitted by inhalation of the conidia of *B. dermatitidis*. The spores enter into the lungs, and are engulfed by alveolar macrophages, where they get converted into yeast phase. This yeast expresses a 120-kDa glycoprotein called **BAD-1** (*B. dermatitidis* adhesin-1) which is an essential virulence factor and also a major inducer of cellular and humoral immune responses.

Clinical Manifestations

Acute pulmonary blastomycosis is the most common form. Extrapulmonary manifestations may also occur such as:

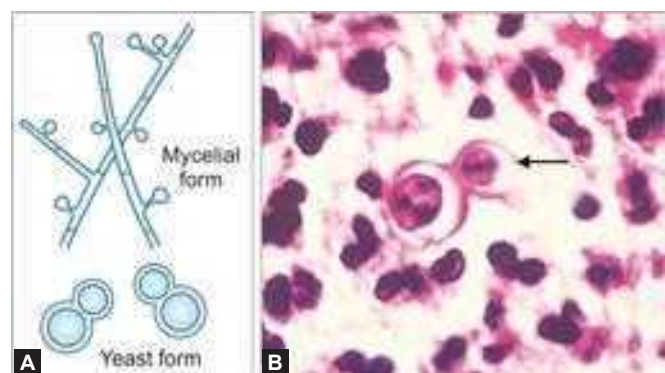
- ❖ Skin involvement is the most common extrapulmonary form; characterized by either verrucous (more common) or ulcerative type of skin lesions
- ❖ Osteomyelitis may develop along with contiguous soft-tissue abscesses and draining sinuses
- ❖ Prostate and epididymis involvement in men
- ❖ Central nervous system (CNS) involvement has been reported in ~40% of AIDS patients. Brain abscess is the usual presentation, followed by cranial or spinal epidural abscess and meningitis.

Epidemiology

Like histoplasmosis, blastomycosis is also endemic in North America, particularly in states bordering the Ohio River and Mississippi River.

Laboratory Diagnosis

- ❖ **Histopathological staining** of the tissue biopsy specimens reveals thick-walled round yeast cells of 8–15 μm size with single broad-based budding (Figure of 8 appearance) (Figs 38.5A and B)
- ❖ **Culture media** such as SDA, blood agar and BHI agar are inoculated. At 25°C, mycelial form containing hyphae with small pear-shaped conidia are produced; whereas at 37°C mold to yeast conversion takes place
- ❖ **Skin test:** It is done to demonstrate delayed type hypersensitivity to blastomycin antigen
- ❖ **Antibody detection:** Immunodiffusion test specific for *B. dermatitidis* has been developed against yeast phase antigens such as antigen—A, BAD-1 and ASWS antigen (alkali soluble water soluble)
- ❖ **Antigen detection assay** to detect *Blastomyces* antigen in urine (more sensitive) and in serum is commercially available



Figs 38.5A and B: *Blastomyces*: **A.** Schematic (Mycelial and yeast forms); **B.** Histopathological stain (arrow showing) broad-based budding yeast cells (Figure of 8 appearance).

Source: B. Public Health Image Library/ID#:493/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Molecular methods**, including DNA probe hybridization and real time PCR are available.

TREATMENT

Blastomycosis

Liposomal amphotericin B is the drug of choice in most of the cases. Itraconazole can be given in immunocompetent patients with mild pulmonary or non-CNS extrapulmonary blastomycosis.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis (also called desert rheumatism or Valley fever or California fever), is a systemic fungal disease caused by a dimorphic soil dwelling fungus—*Coccidioides* which has two species, *C. immitis* and *C. posadasii*.

Pathogenesis

Coccidioides is transmitted by inhalation of arthroconidia. In lungs, they enlarge, become rounded, and develop internal septations to form large sac like structures of size up to 200 µm called **spherules**, that encompass numerous endospores. Spherules may rupture and release packets of endospores that can disseminate and develop into new spherules. If returned to artificial media or the soil, spherules revert back to the mycelial stage.

Clinical Manifestations

Most patients are asymptomatic (60%). In remainders, pulmonary coccidioidomycosis is the most common form; presents as pneumonia, cavities, pleural effusion or nodule formation.

- ❖ Skin lesions such as rashes or erythema nodosum and arthritis with joint pain may appear secondary to pulmonary infection particularly in women
- ❖ Disseminated form: Males and persons with low CMI (HIV infected patients with CD4+ T cell count <250/µL) are at higher risk. Common sites for dissemination include skin, bone, joints, soft tissues, and meninges.

Epidemiology

It is endemic in certain parts of Arizona, California, Nevada, New Mexico, Texas, Utah and northern Mexico.

Laboratory Diagnosis

- ❖ **Histopathological staining** (H and E stain, PAS or GMS) of sputum or tissue biopsy specimens demonstrates spherules which are large sac like structures (20–80 µm size), have thick, double refractile wall, and are filled with endospores (Figs 38.6A and C)
- ❖ **Cultures** on SDA produces mycelial growth, described as fragmented hyphae consisting of **barrel-shaped arthrospores** with alternate cells distorted (empty cells) (Figs 38.6B and D):
 - *Coccidioides* differs from other dimorphic fungi as it grows as mold at both 25°C and 37°C in usual culture media. It forms spherules at 37°C in certain special culture media only
 - Cultures are highly infectious; may lead to accidental inhalation of spores in laboratories, require biosafety level-3 precautions.
- ❖ **Serology:** Antibodies are detected by immunodiffusion test and CFT
- ❖ **Skin test:** It is done by using fungal extracts (coccidioidin or spherulin); if produces at least a 5 mm induration within 48 hours after injection (delayed hypersensitivity reaction) indicates past infection.

TREATMENT

Coccidioidomycosis

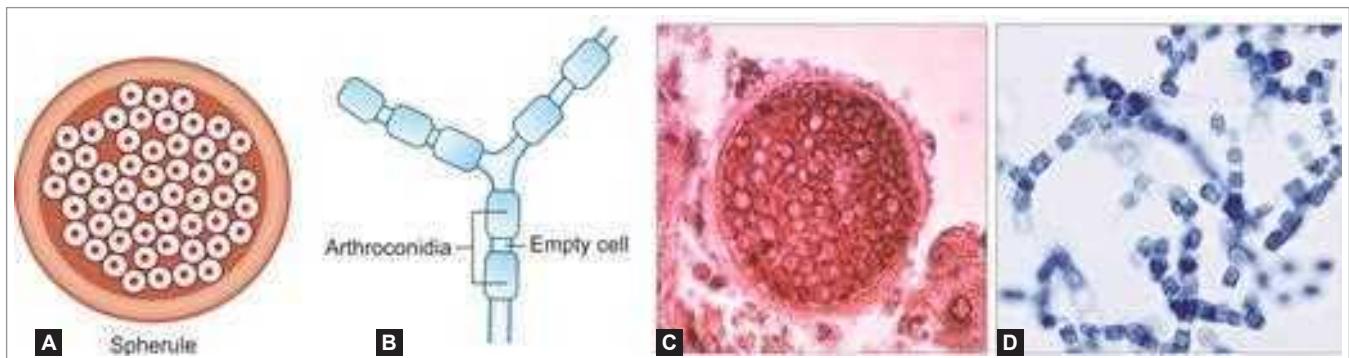
Triazoles such as itraconazole are the drug of choice to treat most cases of coccidioidomycosis, except for diffuse pneumonia with pulmonary sequelae where amphotericin B is recommended.

PARACOCCIDIOIDOMYCOSIS

Paracoccidioidomycosis (also known as South American blastomycosis, Lutz-Splendore-de Almeida disease) is a systemic disease caused by the dimorphic fungus—*Paracoccidioides brasiliensis*.

Pathogenesis and Clinical Manifestations

Transmission is by inhalation of spores, which then transform into the yeast phase in lungs. It occurs as two major forms.



Figs 38.6A to D: *Coccidioides*: **A.** Spherules (schematic); **B.** Hyphae with arthroconidia (schematic); **C.** Spherules (PAS staining); **D.** Hyphae with arthroconidia (LPCB mount).

Source: Public Health Image Library/C. ID#:14499, D. ID#:12196/Centers for Disease Control and Prevention (CDC) Atlanta (with permission).

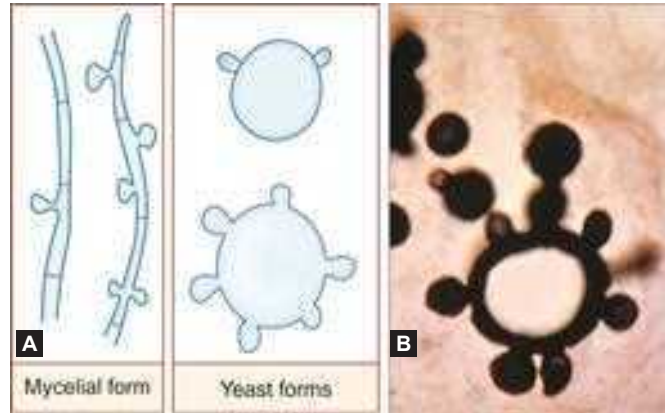
- Acute form (or juvenile type):** It affects young adults under 30 years age. It is a less common variety, but more severe form, manifests as **disseminated infection** involving multiple viscera and is refractory to treatment
- Chronic form (or adult form):** It accounts for 90% of cases and predominantly affects older men. It results from reactivation of quiescent lung lesions
 - It is less severe form, manifested as progressive pulmonary disease affecting lower lobes, with fibrosis
 - Skin, oral mucosal lesions and cervical lymphadenopathy are the other features.

Epidemiology

Paracoccidioidomycosis is endemic in Brazil and other South American countries.

Laboratory Diagnosis

- Histopathological staining** of pus, tissue biopsies or sputum reveals round thick-walled yeasts, with multiple narrow-necked buds attached circumferentially giving rise to **Mickey mouse or pilot wheel** appearance (Figs 38.7A and B)
- Culture** on SDA yields mycelial form at 25°C which converts into yeast phase at 37°C when grown in BHI agar supplemented with blood and glutamine
- Serology:** Antibodies are detected by immunodiffusion, and most recently by ELISA, using gp43 antigen of *P. brasiliensis*



Figs 38.7A and B: Paracoccidioidomycosis: **A.** Schematic representation of mycelial and yeast forms; **B.** Methenamine silver staining shows yeast form (pilot wheel appearance).

Source: B. Public Health Image Library/Dr Lucille K Georg/ID#:527/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- Skin test:** It demonstrates delayed type hypersensitivity response against paracoccidioides antigen.

TREATMENT

Paracoccidioidomycosis

Itraconazole is the treatment of choice for paracoccidioidomycosis, except for the seriously ill patients where amphotericin B is recommended. Sulfonamides are effective, but the response is slow with frequent relapses.

EXPECTED QUESTIONS

I. Write essay on:

- A 29-year-old HIV-infected male presents to the clinic with history of high-grade fever and altered mental status. On examination, his blood pressure was found as 90/60 mm of Hg, and respiratory rate was increased to 28 per minute. Blood cultures yielded creamy white colonies which on Gram stain revealed gram-positive oval budding yeast cells with pseudohyphae.
 - What is the clinical diagnosis and the likely etiological agent?
 - Name the risk factors predisposing this clinical condition.
 - What are the other clinical manifestations caused by this organism?
 - Describe the laboratory diagnosis of this clinical condition in detail.

II. Write short notes on:

- Histoplasmosis.
- Blastomycosis.

Answers

1. b 2. c 3. b 4. b

III. Multiple Choice Questions (MCQs):

- Broad-based budding is seen in:**
 - Histoplasma*
 - Blastomyces*
 - Cryptococcus*
 - Candida*
- Barrel-shaped arthroconidia are seen in:**
 - Histoplasmosis
 - Cryptococcosis
 - Coccidioidomycosis
 - Paracoccidioidomycosis
- Candida* species resistant to azoles is:**
 - C. albicans*
 - C. krusei*
 - C. tropicalis*
 - C. dubliniensis*
- Germ tube test is diagnostic for:**
 - Candida glabrata*
 - Candida albicans*
 - Cryptococcus*
 - Coccidioides immitis*

ANTIBIOTIC RESISTANCE



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause. This is compromising our ability to treat infectious diseases and undermining many advances in medicine.

We must handle antibiotics with care so they remain effective for as long as possible.

WHAT HEALTH WORKERS CAN DO



- 1 Prevent infections by ensuring your hands, instruments and environment are clean
- 2 Keep your patients' vaccinations up to date
- 3 If you think a patient might need antibiotics, where possible, **test to confirm** and find out which one
- 4 Only prescribe and dispense antibiotics when they are **truly needed**
- 5 Prescribe and dispense the **right antibiotic at the right dose for the right duration**

www.who.int/drugresistance

#AntibioticResistance



World Health Organization

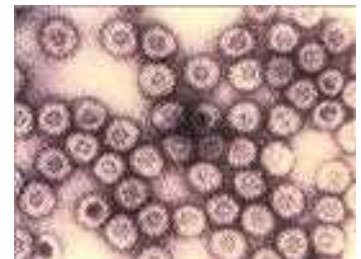
Gastrointestinal (GI) Infections

SECTION

5

SECTION OUTLINE

39. Gastrointestinal Infective Syndromes
40. Food Poisoning: *S. aureus*, *Bacillus cereus*, *Clostridium botulinum* and Others
41. Gastrointestinal Infections due to Enterobacteriaceae: Diarrheagenic *Escherichia coli*, Shigellosis, Nontyphoidal Salmonellosis and Yersiniosis
42. Cholera, Halophilic *Vibrio* and *Aeromonas* Infections
43. Miscellaneous Bacterial Infections of Gastrointestinal System: *Helicobacter*, *Campylobacter* and *Clostridioides difficile* Infections
44. Viral Gastroenteritis: Rotaviruses and Others
45. Intestinal Protozoan Infections: Intestinal Amoebiasis, Giardiasis, Coccidian Parasitic Infections, Balantidiasis, Blastocystosis, and Others
46. Intestinal Helminthic Infections
 - Intestinal Cestode Infections: *Diphyllobothrium*, *Taenia*, *Hymenolepis* and Others
 - Intestinal Trematode Infections: *Fasciolopsis buski*, *Schistosoma mansoni*, *S. japonicum* and Others
 - Intestinal Nematode Infections: *Trichuris*, *Enterobius*, hookworm, *Strongyloides*, *Ascaris* and Others



Prevention of Diarrhoeal Disease



**Safe water/
Adequate sanitation**
Treat water before use and
dispose of waste safely



**Improved
hygiene**
Wash hands when
appropriate



**Routine
vaccination**
Provide rotavirus
vaccine



**Exclusive breastfeeding
for the first six months**



**Good
personal and
food hygiene**



**About how infections
spread**

CHAPTER PREVIEW

- Resident Microbial Flora
- Diarrheal Diseases
- Other Infective Syndromes of GIT

Gastrointestinal (GI) infections are among the most commonly encountered infective syndromes in man (Table 39.1). At the same time, the human gastrointestinal tract (GIT) colonizes a number of resident microbial flora, which provide several beneficiary effect.

RESIDENT MICROBIAL FLORA

Human GIT is colonized by diverse group of normal resident microbial flora.

- ❖ **Upper GIT** contains only sparse flora (streptococci in oral cavity and lactobacilli in stomach)
- ❖ **Lower GIT:** The microbial load gradually increases towards lower part of GIT and is highest in the distal ileum (10^{11} to 10^{12} /g).
 - The large bowel comprises of both anaerobes and aerobes with a ratio of 1,000:1; with *Bacteroides fragilis* (most common) and Enterobacteriaceae such as *E. coli*, *Klebsiella* present predominantly
 - Normal flora also comprises of yeasts (e.g. *Candida*) and parasites (e.g. *Entamoeba coli*).

Normal GI flora has several benefits such as preventing colonization of pathogens in the intestine, synthesizing

various vitamins, modulating the immune system, etc., (discussed in detail in Chapter 7).

DIARRHEAL DISEASES

The diarrheal diseases are one of the leading cause of illness globally; cause significant morbidity and mortality.

- ❖ Worldwide, about 1.7 to 5 billion cases of diarrhea occur per year, with 1.26 million deaths; accounting for the second leading cause of death globally
- ❖ It is more common in developing countries, where young children get diarrhea on an average three times a year
- ❖ There are various clinical types of diarrheal diseases (enlisted in Table 39.1 and described below); caused by a wide variety of infectious agents including bacteria, viruses, and parasites (Table 39.2).

Diarrhea

Diarrhea is defined as passage of three or more loose or liquid stools per day, in excess than the usual habit for that person (World Health Organization).

- ❖ It may be caused by microbial infections, or as a result of other gastrointestinal diseases such as inflammatory bowel diseases, coeliac disease, etc.
- ❖ Acute diarrhea usually lasts for <14 days; most often caused by viral agents, followed by bacterial or parasitic agents
- ❖ Common microbial agents causing diarrhea and the mechanisms involved are summarized in Table 39.2.

Dysentery

Dysentery is characterized by diarrhea with increased blood and mucus, often associated with fever, abdominal pain, and tenesmus (feeling of constant need to pass stools, despite an empty colon) (Table 39.2).

Traveler's Diarrhea

Traveler's diarrhea is the most common travel-related infectious illness.

Table 39.1: Gastrointestinal infective syndromes.

Diarrheal diseases

- Diarrhea
- Dysentery
- Traveler's diarrhea
- Persistent (chronic) diarrhea
- Gastroenteritis
- Food poisoning

Other gastrointestinal infective syndrome

- Acute vomiting
- Necrotizing enterocolitis
- Necrotizing enteritis
- Pseudomembranous enterocolitis
- Tropical sprue
- Peptic ulcer disease
- Infections of other GI structures such as appendicitis, diverticulitis and typhlitis

Table 39.2: Infections agents of acute diarrhea and the underlying mechanism*.			
Mechanism	Features	Examples of pathogens involved	
Non-inflammatory	<p>Location: Proximal small bowel</p> <p>Illness: Watery diarrhea</p> <p>Stool findings:</p> <ul style="list-style-type: none"> No fecal leukocytes Fecal lactoferrin—not increased or mildly increased 	<p>Bacteria (mostly enterotoxin mediated):</p> <ul style="list-style-type: none"> <i>Vibrio cholerae</i> <i>Escherichia coli</i>: <ul style="list-style-type: none"> ➢ Enteropathogenic (nontoxigenic) ➢ Enterotoxigenic ➢ Enteroaggregative <i>Clostridium perfringens</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i> <i>Aeromonas hydrophila</i> <i>Plesiomonas shigelloides</i> <p>Viruses:</p> <ul style="list-style-type: none"> Rotavirus Norovirus Enteric adenoviruses- 40,41 Caliciviruses Astrovirus <p>Fungi: Microsporidia</p>	<p>Parasites (Protozoa):</p> <ul style="list-style-type: none"> <i>Giardia lamblia</i> <i>Cryptosporidium parvum</i> <i>Cyclospora cayetanensis</i> <i>Cystoisospora belli</i> <i>Blastocystis hominis</i> <p>Parasites (Helminths):</p> <ul style="list-style-type: none"> <i>Ascaris lumbricoides</i> Hookworm <i>Strongyloides stercoralis</i> <i>Enterobius vermicularis</i>** <i>Trichinella spiralis</i> <i>Taenia saginata</i>, <i>T. solium</i> <i>Hymenolepis nana</i> <i>Dipylidium caninum</i> <i>Diphyllobothrium latum</i> <i>Fasciolopsis buski</i>
Inflammatory (invasion or cytotoxin)	<p>Location: Colon or distal small bowel</p> <p>Illness:</p> <ul style="list-style-type: none"> Dysentery or Inflammatory diarrhea <p>Stool findings:</p> <ul style="list-style-type: none"> Fecal pus cells (polymorphonuclear leukocytes)—increased Fecal lactoferrin—markedly increased 	<p>Predominantly dysentery:</p> <ul style="list-style-type: none"> <i>Shigella</i> species <i>Campylobacter jejuni</i> Enterohemorrhagic <i>E. coli</i> Enteroinvasive <i>E. coli</i> <i>Vibrio parahaemolyticus</i> <p>Predominantly inflammatory diarrhea:</p> <ul style="list-style-type: none"> Non-typhoidal salmonellae <i>Yersinia enterocolitica</i> <i>Listeria monocytogenes</i> <i>Clostridioides difficile</i> <i>Plesiomonas shigelloides</i> <i>Klebsiella oxytoca</i> 	<p>Parasite: (predominantly dysentery)</p> <ul style="list-style-type: none"> <i>Entamoeba histolytica</i> <i>Balantidium coli</i> <i>Trichuris trichiura</i> <i>Schistosoma mansoni</i> <i>Schistosoma japonicum</i>
Penetrating	<p>Location: Distal small bowel</p> <p>Illness: Enteric fever</p> <p>Stool findings: Fecal mononuclear leukocytes (↑)</p>	<p><i>Salmonella</i> Typhi (enteric fever)*</p> <p><i>Yersinia enterocolitica</i> (typhoid like illness)</p>	

*Note: There is a number of organisms transmitted through the enteric route, but predominantly cause extraintestinal manifestations and therefore are discussed in the respective systems where they predominantly cause infection; for example, *Salmonella* Typhi, under bloodstream infections (Chapter 30).

***Enterobius vermicularis* causes perianal pruritus, not diarrhea.

- ❖ **Epidemiology:** Occurs in about 20–50% of people traveling from temperate industrialized countries to tropical regions of Asia, Africa, and Central and South America
- ❖ **Clinical presentation:** Most cases begin within the first 3–5 days; characterized by a sudden onset of abdominal cramps, anorexia, and watery diarrhea. The illness is generally self-limited, lasting for 1–5 days
- ❖ **Microbial agents** causing traveler's diarrhea are listed in Table 39.3. Overall, enterotoxigenic *Escherichia coli* is the most common agent, followed by enteroaggregative *E. coli*. *Campylobacter jejuni* is more common in Asia. Norovirus diarrhea is associated with traveling on cruise ships.

Persistent and Chronic Diarrhea

Diarrhea that lasts for ≥ 14 days (usually 2–4 weeks) is considered persistent. Chronic diarrhea usually lasts for >4 weeks. May result from infections due to various organisms.

- ❖ Parasites (e.g. *Cryptosporidium*, *Cyclospora*, *Entamoeba histolytica*, *Giardia*) account for a major cause of chronic diarrhea
- ❖ Bacteria (e.g. *Aeromonas*, *Campylobacter*, *Clostridium difficile*, *Plesiomonas*)
- ❖ Viruses such as cytomegalovirus, common in immunocompromised host
- ❖ Fungi such as microsporidia, common in immunocompromised host.

Chronic diarrhea may also result from various noninfectious etiologies such as pancreatic disorders,

Etiologic agent	Comments
Bacteria (50–75%)	
Enterotoxigenic <i>E. coli</i> (10–45%)	Single most important agent
Enterotoxigenic <i>E. coli</i> (5–35%)	Emerging enteric pathogen with worldwide distribution
<i>Campylobacter jejuni</i> (5–25%)	More common in Asia
<i>Shigella</i> , Enteroinvasive <i>E. coli</i>	Major cause of dysentery
Non-typhoidal salmonellae	Common agent in India
Others	Including <i>Aeromonas</i> , <i>Plesiomonas</i> , and <i>Vibrio cholerae</i>
Viruses (<20%)	
Norovirus (<10%)	Associated with cruise ships
Rotavirus (<5%)	Common among children
Parasites (0–10%)	
	<i>Giardia lamblia</i> , <i>Cryptosporidium</i> , <i>Entamoeba histolytica</i> , <i>Cyclospora</i>

intestinal disorders (e.g. colitis, Crohn's disease, irritable bowel syndrome) or tumors.

Gastroenteritis

Gastroenteritis or infectious diarrhea may be defined as inflammation of the mucous membrane of the stomach and intestine resulting in combination of diarrhea, vomiting and pain abdomen with or without mucus or blood in stool, fever or dehydration.

Food Poisoning

Food poisoning refers to an illness acquired through consumption of food or drink contaminated either with microorganisms, or their toxins. It is discussed in Chapter 40.

Pathogenic Mechanisms

Enteric pathogens have developed a variety of strategies to overcome host defenses (Table 39.4).

Inoculum Size

Enteric pathogens differ from each other in their infective dose (minimum dose required to initiate the infection). For example:

- ❖ *Shigella*, enterohemorrhagic *E. coli*, *Giardia*, or *Entamoeba histolytica*: 10–100 bacteria or cysts
- ❖ *Vibrio cholerae*: 10^5 – 10^8 bacilli
- ❖ *Salmonella*: 10^3 – 10^5 bacilli.

Adherence

Adherence to intestinal mucosa helps the organism to compete with the normal bowel flora and thereby colonizing the intestinal mucosa. This is crucial for the pathogenesis of many diarrheal agents such as enteropathogenic *E. coli*,

Toxin production		
Enterotoxins Cholera toxin <i>Vibrio parahaemolyticus</i> <i>E. coli</i> • LT and ST of ETEC • EAST of EAEC • VT of EHEC <i>Clostridioides difficile</i> (toxin A) <i>Aeromonas</i> Rotavirus (NSP4) <i>Campylobacter jejuni</i>	Cytotoxins <i>Shigella dysenteriae</i> type 1 Enterohemorrhagic <i>E. coli</i> <i>Clostridioides difficile</i> (toxin B)	Neurotoxins <i>Staphylococcus aureus</i> enterotoxin <i>Bacillus cereus</i> toxin <i>Clostridium botulinum</i> toxin
Attachment within or close to mucosal cells	Invasion of intestinal epithelium	
<i>E. coli</i> • Enteropathogenic • Enterohemorrhagic <i>Cryptosporidium</i> species <i>Cyclospora</i> species <i>Cystoisospora</i> species Rotavirus Norovirus	<i>Shigella</i> species Enteroinvasive <i>E. coli</i> <i>Campylobacter jejuni</i> <i>Yersinia enterocolitica</i> <i>Plesiomonas shigelloides</i> <i>Entamoeba histolytica</i> <i>Balantidium coli</i>	

Abbreviations: LT, heat labile toxin; ST, heat stable toxin; ETEC, Enterotoxigenic *E. coli*; EAST, Enterotoxigenic *E. coli* heat-stable enterotoxin; EAEC, Enterotoxigenic *E. coli*; VT, verocytotoxin; EHEC, Enterohemorrhagic *E. coli*; NSP4, non-structural protein-4.

enterohemorrhagic *E. coli*, enterotoxigenic *E. coli* and *V. cholerae* (Table 39.4).

Toxin Production

Enteric organisms can produce variety of toxins, which are implicated in pathogenesis of diarrhea (Table 39.4). These include:

- ❖ **Enterotoxins:** Cause watery diarrhea by acting directly on the secretory mechanisms in the intestinal mucosa
- ❖ **Cytotoxins:** Cause destruction of mucosal cells, leading to inflammatory diarrhea
- ❖ **Neurotoxins:** Act directly on the central nervous system producing vomiting.

Invasion

In addition to production of toxins, bacterial invasion is another mechanism by which destruction of intestinal mucosal cells takes place resulting in dysentery (Table 39.4).

Predisposing Factors

Alterations of the host defense mechanisms can promote the diarrheal diseases.

- ❖ **Suppression of the normal flora:** Leads to loss of protective effect of intestinal microbiota (they usually prevent colonization by potential enteric pathogens)
- ❖ **Neutralization of gastric acidity:** Promote the acid labile pathogens (e.g. *V. cholerae*)

- ❖ **Inhibition of intestinal motility:** Interfere with the clearance of bacteria from the small intestine
- ❖ **Age:** Children (<5 years) are more likely to contract most of the diarrheal diseases (e.g. rotavirus) than adults. Children are most commonly affected in the weaning period (called as **weaning diarrhea**). This may be because of unhygienic food preparation during weaning. Another reason is, breastfed infants are usually protected due to maternal antibodies. However, older children and adults are more commonly infected with *Norovirus* diarrhea
- ❖ **Location:** Closed and semi-closed communities, including day-care centers, schools, residential facilities, and cruise ships are among the important settings for outbreaks of diarrheal diseases
- ❖ **Antibiotic-associated:** Patients on prolonged antibiotic course are more likely to develop *C. difficile* infection leading to diarrhea (Chapter 43)
- ❖ **Impaired host immunity:** Immunocompromised hosts are at a higher risk of developing diarrhea. Individuals with AIDS are at high-risk for various invasive diarrheal illness such as salmonellosis, cryptosporidiosis, etc. Individuals with hypogammaglobulinemia are particularly at higher risk for *C. difficile* colitis and giardiasis
- ❖ **Genetic determinants:** Host genetic variation influences susceptibility to diarrheal diseases. People with blood group O show increased susceptibility to disease due to *V. cholerae*, *Shigella*, *E. coli* O157, and Norovirus.
- ❖ **Hanging drop preparation:** It is done for liquid specimens to demonstrate darting motility of *Vibrio cholerae*
- ❖ **Gram stained smear:** Gram staining of stool specimen is not routinely done because of presence of normal flora. It is recommended only in special situations where the typical morphology would suggest preliminary clue for diagnosis:
 - Presence of gram-negative comma-shaped bacilli: *Vibrio cholerae*
 - Gram-positive budding oval yeast cells in immunocompromised host or in infant—suggestive of *Candida* species.
- ❖ **Modified acid fast staining** (with 0.5-1% sulfuric acid) can be carried out for the detection of oocysts of *Cryptosporidium*, *Isospora* and *Cystoisospora*
- ❖ **Electron microscopy** of stool specimen can be performed for detection of morphology of specific viruses causing gastroenteritis
 - Rotaviruses appear as spokes grouped around the hub of a wheel
 - Astroviruses have star-like morphology
 - Coronaviruses have cup-like depressions on the capsid surface.

Bacterial Culture (Table 39.5)

Laboratory Diagnosis

Specimen Collection

Fecal specimen (containing mucus flakes) is collected in a sterile screw capped wide mouthed container. In carriers, a rectal swab may be collected.

- ❖ Specimens should be transported to the laboratory within 1 hour
- ❖ If a delay of longer than 1 hour is anticipated, the fecal specimen should be collected in transport media like Cary-Blair medium, or alkaline peptone water (if cholera is suspected).

Macroscopy

The following macroscopic appearances are noted:

- ❖ Color of the specimen
- ❖ **Consistency** of the specimen—formed, semiformed or liquid
- ❖ Presence of blood (suggestive of dysentery), mucus or pus (suggestive of inflammatory diarrhea)
- ❖ Presence of adult parasitic forms, e.g. *Enterobius*, *Ascaris*, or *Taenia* segments.

Microscopy

- ❖ **Wet mount preparation** in saline or iodine is done for detection of pus cells, RBCs and detection of parasitic cysts, trophozoites, eggs or larvae (Table 39.5)

- ❖ **Culture media:** Fecal specimen should be inoculated onto the following media:
 - Enrichment broth: Selenite F broth and alkaline peptone water
 - Mildly selective medium: MacConkey agar
 - Highly selective medium such as: DCA (deoxycholate citrate agar), XLD (xylose lysine deoxycholate) agar and TCBS (thiosulfate citrate bile salt sucrose) agar.
 - Sorbitol MacConkey agar, when an outbreak of *E. coli* O157 is suspected.
- ❖ **Identification:** Identification of the enteric pathogens is made by performing either conventional biochemical tests or automated methods such MALDI-TOF or VITEK. Then serotyping is performed with specific group or type specific antisera
- ❖ **Antimicrobial susceptibility test:** It is done to choose appropriate drug for treatment. Disk diffusion test is carried out on Mueller-Hinton agar.

Tissue Culture

This is carried out for the detection of enteric viruses and also for some diarrheagenic *E. coli*. Enterotoxigenic *Escherichia coli* (ETEC) penetrates HeLa and HEP-2 cell line, whereas verocytotoxin of enterohemorrhagic *Escherichia coli* (EHEC) is detected by its cytotoxic effect on Vero cell line.

Antigen Detection

Various test formats are available to detect microbial antigens in stool.

- ❖ ELISA is available for detection of rotavirus antigen in stool

Table 39.5: Identification features/ detection methods of common organisms causing acute diarrhea or dysentery.		
Enteric bacteria	Presentation	Identification features
<i>Vibrio cholerae</i> (Figs 42.3 and 42.4B)	Watery diarrhea	Darting motility Coma-shaped gram-negative bacilli in culture smear TCBS agar: Sucrose fermenting yellow colored colonies Identification: By conventional biochemical tests or automated methods like VITEK or MALDI-TOF Agglutinates with <i>Vibrio cholerae</i> O1 antisera and ogawa antisera (this is the most common pattern; though other serotypes are also present)
<i>Shigella</i>	Dysentery	Gram-negative bacilli, non-motile MAC or DCA: Non-lactose fermenting translucent colonies XLD: Red colonies without black center Identification: By conventional biochemical tests or automated methods like VITEK or MALDI-TOF Agglutinates with <i>Shigella</i> polyvalent antisera and specific monovalent antisera
Group B <i>Salmonella</i>	Inflammatory diarrhea	Gram-negative bacilli, motile MAC: Non-lactose fermenting translucent colonies DCA: Non-lactose fermenting colonies with black center XLD: Red colonies with black center Identification: By conventional biochemical tests or automated methods like VITEK or MALDI-TOF Agglutinates with <i>Salmonella</i> poly-O antisera and serotype (O ₄) specific antisera
Viral agents	Diarrhea	Agents: Rotavirus, Norovirus, Adenovirus 40, 41, etc. • Detection of viral particles in stool specimen by electron microscopy • Detection of viral antigen by ELISA or • Detection of nucleic acid (RNA or DNA) by PCR in stool specimen
Intestinal parasites	Presentation	Stool microscopy detects
<i>Entamoeba histolytica</i>	Dysentery	• Trophozoites and/or quadrinucleated round cyst • Detection of specific antigen (e.g. lectin)/specific genes in stool
<i>Giardia intestinalis</i>	Fatty diarrhea	Trophozoites (tear drop-shaped binucleated) with four pairs of flagella and/or Tetra-nucleated oval cyst with a central axoneme
<i>Trichuris</i>	Dysentery	Barrel-shaped eggs with mucus plugs at both ends, bile stained
<i>Enterobius vermicularis</i>	Nocturnal anal pruritus	Plano-concave egg containing larva, nonbile stained
<i>Ascaris lumbricoides</i>	Malabsorption	Fertilized egg: Round-oval, thick albumin coat, floats in saturated saline, bile stained Unfertilized egg: Elongated, thin albumin coat, does not float in saturated saline, bile stained
Hookworm	Diarrhea, anemia	Egg: Oval, contains segmented ovum with four blastomeres, nonbile stained
<i>Strongyloides</i>	Diarrhea	Detection of rhabditiform larva in stool microscopy

Abbreviations: TCBS, thiosulfate citrate bile salts sucrose agar; MAC, MacConkey agar; DCA, deoxycholate citrate agar; XLD, xylose lysine deoxycholate agar; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

- ❖ Rapid tests such as immunochromatographic test is available for simultaneous detection of antigens of three parasites *Entamoeba histolytica*, *Giardia* and *Cryptosporidium* in stool
- ❖ Latex agglutination test is available to detect *E. coli* O157 antigen in stool
- ❖ Rapid test is available to detect *C. difficile* antigens (glutamate dehydrogenase and toxin A/B) in stool.

Molecular Methods

Several molecular test formats are available for detection of gastrointestinal pathogens.

- ❖ Polymerase chain reaction (PCR) assays can be carried out for the detection specific genes of enteric pathogens
- ❖ **BioFire FilmArray:** It is a fully automated commercial nested multiplex PCR. Its gastrointestinal panel is used to detect common bacterial, viral, parasitic diarrheal pathogens.

Other Methods

- ❖ **Methylene blue preparation** can be performed in unformed stool to detect fecal leucocytes, which is suggestive of inflammatory diarrhea
- ❖ **Fecal lactoferrin** detection is also suggestive of inflammatory diarrhea. Fecal lactoferrin is an iron-binding protein found inside the neutrophils. The amount of lactoferrin released by neutrophils has been shown to correlate with the severity of intestinal inflammation.

TREATMENT

Diarrhea

Treatment depends up on the severity.

- ❑ Fluid therapy (rehydration) is the main stay of treatment
- ❑ Anti-motility agents and adsorbents may be considered in moderate-to-severe diarrhea
- ❑ Empiric antibiotic therapy is required only for severe diarrhea (Table 39.6).

Table 39.6: Treatment of diarrheal diseases (adapted from Sanford guideline).

Types of diarrhea	Definition	Treatment
Mild diarrhea	1–2 unformed stools per day Minimal symptoms No interference with daily routine activities	Hydration: Fluids Lactose-free diet and avoid caffeine No antibiotics required
Moderate diarrhea	3–5 unformed stools per day With/without symptoms, Interferes with daily routine activities	Fluids + Anti-motility agents (loperamide) + Adsorbents (bismuth subsalicylate) No antibiotics required
Severe diarrhea	≥6 unformed stools per day ± temperature ≥101°F, tenesmus, blood or fecal leukocytes	Empirical: Ciprofloxacin or levofloxacin for 3–5 days Alternatives: • Azithromycin (for 3 days) for <i>Campylobacter</i> • Metronidazole or vancomycin for 10–14 days (for <i>C. difficile</i>)

OTHER INFECTIVE SYNDROMES OF GIT

1. Acute Nausea and Vomiting

Although nausea and vomiting are a part of manifestations of many of the diarrheal illnesses, there are few infectious conditions where acute nausea and vomiting are the main clinical features.

- ❖ **Food poisoning** due to agents with short incubation period (*S. aureus* and *B. cereus* emetic type), chiefly presents as acute onset of nausea and vomiting within 6h of ingestion of food contaminated with toxins (Chapter 40)
- ❖ **Winter vomiting disease** or also called gastric flu, is a clinical condition presenting with abdominal discomfort, vomiting and/or diarrhea. It commonly occurs in winter months in temperate climates. Although etiologic agents are rarely identified, studies have shown that certain caliciviruses such as Noroviruses (Norwalk virus) and Sapoviruses have been commonly implicated (Chapter 44).

2. Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is a fulminant disease that affects typically the premature infants and accounts for major cause of mortality in the low-birth-weight infants.

- ❖ It is characterized by necrosis of the bowel wall with mucosal sloughing; the most common site being terminal ileum
- ❖ This devastating syndrome often leads to intestinal perforation, peritonitis, and bacteremia
- ❖ The agents that are commonly implicated include *Pseudomonas*, *Klebsiella*, certain *E. coli* strains (EPEC serotype O111:B4), *Salmonella*, *Clostridium perfringens* and *C. butyricum*.

3. Necrotizing Enteritis

It is also called as enteritis necroticans (gas gangrene of the bowel). It is a life-threatening condition characterized by ischemic necrosis of the jejunum and gas in the tissue plane.

- ❖ It is also known as **pigbel** in Papua New Guinea and **darmbrand** in Germany
- ❖ It is caused by *C. perfringens* type C strains, producing β -toxin.

Clostridium perfringens predominantly causes skin and soft tissue infection (gas gangrene), discussed in Chapter 53.

4. Pseudomembranous Enterocolitis

This condition is produced by *Clostridioides difficile*, which typically occurs in patients on prolonged therapy with broad spectrum antimicrobials. It is characterized by formation of pseudomembrane on colonic mucosa; which are whitish-yellow plaque of size ranging from 1 to 2 mm spread over the colonic mucosa (Chapter 43).

5. Tropical Sprue

It is a malabsorption disease commonly found in tropical regions, characterized by malabsorption of at least two distinct nutrients, abnormal duodenal histopathology (abnormal flattening of the villi and inflammation of the endothelial lining) and weight loss.

- ❖ **Epidemiology:** It is most frequent in Asia and the Caribbean islands, common in adults than children; usually affects long-term travelers to endemic regions
- ❖ **Etiology:** Though the etiology is not clear, there is strong presumption that it is caused by enteric infections, perhaps in individuals predisposed by some nutritional deficiency such as folic acid. Patients often show increased small bowel growth with various gram-negative rods, including *Alcaligenes*, *Enterobacter*, *Klebsiella*, *Escherichia coli*, and *Enterobacter*, etc.
- ❖ **Treatment** includes folic acid and tetracycline.

6. Esophagitis

Infections in the mucosa of esophagus (esophagitis) can cause painful or difficult swallowing or the sensation that something is lodged in the throat while swallowing.

- ❖ Individuals who have esophagitis usually have local or systemic underlying illnesses such as hematologic malignancies or HIV infection, or they are receiving immunosuppressive therapy
- ❖ The most common etiologic agents are *Candida* spp. (primarily *C. albicans*), herpes simplex virus, and cytomegalovirus.

7. Gastritis

Gastritis refers to inflammation of the gastric mucosa. This illness is associated with nausea and upper abdominal pain; vomiting, burping, and fever may also be present.

- ❖ *Helicobacter pylori* is associated with majority of cases, recovered from gastric biopsy (Chapter 43)
- ❖ *H. pylori* is also the causative agent of peptic ulcer disease (formation of ulcers in stomach and duodenum) and is a significant risk factor for the development of gastric cancer
- ❖ Peptic ulcer disease presents with epigastric pain with burning sensation. It is the most common symptom—classically occurs in an empty stomach with gastric ulcers and 2-3 hours after food intake in duodenal ulcers.

8. Appendicitis

Appendicitis is acute inflammation of the vermiform appendix, leading to complications such as intestinal obstruction, perforation, peritonitis, and intra-abdominal abscesses.

- ❖ It usually affects adolescence and early adulthood
- ❖ The infection is usually polymicrobial, frequently involving aerobic and anaerobic gram-negative bacilli
- ❖ Diagnosis is usually made clinically, supported by ultrasonography
- ❖ Surgical removal of the appendix, provides the definitive treatment. Adjunctive use of broad-spectrum antibiotics may also be required.

9. Diverticulitis

It is characterized by inflammation of abnormal pouches—diverticula—which can develop in the wall of the large intestine. It may be associated with polymicrobial infection.

10. Typhlitis

Also called as neutropenic enterocolitis. It refers to inflammation of the cecum, associated with polymicrobial infection such as enteric aerobic and anaerobic

organisms, including *Clostridium*, *Enterococcus*, *Enterobacteriaceae*, and *Pseudomonas aeruginosa*. Use of cytotoxic chemotherapeutic agents is the most common predisposing factor.

11. Proctitis

Proctitis is the inflammation of the rectum; presents with itching and mucous discharge per rectum, which subsequently progresses to formation of ulcers and abscesses in the rectum.

- ❖ The majority of infections are sexually transmitted through anal intercourse (Chapter 77)
- ❖ *Chlamydia trachomatis*, herpes simplex virus, *Treponema pallidum*, and *Neisseria gonorrhoeae* are the common etiologic agents.

12. Whipple's Disease (*Tropheryma whipplei*)

It is a rare disease affecting small intestine and also other organs such as CNS and endocardium; caused by a gram-positive actinomycetes; called *Tropheryma whipplei*.

- ❖ **Manifestation:** Involves impaired breakdown of foods, such as fats and carbohydrates, and decreased ability to absorb nutrients. Common symptoms include fever, abdominal pain, diarrhea, weight loss and migratory polyarthralgia. Mesenteric lymph nodes are primarily involved
- ❖ **Laboratory diagnosis:** Histopathological staining of intestinal biopsy shows prominent macrophage infiltration, fat deposition and characteristic pathognomonic vacuoles within the macrophage containing periodic acid-Schiff (PAS) stain positive bacilli. PCR targeting 16S ribosomal RNA can be done to identify the bacilli. Culture of *T. whipplei* has been unsuccessful
- ❖ **Treatment:** Comprises of doxycycline and hydroxychloroquine; given for 1 year, followed by lifelong doxycycline. Relapse may be seen up to 40% of cases which receive incomplete treatment (<1 year).

EXPECTED QUESTIONS

I. Write essay on:

Case scenario-1: A 6-year-old boy developed severe watery diarrhea (12–15 times) and vomiting since 2 days. Stool collected has a rice water type of appearance. On inquiry, it was found that two other members of same family and few children of the same locality also suffered from similar presentation last week.

Case scenario-2: In an outpatient department, a 2-year-old child presented with tenesmus, abdominal pain and passage of bloody diarrhea with mucus, eight times for the past 2 days.

In both case scenarios, stool specimens were collected in sterile containers and sent for microscopy, and culture.

Questions:

1. What is your probable clinical diagnosis?
2. What are the etiological agents, pathogenesis and clinical manifestations?
3. Describe the laboratory diagnosis in detail.
4. What are the treatment modalities according to the etiological agents?

CHAPTER PREVIEW

- Etiology
- Food Poisoning with Onset within 1–6 Hours
- Food Poisoning with Onset within 8–16 Hours
- Food Poisoning with Onset >16 Hours
- Botulism
- Mycotoxicoses

INTRODUCTION

Food poisoning refers to an illness acquired through consumption of food or drink contaminated either with microorganisms, or their toxins. It usually results in common-source outbreak of diarrhea.

- ❖ Food-borne illness is a significant public health problem. It is a major cause of morbidity and an infrequent cause of mortality
- ❖ Globally, an estimated 600 million (1 in 10) are affected with food-borne illness and 4.2 lakh die every year
- ❖ Children under 5 years of age carry 40% of the foodborne disease burden, with 1.25 lakh deaths every year

- ❖ Diarrheal diseases are the most common illnesses resulting from the consumption of contaminated food, affecting 550 million people with 2.3 lakh deaths every year.

ETIOLOGY

The microbial causes of food poisoning have been listed in Table 40.1. The various etiological agents have specific food source and have different incubation time which can be grouped into <6 hr, 8–16 h, >16 h.

Several non-microbial agents can cause food poisoning such as capsaicin (found in hot peppers), a variety of toxins found in fish and shellfish, and some chemical poisons.

Table 40.1: Microbial agents of food poisoning.

Organisms	Symptoms	Common food sources
1–6 h incubation period		
<i>Staphylococcus aureus</i>	Vomiting, diarrhea, abdominal cramps	Ham, poultry, salad, mayonnaise, pastries, dairy products
<i>Bacillus cereus</i>	Vomiting, diarrhea, abdominal cramps	Fried rice
8–16 h incubation period		
<i>Clostridium perfringens</i>	Abdominal cramps, diarrhea	Beef, poultry, legumes, gravies
<i>B. cereus</i>	Abdominal cramps, diarrhea	Meats, vegetables, dried beans, cereals
>16 h incubation period		
<i>Vibrio cholerae</i>	Watery diarrhea	Shellfish, water
Enterotoxigenic <i>E. coli</i>	Watery diarrhea	Salads, cheese, meat, water
Enterohemorrhagic <i>E. coli</i>	Bloody diarrhea	Ground beef, salami, milk, raw vegetables, apple juice
Non-typhoidal salmonellae	Inflammatory diarrhea	Meat, eggs, milk, juice, raw fruits and vegetables
<i>Shigella</i> species	Dysentery	Potato, egg salad, lettuce, raw vegetables
<i>Vibrio paraahaemolyticus</i>	Dysentery	Raw or undercooked shellfish, particularly oysters
<i>Campylobacter jejuni</i>	Inflammatory diarrhea	Poultry, raw milk or water
<i>Clostridium botulinum</i> *	Flaccid paralysis, diplopia, dysphagia	Homemade improperly canned food, and honey (infants)
<i>Listeria monocytogenes</i>	Fever and myalgia (pregnant women)	Soft cheeses, raw sprouts, meats, seafood, and milk
Norovirus	Watery diarrhea, vomiting, abdominal cramps	Salads, fresh fruits, shellfish (such as oysters), or water
<i>Cyclospora</i>	Watery diarrhea, abdominal cramps	Raw fruits or vegetables and herbs
Mycotoxicoses (6–24h)	Depends on type of fungal toxins, e.g. aflatoxin- causes hepatoma	Nuts, maize, wheat, cereals, etc.

*In food botulism, the symptoms can begin as early as 6 hours after or up to 10 days later.

FOOD POISONING WITH ONSET WITHIN 1–6 HOURS

The pathogens which liberate preformed toxins in food have a short incubation period. The symptoms of food poisoning can commence within 1–6 h of consumption of food as the toxins are already present in food. The classical examples include, *S. aureus* enterotoxin and *Bacillus cereus* emetic toxin.

Staphylococcal Food Poisoning

Staphylococcal food poisoning is usually caused by contamination of food from infected human carriers. If food is left for a long time after cooking, the organisms get an opportunity to multiply and produce enterotoxin.

- ❖ **Enterotoxin** is expressed by nearly 50% of *S. aureus* strains. It is a **preformed toxin** (secreted in food before consumption) so that it can act rapidly. As a result, the incubation period is short (1–6 hours)
- ❖ **Site of action:** The toxin stimulates the vagus nerve and the vomiting center of the brain. It also appears to stimulate the intestinal peristaltic activity
- ❖ **Symptoms:** Staphylococcal food poisoning is characterized by nausea, vomiting, occasionally diarrhea, hypotension, and dehydration; however, there is no fever. Symptoms generally resolve within 8–10 hours
- ❖ **Most common source** of infection is a food handler, who is a carrier of *S. aureus*. There is no secondary spread
- ❖ **Most common food items** involved are ham, poultry, salad, mayonnaise, pastries, and dairy products
- ❖ Staphylococcal enterotoxin is a heat stable toxin and is resistant to gastric juice
- ❖ **Serotyping:** Enterotoxins can be typed into 15 serotypes (A–E, G–P); all act as superantigens
 - Type A is most common to cause food poisoning
 - Serotype-F does not cause food poisoning; but causes toxic shock syndrome.
- ❖ **Detection of enterotoxin** in food is carried out by ELISA or latex agglutination test or by detecting enterotoxin gene by multiplex PCR (polymerase chain reaction)
- ❖ **Treatment** is entirely supportive by correcting fluid and electrolyte imbalance.

Bacillus cereus Infections

It is a normal habitant of soil, also widely isolated from food items such as vegetables, milk, cereals, spices, meat and poultry.

Clinical Manifestations

Food poisoning: *B. cereus* is an important agent of food poisoning in man; mediated by producing two types of toxins—diarrheal toxin (causes diarrheal type of food poisoning) and emetic toxin (causes emetic type of food poisoning) (Table 40.2).

- ❖ **Emetic toxin:** It is a heat stable preformed toxin, resembling *S. aureus* enterotoxin

Table 40.2: Differences between diarrheal type and emetic type of food poisoning of *Bacillus cereus*.

<i>Bacillus cereus</i>	Diarrheal type	Emetic type
Incubation period	8–16 hours	1–5 hours
Toxin	Secreted in intestine (Similar to <i>E. coli</i> heat labile enterotoxin)	Preformed toxin (formed in diet, similar to <i>S. aureus</i> enterotoxin)
Heat	Heat labile	Heat stable
Food items contaminated	Meat, vegetables, dried beans, cereals	Rice (Chinese fried rice)
Clinical feature	Diarrhea, fever, abdominal cramps	Vomiting, abdominal cramps
Serotypes involved	2, 6, 8, 9, 10, 12	1, 3, 5

- It acts immediately on intestine so that the incubation period of food poisoning is short (1–6 hours)
- It is associated with the consumption of contaminated fried rice with emetic toxin
- The heat-resistant spores of *B. cereus* can survive boiling. If cooked rice is not refrigerated, the spores can germinate and produce toxin. Frying before serving may not destroy the preformed, heat-stable toxin
- The symptoms are similar to that of *S. aureus* food poisoning.
- ❖ **Diarrheal toxin:** Organism secretes this toxin only after entering into the intestine, hence the incubation period is longer (8–16 hours).

The other manifestations of *B. cereus* include:

- ❖ **Ocular disease:** It causes severe keratitis and panophthalmitis following trauma to the eye that may lead to loss of vision
- ❖ **Systemic infections:** It rarely causes systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia. The presence of a medical device or intravenous drug use predisposes to these infections.

Laboratory Diagnosis

Bacillus cereus can be isolated from feces by using selective media such as MYPA (mannitol, egg yolk, polymyxin B, phenol red and agar). It is a gram-positive spore bearing motile bacillus.

TREATMENT

Bacillus cereus Infections

Bacillus cereus is susceptible to clindamycin, erythromycin, vancomycin and tetracycline. It is resistant to penicillin (by producing β -lactamase) and trimethoprim.

FOOD POISONING WITH ONSET WITHIN 8-16 HOURS

These organisms produce toxin in the intestine after consumption of the food and therefore have a longer incubation period.

- ❖ ***B. cereus*** (diarrheal type of food poisoning): It is mediated by the diarrheal toxin which resembles to *E. coli* heat labile enterotoxin. Here, diarrhea and abdominal cramps are characteristic but vomiting is uncommon (Table 40.2)
- ❖ ***Clostridium perfringens*** food poisoning: It has a slightly longer incubation period (6–24 h) and results from the survival of heat-resistant spores in inadequately cooked beef meat, poultry, or legumes
 - After ingestion, toxin is produced in the intestinal tract, causing moderately severe abdominal cramps and diarrhea; vomiting and fever are uncommon
 - The illness usually begins suddenly, is self-limited and lasts for less than 24 hours
 - *C. perfringens* mainly causes skin and soft-tissue infections (gas gangrene) (Chapter 53).

FOOD POISONING WITH ONSET >16 HOURS

There is a number of organisms for which the manifestation occurs late, >24 h after consumption of contaminated food (Table 40.1).

- ❖ ***Vibrio cholerae***: Symptoms begin 1–4 days after exposure; characterized by watery diarrhea, nausea, abdominal cramps, vomiting, fever and chills. Common food sources include contaminated drinking water or food such as seafood, raw fruits and vegetables (Chapter 42)
- ❖ **Enterotoxigenic *E. coli***: Causes watery diarrhea after 3–4 days of exposure; associated with contaminated salads, cheese, meat and water (Chapter 41)
- ❖ **Enterohemorrhagic *E. coli***: Causes bloody diarrhea; associated with contaminated ground beef, salami, raw milk, raw vegetables and apple juice. Around 5–10% of infected people develop a life-threatening complication (Chapter 41)
- ❖ ***Salmonella* (non-typhoidal)**: Symptoms begin 6 hours – 6 days after exposure; characterized by inflammatory diarrhea, fever, abdominal cramps and vomiting. Common food sources include contaminated chicken, turkey, and meat, eggs, unpasteurized (raw) milk, juice, raw fruits and vegetables (Chapter 41)
- ❖ ***Shigella***: Causes dysentery; associated with contaminated potato, egg, salad, and raw vegetables (Chapter 41)
- ❖ ***Campylobacter***: Symptoms begin 2–5 days after exposure: characterized by diarrhea (often bloody), abdominal cramps and fever. Common food sources include raw or undercooked poultry, raw (unpasteurized) milk, and contaminated water (Chapter 43)
- ❖ ***Vibrio parahaemolyticus***: Causes dysentery; associated with contaminated sea foods such as shellfish, particularly oysters (Chapter 42)
- ❖ ***Listeria***: Symptoms begin 1–4 weeks after the exposure, usually affects pregnant women who develop fever and other flu-like symptoms, such as fatigue and muscle aches. Elderly people may develop meningitis
 - Common food sources include soft cheeses, raw sprouts, meat, seafood, and raw milk
- Infections during pregnancy can lead to serious meningitis and sepsis in newborns (Chapter 71).
- ❖ **Norovirus**: Symptoms begin 12–48 hours after the exposure; characterized by diarrhea, abdominal pain, nausea and vomiting. Common food sources include contaminated salad, fresh fruits, shellfish (such as oysters), or water. Other sources include the infected person; touching contaminated surfaces (Chapter 44)
- ❖ ***Cyclospora***: Symptoms begin 1 week after the exposure; characterized by watery diarrhea, loss of appetite, and weight loss, abdominal pain, bloating, increased gas, nausea, and fatigue. Common food sources include raw fruits or vegetables and herbs
- ❖ **Botulinum food poisoning**: It is associated with consumption of homemade improperly canned or fermented food/beverages and honey (infants); contaminated with botulinum toxin of *Clostridium botulinum* (described subsequently in this chapter)
- ❖ **Mycotic poisoning** is a rare cause of food poisoning, caused by fungi (described subsequently in this chapter).

LABORATORY DIAGNOSIS (FOOD POISONING)

Meticulous history taking regarding the ingestion of specific foods and the time of onset of diarrhea after a meal can provide clues to the bacterial cause of the illness. Vomitus, stool or the suspected food materials are the ideal specimens.

Processing of Food Specimens

Viable Plate Count (Direct Quantification)

Viable plate count (or standard plate count) is the standard method for direct quantitative culture.

- ❖ **Food sampling**: 10 g of food material is taken in a sterile container and is homogenized in 90 mL of sterile diluent, e.g. Ringer's solution
- ❖ **For the food contaminated only on its surface**, such as intact vegetable or fruit, 100 g of food is taken in a sterile container containing 100 mL of sterile water and then shaken well so that all the bacteria present on its surface will come out and are dissolved in water
- ❖ **Food processing**: Serial dilutions of homogenate or diluent is made, and then plated onto appropriate medium.

Pre-enrichment Culture

Pathogenic bacteria from suspected foods can also be isolated by agar culture after pre-enrichment (which helps in recovery of any damaged organisms), followed by selective enrichment promoting the growth of the target organism.

Toxin Detection

- ❖ ELISA-based formats or latex agglutination test are available for the detection of enterotoxins in stool
- ❖ PCR for detection of genes coding for enterotoxins.

T TREATMENT**Food poisoning**

The mainstay of treatment is adequate rehydration and electrolyte supplementation. This can be achieved with either an oral rehydration solution (ORS) or intravenous solutions (e.g. isotonic sodium chloride solution, Ringer lactate solution).

- ❑ Other symptomatic treatment can be given such as absorbents (e.g. aluminum hydroxide) and antisecretory agents (e.g. bismuth subsalicylate)
- ❑ Antitoxin such as heptavalent botulism equine serum antitoxin can be given if food botulism is suspected
- ❑ Antibiotics such as penicillin or metronidazole may be attempted for botulism
- ❑ **Diet:** During episodes of acute diarrhea, patients often develop an acquired disaccharidase deficiency due to washout of the brush-border enzymes. For this reason, avoiding milk, dairy products, and other lactose-containing foods are advisable
- ❑ **Antibiotics:** Usually antibiotics do not play much role. It is mainly indicated for shigellosis, where fluoroquinolone (e.g. ciprofloxacin) is the first-line agent. In suspected cholera, azithromycin is the drug of choice.

BOTULISM

Clostridium botulinum is a gram-positive spore-forming obligate anaerobic bacilli. It produces a powerful toxin called botulinum toxin, which causes **botulism**, a rare disease manifested as various clinical syndromes ranging from food poisoning, wound infection to infant botulism.

- ❖ The term is derived from Latin word *botulus*, meaning sausage; as poorly cooked sausages were formerly associated with food poisoning
- ❖ It is ubiquitous in nature, widely distributed as saprophyte in soil, animal manure, vegetables and sea mud.

Pathogenesis

C. botulinum is non-invasive. Its pathogenesis is due to production of powerful neurotoxin 'botulinum toxin' (BT), probably the most toxic substance known to be lethal to mankind.

- ❖ **Serotype:** Botulinum toxin can be typed into eight serotypes—A, B, C1, C2, D, E, F and G
 - Serotypes A, B, E commonly cause human disease; most severe being serotype A
 - All serotypes produce neurotoxin; except C2 which produces an enterotoxin
 - BT types C and D are bacteriophage coded.
- ❖ BT differs from other exotoxins, as it is produced intracellularly, not secreted and appears outside only after autolysis of bacterial cell
- ❖ Toxin is synthesized initially as a nontoxic protoxin. It requires trypsin or other proteolytic enzymes to convert it into active form
- ❖ **Mechanism of action of BT:** It produces flaccid paralysis

Mechanism of Action of Botulinum Toxin (BT)

After entry (either ingested, inhaled, or produced in a wound), botulinum toxin is transported via blood to peripheral cholinergic nerve terminals.

- ❑ The most common nerve terminal sites are neuromuscular junctions, postganglionic parasympathetic nerve endings, and peripheral ganglia. It does not affect the CNS
 - ❑ BT binds to acetylcholine receptors on the nerve terminals at neuromuscular junction, which results in blockage of release of the acetylcholine, leading to flaccid paralysis.
- ❖ **Therapeutic uses:** As BT produces flaccid paralysis, it can be used therapeutically for the treatment of spasmodic conditions such as strabismus, blepharospasm and myoclonus
 - ❖ Botulinum toxin is also produced by other clostridia such as *C. butyricum*, *C. baratti* and *C. argentinense*
 - ❖ **Recovery:** Blocking of acetylcholine release is permanent, but the action is short lasting as the recovery occurs in 2–4 months, once the new terminal axons sprout
 - ❖ Spores do not produce toxins. Toxin production, therefore, requires spore germination, which occurs in anaerobic atmosphere. Spores do not normally germinate in adult intestine, however may germinate in the intestine of infants.

Clinical Manifestations

The manifestations of botulism are due to decreased acetylcholine in cranial nerve and parasympathetic nerve terminals. Manifestations appear after an incubation period of 18–36 hours; which include:

- ❖ Diplopia, dysphasia, dysarthria
- ❖ Descending symmetric **flaccid paralysis** of voluntary muscles
- ❖ ↓Deep tendon reflexes
- ❖ Constipation
- ❖ There is no sensory or cognitive deficits
- ❖ Respiratory muscle paralysis, may lead to death.

Types of Botulism

- ❖ **Food-borne botulism:** It results from consumption of foods contaminated with preformed botulinum toxin
 - Most common source: Homemade improperly canned or fermented food and beverages
 - Most cases are sporadic; outbreaks are rare.
- ❖ **Wound botulism:** It results from contamination of wounds with *C. botulinum* spores. It presents like food-borne botulism except for absence of gastrointestinal features. It can be recurrent in injection drug users (e.g. black tar heroin)
- ❖ **Infant botulism:** It is the most common type; accounts for 75% of total cases. It results from ingestion of contaminated food (usually **honey**) with spores of *C.*

botulinum in children ≤ 1 year of age. Spores germinate in the intestine releasing the toxin

- Manifestations include inability to suck and swallow, weakened voice, ptosis, floppy neck, and extreme weakness (hence called **floppy child syndrome**)
 - It is self-limiting, managed by supportive care and assisted feeding
 - Rarely, it progresses to generalized flaccidity, respiratory failure and sudden death.
- ❖ **Adult intestinal botulism:** Rarely, in patients with suppressed normal flora, the colonized clostridial spores may germinate producing toxin; as in infant botulism
 - ❖ **Iatrogenic botulism:** It results from injection of overdose of the toxin while used for therapeutic purpose
 - ❖ **Inhalational botulism:** Aerosolization of spores may occur as in act of bioterrorism.

Laboratory Diagnosis

Diagnosis of botulism includes isolation of the bacilli and demonstration of the toxin.

Isolation of the Bacilli

- ❖ Gram staining of smears made from suspected food or feces-reveals gram-positive, non-capsulated bacilli with subterminal, oval, bulging spores
- ❖ It is motile by peritrichate flagella
- ❖ **Isolation:** Culture is done on blood agar or Robertson's cooked meat (RCM) broth. In RCM broth, they can be either proteolytic and saccharolytic; turning the meat particles into black and pink respectively
- ❖ Growth on culture media may be confirmed by Gram staining and biochemical tests or by automated methods such as MALDI-TOF
- ❖ Serotyping is done with type specific antisera

- ❖ Mere presence of bacilli in food or feces is of less significance. Toxin demonstration is more meaningful.

Toxin Demonstration (Mouse Bioassay)

Toxins can be detected in the specimens (serum, stool, wound material) or in samples of ingested foods.

- ❖ Specimens are injected into mouse, that develops paralysis in 48 hours; which can be inhibited by prior administration of specific antitoxin
- ❖ The sensitivity of the mouse bioassay varies inversely with the time elapsed between onset of symptoms and sample collection.

TREATMENT

Botulism

- Meticulous intensive care support is needed (such as mechanical ventilation, if respiratory paralysis develops)
- **Botulinum antitoxin:** It should be administered immediately on clinical suspicion, without waiting for laboratory confirmation. Earlier the administration, better is the cure rate because antitoxin can neutralize the unbound free toxin molecules. However, once toxin binds to nerve endings, antitoxin has no role
- In wound botulism, suspected wounds and abscesses should be cleaned, debrided, and drained promptly. Antibiotics such as penicillin or metronidazole may be useful.

MYCOTOXICOSES

Mycotic poisoning is a rare cause of food poisoning, caused by fungi. The disease can be classified into two varieties:

1. **Mycotoxicosis:** Refers to the disease produced by consumption of food contaminated with toxins liberated by certain fungi (Table 40.3)
2. **Mycetism:** Refers to the toxic effects produced by eating poisonous fleshy fungi; usually different types of mushrooms (Table 40.4).

Table 40.3: Features of common mycotoxicosis.

Mycotoxin	Produced by fungal species	Source	Clinical condition
Aflatoxin	<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i> , <i>A. nomius</i> <i>Penicillium puberulum</i>	Nuts, maize	Hepatoma, hepatitis Indian childhood cirrhosis Reye's syndrome
Fumonisin	<i>Fusarium moniliforme</i>	Maize	Equine leukoencephalomalacia Porcine pulmonary edema Carcinoma esophagus
Trichothecenes	<i>Fusarium graminearum</i>	Maize, wheat, sorghum	Alimentary toxic aleukia Biological warfare (yellow rain)
Ochratoxin	<i>Aspergillus ochraceus</i> , <i>A. niger</i> <i>Penicillium verrucosum</i>	Cereals, bread	Nephropathies (Balkan endemic nephropathy)
Cyclopiazonic acid	<i>Aspergillus flavus</i> , <i>A. versicolor</i> , <i>A. oryzae</i> <i>Penicillium cyclopium</i>	Groundnut, corn	Kodua poisoning Co-contaminant with aflatoxin
Zearalenones	<i>Fusarium graminearum</i>	Wheat, maize	Genital disorder in pigs

Table 40.4: Features of common mycetism.

Mushroom poisoning	Produced by fungal species	Source	Clinical condition
Ergot alkaloid	<i>Claviceps purpurea</i>	Rye flour	St. Anthony's fire
Coprine poisoning	<i>Coprine atramentarius</i>	Butter	Antabuse like reaction
Muscarine	<i>Inocybe fastigiata</i>	Food	Cholinergic effect
Ibotenic acid, muscimol	<i>Amanita pantherina</i>	Edible mushroom	Abdominal pain, vomiting, diarrhea
Cyclopeptide	<i>Amanita phalloides</i>	Edible mushroom	Hepatocellular failure, green death cap

CHEMICAL CAUSES OF FOOD POISONING

There are several non-microbial agents that can cause food poisoning such as capsaicin (found in hot peppers), variety of toxins found in fish and shellfish, ciguatera, histamine-fish poisoning (scombroid), and some chemical poisons (e.g. heavy metals).

Scombroid Food Poisoning

Morganella morganii, a commensal in sea fish can breakdown histidine present in sea fish into histamine, which can cause food poisoning following seafood intake. Proper storage of fish in freezer (<16°C) can prevent this condition. It is a gram-negative bacillus, belongs to Enterobacteriaceae family.

EXPECTED QUESTIONS

I. Write essay on:

- A group of patients presented to the emergency department with chief complaints of fever, vomiting and diarrhea. All of them had attended a birthday party 4–6 hours back.
 - What is your probable clinical diagnosis?
 - What are the etiological agents, pathogenesis and clinical manifestations?
 - Describe the laboratory diagnosis in detail.
 - What are the treatment modalities according to the etiological agents?

II. Write short notes on:

- Bacillus cereus* food poisoning.
- Food botulism.

III. Multiple Choice Questions (MCQs):

- Food poisoning associated with contaminated Chinese fried rice:**

- Staphylococcus aureus*
- Bacillus cereus*
- Clostridium perfringens*
- Vibrio cholerae*

2. Scombroid food poisoning is due to:

- Morganella morganii*
- Staphylococcus aureus*
- Clostridium perfringens*
- Vibrio cholerae*

3. *Staphylococcus aureus* enterotoxin, all are true, except:

- Preformed toxin
- Incubation period is short (1–6 hours)
- The toxin stimulates the vagus nerve and the vomiting center of the brain
- Treatment is mainly by early institution of antibiotics

Answers

1. b 2. a 3. d

Gastrointestinal Infections due to Enterobacteriaceae

CHAPTER 41

CHAPTER PREVIEW

- Family Characters (General Properties)
- *Shigella*
- Non-GI Pathogens
- *Escherichia coli*
- Non-typhoidal Salmonellae

Family Enterobacteriaceae includes the commensal bacteria in the human intestine called **coliform bacilli**, include such as *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Morganella*, *Providencia* and *Citrobacter*, etc. and also include some enteric pathogens, such as *Shigella* and *Salmonella*.

FAMILY CHARACTERS (GENERAL PROPERTIES)

Members of the family Enterobacteriaceae should have the following properties:

- ❖ They are gram-negative bacilli
- ❖ Aerobes and facultative anaerobes
- ❖ Nonfastidious, can grow in basal media like nutrient agar
- ❖ Ferment glucose to produce acid with or without gas
- ❖ Reduce nitrate to nitrite
- ❖ They produce catalase (except *Shigella dysenteriae* type-1)
- ❖ They do not produce oxidase
- ❖ They are generally motile with peritrichous flagella, except for some members which are nonmotile, such as *Shigella* and *Klebsiella*.

Classification

- ❖ **The oldest method of classification** of the family Enterobacteriaceae was based on fermentation of lactose on MacConkey agar (Table 41.1). It is still the most widely used classification, has a great practical application in laboratories to differentiate various members
- ❖ **Ewing's classification:** Though several classification schemes were proposed for Enterobacteriaceae, the **Ewing's classification** was the most popular. It classifies the family Enterobacteriaceae into eight tribes; each tribe further comprises of several genera (Table 41.2). Use of tribe has a great impact in the laboratory for easy identification, as genera under each tribe share common properties
- ❖ **Newer method of classification:** After the availability of molecular methods, the taxonomy is further changed. In 2016, the order 'Enterobacterales' was given the priority and several new families within the Enterobacterales

were proposed, consisting of species that were formerly members of the family Enterobacteriaceae. However, Ewing's classification is still commonly followed.

The members of Enterobacteriaceae that are gastrointestinal (GI) pathogens are discussed below. The members which mainly cause non-GI manifestations are discussed in the respective systems which they principally infect.

ESCHERICHIA COLI INFECTIONS

It was described first by Escherich in 1885. *E. coli* is the most important species encountered as human pathogen.

Table 41.1: Classification of family Enterobacteriaceae based on lactose fermentation.

Groups	Colonies on MacConkey agar	Examples
Lactose fermenters (LF)	Ferment lactose, produce pink colonies	<i>Escherichia</i> <i>Klebsiella</i>
Non-lactose fermenters (NLF)	Do not ferment lactose, produce pale or colorless colonies	<i>Salmonella</i> , <i>Shigella</i> , <i>Proteus</i> and <i>Yersinia</i>
Late lactose fermenters (LLF)	Ferment lactose late, produce pink colonies only after 2 days of incubation	<i>Shigella sonnei</i>

Table 41.2: Ewing's classification of family Enterobacteriaceae.

Tribe	Genus
Tribe I: Escherichieae	<i>Escherichia</i> , <i>Shigella</i>
Tribe II: Edwardsielleae	<i>Edwardsiella</i>
Tribe III: Salmonelleae	<i>Salmonella</i>
Tribe IV: Citrobactereae	<i>Citrobacter</i>
Tribe V: Klebsielleae	<i>Klebsiella</i> , <i>Enterobacter</i> , <i>Hafnia</i> , <i>Serratia</i> , <i>Pantoea</i>
Tribe VI: Proteeae	<i>Proteus</i> , <i>Morganella</i> , <i>Providencia</i>
Tribe VII: Yersinieae	<i>Yersinia</i>
Tribe VIII: Erwinieae	<i>Erwinia</i>

- ❖ It is also the most common aerobe to be harbored in the gut of humans and animals
- ❖ After excreted in feces, it remains viable only for some days in the environment. Hence, detection of *E. coli*, especially a variant called **thermotolerant *E. coli*** (survives at 44°C) is taken as an indicator of recent contamination of drinking water with human or animal feces (Chapter 27)
- ❖ Other species are less important as human pathogens. These include *E. fergusonii*, *E. hermannii* and *E. vulneris* which are rarely isolated from clinical specimens.

Virulence Factors of *E. coli*

Virulence factors of *E. coli* may be grouped into surface antigens and toxins.

Surface antigens

E. coli possesses four surface antigens—(1) somatic (O), (2) flagellar (H), (3) capsular antigens (K), and (4) fimbrial antigen.

- Serotyping of *E. coli* is based on agglutination with the specific antisera directed against each surface antigen
- So far more than 174 O serotypes, 100 K serotypes and 53 H serotypes of *E. coli* have been recognized
- The strain of *E. coli* is designated based on the serotype number of its antigens; for example, O121:K37:H8.

1. **Somatic or O antigen:** It is a side chain present on lipopolysaccharide (LPS) antigen. It is a major surface antigen; induces antibody formation
2. **Flagellar or H antigen** (H from *Hauch*, meaning film of breath): It is responsible for bacterial motility and therefore contributes to their virulence
3. **Capsular or K antigen** (K for Kapsel, German for capsule):
 - It is the polysaccharide capsular antigen present on the envelope or microcapsule
 - When present, it encloses O antigen and renders the strain inagglutinable by O antiserum

Contd...

Contd...

- It may contribute to virulence by inhibiting phagocytosis
- It is expressed by only few strains of *E. coli*; e.g. those causing neonatal meningitis, pyelonephritis and septicemia.

4. **Fimbrial antigen** (pilus) is the organ of adhesion, helps in attachment and colonization. Various fimbrial antigens are:
 - **CFA** (colonization factor antigen): It is a type of fimbriae expressed by enterotoxigenic *E. coli*
 - **Mannose resistant fimbriae:** These are expressed by uropathogenic *E. coli* and help in colonization of these strains onto uroepithelial cells
 - **P fimbriae:** Bind specifically to the P blood group antigens present on human RBCs and uroepithelial cells.

Toxins

The exotoxins secreted by *E. coli* are of several types:

- **Enterotoxins:** They are produced by diarrheagenic strains of *E. coli*. They are of three types; heat labile toxin, heat stable toxin and verocytotoxin (all have been described in detail in Table 41.3)
- **Cytotoxic necrotizing factor 1 (CNF1) and secreted autotransporter toxin (SAT):** They are cytotoxic to bladder and kidney cells; act as virulence factor for pathogenesis of UTI.

Clinical Manifestations

E. coli is one of the most common pathogen encountered clinically and has been associated with various manifestations.

- ❖ **Urinary tract infection (UTI):** It is caused by uropathogenic *E. coli* (UPEC). UPEC accounts for 70–75% of all cases of UTI; discussed in Chapter 76
- ❖ **Diarrhea:** It is caused by six pathotypes of diarrheagenic *E. coli* (described later)
 1. Enteropathogenic *E. coli* (EPEC)
 2. Enterotoxigenic *E. coli* (ETEC)

Table 41.3: Various properties of enterotoxins of *Escherichia coli*.

LT (heat-labile toxin)	ST (heat-stable toxin)	Verocytotoxin or Shiga toxin
Produced by: Enterotoxigenic <i>E. coli</i>	Produced by: Enterotoxigenic <i>E. coli</i>	Produced by: Enterohemorrhagic <i>E. coli</i>
It resembles cholera toxin in its structure and function, but it is less potent than the latter Mechanism of action: It has 2 peptide fragments: A and B Fragment B: It is the binding fragment; binds to GM1 ganglioside receptors present on the intestinal epithelium following which A fragment is internalized Fragment A: It is the active fragment, causes ADP ribosylation of G protein → activates adenylate cyclase → results in the intracellular accumulation of cyclic AMP → leads to increased outflow of water and electrolytes into the gut lumen, with consequent diarrhea	Mechanism of action: It binds to the guanylate cyclase C → increased production of cyclic GMP → fluid accumulation in gut lumen → diarrhea	It is so named because it is cytotoxic to Vero cell lines Mechanism of action: It has two fragments: A and B Fragment B binds to a globotriosyl-ceramide (Gb3) receptor on intestinal epithelium Fragment A is the active fragment. It inhibits protein synthesis by inhibiting 60S ribosomal subunit
Plasmid-coded	Plasmid-coded	Bacteriophage-coded
Detection of LT: Toxin detection: by latex agglutination, ELISA. Molecular methods: PCR detecting gene coding for LT	Detection of ST: Same as for LT	Detection of VT: Serologically—latex agglutination, ELISA Molecular methods—using specific DNA probe Cytotoxicity on Vero and HeLa cell lines

Abbreviations: AMP, adenosine monophosphate; GMP, guanosine monophosphate.

3. Enteroinvasive *E. coli* (EIEC)
4. Enterohemorrhagic *E. coli* (EHEC)
5. Enteroaggregative *E. coli* (EAEC)
6. Diffusely adherent *E. coli* (DAEC).

❖ **Other infections:**

- Abdominal infections: *E. coli* is the most common cause of both primary bacterial peritonitis (occurs spontaneously) and secondary bacterial peritonitis (occurs secondary to intestinal perforation leading to spillage of commensal *E. coli* from intestine). It also causes visceral abscesses, such as hepatic abscess
- Pneumonia (especially in hospitalized patients—ventilator-associated pneumonia)
- Meningitis (especially neonatal meningitis)
- Wound and soft tissue infection such as cellulitis and infection of ulcers and wounds, especially in patient with diabetic foot
- Bacterial prostatitis (most common cause)
- Osteomyelitis
- Endovascular infection and bacteremia.

LABORATORY DIAGNOSIS

Escherichia coli Infections

- **Sample collection:** Depends on the site of infection—urine, stool, pus, wound swab, blood, CSF, etc.
- **Direct smear:** Gram-negative bacilli, and pus cells
- **Culture:**
 - Blood agar: Gray, moist colonies
 - MacConkey agar: Flat, pink LF colonies
- **Culture smear and motility:** Motile gram-negative bacilli
- **Identification:**
 - Catalase positive and oxidase negative
 - ICUT tests: Indole (+), Citrate (-), Urease (-), TSI: A/A, gas (+), H₂S (-)
 - Automated systems such as MALDI-TOF or VITEK
- **Antimicrobial susceptibility testing**

Laboratory Diagnosis

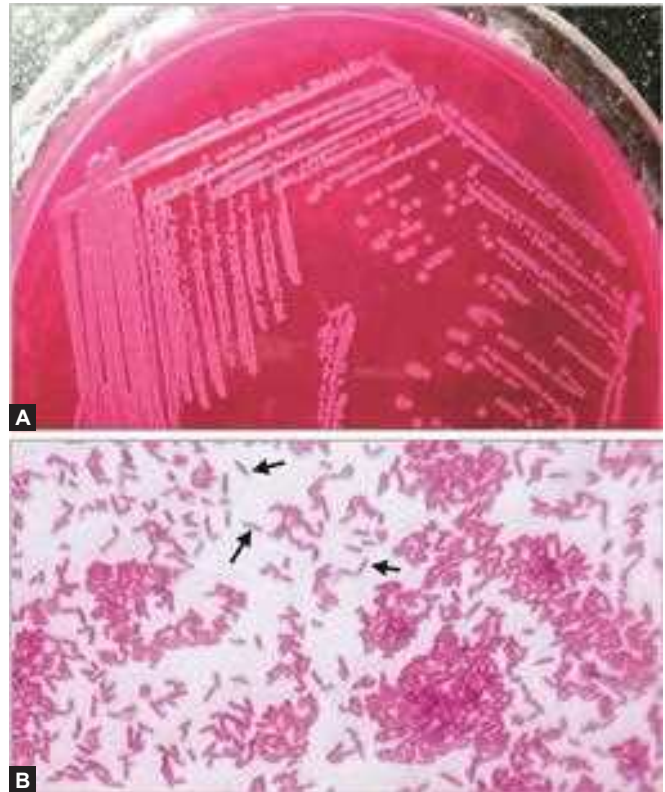
The laboratory diagnosis of UTI has been described in Chapter 76. Diagnostic methods specific for diarrheagenic *E. coli* has been described under diarrheagenic *E. coli*, later in this chapter.

- ❖ **Sample collection:** It depends on the site of infection (Table 41.4)

Table 41.4: Sample collection in *E. coli* infections.

Specimens collected	Disease
Pus, exudates and wound swab	Cellulitis or wound infection
Urine (midstream)	Urinary tract infections (UTI)
Stool	Diarrhea
Cerebrospinal fluid (CSF)	Meningitis
Peritoneal exudate	Peritonitis
Sputum	Pneumonia
Tracheal aspirate	Ventilator-associated pneumonia
Blood	Bacteremia

- ❖ **Direct smear of specimen by Gram staining:** *E. coli* is gram-negative, straight rod, arranged singly. Plenty of pus cells are also found in direct smear
- ❖ **Culture:** *E. coli* is an aerobe and facultative anaerobe. It grows on ordinary culture media at an optimum temperature of 37°C in 18–24 hours. The culture media used are as follows:
 - **Blood agar:** Colonies are gray moist and occasionally β hemolytic (mainly UPEC strains)
 - **MacConkey agar:** It produces moist, **flat and pink** colonies due to lactose fermentation (Fig. 41.1A).
- ❖ **Culture smear and motility testing:** Culture smear of the colonies shows gram-negative bacilli arranged singly (Fig. 41.1B). Hanging drop reveals motile bacilli (by peritrichate flagella)
- ❖ **Identification:** Identification of *E. coli* from colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as described below
 - *E. coli* is catalase positive and oxidase negative
 - **ICUT tests:** Indole test (positive), citrate test (negative), urease test (negative) and TSI (triple sugar iron agar) test shows acid/acid, gas present, H₂S absent.
- ❖ **Antimicrobial susceptibility testing:** It is necessary to administer appropriate antibiotics



Figs 41.1A and B: A. Flat pink lactose fermenting colonies of *E. coli* on MacConkey agar; B. Slender gram-negative bacilli (arrows showing).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- It is performed either by disk diffusion method on Mueller-Hinton agar or by automated MIC-based method (e.g. VITEK)
- *E. coli* can rapidly develop resistance to multiple drugs. Many strains of *E. coli* are producers of β -lactamases such as ESBL (extended spectrum β -lactamases), AmpC β -lactamases and MBL (metallo- β -lactamases).

TREATMENT

E. coli infections (other than diarrhea)

Treatment is essentially based up on antimicrobial susceptibility test report.

- Majority of *E. coli* acquired in hospitals are multi-drug resistant (MDR); resistant to ≥ 3 classes of antimicrobials such as aminocillins, third generation cephalosporins, quinolones, cotrimoxazole, etc. However, if found susceptible, then these agents may be preferred
- Carbapenems, amikacin or β -lactam/ β -lactamase inhibitor combinations (BL/BLIs) such as piperacillin-tazobactam or cefoperazone-sulbactam are usually the agent of choice for hospital acquired MDR *E. coli* infections
- Polymyxins, fosfomycin or tigecycline are the next line antimicrobials for carbapenem resistant isolates (e.g. carbapenamase producing isolates). In India, prevalence of **carbapenem resistant *E. coli*** is reported to be around 11-27% (2019)
- In healthcare facilities with high prevalence of MDR *E. coli*, empirical treatment should be started with higher spectrum antimicrobial such as carbapenem. The therapy can be tailored based on the susceptibility report.

Diarrhea (Diarrheagenic *E. coli*)

Diarrheagenic *E. coli* are antigenically distinct from the commensal *E. coli* which colonize the intestine. Only few serotypes of *E. coli* which express the enterotoxin or other virulence mechanisms can cause diarrhea.

- ❖ The WHO estimated that >300 million illnesses and nearly 200,000 deaths are caused by diarrheagenic *E. coli* globally each year
- ❖ There are six pathotypes of diarrheagenic *E. coli*, as described below.

Enteropathogenic *E. coli* (EPEC)

EPEC frequently causes **infantile diarrhea** (outbreaks) and occasionally cause sporadic diarrhea in adults.

- ❖ Person-to-person spread is seen
- ❖ It is nontoxigenic and noninvasive
- ❖ Mechanism of diarrhea includes:
 - **Adhesion** to intestinal mucosa, mediated by plasmid coded **bundle-forming pili**, which form cup-like projections called **pedestals**
 - **A/E lesions** (attaching and effacing lesions): These are typical lesions produced on the intestinal epithelium; which leads to disruption of brush border epithelium causing increased secretion and watery diarrhea.

Enterotoxigenic *E. coli* (ETEC)

ETEC is the most common cause of **traveler's diarrhea** causing 25-75% of cases.

- ❖ It causes acute watery diarrhea in infants and adults
- ❖ It is toxigenic, but not invasive
- ❖ **Pathogenesis of ETEC is by:**
 - **Attachment** to intestinal mucosa is mediated by fimbrial protein called **CFA** (colonization factor antigen)
 - **Toxin production**—(1) heat-labile toxin (acts by \uparrow cAMP), (2) heat-stable toxin (acts by \uparrow cGMP).
- ❖ **Diagnosis:** Detection of toxins is the mainstay of diagnosis (refer to Table 41.3).

Enteroinvasive *E. coli* (EIEC)

EIEC is biochemically, genetically and pathogenically closely related to *Shigella*.

- ❖ **Pathogenesis:** EIEC is not toxigenic, but invasive. The epithelial cell invasion is mediated by a plasmid-coded antigen called **virulence marker antigen (VMA)**
- ❖ **Manifestations:** These include ulceration of bowel, dysentery (diarrhea with mucus and blood, called bacillary dysentery resembling shigellosis)
- ❖ **Diagnosis:**
 - Detection of VMA by ELISA
 - HeLa cell invasion assay
 - Compared with other *E. coli* strains, EIEC are biochemically atypical being nonmotile, and lactose nonfermenters.

Enterohemorrhagic *E. coli* (EHEC)

EHEC is prevalent mainly in industrialized countries; in contrast to other diarrheagenic *E. coli* which are common in developing regions.

- ❖ **Serotypes** associated with EHEC: O157:H7 is the most common serotype. However other strains may account for up to 50% of EHEC infections; among which O104:H4 strain of EAEC is important
- ❖ EHEC is usually transmitted by contaminated food, i.e. consumption of lettuce, spinach, sprouts and under-cooked **ground beef**. The recent outbreak of EHEC (in 2020), was reported in United States, was due to consumption of clover sprouts contaminated with *E. coli* O103
- ❖ **Low infective dose:** The infective dose of EHEC is very low. Only few organisms ($<10^2$ bacilli) are required to initiate the infection
- ❖ **Pathogenesis:** EHEC secretes a toxin called **verocytotoxin** or **Shiga toxin**; therefore, EHEC is also called Shiga toxin producing *E. coli* (STEC)
 - It resembles with Shiga toxin produced by *Shigella dysenteriae* type 1
 - It acts by inhibiting the protein synthesis by inhibiting 60S ribosome.
 - It is of two types—Stx1 and Stx2.

- ❖ **Manifestations:** Shiga toxin has a predilection for endothelial cells causing capillary microangiopathy which leads to:
 - **HC (hemorrhagic colitis):** It manifests as gross bloody diarrhea, abdominal pain and fecal leukocytosis but no fever
 - **Hemorrhagic uremic syndrome (HUS):** It is characterized by injury to small vessels of the kidney and brain, which can lead to bloody diarrhea, thrombocytopenia, renal failure and encephalopathy but without fever. It is more common in children. HUS has 10% mortality and up to 50% of cases may retain some degree of permanent renal or CNS damage.
- ❖ **Diagnosis:**
 - **Special culture media** such as Sorbitol MacConkey agar (does not ferment sorbitol) and Rainbow agar are used for EHEC
 - **Toxin detection:**
 - ◆ Demonstration of cytotoxicity in Vero cell lines (gold standard method)
 - ◆ Fecal toxin detection by ELISA or rapid tests.
 - **DNA PCR probes:** Detects genes coding for Stx1 and Stx2 in feces.

Enteroaggregative *E. coli* (EAEC)

It is so named because it adheres to HEp-2 cells in a distinct pattern, layering of the bacteria aggregated in a stacked-brick fashion. Most strains are “O” untypeable but “H” typeable.

- ❖ **Pathogenesis:**
 - Intestinal colonization is mediated by aggregative adhesion fimbriae I (regulated by *aggR* gene)
 - It also produces **EAST 1 toxin** (enteroaggregative heat stable enterotoxin 1).
- ❖ **Manifestations:** Persistent and acute diarrhea are commonly seen; especially in developing countries. It can also cause traveler’s diarrhea and persistent diarrhea in infants
- ❖ **Diagnosis** is made by—(i) detection of *aggR* and *aatA* gene by PCR, and (ii) HEp-2 adherence test (gold standard).

E. coli O104: H4

It is an enteroaggregative strain that has caused major outbreaks in Germany in 2011. One peculiar feature of this strain is, it produces Shiga toxin and can cause HUS.

Diffusely-adherent *E. coli* (DAEC)

DAEC is characterized by:

- ❖ Ability to adhere to HEp-2 cells in a diffuse pattern
- ❖ Expresses diffuse adherence fimbriae which contribute to the pathogenesis
- ❖ DAEC is capable of causing diarrheal disease, primarily in children aged 2–6 years.

TREATMENT

Diarrheagenic *E. coli*

The mainstay of treatment is fluid replacement. The use of antimicrobials should generally be avoided. The following are the situations where antibiotics may be considered.

- ❑ **Traveler’s diarrhea (ETEC or EAEC):** Only for severe traveler’s diarrhea, azithromycin is indicated. Rifaximin, an oral non-absorbable antibiotic can also be given
- ❑ **EIEC:** Although the infection is self-limited, antibiotics such as azithromycin is indicated as it fastens the recovery, particularly in severe cases
- ❑ **EHEC and *E. coli* O104: H4:** Antibiotics such as cotrimoxazole, beta-lactams, metronidazole should be avoided as they precipitate HUS. If treatment has to be started (e.g. if positive blood culture), azithromycin is the preferable option
- ❑ **EAEC:** Only in immunocompromised patients, ciprofloxacin for 3-7 days is recommended.

SHIGELLA INFECTIONS (SHIGELLOSIS)

Shigella, the most important agent of bacillary dysentery, is named after Japanese microbiologist Kiyoshi Shiga who isolated the first member, *S. dysenteriae* serotype-1 (the *Shiga bacillus*) in 1896 from epidemic dysentery.

Classification

Based on a combination of biochemical and serological characteristics (O antigen), shigellae are classified into four species—*S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. Based on O antigen, each species is further typed into several serotypes.

- ❖ *S. dysenteriae* has 15 serotypes; whereas *S. flexneri* and *S. boydii* have 8 and 19 serotypes respectively
- ❖ *S. sonnei* is antigenically homogeneous and has only one serotype. However, it can be typed by colicin typing into 26 colicin types.

Pathogenesis

Shigella is one of the important cause of bacillary dysentery.

- ❑ **Mode of transmission:** Infection occurs by ingestion through contaminated fingers (most common), food, and water or rarely flies. It can also be transmitted sexually (homosexuals)
- ❑ **Minimum infective dose:** As low as 10–100 bacilli are capable of initiating the disease, probably because of their ability to survive in gastric acidity
- ❑ **Entry via M cell:** Bacilli enter the mucosa via M cells and are then engulfed by macrophages. Subsequently, the macrophages release the bacilli, which induce the recruitment of inflammatory cells to the infected site releasing cytokines which in turn cause acute colitis—the hallmark of shigellosis
- ❑ Invasion is determined by a **large virulence plasmid**, which codes for important virulence factors such as **ipa proteins** and **type III secretion system**
- ❑ **Direct cell-to-cell spread:** Shigellae spread directly from one host cell to the other by inducing actin polymerization of host cells, mediated by *IcsA* proteins

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- ❑ **Exotoxins:** Some serotypes of *Shigella* also produce enterotoxins.
 - **Shigella enterotoxin (ShET):** It is structurally similar to cholera toxin and is found essentially in *S. flexneri 2a*
 - **Shiga toxin:** It is a cytotoxin, produced by *S. dysenteriae* type 1. It is structurally and functionally similar to verocytotoxin of EHEC. It inhibits protein synthesis by inhibiting 60S ribosome. It enhances local vascular damage of intestine as well as internal organs, such as kidney and brain.
- ❑ **Endotoxin:** It acts similar to any other gram-negative endotoxin and induces intestinal inflammation and ulcerations.

Clinical Manifestations

Shigellosis typically evolves through five phases:

1. **Incubation period:** It usually lasts for 1–4 days
2. **Initial phase** is characterized by watery diarrhea with fever, malaise, anorexia and vomiting
3. **Phase of dysentery:** It is characterized by frequent passage of bloody mucopurulent stools with increased tenesmus and abdominal cramps. Endoscopy shows an edematous and hemorrhagic mucosa, with ulcerations and overlying exudates. Most of the cases are self-limiting
4. **Phase of complication:** It is commonly seen with children less than 5 years age
 - **Intestinal** complications such as toxic megacolon, perforations and rectal prolapse
 - **Metabolic** complications, such as hypoglycemia, hyponatremia, and dehydration
 - **Ekiri syndrome** or toxic encephalopathy: It is a metabolic complication of shigellosis; manifests as altered consciousness, seizures, delirium, abnormal posturing and cerebral edema
 - Bacteremia is rare and can lead to meningitis and pneumonia. Rarely, cases of vaginitis and keratoconjunctivitis have been reported.
5. **Postinfectious phase:** Patients expressing HLA-B27, develop an autoimmune reaction months after shigellosis; characterized by reactive arthritis, ocular inflammation and urethritis. It is seen only after *S. flexneri* infection (occurs in 3% of cases).

Epidemiology

Risk factors for shigellosis include overcrowding, poor hygiene and children less than 5 years.

- ❖ It tends to occur as epidemics in developing countries such as Indian subcontinent and sub-Saharan Africa
- ❖ *S. flexneri* accounts for maximum number of cases (60%) in the developing areas including India, whereas *S. sonnei* is more prevalent in developed and industrialized world, accounting for 77% of cases
- ❖ Cases caused by *S. dysenteriae* type-1 are associated with high mortality. It usually causes epidemics of dysentery, particularly in refugee camps
- ❖ Humans are the natural host and cases are the only source of infection. Chronic carriage is rare except in malnourished children or AIDS patients
- ❖ **World:** Shigellosis is the most communicable disease among bacterial causes of diarrhea. Worldwide, 163.2 million cases of bacillary dysentery with 5–11 lakh deaths occur annually, of which majority are from developing countries. Children (<5 years) accounts for nearly 69% of the cases
- ❖ With improved sanitation, the incidence of shigellosis is decreasing. However, the worrisome part of the present day is development of drug resistance among the *Shigella* strains.

Laboratory Diagnosis

Fresh stool is the ideal specimen. Rectal swabs are not satisfactory. Specimens should be transported immediately or sent in transport media such as Sach's buffered glycerol saline.

- ❖ **Wet mount preparation** of feces shows large number of pus cells, erythrocytes and macrophages
- ❖ **Culture:** To inhibit the commensals, fecal specimen is inoculated simultaneously into enrichment broth and selective media
 - **Enrichment broth** such as Selenite F broth, tetrathionate broth and gram-negative broth are used
 - **Selective media such as:**
 - ◆ **Mildly selective media:** On MacConkey agar, the growth appears as translucent and non-lactose fermenting pale colonies
 - ◆ **Highly selective medium** contains higher concentration of bile salts as inhibitory agent. Examples include: (i) **DCA** (Deoxycholate citrate agar), producing colorless NLF colonies and (ii) **XLD agar** (Xylose lysine deoxycholate) producing red colonies without black center.
- ❖ **Culture smear and motility testing:** Gram stained smear of colonies reveal short, gram-negative bacilli. They are nonmotile, noncapsulated and nonsporing
- ❖ **Identification:** Identification of *Shigella* from colonies is made either by automated identification systems such as VITEK; or by conventional biochemical tests as described below:
 - *Shigella* is catalase positive (except *S. dysenteriae* serotype-1) and oxidase negative
 - **ICUT tests:** Indole test (negative), citrate test (negative), urease test (negative) and TSI (triple sugar iron agar) test shows alkaline/acid, gas absent, H₂S absent
 - All *Shigella* species are mannitol fermenters; except *S. dysenteriae*

- Automated method, MALDI-TOF poorly differentiates *Shigella* from *E. coli* as they share the same ribosomal proteins.
- ❖ **Serotyping:** Because of biochemical variations, identification of *Shigella* is always confirmed by slide agglutination with polyvalent antisera (genus specific). Then, the species identification can be done by using group specific antisera specific for serogroups. Serotypes under each species are further detected by using type specific antisera
- ❖ **Colicin typing** can be done for *S. sonnei*
- ❖ **Antimicrobial susceptibility testing** is done on Mueller Hinton agar by disk diffusion test.

TREATMENT

Shigellosis

Because of the prompt transmissibility, current recommendation is that every case of shigellosis should be treated with antibiotics.

- Ciprofloxacin is the drug of choice
- Alternative drugs which are effective are ceftriaxone, azithromycin, pivmecillinam and some fifth-generation quinolones
- Duration of treatment is about 3 days except for:
 - *S. dysenteriae* type 1 infection—5 days
 - Infections in immunocompromised patients—7–10 days
- Oral rehydration solution (ORS) should be started for correction of dehydration and nutrition should be started as soon as possible after the completion of initial rehydration.

Prevention

Being highly infectious; strict infection control measures of contact precaution should be followed (Chapter 21).

- ❖ Handwashing after handling of children's feces and before handling of food is highly recommended
- ❖ Stool decontamination (e.g. with sodium hypochlorite) has proven useful
- ❖ No vaccine against shigellosis is currently available, though several clinical trials are being conducted.

EDWARDSIELLA INFECTIONS

Edwardsiella is a commensal in the gut of reptiles and fishes. Human infection is rare; associated with ingestion of inadequately cooked aquatic animals or due to snake-related injury.

- ❖ *E. tarda* is the most frequently isolated species of *Edwardsiella* in clinical specimens
- ❖ It is associated with gastroenteritis (most common presentation) followed by extraintestinal manifestations such as septic shock, liver abscess and infections related to trauma and aquatic environment
- ❖ **Identification** is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests
 - Ferments fewer sugars (only glucose and maltose)
 - Non-lactose fermenter
 - Produces H₂S.

Treatment: Unlike *E. coli*, *E. tarda* is usually susceptible to most antimicrobials used for gram-negative bacilli such as quinolones or cephalosporins.

SALMONELLA INFECTIONS (SALMONELLOSIS)

Salmonellae are antigenically complex; comprise of six species; which in turn comprise of several serotypes based on their surface antigens. The human serotypes belong to *Salmonella enterica* subspecies *enterica*; which can be further divided into two clinical groups:

1. **Typhoidal salmonellae:** Include serotypes *S. Typhi* and *S. Paratyphi A, B, and C*. They are restricted to human hosts, in whom they cause **enteric fever (typhoid/paratyphoid fever)**; characterized principally by bacteremia and multisystem involvement and therefore, are discussed under bloodstream infections (Chapter 30)
2. **Non-typhoidal salmonellae or NTS:** The remaining serotypes can colonize the intestine of a broad range of animals, including mammals, reptiles, birds and insects. They also infect humans causing food-borne gastroenteritis and occasionally septicemia.

Non-typhoidal Salmonellae

Non-typhoidal salmonellae (NTS) include the pathogenic salmonellae other than *S. Typhi* and *S. Paratyphi A, B and C*. Majority of infections due to NTS are caused by *S. Typhimurium* and *S. Enteritidis* followed by *S. Newport*, *S. Javiana*, *S. Heidelberg*, *S. Choleraesuis* and *S. Dublin*.

Non-typhoidal Salmonellae vs Typhoidal Salmonellae

Non-typhoidal salmonellae differ from typhoidal salmonellae in many respects:

- ❖ **Zoonotic:** NTS can be acquired from multiple animal reservoirs (whereas the typhoidal salmonellae are strictly human pathogens)
- ❖ **Transmission** of NTS is most commonly associated with animal food products, especially eggs, poultry, undercooked ground meat and dairy products (typhoidal salmonellae are mainly water-borne)
- ❖ **Resistance:** Compared to other enteric gram-negative pathogens, salmonellae are relatively resistant to many environmental factors, such as drying, salting, smoking and freezing. This explains why they survive in diverse range of foods
- ❖ **Seasonality:** Transmission of NTS is highest during the rainy season in tropical climates and during the warmer months in temperate climates, coinciding with the peak in food-borne outbreaks
- ❖ **Prevalence:** NTS are widely prevalent in developed as well as developing countries (typhoidal salmonellae are mainly confined to developing countries). Worldwide, NTS causes >90 million enteric infections and 1.5 lakh deaths annually

- ❖ **Outbreaks** of NTS are common in hospitals (typhoidal salmonellae outbreaks are community based)
- ❖ **Pathogenesis** is similar to that of enteric fever except that in NTS gastroenteritis, there is massive neutrophil infiltration into intestinal mucosa (in contrast to enteric fever, where there is mononuclear cells infiltration).

Clinical Manifestations

- ❖ **Gastroenteritis:** Infection with NTS most often results in gastroenteritis—characterized by nausea, vomiting, watery diarrhea, fever and onset of abdominal cramps 6–48 hours after the ingestion of contaminated food (gastroenteritis is uncommon in typhoidal salmonellae)
 - Gastroenteritis caused by NTS is usually self-limited. Diarrhea resolves within 3–7 days
 - Patients excrete the organism in stool for up to 4–5 weeks after infection. Less than 1% of cases become chronic carriers and excrete bacilli for >1 year.
- ❖ **Bacteremia:** Up to 8% of patients with NTS gastroenteritis develop into bacteremia which leads to either endovascular infection or seedling to various organs leading to metastatic localized infection. Risk factors for bacteremia include:
 - NTS serotype: Most common being *S. Choleraesuis* (source—pig) and *S. Dublin* (source—cattle)
 - Age: Infants and elderly people are at higher risk
 - HIV and other conditions with low immunity.
- ❖ **Endovascular infections**, such as endocarditis and arteritis, occur rarely in people with pre-existing valvular heart disease
- ❖ **Metastatic localized infections** such as:
 - Intra-abdominal infections, such as hepatic or splenic abscesses or cholecystitis
 - NTS meningitis: Common in adults with HIV infection and in infants
 - Pulmonary infections, such as lobar pneumonia and lung abscess
 - UTI (pyelonephritis and cystitis) in people with underlying renal stones or urinary tract abnormality
 - Genital tract infections include ovarian, testicular abscesses, prostatitis and epididymitis
 - *Salmonella* osteomyelitis: It is commonly associated with sickle cell disease, preexisting bone disease or hemoglobinopathies
 - **Reactive arthritis** (Reiter's syndrome) seen in persons with HLA-B27 histocompatibility antigen.

TREATMENT

Non-typhoidal salmonellosis

In contrast to enteric fever where antibiotics are started as early as possible, in uncomplicated NTS gastroenteritis, the treatment is conservative with fluid replacement.

- Antibiotic use is associated with increased rates of carriers and relapse, hence it is limited only to invasive NTS infection or severe gastroenteritis having higher risks of developing invasive infection

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TREATMENT

Non-typhoidal salmonellosis

- Drugs given are similar to that of enteric fever
 - Ciprofloxacin is given for preemptive treatment or severe gastroenteritis
 - Ceftriaxone is indicated for bacteremia and invasive infections.

Drug Resistance

NTS are more drug resistant than typhoidal salmonellae.

- ❖ MDR strains of NTS are resistant to more than 5 drugs—ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines (abbreviated as **ACSSuT**)
- ❖ Increased use of ceftriaxone and ciprofloxacin to treat MDR strains leads to emergence of resistance to ceftriaxone (due to production of AmpC β -lactamases) and ciprofloxacin (due to point mutation in DNA gyrase genes).

YERSINIA INFECTIONS

The tribe Yersiniaceae comprises of genus *Yersinia* which contains three well-established human pathogens.

- ❖ *Yersinia pestis*: It was isolated for the first time by Alexandre Yersin in 1894 in Hong Kong. It is the agent of **plague**; a fulminant systemic zoonosis, transmitted from rodents by arthropod vector (the rat flea). It is discussed in Chapter 81
- ❖ *Y. pseudotuberculosis* and *Y. enterocolitica*—both cause **yersiniosis**.

Yersiniosis

Yersiniosis is a zoonotic infection caused by enteropathogenic *Yersinia* species, i.e. *Y. enterocolitica* or *Y. pseudotuberculosis*.

- ❖ Pigs and other wild and domestic animals are the usual hosts
- ❖ Human infection occurs due to consumption of contaminated food such as raw pork, milk, etc.
- ❖ It is most common in childhood and in colder climates
- ❖ Patients present with abdominal pain and sometimes with diarrhea.

Geographical Distribution

- ❖ *Y. enterocolitica* is found worldwide, most commonly in Northern Europe and America
- ❖ Outbreaks of *Y. pseudotuberculosis* are generally rare, have been reported from Finland.

Serogrouping

- ❖ *Y. enterocolitica* is further characterized biochemically (six biotypes) and antigenically (60 serotypes, based on somatic O antigen). Worldwide, most clinical infections are associated with serogroups O:3 and O:9

- ❖ *Y. pseudotuberculosis* can be further differentiated into six serotypes (1 to 6) based on somatic O and flagellar H antigens.

Clinical Manifestations

Overall, *Y. enterocolitica* is more frequently reported clinically than *Y. pseudotuberculosis*.

- ❖ **Self-limited gastroenteritis** (diarrhea with or without blood) occurs in younger children
- ❖ **Intestinal complications** occur in older children, characterized by terminal ileitis (mostly in *Y. enterocolitica*) and mesenteric adenitis. Patients present with acute pain abdomen, may mimic **pseudoappendicitis**
- ❖ **Septicemia:** It is seen typically in adults, characterized with fever and leukocytosis. It usually occurs in patients with coexisting diabetes mellitus, liver disease and iron overload
- ❖ **Post-infective phenomena** (in adults) occurs commonly with *Y. enterocolitica*. It occurs as a result of autoimmune activity, initiated by the deposition of bacterial non-viable components in joints and other sites. Manifestations include:
 - **Reactive arthritis**—mostly associated in persons positive for HLA-B 27
 - **Erythema nodosum:** It occurs independently without any link to HLA-B 27 phenotype
 - **Graves' disease**—*Y. enterocolitica* contains an antigen similar to thyroid-stimulating hormone (TSH) binding site. However, whether this cross-reactivity has any significant role in Graves' disease remains unclear.
- ❖ **Super antigen:** Some strains of *Y. pseudotuberculosis* express a super antigen **mitogen**, which has caused scarlet-like fever in Russia, similar illness in Japan (**Izumi-fever**) and has been linked to the pathogenesis of idiopathic acute systemic vasculitis of childhood called **Kawasaki's disease**.

Laboratory Diagnosis

Culture Isolation

- ❖ **For isolation from blood:** Blood culture bottles (BHI broth) should be used
- ❖ **For isolation from lymph nodes aspirate:** Culture is done on conventional media (blood agar, nutrient agar and MacConkey agar)
 - **Blood agar:** They produce granular translucent colonies with a beaten copper surface, non-hemolytic colonies
 - **MacConkey agar:** Growth of *Y. pseudotuberculosis* is poor. *Y. enterocolitica* grows well and produces lactose non-fermenting pale colonies.
- ❖ **For isolation from feces, food or soil:** Selective media should be used, such as:
 - Deoxycholate citrate agar
 - MacConkey agar

- **Yersinia CIN agar** (Cefsulodin-irgasan-novobiocin): Typical **dark red bull's eye** appearing colonies are formed in 24 hours.

- ❖ **Incubation:** Plates should be incubated at 25°C and 37°C to differentiate from most of the other pathogens which grow only at 37°C
- ❖ **Cold enrichment** can also be done by incubating in phosphate-buffered saline at 4°C for 3 weeks.

Identification

Y. enterocolitica and *Y. pseudotuberculosis* show the following properties by which they can be differentiated from *Y. pestis*:

- ❖ **Differential motility:** They are motile at 22°C (but not at 37°C)
- ❖ **Cold enrichment:** Growth improves on refrigeration (4°C)
- ❖ Automated identification systems such as MALDI-TOF can also be used for accurate species identification.

Serology

Antibodies can be detected by agglutination or ELISA using serotype specific O-antigen types. In *Y. pseudotuberculosis* infection, antibodies appear early during acute phase of illness; whereas *Y. enterocolitica* specific agglutinating antibodies are more likely to be found in convalescent sera.

TREATMENT

Yersiniosis

Most cases of diarrhea are self-limiting. Treatment is required only for systemic infections such as in case of septicemia.

- ❑ Fluoroquinolone (ciprofloxacin) or third-generation cephalosporins (cefotaxime) are effective
- ❑ *Y. enterocolitica* strains nearly always produce β -lactamases but not *Y. pseudotuberculosis* strains.

PLESIOMONAS

Plesiomonas is an oxidase positive, motile, fermenting gram-negative bacillus, recently has been included in the family Enterobacteriaceae, based on DNA hybridization studies (previously classified under Vibrionaceae family). *P. shigelloides* is the only species. It is so named because it is antigenically related to *S. sonnei*

- ❖ It is found as saprophyte in water and soil, and also as a commensal in animal and rarely in human intestine
- ❖ **Human infection:** It rarely causes gastroenteritis which may be severe in immunocompromised patients. Extraintestinal manifestations include rare cases of meningitis, septicemia, cellulitis and septic arthritis.

INFECTIONS DUE TO NON-GI PATHOGENS

The following are the members of Enterobacteriaceae family which mainly cause extra-intestinal manifestations and therefore discussed in the respective systems which they principally infect.

- ❖ **Uropathogenic *E. coli* (UPEC):** It accounts for the most common cause of UTI; discussed in Chapter 76

- ❖ **Typhoidal salmonellae:** They include serotypes *S. Typhi* and *S. Paratyphi* A, B, and C. They cause **enteric fever**; discussed under bloodstream infections (Chapter 30)
- ❖ ***Klebsiella* species:** They are usually found as commensals in human intestines and as saprophytes in soil. It has three species—*K. pneumoniae*, *K. oxytoca* and *K. granulomatis*. *K. pneumoniae* further comprises of three subspecies
 - *K. pneumoniae* subspecies *pneumoniae* is the most pathogenic among all. It is responsible for severe lobar pneumonia (Chapter 61), UTI and pyogenic infections. Some strains can rarely cause diarrhea and have been shown to produce an *E. coli* like heat stable enterotoxin
 - *K. pneumoniae* subspecies *ozaenae* and *rhinoscleromatis* are pathogens of nasal cavity and have been discussed in Chapter 78
 - The infections produced by *K. oxytoca* are similar to that of *K. pneumoniae* subspecies *pneumoniae*
 - *Klebsiella granulomatis*: It is the causative agent of a sexually transmitted infection called granuloma inguinale or donovanosis (Chapter 77).
- ❖ ***Citrobacter* species:** They are mostly environmental contaminants, occasionally cause urinary tract, gallbladder and middle ear infections and neonatal meningitis (Chapter 76)
- ❖ ***Serratia* species:** *Serratia marcescens* is the medically important species; found as saprophyte in the environment. However, it is being increasingly reported in various **nosocomial infections**, such as meningitis, endocarditis, septicemia, urinary, respiratory and wound infections (Chapter 61)
- ❖ **Tribe Proteaceae:** It comprises of three genera: *Proteus*, *Morganella* and *Providencia*. They are part of commensals in human intestine. However, they can cause nosocomial outbreaks of UTI, wound infections, etc. (Chapter 76). *Morganella morganii* is a commensal in sea fish; can cause food poisoning following seafood intake, called scombroid food poisoning (Chapter 40)
- ❖ ***Yersinia pestis*:** It is the causative agent of **plague**; a fulminant systemic zoonosis, transmitted from rodents by arthropod vector (the rat flea) (Chapter 81).

EXPECTED QUESTIONS

- I. **Write essay on:**
1. List the diarrheagenic *Escherichia coli*. Discuss the pathogenesis and laboratory diagnosis of diarrheagenic *E. coli*.
- II. **Write short notes on:**
1. Nontyphoidal salmonellae
 2. Pathogenesis of shigellosis.
- III. **Multiple Choice Questions (MCQs):**
1. **Traveler's diarrhea is caused by:**
 - a. ETEC
 - b. EHEC
 - c. EPEC
 - d. EIEC
 2. **Culture media used for EHEC O157: H7 is:**
 - a. O7 culture
 - b. Sorbitol MacConkey media
 - c. XLD agar
 - d. Deoxycholate media
 3. **Which of the following *Shigella* species is mannitol nonfermenter?**
 - a. *S. sonnei*
 - b. *S. boydii*
 - c. *S. dysenteriae*
 - d. *S. flexneri*
 4. **A 20-year-old man presented with hemorrhagic colitis. The stool sample grew *Escherichia coli* in pure culture. The following serotype of *E. coli* is likely to be causative agent:**
 - a. O157:H7
 - b. O159:H9
 - c. O107:H7
 - d. O55:H7
 5. **Most common cause of infantile diarrhea in developing country is:**
 - a. EHEC
 - b. ETEC
 - c. EPEC
 - d. EIEC
 6. **All are catalase positive, except:**
 - a. *Shigella flexneri*
 - b. *Shigella boydii*
 - c. *Shigella dysenteriae* type 1
 - d. *Shigella sonnei*
 7. **In tropical countries (India) bacillary dysentery is caused by:**
 - a. *Shigella dysenteriae*
 - b. *Shigella flexneri*
 - c. *Shigella sonnei*
 - d. *Shigella boydii*

Answers

1. a 2. b 3. c 4. a 5. c 6. c 7. b

Cholera, Halophilic Vibrio and Aeromonas Infections

CHAPTER 42

CHAPTER PREVIEW

■ Cholera

■ Halophilic *Vibrio* Infections

■ *Aeromonas* Infections

Vibrios are curved gram-negative bacilli that are actively motile by means of single polar flagellum. The name '*Vibrio*' is derived from its characteristic vibratory motility.

- ❖ Robert Koch isolated the organism in 1886, and named it as Komma bacillus; due to its characteristic **curved** or **comma-shaped** appearance
- ❖ **Habitat:** Vibrios are ubiquitous, found worldwide. Being salt loving, the natural habitat of vibrio is the marine environments (sea water and sea food), surface waters, river and sewage
- ❖ Among the several species of *Vibrio* infecting man, the most important is *V. cholerae* that causes a devastating acute diarrheal disease '**cholera**' and has been responsible for seven global pandemics and several epidemics over the past two centuries.

CHOLERA

Classification of Vibrios

Based on Salt Requirement

A unique property exhibited by all vibrios, is their growth is being stimulated in presence of salt. However, the optimum salt concentration required, varies among different vibrios. Accordingly, they are classified into:

- ❖ **Nonhalophilic vibrios:** They can grow without salt, but 1% salt is optimum for their growth. They cannot grow at higher salt concentrations. Examples include *V. cholerae* and *V. mimicus*
- ❖ **Halophilic vibrios:** They cannot grow in the absence of salt. They can tolerate and grow at higher salt concentration of up to 7–10%. Examples include *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*.

Gardner and Venkatraman Classification

This classification of *V. cholerae* (1935) was based on serogrouping, biotyping, serotyping and phage typing; which was later on updated by several researchers. Such typing schemes are of great epidemiological importance in tracking the outbreaks by finding out the relatedness

between the isolates in different clinical specimens (Fig. 42.1).

Serogrouping

Based on somatic O antigen, *V. cholerae* can be grouped into more than 200 serogroups or serovars (updated by the most widely used Sakazaki typing scheme).

- ❖ **O1 serogroup:** Among all serogroups, O1 was responsible for all pandemics and most of the epidemics of cholera. Strains belonging to O1 serogroup are agglutinated by O1 antisera
- ❖ **NAG vibrios:** Serogroups other than O1 were not agglutinated by O1 antiserum and were called non-agglutinable or NAG vibrios. They were thought to be non-pathogenic, hence also named as **non-cholera vibrios (NCV)**. Later on, it was observed that many other serogroups are pathogenic to man and are agglutinable with their respective antisera. Hence these terms such as NAG or NCV are no longer in use
- ❖ **O139 serogroup:** It was identified in 1992 and since then it has caused several epidemics and outbreaks of cholera in the coastal regions of India and Bangladesh

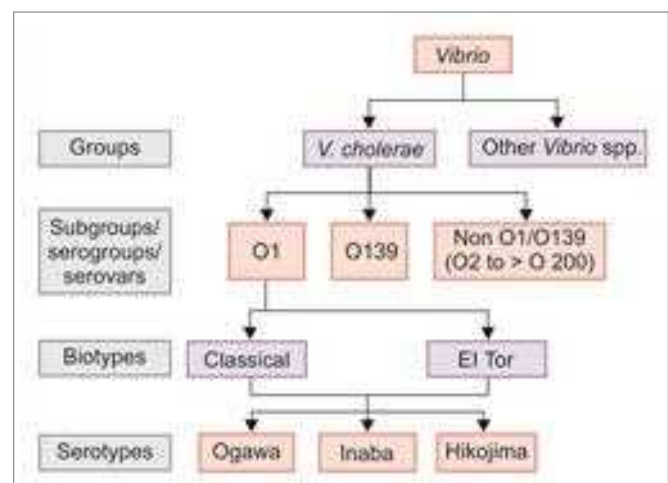


Fig. 42.1: Gardner and Venkatraman classification of *V. cholerae*.

- ❖ **Non O1/O139 serogroups:** They have occasionally caused sporadic outbreaks of diarrhea and extraintestinal manifestations, but have never caused epidemic cholera so far.

Biotyping

Serogroup O1 has two biotypes—(1) classical, and (2) El Tor; differentiated by various biochemical reactions and their susceptibility to polymyxin B and bacteriophages (Table 42.1).

- ❖ **Classical biotype:** It was responsible for the first six pandemics of cholera worldwide. It was highly virulent and had caused several deaths
- ❖ **El Tor biotype** replaced the classical biotype by 1961 and caused the seventh pandemic of cholera. It was first identified by Gotschlich (1905) at a quarantine camp on the Sinai Peninsula in El Tor, Egypt
- ❖ **Currently,** almost all outbreaks or epidemics of cholera are due to biotype El Tor, although occasional classical isolates are still seen
- ❖ **El Tor variants:** The distinction between classical and El Tor has faded over time. Many El Tor isolates of current days show properties of classical vibrios and are called as El Tor variants or El Tor hybrids. Examples of such variants include Matlab variants and Mozambique variants.

Serotyping

O1 serogroup can further be divided into three serotypes—(1) Inaba, (2) Ogawa, and (3) Hikojima; based on minor antigenic differences of O antigen.

- ❖ Ogawa is the most common serotype isolated from clinical samples followed by Inaba
- ❖ However during epidemics, shifting between serotypes can take place, more common being Ogawa to Inaba shift which occurs due to mutations in *rfbT* gene
- ❖ Hikojima represents an unstable transitional state; where both Inaba and Ogawa antigens are expressed.

Phage Typing

El Tor and classical biotypes can also be differentiated based on their susceptibility to different lytic bacteriophages.

Biotypes of <i>V. cholerae</i> O1	Classical biotype	El Tor biotype
β-hemolysis on sheep blood agar	Negative	Positive
Chick erythrocyte agglutination	Negative	Positive
Polymyxin B (50 IU)	Susceptible	Resistant
Group IV phage susceptibility	Susceptible	Resistant
El Tor Phage V susceptibility	Resistant	Susceptible
VP (Voges-Proskauer) test	Negative	Positive
Cholera toxin gene	CTX-1	CTX-2

- ❖ Basu and Mukherjee phage typing was the most widely used scheme to differentiate O1 biotypes
- ❖ It was later updated by Chattopadhyay (1993), and then it has been expanded to include O139 strains (Chakrabarti, 2000).

Pathogenesis of Cholera

Pathogenesis of cholera is toxin-mediated. Both *V. cholerae* O1 and O139 are capable of producing cholera toxin, thus resulting in cholera.

- ❖ **Mode of transmission:** *V. cholerae* is transmitted by ingestion of contaminated water or food
- ❖ **Infective dose:** Since *V. cholerae* is extremely acid-labile; a high infective dose of 10^8 bacilli is required to bypass the gastric barrier
- ❖ **Factors promoting transmission:** These include all those conditions where gastric acidity is reduced, such as hypochlorhydria, use of antacids, etc.
- ❖ **Crossing of the protective layer of mucus:** In the small intestine, vibrios penetrate the mucous layer and reach near the epithelial cells, which may be achieved by:
 - Its highly active motility
 - Secreting mucinase and other proteolytic enzymes
 - Secreting hemagglutinin protease (cholera lectin): It cleaves the mucus and fibronectin.
- ❖ **Adhesion and colonization:** The next step in the pathogenesis is, its adhesion to the intestinal epithelium which is facilitated by a special type IV fimbria called **toxin-coregulated pilus (TCP)**
- ❖ **Cholera toxin (CT):** Once established in the human small intestine, the organism produces a powerful enterotoxin called cholera toxin. It resembles heat-labile toxin (LT) of *E. coli* in its structure and function, but it is more potent than the latter

Mechanism of Action of Cholera Toxin

The toxin molecule consists of two peptide fragments—A and B (Fig. 42.2)

- **Fragment B** is the binding fragment; binds to GM1 ganglioside receptors present on the intestinal epithelium, following which A fragment is internalized
- **Fragment A** is the active fragment, causes ADP ribosylation of G protein → upregulates the activity of adenylate cyclase → results in the intracellular accumulation of cyclic adenosine monophosphate (cAMP).

❖ Increase in cyclic AMP leads to:

- In small intestine, cyclic AMP inhibits the absorptive sodium transport system in villus cells and activates the secretory chloride transport system in crypt cells, which lead to the accumulation of sodium chloride in the intestinal lumen
- Water moves passively into the bowel lumen to maintain osmolality which leads to the accumulation of isotonic fluid that results in watery diarrhea

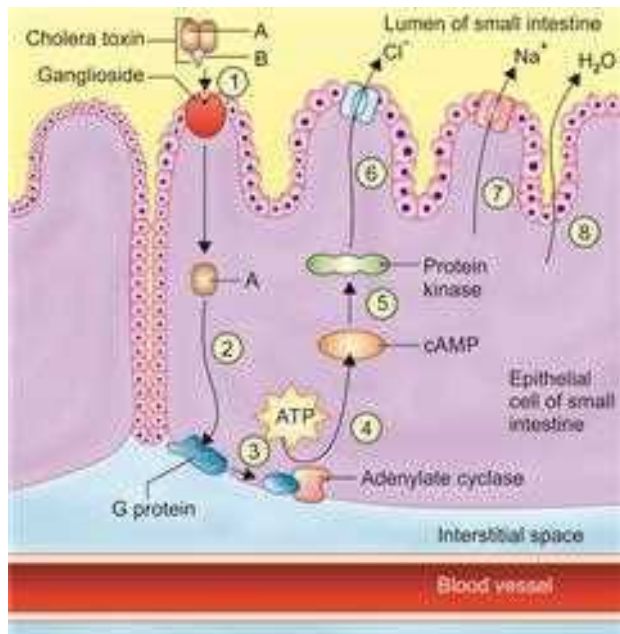


Fig. 42.2: Mechanism of action of cholera toxin.

Abbreviations: cAMP, cyclic adenosine monophosphate; ATP, adenosine triphosphate.

- Loss of fluid and electrolytes leads to shock (due to profound dehydration) and acidosis (due to loss of bicarbonate).
- ❖ **Gene for cholera toxin (CTX):** Cholera toxin is phage coded; i.e. it is encoded by genome of a filamentous bacteriophage (CTX) which is integrated as prophage into the *V. cholerae* chromosome. This phage genome also encodes for TCP, accessory colonization factors, and other regulator genes
- ❖ **Other virulence factors include:**
 - Zona occludens toxin: It disrupts the tight junctions between mucosal cells
 - Accessory colonization factors: They help in adhesion and colonization
 - Bacterial endotoxin (LPS): Unlike other gram-negative bacilli, the LPS of *V. cholerae* does not contribute to the pathogenesis of cholera. However, it is immunogenic, and is included as a component in killed vaccines.

Clinical Manifestations of Cholera

V. cholerae O1 or O139 infections produce a range of clinical manifestations such as:

1. Asymptomatic infection (75% of cases)
2. Mild diarrhea or cholera (20% of cases)
3. Sudden onset of explosive and life-threatening diarrhea (*cholera gravis*, in 5% of cases).

Incubation period varies from 24 to 48 hours. The common manifestations include:

- ❖ **Watery diarrhea:** Cholera characteristically begins with the sudden onset of painless watery diarrhea that may quickly become voluminous

Table 42.2: Progression of clinical manifestations in relation to fluid loss.

Fluid loss	Symptoms
<5%	Increased thirst
At 5–10%	<ul style="list-style-type: none"> • Postural hypotension • Weakness • Tachycardia • Decreased skin turgor
At >10%	Renal failure (due to acute tubular necrosis) and fluid loss result in: <ul style="list-style-type: none"> • Oliguria • Weak or absent pulses • Sunken eyes • Sunken fontanelles in infants • Wrinkled (“washerwoman”) skin • Somnolence and coma

- ❖ **Rice water stool:** The stool is typically non-bilious, slightly cloudy and watery with mucus flakes and a fishy, inoffensive odor. Being noninvasive, there is no associated blood or pus cells in stool. It often resembles the water in which rice has been washed
- ❖ Vomiting may be present but fever is usually absent
- ❖ Muscle cramps may occur due to electrolyte imbalance
- ❖ **Progression** of clinical manifestations is directly proportional to the fluid loss, which results in loss of body weight (Table 42.2).

Epidemiology

History of Pandemics

Cholera can occur in many forms—sporadic, limited outbreaks, endemic, epidemic or pandemic.

- ❖ **Homeland:** The **delta region** of the Ganges and Brahmaputra in West Bengal (India) and Bangladesh was known to be the homeland of cholera since ancient times
- ❖ **Till early nineteenth century**, cholera was virtually confined to its homeland, causing large epidemics periodically
- ❖ **First six pandemics** occurred between 1817 and 1923. All were caused by the classical biotype of *V. cholerae* which had spread from Bengal to involve most of the world; resulted in several thousands of deaths
- ❖ After the end of the 6th pandemic, **from 1923 to 1961** cholera was largely restricted to its homeland
- ❖ **Seventh pandemic:** It had started in 1961 and it differed from the first six pandemics in many ways
 - It was the only pandemic that originated outside India, i.e. from Indonesia (Sulawesi, formerly Celebes Island) in 1961. India was affected in 1964 and the whole world was encircled by 1991
 - It was the only pandemic to be caused by El Tor biotype which had largely replaced the classical biotype by that time
 - El Tor produced a much milder cholera; however, El Tor infection was associated with more carrier rate

than the classical. This is due to the fact that El Tor is much hardier than the classical vibrios and capable of surviving in the environment much longer

- This accounted for rapid spread of El Tor, involving the entire globe including some parts such as Central and South American countries, Australia and other affluent countries which were never affected before.

O139 (Bengal Strain)

It was isolated first from Chennai in 1992. Since it was not agglutinated by any of the antisera available at that time (O1 to O138), it was designated as a new serogroup O139 or the **Bengal strain** as it spread rapidly along the coastal region of Bay of Bengal up to West Bengal, then to the adjacent areas of Bangladesh.

- O139 appears to be a derivative of O1 El Tor, but differs from the latter in having a distinct LPS and being **capsulated**. As a result, it is invasive, can cause bacteremia and extraintestinal manifestations
- There is no cross-protection between O1 and O139
- O139 had caused large-scale outbreaks of clinical cholera and spread rapidly across almost 11 Asian countries and became a threat to cause the next pandemic
- However, by 1994 the fear had come down and once again the O1 El Tor became dominant and largely replaced O139
- Currently, O139 still causes a minority of cases in India and Bangladesh.

Current Situation

In the World

Cholera is a notifiable disease. However, it is often under reported, hence the true incidence is unknown.

- ❖ WHO has estimated that more than 1.3-4 million cases of cholera occur every year, resulting in 21,000 to 1.4 Lakh deaths annually
- ❖ During 2017, 12.2 lakh cases were notified to WHO, including 5,654 deaths. The number of cholera cases decreased globally by 60% in 2018 (4.9 lakh cases and 2,990 deaths)
- ❖ Several outbreaks have been recently reported such as from Yemen in the year 2016-2018 (large scale outbreak with >6 lakh cases and >2000 deaths reported), Nigeria in 2017, Zimbabwe (2018-19), Algeria (2018) and Somalia >10,500 cases in 2017-20
- ❖ The majority of cases are due to O1 El Tor. However, occasional cases may occur due to O139 and classical biotype, especially in Bangladesh.

Ending cholera: A Roadmap to 2030—In 2017, WHO launched this strategy for cholera control; which aims to reduce cholera deaths by 90% and to eliminate cholera in as many as 20 countries by 2030.

In India

The situation has greatly changed in India both geographically as well as in terms of number of cases and deaths.

- ❖ West Bengal is no longer the homeland; almost all the states have been affected
- ❖ Both morbidity (number of cases) and mortality (deaths) have greatly reduced
 - In 2016, about 841 cholera cases were reported, in contrast to more than 1,76,307 cases with 86,997 deaths in 1950
 - In early 2020, there was an outbreak occurred in Bangalore, infecting >17 cases.
- ❖ El Tor dominance continues, while O139 causes minority of cases
- ❖ **NICED:** National reference Center for cholera in India is located at National Institute of Cholera and Enteric Diseases (NICED), Kolkata.

Epidemiological Determinants

- ❖ **Reservoir:** Humans are the only reservoir of infection. There is no known animal reservoir
- ❖ **Source:** The source of the infection may be either asymptomatic cases or carriers
- ❖ **Carriers:** Asymptomatic carriers play an important role in transmitting cholera over long distances
 - Although carriage usually is short-lived, a few individuals may excrete the organisms for a prolonged period
 - In general, biotype El Tor has more carrier rate than classical. The case-carrier ratio is 1: 50 for the classical biotype and 1:90 for the El Tor biotype
 - Patients when effectively treated do not become carriers after they recover, but can be reinfected if exposed again.
- ❖ **Cholera season:** Maximum transmission is associated with high temperatures, heavy rainfall and flooding, but cholera can occur throughout the year
- ❖ **Other factors** that promote transmission include poor sanitation, poverty, overcrowding, population mobility (as occurs in pilgrimages, fairs, festivals and marriages)
- ❖ **Factors determining severity** of the disease include:
 - Lack of pre-existing immunity
 - Persons with '**O'** blood group are at greater risk of severe disease if infected, while those with type AB blood group are at least risk. The reason is not clear.
 - Malnutrition
 - People with low immunity (e.g. HIV infected people).
- ❖ **Age:** During inter epidemic period, all the age groups are affected equally, however during epidemics it affects more number of children
- ❖ **Habitat:** *V. cholerae* is a natural inhabitant of coastal sea salt water and brackish estuaries, where the organism can persist for long periods, particularly in association with small crustaceans, such as copepods, crabs or plankton
- ❖ **Persistence of *V. cholerae*:**
 - During epidemics, it is maintained by carriers and subclinical cases

- In inter epidemic period, it is maintained in sea water, crustaceans and planktons.
- ❖ **Resistance**
 - *V. cholerae* is acid-labile but stable to alkali
 - It is heat-labile (killed within 30 minutes by heating at 56°C or within few seconds by boiling), but stable to refrigeration and can remain in ice for 4–6 weeks
 - Drying and sunshine can kill the bacilli in few hours
 - It is susceptible to disinfectants, such as cresol and bleaching powder (6 mg/L)
 - In general, biotype El Tor is more resistant than classical.

LABORATORY DIAGNOSIS

Cholera

- ❑ **Specimens:** Watery stool or rectal swab (for carriers)
- ❑ **Transport media:** VR medium, Cary-Blair medium
- ❑ **Direct microscopy**
 - Gram-negative rods, short curved comma-shaped (fish in stream appearance)
 - Hanging drop-demonstrates darting motility
- ❑ **Culture**
 - Enrichment broth: Alkaline peptone water, Monsur's taurocholate tellurite peptone water
 - Selective media: Bile salt agar, Monsur's GTTT agar, TCBS agar (yellow colonies)
 - MacConkey agar—produces translucent NLF colonies
- ❑ **Culture smear and motility testing**—reveals
 - Short curved gram-negative bacilli and
 - Darting motility
- ❑ **Identification**
 - Catalase and oxidase positive
 - ICUT: Indole (+), Citrate (+/-), Urease (-), TSI:A/A, gas (-), H₂S (-)
 - String test positive
 - It produces hemodigestion on blood agar
 - Automated systems such as MALDI-TOF and VITEK
- ❑ **Biotyping:** To differentiate classical and El Tor
- ❑ **Serogrouping:** To differentiate O1 and O139
- ❑ **Serotyping:** To differentiate Ogawa, Inaba and Hikojima serotypes of serogroup O1
- ❑ **Antigen detection** by cholera dipstick assay
- ❑ **Molecular method**—multiplex PCR detecting common diarrheal pathogens
- ❑ **Antimicrobial susceptibility testing.**

Laboratory Diagnosis

Specimens

- ❖ Freshly collected watery stool is the specimen of choice for acute cases. Ideally, it should be collected before starting the antibiotics
- ❖ Rectal swab is the preferred specimen for convalescent patients or carriers.

Transport/Holding Media

Specimens should be transported immediately to the laboratory. If delay is expected, stool (1–3 mL) or rectal

swabs may be inoculated in 10–20 mL of one of the following transport media:

- ❖ Venkatraman-Ramakrishnan (VR) medium
- ❖ Alkaline salt transport medium
- ❖ Cary-Blair medium: It is also useful for *Salmonella* and *Shigella*
- ❖ Autoclaved sea water.

Direct Microscopy

- ❖ **Gram staining** of mucus flakes of feces reveals short curved **comma-shaped** gram-negative rods, arranged in parallel rows, which is described by Koch as **fish in stream** appearance (Fig. 42.3)
- ❖ **Motility testing by hanging drop method:** They are actively motile frequently changing their direction, described as **darting motility** (dart means a small, slender, pointed missile which shows sudden, rapid movement when thrown at a target). It is also described as shooting star or swarming gnats motility.

Culture

V. cholerae is strongly aerobic, non-fastidious; grows well on ordinary media, such as nutrient agar.

- ❖ However to inhibit the commensals, fecal specimen should be inoculated simultaneously onto enrichment broth and selective media
- ❖ These media contain salt (0.5–1%), which stimulates its growth and have alkaline pH, which allows *V. cholerae* to grow, while inhibiting the fecal commensals.

Enrichment Broth

Fecal specimen is inoculated onto enrichment broth, following which they are incubated for 4–6 hours. Thereafter a subculture is made onto another selective medium (as



Fig. 42.3: *Vibrio cholerae* (Gram stain): Curved comma-shaped gram-negative rods (fish in stream appearance).

Source: Public Health Image Library, ID#:5324/Centers for Disease Control and Prevention (CDC) (with permission).

listed below). Prolonged incubation of the broths should be avoided as the commensals may overgrow.

- ❖ Alkaline peptone water (APW)
- ❖ Monsur's taurocholate tellurite peptone water.

Selective Media

Stool specimen is directly inoculated on to a selective medium and the plate is incubated at 37°C for 24 hours.

- ❖ **TCBS agar:** It contains thiosulfate, citrate, bile salts (as inhibitor), sucrose and has pH of 8.6. This is widely used at present (Fig. 42.4B). *V. cholerae* can ferment sucrose and therefore produce large yellow colonies
- ❖ **Alkaline bile salt agar (BSA):** *V. cholerae* produces translucent **oil drop** colonies
- ❖ **Monsur's gelatin taurocholate trypticase tellurite agar (GTTTA):** *V. cholerae* produces translucent colonies with a grayish black center and a turbid halo. Classical biotypes grow better on this medium than on TCBS agar
- ❖ **MacConkey agar:** When not sure about the type of enteric pathogen present in feces, MacConkey agar can be included in the panel. Being a mildly selective medium, it also supports other enteric pathogens such as *Shigella* and *Salmonella*. Colonies of *V. cholerae* are translucent and pale which may become pink on prolonged incubation (due to late lactose fermentation).

Culture Smear and Motility Testing

- ❖ Culture smear of the colonies reveals short curved gram-negative bacilli
- ❖ Hanging drop shows typical darting motility.

Identification

Identification is made either by automated systems such as MALDI-TOF or VITEK; or by conventional biochemical tests. The key biochemical properties include:

- ❖ Catalase and oxidase positive
- ❖ **ICUT test**—shows the following reactions:
 - Indole test—positive
 - Citrate test—variable

- Urease test—negative
- TSI (triple sugar iron agar test)—Being sucrose fermenter, it shows acid/acid, gas absent, H₂S absent.
- ❖ **Hemodigestion:** On blood agar, it causes nonspecific lysis of blood cells, seen as greenish clearing around the main inoculum (Fig. 42.4A)
- ❖ **String test:** When a colony of *Vibrio* is mixed with a drop of 0.5% sodium deoxycholate on a slide, the suspension loses its turbidity, and becomes mucoid. When tried lifting the suspension with a loop, it forms a string (Fig. 42.4C).

Biotyping

The classical and El Tor biotypes can be differentiated by various biochemical tests, susceptibility to polymyxin B and bacteriophages (refer Table 42.1).

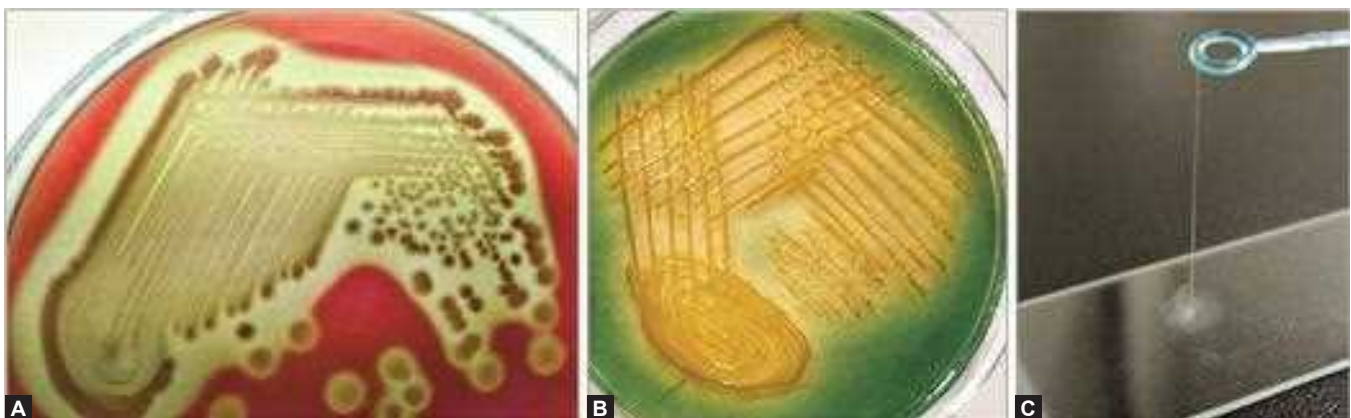
Serogrouping

Species identification is always confirmed by agglutination test done on a slide with *V. cholerae* polyvalent O antisera:

- ❖ **Specific serogroups** can be identified by using group-specific antisera. First the colony is tested with O1 antisera → If found negative, then tested with O139 antisera
- ❖ **Serotyping:** If agglutinated with O1 antisera, then the serotyping is done by testing simultaneously with Ogawa and Inaba antisera:
 - If agglutinated with Ogawa antisera—it is designated as Ogawa serotype
 - If agglutinated with Inaba antisera—it is designated as Inaba serotype
 - If agglutinated with both Ogawa and Inaba antisera—it is designated as Hikojima serotype.

Antigen Detection

A point-of-care antigen-detection test called—cholera dipstick assay is commercially available. It is useful in the fields, where laboratory facilities are unavailable.



Figs 42.4A to C: A. *Vibrio cholerae* on blood agar (hemodigestion); B. TCBS agar with yellow colored colonies of *Vibrio cholerae*; C. String test.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Molecular Method

Molecular methods such as PCR can directly detect *V. cholerae* specific genes in stool. BioFire FilmArray is an automated multiplexed PCR assay available commercially. Its gastrointestinal (GI) Panel can simultaneously detect 22 different enteric pathogens directly from stool specimens including *V. cholerae*.

Antimicrobial Susceptibility Testing (AST)

As there is increasing trend of drug resistance in *Vibrio cholerae*, AST should be performed for guiding therapy. It is done on Mueller Hinton agar by disk diffusion test.

TREATMENT

Cholera

Fluid replacement: It is the most important measure for management of the cholera patient. It should be prompt and adequate to correct hypovolemia and thereafter to be maintained to replace the ongoing fluid losses

- ❑ **In mild to moderate fluid loss:** Oral rehydration solution (ORS) should be given
- ❑ **In severe cases:** Intravenous fluid replacement with Ringer's lactate (or normal saline) should be carried out till the consciousness arrives, thereafter replaced by ORS.

Antibiotics have a minor role as the pathogenesis is mainly toxin mediated

- ❑ Although not necessary for cure, use of antibiotic may decrease the duration and volume of fluid loss and hastens clearance of the organism from the stool, thus prevents the development of carrier stage
- ❑ The WHO recommends the use of antibiotics to only severely dehydrated patients, although wider use is not contraindicated
- ❑ **Drug of choice:** Macrolides such as azithromycin or erythromycin are the drugs of choice for adults, children and also in pregnancy. Alternatively for adults, doxycycline or tetracycline or ciprofloxacin can be given in areas with confirmed susceptibility.

Prevention

General Measures

General measures include:

- ❖ Provision of safe water
- ❖ Improved sanitary disposal of feces
- ❖ Proper food sanitation
- ❖ Prompt outbreak investigation and taking necessary steps to reduce transmission
- ❖ Notification: Cholera is a notifiable disease locally and nationally, hence the cases should be notified
- ❖ Infection control measures (of contact precaution) such as hand hygiene is crucial to limit the spread of the disease (see Chapter 21).

Chemoprophylaxis

Tetracycline is the drug of choice; alternative drug includes doxycycline. It is indicated to household contacts, only during epidemic. Mass antibiotic prophylaxis may contribute to the emergence of resistance; Hence WHO

does not recommend antibiotic prophylaxis against cholera for travelers coming from or going to cholera endemic countries.

Vaccine

Injectable Killed Vaccines

They are no longer in use, as they provide little protection, cause adverse effects and fail to induce a local intestinal mucosal immune response.

Oral Cholera Vaccines

Oral cholera vaccines (OCV) are currently in practice. Two types of oral vaccines are available.

1. Killed whole-cell vaccine:

❖ **Whole-cell (WC) vaccine:** It is composed of killed whole cells of *V. cholerae* O1 and O139

- **Formulations:** Two formulations are available: Shanchol (India) and Euvichol (South Korea)
- **Schedule:** Two doses are given orally, with minimum of two weeks gap, for all individuals >1 year age
- **Protection:** It provides protection against cholera for 3 years.

❖ **Whole-cell recombinant B subunit vaccine (WC/rBS):** In addition to the composition of WC vaccine, it has recombinant cholera toxin B subunit

- **Formulation:** It is available commercially as Dukoral
- **Schedule:** Two doses are given orally, with minimum of one week gap. A third dose is given for children aged 2-5 years. It is given for all individuals >2 year age
- **Protection:** It provides protection against cholera for 2 years.

WHO recommends to use vaccine during epidemics and outbreaks in the community but not during inter epidemic period.

2. **Oral live attenuated vaccines (OCV):** They use mutant strains that lack the gene encoding for cholera toxin.

❖ **CVD 103-HgR:** It is commercially available as *Vaxchora*; given as single oral dose

- **Indication:** It is recommended for adults of age 18-64 years, traveling to an area with active cholera transmission. It is also indicated to limit the spread of infection during an outbreak
- **Protection:** It gives 90% protection at 10 days after vaccination; which lasts for 3-6 months.

Non O1/O139 *V. cholerae*

They may resemble biochemically to *V. cholerae* O1/O139, but do not agglutinate with O1 or O139 antisera.

Clinically, they differ from O1/O139 strains as follows:

- ❖ **Gastroenteritis:** Several outbreaks of gastroenteritis following sea food consumption (raw oysters) have been reported from Mexico and other places
 - Stool is watery or partly formed, less voluminous and bloody or mucoid

- Many cases have abdominal cramps, nausea, vomiting and fever
- However, they never cause epidemic cholera
- Treatment is same as that of cholera. Fluid replacement is the most crucial step. Antibiotics can be given in severe dehydration.
- ❖ **Extraintestinal manifestations:** Such as otitis media, wound infection and bacteremia (in patients with liver disease) have been reported sporadically
 - Most of these infections are acquired by occupational or recreational exposure to seawater
 - Antibiotics are often indicated. Most strains are sensitive to tetracycline, ciprofloxacin and third-generation cephalosporins.

HALOPHILIC VIBRIO INFECTIONS

Halophilic vibrios can withstand higher salt concentration (>6%) in contrast to *V. cholerae*, which can tolerate up to 6%. They are widespread in marine environments. Cases tend to occur during late summer and early rain fall, when the bacterial counts are highest in the water.

Vibrio parahaemolyticus Infections

Though *V. parahaemolyticus* was first reported from Japan (1953), the incidence of infection has greatly increased in several countries including Japan since 1993. In India, it has been reported from Kolkata.

Clinical Manifestations

- ❖ Food-borne gastroenteritis is the most common presentation, occurs following raw or uncooked sea food (e.g. oyster) intake. It commonly presents as watery diarrhea or rarely as dysentery with abdominal cramps
- ❖ Extraintestinal manifestations such as wound infection, otitis and sepsis are rare.

Laboratory Diagnosis

Laboratory diagnosis of *V. parahaemolyticus* is carried out similar to that followed for *V. cholerae*. The distinct properties are as follows:

- ❖ **Morphology:** It is capsulated, shows bipolar staining in fresh isolates and pleomorphism in older cultures
- ❖ Motile by peritrichous flagella (but it does not show darting motility)
- ❖ On TCBS, agar it produces green colonies (sucrose non-fermenter)
- ❖ **Kanagawa phenomenon:** It causes β -hemolysis on Wagatsuma agar (a special type of high salt blood agar)
- ❖ **Swarming:** It swarms on blood agar
- ❖ Urease test is positive in few strains
- ❖ **Salt tolerance test:** It can resist maximum of 8% NaCl
- ❖ Identification can also be made by automated methods such as MALDI-TOF and VITEK.

TREATMENT

V. parahaemolyticus infections

- ❑ Most of the gastroenteritis is self-limiting and treatment is same as that of cholera
- ❑ Indications for antibiotic use: Severe gastroenteritis or extraintestinal manifestations associated with underlying diseases, such as diabetes, pre-existing liver disease, iron-overload states, or immunosuppression
- ❑ Doxycycline or macrolide are the drug of choice
- ❑ For proven bacteremia, doxycycline plus ceftriaxone is recommended.

Vibrio vulnificus Infections

Though rare, *V. vulnificus* produces the most severe infection among the *Vibrio* species.

Clinical Manifestations

It can cause two distinct syndromes:

1. **Primary sepsis:** Usually occurs in patients with underlying liver disease and iron overload or rarely in renal insufficiency and immunosuppression
2. **Primary wound infection:** It is characterized by painful erythematous swelling or cellulitis or even vesicular, bullous or necrotic lesions, generally affects people without underlying disease (*Vulnificus* is Latin word for “wound maker”).

Laboratory Diagnosis

V. vulnificus can be cultured from blood or cutaneous lesions. It ferments lactose, which differentiates it from all other vibrios. Identification can also be made by automated methods such as MALDI-TOF and VITEK.

TREATMENT

Vibrio vulnificus infections

Early antibiotic institution, wound debridement, and general supportive care are the keys to recovery. *V. vulnificus* is sensitive in vitro to a number of antibiotics, including tetracycline, fluoroquinolones, and third-generation cephalosporins.

Vibrio alginolyticus Infections

V. alginolyticus can occasionally cause eye, ear and wound infections.

- ❖ Few cases of otitis externa, otitis media and conjunctivitis have been reported
- ❖ It rarely causes bacteremia in immunocompromised hosts
- ❖ It is the most salt-tolerant *Vibrio* and can grow at salt concentrations of more than 10%
- ❖ Identification can also be made by automated methods such as MALDI-TOF and VITEK
- ❖ Disease is usually self-limiting. Severe infections respond well to antibiotics (tetracycline) and drainage.

AEROMONAS INFECTIONS

Aeromonas was earlier placed in the family Vibrionaceae; however, it has now been assigned to a separate family, Aeromonadaceae. *A. hydrophila* causes **red leg disease** in frog.

Pathogenicity of *Aeromonas* in humans is mainly related to:

- ❖ Tissue adherence mediated by adhesions such as S-layer and fimbriae
- ❖ Capsular polysaccharide (prevents the bacilli from phagocytosis)
- ❖ Exotoxins, such as aerolysin, phospholipases, hemolysins, enterotoxin and cytotoxin similar to Shiga toxin
- ❖ Endotoxin or LPS.

Clinical manifestations: Over 85% of the human infections are caused by *A. hydrophila*, *A. caviae* and *A. veronii*. Most of the other species are mainly isolated from environmental sources and animals. Various manifestations include:

- ❖ Gastroenteritis (watery diarrhea, vomiting, fever and rarely dysentery) and peritonitis
- ❖ Musculoskeletal and wound infections
- ❖ Bacteremia in immunocompromised adults and infants
- ❖ Respiratory tract infections, such as epiglottitis, pharyngitis and pneumonia.

Laboratory diagnosis: Identification of *Aeromonas* up to species level from colonies is made either by automated identification systems such as MALDI-TOF and VITEK or by various conventional biochemical tests (e.g. oxidase and catalase positive).

TREATMENT

Aeromonas infections

Aeromonas is susceptible to ciprofloxacin and levofloxacin. Alternatively, cotrimoxazole and cefepime can be given. However, the plasmid mediated drug resistance has been reported including β -lactamase production.

EXPECTED QUESTIONS

I. Write essay on:

1. A 4-year-old boy developed severe watery diarrhea and vomiting. The stool collected has a rice water type of appearance. It was sent for bacteriological analysis.
 - a. What is the probable etiological diagnosis of this condition?
 - b. Describe in detail the pathogenesis of this condition.
 - c. Add a note on its laboratory diagnosis.

II. Write short notes on:

1. Prophylaxis against cholera.
2. Halophilic vibrios.

III. Multiple Choice Questions (MCQs):

1. Which of the following media can be used as transport medium for vibrios?
 - a. Selenite F broth
 - b. Nutrient broth
 - c. Tetrathionate broth
 - d. Venkatraman–Ramakrishnan medium
2. All of the following tests can differentiate between classical and El Tor biotypes of *V. cholerae*, except:
 - a. β -hemolysis on sheep blood agar
 - b. Chick erythrocyte agglutination
 - c. Growth on TCBS agar
 - d. Polymyxin B (50 IU)
3. Pathogenesis of *V. cholerae* involves one of the following second messenger systems:
 - a. cGMP
 - b. cAMP
 - c. Ca^{2+}
 - d. IP3
4. Selective media for *Vibrio cholerae*:
 - a. TCBS
 - b. Mannitol salt agar

- c. Robertson cooked meat medium
- d. Modified Thayer Martin medium

5. All of the following *Vibrio* species are halophilic, except:

- a. *V. cholerae*
- b. *V. parahaemolyticus*
- c. *V. alginolyticus*
- d. *V. vulnificus*

6. Gardner and Venkatraman classification—which of the following is a biotype of *Vibrio*?

- a. Ogawa
- b. Inaba
- c. Hikojima
- d. El Tor

7. O139 (Bengal strain)—all are true, except:

- a. Capsulated
- b. Toxigenic
- c. Clinically similar to El Tor
- d. More common than El Tor

8. *V. cholerae*—all are true, except:

- a. Acid-labile but stable to alkali
- b. Heat-labile
- c. Classical is more resistant than El Tor
- d. 'O' blood group are affected more frequently

9. All are selective media for *V. cholerae*, except:

- a. Alkaline peptone water
- b. Alkaline bile salt agar
- c. TCBS agar
- d. Monsur's agar (GTTTA) medium

10. Which of the following confirms the isolate of *V. cholerae* as Hikojima serotype?

- a. If agglutinated with Ogawa antisera
- b. If agglutinated with Inaba antisera
- c. If agglutinated with Hikojima antisera
- d. If agglutinated with both Ogawa and Inaba antisera

Answers

1. d 2. c 3. b 4. a 5. a 6. d 7. d 8. c 9. a 10. d

Miscellaneous Bacterial Infections of Gastrointestinal System

CHAPTER 43

CHAPTER PREVIEW

■ Campylobacteriosis

■ *Helicobacter* Infections

■ *Clostridioides difficile* Infection

This chapter covers various bacterial infections of gastrointestinal system caused by *Campylobacter*, *Helicobacter* and *Clostridioides difficile*.

CAMPYLOBACTERIOSIS

Campylobacter species cause both diarrheal and systemic diseases. They are motile, nonsporing, microaerophilic, curved gram-negative rods.

Human pathogens fall into two major groups:

1. Primarily diarrheal disease: It is caused by *C. jejuni* (accounting for 80–90% of total cases), and others such as *C. coli*, *C. upsaliensis*, *C. lari*, *C. hyointestinalis*, *C. fetus*
2. Extraintestinal infection: Caused by *C. fetus*.

Epidemiology

- ❖ **Source:** *Campylobacter* species are zoonotic, found in the intestine of many animals (poultry, cattle, sheep and swine) and household pets (including birds, dogs and cats). However, animals are asymptomatic
- ❖ **Mode of transmission:** *Campylobacter* is transmitted by the following routes:
 - By raw or undercooked food products: Ingestion of contaminated poultry (most common), raw (unpasteurized) milk or untreated water
 - Through direct contact with the infected household pets
 - Oral-anal sexual contact.
- ❖ **Infective dose:** The infective dose is small; <500 organisms can cause disease
- ❖ **Age:** Persons of all ages are affected; however:
 - *C. jejuni* infection is common among children
 - In contrast, *C. fetus* infection is the highest in extremes of age.
- ❖ **Developing versus developed countries:**
 - In developing countries, *C. jejuni* infections are hyperendemic, mostly present as asymptomatic infection except for children less than 2 years (usually symptomatic)

- In developed countries, *Campylobacter* is the leading bacterial cause of diarrheal disease, more common than *Shigella* and *Salmonella*.

- ❖ **Seasonality:** Incidence peaks during summer and early autumn.

Pathogenesis

Pathogenesis of *C. jejuni* is due to expression of the following virulence factors:

- ❖ Motility of the strain (possesses single polar flagellum and exhibits darting motility)
- ❖ Capacity to adhere to host tissues
- ❖ Produce toxins, which play a minor role:
 - Enterotoxin (heat-labile, similar to cholera toxin)
 - Cytotoxins (cytotoxic distending toxin, or CDT).
- ❖ Proteinaceous capsule-like structure (S-layer) expressed by *C. fetus*.

Clinical Manifestations

Incubation period of campylobacteriosis varies from 2–4 days. The clinical manifestations seen are as follows:

- ❖ **Intestinal infection:** It is characterized by inflammatory diarrhea, abdominal pain and fever. Degree of diarrhea varies from several loose stools to grossly bloody stools. It is self-limiting; however, relapse is seen in 5–10% of untreated cases
- ❖ **Complications:** It is mainly due to *C. fetus* developing mostly in immunocompromised hosts and at the extremes of age. Common manifestations include bacteremia, sepsis, meningitis, vascular infections (endocarditis, aneurysm, and thrombophlebitis)
- ❖ *Campylobacter* can precipitate the pathogenesis of various other diseases such as:
 - Guillain-Barré syndrome (mainly by *C. jejuni* serotype O19)
 - Irritable bowel syndrome
 - Alpha chain disease, a form of lymphoma that originates in small intestinal mucosa-associated lymphoid tissue

- Reactive arthritis and other rheumatologic manifestations, in persons with the HLA-B27 phenotype.

Laboratory Diagnosis

Freshly collected stool specimen and rectal swab are the preferred specimens.

Direct Microscopy

- ❖ Gram stained smear of feces may show curved gram-negative bacilli, comma or S-shaped or spiral (*gull wing*) shaped (Fig. 43.1A)
- ❖ Dark ground microscopy demonstrates the darting motility of the bacilli.

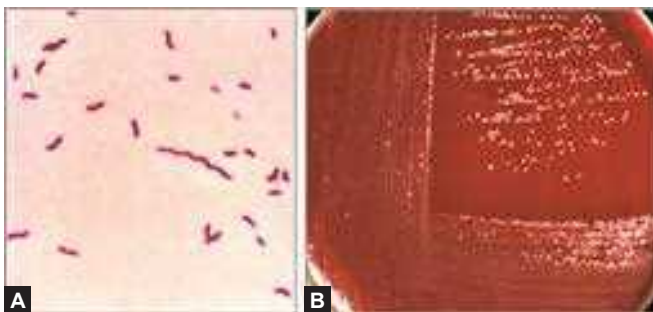
Culture

The culture media for *Campylobacter* are as follows:

- ❖ **Transport medium:** If delay is expected, transport medium such as Cary-Blair medium can be used
- ❖ **Selective media:** Feces or rectal swabs are plated onto selective media such as:
 - Skirrow's selective medium (Fig. 43.1B)
 - Butzler's selective medium
 - Campy BAP selective medium.
- ❖ **Culture conditions:** Inoculated plates are incubated at:
 - Microaerophilic condition (5% oxygen)
 - **Growth at 42°C:** Thermophilic *Campylobacter* species (*C. jejuni*, *C. coli* and *C. lari*) can be differentiated from *C. fetus*, which is nonthermophilic.
- ❖ After 2–5 days of incubation, characteristic **effuse droplet-like colonies** are produced which can be further subjected to conventional biochemical tests or automated systems such as MALDI-TOF or VITEK for species identification.

Molecular Method

Automated multiplexed PCR assay such as, BioFire FilmArray is available. Its gastrointestinal (GI) panel can simultaneously detect 22 different enteric pathogens directly from stool specimens, including *Campylobacter*.



Figs 43.1A and B: *Campylobacter*: A. Gram-negative spiral rods; B. Growth on Skirrow's media.

Source: Public Health Image Library: A. ID#: 6657; B. D#: 3918/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

TREATMENT

Campylobacteriosis

Fluid and electrolyte replacement is the mainstay of treatment. Specific antimicrobial therapy is indicated only for severe infection or for immunocompromised patients. Antibiotics used for treatment are:

- ❑ **Diarrheal disease:** Oral macrolides are the drug of choice (erythromycin or azithromycin). Ciprofloxacin or doxycycline can be used alternatively
- ❑ **Systemic/life threatening infection** (by *C. fetus*): Parenteral imipenem or meropenem plus gentamicin should be started empirically but susceptibility testing then be performed.

HELICOBACTER INFECTIONS

Helicobacter pylori is curved gram-negative rod that colonizes stomach and is associated with peptic ulcer disease and gastric carcinoma.

Pathogenesis

Colonization of the Gastric Mucosa

Man is the only important reservoir host of *H. pylori*. It colonizes the stomach of 50% of the world's human population (30% in developed countries to nearly 80% in developing countries). Children may acquire this organism from parents or from other children. The colonization is favored by the following factors:

- ❖ **Motility:** *H. pylori* is highly motile (conferred by 4 to 8 unipolar flagella), which allows it to remain in the viscous environment of the mucus layer overlying the gastric mucosa
- ❖ **Acid-resistance:** It may be due to:
 - **Urease enzyme:** It produces abundant urease that catalyzes urea hydrolysis to produce ammonia which in turn buffers the gastric acid
 - **Amidase and arginase:** May contribute to the production of ammonia.
- ❖ **Adhesins:** Though most *H. pylori* remain within the mucus layer, a few (~2%) may bind to mucosal epithelium by expressing adhesion molecules such as:
 - Blood group antigen-binding adhesin: Binds to Lewis blood group antigen
 - Adherence-associated lipoprotein.
- ❖ **Resistance to oxidative stress:** *H. pylori* produces many detoxifying enzymes that protect the organism against the effects of oxygen-derived free radicals generated from the bacterium's own metabolism and the inflammatory defences of the host.

Induces Pathological Changes

- ❖ **Vacuolating cytotoxin (VacA):** *H. pylori* secretes VacA that induces the formation of vacuoles in the cytoplasm of epithelial cells
- ❖ **Cytotoxin-associated gene A (CagA):** It helps the bacterium to modulate certain aspects of the host cell's metabolism including:

- Cytoskeletal rearrangements
- Host-cell morphological changes
- Expression of proto-oncogenes
- Release of proinflammatory cytokines from gastric epithelial cells.
- ❖ **Molecular mimicry:** Lipopolysaccharide of *H. pylori* (glycoprotein moiety) is identical to the Lewis blood group antigen expressed on gastric parietal cells which may result in:
 - Immune tolerance by downregulating T cells
 - Induction of autoantibodies that cross-react with mucosal epitopes and contribute to the development of chronic active gastritis.
- ❖ **Alteration in gastric mucus:** LPS also inhibits glycosylation and sulfation of gastric mucus, which may impede its protective function and increase the vulnerability of the epithelial surface to gastric acidity
- ❖ **Host factors:** People with polymorphisms in cytokine genes (e.g. interleukin-1) or genes coding Toll-like receptors are at increased risk of gastric adenocarcinoma
- ❖ **Environmental risk factors**
 - Smoking increases the risk of ulcers and cancer in *H. pylori*-colonized individuals
 - Diets high in salt and preserved foods increase cancer risk, whereas diets high in antioxidants and vitamin C are protective.

Clinical Manifestations

- ❖ **Acute gastritis:** Antrum is the most common site involved, cardiac end is not involved.
 - Antral gastritis: It predisposes to duodenal ulcers
 - Pangastritis: It predisposes to adenocarcinoma of stomach.
- ❖ **Peptic ulcer disease:** 70% of duodenal ulcers and 50% of gastric ulcers are due to *H. pylori*
 - Mechanism of duodenal ulcer: *H. pylori*-induced inflammation inhibits somatostatin producing D cells → ↑ gastrin release → ↑ meal-stimulated acid secretion → induces duodenal ulcer and gastric metaplasia of duodenal mucosa
 - Mechanism of gastric ulcer: Though not clear, however, it is believed that there is hypochlorhydria despite increased gastrin release
 - Epigastric pain with burning sensation: It is the most common presentation; develops either following a meal (as in duodenal ulcer) or in empty stomach (as in gastric ulcer).
- ❖ Chronic atrophic gastritis
- ❖ Autoimmune gastritis
- ❖ Pernicious anemia
- ❖ Adenocarcinoma of stomach
- ❖ Gastric mucosa associated lymphoid tissue (MALT) lymphomas.

Protective Role for *H. pylori*

Colonization of *H. pylori*-induced hypochlorhydria has an inverse relation with the occurrence of:

- ❖ Gastroesophageal reflux disease (GERD)
- ❖ Barrett's esophagus
- ❖ Adenocarcinoma of esophagus
- ❖ Allergic disorders including asthma.

Laboratory Diagnosis

Diagnosis of *H. pylori* infection may be established by invasive and noninvasive methods.

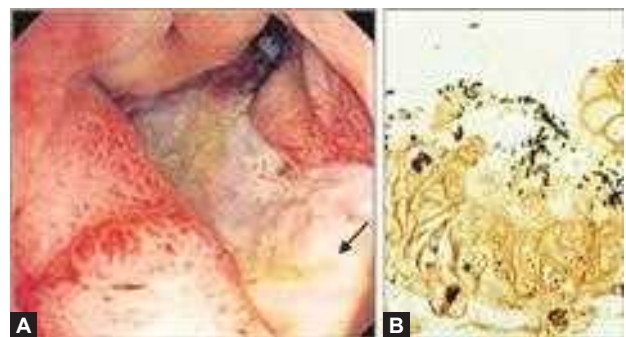
Invasive Tests

Endoscopy-guided multiple biopsies can be taken from gastric mucosa (antrum and corpus) (Fig. 43.2A) and are subjected to:

- ❖ **Histopathology** with Warthin Starry silver staining (Fig. 43.2B). Sensitivity can be improved by the use of immunostaining with anti-*H. pylori* antibody
- ❖ **Microbiological methods**
 - **Gram-staining:** Curved gram-negative bacilli with seagull-shaped morphology
 - **Culture:** It is the most specific test for *H. pylori*; however, it is not sensitive
 - ♦ Commonly used culture media are Skirrow's media and chocolate agar
 - ♦ Plates are incubated at 37°C under microaerophilic condition (5% oxygen).
 - **Identification** is made by automated identification systems such as MALDI-TOF or by conventional biochemical test such as urease test.
- ❖ **Biopsy urease test** (also called **rapid urease test**): It detects the presence of urease activity in gastric biopsies by using a broth that contains urea and a pH indicator. It is rapid, sensitive and cheap.

Noninvasive Tests

- ❖ **Urea breath test:** Patient drinks a solution of urea labeled with the nonradioactive ¹³C and then blows into



Figs 43.2A and B: **A.** Endoscopy shows duodenal ulcer due to *H. pylori* (arrow); **B.** *H. pylori* (black curved rods) colonized on the gastric mucosa, Warthin-Starry staining.

Source: A. Wikipedia; B. Yutaka Tsutsumi, MD, Professor, Department of Pathology, Fujita Health University School of Medicine (with permission).

a tube. If urease produced by *H. pylori* is present, the urea is hydrolyzed and labeled CO₂ is detected in breath samples by mass spectroscopy. Urea breath test is very popular nowadays as it is noninvasive and is:

- Best test to detect live bacteria
 - Most consistent and accurate test
 - Most sensitive, quick and simple
 - Used to monitor treatment response (becomes negative after improvement).
- ❖ **Fecal antigen (coproantigen) assay:** It is a non-invasive test with 90–95% sensitivity and specificity; detects both live and dead bacteria
- Used to monitor treatment response
 - Useful for screening of children.
- ❖ **Antibody (IgG) detection** by ELISA: It is used for:
- Screening before endoscopy
 - Seroepidemiological study.

TREATMENT

H. pylori infection

Treatment for *H. pylori* infection

Treatment of *H. pylori* infection is indicated for: (i) active or past history of peptic ulcer disease, (ii) atrophic gastritis, (iii) MALT lymphoma, (iv) following endoscopic resection of early gastric cancer

- However, treatment is not recommended for asymptomatic colonizers or primary prophylaxis for gastric cancer because of risk of adverse side effects and development of antibiotic resistance
- Usually multidrug regimens are used. Monotherapy is not useful because of inadequate antibiotic delivery to the colonization niche
- Success of treatment depends on: (i) Patient's close compliance with the regimen, and (ii) Use of susceptible antibiotics

Treatment regimen:

- **OBTM quadruple therapy:** Omeprazole + bismuth subsalicylate + metronidazole + tetracycline; given for 14 days
- **OCM or OCA triple therapy:** Omeprazole + clarithromycin + metronidazole or amoxicillin for 14 days; used only if *H. pylori* clarithromycin resistance is low (<15%).

Monitoring Patients during Treatment

- ❖ Perform urea breath test after completion of treatment regimen. If tests positive, repeat the treatment course with the same quadruple regimen
- ❖ If urea breath test still remains positive, treatment is instituted based on culture of endoscopy guided biopsy, followed by antimicrobial susceptibility report.

CLOSTRIDIODES DIFFICILE INFECTION

Clostridium difficile is an obligate anaerobic, gram-positive, spore-forming *bacillus*, responsible for a unique colonic disease—**pseudomembranous colitis** which occurs almost exclusively in association with prolonged antimicrobial use. It was so named due to unusual difficulties involved in the isolation of *C. difficile*. Taxonomically, it is recently placed into a separate genera, *Clostridioides difficile*.

Pathogenesis

Clostridioides difficile is a major cause of healthcare-associated infection; mainly in the Western world. In India, the disease is reported less commonly. It is associated with the following risk factors.

- ❖ **Prolonged hospital stay:** Spores are found widely in nature, particularly in the hospitals and get colonized in the colon of patients
- ❖ **Prolonged antimicrobial use:** This can result in disruption of the normal colonic flora, which enhances the susceptibility to *C. difficile* infection
 - Cephalosporins (e.g. ceftriaxone) are frequently responsible for this condition
 - Other antibiotics, such as clindamycin, ampicillin and fluoroquinolones (ciprofloxacin) are also implicated in hospital outbreaks
 - However, all antibiotics, including vancomycin and metronidazole (which are the drugs of choice in *C. difficile* infection) have been found to carry a risk of infection, if given for prolonged duration.
- ❖ **Toxin production:** Pathogenesis is toxin-mediated. *C. difficile* may be harbored as a commensal in the intestine; however, only the toxigenic strains can cause pseudomembranous colitis
 - It produces two powerful exotoxins—toxin A (enterotoxin) and toxin B (cytotoxin)
 - Both toxins A and B are secreted in the intestine → glycosylate the GTP binding proteins that regulate the cellular actin cytoskeleton → disruption of the cytoskeleton results in loss of cell shape, adherence, and disruption of epithelial cell barrier → leading to diarrhea, and pseudomembrane formation
 - Infants do not develop symptomatic infection because they lack suitable mucosal toxin receptors which usually develop later in life. However, asymptomatic carriage is documented.
- ❖ **Host immune response may determine the outcome of the infection:**
 - Persons developing strong IgG response to toxin A—become asymptomatic carriers
 - Persons with inadequate IgG response to toxin A—develop disease.
- ❖ **Other risk factors:**
 - Suppression of normal flora (normal flora helps in converting primary bile salts to secondary bile salts, which in turn resist the germination of spores)
 - Advanced age (>65 years)
 - Immunosuppression
 - Cancer chemotherapy
 - Gastric acid suppressant medications
 - Malignancies and gastrointestinal surgeries
 - Use of electronic rectal thermometer.
- ❖ **Hypervirulent epidemic strain:** Recently, a **ribotype BI/NAP1/027** has been described. It is known as hypervirulent strain, as it produces higher levels of toxins

and causes severe infection. Fidaxomicin is the drug of choice.

Clinical Manifestations

- ❖ **Diarrhea** is the most common manifestation caused by *C. difficile*. Other manifestations include fever, abdominal pain and leukocytosis. Blood in stool is uncommon
- ❖ **Pseudomembrane:** It is composed of necrotic leukocytes, fibrin, mucus, and cellular debris. It attaches to the underlying mucosa
 - It appears as whitish-yellow plaque of size ranging from 1–2 mm to large enough to spread over the entire colonic mucosa
 - Relapse after treatment is common and seen in 15–30% of cases.

Laboratory Diagnosis

The case definition of *C. difficile* infection comprises of ≥3 unformed stool per day and isolation of the bacilli followed by toxigenicity testing.

- ❖ **Stool culture:** It is done under anaerobic condition at 37°C for 24–48 hours by using *C. difficile* specific selective media such as **CCFA** (cefoxitin cycloserine fructose agar) or **CCYA** (cefoxitin cycloserine egg yolk agar). Stool culture is highly sensitive and specific. However, since *C. difficile* can colonize the GIT, only isolation is not enough to establish the infection. Toxin demonstration is more meaningful
- ❖ **Cell culture cytotoxin neutralization assay:** It is highly specific but not as sensitive as stool culture and also has a long turnaround time
- ❖ **Antigen detection:** Various methods such as rapid tests or enzyme immunoassay are available for the detection of *C. difficile* specific antigens in stool specimen
 - **Toxin A/B:** Its detection in stool indicates presence of toxigenic strains of *C. difficile*
 - **Glutamate dehydrogenase (GDH):** It is present in both toxigenic and non-toxigenic strains, therefore called as *C. difficile* common antigen. Detection of GDH, in absence of toxin A/B indicates presence of non-toxigenic strains of *C. difficile* in specimen.
- ❖ **Molecular methods:** Such as PCR, real time PCR, gene Xpert are available targeting genes coding for *C. difficile*

such as *tcd A* (toxin A), *tcd B* (toxin B) and *tpi* (triose phosphate isomerase) in stool. They are highly sensitive and specific with faster turnaround time

- ❖ **Colonoscopy:** It is highly specific if pseudomembranes are seen; however, the sensitivity is low, when compared with other tests
- ❖ **Histopathology:** The histopathology of colonic pseudomembrane (obtained by colonoscopy) can be performed by hematoxylin and eosin stain. It is highly specific; but the sensitivity is very low.

TREATMENT

C. difficile infection

Antimicrobial therapy

- ❑ **Mild to moderate cases (Initial episode):** Oral vancomycin or fidaxomicin is the DOC (given for 10 days). Oral metronidazole can be given alternatively, but it is less effective.
- ❑ **Fulminant cases:** Oral vancomycin **plus** IV metronidazole **plus** rectal instillation of vancomycin is recommended.
- ❑ **Recurrent cases:** The treatment options are:
 - For first recurrence: Oral vancomycin or fidaxomicin (given for 10 days)
 - For multiple recurrences: Taper-and-pulse regimen—after the 10 days therapy of oral vancomycin (125 mg four times day), the dose can be tapered over 2–8 weeks.

Other modalities of treatment

- ❑ **Intravenous immunoglobulin:** Passively provide antibodies to neutralize the *C. difficile* toxins, primarily toxin A
- ❑ **Fecal microbiota transplant (FMT)** can be considered for multiple recurrent cases. It involves replenishing of the gut flora with donated feces from a screened healthy donor
- ❑ **Fidaxomicin:** It is a macrolide antibiotic, can be used in initial and recurrent cases, and also against hypervirulent strains.

Note: IV vancomycin is not effective for *C. difficile* infection.

Prevention (Infection Control Measures)

Broad spectrum antimicrobials should be stopped at the earliest. Infection control measures of contact precaution (see Chapter 21) should be followed such as:

- ❖ **Strict hand hygiene:** Hand wash is recommended, alcohol based hand rubs are not effective
- ❖ **Isolation:** Patient should be placed at isolation room and transfer should be restricted
- ❖ **Ensure proper disinfection** of floor, surfaces, toilets and other soiled areas using 1% freshly prepared hypochlorite solution.

EXPECTED QUESTIONS

I. Write short notes on:

1. Laboratory diagnosis of *Helicobacter pylori*.
2. *Clostridioides difficile* infection.

II. Multiple Choice Questions (MCQs):

1. **Most sensitive method of diagnosis of *Helicobacter pylori* is:**

- a. Culture
- b. Biopsy urease test
- c. Histopathology
- d. Urea breath test

2. All the following drugs are indicated in *Clostridioides difficile* infection, except:

- a. Oral vancomycin
- b. Fidaxomicin
- c. Oral metronidazole
- d. IV vancomycin

Answers

1. d
2. d

CHAPTER PREVIEW

■ Rotavirus Diarrhea

■ Other Viral Agents of Gastroenteritis

Viral etiology accounts for majority of the acute infectious gastroenteritis worldwide (Table 44.1). Viral gastroenteritis most commonly occurs among children. However persons of all ages can be affected. Several enteric viruses can cause acute gastroenteritis in humans, most common being rotavirus.

ROTAVIRUS DIARRHEA

Rotaviruses are the most common cause of diarrheal illness in children.

Morphology

Rotaviruses measure 60–80 nm in size and possess icosahedral symmetry.

- ❖ Comprise of a triple layered, wheel-shaped capsid (Rota in latin meaning ‘wheel’)
- ❖ Possess segmented dsRNA (11 segments)
- ❖ **Proteins:** There are six structural viral proteins (VP1 to VP7 except VP5) and six non-structural proteins (NSP1-6)
- ❖ **VP6:** Viral protein 6 (VP6) is group-specific

- ❖ **VP7** (forms the outer capsid layer) and **VP4** (forms spikes that emanate through the outer capsid layer) are strong inducers of neutralizing antibodies and are type specific.

Classification of Rotaviruses

Rotaviruses belong to the family Reoviridae; the only virus family to have dsRNA.

Traditional groups: Rotaviruses are further classified into seven major groups (A–G) based on the antigenic composition of the group specific VP6. Most human diarrhea is caused by group A

Binary system of typing: This is the recent classification of rotaviruses which is based on a binary system, i.e. combination of both serotyping and genotyping methods

- ❑ Genotyping method is based on the gene coding for VP4 antigen (a protease-sensitive protein or P-type antigen). Genotype numbers are expressed as P[1], P[2], etc.
- ❑ Serotyping method is based on VP7 antigen (a glycoprotein or G-type antigen). Serotype numbers are expressed as G1, G2, etc.
- ❑ Currently, 19G and 28[P] types are known. The most common type seen in the world as well as in India is **G1P[8]** type, which accounts for nearly 70% of total isolates
- ❑ The diversity among the rotavirus types is more commonly encountered in areas with poor hygiene.

Virus	Genome	Gastroenteritis features
Rotavirus*	Segmented dsRNA	Group A: Most common cause of severe endemic diarrheal illness in children worldwide Group B: Causes outbreaks of diarrhea in adults in China
Caliciviruses		
<i>Norovirus</i>	ssRNA	Causes outbreaks of vomiting and diarrheal illness in all ages (especially in older children and adults)
<i>Sapovirus</i>	ssRNA	Causes sporadic cases and occasional outbreaks of diarrheal illness in infants, young children, and in elderly
Astrovirus	ssRNA	
Adenovirus* (type 40 and 41)	dsDNA	Second most common viral agent of endemic diarrheal illness of infants and young children worldwide

*Clinical severity is maximum.

Pathogenesis

Rotaviruses are transmitted by fecal–oral route, then they progress further to destroy enterocytes of small intestine; however, the gastric and colonic mucosa are spared.

- ❖ They multiply in the cytoplasm of enterocytes and damage their transport mechanisms resulting in secretory diarrhea
- ❖ The non-structural protein-NSP4, acts as enterotoxin and induces secretion by altering epithelial cell function and permeability.

Clinical Manifestations

The incubation period is about 1–3 days. It has an abrupt onset, characterized by vomiting followed by watery diarrhea, fever and abdominal pain.

- ❖ Recovery usually occurs in majority, but a few children may suffer from severe loss of electrolytes and fluids leading to dehydration
- ❖ Infected adults are usually asymptomatic but show seroconversion. However, epidemics or large outbreaks have occurred in adults, especially in closed populations (e.g. geriatric ward)
- ❖ Group B rotaviruses have been implicated in large outbreaks of severe gastroenteritis in adults in China.

Epidemiology

Rotaviruses are the single most important cause of gastroenteritis in young children.

- ❖ Worldwide, about 3–5 billion diarrheal episodes in children occur annually resulting in nearly 1 million deaths especially from sub-Saharan Africa and Southern Asia
- ❖ **In developing countries like India:** Rotavirus illness occurs at a younger age, is less seasonal and more frequently caused by diverse and uncommon serotypes
- ❖ Whereas in a temperate climate, Group A rotavirus causes outbreaks in cooler months
- ❖ Rotavirus causes 8,72,000 hospitalizations, 32,70,000 outpatient visits, and an estimated 78,000 deaths annually in India.

Laboratory Diagnosis

- ❖ **Direct detection of virus:** Feces collected early in the illness is the most ideal specimen. Rotaviruses can be demonstrated in stool by:
 - **Immunoelectron microscopy (IEM):** Rotaviruses have a sharp edged triple shelled capsid; look like the spokes grouped around the hub of a wheel (Fig. 44.1)
 - **Isolation** of rotavirus is difficult. Rolling of tissue cultures may be attempted to enhance replication

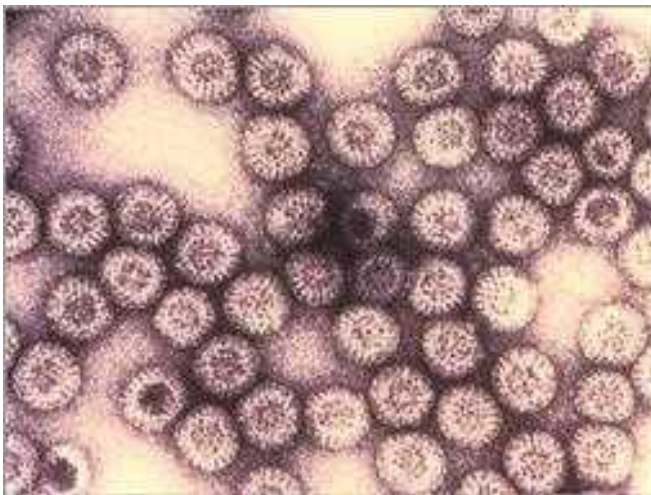


Fig. 44.1: Rotavirus (electron micrograph).

Source: Public Health Image Library, /ID# 15194/Dr Erskine L Palmer/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Detection of viral antigen** in stool by ELISA and latex agglutination-based methods
- ❖ **RT-PCR** is the most sensitive detection method for detection of rotavirus from stool
- ❖ **Typing methods:** G serotypes and P genotypes of rotaviruses can be detected by RNA sequence typing and neutralization test respectively
- ❖ **Serologic tests** (ELISA) can be used to detect the rise of antibody titer. This may be useful for seroprevalence purpose.

TREATMENT

Viral gastroenteritis

Treatment is mainly supportive, to correct the loss of water and electrolytes such as oral or parenteral fluid replacement.

Prevention

Vaccine

Two brands of rotavirus vaccine are available:

Rotavac

It contains live attenuated Rotavirus 116E (G9P[10] strain). It provides cross protection against many types including G1P[8] type.

- ❖ It is manufactured by Bharat biotech, India
- ❖ It is introduced under national immunization schedule of India (2020), in selected states—Andhra Pradesh, Assam, Haryana, Himachal Pradesh, Jharkhand, Madhya Pradesh, Odisha, Rajasthan, Tamil Nadu, Tripura and Uttar Pradesh
- ❖ Three doses (5 drops/dose): Administered orally at 6, 10 and 14 weeks along with DPT and OPV
- ❖ Overall efficacy in first 2 years of life is about 55%
- ❖ Side effects ($\geq 5\%$): Crying, irritability, fever and diarrhea. No vaccine induced intussusception has been reported.

Rotarix

Rotarix contains live attenuated G1P[8] strain; also provides cross protection against G3, G4 and G9. It has to be reconstituted before use. Given as two doses: 1st at 6 week and 2nd dose is given 4 weeks later.

General Preventive Measures

It includes—(1) measures to improve hygiene and sanitation in the community, and (2) contact precautions such as strict hand hygiene to prevent transmission from infected persons (Chapter 21).

OTHER AGENTS OF GASTROENTERITIS

- ❖ **Family Caliciviridae** comprises of four genera; out of which two consists of important agents of human diarrhea—(1) genus *Norovirus*, which includes the Norwalk viruses, and (2) genus *Sapovirus*, which includes the Sapporo-like viruses

- They are icosahedral, 27–40 nm in size; possess **cup-like depressions** (calyx meaning cup), on the capsid surface typically observed under electron microscope. They are single stranded (+)sense RNA and a single major structural protein
- **Norwalk virus** is the most important cause of epidemic viral gastroenteritis in adults
 - ◆ It is common in winter months in temperate climates; therefore called as **winter vomiting disease** or gastric flu
 - ◆ **Symptoms** begin 12–48 hours after the exposure; characterized by diarrhea, abdominal pain, nausea and vomiting
 - ◆ **Common food sources** include contaminated salad, fresh fruits, shellfish (such as oysters), or water. Other sources include the infected person; touching contaminated surfaces.
- **Sapoviruses** cause sporadic cases and occasional outbreaks of diarrheal illness in infants, young children, and the elderly
- Laboratory diagnosis and treatment are similar to that of rotavirus. They are not cultivable.
- ❖ **Adenoviruses (types 40 and 41)** are the second most common viral agents of endemic diarrheal illness of infants and young children worldwide, responsible for 2–12% of all diarrhea episodes in young children
- ❖ **Astroviruses** exhibit a distinctive **star-like** morphology under the electron microscope
 - They are of 28–30 nm size with an icosahedral symmetry and contain a positive sense, ssRNA
 - At least seven serotypes have been identified, of which serotype-1 is the most common
 - Astroviruses cause sporadic cases and occasional outbreaks of diarrhea in infants, young children and in elderly.
- ❖ **Respiratory viruses:** Diarrhea has also been reported as a part of manifestations of certain respiratory viruses such as:
 - SARS coronaviruses
 - Influenza A/H5N1 virus
 - Influenza A/H1N1 virus (the 2009 pandemic strain).
- ❖ **Toroviruses** and **Picobirnaviruses** cause gastroenteritis in a variety of animals, but their role as primary cause of gastroenteritis in humans remains unclear.

EXPECTED QUESTIONS

I. Write short note on:

1. Rotavirus gastroenteritis.

II. Multiple Choice Questions (MCQs):

1. **The most common viral cause of gastroenteritis:**
 - a. Rotavirus
 - b. Norwalk virus
 - c. Adenovirus
 - d. Hepadnavirus
2. **Which is the most important cause of epidemic viral gastroenteritis in adults?**
 - a. Rotavirus
 - b. Norwalk virus
 - c. Adenovirus
 - d. Hepadnavirus
3. **A 6-month child presented with diarrhea and vomiting for three days. Which of the following**

enterotoxin is most likely responsible for the condition?

- a. NSP4
 - b. NSP6
 - c. VP3
 - d. VP7
4. **The most common organism causing diarrhea in adults associated with shellfish ingestion:**
 - a. Astrovirus
 - b. Adeno virus types 40, 41
 - c. Norwalk virus
 - d. Rotavirus
 5. **Rotavirus vaccine is given by which route?**
 - a. Intramuscular
 - b. Intravenous
 - c. Oral
 - d. Subcutaneous

Answers

1. a 2. b 3. a 4. c 5. c

CHAPTER PREVIEW

- Intestinal Amoebiasis
- Intestinal Flagellate Infection (Giardiasis)
- Opportunistic Intestinal Coccidian Parasitic Infections
- Other Intestinal Protozoan Infections

The protozoan parasites which cause intestinal infection include:

- ❖ Intestinal amoebae: *Entamoeba histolytica*
- ❖ Intestinal flagellate: *Giardia lamblia*
- ❖ Opportunistic intestinal coccidian parasites: *Cryptosporidium*, *Cyclospora*, and *Cystoisospora*
- ❖ Others: *Balantidium coli*, *Blastocystis hominis*, *Sarcocystis* and Microsporidia (now considered as fungi).

Entamoeba histolytica and *Balantidium coli* produce dysentery, whereas the other intestinal protozoans produce diarrheal disease.

INTESTINAL AMOEBIASIS

Amoebae (meaning “change”) are single-celled protozoan parasites that constantly change their shape; which is due to presence of an organ of locomotion called as “pseudopodium.” Based on the habitat they are of two types—(i) intestinal amoebae, and (ii) free-living amoebae.

- ❖ **Intestinal amoebae:** *Entamoeba histolytica* is the most important pathogenic intestinal amoeba. Most other species of *Entamoeba* are harmless commensals in human intestine; e.g. *Entamoeba coli*
- ❖ **Free-living amoebae:** They are small, freely living, widely distributed in soil and water and can cause opportunistic infections in humans, affecting central nervous system. Important human pathogenic free-living amoebae are: *Naegleria*, *Acanthamoeba* and *Balamuthia* (Chapter 75).

ENTAMOEBIA HISTOLYTICA INFECTIONS

E. histolytica causes amoebic dysentery (discussed in this chapter) and a wide range of other invasive diseases, including amoebic liver abscess (Chapter 49).

There are three other species of *Entamoeba* such as *E. dispar*, *E. moshkovskii* and *E. bangladeshi* which are morphologically similar to *E. histolytica*; can be differentiated from each other by molecular and other methods described

later. All of them are found as commensal in human intestine; *E. moshkovskii* and *E. bangladeshi* are reported to be associated with infantile diarrhea.

Epidemiology

E. histolytica is worldwide in distribution but more common in tropical and subtropical countries.

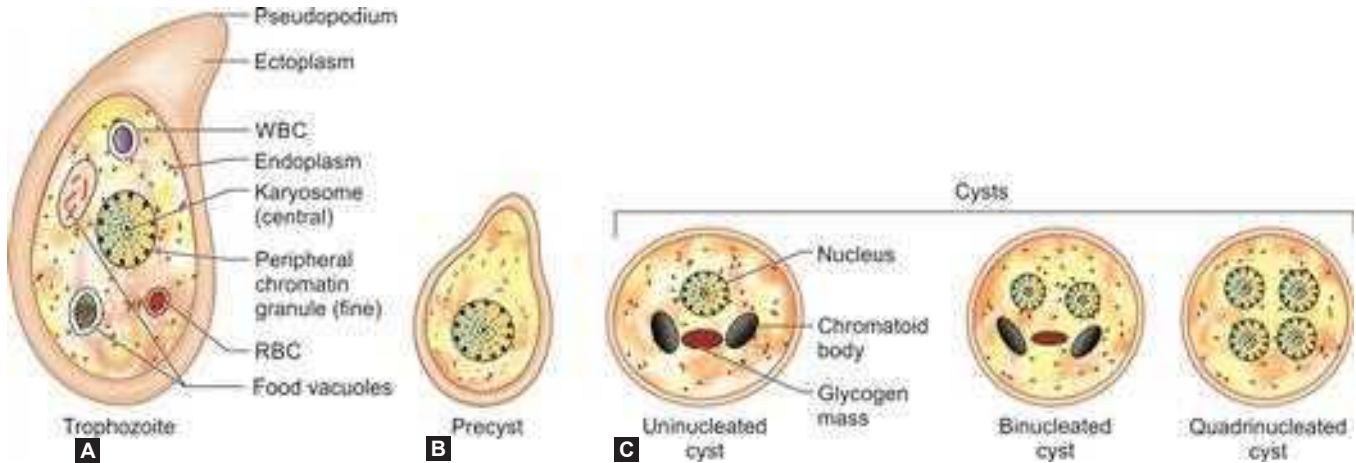
- ❖ The largest burden of the disease occurs in tropics of China, Central and South America, and Indian subcontinents affecting 10% of the world’s population (500 million)
- ❖ It is the third most common parasitic cause of death in the world (after malaria and schistosomiasis)
- ❖ Globally, approximately 50 million cases of intestinal amoebiasis with 110, 000 deaths have been reported annually
- ❖ In India, it has been reported throughout with a prevalence rate of 15% (ranges from 3.6% to 47.4%).

Morphology

E. histolytica exist as three stages (Figs 45.1A to C).

1. **Trophozoite:** It is the invasive form as well as the feeding and replicating form of the parasite found in the feces of patients with active disease
 - It measures 15–20 μm in size; contains a single nucleus
 - It possesses finger like projections called as pseudopodia, which helps in locomotion.
2. **Precyst:** It is the intermediate stage between trophozoite and cyst
3. **Cyst:** It is the diagnostic form of the parasite found in the feces of carriers as well as patients with active disease
 - They measure 12–15 μm in size, contain 1–4 number of nuclei
 - Cysts can be either immature or mature. Immature cyst contains one or two nuclei. Whereas the mature cyst is quadrinucleated and is the infective form of the parasite.

The morphological forms have been described in detail subsequently under laboratory diagnosis.



Figs 45.1A to C: *Entamoeba histolytica* (schematic diagram): **A.** Trophozoite; **B.** Precyst; **C.** Cysts.

Life Cycle (Fig. 45.2)

Host: *E. histolytica* completes its life cycle in a single host, i.e. man.

Infective form: Mature quadri-nucleated cyst is the infective form. However, immature cysts can also be infective.

Mode of transmission: *E. histolytica* is transmitted by—(i) **fecal-oral route** (most common), by ingestion of food or water contaminated with mature quadri-nucleated cysts, (ii) **sexual** (anogenital or orogenital) contact, (iii) very rarely by **vectors** (flies and cockroaches may mechanically transmit the cysts from feces, and contaminate the food and water).

Development in Human Intestine

❖ **Small intestine:** Cysts bypass gastric juice and reach small intestine, where they undergo **excystation**. The cyst wall gets lysed by trypsin and four small trophozoites are released

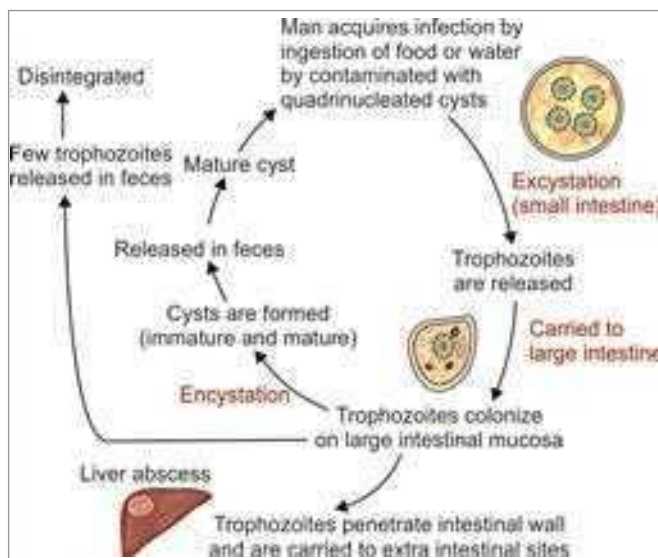


Fig. 45.2: Life cycle of *Entamoeba histolytica*.

- ❖ **Large intestine:** Trophozoites are carried to the ileocecal region of large intestine, where they multiply by binary fission, and then colonize on the intestinal mucosa
- ❖ After colonization, trophozoites show different courses depending on host susceptibility
 - **Asymptomatic cyst passers:** In majority of individuals, trophozoites do not cause any lesion, transform into cysts and are excreted in feces
 - **Amoebic dysentery:** In some individuals, trophozoites adhere to intestinal mucosa producing intestinal ulcers and dysentery
 - **Invasive amoebiasis:** Very rarely, trophozoites invade intestinal mucosa, gain access to the portal veins and migrate to extraintestinal sites, most common site being liver where they cause amoebic liver abscess (Chapter 49).
- ❖ **Excystation:** When the intestinal lesions start healing and patient improves, the trophozoites transform into precysts then into cysts which are liberated in feces. Encystation occurs only in the large intestine. Cysts are never formed once the trophozoites are excreted in stool
- ❖ Cysts released in feces can survive in the environment and become the infective form. Trophozoites are also excreted but get disintegrated either in the environment or by gastric juice when ingested.

Virulence Factors

Trophozoite of *E. histolytica* is the invasive form and possesses many virulence factors that play an important role in the pathogenesis.

- ❖ **Amoebic lectin antigen:** It is a 260-kDa galactose and N-acetylgalactosamine inhabitable surface protein (Gal/NAG lectin), present on the surface of trophozoites. It is the principal virulence factor, helps in **adhesion** by binding to glycoprotein receptors on large intestinal epithelium and vascular endothelium
- ❖ **Other virulence factors include:**
 - **Amoebapore:** It forms pores on the target cell membrane causing leakage of ions

- Cysteine proteases and hydrolytic enzymes: Help in invasion by the destruction of the target tissue
- Neuraminidase and metallocollagenase: Help in invasion.

Pathogenesis

The pathogenesis of intestinal amoebiasis occurs through the following steps.

- ❖ **Colonization:** Trophozoites first colonize the intestinal mucosa; facilitated by the bacterial flora which lower the oxygen tension
- ❖ **Adhesion:** Then the trophozoites penetrate the mucus layer and adhere to the large intestinal mucosa by means of Gal/GalNAc lectin molecules
- ❖ **Flask-shaped ulcers:** Trophozoites produce characteristic flask-shaped ulcerative lesions, (broad base with a narrow neck) in large intestine, most common site being cecum. Ulcers are usually small, round to oval; scattered with intervening normal mucosa. They may be superficial or deep; size ranging from pin head to inches
- ❖ **Invasion:** Amoebae then invade the large intestinal wall; migrate to extraintestinal sites. The invasion is facilitated by cysteine proteases, and hydrolytic enzymes secreted by *E. histolytica*
- ❖ **Intestinal complications:** Trophozoites may produce various intestinal complications.

Complications of Intestinal Amoebiasis

Local migration of trophozoites may produce various intestinal complications (Fig. 45.3).

- ❑ **Fulminant amoebic colitis:** Results from generalized necrotic involvement of entire large intestine, occur

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more commonly in immunocompromised patients and in pregnancy

- ❑ **Amoebic appendicitis:** Results when the infection involves the appendix
- ❑ Intestinal perforation and amoebic peritonitis
- ❑ Toxic megacolon and intussusception
- ❑ **Amoebiasis cutis or cutaneous amoebiasis:** It is a virulent form of amoebiasis, presents as perianal skin ulcers as extension of amoebic colitis; may also be seen following anal intercourse
- ❑ **Amoeboma (amoebic granuloma):** A diffuse pseudotumor like mass of granulomatous tissue found in rectosigmoid region
- ❑ **Chronic amoebiasis:** It is characterized by thickening, fibrosis, stricture formation with scarring.

Clinical Manifestations

Incubation period varies from one to four weeks. Males and females are affected equally. Majority of infections (90%) result in asymptomatic cyst passers. The remaining (10%), develop intestinal amoebiasis; characterized by:

- ❖ **Amoebic dysentery:** Symptoms include bloody diarrhea (up to 10 times per day) with mucus and pus cells, colicky abdominal pain, fever, prostration, and weight loss. Amoebic dysentery should be differentiated from bacillary dysentery (Table 45.1)
- ❖ **Amoebic appendicitis:** Presented with acute right lower abdominal pain
- ❖ **Amoeboma:** It presents as palpable abdominal mass
- ❖ **Fulminant colitis:** Presents as intense colicky pain, rectal tenesmus, more than 20 motions/day, fever, nausea, anorexia and hypotension.

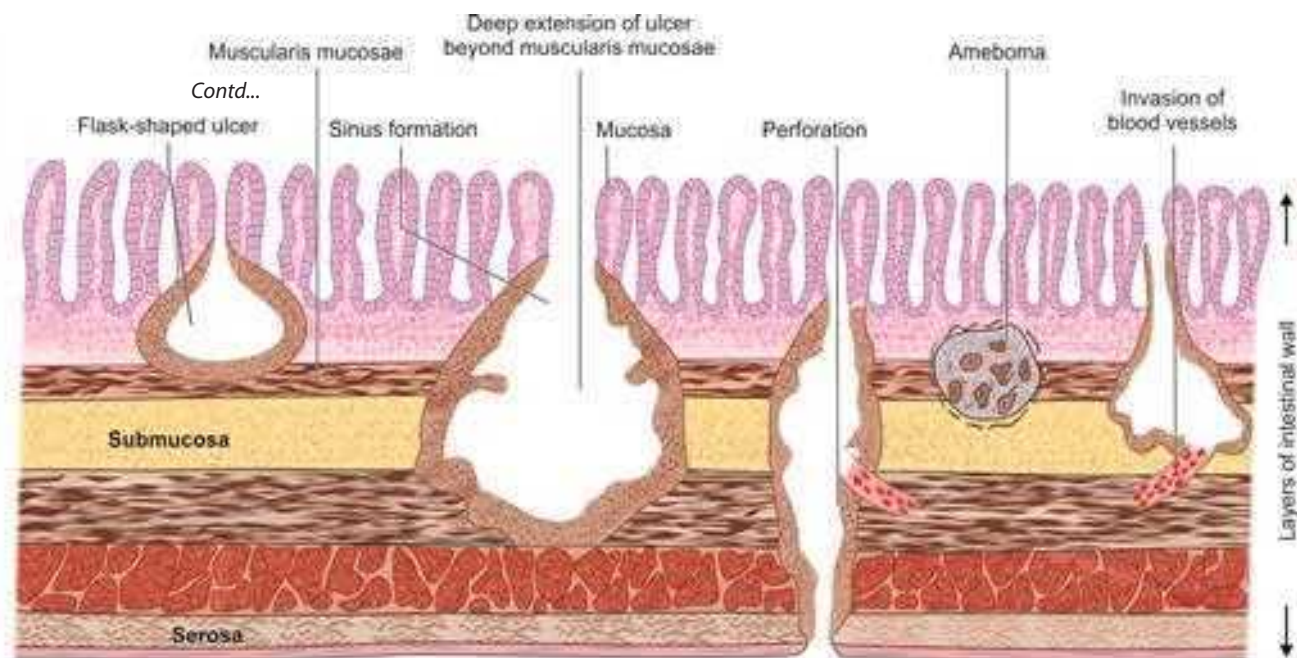


Fig. 45.3: Complications of intestinal amoebiasis (cross-section of intestinal wall).

Table 45.1: Differences in stool characters between amoebic dysentery and bacillary dysentery.

Character	Amoebic dysentery	Bacillary dysentery
Pathology		
Ulcer	Deep	Shallow
Margin	Ragged and undermined	Uniform
Intervening mucosa	Normal	Inflamed
Cellular response	Mononuclear	Polymorphonuclear
Stool macroscopic feature		
Number of motion	6–10/day	> 10/day
Amount	Copious amount	Small quantity
Color	Dark red	Bright red
Odor	Offensive	Odorless
Reaction	Acidic	Alkaline
Consistency	Not adherent to the container	Adherent to the container
Stool microscopic feature		
Red blood cells (RBCs)	In clumps	Discrete or in rouleaux
Pus cells	Few	Numerous
Macrophages	Few	Numerous*
Eosinophils	Present	Absent or rare
Charcot-Leyden crystal	Present	Absent
Pyknotic body**	Present	Absent
Organism detected	<i>E. histolytica</i> cyst or trophozoite	Bacteria (e.g. <i>Shigella</i>)

*Macrophage may contain RBCs, so can be mistaken as trophozoite of *E. histolytica*

**Pyknotic body: Nuclear remains of tissue cells and leukocytes

LABORATORY DIAGNOSIS

Intestinal amoebiasis

- ❑ **Stool microscopy** by wet mount, permanent stains, etc.—detects cysts (round, 1–4 nuclei) and trophozoites (with finger like pseudopodia)
- ❑ **Histology**—Intestinal biopsies stained with PAS or H&E stains reveal trophozoites
- ❑ **Stool culture**—Polyxenic and axenic culture
- ❑ **Stool antigen detection** (copro-antigen)—ELISA (detecting 170-kDa of lectin Ag) and ICT (detecting 29-kDa surface Ag)
- ❑ **Serology**
 - Amoebic antigen—ELISA (170-kDa of lectin Ag)
 - Amoebic antibody—ELISA
- ❑ **Isoenzyme** (zymodeme) analysis
- ❑ **Molecular diagnosis**—Nested multiplex PCR and real time PCR (18S rRNA) and Biofire FilmArray

Laboratory Diagnosis

Sample Collection

Stool is the specimen of choice. Minimum of three stool samples should be collected on alternate day (within 10 days) as amoebae are shed intermittently.

- ❖ Other samples include rectal exudates and rectal ulcer tissues, collected by colonoscopy
- ❖ It should be examined immediately within 1–2 hours of collection or can be preserved in merthiolate iodine or formalin. However, refrigeration is not recommended.

Stool Macroscopy

Stool is foul smelling, copious in amount, dark red in color mixed with blood and mucus and not adherent to the container. It should be differentiated from bacillary dysentery, where the stool is bright red, odorless and adherent to the container (Table 45.1).

Stool Microscopy

Direct examination of stool (from the blood-streaked area) by saline and iodine mount is performed to demonstrate trophozoites and cysts (see highlight box below).

- ❖ Microscopy is poorly sensitive (25–60% with single sample) but the sensitivity increases to ~90% when six stool samples are examined
- ❖ The sensitivity can further be improved by examining the stool samples after concentration by formalin ether sedimentation method
- ❖ Stool can also be examined by staining with permanent stains like trichrome stain and iron hematoxylin stain. Internal structures of cysts and trophozoites are well demonstrated by permanent stains (Figs 45.4B, C and 45.5C)
- ❖ **Reporting:** Cyst and trophozoite of *E. histolytica* are indistinguishable from that of *E. dispar* or *E. moshkovskii* or *E. bangladeshi*, except for the presence of RBCs in trophozoites of *E. histolytica* (which might not be there after dysentery episode is over). So, the report should always be sent as “cyst or trophozoite of *E. histolytica*/*E. dispar*/*E. moshkovskii*/*E. bangladeshi* are found in the stool microscopy”.

Trophozoites

Demonstration of trophozoite in freshly-passed stool is considered as gold standard microscopic test for active infection (Figs 45.1A and 45.4A). They are not found in stool of asymptomatic carriers.

- ❑ **Measures** 15–20 µm, has cytoplasm (divided into a clear ectoplasm and a granular endoplasm) and single nucleus
- ❑ **Motility:** They are actively motile, with finger-like pseudopodia, which exhibit active, unidirectional rapid progressive and purposeful movement
- ❑ **Hematophagous trophozoite:** Presence of ingested RBCs in cytoplasm is a feature *E. histolytica* (being invasive), which differentiates it from *E. dispar*
- ❑ **Nucleus:** It has a central karyosome and fine peripheral chromatin granules lining the nuclear membrane. The space between the karyosome and the nuclear membrane is traversed by spoke like radial arrangement of achromatic fibrils (**cart wheel appearance**). Nucleus is seen as a faint outline in saline mount, but better appreciated in permanent staining (Figs 45.1A, 45.4B to D).
- ❑ Trophozoites are better-appreciated in saline mount (Fig. 45.4A) than iodine mount as iodine kills the trophozoites and motility cannot be appreciated. Therefore, trophozoites should not be reported based on iodine mount.

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Quadrinucleated Cysts

Cysts are found in stool specimens of both patients (with active infection) and carriers. The internal structure of cysts is clearly appreciated by iodine mount than saline mount and even better on permanent staining.

- ❑ Cysts appear round, 12–15 µm in size containing 1–4 nuclei (Figs 45.1C and 45.5A to C). The appearance of nucleus is as described for trophozoites
- ❑ Both mature cysts (contain 4 nuclei) and immature cysts (contain 1 or 2 nuclei) are found in stool
- ❑ Cytoplasm of uninucleated cyst contains few **chromatoid bodies** (aggregation of ribosome) and a large **glycogen mass** (stains brown with iodine).

Histology

Trophozoites can be detected in sigmoidoscopy guided biopsies collected from at least six areas of colonic mucosa and stained with Periodic acid–Schiff (PAS) or H&E stains (Fig. 45.4D), or ideally with peroxidase staining with anti-*E. histolytica* antibodies.

Stool Culture

Culture methods are not routinely used for diagnosis; show 50–70% sensitivity and 100% specificity. They are useful in studying pathogenicity of amoeba, for preparation of

amoebic antigen for serological tests, research and teaching purpose. Various culture media include:

- ❖ **Polyxenic culture:** This culture medium contains bacterial supplement, starch and serum providing nourishment to amoeba. Various culture media used are:
 - National Institute of Health (NIH) media
 - Boeck and Drbohlav egg serum medium containing Locke's solution.
- ❖ **Axenic culture:** It lacks bacterial supplement, e.g. Diamond's medium.

Stool Antigen Detection (Coproantigen)

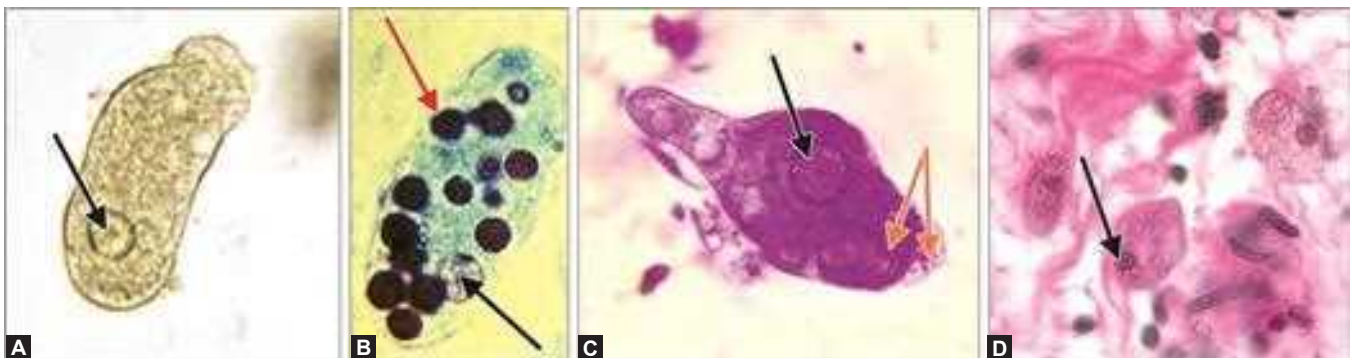
As amoebic antigens get denatured by stool preservatives, only fresh or frozen stool sample should be used. Various tests used to detect antigens in stool are:

- ❖ **ELISA** detecting 170 kDa of lectin antigen: It shows >95% sensitivity and specificity. It can also differentiate pathogenic *E. histolytica* (lectin antigen positive) and nonpathogenic *E. dispar* (lectin antigen negative)
- ❖ **Immunochromatographic test (ICT):** It is a rapid diagnostic test, gives result within 20–30 minutes.

Triage Parasite Panel

It a cartridge based commercially available ICT, shows 83–96% sensitivity and 99–100% specificity. It simultaneously detects three parasitic antigens in stool (Fig. 45.6).

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Figs 45.4A to D: *Entamoeba histolytica* trophozoites: **A.** Saline mount; **B and C.** Trichrome stain, note the single nucleus (black arrow), RBCs (red arrow) and ectoplasm and endoplasm (orange arrows); **D.** Trophozoites in colon tissue stained with H&E.

Source: Swierczynski G, Milanesi B. Atlas of human intestinal protozoa microscopic diagnosis (with permission).



Figs 45.5A to C: Cyst of *E. histolytica*: **A.** Saline mount; **B.** Iodine mount; **C.** Trichrome stained smear. Note the chromatoid body with blunt ends (arrow).

Source: Swierczynski G, Milanesi B. Atlas of human intestinal protozoa microscopic diagnosis.



Fig. 45.6: Triage parasite panel, positive for *E. histolytica*/*E. dispar* antigen.

Source: Alere Diagnostics.

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- ❑ *Giardia lamblia* (alpha-1 giardin antigen)
- ❑ *E. histolytica*/*E. dispar* (29 kDa surface antigen): It cannot differentiate *E. histolytica* from *E. dispar*, as both express this antigen
- ❑ *Cryptosporidium parvum* (protein disulfide isomerase antigen)

Serology

Amoebic Antigen

Amoebic antigen in serum is found only in patients with active infection and disappears after clinical cure. So its presence in serum indicates recent and active infection.

- ❖ ELISA is available detecting 170-kDa of lectin antigen—usually positive in the early stage of the disease (sensitivity of 65%)
- ❖ ELISA is also available detecting various other antigens like—serine rich *E. histolytica* protein (SREHP), lysine rich surface antigen, etc.

Amoebic Antibody

ELISA is available detecting serum antibody (IgG) against lectin antigen. Antibodies appear only in the later stages of intestinal amoebiasis and therefore are not much useful in diagnosis.

Isoenzyme (Zymodeme) Analysis

When isoenzymes of *E. histolytica* are subjected to electrophoresis, several zymodeme patterns can be produced. It can differentiate *E. histolytica* from *E. dispar*, as both produce distinct zymodeme patterns.

However, this method is difficult to perform, technically demanding and time-consuming and therefore not routinely used.

Molecular Diagnosis

Molecular methods have emerged as the gold standard test for the diagnosis of amoebiasis.

- ❖ **Nested multiplex PCR** is available targeting small subunit rRNA genes. It can differentiate *E. histolytica*, *E. dispar* and *E. moshkovskii* with a sensitivity nearing 90% and specificity of 90–100%
- ❖ **Real-time PCR** targeting 18S rRNA gene can be used as an alternative to the conventional PCR. It is more sensitive can quantitate the parasite load and takes less time than conventional PCR
- ❖ **BioFire FilmArray**: It is a fully automated commercial PCR system from bioMérieux. Its gastrointestinal panel is used to detect common bacterial, viral, parasitic (*Cryptosporidium*, *Cyclospora*, *E. histolytica*, *Giardia*) diarrheal pathogens.

Other Nonspecific Findings

- ❖ **Imaging method**: Colonoscopy can be performed to detect collar button or flask shaped amoebic ulcers

- ❖ **Charcot-Leyden crystals** in stool: They are diamond shaped, eosinophilic breakdown products found in stool of some cases
- ❖ **Moderate leucocytosis** in blood.

TREATMENT

Intestinal amoebiasis

Asymptomatic carriage: Treatment helps in reducing passage of cysts in stool and thereby preventing the disease transmission. Luminal agents are used for treatment.

- ❑ Iodoquinol (for 20 days) or
- ❑ Paromomycin (for 10 days)

Amoebic dysentery: Treatment consists of:

- ❑ *Tissue agents*: Metronidazole (for 5–10 days) or tinidazole (for 3 days) plus
- ❑ *Luminal agents* as above

Other measures include fluid and electrolyte replacement and symptomatic treatment.

Prevention

Preventive measures are as follows:

- ❖ Avoiding ingestion of food and water contaminated with human feces
- ❖ Treatment of asymptomatic carriers.

AMOEBAE MORPHOLOGICALLY RESEMBLING *E. HISTOLYTICA*

E. dispar, *E. moshkovskii* and *E. bangladeshi* are the amoebae which are morphologically (both cyst and trophozoite) similar to that of *E. histolytica*.

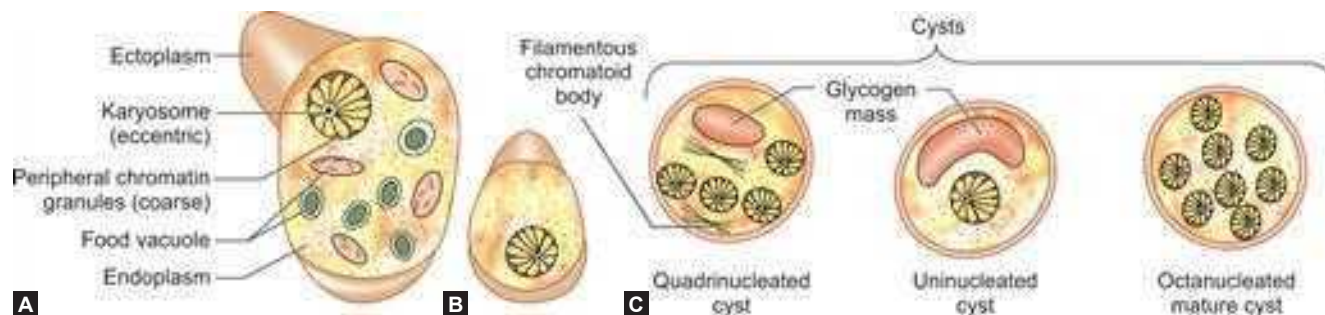
- ❖ *E. dispar* is a non-pathogenic commensal found in human intestine
- ❖ *E. moshkovskii* is primarily a free-living amoeba, found in polluted water, may cause occasionally non-invasive diarrhea in infants
- ❖ *E. bangladeshi* have been recovered from asymptomatic individuals, as well as those with diarrhea
- ❖ **Diagnosis**: They can be differentiated from *E. histolytica* by various methods
 - PCR amplifying small subunit rRNA gene—most reliable method
 - Detection of lectin antigen in stool
 - RBC inside trophozoites—present only in *E. histolytica* (marker of invasiveness)
 - Zymodeme study.

NONPATHOGENIC AMOEBAE

There are many nonpathogenic amoebae that may be found as harmless commensals in human intestine. Examples include *Entamoeba coli*, *E. hartmanni*, *E. gingivalis*, *E. polecki*, *Endolimax nana* and *Iodamoeba butschlii*.

Entamoeba coli

Entamoeba coli is the most common nonpathogenic amoeba that colonizes the large intestine. It is frequently found in the stool samples of healthy individuals and



Figs 45.7A to C: *Entamoeba coli* (schematic diagram): **A.** Trophozoite; **B.** Precyst; **C.** Cyst.

Source: Swierczynski G, Milanesi B. Atlas of human intestinal protozoa microscopic diagnosis (with permission).

should be differentiated from that of *E. histolytica* (Table 45.2 and Figs 45.7 and 45.8).

INTESTINAL FLAGELLATE INFECTIONS

Giardia lamblia is a classic example of intestinal flagellate associated with diarrheal manifestations. It bears flagella as the organ of locomotion.

GIARDIASIS

Giardiasis is caused by *Giardia lamblia*, also known as *G. intestinalis* or *G. duodenalis*. It was first observed by AV Leeuwenhoek in 1681 while examining his own stool. Later it was described and named after Dr F Lambl and Professor A Giard (1859).

Epidemiology

G. lamblia is worldwide in distribution, it is considered as one of the most common parasite—causes both endemic and epidemic intestinal disease and diarrhea.

- ❖ **Geographical area:** More common in warm climate of tropics and subtropics
- ❖ **In India:** Prevalence in children ranges from 0.5–70%
- ❖ **Zoonotic potential:** *Giardia* has low zoonotic potential infecting many mammals. Outbreak of giardiasis reported in Canada has been linked to beavers, hence the disease is also called as **beaver fever**
- ❖ **Genotypes:** *Giardia* has been classified into two genotypes A and B, which are further classified into eight assemblages (A to H). Human infections are caused by assemblages A and B. Assemblage B is common from Southeast Asia including India, whereas assemblage A is more common in Africa.

Morphology

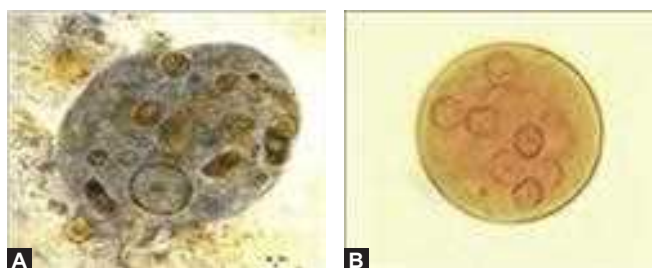
Giardia inhabits in crypts of duodenum and upper part of jejunum in man. It occurs in two forms (Fig. 45.10):

1. **Pear-shaped trophozoite:** It is the pathogenic form and feeding stage of the parasite
2. **Tetra-nucleated oval cyst:** It is the infective form as well as the diagnostic form of the parasite.

Table 45.2: Differences between *Entamoeba histolytica* and *Entamoeba coli*.

	<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>
Trophozoite		
Size	15–20 μm	20–25 μm
Motility	Very active and unidirectional purposeful motility Pseudopodia with finger-like projection	Sluggish, nonpurposeful and aimless motility in any direction Blunt pseudopodia
Cytoplasm	Clearly differentiated to ectoplasm and endoplasm	Not differentiated
Cytoplasmic inclusions	RBC, leukocytes, tissue debris and bacteria	Same except it does not contain RBC
Nucleus	Karyosome is small and central Nuclear membrane is thin and lined by fine chromatin granules	Karyosome is large and eccentric Nuclear membrane is thick and lined by coarse chromatin granules
Cyst		
Size	12–15 μm	15–25 μm
Nucleus	Same as trophozoite	Same as trophozoite
Nuclei	1–4 in number	1–8 in number
Chromatoid body	Thick bars with rounded ends	Filamentous and thread like ends

Abbreviation: RBC, red blood cells.



Figs 45.8A and B: *Entamoeba coli*: **A.** Trophozoite (iron hematoxylin stain) shows nucleus with coarse peripheral chromatin and abundant food vacuoles in the cytoplasm containing fecal debris; **B.** Cyst (iodine mount) containing several nuclei.

Source: Swierczynski G, Milanesi B. Atlas of human intestinal protozoa microscopic diagnosis (with permission).

The morphological forms are described in detail subsequently under laboratory diagnosis.

Life Cycle (Fig. 45.9)

Host: *Giardia* completes its life cycle in one host.

Infective form: Cysts are the infective form.

Mode of transmission: Man acquires infection by ingestion of food and water contaminated with mature cysts or rarely by sexual route (mainly in homosexuals).

Development in Man

- ❖ **Excystation:** Two trophozoites are released from each cyst in the duodenum within 30 minutes of entry
- ❖ **Multiplication:** Trophozoites multiply by longitudinal binary fission in the duodenum
- ❖ **Adhesion:** Trophozoites adhere to the duodenal mucosa by the bilobed adhesive ventral disk
- ❖ In active stage of the disease, the trophozoites are directly excreted in diarrheic stool due to sloughing of intestinal epithelial cells every 72 hours
- ❖ **Encystation:** Gradually when the trophozoites pass down to jejunum, encystation begins, which involves retraction of the flagella into axoneme followed by the formation of the cyst wall. On maturation, nuclei divide to become four. The cysts excreted in feces can survive in the environment and are infective to man.

Pathogenicity

- ❖ **Infective dose:** As few as 10–25 cysts can initiate the infection
- ❖ **Risk factors:** Children are more commonly affected. Other high-risk groups are elderly debilitated persons, poor hygiene, and immunodeficient individuals.

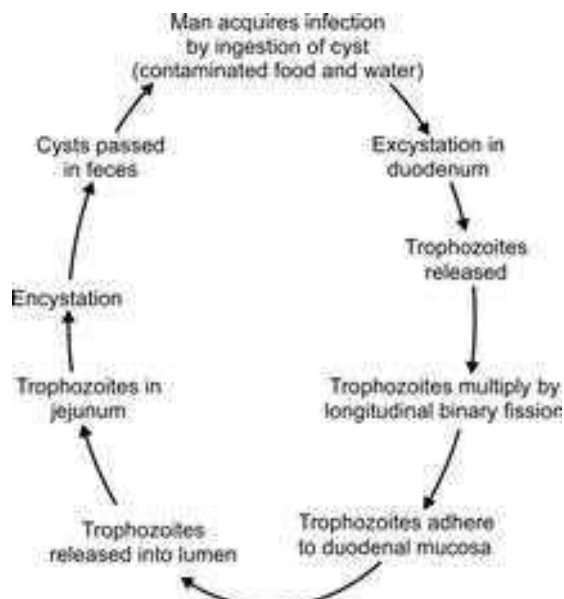


Fig. 45.9: Life cycle of *Giardia lamblia*.

However, it does not appear to be associated with HIV/AIDS

- ❖ **Pathogenic form:** Trophozoites are the pathogenic form, they adhere to the duodenal mucosa, cause disruption of the intestinal epithelium that leads to increased permeability and malabsorption. Trophozoites do not invade the mucosa, but feed on mucus secretions
- ❖ **Malabsorption** may be of various types such as:
 - Malabsorption of fat (steatorrhea)—leads to foul smelling profuse frothy diarrhea
 - Disaccharidase deficiencies (lactose, xylose)—leading to lactose intolerance
 - Malabsorption of vitamin A, B12 and iron
 - Protein losing enteropathy.
- ❖ **Antigenic variation:** *Giardia* undergoes frequent antigenic variations due to a cysteine rich protein on its surface called **variant surface protein (VSP)**. This helps the parasite to evade host immune system; which leads to persistence of infection resulting in chronic and recurrent illness.

Clinical Features

Clinical course of giardiasis can be divided into three stages:

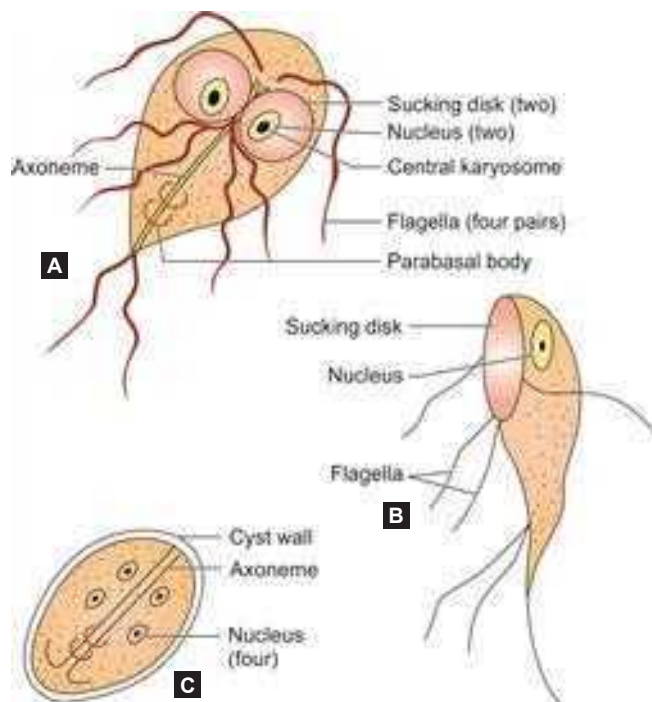
1. **Asymptomatic carriers:** Most infected persons are asymptomatic, harbor the cysts in the gut and spread the infection
2. **Acute giardiasis:** Incubation period varies from 1 week to 3 weeks
 - Common symptoms include diarrhea, abdominal pain, bloating, belching, flatus and vomiting
 - **Fatty diarrhea (steatorrhea):** Diarrhea is often foul smelling with fat, cellular exudate and mucus but no blood
 - *Giardia* is an important cause of traveler's diarrhea.
3. **Chronic giardiasis:** It may present with or without a previous acute symptomatic episode
 - **GI symptoms:** Include recurrent episodes of foul smelling diarrhea, foul flatus, sulfurous belching with rotten egg taste, and profound weight loss leading to growth retardation
 - **Extraintestinal manifestations** have been described, such as urticaria, arthritis, anterior uveitis, salt and pepper retinal changes.

Laboratory Diagnosis

Stool Examination

Stool microscopy is considered as the gold standard for diagnosis of giardiasis, which detects cysts and trophozoites.

- ❖ Trophozoites adhere firmly to the duodenal mucosa by adhesive disk leading to intermittent shedding. Hence, repeated stool examination (at least three samples collected on alternate days within 10 days) should be done



Figs 45.10A to C: *Giardia lamblia* (schematic diagram):
A. Trophozoite front view; **B.** trophozoite lateral view; **C.** Cyst.

- ❖ Pus cells or blood (RBCs) will never be seen in stool microscopy in case of giardiasis. If found; suggests alternative diagnosis
- ❖ **Sensitivity** varies from 60% to 80% with one stool and more than 90% after three stools examination
- ❖ **Concentration techniques** like zinc sulfate floatation or formalin ether sedimentation methods are employed to increase the chance of detection
- ❖ **Duodenal sampling:** If stool examination is negative, then direct duodenal samples like aspirates (obtained by entero-test) or biopsy (done by endoscopy) should be processed
- ❖ **Permanent stains** such as trichrome stain can be used to demonstrate cysts and trophozoites in stool (Figs 45.11C and 45.12B).

Trophozoite

Presence of trophozoites indicates active stage of the disease. They are better appreciated in the saline mount and permanent staining, but not in iodine mount.

- ❑ **Measure** 10–20 μm in length and 5–15 μm in width.
- ❑ **Shape:** In front view, it is pear-shaped (or tear drop or tennis racket shaped) and laterally, it appears as a spoon or sickle shaped (Figs 45.10A, B and 45.12A to D)
- ❑ **Motility:** It has a falling leaf-like motility
- ❑ It is bilaterally symmetrical; bears the following structures (Figs 45.10A):
 - One pair of nuclei
 - Adhesive or sucking disk (bilobed) at its ventral surface
 - Four pairs of flagella
 - Pair of axonemes (intracellular portion of flagella)

Cyst

Giardia cyst is oval-shaped, measures 11–14 μm in length and 7–10 μm in width (Figs 45.10C and 45.11A to D).

- ❑ It contains four nuclei, and axonemes
- ❑ They cannot differentiate active disease from carriers, as they are passed in stool in both.

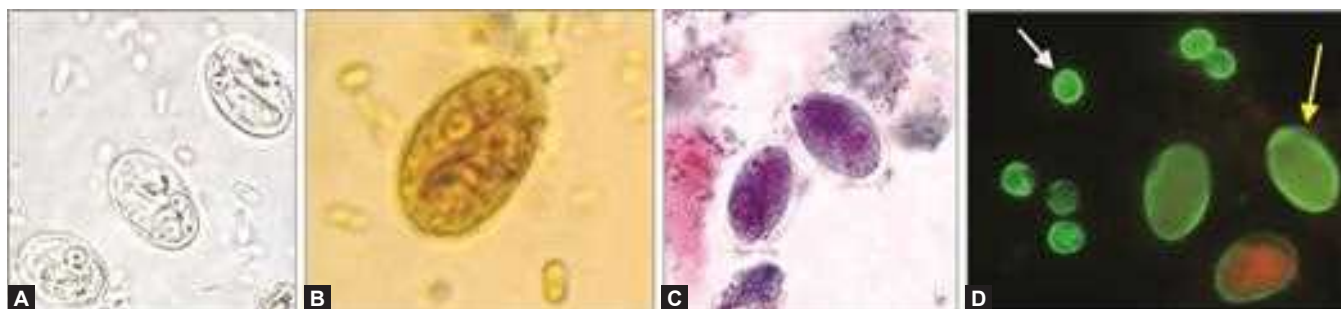
Entero-test (or String Test)

It uses a gelatin capsule attached to a thread containing a weight (Fig. 45.13).

- ❖ One end of the thread is attached to the outer aspect of the patient's cheek, and then, the capsule is swallowed
- ❖ Capsule gets dissolved in stomach releasing the thread which is carried to the duodenum, gets unfolded and takes up the duodenal samples
- ❖ Four hours later, the thread is withdrawn and shaken in saline to release trophozoites which can be detected microscopically by wet mount or permanent stained smear
- ❖ The entero-test is also useful for other upper intestinal parasites such as *Strongyloides*, *Cryptosporidium* and *Clonorchis*.

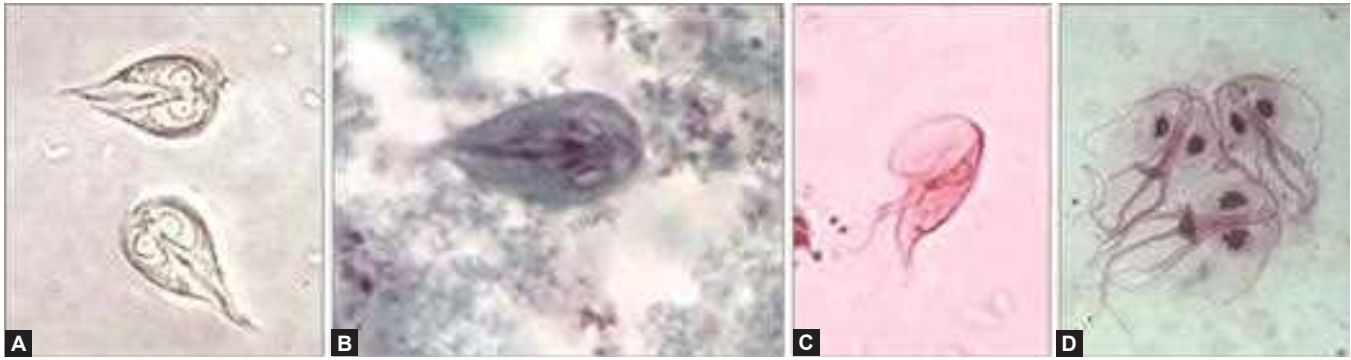
Histopathology

Endoscopy-guided duodenal biopsy tissue can be processed by touch preparation and stained by Giemsa stain to demonstrate the trophozoites (Fig. 45.12D).



Figs 45.11A to D: Cysts of *Giardia lamblia*: **A.** Saline mount; **B.** Iodine mount; **C.** Trichrome stain; **D.** Direct immunofluorescence assay.

Source: A and B. Giovanni Swierczynski, Bruno Milanese "Atlas of human intestinal protozoa Microscopic diagnosis" (with permission); C and D. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Figs 45.12 A to D: Trophozoites of *Giardia lamblia*: **A.** Saline mount front view; **B.** Trichrome stain front view; **C.** Merthiolate iodine formalin (MIF) stain lateral view (spoon shaped); **D.** Giemsa stained mucosal imprint (front view).

Source: A to C. Giovanni Swierczynski, Bruno Milanese. "Atlas of human intestinal protozoa Microscopic diagnosis" (with permission); D. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

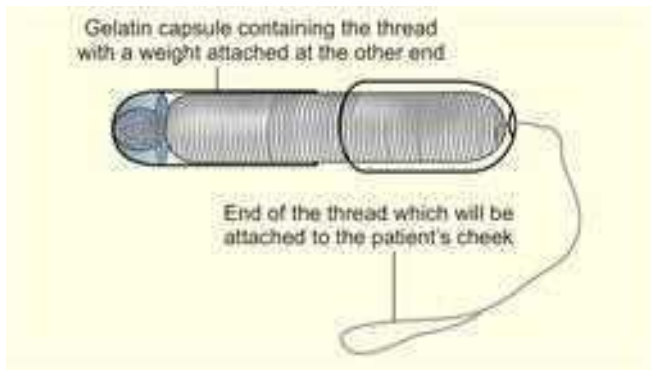


Fig. 45.13: Entero-test equipment showing duodenal capsule.

Antigen Detection in Stool (Coproantigen)

ELISA and direct fluorescent antibody tests (DFA, Fig. 45.11D) are available using labeled monoclonal antibodies against cyst wall protein antigens. Both the tests are highly sensitive (90–100%) and specific (99–100%). They are very useful in microscopy negative samples and also in outbreak situations.

Rapid ICT (e.g. triage parasite panel) has been developed that simultaneously detect antigens of *Giardia* (alpha-1 giardin antigen), *Entamoeba histolytica* and *Cryptosporidium* with comparable sensitivity and specificity like ELISA (Fig. 45.6).

Antibody Detection

Both indirect fluorescent antibody (IFA) and ELISA formats are developed to detect antibodies in the serum.

- ❖ But unlike microscopy and antigen detection, presence of antibody cannot differentiate recent and past infection
- ❖ Hence, serology is only helpful for epidemiological purpose for estimating the prevalence of infection.

Culture

Giardia can be cultivated in axenic media like Diamond's media used for *E. histolytica*. Culture is done for research purpose and to prepare the antigens.

Molecular Methods

Detection of *Giardia* nucleic acid in stool and environmental samples (water) by polymerase chain reaction (PCR) or by gene probes is highly sensitive and specific. PCR can detect as low as 1–2 cyst(s) in sample.

- ❖ **BioFire FilmArray:** It is a fully automated commercial PCR system from bioMérieux. The gastrointestinal panel is used to detect common bacterial, viral, parasitic (*Cryptosporidium*, *Cyclospora*, *E. histolytica*, *Giardia*) diarrheal pathogens
- ❖ **Molecular typing** can detect genotype and assemblages; performed by sequencing several genes, such as the glutamate dehydrogenase (*gdh*), and β -giardin (*bg*).

Radiological Finding

- ❖ **Fluoroscopy** may reveal hypermotility at the duodenal and jejunal levels
- ❖ **X-ray after barium meal** may reveal non-specific irregular mucosal thickening with large dilated loops of hypotonic bowel (positive in 20% of cases). Barium meal may interfere with the stool examination. So, stool samples should be collected before the barium meal.

TREATMENT

Giardiasis

- ❑ Tinidazole (2g once orally) is considered as the drug of choice
- ❑ Metronidazole (for 5 days) or nitazoxanide (for 3 days) is given alternatively
- ❑ Furazolidone is given to children and auranofin, paromomycin can be given in pregnancy
- ❑ In patients with AIDS and hypogammaglobulinemia, giardiasis is often refractory to treatment. Prolonged therapy with metronidazole (for 21 days) has been successful
- ❑ Metronidazole resistance has been reported in *Giardia*. Auranofin is shown to be effective.

Prevention

Giardiasis can be prevented by:

- ❖ Improved food and personal hygiene

- ❖ Boiling or filtering of potentially contaminated water
- ❖ Treatment of asymptomatic carriers
- ❖ No vaccine is currently available.

OTHER INTESTINAL FLAGELLATES OF MINOR IMPORTANCE

There are a number of intestinal flagellates which are harmless commensals of human gastrointestinal tract.

- ❖ *Trichomonas tenax* (mouth)
- ❖ *Pentatrichomonas hominis* (large intestine)
- ❖ *Chilomastix mesnili* (large intestine)
- ❖ *Enteromonas hominis* (large intestine)
- ❖ *Retortamonas intestinalis* (large intestine)
- ❖ *Dientamoeba fragilis*: It is an amoeboflagellate; differs from other flagellates as it bears internal flagellum. It is a harmless commensal in large intestine, occasionally has been reported in association with mucous diarrhea.

OPPORTUNISTIC INTESTINAL COCCIDIAN PARASITIC INFECTIONS

Cryptosporidium, *Cyclospora*, and *Cystoisospora* are the intestinal coccidian parasites that can cause opportunistic infections in HIV infected patients producing profuse watery diarrhea. They can be diagnosed by detection of acid fast oocysts in stool microscopy.

- ❖ *Cryptosporidium* species infecting man is now classified as two separate species, *C. parvum* (mammals, including humans) and *C. hominis* (primarily humans). Other species infect wide range of mammals, but not man
- ❖ *Cyclospora cayetanensis* is the most recently described coccidian parasite, as human intestinal pathogen
- ❖ *Isospora belli* has been recently renamed as *Cystoisospora belli*. *C. belli* is the only species that infects man. No other animal reservoir is known.

MORPHOLOGY

The life cycle of coccidian parasites passes through various stages such as oocyst, trophozoite, schizont (meront), merozoite, and zygote.

Oocyst

Oocyst is the infective form to man as well as the diagnostic form excreted in the feces.

- ❖ It is round to oval, surrounded by a cyst wall and bears sporozoites (Figs 45.15A to C)
- ❖ The oocysts are acid fast in nature but do not stain by iodine
- ❖ They are extremely resistant to routine chlorination, heat and other disinfectants
- ❖ In *Cryptosporidium*, two types of oocysts are demonstrated—(1) thick walled, and (2) thin walled.

LIFE CYCLE

The life cycle of *Cryptosporidium* is discussed first (Fig. 45.14). It completes its life cycle in single host (man or other animals).

Infective stage: Sporulated oocysts (both thick-walled and thin-walled) are the infective form of the parasite.

Mode of Transmission: Man acquires infection by:

- ❖ **Ingestion** of food and water contaminated with feces containing thick-walled oocysts
- ❖ **By autoinfection:** Thin-walled oocysts can infect the same host through contaminated fingers.

Development in Man

- ❖ **Excystation:** Oocyst gets dissolved in the small intestine, releasing four sporozoites
- ❖ **Invasion:** Sporozoites invade the brush border epithelium of the small intestine and lie inside a parasitophorous vacuole near the microvilli surface, within which all the stages of development take place
- ❖ **Schizogony:** The sporozoites subsequently differentiate into trophozoites, which further develop into a schizont (or meront). Schizont undergoes asexual multiplication (schizogony) to produce merozoites
- ❖ **Gametogony:** The merozoites undergo gametogony and transform into sexual forms (microgamete and macrogamete)
- ❖ **Sporogony:** Fertilization takes place between microgamete and macrogamete to produce the zygote

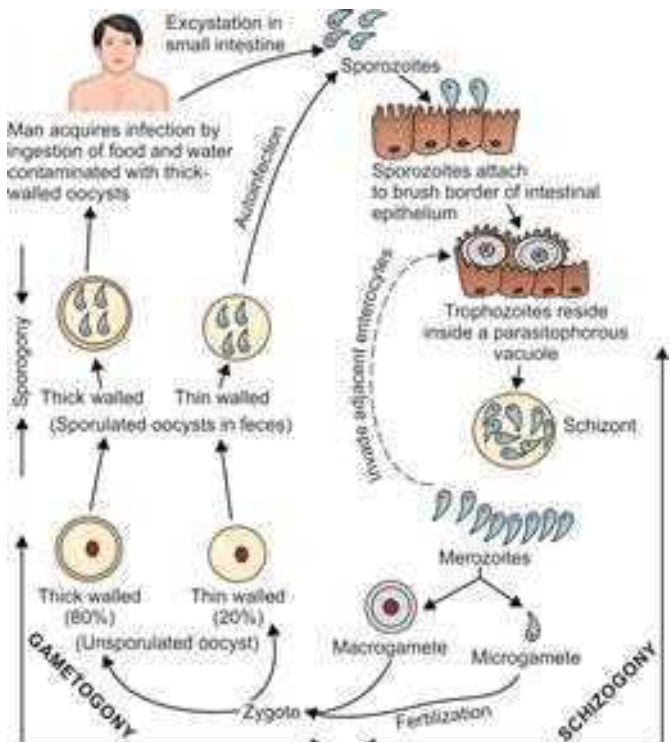


Fig. 45.14: Life cycle of *Cryptosporidium*.

- Subsequently, zygotes transform into both thick-walled oocysts (80%) and thin-walled oocysts (20%). Then the oocysts undergo sporogony to produce four sporozoites
- Sporulated oocysts are excreted in the feces; which are now infective to the same person (thin-walled) and other individuals (thick-walled).

The life cycle of *Cyclospora* and *Cystoisospora* are similar to *Cryptosporidium*, with the following differences.

- ❖ Autoinfection is not observed
- ❖ The oocysts released in the human feces are unsporulated
- ❖ The sporulation of oocyst takes place in the soil (environment); whereas in *Cryptosporidium*, the sporulation of oocyst takes place in the human intestine.

EPIDEMIOLOGY

Although infection with intestinal coccidian parasites is seen worldwide, it is more prevalent in tropical and subtropical climates of developing countries from South America, Africa, and Southeast Asia including India. The highest prevalence is reported from Haiti.

- ❖ **Risk factors:** It is more common in area with poor sanitation and HIV infected individuals
- ❖ **Peak age:** Infants and children are commonly affected
- ❖ **Source of infection** includes rainwater lodges and swimming pool recreational water, or contaminated food (e.g. imported foods including raspberries, basil and mesclun lettuce)
- ❖ *C. parvum* is zoonotic, common in rural areas and *C. hominis* is mainly a human parasite, more commonly seen in an urban setting. Whereas for *Cystoisospora*, humans are the only host; there is no animal reservoir
- ❖ A massive outbreak of cryptosporidiosis occurred in Milwaukee, USA in 1993 affecting >4 lakh people; transmitted through the contaminated public water supply.

PATHOGENESIS

The pathogenesis of intestinal coccidian parasites involves the following steps.

- ❖ **Excystation:** The oocyst secretes proteases and aminopeptidases which facilitate excystation in small intestine, releasing sporozoites
- ❖ **Attachment:** Sporozoites attach to the brush border epithelium of the small intestine (ileum). It is facilitated by virulence factors such as **CP47** (47 kDa *Cryptosporidium* protein)
- ❖ **Penetration:** Discharges from the apicomplex present in the anterior end of the sporozoites help in invasion
- ❖ **Vacuole:** Following penetration, the parasite forms a parasitophorous vacuole near the microvilli surface of the host cells
- ❖ **Cytokines:** Then, the parasite activates the host immune system to release cytokines like tumor necrosis factor (TNF)- α , IL-8, prostaglandins, etc. which induce inflammation

- ❖ These factors increase intestinal secretion of chloride and water and decrease the sodium absorption leading to fluid loss and diarrhea.

Cryptosporidiosis occurs more frequently than cyclosporiasis and cystoisosporiasis. This may be attributed to various factors.

- ❖ Low infective dose of *Cryptosporidium* (10–100 oocysts can initiate the infection)
- ❖ Small size of the oocyst (4–6 μm)
- ❖ Oocysts are resistant to the available drugs and disinfectants including chlorination
- ❖ Large animal and human reservoir
- ❖ Zoonotic contact.

CLINICAL FEATURES

Immunocompetent Hosts

The majority of infections remain asymptomatic. Some individuals develop symptoms after an average incubation period of about 1 week.

- ❖ Watery non-bloody diarrhea is the most common presentation. Other features like abdominal pain, nausea, anorexia, fever, and/or weight loss may be present
- ❖ Self-limiting, subsides within 1–2 weeks
- ❖ They can cause 2–6% cases of traveler's diarrhea, associated with traveling to developing countries

Immunocompromised Hosts (e.g. AIDS)

Host immune status is not a primary factor for initiating the infection, but plays an important role in determining the length and severity of the illness once the infection is established. HIV/AIDS is the most important immunocompromised condition.

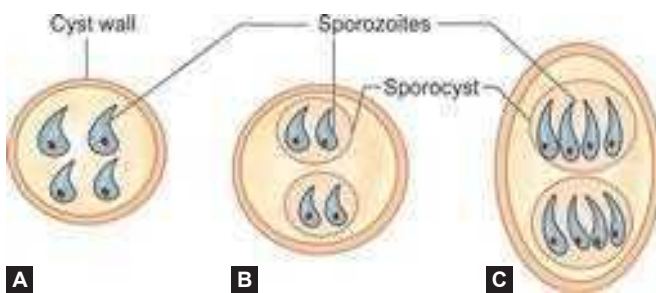
- ❖ It produces a **chronic, persistent profuse diarrhea** (1–25 L/day), leading to significant fluid and electrolyte loss (resembling cholera and diarrhea). Severe weight loss, wasting and abdominal pain may be seen
- ❖ **Extraintestinal manifestations** are common such as biliary tract infection (sclerosing cholangitis), respiratory tract infection, and pancreatitis
- ❖ The disease is more severe in patients with AIDS when CD4+ T cell counts become **<100/ μL**
- ❖ Other immunocompromised conditions include severe combined immunodeficiency syndrome, hematological malignancies and solid organ transplant recipient
- ❖ Disease is more severe in *Cryptosporidium* than *Cyclospora* and *Cystoisospora*, which is attributed to autoinfection seen in the former—a key factor for maintaining the infection, resulting in chronic persistent diarrhea.

LABORATORY DIAGNOSIS

Direct Microscopy (Stool Examination)

Stool microscopy remains the most common method employed for diagnosis of intestinal coccidian parasitic infections.

- ❖ **Sample collection:** Three stool samples should be collected. Rarely (in HIV positive patients), sputum, bronchial wash, duodenal or jejunal aspirate can be collected
- ❖ **Direct wet mount:** Direct wet mounting from the mucus plug of the stool sample is done to demonstrate highly refractile, double-walled oocyst (see highlight box below)
- ❖ **Concentration technique:** If the oocyst load is less, then various techniques are used to concentrate the stool sample
 - Flootation technique like Sheather's sugar floatation technique (widely used for coccidian parasites), or saturated salt floatation technique
 - Sedimentation technique like formalin ether sedimentation technique.
- ❖ **Acid-fast staining:** The oocysts are acid-fast to 0.5–1% sulfuric acid or acid alcohol and appear as red color oocysts against blue background
 - Kinyoun's method (cold acid-fast staining) is the method of choice
 - The sensitivity of acid-fast staining is low and it requires a minimum concentration of 50,000 and 500,000 oocysts/mL of liquid stool and formed stool respectively (Figs 45.16A, 45.17C and 45.18B).
- ❖ **Direct fluorescent antibody staining:** It is available for cryptosporidiosis. The oocysts can be detected by using fluorescent labeled monoclonal antibody directed against cyst wall antigens
 - This test is more sensitive (10 times) and specific than acid-fast staining
 - It is also useful to detect oocyst from water and other environmental samples
 - Currently, this method is considered as the gold standard test for cryptosporidiosis (Fig. 45.16B).
- ❖ **Autofluorescence:** Oocysts of *Cyclospora* fluoresce when viewed under ultraviolet epifluorescence microscopy. It is a rapid and sensitive method, although not specific (Fig. 45.17B). *Cystoisospora* oocysts also show autofluorescence, but this property is not consistent like in *Cyclospora*
- ❖ **Phase contrast microscopy** is also useful (Fig. 45.18C).



Figs 45.15A to C: Sporulated oocysts (schematic diagram) of **A.** *Cryptosporidium*; **B.** *Cyclospora*; **C.** *Cystoisospora*.

Oocyst

The oocysts of intestinal coccidian parasites are highly refractile, double walled and acid-fast. Differences between *Cryptosporidium*, *Cyclospora* and *Cystoisospora* have been described in Table 45.3.

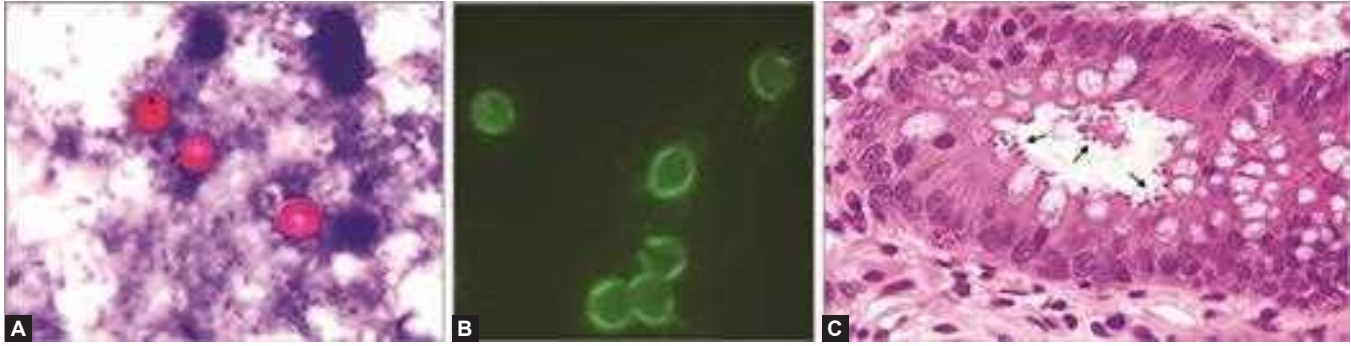
- ***Cryptosporidium*:** The oocyst is round, measures 4–6 μm size, contains four sporozoites (Figs 45.15A, 45.16A to C)
- ***Cyclospora*:** The oocyst is round, measures 8–10 μm size, contains two sporocysts, each comprising two sporozoites. (Figs 45.15B, 45.17A to D)
 - The oocysts are variably acid fast (i.e. 50% of oocysts are acid fast and the rest are nonacid fast)
 - Oocysts also exhibit autofluorescence
- ***Cystoisospora*:** The oocyst is oval and larger, measuring 20–33 μm length; contains two sporocysts, each comprising four sporozoites (Figs 45.15C, 45.18A to D)

Histopathology

Various developmental stages of the coccidian parasites can be demonstrated from the intestinal biopsy specimens (jejunum).

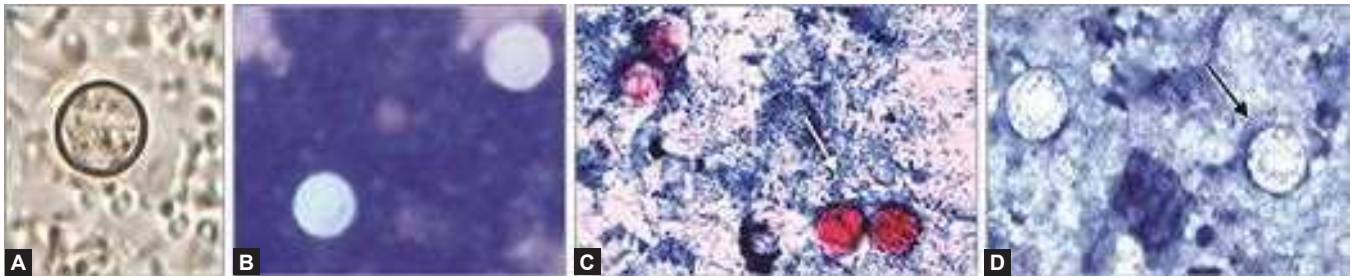
Table 45.3: Differences between *Cryptosporidium*, *Cyclospora* and *Cystoisospora*.

Property	<i>Cryptosporidium</i>	<i>Cyclospora</i>	<i>Cystoisospora</i>
Infective form	Sporulated oocyst	Sporulated oocyst	Sporulated oocyst
Diagnostic form	Sporulated oocyst	Unsporulated oocyst	Unsporulated oocyst
Outbreaks	Frequent, large	Common, large	Occasional, small
Zoonotic potential	Yes	Uncertain	No
Oocyst size	4–6 μm	8–10 μm	20–33 μm
Oocyst shape	Round	Round	Oval
Sporulated oocyst contain	Four sporozoites	Two sporocysts, each having two sporozoites	Two sporocysts, each having four sporozoites
Acid-fastness	Uniformly acid-fast	Variable acid-fast	Uniformly acid-fast
Autofluorescence	No, but can be stained with fluorescent dye	Autofluorescence ++	Autofluorescence +/-
Sporulation of the oocyst	Occurs inside the host cells (enterocytes)	Occurs in soil (environment)	Occurs in soil (environment)
Treatment	Nitazoxanide	Cotrimoxazole	Cotrimoxazole



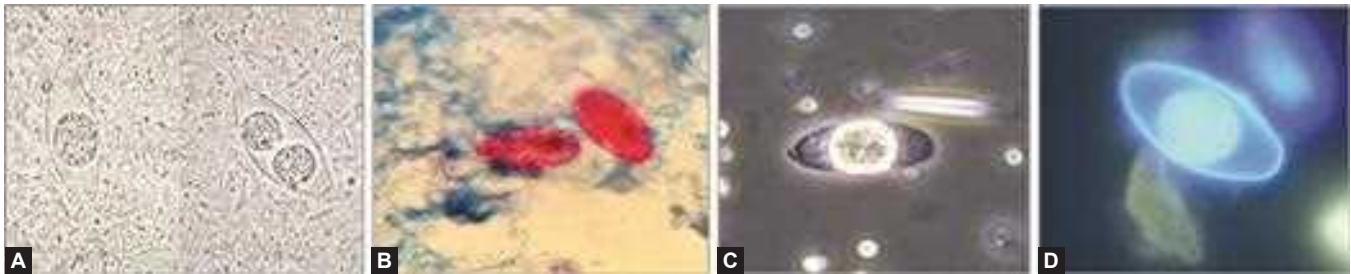
Figs 45.16A to C: *Cryptosporidium* species: **A.** Acid-fast stain shows red color oocysts against blue background; **B.** Direct fluorescent antibody staining shows brilliant green fluorescent oocysts; **C.** Hematoxylin and eosin stain of intestinal biopsy shows numerous oocysts at the luminal surface of the intestinal crypts (marked by arrows).

Source: Swierczynski G, Milanesi B. Atlas of human intestinal protozoa Microscopic diagnosis (with permission).



Figs 45.17A to D: *Cyclospora* species: **A.** Saline mount preparation showing unsporulated oocyst; **B.** Epifluorescence microscopy showing autofluorescent oocysts; **C.** Acid-fast oocysts; **D.** Non-acid fast oocysts (variable acid-fastness).

Source: A. Dr Anand Janagond, Department of Microbiology, S Nijalingappa Medical College, Bagalkot, Karnataka; B to D. Swierczynski G, Milanesi B. Atlas of human intestinal protozoa microscopic diagnosis (with permission).



Figs 45.18A to D: *Cystoisospora belli*: **A.** Saline mount preparation shows left—unsporulated oocyst and right—sporulated oocyst; **B.** Modified acid-fast stain showing unsporulated oocysts; **C.** Phase contrast microscopy showing unsporulated oocyst; **D.** Fluorescent stained smears showing unsporulated oocyst.

Source: B. Dr Anand Janagond, Department of Microbiology, S Nijalingappa Medical College, Bagalkot, Karnataka (with permission); A, C and D. Swierczynski G, Milanesi B. Atlas of human intestinal protozoa Microscopic diagnosis (with permission).

When stained by H&E stain, *Cryptosporidium* appears as 1–3 μm basophilic round bodies within the cell membrane of enterocytes called as “blue beads” (Fig. 45.16C).

Antigen Detection from Stool

It is available for cryptosporidiosis.

- ❖ **ELISA** has been developed to detect *C. parvum* specific coproantigen (oocyst antigen) from stool; shows a sensitivity ranging from 66% to 100% with excellent specificity

- ❖ **ICT** is also available (e.g. Triage parasite panel) for simultaneous detection of antigens of *Cryptosporidium* (detecting protein disulfide isomerase antigen), *Giardia* and *E. histolytica*. It shows sensitivity (83–96%) and specificity (99–100%) (Fig. 45.6).

Antibody Detection

Methods such as ELISA are available detecting antibodies in patient’s serum against oocyst antigens of *Cryptosporidium*

or *Cyclospora*. This is useful for sero-epidemiological purpose, not for routine diagnosis.

Molecular Diagnosis

Molecular methods such as PCR or real-time PCR can be performed to detect specific genes from both clinical and environmental samples targeting genes such as 18S rRNA or small subunit rRNA.

- ❖ Molecular methods are more sensitive, takes less time and can differentiate *Cryptosporidium*, *Cyclospora* and *Cystoisospora*. It can also differentiate various genotypes of *Cryptosporidium* which plays an important role in outbreak situations
- ❖ **BioFire FilmArray:** It is a fully automated commercial PCR system from bioMérieux. The gastrointestinal panel is used to detect common bacterial, viral, parasitic (*Cryptosporidium*, *Cyclospora*, *E. histolytica*, *Giardia*) diarrheal pathogens.

Other Methods

- ❖ Low CD4-T lymphocyte count (especially in HIV positive patient)
- ❖ Fecal leukocyte marker—lactoferrin is increased in 75% of cases indicating increase pus cells in feces.

TREATMENT

Intestinal coccidian infections

Mild cases

Self-limiting, requires fluid replacement like ORS. Lactose-free glutamine supplemented diet is preferred for cryptosporidiosis.

Severe cases

- ❑ **Cryptosporidiosis:** Nitazoxanide is given to adults (for 3 days). It is not effective in HIV-infected patients. Paromomycin can be given as an alternate
- ❑ **Cyclosporiasis and cystoisosporiasis:** Treated with cotrimoxazole (for 7-10 days). HIV-infected patients may experience relapses and may require long-term suppressive maintenance therapy
- ❑ Patients who cannot tolerate cotrimoxazole may be treated with ciprofloxacin or nitazoxanide.

Prevention

- ❖ Requires minimizing exposure to infectious oocysts in human or animal feces
- ❖ Proper hand washing, use of submicron water filters, use of appropriate disinfectants/sterilants to kill the oocysts (such as hydrogen peroxide, steam sterilization), improved personal hygiene are some of the efforts to prevent transmission.

OTHER INTESTINAL PROTOZOAN INFECTIONS

Other protozoan parasites which can cause intestinal disease in man include *Balantidium coli*, *Sarcocystis* spp., and *Blastocystis hominis*. Involvement of intestinal muscles

occurs in chronic Chagas's disease caused by *Trypanosoma cruzi*.

BALANTIDIASIS

Balantidiasis is an intestinal infection caused by a protozoan parasite *Balantidium coli*. It is the largest protozoan and the only ciliated parasite of humans. It produces intestinal disease similar to amoebic dysentery.

- ❖ **Habitat:** It resides in the large intestine, similar to *E. histolytica*
- ❖ **Host:** Pig (main reservoir) and other animals are the primary hosts. Man is an accidental host
- ❖ **Morphology:** It exists in two forms—(1) trophozoite (found in the dysenteric stool), (2) cyst (found in carriers and chronic cases). Both forms are binucleated having a large macronucleus and a small micronucleus
- ❖ **Life cycle:** Man gets infection by ingestion of food and water contaminated with cysts (infective form)
 - Cysts transform into trophozoites, which then multiply in the large intestine by both asexual mode and sexual mode (conjugation)
 - Both trophozoites and cysts are excreted in the feces. Trophozoites disintegrate but the cysts are resistant and are infective to man and pig.
- ❖ **Clinical features:** Majority of infected individuals become asymptomatic carriers. A small number of people develop acute dysenteric disease
 - Characterized by profuse diarrhea and dysentery with mucous and blood, weeks to months later. Other features include fever, nausea, vomiting and abdominal pain
 - The disease is similar to amoebic dysentery except that, it is less severe, invasion is limited to muscularis mucosa; intestinal ulcers are superficial and extraintestinal involvement occurs very rarely.
- ❖ **Epidemiology:** *B. coli* is worldwide in distribution particularly in tropical and subtropical countries where pig to human contact is more. However, it is rare in India
- ❖ **Laboratory diagnosis:** Repeated stool examination should be done as the parasite is excreted intermittently. Trophozoites are appreciated in the saline mount, whereas internal structures of cysts are seen more clearly in iodine mount.

Trophozoites

They are detected in acute disease (dysenteric stool) (Figs 45.19A, 45.20A)

- ❑ Oval shaped, 50–100 µm in length and 40–70 µm in breadth
- ❑ Exhibit rotatory motility due to presence of **cilia** covering the whole body
- ❑ Bi-nucleated, having a kidney shaped large macronucleus and a small micronucleus.

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Cysts

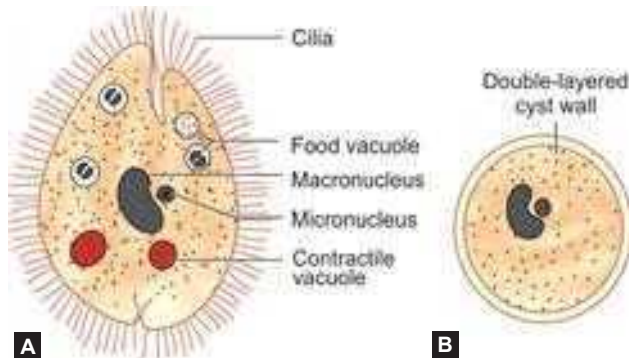
They are seen in chronic cases or in carriers. The cyst is round shaped, measures 50–70 μm in size, surrounded by a cyst wall and has two nuclei (Figs 45.19B, 45.20B).

Histopathology: Histopathological staining of the biopsy tissue or scrapping of the ulcers taken by sigmoidoscopy reveals a cluster of trophozoites, cysts and lymphocytic infiltration in the submucosa.

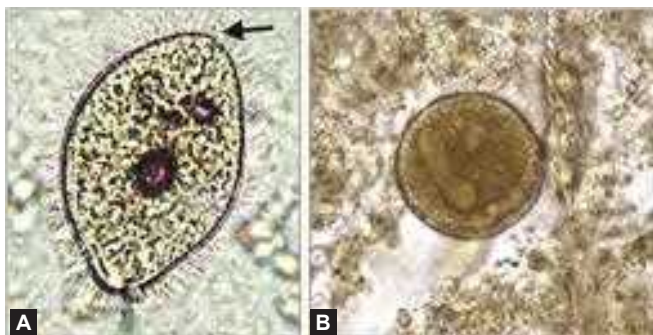
- ❖ **Treatment:** Tetracycline is the drug of choice, given for 10 days. Alternatively, metronidazole can be given
- ❖ **Prevention:** Balantidiasis can be prevented by:
 - Treatment of carriers shedding the cysts
 - Hygienic rearing of pigs and prevention of pig to human contact
 - Prevention of contamination of food or water with pig and human feces.

BLASTOCYSTOSIS

Blastocystosis is an infection caused by a protozoan parasite *Blastocystis hominis*. It resides in the intestine of many animals including humans. It was considered as the most common commensal protozoa of intestine; however, recently its pathogenic role has been described.



Figs 45.19A and B: Morphology of *Balantidium coli* (schematic diagram): **A.** Trophozoite; **B.** cyst.



Figs 45.20A and B: *Balantidium coli*: **A.** Saline wet mount preparation shows trophozoite with cilia; **B.** Iron hematoxylin stain shows cyst with prominent macronucleus.

Source: Giovanni Swierczynski, Bruno Milanese. "Atlas of human intestinal protozoa Microscopic diagnosis" (with permission).

- ❖ **Taxonomy:** It is now classified under Kingdom Chromista; but for the sake of familiarity, it is discussed under Protozoa
- ❖ **Morphology:** *Blastocystis* species shows great morphological variations; occur in six forms—vacuolar, avacuolar, multi-vacuolar, amoeboid, granular and cyst forms
- ❖ **Life cycle:** Cysts are the infective form, transmitted by feco-oral route
 - Cysts develop into vacuolar forms in the intestine, then multiply and transform into other forms and vice versa. The vacuolar forms transform back to cysts
 - All forms can be shed in the feces, however only cysts can survive in the environment.
- ❖ **Pathogenesis:** Most infected individuals are asymptomatic carriers. Few present with symptoms of irritable bowel syndrome (such as abdominal pain, bloating and flatulence) and diarrhea. However, its pathogenicity is doubtful. Ruling out other enteric parasites is necessary before attributing symptoms to *Blastocystis*
- ❖ **Laboratory diagnosis:** Stool microscopy by permanent stained smear (trichrome stain) is the procedure of choice; which demonstrates various forms of *Blastocystis*
 - **Vacuolar forms** are the most common forms seen in stool microscopy. They measure 5–15 μm in size with 1–4 nuclei and a large vacuole in the cytoplasm (Figs 45.21A to C)
 - **Other diagnostic methods** include antibody detection by ELISA and molecular methods such as PCR targeting small subunit rDNA.
- ❖ **Treatment:** Metronidazole is found to be effective used alone or in combination with paromomycin or nitazoxanide.

INTESTINAL SARCOCYSTOSIS

It is a zoonotic parasitic infection caused by *Sarcocystis hominis* (infects cattle) and *S. suihominis* (infects pigs). Human infection is extremely rare.

- ❖ **Host:** Dogs and cats (and accidentally man) are definitive hosts; whereas cattle and pigs serve as intermediate hosts



Figs 45.21A to C: *Blastocystis* species showing vacuolar forms: **A.** Saline wet mount; **B.** Iodine mount; **C.** Trichrome stain.

Source: A to C. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Note: Occasionally, man acts as intermediate host; which leads to development of muscular sarcocystosis (Chapter 57).

- ❖ **Life cycle:** Infection is transmitted to man by ingestion of beef or pork infected with sarcocysts, which transform to oocysts in the intestine that are released in feces
- ❖ **Clinical features:** It is usually asymptomatic but patient may develop nausea, vomiting, abdominal pain and diarrhea
- ❖ **Diagnosis:** It is diagnosed by stool examination demonstrating the oocysts
- ❖ **Treatment:** No specific treatment is available. Infection is generally self-limited.

CHAGAS' DISEASE

In chronic Chagas' disease, the causative agent, *Trypanosoma cruzi* multiplies in the muscles of GIT, leading to megaesophagus (manifested as dysphagia, chest pain, and regurgitation) and megacolon (manifested as abdominal pain and chronic constipation) (Chapter 36).

FUNGI CAUSING INTESTINAL INFECTIONS

MICROSPORIDIOSIS

Microsporidia are lower eukaryotic, spore forming obligate intracellular parasites, cause opportunistic infection in HIV-infected patients.

- ❖ **Taxonomic status:** Microsporidia were once classified under parasite; now considered to be evolved from the fungi, being most closely related to zygomycetes
- ❖ **Classification based on their habitat:** Microsporidia include over 170 genera infecting a broad range of vertebrates and invertebrates. However, only few are found to infect man, producing various manifestations ranging from intestinal, musculoskeletal and ocular infections (Table 45.4)
- ❖ **Spore:** Microsporidia exist in nature as spores. Spore is highly resistant extracellular form; survives in the

Table 45.4: Classification and clinical manifestations of microsporidia.

Genus	Species	Habitat and infections
<i>Enterocytozoon</i>	<i>E. bieneusi</i>	Most common species infecting man Small intestine: Enteric infection (diarrhea, acalculous cholecystitis) Rarely cause nasal polyp, infections of bile duct, liver and respiratory system It has zoonotic potential. More than 90 genotypes are described; only 17 infect humans, most common being group 1
<i>Encephalitozoon</i>	<i>E. intestinalis</i>	Second most common species infecting man Small intestine: Enteric infection
	<i>E. cuniculi</i>	Extraintestinal infections such as: <ul style="list-style-type: none"> • Disseminated systemic infection • Eye: Keratoconjunctivitis • Sinuses: Chronic sinusitis • CNS: Brain abscess • Heart: Myocarditis and endocarditis • Respiratory and genitourinary tract infections
	<i>E. hellem</i>	Disseminated infection involving eyes (keratoconjunctivitis), urinary tract and respiratory tract Transmission is from birds
<i>Pleistophora</i>	<i>P. ronneae</i>	Skeletal muscle (myositis)
<i>Trachipleistophora</i>	<i>T. hominis</i>	Myositis, sinusitis and conjunctivitis
	<i>T. anthropophthera</i>	Infects brain, heart and kidney
<i>Brachiola</i>	<i>B. vesicularum</i>	Skeletal muscle and corneal stroma (corneal ulcer)
<i>Anncaliia</i>	<i>A. connori</i>	Eye: Corneal stroma Smooth and cardiac muscle infections
	<i>A. algerae</i>	Eyes, muscle and possible disseminated infection Mosquito-borne transmission documented
<i>Nosema</i>	<i>N. oculorum</i>	Eye: Corneal stroma
<i>Vittaforma</i>	<i>V. corneae</i>	Eye: Corneal stroma and rarely urinary tract (UTI) Water-borne transmission has been documented
<i>Tubulinosema</i>	<i>T. acridophagus</i>	Systemic disseminated infection Grasshoppers are the non-human host
<i>Microsporidium</i>	<i>M. ceylonensis</i>	Eye: Corneal stroma (corneal ulcer)
	<i>M. africanum</i>	Eye: Corneal stroma (corneal ulcer)

Note: Most of the species are zoonotic having various animal reservoirs.

environment. It is also the infective stage. It enters into the host cell via a polar tube within a spore

- ❖ **Life cycle:** Humans acquire infection by ingestion (or rarely inhalation or ocular contact) of spores of microsporidia
 - Spores come in contact with the host cell (e.g. **enterocyte**) and inject its cytoplasm (called sporoplasm) with help of polar tube
 - They multiply inside host cells first by asexual mode producing meronts, and then by sexual mode (sporogony) producing sporonts that subsequently develop into spores.
- ❖ **Epidemiology:** Microsporidia infections have been increasingly reported in AIDS patients from North America, Western Europe and Australia. In India, few clusters of cases have been reported so far.

Pathogenesis and Clinical Feature

Microsporidia mainly cause opportunistic infections

- ❖ In HIV-infected patients, when their CD4 T cell count falls below 100 μ L or
- ❖ In any other patients with immunosuppression like recipients of organ transplant.

Depending up on the species causing infection, clinical presentation of microsporidiosis may be of various types (Table 45.4):

- ❖ **Enteric infections:** Agents include *Enterocytozoon* and *Encephalitozoon*
- ❖ **Ocular infections:** Agents include *Nosema*, *Vittaforma*, *Microsporidium*, and others (Chapter 78)
- ❖ **Myositis** (infection of muscles): Agents include *Pleistophora*, *Trachipleistophora*, *Brachiola*, and *Anncaliia* (Chapter 57)
- ❖ Disseminated infections by *Tubulinosema*.

Laboratory Diagnosis

Light Microscopy for the Spore Detection

Various samples can be collected like stool, small intestinal contents (collected by Entero-test), corneal smear or small intestinal biopsies, sputum, urine, etc.

- ❖ **Modified trichrome stain (MTS):** It is the recommended stain for microsporidia (Fig. 45.22A). Microsporidia appear as red oval refractile spores against a blue background
- ❖ **Modified acid fast stain:** Microsporidia spores are acid-fast to 1% acid alcohol; appear red, 1–1.5 μ m size (Fig. 45.22B)
- ❖ **Gram stain:** Microsporidia spores stain gram-positive
- ❖ **Other stains:** Giemsa stain (Fig. 45.22C), periodic acid Schiff stain (PAS), Gram chromotrope stain and fluorochrome stain like calcofluor white stain can be used.

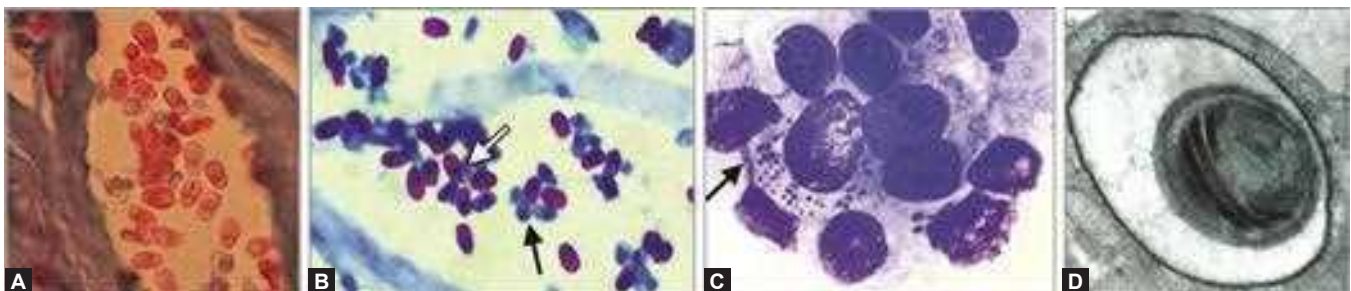
Electron Microscopy

It is considered as the **gold standard** method for the diagnosis of microsporidiosis (Fig. 45.22D).

- ❖ It helps in identifying microsporidia to the genus and species level
- ❖ It is highly specific, but lacks sensitivity. It is also time consuming, labor-intensive and expensive.

Other Methods

- ❖ **Cell culture:** Microsporidia have been successfully cultivated in a number of cell lines including monkey kidney cells (Vero), human fetal lung fibroblast cell line. Its use in routine clinical diagnosis is limited as it is time consuming and laborious
- ❖ **Antibody assays:** Various methods like indirect immunofluorescence, ELISA and western blot are available to detect antibodies. They are not very useful as they lack specificity
- ❖ **Antigen assays:** Direct antibody fluorescent test (DAF) is available for detecting the antigens on Microsporidia spores
- ❖ **Molecular methods:** PCR-based methods have been developed, targeting different genes like small subunit gene of rRNA for diagnosis and speciation of Microsporidia
- ❖ **In situ hybridization** has been established for the detection of *E. bienersi* in humans by using probes directed



Figs 45.22A to D: Microsporidia spores: **A.** Modified trichrome stain; **B.** Acid-fast stain (showing red stained mature spores and blue stained (nonacid-fast) immature or degenerating spores); **C.** Duodenal biopsy (Giemsa stain) shows numerous spores inside the enterocyte (marked by arrow); **D.** Electron microscopic picture.

Source: A. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission); B and D. Savitri Sharma, LV Prasad Eye Institute, Hyderabad; C. Giovanni Swierczynski, Bruno Milanese. "Atlas of human intestinal protozoa Microscopic diagnosis" (with permission).

against the small subunit rRNA of *E. bienersi*, present directly in the biopsy specimens.

T TREATMENT**Microsporidiosis**

- ❑ **Albendazole** is effective for the treatment of enteric, muscular and ocular microsporidiosis. It is given 400 mg twice daily for 2–4 weeks. Relapse may be seen in some cases
- ❑ **Other alternative drugs** which are tried include: Octreotide, nitazoxanide, fumagillin and itraconazole

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T TREATMENT**Microsporidiosis**

- ❑ **Nutritional therapy:** To reduce malabsorption in case of enteric microsporidiosis
- ❑ **Topical agents** can be applied for the corneal lesions like topical itraconazole, metronidazole and topical propamidine
- ❑ **Control of AIDS** by antiretroviral therapy (ART) is important to reconstitute the immune system and to prevent remissions.

EXPECTED QUESTIONS**I. Write essay on:**

1. A 17-year-old boy presented with bloody diarrhea with mucus and pus cells, colicky abdominal pain, fever, and prostration. The wet mount examination of the stool sample was performed which showed trophozoites of 5–20 µm, actively motile, with finger-like pseudopodia.
 - a. What is the etiological diagnosis?
 - b. Describe the pathogenesis and clinical manifestations produced.
 - c. What are the various diagnostic modalities?
 - d. How will you treat this condition?
2. A 46-year-old female patient infected with HIV presented to the casualty with severe profuse diarrhea, with a frequency of 15 times a day for the past 10 days. She also complained of weight loss and abdominal pain. The stool specimen was subjected for modified acid-fast staining, which revealed round sporulated oocysts (4–6 µm in size), containing four sporozoites.
 - a. Identify the etiological agent.
 - b. Write briefly about the life cycle of the etiological agent.
 - c. What are the various diagnostic modalities?
 - d. How will you treat this clinical condition?

II. Write short notes on:

1. A 3-year-old boy presented with recurrent episodes of foul smelling diarrhea, foul flatus, sulfurous belching and profound weight loss. The wet mount examination of the stool sample revealed pear-shaped trophozoites with falling leaf-like motility. What is the etiological diagnosis and what are the various diagnostic modalities?
2. Infections caused by Microsporidia.

III. Multiple Choice Questions (MCQs):

1. **Infective form and invasive form of *Entamoeba histolytica* are:**

- a. Trophozoite and quadrinucleated cyst
 - b. Quadrinucleated cyst and trophozoite
 - c. Both trophozoite
 - d. Both quadrinucleated cyst
2. **Sporulated oocyst of *Cystoisospora belli* contains totally:**
 - a. One sporocyst and two sporozoites
 - b. One sporocyst and four sporozoites
 - c. Two sporocysts and four sporozoites
 - d. Two sporocysts and eight sporozoites
 3. **Sporulated oocyst of *Cyclospora cayetanensis* contains:**
 - a. One sporocyst and two sporozoites
 - b. One sporocyst and four sporozoites
 - c. Two sporocysts and four sporozoites
 - d. Two sporocysts and eight sporozoites
 4. **Which statement is false about *Cryptosporidium* species?**
 - a. Developmental stages of the parasite occur inside a parasitophorous vacuole
 - b. High infective dose
 - c. Large number of animal reservoirs
 - d. It causes diarrhea in AIDS patients
 5. **Intermediate host for *Sarcocystis suis hominis* is:**
 - a. Pig
 - b. Dog
 - c. Man
 - d. Cattle
 6. **Microsporidia spores are better stained by which of the following stain?**
 - a. Albert stain
 - b. H and E stain
 - c. Modified trichrome stain (MTS)
 - d. Iodine stain
 7. **Which of the following bears two nuclei named as macronucleus and micronucleus?**
 - a. *Balantidium coli*
 - b. *Dientamoeba fragilis*
 - c. *Cystoisospora belli*
 - d. *Entamoeba coli*

Answers

1. b 2. d 3. c 4. b 5. a 6. c 7. a

CHAPTER PREVIEW

- Intestinal Cestode Infections
- Intestinal Trematode Infections
- Intestinal Nematode Infections

Intestinal helminthic infections contribute a significant proportion of gastrointestinal (GI) infections. Nevertheless, majority of helminths (cestodes, trematodes and nematodes) cause infections of GIT.

- ❖ **Intestinal cestodes:** *Diphyllobothrium*, *Taenia saginata*, *T. solium*, *Hymenolepis nana* and *Dipylidium caninum*
- ❖ **Intestinal trematodes**
 - **Intestinal flukes**, e.g. *Fasciolopsis buski*
 - **Blood flukes** such as *Schistosoma mansoni* and *S. japonicum* reside in mesenteric venous plexus of GIT, and cause various GI symptoms including dysentery.
- ❖ **Intestinal nematodes:** Include
 - Small intestinal nematodes—*Ascaris*, hookworm and *Strongyloides*
 - Large intestinal nematodes such as *Trichuris* and *Enterobius*.
- ❖ **Intestinal nematodes of lower animals** rarely infect man. Examples include *Toxocara*, *Angiostrongylus*, etc.

INTESTINAL CESTODE INFECTIONS

Cestodes are long, segmented, flattened dorsoventrally, tape like worms, therefore also called as tapeworms. Based on habitat, they are classified into two groups:

1. **Intestinal cestodes:** Here, the adult worms inhabit in human Intestine. Examples include:
 - *Diphyllobothrium* species
 - *Taenia solium* and *Taenia saginata* causing intestinal taeniasis
 - *Hymenolepis* species
 - Less common: *Taenia asiatica*, *Hymenolepis diminuta* and *Dipylidium caninum*.
2. **Somatic/tissue cestodes:** Here, the larvae are found in the human muscles or organs. Examples include:
 - *Taenia solium*—causes cysticercosis affecting CNS, muscle and eye (Chapter 75)
 - *Echinococcus* species—the agent of hydatid disease affecting liver (Chapter 49).

Life cycle of cestodes pathogenic to man, has been discussed in Table 46.1.

MORPHOLOGICAL FORMS

In general, helminths exist in three morphological forms—(1) adult form (or the worm), (2) larval form, and (3) eggs.

Adult Worm

Adult worm is long, segmented, dorsoventrally flattened; varies in length from few millimeters to several meters. It inhabits in the intestine of men and animals. Adult worm consists of three parts (Fig. 46.1).

1. **Head or scolex:** It is the organ of attachment, helps in attachment to intestinal mucosa by virtue of bearing four cup like muscular structures called suckers (or acetabula)
 - In some species like *T. solium* and *H. nana*, scolex has a beak like apical protrusion called as rostellum, which may be armed with hooklets (these species are called as armed tapeworms)
 - In *Diphyllobothrium*, the scolex does not possess suckers but it bears a pair of longitudinal grooves called as bothria by which it attaches to small intestine.
2. **Neck:** Next to head, the portion is called as neck from which the segments (proglottids) arise
3. **Strobila** (body or trunk): It consists of a number of segments (or proglottids). The length of the tapeworm varies based on the number of segments. Based on the reproductive organs they bear, proglottids can further be grouped into three types:
 - i. **Immature segments:** Here, male and female reproductive organs are not differentiated
 - ii. **Mature segments:** Cestodes are hermaphrodites or monoecious, i.e., contain male and female organs in the same segment, male organs appear first
 - iii. **Gravid segments or fertilized segments:** Following fertilization, the uterus gets filled with eggs. Other organs are atrophied.

Cestodes	Host		Mode of transmission	Infective form	Diagnostic form	Organs affected
	Definitive	Intermediate				
<i>Taenia saginata</i>	Man	Cattle	Ingestion	Cysticercus bovis	Embryonated eggs	GIT
<i>Taenia solium</i> (intestinal taeniasis)	Man	Pig	Ingestion	Cysticercus cellulosae	Embryonated eggs	GIT
<i>Taenia solium</i> (cysticercosis)	Man	Man	Ingestion, autoinfection	Embryonated eggs	Cysticercus larvae	CNS, muscle, eye
<i>Echinococcus granulosus</i>	Dog	Man	Ingestion	Embryonated eggs	Hydatid cyst (larva)	Liver
<i>Hymenolepis nana</i>	Man	-	Ingestion, autoinfection	Embryonated eggs	Embryonated eggs	GIT
<i>Diphyllobothrium latum</i>	Man	1st- Cyclops, 2nd- Fish	Ingestion	Plerocercoid larvae	Operculated eggs	GIT

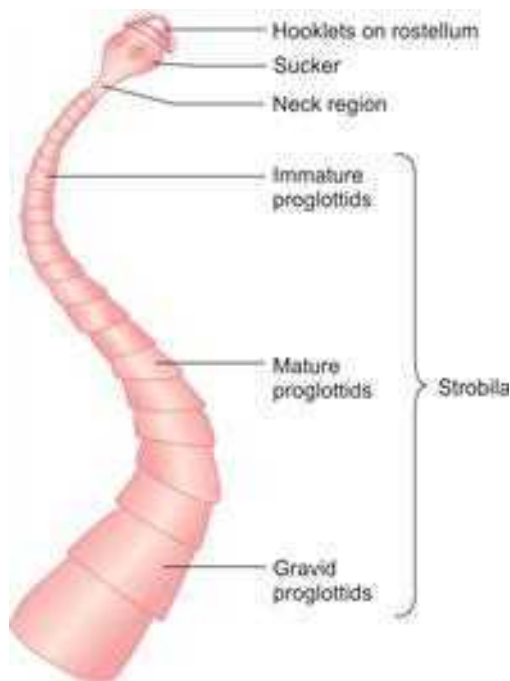


Fig. 46.1: Adult worm of cestode (schematic diagram).

Eggs

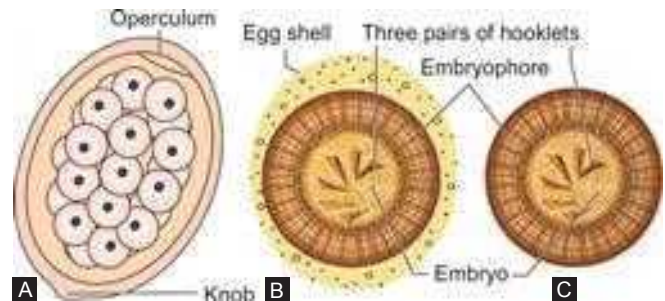
Eggs are formed following fertilization, fill the gravid proglottids and are subsequently released in feces—considered as the diagnostic form (Figs 46.2A to C).

- ❖ In most cestodes, the eggs are round to oval, consist of an embryo (or oncosphere) with six hooklets, surrounded by radially striated embryophore (Fig. 46.2C). A thin outer egg shell may be present initially, which eventually gets lost (Fig. 46.2B)
- ❖ In *D. latum*, eggs are ovoid, and operculated (Fig. 46.2A).

Larva

Embryonated eggs undergo further development to form larvae. Most cestodes have only one larval stage except *D. latum*.

- ❖ In *Taenia*, the larval stage is called as **cysticercus**
- ❖ In *Hymenolepis*, the larval stage is called as **cysticercoid**



Figs 46.2A to C: Schematic diagrams of eggs of cestodes: A. *D. latum*; B. Other cestodes; C. Other cestodes after the loss of egg shell.

- ❖ In *Echinococcus*, it is called as **hydatid cyst**
- ❖ In *Diphyllobothrium* there are three larval stages—coracidium (first stage), proceroid (second stage) and plerocercoid (third stage).

INTESTINAL TAENIASIS

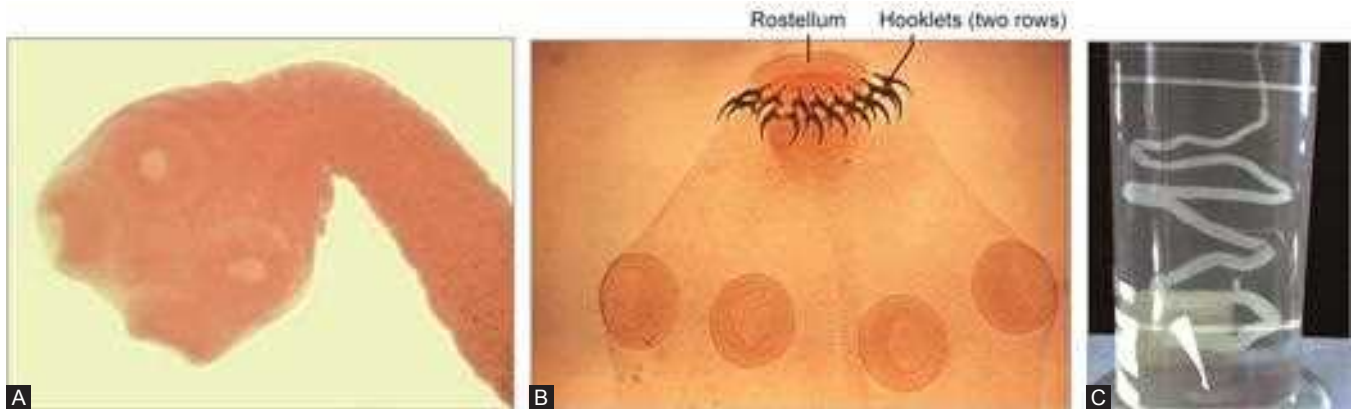
Two important pathogenic species are *T. saginata* and *T. solium*. They cause two types of manifestations in humans

- ❖ Intestinal taeniasis—caused by both *T. saginata* and *T. solium*
- ❖ Cysticercosis—caused by only *T. solium*. It infects various tissues such as CNS, eyes and muscles (Chapter 75).

Morphology

Taenia exists in three morphological forms.

- Adult worm:** It is similar to other cestodes described earlier; comprises of head or scolex, neck and a body divided into several proglottids (Fig. 46.3C).
 - The scolex bears four cup like muscular suckers which helps in attachment (Figs 46.3A and B)
 - In *T. solium*, the scolex has a beak like apical protrusion called as rostellum. The rostellum is armed with two rows of hooklets (hence called as armed tapeworm) (Fig. 46.3B).
- Eggs** contain embryo or oncosphere which contains three pair of hooklets, surrounded by an embryophore (Figs 46.2B and C)



Figs 46.3A to C: A. Carmine-stained scolex of *T. saginata*; B. Carmine-stained scolex of *T. solium*; C. Adult worm of *Taenia* species.

Source: A. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission); B. Public Health Image Library, ID#: 5262, Centers for Disease Control and Prevention (CDC), Atlanta (with permission); C. Head, Department of Microbiology, Meenakshi Medical College, Chennai (with permission).

3. **Larvae:** Cysticercus is the larval stage of *Taenia*. It contains a muscular organ with bladder like sac. It is called as:

- Cysticercus bovis in *T. saginata*
- Cysticercus cellulosae in *T. solium*.

Life Cycle (Intestinal taeniasis)

Life cycle of *Taenia* passes through two hosts (Fig. 46.4). Man is the definitive host; whereas the intermediate host is cattle for *T. saginata* (hence called beef tapeworm) and pigs for *T. solium* (hence called pork tapeworm).

❖ **Transmission:** Man acquires infection by ingestion of contaminated undercooked beef or pork containing the

larvae, i.e. cysticercus bovis or cysticercus cellulosae (infective form)

❖ **Human GIT:** The larvae develop into adult worms (in 10-14 weeks) in human intestine, which undergo self-fertilization within the segments to produce eggs, that are released into feces (diagnostic form)

❖ **Intermediate hosts (cattle or pigs):** Eggs are ingested by cattle or pigs while grazing the field. Eggs penetrate the intestinal wall and migrate to skeletal muscles via blood, where they transform into larvae (cysticercus) that get encysted and deposited as cysts. This is the infective form and the cycle is repeated. This takes around 7-10 weeks of time.

Note: The life cycle of *T. solium* causing cysticercosis (in man) is different from that of intestinal taeniasis (Chapter 75).

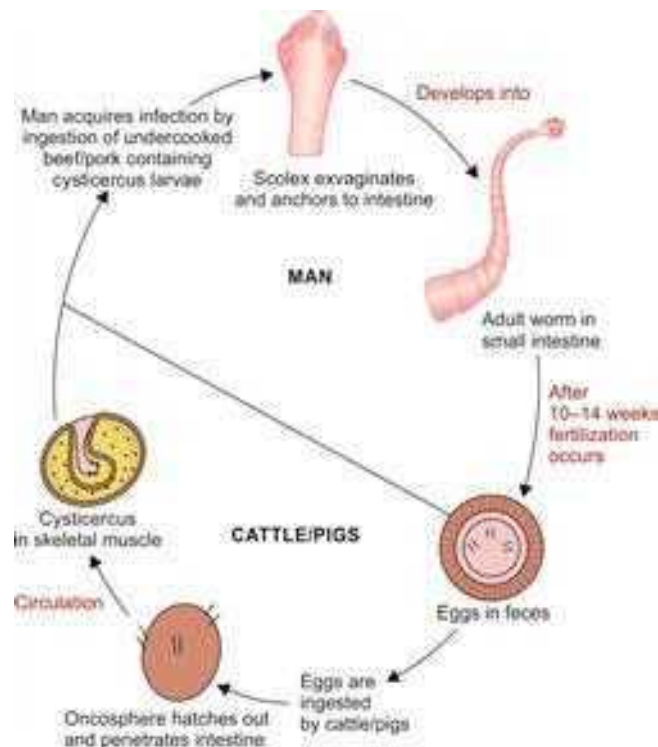


Fig. 46.4: Life cycle of *Taenia saginata* and *T. solium* causing intestinal taeniasis.

Epidemiology

T. saginata infection is common in cattle breeding areas of the world. The areas with the highest prevalence (up to 27%) include Central Asia, Central and East Africa.

T. solium intestinal infection is endemic in Mexico, Central America, South America, Africa, Southeast Asia, India, Philippines, and Southern Europe. However, it is less frequently reported from the Muslim countries (as pork eating is not allowed).

Clinical Manifestations

Majority of cases are asymptomatic; patients may become aware of infection by noticing the passage of proglottids in their feces.

- ❖ Common symptoms include mild abdominal pain, nausea, loss of appetite and change in the bowel habit
- ❖ Perianal discomfort or pruritus may be felt (when proglottids are discharged).

Laboratory Diagnosis

Stool Examination

Wet mount examination of stool is carried out to demonstrate the characteristic eggs and less often proglottids of *Taenia* species.

- ❖ Multiple stool examination and concentration techniques (formol-ether sedimentation) can be followed to increase the detection rate
- ❖ Anal swabs (cellophane swabs used for *Enterobius*) can be used to collect fecal matter and is superior for the detection of eggs than in the stool
- ❖ **Eggs** of *T. saginata* and *T. solium* are morphologically similar except that eggs of *T. saginata* are acid fast (Table 46.2)
 - Egg is round 31–43 μm size and consists of an embryo with six hooklets surrounded by an embryophore (Figs 46.2B and C)
 - Eggs are bile-stained; do not float in saturated salt solution.
- ❖ **Proglottids** of *T. saginata* and *T. solium* can be differentiated by lateral branches in uterus, accessory lobe in ovary, vaginal sphincter and expulsion of segments (singly or in chain) (Table 46.2)
- ❖ **Scolex** can be detected in feces very rarely. *T. solium* scolex is armed with rostellum and hooklets.

Taenia Specific Antigen Detection in Stool

ELISA has been developed to detect *Taenia* specific antigen (coproantigen) in stool by using polyclonal *Taenia* antibodies.

- ❖ **Advantages:** This test has many utilities:
 - Claims more sensitive than stool examination
 - Can detect *Taenia* carriers; is useful for the control of this zoonotic infection
 - Prognosis: It can be used for treatment follow-up; becomes negative after 30 days of effective treatment.
- ❖ **Disadvantage:** It cannot differentiate between *T. saginata* and *T. solium*.

Molecular Methods

PCR targeting mitochondrial DNA followed by sequencing is available; which can distinguish between *Taenia* species.

TREATMENT

Intestinal taeniasis

- ❑ **Praziquantel (drug of choice):** Single dose of (10 mg/kg) is highly effective
- ❑ Niclosamide (2 g) is also effective but is not widely available.

Prevention

Intestinal taeniasis can be prevented by:

- ❖ Adequate cooking of beef or pork viscera
 - Thorough cooking at 65°C for 5 minutes
 - Refrigeration at 4°C for >30 days
 - Salting and pickling are not effective.
- ❖ **Effective fecal disposal** to prevent infection to cattle and pigs.

Taenia saginata asiatica (Asian Tapeworm)

It is a subspecies of *T. saginata*; causes intestinal taeniasis. It is morphologically similar to *T. saginata* except that

Table 46.2: Differences between *Taenia saginata* and *T. solium*.

Features	<i>Taenia saginata</i>	<i>Taenia solium</i>
Adult worm		
Length	4–6 meters	2–4 meters
Head/scolex	<ul style="list-style-type: none"> • Large and quadrangular • Four suckers present • No rostellum, No hooklets 	<ul style="list-style-type: none"> • Small and globular • Four suckers present • Bears rostellum with two rows of hooklets • Hence called as armed tapeworm
Proglottids		
No. of proglottids	1,000–2,000	800–1,000
Uterus	Bears in 15–20 lateral branches	Bears in 7–13 lateral branches
Lobes of ovary	Two, no accessory lobe	Three–two lobes with an accessory lobe
Vaginal sphincter	Present	Absent
Expulsion of segments	Expelled singly in the feces	Expelled in chain of 5–6 segments
Larva		
	Cysticercus bovis present in cattle's muscle, but not in man	Cysticercus cellulosae present in pig's muscle and also in man (muscle, eye and brain)
Egg		
	Acid fast, 31–43 μm size	Non-acid fast, 31–43 μm size
Life cycle		
Disease	Causes intestinal taeniasis	Causes intestinal taeniasis and cysticercosis
Host	Definitive host: Man Intermediate host: Cattle	<ul style="list-style-type: none"> • For intestinal taeniasis—definitive host is man, intermediate host is pig • For cysticercosis—man acts both as definitive and intermediate hosts
Infective form	Larva (cysticercus bovis)	<ul style="list-style-type: none"> • For intestinal taeniasis—larva (<i>C. cellulosae</i>) • For cysticercosis—egg
Diagnostic form	Eggs in feces	<ul style="list-style-type: none"> • For intestinal taeniasis—eggs in feces • For cysticercosis—larva deposited in tissue
Mode of transmission	Ingestion of contaminated beef	<ul style="list-style-type: none"> • For intestinal taeniasis—ingestion of contaminated pork • For cysticercosis—(i) contaminated food and water and (ii) auto-infection

intermediate host is pig (not cow), where cysticerci are located primarily in the liver (not in the muscle).

- ❖ Man acquires infection through ingestion of pig liver containing infected cysticerci
- ❖ Human infection has been reported from Taiwan and other Asian countries like Korea and China, but not reported from India, yet

- ❖ It is differentiated from *T. saginata* by PCR targeting mitochondrial DNA followed by sequencing.

HYMENOLEPIASIS

Hymenolepiasis is caused by *Hymenolepis nana*, which is the smallest cestode (2.5–4 cm in length) infecting man, hence also called as dwarf tapeworm.

- ❖ **Life cycle** (Fig. 46.5): Man is the only host. Eggs are the infective form. Man acquires the infection by ingestion of food and water contaminated with eggs or by autoinfection with the eggs released in their small intestine

- **Direct cycle:** After ingestion, in the small intestine eggs hatch out and develop into cysticercoid larvae and subsequently into adult worms. Adult worms, when fully mature undergo fertilization to produce eggs that are released in feces (diagnostic form). Eggs are infective to man and the cycle continues
- **Indirect cycle:** Rarely, man acquires infection by accidental ingestion of rat flea containing the cysticercoid larvae, which subsequently develop into adult worms and the cycle continues.

- ❖ **Clinical manifestations:** *H. nana* infection is usually asymptomatic. When the worm burden exceeds, patients develop symptoms like anorexia, abdominal pain, headache, dizziness and diarrhea with mucus
- ❖ **Epidemiology:** *H. nana* is considered as the most common tapeworm infection throughout the world infecting 50–75 million of people. The overall prevalence ranges from 0 to 4% with higher prevalence in children (16%)
- ❖ **Laboratory diagnosis:** Stool microscopy detecting the characteristic eggs confirms the diagnosis. Stool concentration can be done if the egg load is less. PVA (polyvinyl-alcohol) should not be used for preservation as it distorts the morphology

Eggs of *H. nana* (Figs 46.6A and B)

Egg is round to slightly oval in shape, 30–47 μm size

- It has two membranes that surrounds an embryo with six hooklets. Space between the two membranes is filled with yolk granules
- **Polar filaments:** Both the poles of embryophore are thickened from which four to eight polar filaments emerge
- **Non-bile stained (colorless in saline mount):** It is the only cestode egg that is not stained by bile when passed through the intestine
- Eggs are the infective form as well as the diagnostic form of the parasite.

- ❖ **Treatment:** Praziquantel (25 mg/kg once) is the treatment of choice, since it acts against both the adult worms and the cysticercoid larvae. Nitazoxanide or niclosamide may be used alternatively
- ❖ **Prevention:** Good personal hygiene and improved sanitation can control the disease.

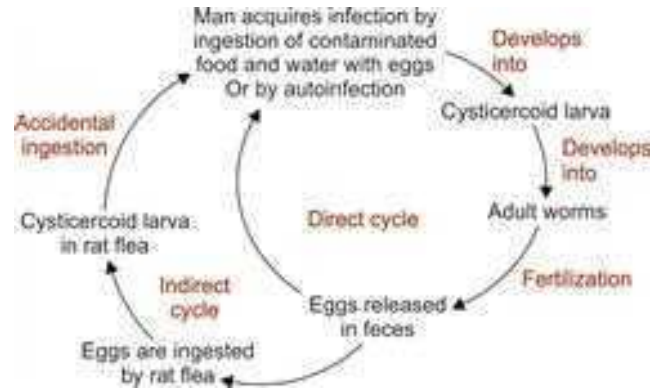
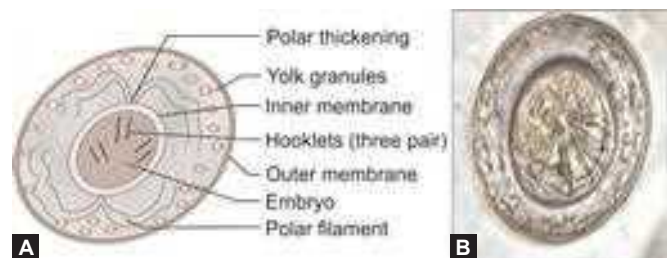


Fig. 46.5: Life cycle of *Hymenolepis nana*.



Figs 46.6A and B: Egg of *Hymenolepis nana*: A. Schematic; B. In saline mount (non-bile stained).

Source: B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Hymenolepis diminuta Infection

H. diminuta is also called as rat tape worm. Rodents are the definitive host and insects are the intermediate host. Human infection is rare, occurs through indirect cycle (as described for *H. nana*). Eggs are larger 70–85 × 60–80 μm, do not bear polar filaments and are bile stained.

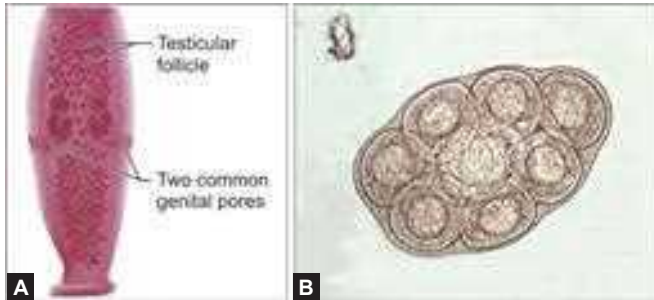
DIPYLIDIUM CANINUM INFECTION

D. caninum is a common tapeworm of dogs and cats; rarely infects man producing GI symptoms such as loss of appetite, diarrhea, pruritus ani, abdominal pain, etc.

- ❖ **Host:** Definitive hosts are dogs and cats (rarely men). Intermediate hosts are insects (fleas)
- ❖ **Transmission:** Man acquires infection by ingestion of flea containing cysticercoid larva
- ❖ **Diagnosis:** Eggs are detected in feces, 25–40 μm size, present in groups of 15 (egg packets). Proglottids are typically barrel-shaped and contain two genital pores hence also called as double pored tapeworm (Figs 46.7A and B).

DIPHYLLOBOTHRIASIS

Diphyllobothriasis is an intestinal parasitic infection, caused by a cestode *Diphyllobothrium latum*, also known as **fish tapeworm**—is the largest known parasite found in human intestine.



Figs 46.7A and B: *Diphylidium caninum*: **A.** Proglottids with two genital pores (carmine stained); **B.** Egg packets (in saline mount).
Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Morphology

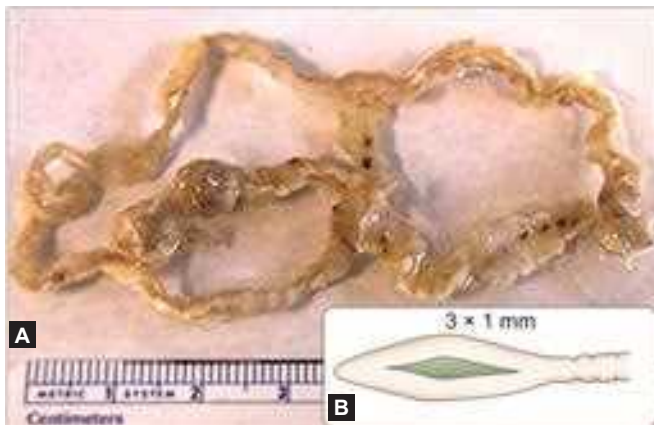
D. latum has three morphological forms.

- 1. Adult worm:** Long, measures up to >15 meters with over 3,000 proglottids. The head/scolex bears two grooves or bothria (Figs 46.8A and B)
- 2. Operculated egg**
- 3. Larvae:** Occurs in three stages—first stage (coracidium), second stage (proceroid larva) and third stage (plerocercoid larva).

Life Cycle (Fig. 46.9)

Life cycle of *D. latum* passes through three hosts. Humans are the definitive host. There are two intermediate hosts; first (*Cyclops*) and second (fish).

- ❖ **Transmission:** Humans get infection by ingestion of undercooked fish containing third stage plerocercoid larva (infective form)
- ❖ **Human GIT:** Plerocercoid larva develops into adult worm in human small intestine; which sexually matures and fertilization takes place to produce eggs that are released in feces (diagnostic form)
- ❖ **In Cyclops:** Eggs in water transform into L₁ larva (coracidium), which infects (*Cyclops*). In *Cyclops*,



Figs 46.8A and B: *Diphyllobothrium latum*: **A.** Section of an adult worm containing many proglottids; **B.** Scolex (schematic) with two bothria (or grooves).

Source: A. DPDx Image Library, Centers for Disease Control and Prevention (CDC) Atlanta (with permission).

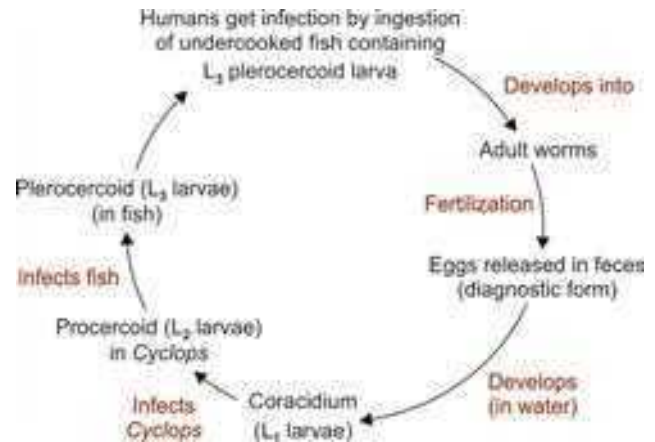


Fig. 46.9: Life cycle of *Diphyllobothrium latum*.

L₁ larva develops into L₂ (proceroid) larva, which infects fish

- ❖ **In fish,** L₂ larva develops to form L₃ (plerocercoid) larva which becomes the infective form.

Clinical Features

Most of *D. latum* infections are asymptomatic. Few individuals develop symptoms.

- ❖ **Minor manifestations** may include abdominal discomfort, diarrhea, vomiting, weakness and weight loss or rarely acute abdominal pain due to intestinal obstruction, cholangitis or cholecystitis
- ❖ **Vitamin B12 deficiency:** The adult worm causes dissociation of the vitamin B12-intrinsic factor complex within the gut lumen, which leads to a decrease in absorption of B12 at ileum
 - Vitamin B12 deficiency leads to development of **megaloblastic anemia** and some people may exhibit neurologic sequelae like paresthesia
 - This effect has been noted only in Scandinavias (exclusively in Finland), where up to 2% of infected patients, especially the elderly, have megaloblastic anemia. This may be attributed to genetic predisposition of the individuals.

Epidemiology

Though *D. latum* infection occurs worldwide; high incidence has been reported from Scandinavia countries (such as Russia and Finland) and North America. *D. latum* is very rare in India. Three cases have been reported so far (Vellore 1998, Pondicherry 2007 and Karimnagar 2011).

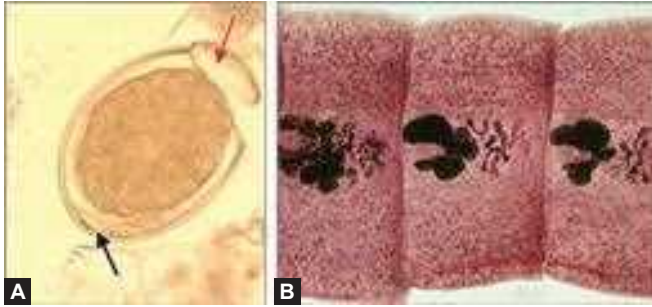
Laboratory Diagnosis

Stool examination reveals characteristic eggs and sometime segments of adult worm.

Eggs and Proglottids (*D. latum*)

Eggs are operculated, with a knob at the other end (Figs 46.2A and 46.10A). Eggs are bile-stained; do not float in saturated salt solution

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Figs 46.10A and B: *Diphyllobothrium latum*: **A.** Egg—note the operculum (red arrow) and posterior knob (black arrow); **B.** Carmine-stained gravid proglottids (broader than longer, rosette-shaped ovaries).

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

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Proglottids have two characteristic features which help in identification (Fig. 46.10B):

- ❑ They are released in chain of segments; not in single
- ❑ They are wider than long (3 × 11 mm) (*latum* meaning broader)

Other diagnostic methods include:

- ❖ **Molecular method:** Species identification is reliably done by PCR followed by sequencing
- ❖ **Blood examination** reveals eosinophilia and evidence of megaloblastic anemia such as:
 - ↑ Mean corpuscular volume and mean corpuscular hemoglobin
 - Normal mean corpuscular hemoglobin concentration
 - Macrocytes (enlarged RBCs).

TREATMENT

Diphyllobothriasis

- ❑ Praziquantel (5–10 mg/kg once) is highly effective (drug of choice)
- ❑ Niclosamide is given alternatively
- ❑ Parenteral vitamin B12 should be given if B12 deficiency is manifested.

Prevention

Preventive measures include thorough cooking of fish or freezing. *D. latum* infection has been called the **Jewish housewives' disease**, as they acquire infection by tasting the uncooked fish while preparing.

- ❖ Proper cooking of fish (5 minutes at 55°C)
- ❖ Deep freezing (–20°C for 7 days, or –35°C for 15 hours) of fish should be followed for people who eat raw fish.

INTESTINAL TREMATODE INFECTIONS

INTRODUCTION

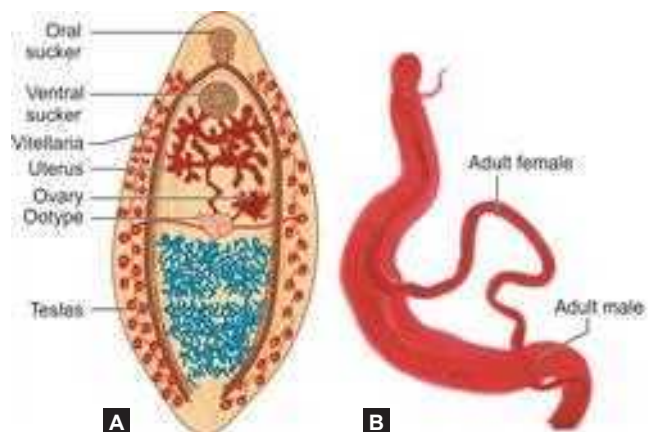
Trematodes (or flukes) are unsegmented, leaf-like and flat worms. Based on the habitat, trematodes are classified as follows:

- ❖ **Intestinal flukes**—e.g. *Fasciolopsis buski*. It resides in intestine, may cause various GI symptoms. It is discussed in this chapter
- ❖ **Blood flukes**—e.g. *Schistosoma*. They reside in venous plexus of various viscera
 - *S. mansoni* and *S. japonicum*—reside in venous plexus of GIT, cause various GI symptoms including dysentery. Therefore, they are discussed in this chapter
 - *S. haematobium*—resides in venous plexus of bladder; causes urinary schistosomiasis and carcinoma of bladder (discussed in Chapter 76).
- ❖ **Hepatic flukes**—e.g. *Fasciola hepatica* and *F. gigantica* in liver; *Clonorchis* and *Opisthorchis* in bile duct (Chapter 49)
- ❖ **Lung flukes**—e.g. *Paragonimus westermani*. It resides in lungs, may produce endemic hemoptysis (Chapter 69).

Morphology

Trematodes exist in three morphological forms—adult worm, egg and larva.

- ❖ **Adult worm:** The adult worms are unsegmented and flattened dorsoventrally (Fig. 46.11A), but some have thick fleshy bodies (schistosomes) (Fig. 46.11B)
 - They range from 1 mm to ~60 mm, possess two **suckers** (as the organ of attachment), an incomplete digestive system, nervous system and excretory system
 - Most trematodes are hermaphrodites or monoecious (male and female organs present in same worm), except schistosomes which are diecious (sexes are separate).
- ❖ **Eggs:** Trematodes are oviparous, i.e. they lay eggs; which develop into larvae later in the environment
 - The eggs of all the trematodes are characteristically operculated except that of schistosomes
 - *Schistosoma* eggs are non-operculated and bear a spine.
- ❖ **Larvae:** Most trematodes have five larval forms such as miracidium, sporocyst, redia, cercaria, and metacercaria.



Figs 46.11A and B: Adult worm of: **A.** *Fasciolopsis buski* (schematic); **B.** *Schistosoma* (The thin female worm resides in the gynecophoric canal of the thicker male worm).

Source: B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Trematodes*	Organ affected	Host		Mode of transmission	Infective form	Diagnostic form
		Definitive	Intermediate			
<i>Schistosoma</i>		Man	Snail	Skin penetration	Cercaria larvae	Non-operculated eggs with spine (Figs 46.14A to C)
<i>S. haematobium</i>	Bladder					
<i>S. mansoni</i>	Intestine					
<i>S. japonicum</i>	Intestine					
<i>Fasciolopsis buski</i>	Intestine	Man	1st- Snail 2nd- Aquatic plants	Ingestion	Metacercaria larvae	Operculated eggs
<i>Fasciola hepatica</i>	Liver					
<i>Clonorchis</i> spp., <i>Opisthorchis</i> spp.	Bile duct	Man	1st- Snail, 2nd- Fish	Ingestion	Metacercaria larvae	Operculated eggs
<i>Paragonimus</i> spp.	Lungs	Man	1st- Snail, 2nd- Crab	Ingestion	Metacercaria larvae	Operculated eggs

*Trematodes are hermaphrodites (male and female organs present in same worm), except schistosomes which are diecious (sexes are separate).

Schistosomes have only three larval stages miracidium, sporocyst and cercaria.

Life Cycle

The life cycle of most trematodes are similar except for *Schistosoma* which has a different life cycle (Table 46.3).

Life Cycle of All Trematodes Except Schistosomes (Fig. 46.12)

Host: Most trematodes (except *Schistosoma*) complete their life cycle in three different hosts, one definitive host (man) and two intermediate hosts.

- ❖ The first intermediate host is fresh water snail or mollusk
- ❖ Second intermediate host is either aquatic plant or fish
 - Aquatic plant: for *Fasciola* and *Fasciolopsis*
 - Cray fish or crab: for *Clonorchis*, *Opisthorchis* and *Paragonimus*.
- ❖ **Infective form:** Metacercaria larva is the infective form for all trematodes (except *Schistosoma*)
- ❖ **Modes of transmission:** Humans acquire infection by eating the second intermediate host (water plant or cray

fish or crab), carrying the infective form, the metacercaria larvae

- ❖ **In man (migrate to habitat):** Larvae migrate to their respective habitat
 - Intestine (for *Fasciolopsis*) or
 - Liver (for *Fasciola hepatica* and *F. gigantica*), bile duct (for *Clonorchis* and *Opisthorchis*) or
 - Lungs (for *Paragonimus*).
- ❖ **In man (produce eggs):** Larvae develop into adult worms, sexually mature and produce eggs (diagnostic form) that are passed either in sputum (for *Paragonimus*) or feces (for other trematodes such as for *Fasciola*, *Fasciolopsis*, *Clonorchis* and *Opisthorchis*)
- ❖ **In water:** The eggs mature and hatch to release miracidium larvae which infect the snails
Note: In *Clonorchis* and *Opisthorchis*, the eggs hatch to release miracidium only after ingestion by suitable snail host.
- ❖ **In first intermediate host (snails):** The miracidium larvae undergo various larval stages of developments such as sporocysts → redia → finally into cercaria larvae
- ❖ **In second intermediate host:** The cercaria larvae escape from snails and infect the second intermediate hosts such as plant or fish/crab, where they develop into metacercaria larvae, which are infective form to man and thus the life cycle continues.

Life Cycle of Schistosomes (Fig. 46.13)

Schistosomes are higher trematodes, their life cycle differs considerably from other trematodes (Table 46.3).

- ❖ **Host:** Schistosomes complete their life cycle in two different hosts—one definitive host (man) and one intermediate host (fresh water snail). There is no second intermediate host
- ❖ **Infective form:** Cercaria larvae are the infective form, which are present freely in water
- ❖ **Mode of transmission:** Humans acquire infection by skin penetration of cercaria larvae (infective form) present freely in water

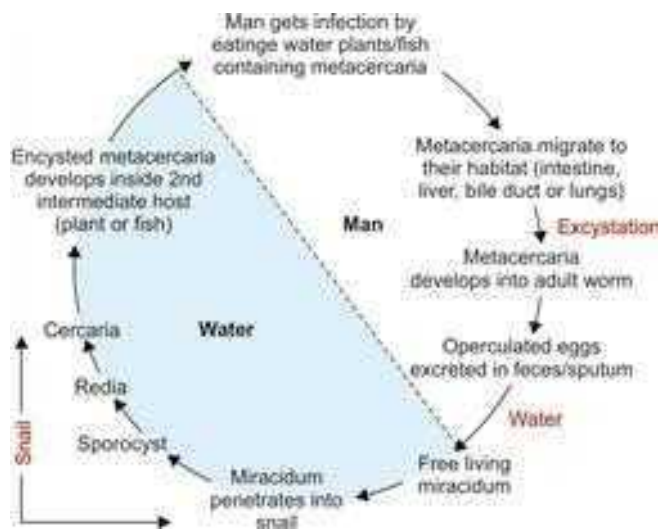


Fig. 46.12: Life cycle of all trematodes (except schistosomes).

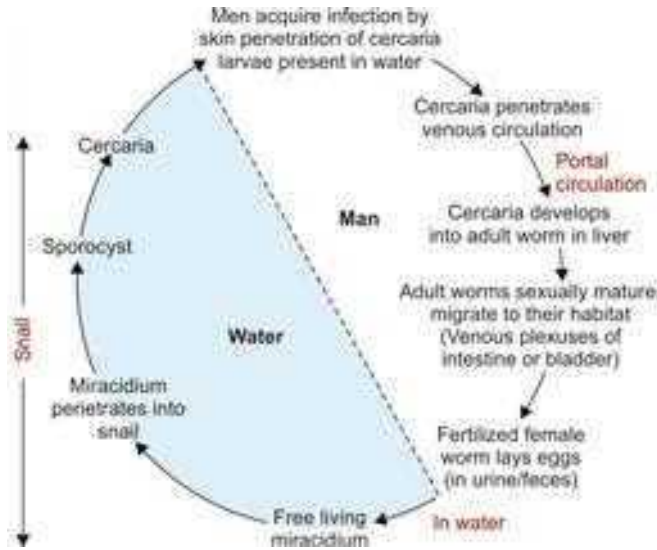


Fig. 46.13: Life cycle of schistosomes.

- ❖ **In man (develop into adult worms):** After penetrating the intact epidermis, cercariae develop into next stage larvae, **schistosomula**. They travel via dermal veins to lungs, later to systemic circulation and finally enter portal circulation. In liver sinusoids they develop into adult worms. Adult worms sexually mature (as male and female) and migrate to their habitat (venous plexuses of intestine or urinary bladder)
 - Intestine (for *S. mansoni* and *S. japonicum*)
 - Kidney and bladder (for *S. haematobium*).
- ❖ **In man (produce eggs):** Fertilized female worms lay eggs in these venous plexuses. Eggs are the diagnostic form; passed either in feces (for *S. mansoni* and *S. japonicum*) or urine (for *S. haematobium*)
- ❖ **In water:** The eggs mature and hatch to release miracidium larvae which infect the snails
- ❖ **In intermediate host (snails):** The miracidium larvae develop into sporocyst larvae → finally into cercaria larvae, which are infective form to man and thus the life cycle continues.

TREATMENT

Trematode infections

Praziquantel is the drug of choice for all trematode infections except for *Fasciola*, where triclabendazole is recommended.

SCHISTOSOMIASIS

Schistosomiasis is the second most devastating tropical parasitic disease (after malaria), affecting more than 200 million people residing in rural and agricultural areas. Schistosomes are known as **blood flukes** as they live in vascular system of humans and other vertebrate hosts. The important schistosomes that parasitize humans are:

- ❖ *S. mansoni* and *S. japonicum*—reside in mesenteric venous plexus (of GIT), cause various GI symptoms including dysentery. *S. mansoni* is prevalent in Africa,

whereas *S. japonicum* is found in Asia. They are discussed in this chapter

- ❖ *S. haematobium*: Found in Africa. It resides in venous plexus of bladder; causes urinary schistosomiasis and carcinoma of bladder (discussed in Chapter 76).

Morphology and Life Cycle

The general morphology and life cycle of schistosomes differ considerably from other trematodes, as described earlier (Table 46.3 and Fig. 46.13).

- ❖ **Adult worm:** Adult worms live in the venous plexuses of definitive hosts (hence called as blood flukes). Male worm possesses a sex canal (gynecophoric canal) in which the female worm reposes (Fig. 46.11B)
 - *S. mansoni* adult worms reside in mesenteric veins draining sigmoidorectal region
 - Adult worms of *S. japonicum* reside in the mesenteric veins draining the ileocecal region.
- ❖ **Eggs:** Schistosomes lay non-operculated eggs with a spine like projection (Figs 46.14A to C)
- ❖ **Larvae:** Various larval forms are—miracidium, sporocysts (first and second generations), and cercaria. There are no rediae and metacercariae stages.

Schistosoma mansoni

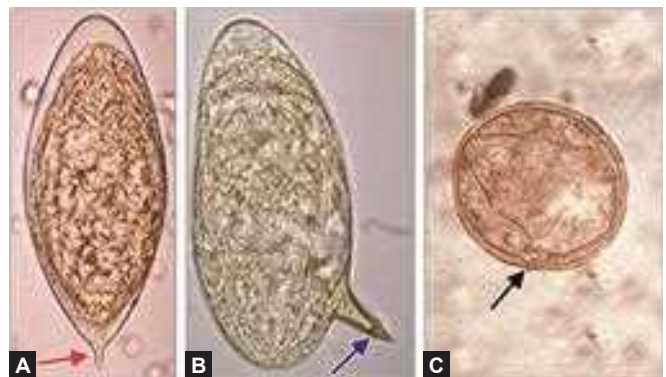
S. mansoni produces intestinal schistosomiasis in humans, first identified by Sir Patrick Manson in West Indies.

Epidemiology

S. mansoni infection is common in 54 countries; from African countries including Madagascar, South America (Brazil and Argentina), Caribbean Islands (West Indies) and Arabian Peninsula. No cases have been reported from India so far.

Pathogenesis and Clinical Features

In general, the pathogenesis of mansonian schistosomiasis occurs in three stages.



Figs 46.14A to C: *Schistosoma* eggs: A. *S. haematobium*; B. *S. mansoni*; C. *S. japonicum*.

Source: A. ID# 4843, B. ID# 4841, C. ID#4842. Public Health Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Cercarial Dermatitis

After 2 or 3 days of skin penetration of cercaria larvae, an itchy maculopapular rash develops on the affected areas of the skin called as cercarial dermatitis (**swimmer's itch**). This is also observed in *S. japonicum* infection.

Acute Schistosomiasis (Katayama Syndrome)

It occurs within 4–8 weeks of infection, especially when the schistosomes start producing eggs. It is less common in endemic area.

- ❖ The antigens (released from the eggs) and the adult worms stimulate the host humoral response, leading to the formation of immune complexes and serum sickness like illness called **Katayama fever**
- ❖ It is characterized by fever, generalized lymphadenopathy, and hepatosplenomegaly. Parasite-specific antibodies may be detected. There is a high peripheral blood eosinophilia.

Chronic Schistosomiasis

After eggs are produced, they are trapped in the small venules and are carried from intestine through portal circulation into liver and other parts of the body.

- ❖ The eggs are deposited in the intestinal wall and other sites such as liver, lungs, brain, and spinal cord
- ❖ Soluble antigens liberated from eggs induce inflammatory reactions, that lead to granuloma formation around the eggs in the intestine and other visceral sites.

Intestinal followed by hepatosplenic disease are the most common form of chronic schistosomiasis.

- ❖ **Intestinal disease:** Fibrosis and thickening occurs in the intestinal wall along the entire length of colon and rectum. Patient may present with diarrhea or dysentery
- ❖ **Hepatosplenic disease:** Granuloma formation and fibrosis in liver (called as Symmers pipestem fibrosis) impedes the portal blood flow leading to portal hypertension, hepatomegaly, splenomegaly and gastric varices
- ❖ **Pulmonary involvement** leads to pulmonary hypertension and right sided heart failure
- ❖ **Neuroschistosomiasis** involving brain and spinal cord (Chapter 75)
- ❖ **Kidney:** Nephrosclerosis and kidney failure may occur due to circulating immune complexes deposited in glomerular membrane
- ❖ **Secondary bacterial infection** can occur, especially with *Salmonella* species and *Staphylococcus aureus*. *S. aureus* colonizing in liver can cause liver abscess.

Laboratory Diagnosis

Stool Microscopy

In acute cases, eggs can be detected in stool by microscopy.

Eggs of *S. mansoni*

Measure 114–180 μm \times 45–73 μm , non-operculated and have characteristic lateral spine (Fig. 46.14B).

In chronic cases or in patients with low worm burden, the number of eggs excreted in stool is less and intermittent. Hence, the following ways can be employed to increase the sensitivity.

- ❖ Multiple stool specimens should be examined
- ❖ Stool concentration techniques by centrifugal sedimentation should be followed
- ❖ **Hatching test:** This involves hatching of motile miracidia from the eggs when stool specimen is diluted in water and beam of light is passed through the water at the top; which confirms the viability of the parasite
- ❖ The quantitation of eggs in stool specimens can be done by Kato thick smear technique.

Other methods include:

- ❖ Histopathological demonstration of lateral spined eggs in biopsy material from rectal mucosa confirms the diagnosis of schistosomiasis
- ❖ Egg shell of *S. mansoni* is **acid-fast** and can be stained by modified Ziehl-Neelsen stain.

Antigen Detection

Antigen detection is useful for assessing the severity of disease and to monitor the efficacy of treatment.

- ❖ **ELISA** is available to detect circulating cathodic (CCA) and circulating anodic antigens (CAA) of schistosomes in the serum and urine
- ❖ **Dipstick test** is available for detecting CCA in urine; showed sensitivity of 92%.

Antibody Detection

Antibody detection tests are less useful as they cross-react with other helminth infections, become positive slowly and remain positive even after successful treatment.

- ❖ Various tests available are cercaria-Huller reaction, ELISA and western blot
- ❖ They detect antibodies by employing antigens such as soluble adult worm or egg antigens.

TREATMENT

S. mansoni infection

Praziquantel is the drug of choice; given 20 mg/kg/dose, two doses in single day. Oxamniquine is also very effective.

Prevention

Preventive measures for schistosomiasis include:

- ❖ Proper disposal of human excreta
- ❖ Eradication of snails by using molluscicides such as metal salts (iron or aluminum sulfate), metaldehyde, methiocarb and acetylcholine esterase inhibitors
- ❖ Treatment of infected persons.

Schistosoma japonicum

It is the most pathogenic species among the schistosomes. It is the only schistosome species that shows zoonotic transmission.

Epidemiology

S. japonicum infection occurs most commonly in China, Indonesia and Philippines. It is eradicated from Japan since 1960. Children of 5–10 years of age are commonly affected. No cases have been reported from India so far.

Pathogenesis and Clinical Features

Pathogenesis is almost similar to that caused by *S. mansoni*. However, the disease is more severe because of the higher egg production and smaller size of the eggs (easy dissemination). The manifestations seen are as follows.

- ❖ Cercarial dermatitis and Katayama fever, intestinal and hepatosplenic disease can occur, as described under *S. mansoni*. Left lobe of liver is the most common site involved
- ❖ **Cerebral schistosomiasis:** CNS involvement is more marked in infection with *S. japonicum* than other schistosomes. It involves brain, causing acute encephalitis (Chapter 75)
- ❖ **Carcinoma:** Both colorectal carcinoma and liver carcinoma (and cirrhosis) have been reported from people of China and Japan infected with *S. japonicum*
- ❖ Chronic secondary infection with *Salmonella* species and hepatitis B virus have been associated with *S. japonicum*.

Laboratory Diagnosis

The diagnostic methods of *S. japonicum* is similar to that of *S. mansoni*. Stool microscopy reveals characteristic eggs.

Eggs of *S. japonicum*

Relatively smaller (70–100 µm length × 50–65 µm width), more spherical than those of other schistosomes and have rudimentary lateral spine (may be absent in some strains) (Fig. 46.14C).

The additional diagnostic methods specific for *S. japonicum* are described here.

- ❖ **Pyrosequencing** assay has been designed to detect DNA specific to *S. japonicum* and *S. mekongi* in fecal samples and in infected snails
- ❖ **Magnetic fractionation method:** Here, the magnetic microspheres are used which bind to the eggs in stool specimen and form egg—microsphere conjugate. *S. japonicum* eggs have higher affinity to bind microspheres than *S. mansoni* eggs due to higher localization of iron in its egg shell. This test is useful for screening of large volume of samples with a good diagnostic sensitivity.

TREATMENT

S. japonicum infection

Praziquantel is given 20 mg/kg/dose, three doses in single day.

Other intestinal schistosomes, that rarely infect man include:

- ❖ *S. intercalatum* remains endemic in Africa; causes disease similar to *S. mansoni* like intestinal, hepatosplenic manifestations and secondary *Salmonella* infections
- ❖ *S. mekongi* infection occurs in the Mekong river basin of Laos, Thailand and Cambodia. Clinically, it resembles to

S. japonicum infection, with intestinal, hepatosplenic and brain involvement. Man and dogs are the definitive hosts.

Elimination of Schistosomiasis

The WHO is currently moving towards elimination of schistosomiasis as a public health problem in Africa by 2020 and globally by 2025.

This may be achieved through treatment of cases using praziquantel to prevent morbidity in later life and also through mass drug administration in some places (Egypt and China).

FASCIOLPSIASIS

Fasciolopsiasis is infection with the intestinal fluke *Fasciolopsis buski*—also known as **giant intestinal fluke**. It is the largest and the most common intestinal fluke infecting man.

Epidemiology

F. buski is mainly endemic in Southeast Asian countries such as India, China, Pakistan, Bangladesh, Thailand and Malaysia.

- ❖ **India:** In India, most of the cases have been reported from Eastern Uttar Pradesh, Bihar, West Bengal and Assam and Maharashtra
- ❖ **Phulwaria endemic foci:** There is an emergent endemic focus of fasciolopsiasis in Phulwaria village, Bihar; 118 cases were detected during 2015.

Life Cycle

The life cycle of *F. buski* is similar to other trematodes (except *Schistosoma*); as described above (Fig. 46.12).

- ❖ **Host:** It has one definitive host (pig or man) and two intermediate hosts (first—snail, second—aquatic plants)
- ❖ **Modes of transmission:** Humans acquire infection by eating contaminated water plants, carrying the infective form, the metacercaria larvae.

Pathogenesis

The main pathogenesis is due to the traumatic and obstructive damage to the intestine.

- ❖ **Light infection:** It may be asymptomatic or its attachment to intestinal mucosa leads to local inflammation, ulcerations with mucus and blood in stool
- ❖ **In severe infection:** There may be partial obstruction of intestinal tract
- ❖ Malabsorption and protein losing enteropathy may be seen with profuse yellowish green stool
- ❖ Marked eosinophilia and leukocytosis are commonly observed.

Laboratory Diagnosis

Detection of large number of operculated eggs in the stool sample gives probable diagnosis of *F. buski*.

- ❖ Eggs are large (130–140 µm × 80–85 µm size), operculated and bile stained; morphologically similar to the eggs of *F. hepatica* (Fig. 46.15B)



Figs 46.15A and B: *Fasciolopsis buski*: **A.** Adult worm; **B.** Egg (saline mount).

Source: A and B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ Sedimentation methods are recommended for stool concentration when worm load is less
- ❖ Definitive diagnosis can be done only after identification of the adult worm (Figs 46.11A and 46.15A).

TREATMENT

Fasciolopsiasis

- ❑ Praziquantel is the drug of choice. It is given as 25 mg/kg, three doses in 1 day
- ❑ Niclosamide is given alternatively.

Other intestinal trematodes that usually infect animals and less commonly infect man include—*Gastrodiscoides hominis*, *Watsonius watsoni*, *Heterophyes heterophyes*, *Metagonimus yokogawai* and *Echinostoma ilocanum*.

INTESTINAL NEMATODE INFECTIONS

Nematodes are probably the most widespread animal group occurring in the world. Many of them are nonpathogenic and exist as free-living forms in fresh or marine water and soil, while few of the species can be pathogenic and exist as parasitic form in humans, animals and plants.

INTRODUCTION TO NEMATODES

Classification based on Habitat

Based on their habitat, nematodes infecting humans are classified into two groups.

Intestinal Nematodes

Intestinal nematodes include five common parasites.

- ❖ Small intestinal nematodes—*Ascaris*, hookworm and *Strongyloides*
- ❖ Large intestinal nematodes—*Trichuris* and *Enterobius*.

Tissue or Somatic Nematodes

Somatic nematodes reside in various tissues.

- ❖ **Filarial nematodes:** They comprise of several vector-borne parasites
 - *Wuchereria bancrofti* and *Brugia malayi* cause lymphatic filariasis (Chapter 37)
 - *Loa loa*, *Onchocerca volvulus*, and *Mansonella* cause cutaneous filariasis (Chapter 57).
- ❖ *Dracunculus medinensis*: Causes Guinea-worm disease; presents as painful cutaneous blisters (Chapter 57)
- ❖ *Trichinella spiralis*: Produces profuse watery diarrhea, followed by myalgia due to deposition of encysted larvae in muscles (Chapter 57).

Apart from the above list, there are several nematodes of lower animals that rarely cause intestinal and somatic disease in man.

Classification based on they Lay Egg or Larva

Nematodes can be classified into three groups based on they lay eggs or larvae after fertilization:

1. **Oviparous:** Following fertilization, the female worms produce eggs that take some time to hatch out to form larvae in the environment
 - Most of the intestinal nematodes are oviparous except for *Strongyloides*. Examples include hookworm, *Ascaris*, *Trichuris*, *Enterobius*, etc.
 - Here, the diagnosis is made by detection of eggs in feces.
2. **Viviparous:** Female worms directly give birth to larvae; there is no egg stage
 - Most of the somatic nematodes are viviparous. Examples include filarial worms, *Trichinella* and *Dracunculus*
 - Here, the diagnosis is made by detection of larvae in tissues or blood.
3. **Ovoviviparous:** Here, the female worms lay eggs containing larvae; which immediately hatch out
 - Example includes *Strongyloides* species
 - The larvae are the diagnostic form detected in stool examination.

Morphology (Nematodes)

Nematodes pass through six developmental stages (Fig. 46.16) adult worm, egg stage and four larval stages (L_1 – L_4). Each larval stage transforms to the next by shedding of the cuticle (called as **molting**).

Adult Worm

Nematodes are elongated, cylindrical or filariform in shape with both the ends pointed (Fig. 46.17).

- ❖ **Size:** Variable in size, ranging from less than 5 mm (hookworm, *Trichinella* and *Strongyloides*) to as long as one meter (*Dracunculus*). Female worms are longer than male worms

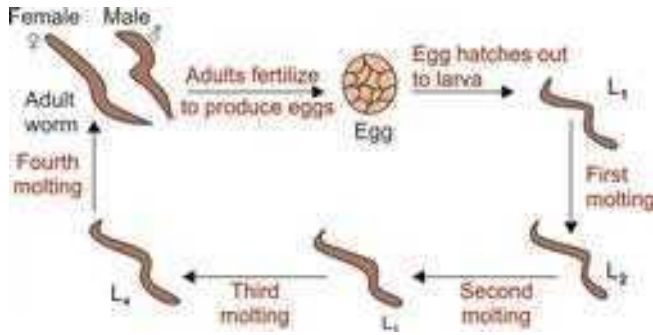


Fig. 46.16: Developmental stages of nematodes.

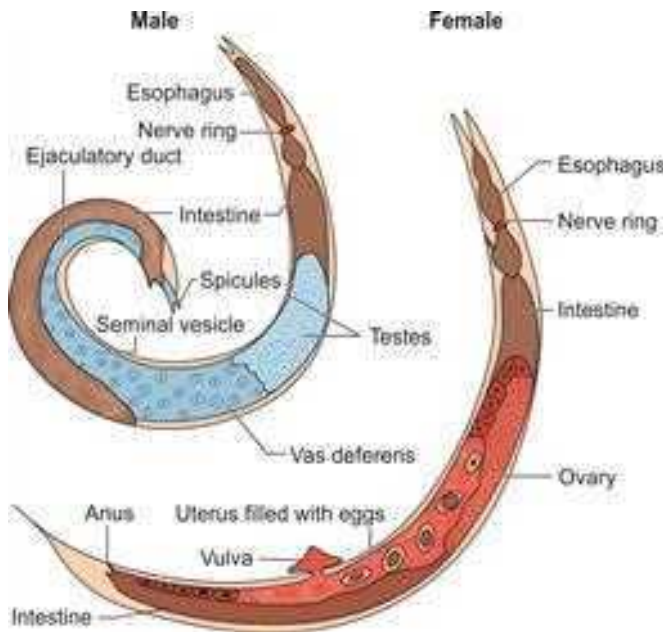


Fig. 46.17: Adult male and female nematode (schematic diagram).

- ❖ **Organs:** Body is bilaterally symmetrical, surrounded by a body wall containing various organs such as alimentary canal, body cavity, nervous system, excretory system and reproductive organs
- ❖ They are dioecious (bisexual), i.e. male and female worms are different (Fig. 46.17).

Life Cycle

Intestinal nematodes complete their life cycle in one host (man); discussed under the respective parasite subsequently in this chapter.

- ❖ *Trichuris*, *Enterobius* and *Ascaris* have considerable similarities in their life cycle such as mode of transmission (ingestion), infective form (embryonated eggs) and diagnostic form (eggs). In addition, *Ascaris* larvae have a migratory phase in the lungs
- ❖ The life cycles of *Strongyloides* and hookworm also have similarities such as mode of transmission (skin penetration) and infective form (L_3 larva).

TRICHURIASIS

Trichuriasis is also known as **whipworm** infection; caused by the intestinal nematode *Trichuris trichiura*. It is a soil-transmitted helminth (see the highlight box, subsequently in this chapter). *Trichuris* has several other species, which rarely infect animals.

Epidemiology

Trichuriasis is worldwide in distribution, mainly in warm and moist climate similar to ascariasis.

- ❖ Children are commonly affected
- ❖ Global prevalence in humans is about 604 million.

Morphology

Similar to other nematodes, *T. trichiura* exists in three forms: adult, larvae (four stages) and egg. The adult worm resembles to a handle of a whip, (therefore called as **whipworm**).

Life Cycle (Fig. 46.18)

Humans are the only host.

- ❖ **Mode of transmission:** Men (usually children) acquire infection by ingestion of contaminated food and water containing embryonated egg (infective form)
- ❖ **Human GIT:** Eggs hatch out in the small intestine releasing the L_2 larva, which migrate to large intestine and molt twice to transform into adult worms
 - The female worms following fertilization start laying unembryonated eggs, that are released in feces (diagnostic form)
 - Each female worm can lay 14,000–20,000 eggs per day for 1–3 years.
- ❖ **Embryonation:** The eggs passed in the feces become embryonated (i.e. molt twice to produce L_2 larvae within

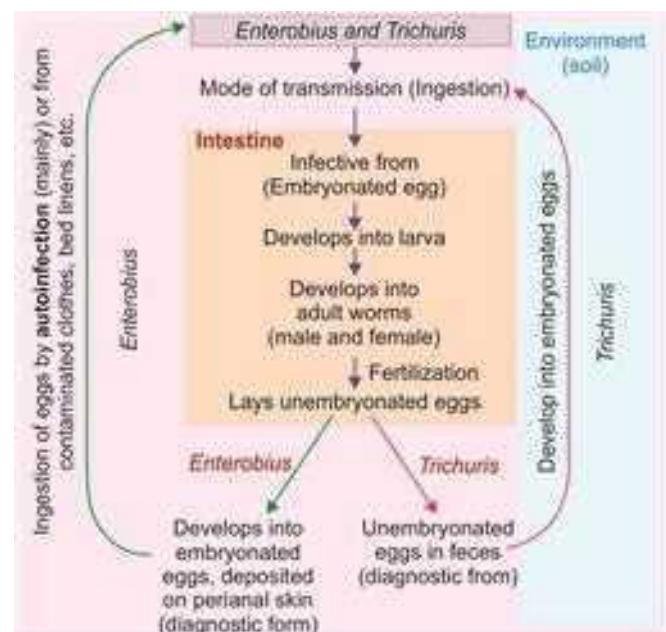


Fig. 46.18: Life cycles of *Trichuris* and *Enterobius*.

the eggshell) in warm (25°C) and moist environment. Embryonated eggs are infective to man and thus the life cycle continues.

Pathogenicity and Clinical Feature

Incubation period varies from 70 to 90 days. Most infected individuals are asymptomatic, with or without having eosinophilia.

In people with heavy infections: Adult female worms get buried in the large intestinal mucosa, that leads to:

- ❖ Mechanical distortion: Leading to inflamed, edematous, and friable mucosa
- ❖ Allergic response by the host: Large number of macrophages infiltrate in the lamina propria that produce tumor necrosis factor- α (TNF- α).

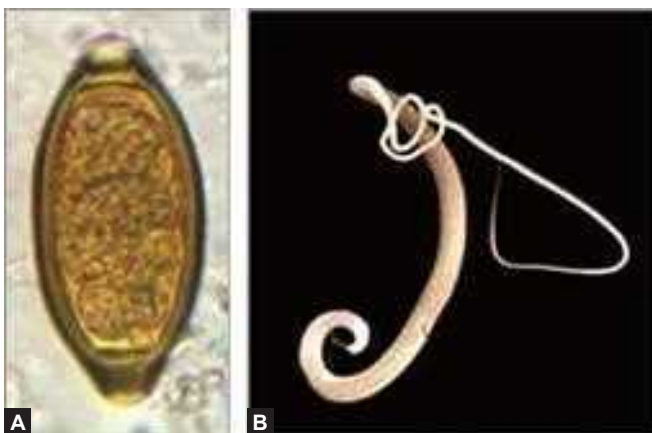
Common manifestations include:

- ❖ Abdominal pain, anorexia, etc.
- ❖ *Trichuris dysentery syndrome*—bloody or mucoid diarrhea resembling inflammatory bowel disease
- ❖ Iron deficiency anemia—occurs as a result of blood loss; not due to blood ingestion as seen in hookworm
- ❖ Recurrent rectal prolapse—occurs due to heavy worm load in the rectum
- ❖ Malnutrition leading to growth retardation and impaired cognitive function—due to the release of anti-inflammatory cytokines induced by *Trichuris* species.

Laboratory Diagnosis

Stool Examination

Stool examination is carried out either by direct wet mount or following concentration of the stool, which reveals characteristic egg and occasionally adult worm. Single stool specimen is sufficient for diagnosis of symptomatic cases (Figs 46.19A and B) as the level of egg output is usually high.



Figs 46.19A and B: *Trichuris trichiura*: **A.** Egg in saline mount (bile stained); **B.** Adult female worm.

Source: A. Dr Anand Janagond, Department of Microbiology, S. Nijalingappa Medical College, Bagalkot, Karnataka (with permission); B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Trichuris trichiura (eggs and adult worm)

Eggs: Measure 50 × 22 μ m, barrel-shaped, with mucus plugs at the ends. Eggs are bile-stained and float in saturated salt solution (Fig. 46.19A)

Whip shaped adult worms of 3–5 cm long, are occasionally seen on proctoscopy (Fig. 46.19B). Anterior three-fifth is thin, hair like, coiled (like rope of a whip) and posterior two-fifth is short and thick.

Other Findings

- ❖ Peripheral blood eosinophilia (<15%)
- ❖ Increased serum IgE level.

TREATMENT

Trichuriasis

- ❑ Mebendazole (500 mg once) or albendazole (400 mg daily for three doses) is safe and moderately effective for treatment, with cure rates of 70–90%
- ❑ Ivermectin (200 mg/kg daily for three doses) is also safe but is less effective.

Prevention

Trichuriasis can be prevented by improved personal hygiene, proper disposal of feces and improved nutrition with dietary iron.

ENTEROBIASIS

Enterobiasis is a common parasitic infection in children caused by the intestinal nematode, *Enterobius vermicularis*. It is also called as **pinworm** or **threadworm** infection (as the adult worm of *Enterobius* is small, white and thread-like).

Epidemiology

Globally, around 209 million people are infected by pinworms; most common age group being school children.

- ❖ People carry the infection for years together due to auto-infective cycles
- ❖ **It has been said that:** “You had the infection as a child, you have it now and you will again get it when you have children”
- ❖ **Factors promoting infection:** Overcrowding and impaired hygiene, poor personal care (nail biting or inadequate hand washing).

Life Cycle (Fig. 46.18)

Humans are the only host. Embryonated eggs are infective to man.

- ❖ **Mode of transmission:** Infection occurs via:
 - **Autoinfection:** It is of two types—(i) *exogenous autoinfection*, by transferring eggs to the mouth with hands that have scratched the perianal area, (ii) *endogenous autoinfection*, by retrograde migration of the larva hatched from the eggs in the perianal skin.

- Through **ingestion of eggs** in the environment (e.g. surfaces, clothes, bed linens, etc.) through contaminated fingers.
- ❖ **Development in man:** Eggs usually contain the fully developed larvae. Larvae hatch out from eggs in the cecum and then develop into adult worms
- ❖ After fertilization, the gravid female worms migrate to large intestine (rectum, colon) and start laying eggs on the perianal skin. Adult female worms usually lay 10,000 eggs/day
- ❖ The eggs are embryonated and are the infective stage to man and the life cycle continues.

Pathogenicity and Clinical Features

Most of the infections are asymptomatic. Few develop clinical disease.

- ❖ **Age and sex:** Females, children and young adults are often symptomatic than males and older people
- ❖ **Cardinal symptoms:** Perianal pruritus often worse at night as a result of the nocturnal migration of the female worm
 - The worms may be found in undergarments and can also be found lying in the buttock area of infected children
 - Repeated scratching is the main reason of contaminated finger; which causes autoinfection.
 - Excoriation of the perianal skin and bacterial superinfection may occur (due to continuous scratching of the skin)
 - Abdominal pain and weight loss (may be seen in heavy infections).
- ❖ **Migration of the worm:** Rarely, pinworms invade the female genital tract, causing vulvovaginitis and pelvic or peritoneal granulomas. Other sites involved are urinary tract, peritoneal cavity, lungs and liver
- ❖ Eosinophilia is inconsistent.

Laboratory Diagnosis

Microscopy of perianal skin samples is the test of choice which detects characteristic eggs. Specimen is collected by two methods.

- ❖ **Cellophane tape** is applied onto the perianal region and then the tape is mounted with a drop of saline on a clear glass slide (Fig. 46.20A)
- ❖ **NIH swab:** Alternatively, NIH swab can be used, devised in National Institute of Health, USA. It consists of a glass rod attached to a cellophane tape by a rubber band. The cellophane part of the glass rod is rolled over the perineal and perianal skin area to collect the sample (Fig. 46.20E). There are few other points to consider while specimen collection for enterobiasis.
- ❖ **Number of specimens:** A series of 4–6 consecutive tapes may be necessary as the female worms migrate intermittently
- ❖ **Timing:** Samples should be collected when the chance of egg deposition is more such as late in the evening, when the patient has been sleeping for several hours, or first thing in the morning
- ❖ The female worms lay eggs in the perianal area; not in the rectum. Hence, eggs are rarely detected by stool examination; around 5% of cases
- ❖ The adult female worms may occasionally be found in the feces or crawling onto the perianal skin (Figs 46.20B and C).

Eggs of *Enterobius*

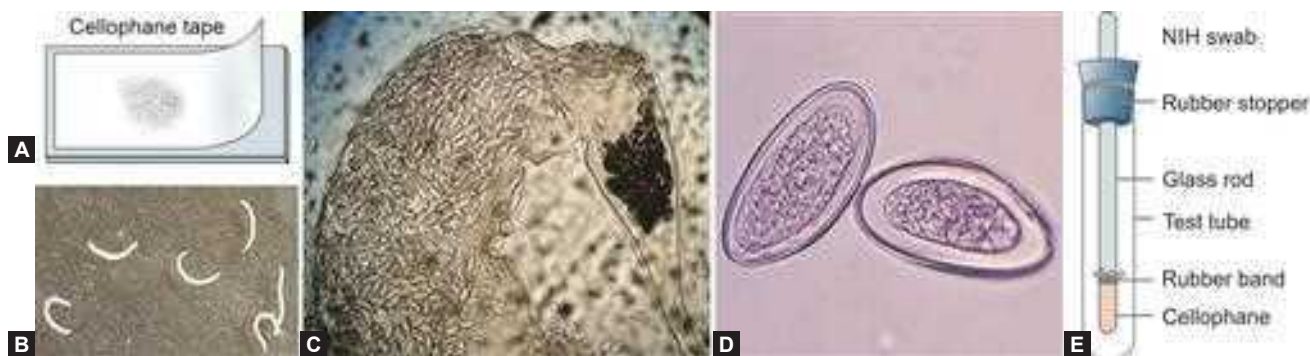
Eggs are planoconvex (one side is convex and the other side is flat), measure 50–60 μm long (Fig. 46.20D)

- ❑ Embryonated egg when freshly passed contains a tadpole shaped larva inside
- ❑ Non-bile-stained, colorless in saline mount
- ❑ Floats in saturated salt solution.

TREATMENT

Enterobiasis

- ❑ One of the following drugs can be given:
 - Mebendazole (100 mg once) or
 - Albendazole (400 mg once) or
 - Pyrantel pamoate (11 mg/kg once; maximum, 1 g)
- ❑ The same treatment should be repeated after 2 weeks
- ❑ Treatment of household members is advocated to eliminate asymptomatic reservoirs of potential reinfection.



Figs 46.20A to E: *Enterobius vermicularis*: **A.** Cellophane tape; **B.** Adult worms (actual size); **C.** Adult female worm containing numerous eggs; **D.** Planoconvex eggs; **E.** NIH swab method (schematic).

Source: B and C. Head, Department of Microbiology, Meenakshi Medical College, Chennai; D. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Prevention

Total prevention is neither realistic nor possible as the transmission is so common; aided by autoinfection. Improving personal hygiene such as proper washing of bed clothes, keeping nail short and clean, frequent hand washing are the key measures to contain the transmission.

ASCARIASIS

Ascariasis is an infection of the small intestine caused by *Ascaris lumbricoides*. It is the largest nematode parasitizing the human intestine. It is commonly called as **roundworm**. It is a soil-transmitted helminth (see the highlight box, subsequently in this chapter).

Epidemiology

A. lumbricoides is cosmopolitan in distribution, mainly affecting tropical countries including India.

- ❖ It is estimated that 807–1200 million people are infected globally, of which 120–250 million are symptomatic
- ❖ Transmission typically occurs through fecally contaminated soil and is due to either lack of sanitary facilities or use of human feces as fertilizer
- ❖ Clay soils are most favorable for the development of *Ascaris* eggs (in contrast to moist porous soil required for hookworm)
- ❖ **Risk factors:** Children (most important disseminator of the disease) and malnutrition.

Morphology

Similar to other nematodes, *Ascaris* exists in three forms: adult, larvae (four stages) and egg. Adult worm is cylindrical and measures 15–31 cm. The female worms liberate two types of eggs—(1) fertilized eggs, and (2) unfertilized eggs.

Life Cycle (Fig. 46.21)

Ascaris involves only one host (man). Embryonated eggs containing the L₂ larvae are the infective form.

- ❖ **Mode of transmission:** Ingestion of embryonated eggs from the contaminated soil, food and water
- ❖ **Migratory phase:** Following ingestion, the eggs hatch out to liberate the L₂ larvae, which molt once to form L₃.
 - The L₃ larvae penetrate the intestine, reach right side of the heart via portal circulation, then enter lungs via pulmonary capillaries, where they molt once to form L₄
 - The L₄ larvae in the lungs migrate up to reach pharynx and finally are swallowed to re-enter the intestine.
- ❖ **Intestinal phase:** The L₄ larvae undergo final molt to develop into adult worms in the small intestine
 - Following fertilization, the female worms start laying the fertilized eggs which are passed in the feces. Sometimes, before mating, the female worms may directly lay the unfertilized eggs
 - A gravid female can lay 2.4 lakh eggs/day.

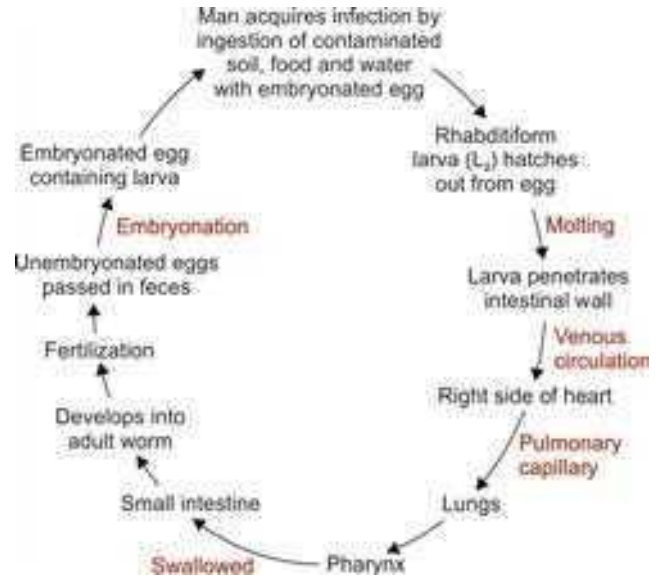


Fig. 46.21: Life cycle of *Ascaris lumbricoides*.

- ❖ **Development in soil:** The fertilized eggs molt twice become embryonated (carrying L₂ larvae), which is infective to man and the life cycle continues. It occurs within 2 weeks under suitable conditions such as warm and clay soil, 22–30°C and 40% humidity
 - The unfertilized eggs cannot develop further, are not infective and disintegrate in some time
 - *Ascaris* embryonated eggs survive for as long as 15 years as they are highly resistant due to the characteristic thick eggshell. *Ascaroside*, a lipoprotein present in the eggshell is responsible for its resistance to disinfectants.

Pathogenesis and Clinical Feature

Pathogenesis caused by *Ascaris* infection is attributed to (i) the host immune response, (ii) migration of larva, (iii) mechanical obstruction by the adult worms, and (iv) nutritional deficiencies due to the presence of adult worms. The incubation period is about 60–70 days; however the pulmonary symptoms can be earlier.

Pulmonary Phase

It results from migrating larvae in the lungs, which provoke an immune-mediated hypersensitivity response.

- ❖ Symptoms are observed in the second week after the ingestion of eggs; characterized by non-productive cough, chest discomfort and fever
- ❖ **Eosinophilic pneumonia (Loeffler's syndrome):** In severe cases, patients develop dyspnea and a transient patchy infiltrates seen in the chest X-ray along with transient peripheral eosinophilia.

Intestinal Phase

It results due the effect of adult worm in the intestine.

- ❖ **Asymptomatic:** Most people with mild *Ascaris* infections are asymptomatic

- ❖ **Malnutrition and growth retardation:** Robbing the nutrition from the host may result in chronic malnutrition and growth retardation (in children <5 years). It is often associated with impairment of educational performance, language learning, social, gross motor, and fine motor skills in children
- ❖ **Intestinal complications:** A large bolus of entangled worms can cause intestinal obstruction, rarely perforation, **intussusception**, or volvulus. It is usually seen in age group >5 years and presents as acute pain abdomen
- ❖ **Extraintestinal complications:** Larger worms can enter and occlude the biliary tree, causing biliary colic, cholecystitis, pancreatitis, or (rarely) intrahepatic abscesses. Wandering worms may migrate to pharynx and can cause respiratory obstruction or may block the eustachian tube
- ❖ **Allergic manifestations** like fever, urticaria, angioneurotic edema and conjunctivitis may occur due to toxic fluid (ascaron or ascarase) released by the adult worm.

Laboratory Diagnosis

Detection of the Parasite

Egg Detection (Stool Examination)

Both fertilized and unfertilized eggs can be detected by stool examination by saline and iodine wet mount.

- ❖ Concentration techniques by sedimentation method should be done if direct stool microscopy is negative
- ❖ Floatation method for stool concentration is not preferred as unfertilized eggs do not float on saturated salt solution.

Eggs of *Ascaris* (Figs 46.22 and 46.23)

Fertilized eggs (Fig. 46.23A)

Round to oval, measure 45–75 μm \times 35–50 μm

- ❑ Surrounded by a thick mamillated, albuminous coat
- ❑ Contains a large unsegmented ovum of granular mass with clear crescentic space at both the poles
- ❑ Bile-stained, appear golden brown in saline mount
- ❑ Floats in saturated salt solution

Contd...

Unfertilized eggs (Fig. 46.23B)

Elongated, measure 85–95 μm \times 43–47 μm

- ❑ Albuminous coat is thin, distorted and scanty
- ❑ Contains an unsegmented, small atrophied ovum with a mass of disorganized highly refractile granules and no crescentic space at poles
- ❑ Bile-stained, appear golden brown in saline mount
- ❑ Does not float in saturated salt solution

Decorticated eggs (Fig. 46.23C)

Sometimes, the fertilized eggs lose the thick mamillated albuminous coat and called as decorticated eggs.

Adult Worm Detection

Occasionally, adult worms may be detected in stool or sputum of the patients by naked eye (Fig. 46.24)

- ❖ Barium meal X-ray of the GIT may demonstrate the adult worms in the intestine. When two worms are lying parallel, gives *trolley car lines* appearance in X-ray
- ❖ Ultrasound or cholangiopancreatography should be done to detect the adult worms in extraintestinal sites.

Larva Detection

During the early pulmonary migratory phase, larvae can be found in sputum or gastric aspirates before the eggs appear in the stool.

Serology

Antibody detection (by ELISA and other formats) though sensitive; cannot differentiate between recent and past

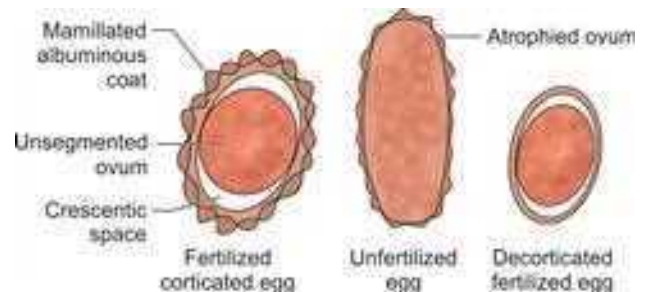
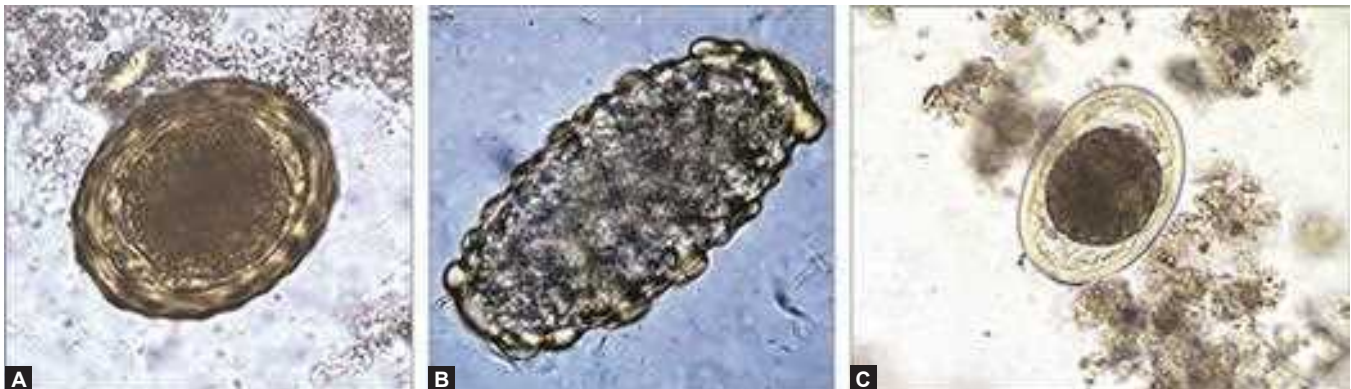


Fig. 46.22: Eggs of *Ascaris lumbricoides* (schematic diagram).

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Figs 46.23A to C: Eggs of *Ascaris lumbricoides*: **A.** Fertilized eggs; **B.** Unfertilized eggs; **C.** Decorticated eggs.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

infection. It also cross-reacts with other helminthic infections. It is mainly useful for seroepidemiological purposes and for assessing transmission in areas aiming for elimination.

Molecular Method

PCR assay has been developed targeting internal transcribed spacer region (ITS1) or cytochrome oxidase-1 of *Ascaris* egg in the stool. Multiplex PCR can simultaneously differentiate *Ascaris*, *Trichuris* and hookworm. Real-time PCR can be used for quantitation of the parasite load in the stool.

Other Methods

- ❖ **Eosinophilia** is prominent during the early lung stage, but disappears later
- ❖ Presence of **Charcot-Leyden crystals** in sputum and stool, a nonspecific finding seen in ascariasis.

TREATMENT

Ascariasis

Antiparasitic drugs

Ascariasis should always be treated early to prevent potentially serious complications.

- ❑ Albendazole (400 mg once) or mebendazole (100 mg twice daily for 3 days or 500 mg once) is recommended. It effectively kills the adult worm, but has limited effect on larval migration phase
- ❑ Alternatively, drugs like ivermectin (150–200 mg/kg once) and nitazoxanide are also effective
- ❑ In pregnancy, pyrantel pamoate is safe

Symptomatic treatment

Partial intestinal obstruction should be managed with nasogastric suction, intravenous (IV) fluid administration but complete obstruction and its severe complications like intussusception require immediate surgical intervention.



Fig. 46.24: Adult worm of *A. lumbricoides*.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Prevention

Same as for other soil-transmitted helminths like hookworm and *Trichuris*. The use of human feces, or “night soil,” for fertilization of crops should be avoided as it is an important source of *Ascaris* (mainly) and other helminthic infections.

HOOKWORM INFECTIONS

Hookworm is one of the important causes of iron deficiency anemia in both tropics and temperate countries. It is one of the soil-transmitted helminths (see the highlight box, subsequently in this chapter).

Classification

Hookworm comprises of several species, which infect humans and animals; out of which only two species are human pathogens; cause intestinal disease.

- ❖ *Ancylostoma duodenale* or old world hookworm
- ❖ *Necator americanus* or new world hookworm or American hookworm.

There are several animal parasites that rarely infect man; cause cutaneous larva migrans—*Ancylostoma braziliensis*, *A. caninum* and *A. ceylanicum* (Chapter 57).

Epidemiology

- ❖ **World:** Hookworm infection is widespread. Globally, nearly 900 million people are infected. *Necator* infection (835 million) is more common than *Ancylostoma* (135 million)
- ❖ **India:** Hookworm infection is widely prevalent in India. More than 200 million people are estimated to be infected in India
 - *N. americanus* is predominant in South India and *A. duodenale* in North India
 - *Necator* is seen in all the states except in Punjab and Uttar Pradesh
 - Heavily infected areas are: Assam (tea gardens), West Bengal, Bihar, Odisha, Andhra Pradesh, Tamil Nadu, Kerala and Maharashtra.
- ❖ **Age and sex distribution:** Males and young adults are commonly affected, but anemia due to iron loss is more severe in children and pregnant women
- ❖ **Endemic index:** *Chandler's index* is used in the epidemiological studies of hookworm disease to estimate the morbidity and mortality in the community due to hookworm infection (which depends much upon the worm load).

Morphology

Similar to other nematodes, hookworm has three morphological forms: adult, larvae (four stages) and egg.

- ❖ *Ancylostoma* and *Necator* can be differentiated by the morphology of adult worm and third stage larva.

Eggs and first stage larvae of both are morphologically indistinguishable

- ❖ Adult worm is small (7–13 mm) in size, has a bent in the anterior end (hence called as hookworm)
- ❖ The adult worm of *Ancylostoma* and *Necator* can be differentiated by buccal capsule with teeth or cutting plate (present in the anterior end) and copulatory bursa (present in the posterior end)
- ❖ L₁ larva is called as rhabditiform larva whereas L₃ stage larva is called as filariform larva.

Life Cycle (Fig. 46.25)

Hookworm involves only one host (man). Third stage filariform (L₃) larva is the infective form.

- ❖ **Mode of transmission:** Through penetration of skin by the L₃ larva; during walking barefoot in dampen soil
- ❖ **Migratory phase:** Following penetration, the L₃ larvae enter into subcutaneous venules and are carried to lungs through venous circulation. Here, they enter into the alveolar space and migrate up to pharynx and finally by swallowing of sputum, they enter GIT
- ❖ **Intestinal phase:** The L₃ larvae molt twice in small intestine to develop into adult worms, which attach to the intestinal mucosa by their teeth in the buccal capsule
 - Following fertilization, female worms lay eggs, which are excreted in the feces
 - A gravid female of *A. duodenale* can lay 25,000–35,000 eggs/day, whereas that of *N. americanus* can lay 6,000–20,000 eggs/day.
- ❖ **Development in soil:** Eggs released in feces are immature, which become embryonated in moist, shady, warm soil
 - L₁ (rhabditiform) larvae hatch out from eggs and then molt twice to develop into L₃ larvae
 - L₃ larvae remain viable in the soil for several weeks and are infective to man. Thus the life cycle is continued.

Pathogenicity

Hookworm has ability to suck blood from the intestinal vessels, which occurs by several ways:

- ❖ Attaching and making cuts in the intestinal wall by buccal capsule and teeth followed by sucking the blood through contraction of their muscular esophagus
- ❖ Secreting hydrolytic enzymes
- ❖ Releasing anticoagulants which help to maintain continuous oozing of blood from the attachment site.

Ancylostoma is more pathogenic than *Necator*, which is because of its larger size, armed with teeth and more migratory. Blood loss in *Ancylostoma* infection is 0.15–0.26 mL/worm/day, compared to *Necator* (0.03 mL/worm/day).

However, cutaneous lesions such as ground itch and dermatitis are more severe in case of *Necator* infection. The skin penetration is facilitated by proteolytic enzymes

and hyaluronidase secreted by hookworm; leading to degradation of collagen and fibronectin.

There is an increased T_H2 response leading to increased antibodies such as IgG4 or IgE levels.

Clinical Features

Affect due to Migrating Larva

- ❖ **Cutaneous lesions:** This may occur in previously sensitized persons (Chapter 57)
 - Infective larvae may provoke pruritic maculopapular dermatitis and rashes (“ground itch”) at the site of skin penetration
 - **Serpiginous tracks** may be formed due to subcutaneous migration of the larva.
- ❖ **Mild transient pneumonitis:** Migrating larvae through the lungs occasionally cause mild transient pneumonitis, asthma and bronchitis; but the severity and frequency of lung manifestation is less compared to ascariasis.

Affect due to Adult Worm in Intestine

Clinical spectrum produced by adult hookworm depends upon the worm load.

- ❖ **Asymptomatic:** Most hookworm infections are asymptomatic
- ❖ **Early intestinal phase (less worm load):** Infected persons may develop epigastric pain, inflammatory diarrhea, or other abdominal symptoms, accompanied by eosinophilia
- ❖ **Late intestinal phase** (chronic infection with heavy worm load): Patients develop iron deficiency anemia and protein energy malnutrition resulting from blood loss. Other features are weakness and shortness of breath and rarely impaired intellectual power and behavioral changes
- ❖ **Wakana disease:** When L₃ larvae of *A. duodenale* are ingested by the oral route, they either migrate to pharynx or develop to adult worm in the intestine
 - Therefore, both gastrointestinal and pulmonary symptoms are observed. Common symptoms include nausea, vomiting, pharyngeal irritation, cough, dyspnea, and hoarseness
 - This disease is not seen with *N. americanus* as their L₃ stage fails to develop after ingestion.

Laboratory Diagnosis

Stool Microscopy

The diagnosis is established by finding of characteristic eggs in the feces.

- ❖ Stool concentration procedures may be required to detect lighter infections
- ❖ In a stool sample that is not fresh, the eggs may hatch out to release rhabditiform larvae (Fig. 46.26C), which need to be differentiated from that of *Strongyloides* (discussed subsequently in this chapter)

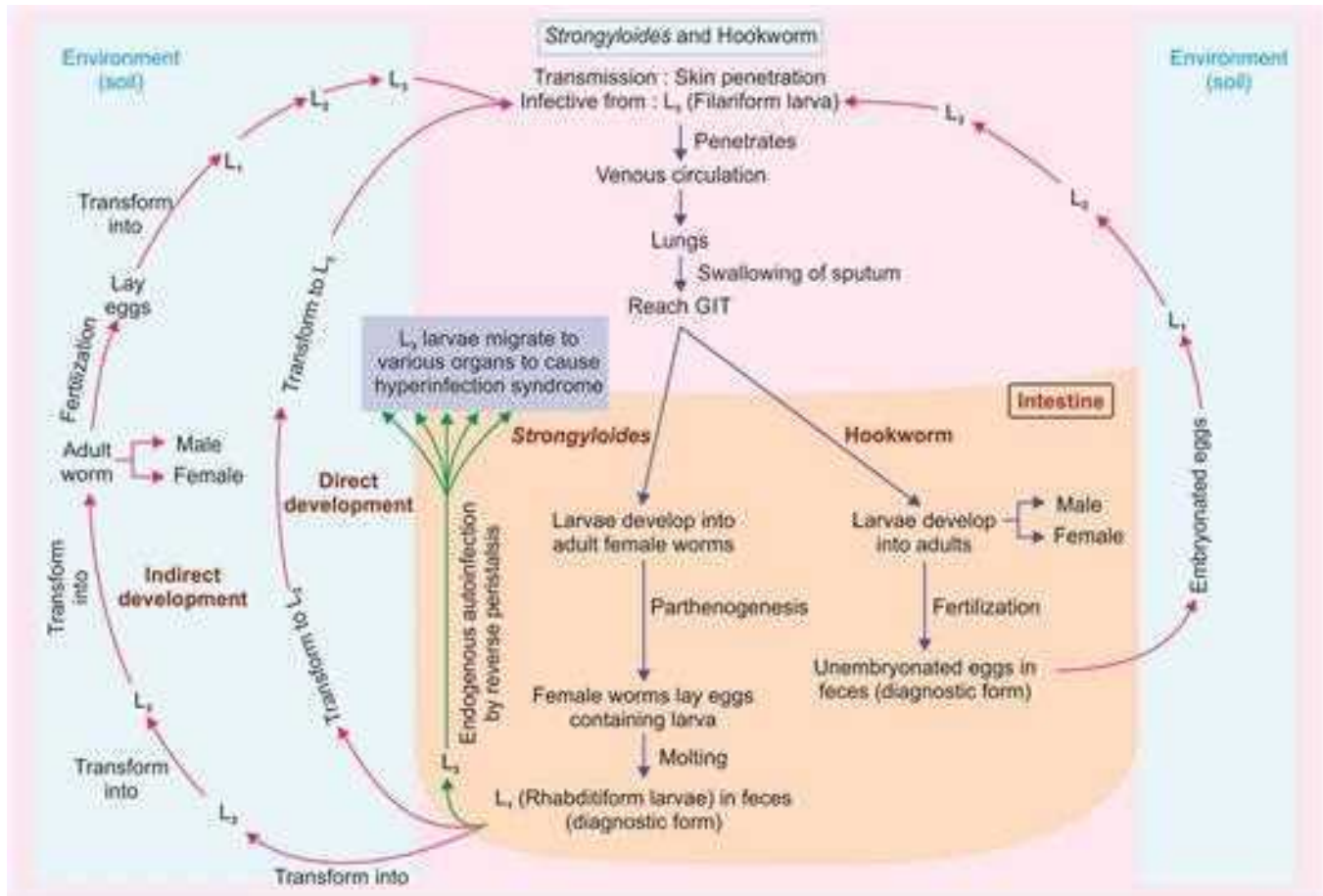


Fig. 46.25: Life cycles of Hookworm and *Strongyloides*.

Eggs of Hookworm (Figs 46.26A and B)

Hookworm eggs are oval, measure $60 \times 40 \mu\text{m}$, surrounded by thin eggshell.

- ❑ Not bile stained, appear colorless in saline mount
- ❑ Ovum (embryo) is segmented; comprises of 4-32 blastomeres
- ❑ There is a clear space between the egg shell and the embryo
- ❑ Floats in saturated salt solution
- ❑ Eggs of both *A. duodenale* and *N. americanus* are morphologically indistinguishable.

❖ **Egg counting:** Number of eggs per gram of stool can be counted to estimate the disease burden in the individual

as well as in the community. The WHO has classified the intensity of infection based on egg count which is directly related to the associated morbidity (Table 46.4). Various methods are:

- Kato Katz technique
- Direct smear method of beaver
- Modified Stoll's dilution egg count method.

Stool Culture

The freshly passed stool samples can be cultured so that the eggs hatch out to develop to L₃ stage filariform larvae



Figs 46.26A to C: Hookworm: A. Egg with four blastomeres; B. Egg with many blastomeres; C. Rhabditiform larva.

Source: A to C. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Table 46.4: Classification of intensity of infection based on WHO guidelines (eggs per gram of stool).

	Light	Moderate	Heavy
<i>Trichuris</i>	1–999	1000–9999	≥10000
<i>Ascaris</i>	1–4999	5000–49999	≥50000
Hookworm	1–1999	2000–3999	≥4000

in 5–7 days, which can differentiate *A. duodenale* from *N. americanus*. Various culture techniques used are:

- ❖ Harada-Mori filter paper tube method
- ❖ Baermann funnel technique
- ❖ Charcoal culture method
- ❖ Agar plate technique (more sensitive).

Molecular Diagnosis

PCR and real-time PCR based assays have been developed targeting genes such as mitochondrial cytochrome oxidase genes. Molecular methods have the advantages such as:

- ❖ Species specific; can differentiate between *Ancylostoma* and *Necator*
- ❖ More sensitive, can detect as low as one copy per 200 mg of stool.

Other Findings

Other findings include: (i) hypochromic microcytic anemia, (ii) eosinophilia, and (iii) hypoalbuminemia.

TREATMENT

Hookworm infections

Antiparasitic

- ❑ Antiparasitic drugs like albendazole (400 mg once) or mebendazole (500 mg once) or pyrantel pamoate (11 mg/kg for 3 days) can be given
- ❑ However, due to the widespread use of the drugs, their efficacy is decreased compared to the past. Resistance to albendazole and mebendazole has also been reported

Symptomatic treatment

- ❑ Mild iron-deficiency anemia can often be treated with oral iron and folic acid
- ❑ Severe hookworm disease with protein loss and malabsorption warrants nutritional support and oral or parenteral iron replacement.

Prevention

General preventive measures include:

- ❖ Improved personal hygiene
- ❖ Proper disposal of feces
- ❖ Improved nutrition with dietary iron
- ❖ Treatment of infected persons.

Soil-transmitted Helminth Infections

Soil-transmitted helminth (STH) infections refer to the intestinal worms infecting humans that are transmitted through contaminated soil such as *Ascaris*, *Trichuris* and hookworm.

- ❑ **Global situation:** Worldwide >1.5 billion people (i.e. 24% of the world's population) are infected with STH infection. Infections are widely distributed in tropical and subtropical

Contd...

areas, with the greatest numbers occurring in sub-Saharan Africa, the America, China and East Asia

- ❑ **High-risk group** for contracting GTH infection include preschool children, school-age children, women of child-bearing age (including pregnant women) and adults in certain high-risk occupations such as tea-pickers or miners
- ❑ **Presentation:** Infected children are nutritionally, intellectually and physically impaired and suffer from malabsorption of nutrients, anemia, diarrhea and dysentery, depending upon the type of helminths infected
- ❑ **Deworming:** It is a periodic prophylactic measure to prevent high-risk people from worm infestation; initiated by World Health Organization (WHO)
 - **The National Deworming Day:** Every year, 10th February and 10th August are celebrated as National Deworming Day in India
 - Deworming should be done once in a year if the prevalence of STH infection is >20% in the community and twice a year if >50%
 - In this day, single-dose albendazole (400 mg) is given to all children aged 1–19 years.
- ❑ **Elimination:** WHO aim is to eliminate the morbidity due to STH infection in children by 2030. The global target is kept as <2% of children have STH infections of moderate and heavy intensity by 2030.

STRONGYLOIDIASIS

Strongyloidiasis caused by an intestinal nematode *Strongyloides stercoralis*. It was known as the “military worm” as it was first found in the feces of French soldiers in Cochin-China in 1876. Human infection is mainly caused by *S. stercoralis* and rarely by *S. fuelleborni*.

Epidemiology

Globally over 600 million people are estimated to be infected by *S. stercoralis*. It is distributed in hot, humid tropical areas. It is particularly common in South-east Asia (including India), Sub-Saharan Africa, and South America (Brazil).

Morphology

Similar to other nematodes, *S. stercoralis* exists in three forms: adult, larvae (four stages) and egg.

- ❖ **Adult worm:** Only female worms are seen in the small intestine of man, measures 2–3 mm long. Male worms are free-living present in environment, but not in human intestine
- ❖ **Eggs:** *Strongyloides* are ovoviviparous, i.e. eggs once laid, immediately hatch out to larvae.

Life Cycle (Fig. 46.25)

S. stercoralis involves only one host (man). Rarely, domestic pets are recognized as reservoir of infection. L₃ larva (filariform) is the infective form.

- ❖ **Mode of transmission:** (1) Penetration of skin by the L₃ larva (by walking barefoot). Larva releases hydrolytic

Contd...

enzymes that helps in penetration; (2) Autoinfection (internal autoinfection)

- ❖ **Migratory phase:** Following penetration, the L₃ larvae enter into subcutaneous venules and are carried to lungs through venous circulation. Here, they enter into the alveolar space and migrate up to pharynx and finally by swallowing of sputum, they enter GIT
- ❖ **Intestinal phase:** The L₃ larvae molt twice in the small intestine to develop into adult female, which are then buried in the intestinal mucosa. However, adult males are not found in human intestine
- ❖ **Laying eggs:** The female worms can directly lay eggs without fertilization, by a process called as **parthenogenesis**
- ❖ Being ovoviviparous, eggs immediately hatch out liberating the rhabditiform (L₁) larvae into the intestinal lumen, which are passed in the feces (diagnostic form) or transformed into (L₃) larvae to cause auto infection

Autoinfection: Some times, the L₁ larvae released in the human intestine do not pass in the feces but develop into filariform (L₃) larvae that eventually penetrate the intestinal wall or perianal skin, enter the venous circulation and reach lungs. Autoinfection is responsible for maintaining the infection as long as 30–40 years and can cause disseminated infection.

- ❖ **Development in environment:** In moist and warm soil, the L₁ larvae molt twice to form the L₃ larvae. Then there are two types of development which takes place
 - **Direct cycle:** The L₃ larvae act as the infective form to man. This cycle usually occurs in temperate climate
 - **Indirect development:** The L₃ larvae molt twice to develop into the adult worms (male and female), which fertilize to produce eggs. Eggs immediately hatch out to L₁ larvae which molt twice to form the infective L₃ filariform larvae. This cycle usually occurs in the tropical climate.

Pathogenesis and Clinical Feature

Effect due to Migrating Larva

- ❖ **Asymptomatic infection:** More than 50% of chronically infected people may be asymptomatic
- ❖ **Rashes:** Some people develop recurrent maculopapular or urticarial rashes that involve primarily the buttocks, perineum, and thighs
- ❖ **Cutaneous larva migrans:** Migrating larvae may produce the pathognomonic serpiginous urticarial rash (commonly on thigh) called as **larva currens** (or racing larva), that advances as fast as 10 cm/hour
- ❖ **Pulmonary symptoms** are uncommon compared to ascariasis and hookworm. It occurs only secondary to underlying chronic obstructive lung disease.

Effect due to Adult Worm and Filariform Larva

- ❖ **Mild to moderate worm load:** Adult worms and larvae traversing the upper small bowel mucosa may produce epigastric pain (resembling peptic ulcer), nausea, diarrhea, constipation, and blood loss
- ❖ **Heavy larva load:** Hyperinfection syndrome and disseminated strongyloidiasis are the important complications, observed in heavy larva load (Table 46.5).

Laboratory Diagnosis

Stool Microscopy

Stool microscopy reveals the characteristic rhabditiform larvae (diagnostic form). Concentration techniques can be performed to increase the yield. Single stool examination is less sensitive (30%) due to irregular and low output of larvae. Hence repeated stool examination (four samples) is required (Fig. 46.27).

Note: Sometime, the hookworm eggs may hatch in the stool releasing the rhabditiform larva which has to be differentiated from that of *S. stercoralis* (see Table 46.6, Figs 46.28A and B).

Table 46.5: Complications of strongyloidiasis.

Hyperinfection syndrome

The underlying cause of hyperinfection syndrome is the repeated autoinfection cycles; which leads to generation of large number of filariform larvae. The larvae penetrate the GIT and migrate to various organs.

- **Risk factors:** Impaired host immunity favors larva multiplication
 - Glucocorticoid therapy is the main risk factor
 - Other risk factors include immunosuppressive conditions such as transplant recipients, hematologic malignancies, and intake of immunosuppressive drugs
 - Hyperinfection syndrome is common in patients coinfecting with human T cell lymphotropic virus type (HTLV-1)
 - Coinfection of *Strongyloides* with HIV is common. However, it is not associated with disseminated strongyloidiasis.
- **Features:** Colitis, enteritis, or malabsorption, and in severe cases disseminated strongyloidiasis may develop.
- **Disseminated strongyloidiasis:**
 - Larvae may invade the GIT and migrate to various organs including CNS, peritoneum, liver, and kidneys
 - Moreover, the passage of enteric flora through disrupted mucosa lead to gram-negative bacterial sepsis, pneumonia, or meningitis which may dominate the clinical course
 - CNS invasion, brain abscess and meningitis are common. Larvae can be seen in the CSF occasionally. CSF examination shows pleocytosis, elevated protein, normal glucose and negative for bacterial culture.
- Eosinophilia is often absent in severely infected patients
- The mortality rate in untreated patients approaches 100% and even with treatment it may exceed 25%.

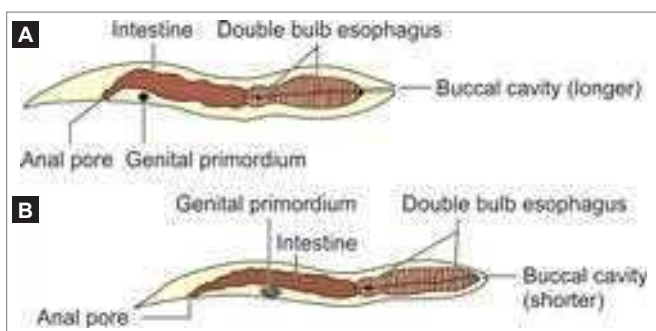


Fig. 46.27: Rhabditiform larva of *Strongyloides stercoralis* (Iodine mount).

Source: Department of Microbiology, Meenakshi Medical College, Chennai; (with permission).

Table 46.6: Differences between rhabditiform larva of hookworm and *Strongyloides stercoralis*.

Rhabditiform larva	Hookworm	<i>Strongyloides</i>
Size	100–150 μm long \times 16 μm width	108–380 μm long \times 14–20 μm width
Mouth (buccal cavity)	Three times longer	Shorter
Genital primordium	Less prominent and small	Prominent and large
Anal pore (subterminal)	80 μm from the posterior end	50 μm from the posterior end



Figs 46.28A and B: Rhabditiform larva (schematic) of: **A.** Hookworm; **B.** *Strongyloides stercoralis*.

Microscopy of Specimens other than Stool

- ❖ **Entero-test:** Sometime duodenal aspirate can be collected by entero-test (Chapter 45) and examined for the presence of larva
- ❖ Disseminated strongyloidiasis can be readily diagnosed by examining stool, sputum, other body fluids, and tissue biopsies, which typically contain high numbers of filariform larvae

Stool Culture

If there is difficulty to differentiate rhabditiform larvae of *Strongyloides* from hookworm, then further confirmation is made by comparing their L₃ larvae; for which stool

culture is performed. When freshly passed stool samples are cultured, L₁ larvae develop into L₃ larvae within 2 days. Various culture techniques used are:

- ❖ Harada-Mori filter paper tube method
- ❖ Baermann funnel technique
- ❖ Agar plate technique (more sensitive)
- ❖ Charcoal culture method.

Serology

ELISA using crude larval antigens has a greater sensitivity (95%) and should be used when microscopic examinations are negative.

- ❖ However, it is less specific because of cross-reactivity with other helminthic infection
- ❖ More so, antibody detection cannot differentiate recent and past infection
- ❖ It can also be used for monitoring the treatment response; however, antibodies disappear slowly following 6–12 months of clinical cure.

LIP assay (Luciferase immunoprecipitation) and **ELISA** have been developed using a 31-kDa recombinant antigen (called NIE antigen).

Coproantigen Detection in Stool

Antigen capture ELISA has been developed using polyclonal rabbit antiserum raised against *Strongyloides ratti* excretory/secretory antigen. It does not show cross-reactivity with other intestinal helminthic infections.

Molecular Diagnosis

Real-time PCR assays are available targeting various genes of *S. stercoralis* such as cytochrome C oxidase gene, 18S rRNA gene in fecal samples. They showed 100% specificity with variable sensitivity.

TREATMENT

Strongyloidiasis

- ❑ Even in the asymptomatic stage, strongyloidiasis must be treated because of the potential for subsequent fatal hyperinfection
- ❑ Ivermectin (200 mg/kg daily for 2 days) is more effective than albendazole (400 mg daily for 3 days)
- ❑ For disseminated strongyloidiasis: Prolonged course of Ivermectin should be given at least 5–7 days or until the parasites are eradicated.

Prevention

Prevention of strongyloidiasis is same as for hookworm and other intestinal nematodes.

Strongyloides fuelleborni

It is a zoonotic parasite affecting monkeys, occasionally causes human infection, called as **swollen belly syndrome**. It is a serious life-threatening condition characterized by diarrhea, respiratory distress and protein losing enteropathy, leading to hypoalbuminemia and edema. It is seen in tropical forest regions of Central and East Africa.

It is diagnosed by detecting the eggs but not larvae in the stool. Thiabendazole is used for treatment.

INTESTINAL NEMATODES OF LOWER ANIMALS THAT RARELY INFECT MAN

There are a number of intestinal nematodes of lower animals that rarely infect man. They are zoonotic, infect various lower animals. Humans are not the natural host for these parasites. Human infections are accidental.

Therefore, they are not able to complete their life cycle in humans as they do in the animal host. Examples include:

- ❖ *Angiostrongylus costaricensis*
- ❖ *Anisakiasis* species
- ❖ *Capillaria philippinensis*—eggs resemble with that of *Trichuris trichiura*
- ❖ *Trichostrongylus* species—also called as pseudo-hookworm as eggs resemble with that hookworm
- ❖ *Oesophagostomum* species
- ❖ *Ternidens deminutus* (or African colon worm).

EXPECTED QUESTIONS

I. Write essay on:

1. A 9-year-old child came to the pediatric OPD with history of passing segments of a worm. The stool examination revealed—round to oval eggs, containing an embryo with three pairs of hooklets, surrounded by radially striated embryophore.
 - a. Identify the disease and the probable causative agent(s).
 - b. Write briefly about the life cycle of the etiological agent(s).
 - c. What are the various diagnostic modalities?
2. A 8-year-old girl came to the pediatric OPD for school health check-up. On examination, she had pallor. Peripheral blood smear revealed microcytic, hypochromic anemia. Stool microscopy (saline mount) showed round to oval non-bile stained egg with segmented ovum (four blastomeres).
 - a. Identify the disease and the causative agent.
 - b. Write briefly about the life cycle of the etiological agent.
 - c. What are the various diagnostic modalities?
 - d. What is deworming strategy?

II. Write short notes on:

1. A young girl presented with history of epigastric pain, nausea, diarrhea and urticarial rash on her lower limbs. Her stool specimen was sent for microscopic examination which revealed motile larvae. Identify the etiological diagnosis and discuss its life cycle.
2. A 3-year-old boy presented with acute abdominal pain, nausea and vomiting. On examination, the child was malnourished. The stool microscopy revealed bile-stained oval eggs with a thick albumin coat.

Identify the disease and draw a labeled diagram of the ova of the causative agent. Mention two complications caused by the adult worm.

3. A 5-year-old girl presented with abdominal discomfort and nausea. Stool examination revealed oval, non-bile stained eggs with polar filaments. Identify the etiological agent and discuss its life cycle.
4. Laboratory diagnosis of Intestinal schistosomiasis.

III. Multiple Choice Questions (MCQs):

1. Which of the following cestode does not have a rostellum and hooks?
 - a. *Echinococcus granulosus*
 - b. *Taenia solium*
 - c. *Taenia saginata*
 - d. *Hymenolepis nana*
2. Which of the following cestode eggs are NOT bile stained?
 - a. *Hymenolepis nana*
 - b. *Diphyllobothrium latum*
 - c. *Echinococcus granulosus*
 - d. *Taenia solium*
3. The larval form of *Hymenolepis nana* is called:
 - a. Hydatid cyst
 - b. Coenurus
 - c. Cysticercus
 - d. Cysticercoid
4. All of the following intestinal nematodes are oviparous, except:
 - a. Roundworm
 - b. *Strongyloides*
 - c. Hookworm
 - d. *Enterobius*
5. Common name of *Trichuris trichiura* is:
 - a. Pinworm
 - b. Roundworm
 - c. Hookworm
 - d. Whipworm

Answers

1. c 2. a 3. d 4. b 5. d



Deworming infants, children and women for better health



World Health Organization

Intestinal parasitic worms (soil-transmitted helminths) are spread through soil, contaminated by human faeces.

Worm infections interfere with children's nutritional uptake and can result in malnourishment, anaemia, and stunted growth.



**Periodic treatment of at-risk populations reduces the intensity of infection.
No individual diagnosis is needed.**

Treatment with what?



Free deworming medicines such as albendazole or mebendazole

Why treat everyone?



To reduce ill health (malnutrition, anaemia, impaired growth); To prevent others from acquiring severe infection

Who should be treated?



Preschool, school-age children and women of reproductive age

Where can treatment be sought?

Schools and community health centres



Global target: To reach 75% of children in need of treatment by 2020

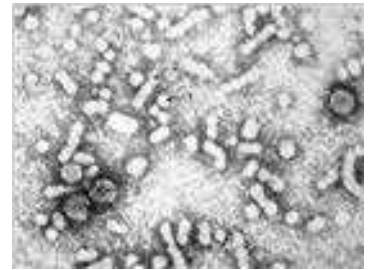
Hepatobiliary System Infections

SECTION

6

SECTION OUTLINE

- 47. Infective Syndromes of Hepatobiliary System and Abdomen
- 48. Viruses Causing Hepatitis
- 49. Parasitic Infections of Hepatobiliary System



**Vaccinate and keep
your life safe from**

Hepatitis B



**Healthcare workers are at highest
risk of contracting Hepatitis B virus**



Infective Syndromes of Hepatobiliary System and Abdomen

CHAPTER 47

CHAPTER PREVIEW

■ Hepatitis

■ Liver Abscess

■ Cholangitis

Major hepatobiliary infections include hepatitis, liver abscess and cholangitis.

HEPATITIS

Hepatitis refers to inflammation of the liver. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or hepatocellular cancer. The causes of hepatitis include both infectious (more common) and non-infectious such as toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases.

The various infectious causes of hepatitis include:

- ❖ **Viral agents causing hepatitis:** They are discussed subsequently in Chapter 48
 - **Hepatitis viruses** (HAV to HEV): They account for the most common cause of hepatitis worldwide
 - **Yellow fever virus:** Causes hepatomegaly, and hemorrhagic fever
 - **Other viruses:** There are many viruses other than hepatitis viruses that can cause sporadic hepatitis, such as cytomegalovirus, Epstein-Barr virus, herpes simplex virus, adenoviruses, rubella virus, hantaviruses, mumps virus and enteroviruses such as Coxsackie viruses. These viruses principally cause infections of other systems and therefore discussed elsewhere.
- ❖ **Parasitic causes of hepatitis:** They are discussed in Chapter 49
 - *Echinococcus granulosus*—infects the liver and forms hydatid cysts in the liver
 - *Trypanosoma cruzi* and *Leishmania* species—may cause hepatomegaly
 - *Entamoeba histolytica*—may cause hepatitis before proceeding into liver abscess
 - *Fasciola hepatica*—can cause hepatomegaly
 - *Clonorchis sinensis*—lives in the bile ducts and causes progressive hepatitis, liver fibrosis and bile duct cancer
 - *Leishmania donovani*, *Schistosoma mansoni* and *S. japonicum*—can cause hepatomegaly
 - *Plasmodium* species: Pass through liver in their life cycle; however hepatic damage does not occur.

- ❖ **Bacterial hepatitis:** Bacterial infection of the liver commonly results in acute hepatitis, granulomatous (or chronic) liver disease or pyogenic liver abscess (described subsequently)

- **Bacterial agents associated with hepatitis** include *Leptospira interrogans*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Bartonella henselae*, *Borrelia burgdorferi*, *Salmonella* species, *Brucella* species and *Campylobacter* species
- **Chronic or granulomatous hepatitis** is seen with infection from mycobacteria species, *Tropheryma whipplei*, *Treponema pallidum*, *Coxiella burnetii*, and *Rickettsia* species.

LIVER ABSCESS

The liver is one of the most common organ to develop visceral abscess. The three major forms of liver abscess, classified by etiology, are as follows:

- ❖ **Pyogenic abscesses** commonly involve enteric bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. These infections are usually polymicrobial in nature (up to 50-80% of the time)
- ❖ **Amoebic abscesses** occur due to *Entamoeba histolytica*, accounts for 10% of cases
- ❖ **Fungal abscesses** most often occur due to *Candida* species, accounts for fewer than 10% of cases.

Pyogenic Liver Abscess

A pyogenic liver abscess is a rare clinical condition characterized by single or multiple collections of pus within the liver; if left untreated, it can be fatal due to development of complications such as peritonitis or sepsis.

- ❖ **Pathogenesis:** Infection of liver may result from local spread from an adjacent site within the peritoneal cavity such as the biliary tract (most common source) or ruptured appendicitis; or it may arise from hematogenous spread of the organism from a distant site. However, in more than 50% of cases, it is cryptogenic (i.e., cause is unknown)
- ❖ **Manifestations:** Fever and right upper quadrant tenderness are the most consistent clinical manifestations

- ❖ **Complications:** Rupture of the liver abscess into adjacent organs or body cavities may result into pleuropulmonary and intra-abdominal abscesses
 - ❖ **Etiology:** Depends on the primary source of infection
 - If infected from the biliary tree, then enteric gram-negative aerobic bacilli (*E. coli* and *Klebsiella*) and enterococci are the common isolates recovered from liver abscess
- Hypervirulent Strains of *K. pneumoniae***
 These strains of *Klebsiella* have been increasingly associated with liver abscess. It is primarily caused by the capsular K1 (or occasionally K2) serotype of *K. pneumoniae* (Chapter 61).
- *Bacteroides fragilis* is the species most frequently isolated from liver abscess if the primary focus is pelvic and other intraperitoneal sources. It is an obligate anaerobic gram-negative bacillus, found as a commensal in human GIT (Chapter 53)
 - If spread by hematogenous source, *S. aureus* or a streptococcal species such as *Streptococcus milleri* group are commonly isolated.
- ❖ **Laboratory diagnosis:** Blood culture is often positive. Culture of the liver aspirate obtained under CT guidance is also useful
 - Increased WBC count, elevated liver enzymes
 - CT scan can be used to locate the abscess.
 - ❖ **Treatment** of liver abscess includes surgical drainage and administration of appropriate systemic antimicrobial therapy, directed at the causative organism.

Amoebic Liver Abscesses

E. histolytica may spread from the large intestine to the liver by either hematogenous route (more common) or direct contagious spread. It is characterized by the presence of anchovy sauce pus in the liver (Chapter 49).

INFECTIONS OF THE BILE DUCT

Cholangitis

Cholangitis refers to the inflammation of the bile duct. The infectious etiology of cholangitis may include bacteria or parasites or viruses.

- ❖ **Bacterial cholangitis** occurs when a microorganism invades the biliary tract and there is associated biliary obstruction (biliary stones); usually polymicrobial in nature. Bacteria that commonly cause cholangitis include *Escherichia coli*, *Klebsiella*, *Enterococcus*, *Enterobacter*, *Pseudomonas*, and anaerobes such as *C. perfringens* and *Bacteroides*
- ❖ **Biliary parasites** cause necrosis, inflammation, fibrosis, strictures, and cholangiectasis of the bile ducts. Common biliary parasites include the trematodes such as *Opisthorchis*, *Clonorchis*, and *Fasciola hepatica*, *Fasciola*

gigantica and cestodes such as *Echinococcus granulosus* and nematode such as *Ascaris*

- ❖ **Viral etiology** of cholangitis may include hepatitis viruses and HIV.

Cholecystitis

Cholecystitis is inflammation of the gallbladder; characterized by symptoms such as right upper abdominal pain, nausea, vomiting, and occasionally fever. Presence of gallstone causes blockade of bile flow, which may be a risk factor for the gallbladder to become infected by bacteria, predominantly *E. coli*, *Klebsiella*, *Streptococcus*, and *Clostridium* species; and viruses such Epstein-Barr virus and HIV.

OTHER INTRA-ABDOMINAL INFECTIONS

Infection of the Peritoneum

Intraperitoneal infections generally arise when the normal anatomic barrier is disrupted.

- ❖ This may result due to variety of causes such as rupture of appendix or diverticulum, weakening of the bowel wall (e.g. in inflammatory bowel disease) or with adjacent organ infection (e.g. pancreatitis)
- ❖ Following anatomical breach, the organisms from the bowel or adjacent organ enter the normally sterile peritoneal space
- ❖ Intraperitoneal infections occur in two stages—peritonitis and abscess formation in the peritoneum and the adjacent organs.

Peritonitis

Peritonitis (inflammation of the peritoneum) is of two types—primary and secondary; both differ in their clinical presentations and etiological agents involved.

Primary (Spontaneous) Bacterial Peritonitis

It usually occurs in conjunction with cirrhosis of the liver or other conditions. There is no apparent primary source of infection.

- ❖ It is mostly caused by enteric gram-negative bacilli such as *E. coli*, however, gram-positive organisms such as streptococci, enterococci, or pneumococci are also associated in some cases
- ❖ It clinically presents with fever (most common), ascites, and abdominal pain.

Secondary Bacterial Peritonitis

In contrast to primary peritonitis, it results from spillage of bacteria from an adjacent intra-abdominal viscus such as intestine.

- ❖ The clinical symptoms may vary according to the primary event—for example, epigastric pain from a ruptured gastric ulcer or right lower quadrant pain in case of appendicitis

- ❖ The organisms implicated are mostly intestinal flora such as enteric gram-negative bacilli (e.g. *E. coli*) and anaerobes (e.g. *Bacteroides fragilis*).

CAPD Peritonitis

Peritonitis may also occur in patients who are undergoing continuous ambulatory peritoneal dialysis (CAPD). Unlike primary and secondary peritonitis, which are caused by endogenous bacteria, CAPD-peritonitis usually involves skin organisms such as *Staphylococcus* species.

Peritoneal Abscess

Peritonitis usually results in gram-negative sepsis; and is highly fatal. In some cases where overt sepsis either does not develop or develops but is not fatal, it can progress further to develop peritoneal abscess. It is more commonly seen with *Bacteroides fragilis* infection (Chapter 53).

Infection of Spleen

Splenic Abscess

Splenic abscesses are much less common than liver abscesses; and mostly develop secondary to haematogenous spread rather than direct contagious spread. Infective endocarditis is the most common primary source and streptococci are the most frequent organism to be recovered from splenic abscess followed by *S. aureus*. Treatment includes splenectomy with adjunctive antibiotics.

Infectious Splenomegaly

Spleen may become immunologically hyperactive in response to the filtering of blood-borne pathogens, which subsequently leads to splenomegaly. It is seen in various infections such as leishmaniasis, malaria, *Schistosoma*

mansoni and *S. japonicum* infection, bacterial endocarditis, infective mononucleosis, HIV, tuberculosis, and also in splenic abscess.

Infection of Pancreas (Pancreatitis)

Majority of the pancreatitis are non-infectious; gallstones and alcoholism being the most common causative agents. About 10% of cases are thought to be caused by infectious microorganisms, which include:

- ❖ **Viruses**—e.g. Mumps, Coxsackie viruses (Coxsackie B4 causes juvenile diabetes), echoviruses, cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, hepatitis viruses, HIV, measles, and rubella virus
- ❖ **Bacteria**—e.g. *Mycoplasma*, *Salmonella*, *Campylobacter* and *M. tuberculosis*
- ❖ **Parasites**—*Ascaris* can cause pancreatitis resulting from the migration of worms in and out of the duodenal papillae. Other parasites include *Clonorchis* species, *P. falciparum*, *Fasciola hepatica*, and hydatid disease
- ❖ **Fungi**—e.g. *Aspergillus* and *Candida*.

Clinical types: Acute pancreatitis may manifest as:

- ❖ Mild interstitial form (80% of cases) or
- ❖ More severe form (necrotizing pancreatitis), seen in 20% of cases. Pancreatic abscess may develop as a late complication of acute necrotizing pancreatitis, occurring more than 4 weeks after the initial attack.

Major manifestations include abdominal pain (cardinal symptom), fever, tachycardia, vomiting, sometimes with anorexia and diarrhea.

TREATMENT

Pancreatitis

Antibiotic is indicated only when bacterial infection is suspected (e.g. abscess). Treatment is based on the culture report of FNAC guided pancreatic specimen.

EXPECTED QUESTIONS

I. Write short notes on:

1. Pyogenic liver abscess.
2. Primary (spontaneous) bacterial peritonitis.

II. Multiple Choice Questions (MCQs):

1. Most common cause of splenic abscess is:

- a. Streptococci
- b. *E. coli*
- c. *Klebsiella*
- d. *Pseudomonas*

2. Hypervirulent strains of *K. pneumoniae* can more frequently cause:

- a. Liver abscess
- b. Spleen abscess
- c. Peritonitis
- d. Appendicitis

3. Continuous ambulatory peritoneal dialysis peritonitis is more frequently caused by:

- a. Staphylococci
- b. *E. coli*
- c. *Klebsiella*
- d. *Pseudomonas*

Answers

1. a 2. a 3. a

CHAPTER PREVIEW

- Hepatitis A
- Hepatitis B
- Hepatitis C
- Hepatitis D
- Hepatitis E
- Yellow Fever

There are a number of viruses that are hepatotropic and can cause hepatitis.

- ❖ **Hepatitis viruses:** Diverse group of viruses, having a common feature of being hepatotropic, to cause viral hepatitis
- ❖ **Yellow fever virus:** They belong to arboviruses, cause hemorrhagic fever and hepatitis
- ❖ **Others:** There are many viruses other than hepatitis viruses that can cause sporadic hepatitis, such as, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, adenoviruses, rubella virus, hantaviruses, mumps virus and enteroviruses such as Coxsackie viruses. These viruses principally cause infections of other systems and therefore discussed elsewhere.

VIRAL HEPATITIS

INTRODUCTION

Hepatitis viruses are heterogeneous group of viruses that are taxonomically diverse (belong to different families) but all are hepatotropic; cause acute inflammation of the liver producing identical histopathologic lesions and similar clinical illness such as fever, nausea, vomiting, and jaundice.

Hepatitis viruses are classified into six types (Table 48.1)—hepatitis A virus to hepatitis G virus except F; abbreviated as HAV, HBV, HCV, HDV, HEV and HGV.

“Hepatitis F” (1994) was proposed for its association with transfusion-associated hepatitis, but further investigations

Table 48.1: Features of hepatitis viruses.

Properties	HAV	HBV	HCV	HDV	HEV
Common name	Infectious hepatitis	Serum hepatitis	Non-A non-B or post-transfusion hepatitis	Delta agent	Non-A non-B enteric transmitted hepatitis
Family	Enterovirus-72 (Picornaviridae)	Hepadnaviridae	Flaviviridae	Unclassified viroid-like	Unclassified Caliciviridae-like
Genus	<i>Hepatovirus</i>	<i>Orthohepadnavirus</i>	<i>Hepacivirus</i>	<i>Deltavirus</i>	<i>Hepevirus</i>
Virion	27 nm, icosahedral	42 nm, spherical	60 nm, spherical	35 nm, spherical	30–32 nm, icosahedral
Envelope	No	Yes (HBsAg)	Yes	Yes (HBsAg)	No
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Stability	Heat and acid-stable	Acid-sensitive	Ether-sensitive, acid-sensitive	Acid-sensitive	Heat-stable
Onset	Abrupt	Insidious	Insidious	Insidious	Abrupt
Age	<ul style="list-style-type: none"> • Children • Young adults 	<ul style="list-style-type: none"> • Young adults • Toddlers and babies 	Any age, but more common in adults	Any age (similar to HBV)	Young adults (20–40 years)
Route	Fecal-oral	<ul style="list-style-type: none"> • Percutaneous (MC) • Sexual • Vertical 	<ul style="list-style-type: none"> • Percutaneous (MC) • Sexual (+/-) • Vertical (+/-) 	<ul style="list-style-type: none"> • Percutaneous (MC) • Sexual (++) • Vertical (+) 	Fecal-oral
IP (days)	15–45 (Average 30)	30–180 (Average 60–90)	15–160 (Average 50)	30–180 (Average 60–90)	14–60 (Average 40)
Fulminant disease	Rare (0.1%)	Rare (0.1–1%)	Rare (0.1%)	Frequent (5–20%)	<ul style="list-style-type: none"> • Usually rare (1–2%) • Pregnancy: 20–40%
Carrier	None	Yes (0.1–30%)	Yes (1.5–3.2%)	Variable	None
Chronicity	None	Occasional (1–10%)	Common (85%)	Common	None
Oncogenic	No	Yes (neonate)	Yes	+/-	No

Contd...

Contd...

Properties	HAV	HBV	HCV	HDV	HEV
Prevalence	High	High	Moderate	Low, regional	Regional
Associated other features	Secondary attack rate 10–20%	HCC, cirrhosis, Autoimmune disorder like AGN, arthritis, PAN	HCC, cirrhosis, Autoimmune disorders like AGN, arthritis, cryoglobulinemia	HCC, cirrhosis, fulminant hepatitis	<ul style="list-style-type: none"> • Secondary attack rate (1–2%) • Rarely seen in western countries
Prognosis	Excellent	Worse with age	Moderate	<ul style="list-style-type: none"> • Acute-good • Chronic-poor 	Good
Prophylaxis	<ul style="list-style-type: none"> • Immunoglobulin • Inactivated vaccine 	<ul style="list-style-type: none"> • HBIG • Recombinant vaccine 	None	HBV vaccine (no vaccine for HBV carriers)	Vaccine (HEV239) (only in China)
Therapy	None	<ul style="list-style-type: none"> • Tenofovir • Telbivudine 	Directly-acting antiviral agents	Interferon ±	None

Abbreviations: MC, most common; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; AGN, acute glomerulonephritis; HBIG, hepatitis B immunoglobulin; IP, incubation period; PAN, polyarteritis nodosa.

failed to confirm the existence of the virus, therefore, it was delisted as a cause for infectious hepatitis.

HEPATITIS A

Hepatitis A is a **vaccine-preventable**, communicable disease of the liver, caused by hepatitis A virus (HAV). Hepatitis A virus belongs to the family Picornaviridae. It was originally designated as “enterovirus 72”, but based on the nucleotide and amino acid sequences, it was later assigned to a new genus, *Hepatovirus* under Picornaviridae family.

Morphology

- ❖ HAV is 27–32 nm in size, spherical particle with icosahedral symmetry, containing a linear ssRNA
- ❖ It has only one serotype, does not cross-react with other hepatitis viruses; however it can be typed into seven genotypes based on the gene sequences.

Mode of Transmission

HAV is transmitted principally by fecal-oral route or by ingestion of contaminated food or water, such as raw or undercooked food and ice or frozen foods.

Epidemiology

Hepatitis A is the most common cause of acute viral hepatitis in children.

- ❖ **Hosts:** Humans are the only host for HAV. However, experimental infection may be induced in chimpanzees
- ❖ **Age:** Children and adolescents (5–14 years of age) are most commonly affected, majority remain **subclinical** (80–95%), but excrete virus in feces for longer time. Adults are more icteric (75–90%) than children with higher mortality rate. Anicteric to icteric cases ratio is about:
 - In children: 12:1
 - In adults: 1:3.
- ❖ **Risk factors:** Poor personal hygiene and overcrowding are the most important risk factors

- In **developing countries** including India with poor personal hygiene and overcrowding, most of the children (90%) are infected with HAV by the age of 10 years. Adults have protective antibodies and are mostly immune to HAV
- However, in **developed countries** with improved hygiene, the incidence is decreasing and there is trend of shift of infection towards the older age.
- ❖ **Outbreaks** are common in summer camps, day care centers, joint families and institutions, neonatal ICUs, and among military troops
- ❖ **Recurrent epidemics** and sudden, explosive epidemics are common and usually result from fecal contamination of a single source (e.g. drinking water, milk or food such as raw vegetables, salad, frozen strawberries, green onions and shellfish). The largest epidemic was reported from Shanghai (1988, >3 lakh cases)
- ❖ **Seasonal incidence:** Though HAV infection is widespread throughout the year, it tends to peak in late rainfall and in early winter
- ❖ **Virus excretion:** Viral excretion in feces may be 2 weeks before to 2 weeks after the appearance of jaundice (however, viremia occurs from –2 weeks to +1 week of jaundice).

Clinical Manifestation

- ❖ Incubation period is about 15–45 days (mean 30 days)
- ❖ Onset is relatively abrupt (subacute)
- ❖ **Clinically**, HAV infection is indistinguishable from other hepatitis viruses; characterized by:
 - Pre-icteric phase (mainly gastrointestinal symptoms such as nausea and vomiting) followed by;
 - Icteric phase or jaundice (dark urine, yellowish sclera and mucus membrane).
- ❖ Complete recovery occurs in most (98%) cases
- ❖ There is no chronic or carriers state
- ❖ **Complications may occur rarely such as:**
 - Fulminant hepatitis; characterized by severe necrosis of hepatocytes

- Relapsing hepatitis; may develop weeks to months after apparent recovery from acute hepatitis
- Cholestatic hepatitis; characterized by protracted cholestatic jaundice and pruritus.

Laboratory Diagnosis

❖ Anti-HAV antibody detection:

- IgM antibodies appear during the acute phase, peak about 2 weeks after the elevation of liver enzymes and disappear within 3–6 months (Fig. 48.1)
- IgG antibodies appear a week after the appearance of IgM and persist for decades

■ Interpretation:

- ◆ Anti-HAV IgM positive—indicates acute infection with HAV
- ◆ Anti-HAV IgG antibody detection in the absence of IgM indicates past infection or recovery
- ◆ ELISA is the method of choice; however many rapid test formats are also available.

❖ **Detection of HAV particles:** HAV appears in stool from -2 to +2 weeks of jaundice. It can also be detected from liver, bile, and blood by immunoelectron microscopy

❖ **HAV antigen detection:** ELISA format is available to detect HAV antigen from stool sample from -2 to +2 weeks of jaundice

❖ **Isolation:** HAV is very difficult to grow in cell line; though various primate cell lines are susceptible. HAV is the only hepatitis virus where isolation has been attempted

❖ **Non-specific findings:** Such as elevated liver enzymes and serum bilirubin level.

- ❖ Hand washing before and after use of toilet
- ❖ Sanitary disposal of infected fecal material by disinfection with 0.5% hypochlorite
- ❖ Purification of drinking water by effective filtration and adequate chlorination (with at least 1 mg/L of residual chlorine)
- ❖ Use of boiled water (boiling for at least 5 minutes) during the outbreaks
- ❖ Vaccination with hepatitis A vaccine (best method of prevention).

Vaccines

The indications to administer HAV vaccine include:

- ❖ All children more than one year of age.
- ❖ Travelers to countries with high or intermediate endemicity of HAV infection
- ❖ Patients with chronic liver disease.

Two types of vaccines are available for human use.

❖ **Formaldehyde inactivated vaccine:** It is prepared from human fetal lung fibroblast cell lines such as MRC-5 and WI 38. It is given to children after 12 months of age. Single dose is administered by intramuscular route (deltoid) followed by booster at 6–12 months gap. Its protective efficacy is about 94%

❖ **Live attenuated vaccine:** It is given as single dose, subcutaneously. It uses H2 and L-A-1 strains of HAV, prepared in human diploid cell line (China).

Both vaccines are highly immunogenic, produce long lasting immunity, possibly life-long.

Human Immunoglobulin (HAV-Ig)

It is extremely useful for post-exposure prophylaxis of **intimate contacts** (household, day care centers) of persons with hepatitis A or to the **travelers**.

- ❖ Dosage of 0.02 mL/kg is recommended which gives protection for about 1–2 months
- ❖ It should be administered as early as possible after exposure (preferably within 2 weeks)
- ❖ However, HAV-Ig is not necessary for those who have already vaccinated, casual contacts (office, factory, school, or hospital), and elderly persons (likely to be immune).

HEPATITIS B

Hepatitis B virus (HBV) is the most widespread and the most important agent among hepatitis viruses. Though it commonly produces an acute self-limiting hepatitis which may be subclinical or symptomatic, it is also capable of causing a range of hepatic complications including chronic hepatitis, fulminant hepatitis, cirrhosis of liver and liver cancer.

- ❖ HBV is the only DNA virus among hepatitis viruses. It was discovered by Blumberg in 1963
- ❖ It belongs to the family Hepadnaviridae, under the genus *Orthohepadnavirus*.

TREATMENT Hepatitis A

There is no specific antiviral drug available against HAV.

Prevention and Containment of Infection

General preventive measures should be attempted to improve hygiene such as:

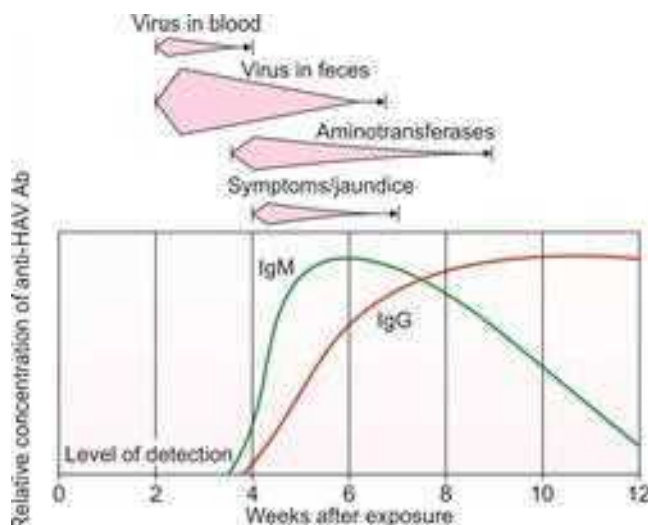


Fig. 48.1: Course of HAV markers with symptoms.

Morphology

Electron microscopy of serum of the patients infected with HBV reveals three morphologic forms (Figs 48.2A and B):

1. **Spherical forms:** Most numerous, small forms measuring 22 nm in diameter. These particles are exclusively made up of HBsAg
2. **Tubular or filamentous forms:** They also have the same diameter, but 200 nm long. They are also exclusively made up of HBsAg
3. **Complete form or Dane particles:** They are less frequently observed. They are larger, 42 nm size spherical virions; made up of:
 - **Outer surface envelope:** HBsAg (Hepatitis B surface antigen)
 - **Inner 27 nm size nucleocapsid:** It consists of core antigen (HBcAg) and pre-core antigen (HBeAg) and partially double stranded DNA.

Viral Antigens

- ❖ **Hepatitis B surface antigen (HBsAg):** HBsAg was previously called **Australia antigen** as it was first observed in the serum of an Australian Aboriginal person (1965)
- ❖ HBsAg is antigenically complex and contains two components—(1) common group reactive antigen ‘a’ epitope and (2) two pairs of type specific antigens d/y and w/r; only one of each pair being present at a time. Thus, there are four subtypes of HBsAg—adw, ayw, adr, and ayr
- ❖ **Hepatitis B core antigen (HBcAg):** HBcAg forms the intracellular core protein. It is not secreted and does not circulate in blood, but can be demonstrated in hepatocytes by immunofluorescence
- ❖ **Hepatitis B precore antigen (HBeAg):** HBeAg is a non-particulate soluble antigen possessing a signal protein which enables it to be secreted. It is therefore present in circulation.

Typing of HBV

Serotypes

HBV is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope protein HBsAg.

- ❖ The immunity is not serotype specific as the dominant ‘a’ antigen is shared by all. But, they are useful for epidemiologic investigations, as all the cases during an epidemic would likely to have the same subtype
- ❖ Serotypes exhibit distinct geographical distribution
 - adw is the predominant subtype in Europe, Australia and America
 - In India, adr is the prevalent subtype in South and East India; whereas ayw is prevalent in Western and Northern India.

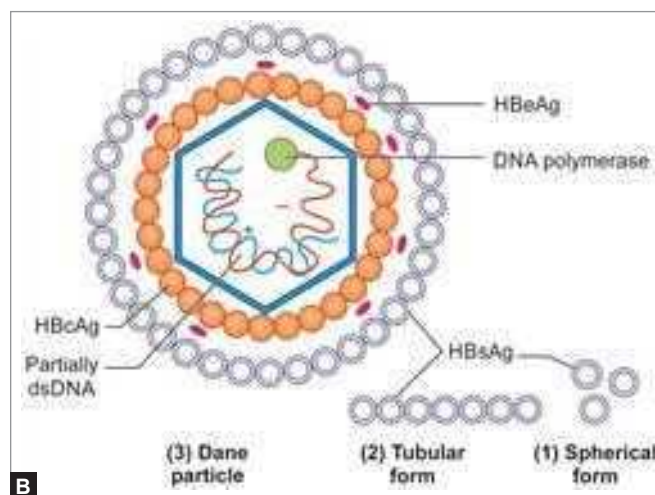
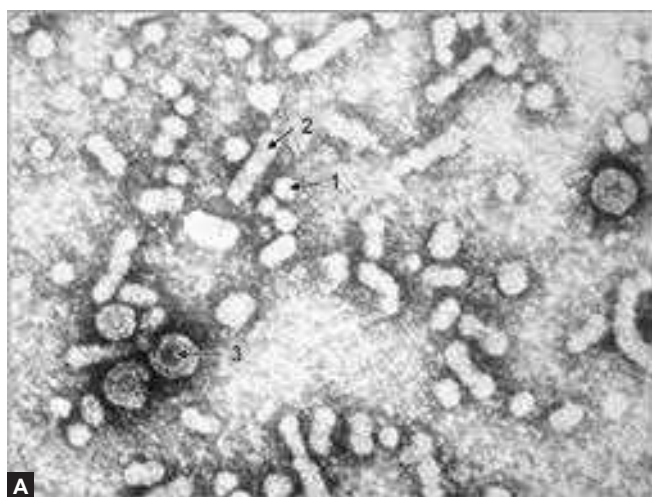
Genotypes

HBV can also be divided into ten genotypes (A-J) according to overall nucleotide sequence variation of the genome. Genotypes D followed by A are prevalent in India.

Viral Genome

Hepatitis B virus genome is compact and consists of four overlapping genes:

1. **S gene:** It has three regions—(i) S gene, (ii) pre-S1 and (iii) pre-S2. They code for surface antigen (HBsAg)
2. **C gene:** It consists of pre-C and C-regions, which code for two nucleocapsid proteins
 - i. Pre-C region—codes for HBeAg
 - ii. C-region—codes for HBcAg.
3. **X gene:** It codes for HBxAg, which can activate the transcription of cellular and viral genes. It may contribute to the pathogenesis of carcinogenesis by binding to p53
4. **P gene:** It is the largest gene and codes for polymerase (P) protein, which has three enzymatic activities—



Figs 48.2A and B: A. Electron microscopic appearance of hepatitis B virus, showing 1-spherical form, 2-tubular form and 3-Dane particle; B. Schematic diagram.

Source: A. ID# 5631/Public Health Image Library/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

(i) DNA polymerase activity, (ii) RNase H activity and (iii) reverse transcriptase activity.

Hepatitis B Virus Mutants

Mutations in various genes of HBV can lead to emergence of mutant strains. Three types of such mutations have been identified, which are as follows:

1. Precore Mutants

Precore mutants have mutations in precore region (which abolishes HBeAg production) or basal core promoter region (down regulates HBeAg production).

- ❖ **Geographical distribution:** Precore mutants have been identified in Mediterranean countries and in Europe
- ❖ Patients infected with precore mutants may be diagnosed late and they tend to have **severe chronic hepatitis** that progresses more rapidly to cirrhosis
- ❖ Such patients do not produce HBeAg, although other viral markers are present as such.

2. Escape Mutants

The escape mutants of HBV have mutations in the S gene (a antigen region) which leads to alteration of HBsAg.

- ❖ Escape mutants are observed in three situations:
 1. Infants born to HBeAg positive mothers
 2. Liver transplant recipients who underwent the procedure for hepatitis B and who were treated with a high-potency human monoclonal anti-HBs preparation
 3. A small proportion of recipients of active and passive immunization, in whom antibody pressure may favor evolutionary change in gene coding *a* antigen.
- ❖ **Underdiagnosed:** Patients infected with escape mutants lack HBsAg and therefore are often underdiagnosed by routine serological test for HBsAg
- ❖ **Vaccine failure:** Escape mutants are capable of causing infection in vaccinated individuals as anti-HBs present in them cannot neutralize these HBsAg negative mutants.

3. YMDD Mutation

Hepatitis B virus infected patients on lamivudine therapy may develop resistance to the drug due to mutation in the YMDD locus present in the HBV reverse transcriptase region of polymerase gene.

Hepatitis B Replication

Replication of HBV is quite different from other DNA viruses as their DNA is partially double-stranded.

- First, the partially dsDNA is converted to fully dsDNA by viral polymerases; which then undergoes transcription to produce mRNA and a pregenomic RNA
- The pregenomic RNA undergoes reverse transcription to form progeny DNA
- HBV mRNA undergoes translation to form viral proteins
- Progeny DNA is encapsidated with viral proteins to form daughter virions.

Transmission

Hepatitis B virus transmission occurs via multiple routes.

- ❖ **Parenteral route:** In developing countries, the most common mode of transmission is via blood and blood products transfusion and needle prick injuries
 - Transmission also occurs by inoculation during surgical or dental procedures or percutaneous inoculation via shared razors and tooth brushes
 - HBV is more infectious than HIV and HCV. As little as 0.00001 mL of blood can be infectious
 - Chance of transmission of HBV following a contaminated needle prick injury is nearly 30% as compared to 3% and 0.3% with HCV and HIV respectively
 - HBV can be transmitted in the absence of visible blood and remains infectious on environmental surfaces for at least 7 days.
- ❖ **Sexual transmission** is found to be the most common route in most developed countries; particularly homosexual males being at higher risk
- ❖ **Vertical (perinatal) transmission:** The spread of infection from HBV carrier mothers to babies appears to be an important mode of transmission particularly in China and Southeast Asia
 - Transmission occurs at any stage; in utero, during delivery (maximum risk) and during breastfeeding
 - Risk is maximum if the mother is HBeAg positive.
- ❖ **Direct skin contact** with infected open skin lesions may transmit the virus, e.g. impetigo (especially in children)
- ❖ Although HBV can survive in mosquitoes; no transmission has been observed.

High-risk groups which are more prone for acquiring infection are:

- ❖ Surgeons, technicians, phlebotomists
- ❖ Paramedical workers
- ❖ Sex workers especially homosexual males
- ❖ Recipients of blood transfusion and organ transplantation
- ❖ Drug addicts.

Epidemiology

Hepatitis B virus infection occurs throughout the world; usually sporadic, but occasional outbreaks can occur in hospitals.

- ❖ **Reservoir of infection:** Humans are the only reservoir of infection, who can be either cases or carriers
 - Cases may be either inapparent or symptomatic
 - Carriers may be temporary (harbor the virus for weeks to months) or persistent/chronic (harbor the virus for >6 months).
- ❖ **Carriers** can also be grouped into:
 - **Simple carriers** (or chronic inactive HBV infection): They are of low infectivity, transmit the virus at a lower rate. They possess low level of HBsAg and no HBeAg
 - **Super carriers** (or Immunotolerant chronic HBV infection): They are highly infectious and transmit

the virus efficiently. They possess higher levels of HBsAg and also have HBeAg, DNA polymerase and HBV DNA.

- ❖ **HBV prevalence:** It is determined based on HBsAg carrier rates. There are three epidemiological patterns observed among various countries:
 1. *Low endemicity:* Carrier rate is less than 2%. It is observed in many countries of American, European regions and Australia. Lowest is recorded for UK and Norway (0.01%)
 2. *Intermediate endemicity:* Carrier rate is between 2 and 8%. It is observed in India, China and many countries of Eastern Mediterranean and Southeast Asian regions
 3. *High endemicity:* Carrier rate is more than 8%. It is observed in many countries of African and Western Pacific regions. Some countries exceed HBV prevalence of >15% such as South Sudan, Kiribati, Swaziland and Solomon Islands.
- ❖ **Situation in the world:** WHO estimates that in 2015, about 257 million people worldwide had HBV infection with a global prevalence of 3.5% and 1.34 million people died from liver cancer and cirrhosis caused by HBV and HCV infections.
 - The African (6.1%) and Western Pacific (6.2%) regions accounted for 68% of global burden
 - 2.7 million persons were coinfecting with HBV and HIV
 - The widespread use of HBV vaccine in infants has led to a considerable reduction in the incidence of new chronic HBV infections.
- ❖ **Situation in India:** India is considered to have an intermediate level of HBV endemicity (4% prevalence); with over 50 million disease burden, which constitutes 11% of the estimated global burden, which is second highest after China (30%)
 - Highest prevalence recorded from Andaman and Arunachal Pradesh
 - Tribal areas, the prevalence is extremely high (19%) than non-tribal populations
 - HBV accounts for 40–50% of hepatocellular carcinoma (HCC) and 10–20% of cirrhosis in India.
- ❖ HBV followed by HCV are the most common cause of:
 - Chronic hepatitis
 - Cirrhosis: HBV accounts for 30% of cirrhosis
 - Hepatocellular carcinoma (HCC): HBV accounts for 45% of HCC
 - Post-transfusion hepatitis: HBV (1:220,000) > HCV (1:18,00,000).
- ❖ **Elimination of viral hepatitis:** The WHO has introduced Global health sector strategy on viral hepatitis (2016–2021) which aims at the elimination of viral hepatitis as a public health threat by 2030 (defined by reducing new infections by 90% and mortality by 65%). **World Hepatitis Day** is celebrated on 28th July every year. Theme observed for the year 2020 is “**Hepatitis-free future**”, with a strong focus on preventing HBV among mothers and newborns
 - ❖ **Resistance:** HBV can be destroyed by hypochlorite and heat (by autoclaving)
 - ❖ **Period of infectivity:** People infected with HBV are said to be infectious as long as the HBsAg is present in blood, i.e. during incubation period (a month before jaundice) up to several months thereafter (occasionally years for chronic carriers)
 - Patients become non-infectious once HBsAg disappears and is replaced by anti-HBs antibody
 - Maximum infectivity is observed when HBeAg is elevated in serum.
 - ❖ **HBV and HIV Co-infection:**
 - The global HBV prevalence in HIV-infected persons is 7.4%. Conversely, about 1% of HBV-infected persons are also infected with HIV. The highest burden (71%) for HIV-HBV coinfection is found in sub-Saharan Africa
 - Although HBV does not alter the progression of HIV, the presence of HIV greatly enhances the risk of developing HBV-associated cirrhosis and liver cancer
 - Antiviral agents such as tenofovir and emtricitabine are effective against HIV and HBV both. Hence they are useful in controlling HIV-HBV coinfection.
 - ❖ **Age:** The outcome (Fig. 48.3) of HBV infection depends on the age. Following HBV infection:
 - Chance of developing acute hepatitis is directly related to the age:
 - ♦ 1% (perinatal)
 - ♦ 10% (early childhood; 1–5 years of age)
 - ♦ 30% (late childhood; after 5 years of age).
 - Chance of developing chronic hepatitis or carrier state is inversely related to age:
 - ♦ 80–90% (perinatal)
 - ♦ 30% (early childhood; 1–5 years of age)
 - ♦ 5% (late childhood; after 5 years of age).
 - **Explanation:** Pathogenesis of HBV infection is immune mediated
 - ♦ Hepatocytes carrying viral antigen are subjected to natural killer cell mediated or CD8 T cell mediated cytotoxicity
 - ♦ Absence of an effective immune system (e.g. infants) leads to carrier state.

Clinical Manifestations

Hepatitis B has an incubation period of about 30–180 days. The onset of infection is slow and insidious.

- ❖ Patients may present with subclinical infection, acute or chronic hepatitis (Fig. 48.3)
- ❖ Clinically, HBV infection is indistinguishable from other hepatitis viruses; characterized by:
 - Pre-icteric phase (predominant gastrointestinal symptoms such as nausea and vomiting) followed by;
 - Icteric phase or jaundice.

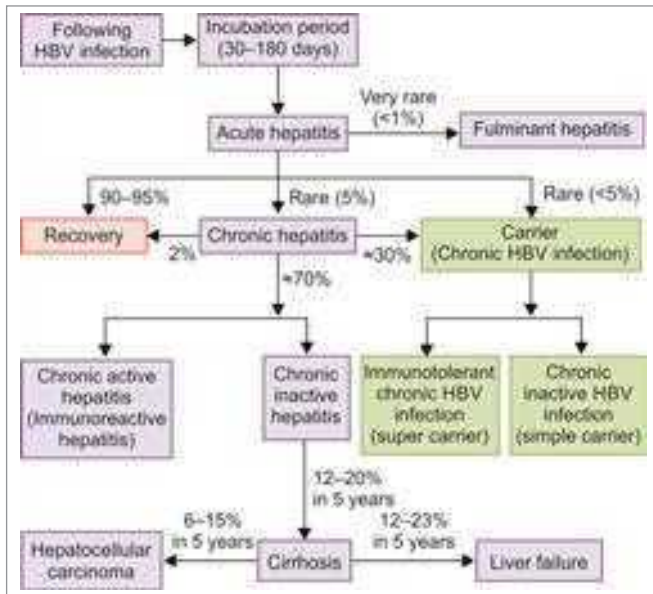


Fig. 48.3: Various outcomes of HBV infection.

- ❖ Clinical outcome may be either development of carrier state or complete recovery
- ❖ **Hepatic complications:** Very few cases may proceed to complications such as fulminant hepatitis or cirrhosis or hepatocellular carcinoma
- ❖ **Extrahepatic complications:** During the prodromal phase, a serum sickness-like syndrome characterized by arthritis, rash, angioedema, and rarely, hematuria and proteinuria may develop in 5–10% of patients. This is due to immune complex deposition.

LABORATORY DIAGNOSIS

Hepatitis B

- ❑ **Antigen markers:** HBsAg, HBeAg and HBcAg
 - **HBsAg:** First marker to appear; elevated in acute, chronic or in carriers
 - **HBeAg:** Indicates active viral replication and high infectivity
 - **HBcAg:** Not detectable in serum; can be detected in hepatocytes by immunofluorescence test
- ❑ **Antibody markers:** Anti-HBs, Anti-HBe and Anti-HBc
 - **Anti-HBc Ab:** IgM indicates acute hepatitis and IgG appears in chronic hepatitis or carriers or after recovery
 - **Anti-HBs Ab:** Marker of recovery or vaccination
 - **Anti-HBe Ab:** Indicates low viral replication and low infectivity
- ❑ **Molecular markers:** HBV DNA detection
 - Indicates active viral replication and high infectivity
 - Viral DNA load helps in monitoring treatment response
- ❑ **Non-specific markers:** Elevated liver enzymes and serum bilirubin; indicates clinically active infection.

Laboratory Diagnosis (Viral Markers of HBV Infection)

Definitive diagnosis of hepatitis B depends on the serological demonstration of the viral markers (Figs 48.3 and 48.4, and Table 48.2).

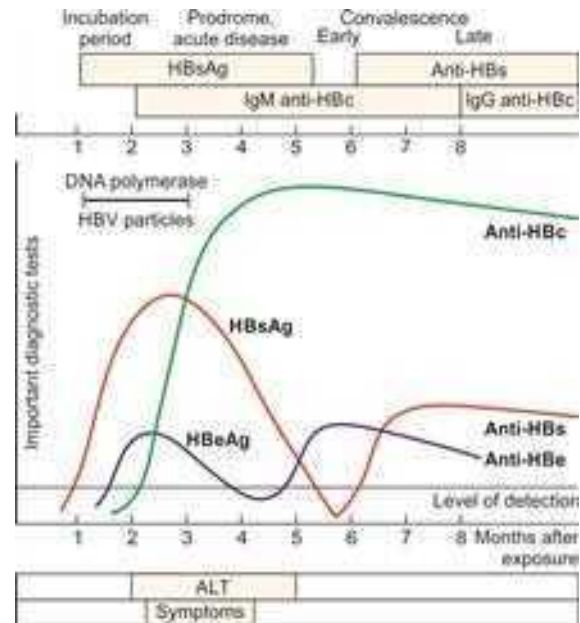


Fig. 48.4: Serological markers of hepatitis B virus in various stages of hepatitis B virus infection.

- ❖ The detection methods available for HBV antigens and antibodies are ELISA, chemiluminescence, ELFA (enzyme-linked fluorescent assay, e.g. Vidas) and rapid methods such as ICT
- ❖ Viral DNA can be detected by PCR (polymerase chain reaction); but quantified by real time PCR
- ❖ HBV does not grow in any conventional culture system.

Hepatitis B Surface Antigen (HBsAg)

HBsAg is the first marker to be elevated following infection; appears within 1–12 weeks (usually between 8 and 12 weeks of infection).

- ❖ It appears during incubation period; 2–6 weeks before the biochemical and clinical evidence of hepatitis
- ❖ Presence of HBsAg indicates onset of infectivity (i.e. patient is capable of transmission of HBV)
- ❖ It remains elevated in the entire duration of acute hepatitis; becomes undetectable 1–2 months after the onset of jaundice. However, it persists rarely beyond 6 months if the disease progresses to chronic hepatitis or carrier state
- ❖ HBsAg is used as an epidemiological marker of hepatitis B infection (i.e. to calculate prevalence of infection).

Hepatitis B Precore Antigen (HBeAg) and HBV DNA

HBeAg and HBV DNA appear concurrently with or shortly after appearance of HBsAg in serum.

- ❖ **They are the markers of:**
 - Active viral replication
 - High viral infectivity (i.e. high transmission rate).
- ❖ However, these markers can be present in either acute, chronic or carrier state; hence, they cannot differentiate between these stages. Their presence just indicates that the virus is actively multiplying, which could be either:

Table 48.2: Interpretation of markers in various stages of hepatitis B infection.

HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Symptoms	Liver enzymes	DNA	Interpretation
+	-	-	-	-	Absent	Normal	+	Early acute hepatitis (incubating)
+	-	IgM	++	-	Present	Elevated	++	Acute hepatitis B, high infectivity
+	-	IgG	++	-	Present	Elevated	++	Chronic hepatitis B, high infectivity (Immunoreactive chronic hepatitis)
+	-	IgG	-	+	Present	Elevated	+	Chronic hepatitis B, low infectivity (Chronic inactive hepatitis)
+	-	IgG	+	-	Absent	Normal	++	Immunotolerant chronic HBV infection (previously called as super carriers)
+	-	IgG	-	+/-	Absent	Normal	+	Chronic inactive HBV infection (previously called as simple carriers)
+	-	IgG	-	-	+/-	Normal/elevated	+	Precore-mutant hepatitis B infection
-	-	IgG	-/+	-/+	+/-	Normal/elevated	+	Escape mutant hepatitis B infection
-	+	IgG	-	+/-	Absent	Normal	-	Recovery
-	+	-	-	-	Absent	Normal	-	Post-vaccination
-	-	IgG (+/-)	-	-	Absent	Normal	+	Occult hepatitis B infection

- Acute active hepatitis
- Chronic active hepatitis
- Or a carrier in whom HBV is actively multiplying and is highly infectious (called **super carriers**).

Hepatitis B Core Antigen (HBcAg)

- ❖ HBcAg is a hidden antigen due to its surrounding HBsAg coat. It is also non-secretory in nature; hence, it cannot be detected in blood
- ❖ However, HBcAg may be detected in hepatocytes by immunofluorescence test.

Anti-HBc IgM (Hepatitis B Core Antibody)

Anti-HBc IgM is the first antibody to be elevated following infection.

- ❖ It appears within first 1–2 weeks after the appearance of HBsAg and lasts for 3–6 months
- ❖ Its presence indicates acute hepatitis B infection
- ❖ It is probably the only marker (sometimes anti-HBcIgG) present during the period between appearance of anti-HBs antibody and disappearance of HBsAg.

Anti-HBc IgG (Hepatitis B Core Antibody)

Anti-HBc IgG appears in late acute stage and remains positive indefinitely whether the patient proceeds to:

- ❖ **Chronic stage** (with persistence of HBsAg, symptomatic and elevated liver enzymes)
- ❖ **Carrier state** (with persistence of HBsAg, but asymptomatic)
- ❖ Recovery (appearance of anti-HBs antibody).

It can also be used as epidemiological marker of HBV infection.

Anti-HBe (Hepatitis B Precore Antibody)

- ❖ Anti-HBe antibodies appear after the clearance of HBeAg and remain elevated for variable period
- ❖ Its presence signifies diminished viral replication and decreased infectivity.

Anti-HBs (Hepatitis B Surface Antibody)

It appears after the clearance of HBsAg and remains elevated indefinitely.

- ❖ Its presence indicates recovery, immunity and non-infectivity (i.e. stoppage of transmission)
- ❖ It is also the only marker of hepatitis B vaccination.

TREATMENT

Hepatitis B

Most acute hepatitis B infections are self-limiting; do not require any specific treatment. In contrast, treatment of chronic hepatitis B has perceived many recent advances. With the advent of newer antiviral drugs, now it is possible to contain the disease.

Indications*

The indications to initiate the treatment for patients with hepatitis B infection are:

- ❑ Acute hepatitis with acute liver failure
- ❑ Chronic active hepatitis (immunoreactive hepatitis, HBeAg positive)
- ❑ Chronic inactive hepatitis (HBeAg negative):
 - HBV DNA >2,000 IU/mL plus ALT ↑ (> normal) plus moderate degree of liver fibrosis
 - HBV DNA >20,000 IU/mL and ALT ↑↑ (>2 times normal); regardless of the degree of liver fibrosis
- ❑ Associated cirrhosis (regardless of ALT level, viral load)
- ❑ Carriers (super or simple) with family history of HCC or cirrhosis
- ❑ Super carriers or immunotolerant hepatitis (here, treatment is indicated if the person's age is >30 years)

Antiviral agents include (also refer Table 48.3):

- ❑ **Antiviral agents** (nucleoside analogues): Tenofovir and telbivudine are the agent of choice currently.
 - Tenofovir and emtricitabine are indicated for HIV-HBV coinfection.
 - Lamivudine, adefovir and entecavir can also be used, but they are less preferred because of high risk of development of resistance.
- ❑ **Pegylated interferon alfa** (used previously; now not in use because of adverse effects)

*Source: EASL guideline 2017 (European Association for the Study of the Liver).

Table 48.3: Treatment options available for chronic hepatitis B infection.

	Interferon alfa	Antiviral agents*
Route and dosage	Subcutaneous, weekly	Oral, daily
Mode	Monotherapy	Monotherapy
Duration	48 weeks	Long-term until HBsAg loss
Side effects	High	Minimal
In decompensated disease, immunocompromised patients	Cannot be given	Can be given
HBV DNA	<2000 IU/mL	Undetectable, i.e. <10 IU/mL**
HBsAg loss or HBeAg seroconversion	Occurs relatively earlier (1 year)	Occurs very slowly
Preference	Was preferred in the past	First choice of treatment currently

*Telbivudine and tenofovir.

**HBV DNA clearance from blood may occur early; however viral DNA may be present in hepatocytes quiescently; which may get activated later. Hence, HBeAg and HBsAg seroconversion (become negative) are considered as more reliable indicators of successful therapy.

Prophylaxis

Active Immunization (Hepatitis B Vaccine)

Hepatitis B vaccine is a recombinant subunit vaccine.

- ❖ The surface antigen (HBsAg) is used as vaccine candidate which is prepared in Baker's yeast by DNA recombinant technology by cloning the S gene into the yeast chromosome
- ❖ **Route of administration:** Vaccine is administered by intramuscular route over deltoid region (in infant- anterolateral thigh)
- ❖ **Dosage:** 10–20 µg/dose (half of the dose is given to children below 10 years)
- ❖ **Schedule:**
 - Recommended schedule for adults: Three doses are given at 0, 1 and 6 months
 - Under national immunization schedule: It is given at 6, 10, 14 weeks (along with DPT vaccine). Additional dose at birth may be given in areas with prevalence of HBV more than 8%
 - Minimum interval between the doses—4 weeks
 - If there is documentaion of partial vaccination (1 or 2 doses): Then do not restart; just complete the vaccine series, regardless of when the last dose was taken.
- ❖ **Marker of protection:** Recipients are said to be protected if they develop seroconversion with an anti-HBsAg antibody titer of ≥ 10 mIU/mL
 - Seroconversion occurs in about 95% of infants, children and young adults. However, among older people (>60 years), the protection is about 65–75%
 - Protection may last for about 30 years or even longer.

- ❖ **Re-vaccination:** If titer remain <10 mIU/mL after first series of vaccination; the HCW is subjected to second series of vaccination (3 doses at 0, 1, 6 months)
- ❖ **Non/low responders:** About 5–10% of individuals do not show seroconversion even after two series of vaccination; i.e., six doses of vaccination. They are called as non-responders. They do not need any further vaccination
- ❖ **Booster doses are not needed:** The health care workers once protected, should not check their titer again or should not take booster vaccines. They remain protective even if the titer falls <10 mIU/mL. This is because the memory cells get stimulated much faster and the titer rises very soon following an exposure to HBV.

Passive Immunization (Hepatitis B Immunoglobulin or HBIG)

- ❖ **Indications:** HBIG is used in the following situations where an **immediate protection** is warranted
 - Acutely exposed to HBsAg positive blood, e.g. surgeons, nurses, laboratory workers
 - Sexual contact of acute hepatitis B patients
 - Neonates borne to hepatitis B carrier mothers
 - Post-liver transplant patients who need protection against HBV infection.
- ❖ **Timing:** Following accidental exposure, HBIG should be started immediately (ideally within hours, but not later than 7 days)
- ❖ **Recommended dose** is 0.06 mL/kg (or 10–12 IU/kg) single dose, given IM
- ❖ HBIG provides a temporary protection for 3–6 months.

Combined Immunization

Combined immunization with **HBIG** and **vaccine** is more efficacious than HBIG alone. It is recommended for **neonates** born to HBV infected mother, where a single injection of 0.5 mL of HBIG is given to the neonate immediately after the birth, followed by full course of vaccine (the first dose being given within 12 hours of birth).

The guideline for post-exposure prophylaxis for hepatitis B is described in detail in Chapter 25.

General Prophylactic Measures

- ❖ Screening of blood bags, semen and organ donors
- ❖ Following safe sex practices (e.g. using condoms, avoiding multiple sex partners)
- ❖ Following safe injection practices—use of the disposable syringes and needles
- ❖ Following safe aseptic surgical practices
- ❖ Health education.

HEPATITIS C

Hepatitis C virus (HCV) is the common cause of post-transfusion hepatitis in developing countries. It was discovered

in 1989 and first labeled as “non-A, non-B hepatitis virus while performing the experiments in chimpanzees.

Morphology

Hepatitis C virus is classified under family Flaviviridae, genus *Hepacivirus*.

- ❖ It is spherical, 60 nm size and enveloped
- ❖ **Nucleic acid:** It contains a positive sense ssRNA
- ❖ **Proteins:** HCV possesses:
 - Three structural proteins: The nucleocapsid core protein C; two envelope glycoproteins (E1 and E2)
 - Six nonstructural (NS) proteins: NS2, NS3, NS4A, NS4B, NS5A, and NS5B
 - One p7 membrane protein: It functions as an ion channel; was earlier considered as NS1.

Genetic Diversity of HCV

Similar to HIV, Hepatitis C virus displays diversity in the RNA genome that occurs because of high rates of mutations seen in the virus which in turn is due to (i) the high replicative activity, and (ii) the lack of proofreading activity of the RNA polymerase.

- ❖ **Genotypes:** HCV is divided into six major genotypes
- ❖ **Subtypes:** Genotypes are further divided into more than 100 subtypes. Within any given patient, the subtypes of HCV circulate as complex closely related viral population known as **quasispecies**
- ❖ The **E2 envelope protein** is the most variable region of the entire HCV genome followed by the non-structural proteins (especially, NS5B encoded RNA polymerase); hence they are more prone to undergo mutations
- ❖ Unfortunately, E2 protein happens to be the target against which most of the neutralizing (protective) antibodies are produced
- ❖ Thus, diversity in the gene coding E2 protein enables the emergent mutant virus strains to escape from host's humoral immunity, which in turn can result in:
 - Establishment of chronic infection
 - Failure of development of effective vaccine.
- ❖ The genotypes also vary from each other in their—
 - Epidemiological distribution:
 - ♦ Genotype 1 and 2 represent the most common variants in Western countries
 - ♦ In India, genotypes 1 and 3 are more prevalent.
 - Susceptibility to antiviral drugs: For example, patients with genotype-1b respond poorly to therapy than other genotypes
 - Duration of treatment needed.

Transmission

Various modes of transmission of HCV are as follows:

- ❖ **Parenteral:** HCV is most commonly transmitted through exposure to infectious blood:
 - Recipients of contaminated blood transfusions, blood products or organ transplantations
 - Contaminated needles and sharps pricks

- Injection drug users.

- ❖ **Vertical transmission** from infected mother to fetus may occur but at much lower rate (6%) than that of HBV (20%)
- ❖ **Sexual transmission** (rare).
Hepatitis C virus **does not spread** through breast milk, food or casual contacts including hugging or kissing.

Clinical Manifestations

Incubation period is about 15–160 days (average 50 days). Following an infection with HCV:

- ❖ **Asymptomatic infection:** Seen in about >75% of cases.
- ❖ **Acute hepatitis:** About 20% of people develop acute hepatitis; characterized by symptoms similar to that of other hepatitis viruses described earlier. Rest patients remain asymptomatic in early stage
- ❖ **Spontaneous clearance:** In about 5–15% of infections, the virus gets cleared spontaneously within 12 weeks
- ❖ **Chronic disease:** About 75–85% directly develop chronic disease; out of which:
 - 60–70% develop chronic hepatitis
 - 5–20% develop cirrhosis
 - 1–5% develop hepatocellular carcinoma (HCV accounts for 25% of total liver cancer).
- ❖ **Extrahepatic manifestations:** Due to deposition of circulating immune complexes (composed of HCV antigens and their antibodies) in extrahepatic sites, various manifestations can set in such as:
 - Mixed cryoglobulinemia
 - Glomerulonephritis
 - Arthritis and joint pain.

Epidemiology

Hepatitis C virus infection occurs worldwide. Every year, 3–4 million people are infected with HCV with more than 3.5 lakhs deaths.

Population prevalence rate:

- ❖ About 3% of the world population has been infected with HCV worldwide with more than 170 million chronic carriers
- ❖ Higher prevalence rates have been documented from Africa (up to 10%) followed by South America and Asia
- ❖ In India, the prevalence of HCV is about 1%.

Laboratory Diagnosis

HCV Antibody Detection Assay (ELISA)

The antibody detection tests have been the standard test for screening and primary testing for HCV diagnosis.

ELISA is the most common platform used; followed by rapid test and chemiluminescence formats.

- ❖ **First and second generation ELISA** were used previously; now obsolete as they are less sensitive
- ❖ **Third generation ELISA:** This has been the standard method for HCV serology. It employs antigens from NS5 region in addition to core, NS3 and NS4 regions

- **Advantages** include: (i) increased sensitivity and specificity (>99%), (ii) not influenced by HCV genotype, (iii) becomes positive in 5 weeks of infection.
- **Disadvantages:** These assays detect IgG antibodies to HCV, hence,
 - ◆ They do not discriminate between active or past infection; for which HCV RNA test is required
 - ◆ Cannot differentiate acute and chronic HCV infection (as IgG appears early and stays long); for which IgG avidity test is performed.

Recombinant Immunoblot Antibody Assay (RIBA)

RIBA was used in the past as a supplementary test to confirm the ELISA result, which works on the principle of western blot; not in use currently.

HCV Core Antigen Assay

Automated quantitative test detecting core antigen has been available recently.

- ❖ This test is less expensive and less time consuming than HCV RNA PCR
- ❖ **Advantages:** Antigen detection assays can be used as an alternative to HCV RNA testing for: (i) diagnosis of active/current infection, (ii) monitoring response to treatment
- ❖ **Disadvantage:** They are less sensitive than RT-PCR; hence not recommended for blood screening purpose.

An **ELISA format** has recently been available that detects simultaneously antigen (capsid Ag) and antibody (to NS3 and NS4 Ag).

Molecular Methods

Real time RT-PCR detecting HCV RNA has been the gold standard method for:

- ❖ Confirmation of active HCV infection: HCV RNA can be detected in blood as early as 2–3 weeks after infection
- ❖ Quantification of HCV RNA for monitoring the response to treatment
- ❖ For determining HCV genotype and subtype (it can also be determined by performing sequence-based method).

Hepatitis C Testing Sequence

CDC recommends a two-step testing: First, anti-HCV antibody test is performed

- ❖ If positive, then test for HCV RNA to identify active infection
- ❖ If negative, then no further action is required, except for immunocompromised patients where HCV RNA should be tested.

Hepatitis C Screening

CDC recommends one-time hepatitis C screening

- ❖ For all aged >18 years and all pregnant women (during each pregnancy) in areas with HCV prevalence (HCV RNA-positivity) is >0.1%
- ❖ For high-risk group, regardless of HCV prevalence—people with HIV, IV drug users, etc.

Biopsy for Staging HCV Infection

It is a 'gold standard' test for staging HCV infection. It is helpful to determine the ideal timing of HCV treatment (in some settings). When biopsy is not obtained, Fibroscan (elastography) is used to assess liver fibrosis.

T TREATMENT

Hepatitis C

The goal of treatment in HCV infection is:

- ❑ To prevent development of complications such as cirrhosis and liver cancer
- ❑ To achieve **sustainable viral response (SVR)** which is defined as undetectable HCV RNA in blood (≤ 15 IU/mL) at the end of treatment.

Interferon alfa plus ribavirin

Earlier, pegylated-interferon (PEG-IFN) alpha plus ribavirin (RBV) was used for treatment of HCV infection. This was associated with high adverse effects with a moderate degree of viral clearance.

Direct-acting antiviral agents (DAAs)

After the discovery of direct-acting antiviral agents (DAAs), the treatment of HCV infection has been revolutionized. DAAs are the now the treatment of choice for HCV infection as they can produce SVR rate up to 90% with minimal side effects.

- ❑ **Three classes of DAAs** are available: NS3/4A (proteases) inhibitors, NS5B (polymerases) inhibitors and NSSA inhibitors (Table 48.4)
- ❑ **Combination therapy:** Currently, the treatment regimen for HCV includes combinations of different DAAs with or without PEG-IFN and ribavirin
 - *Genotype specific:* The drugs to be included in the regimen depends upon the genotype detected. Most DAAs are genotype specific; except sofosbuvir/velpatasvir which are active against all genotypes
 - *Duration:* The duration of treatment regimen is either 12 or 24 weeks; depending upon the HCV genotype and presence or absence of liver cirrhosis.

Predictors of Treatment Response

- ❖ **Genotypes:** HCV genotype 1 and 4 are associated with poor prognosis than other genotypes
- ❖ **Subtype:** 1b shows poor prognosis compared to 1a
- ❖ Stage of disease (presence of cirrhosis) and associated co-infections such as HIV and HBV: associated with poor prognosis
- ❖ **Adherence:** Poor compliance to treatment, is associated with worse prognosis
- ❖ **Viral RNA load:** Higher the viral load (> 800,000 IU/mL), worse is the prognosis
- ❖ **Interleukin 28 B** is a strong inducer of interferon- α . People possessing a subtype of IL 28B (called CC genotype), produce a stronger immune response to HCV infection by inducing IFN- α release; hence have a better outcome
- ❖ **Race:** Caucasians and African Americans lack **CC genotype**, hence show a poor treatment response than that of Asians
- ❖ **Others:** Metabolic disorders such as insulin resistance, obesity metabolic syndrome, steatosis, lack of vitamin D

Table 48.4: Drugs used for hepatitis C virus infection.

Class	Agents
Directly acting antiviral agents (DAAs)	
NS3/4A (proteases) inhibitors	Ends with suffix 'previr' Grazoprevir, Paritaprevir, Simeprevir
NS5B (polymerases) inhibitors	Ends with suffix 'buvir' Dasabuvir, Sofosbuvir
NS5A inhibitors	Ends with suffix 'asvir' Daclatasvir, Ledipasvir, Velpatasvir
Other classes	
Interferons	Pegylated interferon alfa-2a and 2b
Nucleoside inhibitor	Ribavirin

supplement, older age, alcohol consumption—all reduce the chance of responding to HCV therapy.

Prevention

There is no effective vaccine available for HCV. General prophylactic measures are essentially same as that for HBV.

HEPATITIS D

Hepatitis D virus (HDV) is a defective virus; cannot replicate by itself; depends on Hepatitis B virus for its survival.

Morphology

Hepatitis D virus is taxonomically unclassified though resembles viroids. It is small in size (35 nm), consisting of:

- ❖ Circular, negative-sense ssRNA
- ❖ Protein coat is made up of single protein called **hepatitis D antigen (HDAg)**
- ❖ Surrounded by an envelope protein derived from HBsAg from hepatitis B virus; hence, it is called **defective virus**.

Transmission

Transmission is similar to that of HBV and HCV. Parenteral route is the most common mode; followed by sexual and vertical routes.

HDV and HBV Association

The association of HDV with HBV is of two types (Table 48.5):

- ❑ **Co-infection:** It occurs when a person is exposed simultaneously to serum containing both HDV and HBV
 - Hepatitis B virus infection sets in first so that HBsAg becomes available for HDV
 - This is usually a transient and self-limited condition; clinically indistinguishable from acute hepatitis B infection
 - It rarely progresses to chronic stage (1–10%); at a rate similar to that of HBV infection alone
 - Vaccination against hepatitis B can prevent against HDV.
- ❑ **Super-infection:** It occurs when a chronic carrier of HBV is exposed to serum containing HDV. After 30–50 days, this results in disease, which may have two phases:
 - **Acute phase:** This is the first stage, in which HDV replicates actively with high transaminase levels with suppression of HBV

Contd...

- **Chronic phase:** All (100%) patients progress to chronic stage, in which HDV replication decreases, HBV replication increases, transaminase levels fluctuate, and the disease progresses to cirrhosis and hepatocellular carcinoma (HCC). Mortality rate is much higher (>20%). The order of frequency of chronicity of viral hepatitis in decreasing order is: HDV superinfection (100%) > perinatal HBV (90%) > HCV (85%) > HBV and HBV-HDV coinfection (1–10%). However, in terms of absolute number of chronic cases/carriers; HBV accounts for the maximum.

Laboratory Diagnosis

- ❖ **In co-infection:** IgM against both HDAg and HBcAg are elevated, although IgM anti-HDV appears late and is frequently short-lived
- ❖ **In super-infection:** As HBV infection is already established as carrier, IgG anti-HBc will be detected. Anti-HDV would be IgM type initially; but as the patient progresses to chronic state, mixture of IgM and IgG would persist for months or longer
- ❖ Anti-HBc antibody is the key to differentiate between co-infection and super-infection
 - **IgM anti-HBc + IgM anti-HDV:** Indicates co-infection
 - **IgG anti-HBc + mixture of IgM and IgG anti-HDV:** Indicates super-infection.
- ❖ HDV RNA is detectable in the blood and liver just before and in the early days of acute phase of both co-infection and super-infection
- ❖ HBeAg, the marker of active HBV replication may be present in super-infection.

Table 48.5: Differences between Hepatitis D and Hepatitis B virus (HDV-HBV) co-infection and super-infection.

Features	Co-infection	Super-infection
Definition	HBV and HDV infection occurs simultaneously	HDV infection occurs to a carrier of HBV
Patient status	Healthy	HBV carrier
Risk of development of:		
Fulminant disease	More than that of HBV alone	More than that of co-infection
Chronic hepatitis	Rare	Much greater
Cirrhosis	Rare	More
HCC	Rare	More
Mortality	Rare	>20%
Diagnosis	<ul style="list-style-type: none"> • HBsAg • Anti-HBc (IgM) • Anti-HDV (IgM) • HDV RNA 	<ul style="list-style-type: none"> • HBsAg • HBeAg • Anti-HBc (IgG) • Anti-HDV: <ul style="list-style-type: none"> ➤ In acute phase-IgM ➤ In chronic phase-IgG and IgM • HDV RNA

Contd...

Abbreviation: HCC, hepatocellular carcinoma.

Epidemiology

Globally, about 15 million people, i.e., 5% of HBV infected persons are co-infected with HDV.

Hepatitis D virus infection occurs worldwide, but prevalence varies greatly. Surprisingly, HDV is not prevalent in Southeast Asia including India; where HBV carriers are maximum.

Two epidemiologic patterns have been identified:

1. **In endemic areas:** It is transmitted predominantly by non-percutaneous means, especially by close personal contact
2. **In non-endemic areas:** HDV infection is confined to persons frequently exposed to blood and blood products, primarily injection drug users and hemophiliacs who are infected with HBV
 - HDV infection can be introduced into a non-endemic population through IV drug users or by migration of persons from endemic to non-endemic areas
 - Introduction of HDV into non-endemic areas where HBV infection is common may lead to explosive outbreaks of severe hepatitis with high mortality.

TREATMENT

Hepatitis D

Patients with HDV infection can be treated with IFN- α . Treatment for HBV should be continued as described earlier.

Prevention

Vaccination for HBV can also prevent HDV infection. General prophylactic measures are essentially same as that for HBV.

HEPATITIS E

Hepatitis E virus (HEV) causes an enterically transmitted hepatitis primarily occurring in young adults which occurs as epidemics in developing countries.

Morphology

Although HEV resembles caliciviruses, taxonomically, it is distinct from them; hence has been assigned to a unique genus, *Hepevirus*, under the family Hepeviridae.

- ❖ HEV is small (30–32 nm size), non-enveloped with icosahedral symmetry
- ❖ It contains positive-sense, ssRNA and a specific antigen (HEV-Ag)
- ❖ **Genotypes:** HEV has single serotype; however, five genotypes exist in nature, which differ up to 25% in their RNA sequence
 - Only four genotypes have been detected in humans
 - Genotypes 1 and 2 appear to be more virulent
 - Genotypes 3 and 4 are more attenuated and account for subclinical infections.

Clinical Manifestations

Incubation period is about 14–60 days (average 40 days).

- ❖ Most of the patients present as self-limiting acute hepatitis lasting for several weeks followed by complete recovery

- ❖ Fulminant hepatitis may occur rarely in 1–2% of cases; except for the **pregnant women** who are particularly at higher risk (**20%**) of developing fulminant hepatitis
- ❖ There is no chronic infection or carrier state.

Epidemiology

Hepatitis E virus is a zoonotic pathogen affecting various animals such as monkeys, cats, pigs and dogs

- ❖ **Transmission:** It is fecal-orally transmitted via sewage contamination of drinking water or food
- ❖ **Epidemics** of HEV infections have been reported primarily from Asia, Africa and Central America; HEV is the most common cause of acute hepatitis in this zone. WHO estimates that hepatitis E caused approximately 44,000 deaths in 2015; accounting for 3.3% of the mortality due to viral hepatitis
- ❖ Other parts of the world (temperate climate), HEV is uncommon and usually occurs in travelers coming from endemic zone. China reported more than 1 Lakh cases of jaundice during an outbreak in 1986–88
- ❖ **In India,** HEV infection accounts for maximum (30–60%) cases of sporadic acute hepatitis and epidemic hepatitis. The first major epidemic of HEV was reported from New Delhi (1995) where 30,000 people were affected due to sewage contamination of the city's drinking water supply following a flood that occurred in Yamuna river
- ❖ Though it resembles to HAV, the striking features that differentiate HEV from that of HAV are:
 - Secondary attack rate (transmission from infected persons to their close contacts) is rare (1–2%) in HEV, compared to 10–20% in HAV
 - Age: Young adults (20–40 years age) are commonly affected in HEV infection compared to children in HAV infection.

Laboratory Diagnosis

- ❖ **HEV RNA** (by reverse transcriptase PCR) and **HEV virions** (by electron microscopy) can be detected in stool and serum even before the onset of clinical illness
- ❖ **Serum antibody** detection by ELISA:
 - **IgM anti-HEV** appears in serum simultaneously with the increased levels of liver enzymes; indicates acute infection
 - **IgG anti-HEV** replaces IgM in 2 to 4 weeks (once the symptoms resolve) and persists for years; indicates recovery or past infection.

TREATMENT

Hepatitis E

There is no specific antiviral drug available.

Prevention

General measures for prevention and containment of infection are the same as described for HAV. China has produced and licensed first HEV vaccine called '**HEV 239**' using recombinant HEV proteins. However, it is not yet available globally.

HEPATITIS G

Hepatitis G virus (HGV, also referred to as GB virus C) was discovered in 1995.

- ❖ It is related to Hepatitis C virus, belongs to family Flaviviridae, under the genus *Pegivirus*
- ❖ HGV is transmitted by contaminated blood or blood products, or via sexual contact
- ❖ Hepatitis G virus is wrongly named as it is not hepatotropic and does not cause hepatitis. Instead, it replicates in the bone marrow and spleen
- ❖ HGV is associated with acute and chronic liver disease, but it has not been clearly implicated as an etiologic agent of hepatitis
- ❖ It has been classified into six genotypes, each has its own geographical distribution
- ❖ HGV co-infection is observed in 6% of chronic HBV and in 10% of chronic HCV infections
- ❖ **HIV co-infection:** HGV commonly co-infects people infected with HIV (prevalence 35%); but surprisingly this dual infection is **protective against HIV** and patients survive longer.

YELLOW FEVER

INTRODUCTION

Yellow fever is an acute, febrile illness; severe cases are characterized by liver dysfunction which leads to jaundice (hence the name yellow fever), renal dysfunction and hemorrhage, with high mortality.

- ❖ **Taxonomy:** Yellow fever virus is an arbovirus; belongs to family Flaviviridae. It is an enveloped virus, containing ssRNA
- ❖ **Geographical distribution:** Yellow fever is endemic in West Africa and Central South America. It is not found in rest of the World including India
- ❖ **Typing:** At least **seven genotypes** of yellow fever virus have been identified based on genomic sequence, five in Africa and two in South America. However, antigenically it is homogenous, only one serotype is known to exist.

Transmission

- ❖ **Vector:** Humans get the infection by the bite of *Aedes aegypti* or the tiger mosquito
- ❖ **Transmission cycle:** Two major cycles of transmission have been recognized:
 1. **Jungle cycle:** This cycle occurs between monkeys and forest mosquitoes
 2. **Urban cycle:** This cycle occurs between humans and urban mosquitoes (*Aedes aegypti*).

India

Yellow fever has not invaded India yet. However, India has all the potential of developing yellow fever in future

because the vector, *A. aegypti*, is widely distributed here, and India has the tropical climatic condition similar to Africa. Various reasons have been hypothesized to explain the absence of yellow fever in India.

Measures in airport: Government of India has laid down strict guidelines for vigilance and quarantine of the travelers in the international airports

- ❖ Unprotected (i.e. unvaccinated) travelers coming from endemic zone to India will be kept in quarantine for the longest incubation period; i.e. 6 days
- ❖ Breteau index or the *Aedes aegypti* index should be less than one, surrounding 400 meter of an airport.

(Breteau index: No. of containers showing breeding of *Aedes aegypti* larvae/No. of houses surveyed × 1000).

CLINICAL MANIFESTATIONS

Incubation period is about **3–6 days**. Febrile illness occurs in early stage of the disease and characterized by:

- ❖ Presence of fever, chills, headache, dizziness, myalgia, and backache—followed by nausea, vomiting, and relative bradycardia
- ❖ Patient is viremic in this stage and may be a source of infection for mosquitoes.

Severe cases are characterized by:

- ❖ Hemorrhagic manifestations
- ❖ Platelet dysfunction
- ❖ Features of liver involvement (hepatitis):
 - Mid-zonal necrosis and presence of councilman bodies
 - Intranuclear inclusions may be seen inside the hepatocytes called **Torres bodies**
 - Appearance of jaundice.
- ❖ Renal dysfunction
- ❖ Encephalitis occurs very rarely
- ❖ Mortality rate is high (>20%), especially among children and elderly.

LABORATORY DIAGNOSIS

- ❖ **Serology:** IgM ELISA can be done after 3 days of onset of symptoms. It gives false positive result in other flavivirus infections such as dengue, West Nile and Zika, and also in people who are recently vaccinated within 30 days. Hence the positive result should be confirmed with a more specific test (e.g. plaque-reduction neutralization test).
- ❖ **Molecular method:** RT-PCR detecting specific viral RNA (NS5 region) in blood is more confirmatory than serology. It should be performed within 10 days of onset of symptoms, beyond which RNA becomes undetectable in blood.

EPIDEMIOLOGY

It is estimated that about 200,000 cases of yellow fever with 30,000 deaths occur annually worldwide and majority of outbreaks (~90%) occur in Africa.

- ❖ Epidemics usually occur in humid and semi-humid savanna area adjoining a rain forest
- ❖ **Infection to case ratio** ranges from 20:1 in non-outbreak season to 2:1 during epidemic
- ❖ All age groups are susceptible.

TREATMENT**Yellow fever**

Treatment is only by symptomatic care. Preventive measures include vaccination and mosquito control.

YELLOW FEVER 17D VACCINE

It is a live attenuated vaccine, which is prepared in allantoic cavity of chick embryo (hence, it is contraindicated in people having allergy to egg).

- ❖ There is no risk of encephalitis (unlike the previously used mouse brain derived inactivated **Dakar vaccine** which was found to be encephalitogenic)
 - ❖ **In India:** It is prepared in Central Research Institute (CRI), Kasauli
 - ❖ **Strict cold chain** has to be maintained during the transport with a temperature range of -30°C to $+5^{\circ}\text{C}$
- ❖ It is available in **lyophilized form** and has to be reconstituted before use
 - ❖ Reconstitution is usually done with diluents such as **physiological saline**. Once reconstituted, it should be used within $\frac{1}{2}$ hour
 - ❖ **Dosage:** Single dose, given subcutaneously
 - ❖ Vaccine is effective within 7 days of administration, the efficacy lasts for up to 35 years
 - ❖ **Validity of yellow fever vaccine certificate:** Certificate is issued after 10 days of vaccination and renewed (i.e. reimmunization) every 10 years. This is the recommendation followed for international travel
 - ❖ Cholera and yellow fever vaccine interact with each other, hence should not be given together (3 weeks gap to be maintained)
 - ❖ **Contraindications** of yellow fever vaccine include:
 - Children <9 months, (<6 months during epidemic)
 - Pregnancy (except during outbreak)
 - HIV-infected people
 - People with allergy to egg.

EXPECTED QUESTIONS**I. Write essay on:**

1. A 40-year-old male presented with history of loss of appetite, malaise and jaundice of 2 months duration. On examination, there was icterus, hepatomegaly and tenderness in the right hypochondriac region. He gave a history of blood transfusion in the past. On laboratory examination, he was found to be positive for HBsAg.
 - a. What is the most probable etiological diagnosis?
 - b. Discuss in detail about the various laboratory diagnosis of this condition.
 - c. How will you prevent the transmission of this infection?

II. Write short notes on:

1. Yellow fever vaccine.
2. Laboratory diagnosis of hepatitis C virus.

III. Multiple Choice Questions (MCQs):

1. **Perinatal hepatitis B transmission is maximum in:**
 - a. 1st trimester
 - b. 2nd trimester
 - c. 3rd trimester
 - d. During delivery
2. **Which hepatitis virus is associated with highest mortality in pregnancy?**
 - a. Hepatitis A
 - b. Hepatitis B
 - c. Hepatitis C
 - d. Hepatitis E
3. **Hepatitis virus that spreads by fecal-oral route:**
 - a. Hepatitis A
 - b. Hepatitis B
 - c. Hepatitis C
 - d. Hepatitis D
4. **Which is known as Australia antigen?**
 - a. HBsAg
 - b. HBeAg
 - c. HBcAg
 - d. HBV DNA

Answers

1. d 2. d 3. a 4. a

CHAPTER PREVIEW

- Amoebic Liver Abscess
- Human Echinococcosis
- Trematode Infections of Liver
- Toxocariasis
- Rare Parasitic Infections of Liver

Parasites causing hepatobiliary infections include:

- ❖ Protozoa: *Entamoeba histolytica*, causes amoebic liver abscess
- ❖ Cestode: *Echinococcus*, causes human echinococcosis (e.g. hydatid disease)
- ❖ Trematodes such as *Fasciola hepatica*, *F. gigantica*, *Clonorchis*, *Opisthorchis*, *Schistosoma mansoni* and *S. japonicum* (rarely)
- ❖ Nematode: *Toxocara* causing visceral larva migrans.

Apart from this list, there are few other parasitic agents that rarely infect liver (enlisted at the end of this chapter).

AMOEBIC LIVER ABSCESS

Amoebic liver abscess (ALA) is an important cause of space-occupying lesion of the liver, occurs mainly in developing countries; caused by a protozoan parasite, *E. histolytica*.

Pathogenesis

About 2–8% of patients with history of intestinal amoebiasis develop extraintestinal amoebiasis.

- ❖ **Transmission** occurs through ingestion of food and water contaminated with cysts of *E. histolytica*
- ❖ **Attachment:** Cysts develop into trophozoites, which adhere to intestinal mucosa by virtue of Gal/NAG lectin antigen (major virulence factor)
- ❖ **Invasion:** The trophozoites secrete cysteine proteases and hydrolytic enzymes, which help in the invasion of intestinal mucosa
- ❖ **Spread:** In few cases, erosion and necrosis of small intestine are so extensive that the trophozoites gain entry into the portal venous system and carried to extraintestinal sites
- ❖ **Survival:** Resistance to complement-mediated lysis (mediated by Gal/NAG lectin antigen) is a crucial property of *E. histolytica*, critical for its survival in the bloodstream
- ❖ **Liver** is the most common extraintestinal site; followed by lungs, brain, genitourinary tract and spleen

- ❖ **Most common hepatic site** affected is the posterior-superior surface of the right lobe of liver. Abscess is usually single or rarely multiple (Fig. 49.1A)
- ❖ The amoebic trophozoites occlude the hepatic venules; which leads to anoxic necrosis of the hepatocytes
- ❖ **Inflammatory response** surrounding the hepatocytes leads to the formation of abscess
- ❖ **Neutrophils** recruited to the site are lysed by amoebae, leading to release of mediators that contribute to hepatic necrosis
- ❖ **Microscopically**, the abscess wall is comprised of:
 - Inner central zone of necrotic hepatocytes without amoeba
 - Middle zone of degenerative hepatocytes, RBC, few leukocytes and occasionally amoebic trophozoites
 - Outer zone comprised of healthy hepatocytes invaded with amoebic trophozoites.
- ❖ **Anchovy sauce pus:** Liver abscess pus is thick chocolate brown in color. The fluid is acidic and pH 5.2–6.7 and is comprised of necrotic hepatocytes without any pus cells and occasionally amoebic trophozoites (mainly found in last few drops of pus) as amoebae multiply in the wall of abscess (Fig. 49.1B).



Figs 49.1A and B: A. Cross section of the liver showing amoebic liver abscess (right side); B. Anchovy sauce pus aspirated from amoebic liver abscess.

Source: Head, Department of Pathology, Meenakshi Medical College, Chennai (with permission).

Clinical Manifestations

ALA presents with tender hepatomegaly and fever (most consistent features) along with weight loss, sweating and weakness, very rarely jaundice, and cough.

Complications of Amoebic Liver Abscess

With continuous hepatic necrosis, abscess may grow in various direction of the liver discharging the contents into the neighboring organs.

- ❖ Right-sided liver abscess may rupture externally to skin causing skin lesions on the abdominal wall called as **granuloma cutis (amoebiasis cutis)** or rupture into lungs (pulmonary amoebiasis with trophozoites in sputum) or into the right pleura (**amoebic pleuritis**, the most common complication)
- ❖ Rupture of liver abscess below the diaphragm leads to subphrenic abscess and generalized peritonitis
- ❖ Left-sided liver abscess may rupture into the stomach or left pleura or pericardial cavity (**amoebic pericarditis**)
- ❖ Hematogenous spread can occur from liver affecting brain (causing brain abscess and secondary amoebic encephalitis), lungs, spleen and genitourinary organs (causing painful genital ulcers).

Epidemiology

Worldwide, approximately 40–50 million cases of ALA are reported annually, with the majority of infections occurring in developing countries.

- ❖ The highest **prevalence** is found in developing countries in the tropics, particularly in Mexico, India, Central and South America, and tropical areas of Asia and Africa
- ❖ The most common group affected: Young adult males (male to female ratio is 9:1)
- ❖ Risk factors associated with ALA include:
 - Immigrants from endemic areas
 - Crowding and poor hygiene
 - Men who have sex with men (secondary to sexually acquired amoebic colitis)
 - Presence of immunosuppression.

LABORATORY DIAGNOSIS

Amoebic liver abscess

- ❑ **Microscopy**—detects trophozoites (<25% sensitive)
- ❑ **Antigen detection** (in serum, liver pus and saliva)—by ELISA (170-kDa of lectin Ag)
- ❑ **Antibody detection**—ELISA (Ab to 170-kDa lectin Ag)
- ❑ **Molecular diagnosis**—nested multiplex PCR and real-time PCR (detecting 18S rRNA)
- ❑ **Ultrasonography**—detects the site of abscess and its extension

Laboratory Diagnosis of ALA

Microscopy

Microscopy of liver pus can detect trophozoites (but never cyst) with less than 25% sensitivity. However, it confirms the diagnosis.

- ❖ Trophozoites may be found only in the last portion of the aspirated material from the abscess wall, not in the necrotic debris obtained from the center of the abscess
- ❖ Trophozoites measure 15–20 µm, possess a single nucleus; are actively motile, with finger-like pseudopodia (described in Chapter 45, Figure 45.1A)
- ❖ Stool microscopy is not useful.

Antigen Detection

Lectin antigen (170-kDa) is usually absent in stool but can be demonstrated in serum, liver pus and saliva. It has a sensitivity of 70% sensitive, which goes up to 100% when tested before start of treatment.

Antibody Detection

Antibody detection in serum is much more useful in extraintestinal amoebiasis. ELISA is available detecting antibody against 170-kDa lectin antigen. It has sensitivity of 90% and specificity of 85%. It usually becomes negative within 6–12 months of cure.

Molecular Diagnosis

PCR done on amoebic liver pus approaches sensitivity of 100% and specificity of 90–100%. Nested multiplex PCR and real-time PCR can also be done by detecting 18S rRNA.

Imaging Methods

Ultrasonography (USG) of the liver shows the site of the abscess and its extension. **CT and MRI** scan can be done alternatively. USG has a sensitivity of 75–80%, compared to CT scan (88–95%). However, they cannot differentiate ALA from the pyogenic liver abscess.

Nuclear imaging studies is useful for differentiating an amoebic liver abscess from a pyogenic abscess. ALA does not contain leukocytes, they appear as cold lesions, in contrast to pyogenic liver abscesses which appear as hot lesions as they contain leukocytes.

TREATMENT

Amoebic liver abscess

Treatment of ALA consists of a tissue amoebicidal agent (acts on trophozoites in the liver), followed by luminal amoebicidal agent to eradicate the intestinal carriage.

- ❑ **Tissue agents:** Metronidazole (750 mg PO or IV tid for 5–10 days); or tinidazole/ornidazole (2 g PO once); and
- ❑ **Luminal agents:** Such as Iodoquinol (for 20 days) or paromomycin (for 10 days)

Aspiration

Aspiration of the liver abscess content is indicated (1) risk of impending rupture, (2) left lobe liver abscess of >10 cm, (3) no improvement after anti-protozoan therapy for 5–7 days.

Drug Resistance

Metronidazole resistance has been reported in *E. histolytica*; mainly in patients with liver abscess. Multidrug resistance has also been reported against iodoquinol, diloxanide furoate and emetine.

Prevention

Includes avoidance of ingestion of food and water contaminated with human feces and treatment of asymptomatic persons.

HUMAN ECHINOCOCCOSIS

Human echinococcosis is a zoonotic disease that is caused by a cestode of the genus *Echinococcus*. It occurs in 4 forms:

- ❖ Cystic echinococcosis, also known as hydatid disease or hydatidosis, caused by *Echinococcus granulosus*
- ❖ Alveolar echinococcosis, caused by *E. multilocularis*
- ❖ Two forms of neotropical echinococcosis:
 - Polycystic hydatid disease, caused by *E. vogeli*
 - Unicystic hydatid disease, caused by *E. oligarthrus*.

Cystic Echinococcosis

It is caused by *Echinococcus granulosus*; also called as dog tapeworm.

Morphology

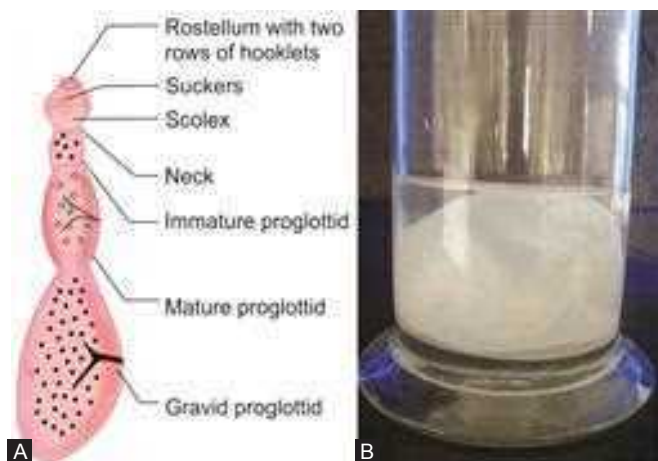
It is a tissue cestode, exists in three morphological forms—adult, larva (called hydatid cyst), and egg.

- ❖ The **adult worm** resides in dog's intestine. It measures 3–6 mm long, consists of head, neck and a strobila or body made up of three proglottids/segments (Fig. 49.2A)
- ❖ The **larval form** is called as hydatid cyst. It is the pathogenic form, forms cystic lesions in liver and other viscera of man and other herbivores (Fig. 49.2B)
- ❖ **Eggs:** *E. granulosus* eggs are morphologically similar to *Taenia* eggs, consists of an embryo with six hooklets surrounded by an embryophore.

Life Cycle (Fig. 49.3)

Host: *E. granulosus* passes its life cycle through two hosts:

1. Definitive host: Dogs and other canine animals



Figs 49.2A and B: *Echinococcus granulosus*: **A.** Adult worm (schematic); **B.** Hydatid cyst, gross specimen.

Source: B. Head, Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka (with permission).

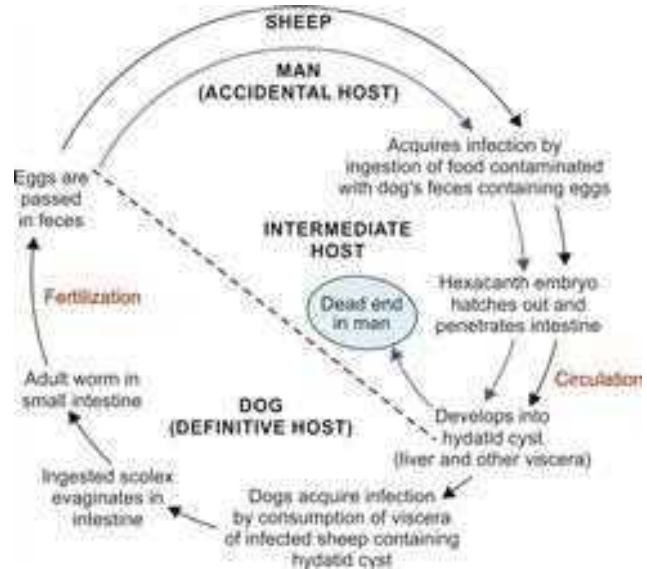


Fig. 49.3: Life cycle of *Echinococcus granulosus*.

2. Intermediate hosts: Sheep and other herbivores are the usual intermediate host. Man acts as an accidental intermediate host (dead end).

Infective form: Eggs are the infective form.

Mode of transmission: Man (and other intermediate hosts) acquires the infection by ingestion of food contaminated with dog's feces containing *E. granulosus* eggs.

Development in Man/Sheep

- ❖ In duodenum, the embryo or oncosphere is released, which penetrates the intestinal wall, enters the portal circulation and carried to the liver (60–70% of cases) or lungs or rarely to other organs
- ❖ Although majority of embryo are destroyed by host immune response, few escape and develop into fluid filled bladder-like cyst called as **hydatid cysts**
- ❖ The hydatid cyst undergoes **maturation** increases in size at a rate of 1 cm/month. Full development takes 10–18 months in sheep
- ❖ This stage is infective to dog and other definitive hosts
- ❖ Man is a dead end, as dogs do not feed on human viscera and therefore the cycle stops there.

Development in Dog

Dog and other canine animals acquire infection by consumption of the contaminated viscera of intermediate hosts (sheep) containing mature hydatid cysts.

The hydatid cyst (larva) transforms into adult worm in dog's intestine. The adult worms sexually mature, self-fertilize to produce eggs which are passed in feces and are infective to man.

Pathogenicity

Pathogenicity is related to the deposition of the hydatid cysts (larval form of the parasite) in various organs.

Hydatid cyst (Figs 49.2B and 49.5A)

It is a fluid-filled bladder-like cyst; unilocular, subspherical in shape and average size measures 5–8 cm (from few mm to >30 cm).

- ❖ **Cyst wall** consists of three layers: outer pericyst (host derived), middle ectocyst and inner endocyst
- ❖ **Brood capsule:** The inner side of the endocyst gives rise to brood capsule, and also secretes the hydatid fluid. The brood capsule contains number of protoscolices (future head)
- ❖ **Hydatid fluid:** It is clear, pale yellow colored fluid, which is antigenic, toxic and anaphylactic
- ❖ **Hydatid sand:** Some of the brood capsules and protoscolices break off and get deposited at the bottom as hydatid sand
- ❖ **Fate:** The hydatid cyst may undergo—(i) spontaneous resolution, or (ii) rupture of the cyst, which may lead to either formation of secondary cysts (carried to other organs) or anaphylactic reaction to the hydatid fluid antigens.

Clinical Features

Infection usually occurs in childhood but gets manifested in adult life.

- ❖ **Site:** Most common site of the cyst is liver (60–70%, right lobe) or lung (20%), followed by kidney, muscle, spleen, soft tissue, brain, bone and others
- ❖ **Asymptomatic:** The cysts grow up to 5–10 cm in size within the first year and can survive for years or even decades, without any symptoms
- ❖ **Symptoms:** Few patients develop symptoms which may be due to:
 - *Pressure effect of the enlarging cyst:* Leads to palpable abdominal mass, hepatomegaly, abdominal tenderness, portal hypertension and ascites
 - *Obstruction:* Daughter cysts may erode into the biliary tree or a bronchus and enter into the lumen to cause cholestasis, cholangitis, and dyspnea
 - *Secondary bacterial infection,* causing pyogenic abscess in liver
 - *Anaphylactic reactions:* Cyst leakage or rupture may be associated with a severe allergic reaction to hydatid fluid antigens; leading to hypotension, syncope and fever.
- ❖ **Outcome of the disease:** It depends on the cyst size and location
- ❖ **Younger children** are more associated with extrahepatic cysts in lungs, brain and orbital sites and multi-organ involvement
- ❖ **Allergy:** Patient sometimes develops hypersensitivity (atopy and anaphylaxis); which is attributed to release of various allergens from the cyst such as 12-kDa AgB (protease inhibitor), Ag5 serine protease, etc.

Epidemiology

E. granulosus is worldwide in distribution.

- ❖ **World:** Higher incidence has been reported from Central Asia (>10 per 1 Lakh population); which may be up to 27 per 1 lakh population in Tajikistan
- ❖ **India:** Hydatid disease has been reported from various places in India like Andhra Pradesh, Tamil Nadu, Chandigarh, Kashmir, Maharashtra and West Bengal
- ❖ **Genotypes:** *E. granulosus* comprises of 10 genotypes; which differ in their intermediate host, and geographic distribution. Genotypes G1–G3 cause 88% of human cases. In India, G1 (sheep strain) is more common, followed by G5 (cattle strain).

LABORATORY DIAGNOSIS**Hydatid disease**

- ❑ **Hydatid fluid microscopy** (direct mount or staining with acid-fast stain)—detects brood capsules and protoscolices
- ❑ **Histological examination** (H & E)—demonstrates cyst wall and attached brood capsules
- ❑ **Antibody detection**—ELISA (using B2t antigen), DIGFA (dot immunogold filtration assay) and western blot
- ❑ **Imaging methods**—X-ray, USG (demonstrates Water lily sign), CT scan, MRI
- ❑ **Molecular methods**—PCR, PCR-RFLP and molecular typing (10 genotypes, most common in India is type 1)
- ❑ **Skin test** (Casoni test)—demonstrates type I hypersensitivity reaction (obsolete now).

Laboratory Diagnosis**Hydatid Fluid Microscopy**

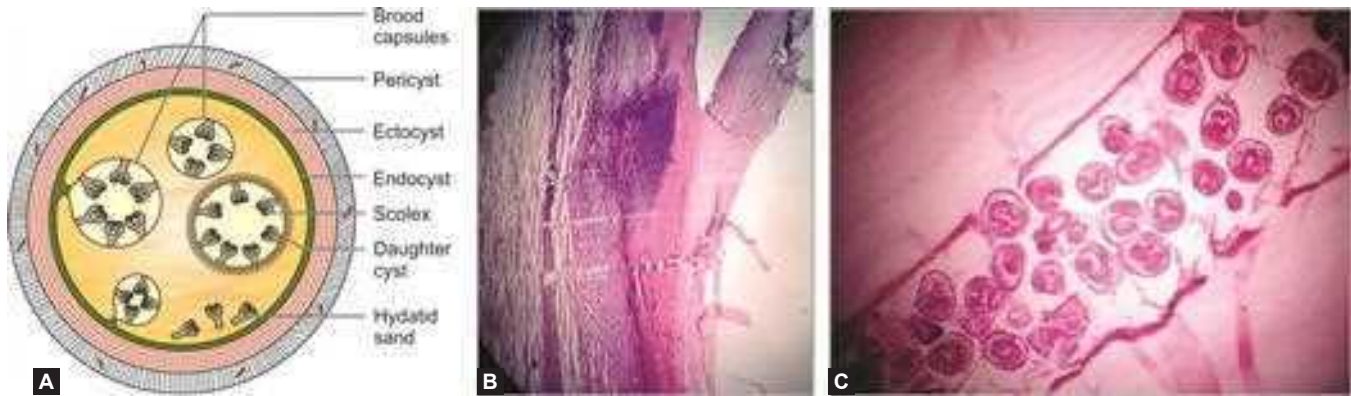
After surgical removal of the cyst (Fig. 49.4), the hydatid fluid is aspirated and examined by direct microscopy or staining with acid-fast stain for presence of hydatid sand.

- ❖ Diagnostic aspiration is not usually recommended because of the risk of fluid leakage which may lead to anaphylaxis or dissemination of infection



Fig. 49.4: Surgically resected hydatid cyst from liver.

Source: Head, Department of Pathology, Meenakshi Medical College, Chennai (with permission).



Figs 49.5A to C: Microscopy of hydatid cyst: **A.** Schematic; **B and C.** Histopathological section (hematoxylin and eosin stain) showing (B) all three layers of cyst wall—pericyst, ectocyst and endocyst, (C) endocyst with attached brood capsules.

Source: B and C. Head, Department of Pathology, Meenakshi Medical College, Chennai (with permission).

- ❖ Drop of centrifuged fluid is placed between two slides and the slides are rubbed over the fluid
- ❖ Hydatid sand is felt as grating of the sand grains in between the slides. The hydatid sand comprises of protoscolices and brood capsules.

Histological Examination

Surgically removed cysts (Fig. 49.4) can be subjected to histopathological stains like Giemsa, hematoxylin and eosin (H & E) and periodic acid–Schiff (PAS) stain to demonstrate the three layers of the cyst wall and attached brood capsules (Figs 49.5A to C).

Antibody Detection

Serodiagnosis of hydatid disease follows a two-step approach; first, a more sensitive test (for screening) such as ELISA or DIGFA (dot immunogold filtration assay) is carried out. If found positive, should be confirmed with more specific test such as immunoblot.

- ❖ **ELISA** using crude *E. granulosus* cyst fluid antigen or recombinant B2t antigen are available. The later shows better results. It is also used as an indicator of cure in surgically treated patients
- ❖ **DIGFA:** Dot immunogold filtration assay (DIGFA) can simultaneously detect serum antibodies against four native antigens; three from *E. granulosus* (cyst fluid, AgB and protoscolex antigens) and one from *E. multilocularis* (Em2 antigen)
- ❖ **Immunoblot (Western blot):** Immunoblot is the most specific serological method; detects antibodies to hydatid cyst fluid antigen or antigen B fragment.

Antibody methods are useful for sero-epidemiological study but cannot differentiate recent and past infection. **Antigen B** is the antigen of choice used for sero-epidemiological study for the detection of antibody.

Patients with liver cysts show better results than extrahepatic cysts. Pulmonary cysts do not produce detectable antibodies. Alveolar echinococcosis shows better results than cystic echinococcosis.

Imaging Methods

Imaging methods play an important role as they are non-invasive methods, which can detect the cysts incidentally in asymptomatic individuals and in seronegative cases.

- ❖ **X-rays:** It is simple, inexpensive, yet useful technique to detect hepatomegaly and calcified cysts and cysts in lungs
- ❖ **Ultrasound (USG):** It is the imaging method of choice because of its low cost and high diagnostic accuracy of 90%
 - It detects both single and multiple cysts
 - **WHO classification of USG imaging:** WHO has classified the USG images of cysts into six types according to its activity (CL and CE1 to CE5) (Table 49.1). This is useful in determining whether the cyst is active or not
 - USG is used to monitor the response to treatment
 - It is also used for epidemiological studies to detect the prevalence of hydatid cyst in population.

Table 49.1: WHO international classification of ultrasound images of hydatid cyst.

Types	Activity	USG finding
CL	Active	Cystic lesion and no visible cyst wall
CE1	Active	Visible cyst wall and internal echoes (snowflake sign)
CE2	Active	Visible cyst wall and internal septation (honeycomb appearance)
CE3	Transitional	Have detached laminar membranes or may be partially collapsed, and floating within the cyst cavity (known as Water lily sign)
CE4	Inactive	Non-homogeneous mass
CE5	Inactive	Cyst with a thick calcified wall

Abbreviations: WHO: World Health Organization; CE- cystic echinococcosis.
Note: This classification is also applicable for CT scan and MRI images.

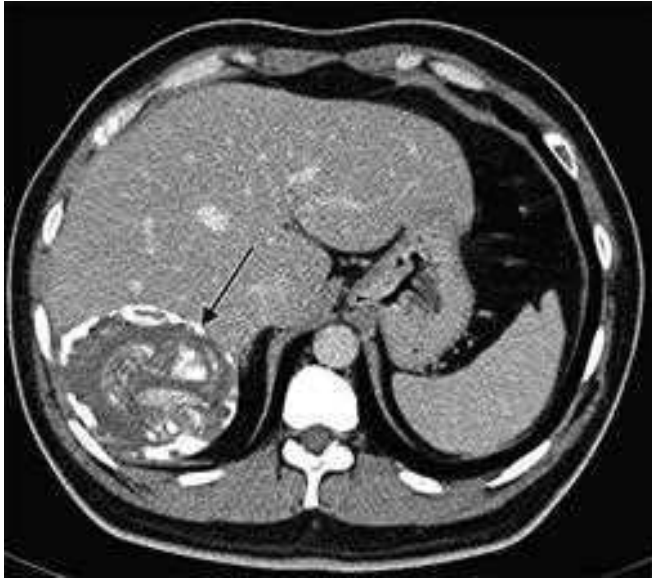


Fig. 49.6: CT scan showing calcified hydatid cyst in the liver.

Source: Dr A Subathra, Department of Radiodiagnosis, JIPMER, Puducherry (with permission).

- ❖ **Computed tomography (CT scan):** It can detect 90–100% of cases (Fig. 49.6)
 - It detects more accurately the number, location of the cyst and the complications
 - It is superior to detect the calcified lesions
 - It is superior to USG to detect smaller cysts, extrahepatic cysts and to differentiate hydatid cyst from other cystic lesions
 - CT scan can also be used as a prognostic marker.
- ❖ **Magnetic resonance imaging (MRI):** It has a higher contrast resolution, which makes cysts clearer. It can be used as an alternate to CT scan. However, it poorly detects the calcified cysts.

Molecular Methods

- ❖ PCR targeting mitochondrial DNA has been developed
- ❖ PCR-RFLP can be used to detect genotypes of *E. granulosus*.

Skin Test (Casoni Test)

It is an immediate hypersensitivity reaction (wheal and flare), which develops following injection of hydatid fluid antigens. It was developed by Casoni in 1911. Now this test is obsolete.

TREATMENT

Hydatid disease

Therapy for cystic echinococcosis is based on viability, size and location of the cyst; guided by USG and overall health of the patient.

PAIR (puncture, aspiration, injection and re-aspiration)

It is an alternative method recommended instead of surgery. It involves four basic steps:

1. Percutaneous puncture of the cyst
2. Aspiration of 10–15 mL of cyst fluid

Contd...

TREATMENT

Hydatid disease

3. Infusion of scolicidal agents like hypertonic saline, cetrimide, or ethanol
 4. Re-aspiration of the fluid after 5 minutes
- PAIR claims higher cure rate, less recurrence rate, less complications and hospitalization compared to surgery
PAIR is recommended for a single hepatic cyst (CE1 lesion and uncomplicated CE3 lesion)
PAIR is contraindicated for:
- ❑ Superficially located cysts (because of the risk of rupture)
 - ❑ CE2: Cyst with multiple thick internal septal division (honeycomb appearance)
 - ❑ Inaccessible cyst or extrahepatic cysts
 - ❑ Cysts communicating to the biliary tree
 - ❑ CE4 and CE5 lesions: These are inactive lesions; should be managed with observation only

Surgery

- ❑ Though surgery is the definitive method of treatment, it should be reserved for:
 - Cases where PAIR is contraindicated or refractory
 - Secondary bacterial infection
 - Advanced disease
- ❑ Disadvantages of surgery are high recurrence rate (2–25%) and postoperative complications (10–25%)
- ❑ Preoperative use of albendazole is effective in reducing size and to prevent recurrence

Antiparasitic agents

Albendazole is the drug of choice, given to prevent recurrence and to reduce the size of the cyst before surgery or PAIR and is given at 15 mg/kg daily in two divided doses; 1 week before to 4 weeks after the procedure

- ❑ Complicated and multivesicular cysts may require longer duration
- ❑ Pulmonary cyst, preoperative albendazole should be avoided; praziquantel is given alternatively

Percutaneous thermal ablation

It is a noninvasive method, involves percutaneous radiofrequency ablation of the germinal layer of the cysts.

Prevention

Cystic echinococcosis can be prevented by:

- ❖ Administering praziquantel to infected dogs
- ❖ To improve personal hygiene to reduce contamination of food and water with dog's feces
- ❖ Vaccinating the sheep
- ❖ Limitation of stray dog population.

Alveolar Echinococcosis

Echinococcus multilocularis is the agent of alveolar hydatid disease.

- ❖ **Life cycle:** Life cycle is similar to that of *E. granulosus*. Only the hosts are different
 - Definitive host: Foxes and wolves (and also dogs and cats)
 - Intermediate hosts: Small wild rodents like squirrels, voles, mice, etc. Man is an accidental intermediate host.
- ❖ **Clinical features:** It produces alveolar (or multilocular) hydatid disease. So named because the cysts have

Contd...

multiple locules resembling lung alveoli. The cysts are usually sterile, do not produce brood capsule and protoscolices

- **Liver** is the most common organ affected (98% of cases)
- **Symptoms** developed are similar to that of *E. granulosus* such as hepatomegaly and portal hypertension
- Cyst has an ability to migrate rapidly to other organs mimicking a malignant tumor. However, there is no malignant potential. The rapid invasion is due to a *surface protein 14-3-3* found on the cyst wall.
- ❖ **Geographical distribution:** This disease is found more frequently in Russia, Kazakhstan, China, South-Central Europe and North America
- ❖ **Laboratory diagnosis:** It is similar to as described for cystic echinococcosis
 - *Imaging methods* can detect the number and size of the cyst, extension of the lesion
 - *Antibody detection tests:* Different ELISA formats are available using cyst fluid antigen, recombinant Em-10 and Em-18 antigens. These tests are sensitive but gives false positive results, in cross-reacting *E. granulosus* infection
 - *Histopathology:* Detects the multi-loculated sterile cyst and associated necrosis
 - *Molecular method:* PCR can differentiate various *Echinococcus* species.
- ❖ **Treatment:** The treatment modalities are as described for *E. granulosus*.

Neotropical Echinococcosis

It comprises of two forms—polycystic hydatid disease (caused by *E. vogeli*) and unicystic hydatid disease (caused by *E. oligarthrus*).

- ❖ **Host:** Wild felids like wild cats, jaguars and pumas (*E. oligarthrus*) or bush dogs (*E. vogeli*) are the definitive host, whereas rodents are the intermediate host
- ❖ **Clinical features:** *E. vogeli* infects most commonly liver (80%) followed by lungs and other viscera. Symptoms are similar to cystic echinococcosis
- ❖ **Epidemiology:** Till date, over 200 cases of polycystic hydatid disease have been reported, mainly from South America. Only three cases of *E. oligarthrus* are reported so far
- ❖ Laboratory diagnosis and treatment are similar to that of *E. granulosus*.

TREMATODE INFECTIONS OF LIVER

Fasciola, *Clonorchis*, *Opisthorchis* are together called as liver flukes. *Fasciola* infect liver and bile duct, whereas the others infect only the bile duct.

Life Cycle

Life cycle of liver flukes is similar to as described for other trematodes, except schistosomes (Chapter 46, Figure 46.12).

- ❖ **Morphology:** Similar to other trematodes, they have adult worm (leaf-like), operculated eggs, and larvae (in five stages)
- ❖ **Hosts:** Liver flukes have three hosts
 - **Definitive host:** Humans are the definitive host. Other animals can also act as definitive host such as—sheep for *F. hepatica* (also called as sheep liver fluke) and dogs and cats for *Clonorchis* and *Opisthorchis*
 - **Intermediate hosts:** Snails are the first intermediate host, whereas the second intermediate host is aquatic plant (for *Fasciola*) and cray fish (for *Clonorchis*, and *Opisthorchis*)
- ❖ **Transmission:** Man gets infection by ingestion of second intermediate host carrying metacercaria larvae (infective form)
- ❖ The larvae excyst and penetrate through the intestinal wall to migrate to their habitat (liver or bile duct), where they develop into adult worms
- ❖ Adult worms undergo fertilization to produce eggs. Eggs are passed from the bile duct or liver to intestine and excreted in feces (diagnostic form).

Fascioliasis

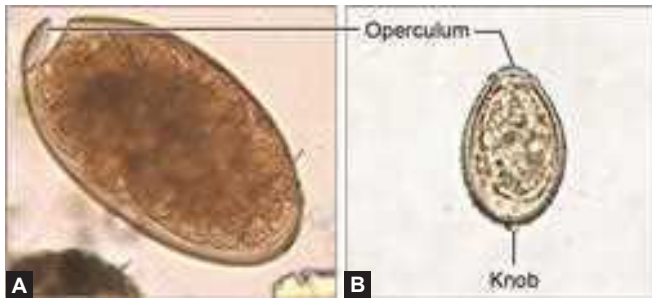
Fascioliasis (caused by *Fasciola hepatica*), although reported worldwide is particularly endemic in sheep-raising countries such as Peru, Bolivia, and Chile. In India, it is extremely rare.

Clinical Features

- ❖ **Liver:** Migration of metacercaria larvae into liver causes fever, right upper quadrant pain, hepatomegaly and eosinophilia. Liver parenchyma is inflamed with formation of multiple subcapsular abscesses (called as **liver rot**)
- ❖ **Bile duct:** The adult worm can cause obstruction of the bile duct and dilatation of the biliary tract and biliary cirrhosis. It does not cause malignancies
- ❖ **Halzoun or Marrara syndrome:** In endemic areas (e.g. Lebanon or Sudan) where uncooked goat and sheep livers may be eaten, the adult worms may attach to the pharyngeal wall; causing severe pharyngitis and laryngeal edema.

Laboratory Diagnosis

- ❖ **Stool microscopy** reveals characteristic operculated eggs, measuring 130–150 μm \times 63–90 μm in size (Fig. 49.7A). Concentration techniques (by sedimentation methods) can be followed to increase the sensitivity. Floatation methods are not useful. Egg of *F. hepatica* is similar to that of *Fasciolopsis buski*
- ❖ **Antibody detection:** ELISA or western blot are available to detect serum antibodies against excretion secretion antigen. It helps in the early diagnosis before the eggs are detected in stool, useful for the seroepidemiological study and to monitor the response to treatment



Figs 49.7A and B: Saline mount showing operculated eggs of: **A.** *Fasciola hepatica*; **B.** *Clonorchis* or *Opisthorchis*.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Molecular methods:** Various PCR-based methods are available to detect *F. hepatica* specific genes in stool specimens
- ❖ **Imaging methods** like ultrasound, CT scan or MRI can be employed to detect the lesions in the liver.

Fasciola gigantica

F. gigantica is closely related to *F. hepatica*. It is a common parasite of herbivores like cattle; human infection is rare. Eggs of *F. gigantica* are morphologically similar to that of *F. hepatica* and but larger in size. Rest of life cycle, clinical feature, laboratory diagnosis and treatment are similar to that of *F. gigantica*.

Clonorchiasis and Opisthorchiasis

Clonorchis sinensis (called as Chinese or oriental liver fluke) is found primarily in Eastern Asia like China, Korea, Japan and Malaysia.

Opisthorchis viverrini has been reported from Southeast Asia, mainly from Laos, Thailand and Cambodia. In India, they are not reported yet. *Opisthorchis felineus* (cat liver fluke) is another species, that is limited to Central and Eastern Europe, Russia and Kazakhstan.

Clinical Features

In chronic infection with heavy worm burden, they cause mechanical obstruction of the bile duct and irritation due to toxin released by the flukes leads to cholangitis, dilatation of the bile duct, marked ductal epithelial hyperplasia, and fibrosis leading to cholangiocarcinoma (bile duct carcinoma).

- ❖ They inhibit tumor suppressor genes (p53) and release of cytokines such as IL-6 and TNF- α (factors associated with carcinogenesis)
- ❖ In addition, *O. viverrini* can also cause hepatocellular carcinoma; reported mainly from the Northeastern Thailand.

Laboratory Diagnosis

- ❖ **Stool microscopy:** Demonstration of the characteristic flask-shaped eggs (measures 28–35 μm \times 12–19 μm) in the stool establishes the diagnosis. Eggs of *Clonorchis*

and *Opisthorchis* are morphologically indistinguishable (Fig. 49.7B).

- Microscopy of the duodenal aspirate is more sensitive than stool microscopy. Entero-test can be done to take duodenal contents (similar to that is done for *Giardia*)
- Formalin ether concentration should be done when egg burden is low.
- ❖ **Antibody detection:** ELISA using recombinant propeptide of cathepsin L proteinase is available for detection of specific IgG4 antibodies against *C. sinensis*. It is used to evaluate the level of infection and for monitoring the treatment response
- ❖ **Antigen detection:** ELISA is also available for the detection of circulating antigen in the serum. Detection of antigen is more useful as it indicates current infection
- ❖ A **multiplex PCR** has been developed to detect *Clonorchis* and *Opisthorchis* simultaneously. It is rapid with high sensitivity and specificity.

TREATMENT

Liver fluke infections

- ❑ Triclabendazole (10 mg/kg once) is the drug of choice for fascioliasis
- ❑ Praziquantel (25 mg/kg, three doses in 1 day) is the drug of choice for clonorchiasis and opisthorchiasis.

Prevention (Liver Fluke)

Common preventive measures include sanitary disposal of sewage, and control of snail hosts

- ❖ Fascioliasis can be prevented by avoidance of consumption of alfalfa juice, raw water plants and cleaning them before use
- ❖ Clonorchiasis and opisthorchiasis can be prevented by avoidance of eating raw or undercooked fresh water fish.

Hepatosplenic Schistosomiasis

Lodging of *Schistosoma mansoni* and *S. japonicum* eggs in the liver can lead to granuloma formation and fibrosis (called as **Symmers pipestem fibrosis**).

- ❖ Fibrosis impedes the portal blood flow leading to portal hypertension, hepatomegaly, splenomegaly (enlarged and hard) and gastric varices
- ❖ *S. mansoni* is increasingly associated with hepatitis C virus; particularly in Egypt and accelerates the occurrence of chronic hepatitis and cirrhosis in these patients
- ❖ Hepatitis B virus infection has been associated with *S. japonicum*.

TOXOCARIASIS

Toxocariasis is caused by a less common zoonotic nematodes, *Toxocara* species that rarely infect humans mainly affecting the liver.

- ❖ Two important species are *T. canis* (dog roundworm) and *T. cati* (cat roundworm)

- ❖ They are the primary agents causing visceral larva migrans in man.

Larva Migrans

There are a number of nematodes of lower animals that occasionally infect man.

- ❑ **In lower animals:** The life cycle of most of these nematodes involve ingestion or skin penetration by the larval stage (or eggs). The eggs develop into larvae. Then the larvae migrate to the intestine, lungs or other organs where they develop into adult worms.
- ❑ **In humans:** Larvae of these lower animal nematodes when accidentally infect man, they are not able to complete their normal development (because humans are the unusual host for them) and their life cycle gets arrested. The larvae wander around aimlessly in the body. This is called as **larva migrans (LM)**.

Two types of larva migrans exists:

1. **Cutaneous larva migrans:** Also called as **creeping eruption**. Larva migration occurs in skin and subcutaneous tissue (discussed in Chapter 57)
2. **Visceral larva migrans:** Larva migrate to viscera, following which the life cycle gets arrested. It is primarily caused by infection with *Toxocara* but less frequently caused by other helminths such as:
 - *Angiostrongylus cantonensis*: Causes eosinophilic meningitis (Chapter 75)
 - *Angiostrongylus costaricensis*: Causes abdominal infection
 - *Gnathostoma spinigerum*: Creeping eruption, eosinophilic meningoencephalitis and ocular infection
 - *Anisakis*: Causes eosinophilic granuloma of bowel
 - *Baylisascaris procyonis*: Eosinophilic meningoencephalitis.

Life Cycle (Arrested) and Pathogenesis

Felines are the natural host for *Toxocara*. Humans act as abnormal host.

- ❖ Transmission to man occurs through ingestion of embryonated eggs (infective form) contaminated in soil
- ❖ Larvae hatch out from the eggs in the intestine, penetrate the intestinal wall and carried via the portal circulation to the liver. The larvae may remain in the liver or migrate to other organs like lungs or eye
- ❖ Since humans are the unusual host, further development of the larvae does not take place. Instead, the larvae get encapsulated in the dense fibrous tissue in liver (most common site) or lungs or eyes; or may continue to wander around the body producing granuloma.

Clinical Features

- ❖ **Visceral larva migrans (VLM):** The liver is the most frequently involved organ (hepatosplenomegaly). But any organ can be affected. Usually younger children (around 3 years) are affected. Other features include

lymphadenopathy, lung involvement, skin lesions (urticaria and nodules) and seizures (result from cerebral involvement)

- ❖ **Ocular larva migrans (OLM):** The most common cause of OLM is *Toxocara* larva. Unilateral painless chorioretinal granuloma in the posterior pole is the most common presentation.

Laboratory Diagnosis

Diagnosis is often difficult and mainly stay on:

- ❖ **Serology:** ELISA employing excretory secretory antigen of larva of *T. canis* is highly sensitive and specific. It can confirm the infection but may also be elevated in asymptomatic patients
- ❖ Biopsy of the tissue from liver, lungs, brain may occasionally reveal the larvae; however, biopsy is usually not recommended
- ❖ Blood eosinophilia.

TREATMENT

Toxocarasis

The recommended regimen includes albendazole for 5 days or mebendazole for 21 days with glucocorticoid.

RARE PARASITIC INFECTIONS OF LIVER

Capillaria hepatica

C. hepatica is a parasite of rodents. Human infection is rare; reported from West Africa. Transmission occurs through accidental ingestion of contaminated soil containing eggs.

- ❖ **Clinical features:** Ranges from hepatitis, hepatomegaly, peritonitis and eosinophilia
- ❖ **Laboratory diagnosis:** Detection of characteristic barrel-shaped eggs in the liver parenchyma, which are similar to the eggs of *Trichuris* and other *Capillaria* species.
- C. philippinensis* and *C. aerophila* are the other species affecting GIT and respiratory systems respectively.

Other parasites that occasionally infect liver include:

- ❖ *Plasmodium*: Its life cycle has a pre-erythrocytic stage, infecting the liver. However, it does not cause liver damage and there are no hepatic symptoms
- ❖ Visceral leishmaniasis may occasionally present as hepatomegaly
- ❖ *Enterocytozoon bieneusi* may infect liver occasionally
- ❖ *Balantidium coli* may produce liver abscess
- ❖ *Enterobius*: Migration of the adult worm may occur to liver
- ❖ **Disseminated strongyloidiasis:** May infect liver, although CNS is the primary site
- ❖ **Occult filariasis:** Occasionally, microfilariae are entrapped in organs like spleen, liver and lymph node leading to hepatosplenomegaly and lymphadenopathy. This is also called as **Meyers Kouwenaar Syndrome**. (Chapter 69).

EXPECTED QUESTIONS

I. Write essay on:

1. A 42-year-old female patient was brought to the emergency department with complaints of sudden episode of high-grade fever and acute pain in the right hypochondrium. She had a history of dysentery and jaundice for the last two months. Ultrasound scan of the abdomen revealed enlarged liver with acute peritonitis. Pus aspirated from liver was thick chocolate brown in color. Microscopy of liver pus revealed necrotic hepatocytes without any pus cells.
 - a. Identify the clinical condition and the probable causative agent.
 - b. What are the complications seen in this condition?
 - c. What are the various diagnostic modalities?
2. A 24-year-old woman presented with complaints of pain in the right hypochondrium. Ultrasonography revealed a single space occupying cystic lesion in the right lobe of the liver. The cyst was removed surgically and subjected to histopathological examination, which revealed three layered cyst wall with attached brood capsules.

- a. Identify the disease and the causative agent.
- b. Write briefly about the life cycle of the etiological agent.
- c. What are the various diagnostic modalities?

II. Write short notes on:

1. Visceral larva migrans.
2. Fascioliasis.

III. Multiple Choice Questions (MCQs):

1. Definitive host for *Echinococcus* is:

- a. Man
- b. Dog
- c. Sheep
- d. Pig

2. Alveolar hydatid disease is caused by:

- a. *Echinococcus granulosus*
- b. *Echinococcus vogeli*
- c. *Echinococcus oligarthus*
- d. *Echinococcus multilocularis*

3. Which of the following is not a liver fluke?

- a. *Clonorchis sinensis*
- b. *Opisthorchis viverrini*
- c. *Fasciola hepatica*
- d. *Fasciolopsis buski*

Answers

1. b 2. d 3. d

Skin, Soft Tissue and Musculoskeletal System Infections

SECTION

7

SECTION OUTLINE

50. Infective Syndromes of Skin, Soft Tissue and Musculoskeletal Systems
51. Staphylococcal Infections
52. Beta-hemolytic Streptococcal Infections
53. Gas Gangrene (*Clostridium perfringens*) and Infections due to Non-sporing Anaerobes
54. Leprosy
55. Miscellaneous Bacterial Infections of Skin and Soft Tissues: Anthrax (*Bacillus anthracis*), Actinomycosis, Nocardiosis, Non-venereal Treponematoses and Others
56. Viral Exanthems and Other Cutaneous Viral Infections
57. Parasitic Infections of Skin, Soft Tissue and Musculoskeletal System
58. Fungal Infections of Skin, Soft Tissue and Musculoskeletal System



For Multidrug Resistant Organisms (MDROs) in Hospital



CONTACT PRECAUTIONS EVERYONE MUST:



Clean their hands, including before entering and when leaving the room.

PROVIDERS AND STAFF MUST ALSO:



Put on gloves before room entry.
Discard gloves before room exit.



Put on gown before room entry.
Discard gown before room exit.

**Do not wear the same gown and gloves
for the care of more than one person.**



Use dedicated or disposable equipment.
Clean and disinfect reusable equipment
before use on another person.

Infective Syndromes of Skin, Soft Tissue and Musculoskeletal Systems

CHAPTER 50

CHAPTER PREVIEW

■ Skin and Soft Tissue Infections (SSTIs)

■ Musculoskeletal Infections

INTRODUCTION

Approximately 15% of all patients who seek medical attention have some skin diseases or lesions, out of which many are infectious. Before discussing various skin and soft tissue infections, understanding the structure and function of skin is important, so that it can be related to the pathogenesis of the disease.

Anatomy of Skin

The skin comprises of epidermis, dermis and subcutaneous tissues (Fig. 50.1).

- ❖ The **epidermis** is made up of several layers of squamous epithelium. From superficial to deep, the layers are called as stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, stratum germinativum (also called stratum basale)
- ❖ The **dermis** is composed of dense connective tissue rich in blood and nerve endings, and is the site of

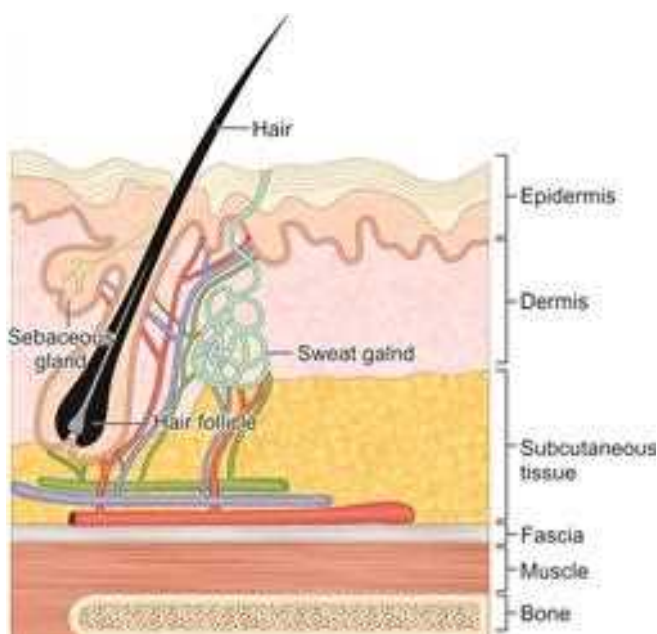


Fig. 50.1: Structure of skin and subcutaneous tissue.

origin for some superficial hair follicles and sebaceous glands

- ❖ The **subcutaneous tissue** contains loose connective tissue and is rich in fat. Deeper hair follicles and sweat glands originate in this layer
- ❖ **Fascial layer** is present below the subcutaneous tissue. It is made up of sheets or bands of fibrous tissue covering the muscles, ligaments, and other connective tissues.

Physiology of Skin

The skin is the body's largest and thinnest organ. It performs various functions.

- ❖ It serves as an anatomical barrier and protects the internal organs from the external environment
- ❖ Its other functions include the control of body temperature, excretion of water and salts, synthesis of important chemicals and hormones, and also acting as a sensory organ
- ❖ The skin's normal microbial flora, pH, and chemical defenses (high salt and acidic environment) also help to prevent colonization by many pathogens
- ❖ Normal resident microbial flora of skin include coagulase-negative staphylococci including *S. epidermidis*, micrococci, diphtheroids, and anaerobes such as *Propionibacterium acnes*.

SKIN AND SOFT TISSUE INFECTIONS (SSTIs)

Skin and soft tissue infections (SSTIs) constitute a major infective syndrome. SSTIs can arise from invasion of organisms through skin due to breach in the anatomical barrier or from the hematogenous route, secondary to any systemic infection.

Clinical Manifestations

Various terms are used to describe skin manifestations, which differ from each other by their clinical appearance. The terms most frequently used to describe manifestations of skin infections are listed in Table 50.1; which can be classified into:

Table 50.1: Clinical manifestations of skin lesions.		
Primary skin lesions and their description		Common organisms/disease implicated
Macule	Flat, nonpalpable discoloration of skin (≤ 5 mm size). If size exceeds 5mm, is called as a patch	Dermatophytes, <i>T. pallidum</i> (secondary syphilis) Viral rashes (e.g. enteroviruses)
Papule	Elevated palpable solid lesion, usually ≤ 5 mm in size	Molluscum contagiosum Scabies, Warts (human papillomavirus)
Nodule	Elevated palpable solid lesion, usually >5 mm in size. If size is >2 cm, then it is called as a plaque	<i>Corynebacterium diphtheriae</i> , <i>Sporothrix schenckii</i> Post kala-azar dermal leishmaniasis <i>Mycobacterium marinum</i> , <i>Nocardia</i> species
Vesicle	Fluid-filled blister of size ≤ 5 mm	HSV, VZV, Molluscum contagiosum Hand-foot-mouth disease (Coxsackievirus A16) <i>Rickettsia akari</i> (Rickettsialpox)
Bulla	Fluid-filled blister of size >5 mm	<i>Clostridium</i> species (gas gangrene), HSV <i>S. aureus</i> (bullous impetigo and scalded skin syndrome) <i>Vibrio vulnificus</i> and other gram-negative rods
Pustule	Pus filled lesion of size ≤ 5 mm	<i>Candida</i> , <i>S. aureus</i> (folliculitis), HSV <i>S. pyogenes</i> and <i>S. aureus</i> (impetigo), Dermatophytes
Abscess	Pus filled lesion of size > 5 mm	<i>S. aureus</i> , <i>S. pyogenes</i> and others
Petechiae	Extravasation of blood into skin, of size <2 mm	HIV, hepatitis C, EBV, rubella, CMV and RMS fever
Purpura	Extravasation of blood into skin, of size 2 mm–1 cm	CMV, endocarditis, meningococemia, mononucleosis, RMS fever, scarlet fever, sepsis, viral hemorrhagic fevers
Ecchymosis	Extravasation of blood into skin, of size >1 cm	Ebola and Marburg viral hemorrhagic fevers
Secondary skin lesions and their description		Common organisms implicated
Scales	Flakes arising from horny layer	Dermatophytes
Ulcer	A lesion with loss of epidermis and dermis	<i>Mycobacterium leprae</i> (leprosy) <i>Mycobacterium ulcerans</i> (Buruli ulcer) <i>Yersinia pestis</i> (bubonic plague) <i>Haemophilus ducreyi</i> (chancroid) <i>T. pallidum</i> (hard chancre)
Eschar	It is black dead tissue formed in a full-thickness wound	<i>B. anthracis</i> (cutaneous anthrax), scrub typhus, rickettsialpox also seen following burn injury
Crusted lesions	Scale like lesion, composed of dried blood and tissue fluid	Bullous impetigo/ecthyma (<i>S. aureus</i>) Impetigo contagiosa (<i>S. pyogenes</i>) Ringworm (dermatophytes), Cutaneous leishmaniasis

Abbreviations: RMS, Rocky Mountain spotted fever; CMV, cytomegalovirus; HSV, Herpes simplex virus; VZV, Varicella-zoster virus; EBV- Epstein-Barr virus.

- ❖ **Primary skin lesions:** They are the direct result of underlying disease process and are of diagnostic importance. Examples include macule, papule, nodule, plaque, vesicle, bulla, pustule, abscess, purpura, petechiae and ecchymosis
- ❖ **Secondary skin lesions:** They evolve from primary lesions by self-trauma or altered keratinization. Common examples include scales, ulcers, eschars, and crusted lesions.
- ❖ Wound infections such as surgical site infection, bite wound infection, burn-wound infection and infections of sinuses and fistula
- ❖ SSTI due to vascular injury and neuropathy: Diabetic foot ulcer and decubitus ulcer
- ❖ Cutaneous manifestations of systemic diseases
- ❖ Lymphadenitis and lymphangitis.

Infection of Dermis and Epidermis

Occurs due to a traumatic breach in the skin which results in the inoculation of microorganisms. Various types are erysipelas, erythrasma, erysipeloid, impetigo, and cellulitis (Table 50.2).

Infection of Skin Appendages (Table 50.3)

Classifications of SSTIs

SSTIs can be classified into various types.

- ❖ Infection of dermis and epidermis
- ❖ Infection of skin appendages such as hair follicle, sebaceous gland, sweat gland and nail
- ❖ Infection of fascia (e.g. necrotizing fasciitis)
- ❖ Infection associated with hair: Folliculitis, furuncle, carbuncle

Superficial epidermis		Organisms
Ringworm or tinea infection	Because of their ability to utilize keratin, they infect keratinized layer of epidermis (skin), hair and nail. They do not invade beyond the superficial layer of the skin <ul style="list-style-type: none"> The skin lesions appear as annular or ring-shaped pruritic, scaly with central clearing and raised edges Scaling, erythema or rarely bister formation may occur 	Dermatophytes <ul style="list-style-type: none"> <i>Trichophyton</i>—infects skin, hair and nail <i>Microsporum</i>—infects skin and hair <i>Epidermophyton</i>—infects skin and nail
Deep epidermis and dermis		
Impetigo	Erythematous (red) lesions that may be either non-bullous (more common) or bullous; that ruptures and develops into honey-colored crusts Commonly affects young children; most commonly found on the face	<i>S. pyogenes</i> (common agent) and <i>S. aureus</i> (for bullous impetigo)
Erysipelas	Non-necrotizing inflammation of dermis and subcutaneous tissue Lesions are painful, red, swollen, and indurated with a distinct border Patients may also have fever and regional lymphadenopathy	<i>S. pyogenes</i> (common agent) and <i>S. aureus</i> (occasionally)
Erythrasma	Chronic infection of the keratinized layer of the epidermis; lesions are dry, scaly, itchy, and discolored (reddish brown)	<i>Corynebacterium minutissimum</i> (common in diabetics)
Erysipeloid	Purplish-red, nonvesiculated skin lesion with an irregular, raised border; the lesions itch and burn; fever and other systemic symptoms are uncommon	<i>Erysipelothrix rhusiopathiae</i>
Cellulitis	Diffuse, spreading infection involving the deeper layers of the dermis; lesions are ill-defined, flat, painful, red, and swollen; patients have fever, chills, and regional lymphadenopathy	Common: <i>S. pyogenes</i> , <i>S. aureus</i> Less common: <i>Aeromonas</i> , <i>Vibrio</i> spp., and <i>H. influenzae</i>

Infections of hair follicle		
Folliculitis	Superficial infection of the hair follicle, presents as papule or pustule	<i>Staphylococcus aureus</i> (most common) Hot-tub folliculitis (<i>Pseudomonas aeruginosa</i>)
Furuncle (or boil)	Deeper infections of the hair follicles, that begins as a red nodule and then becomes painful and full of pus	<i>Staphylococcus aureus</i>
Carbuncle	Furuncles that coalesce and spread more deeply into the dermis and subcutaneous tissues; they usually have multiple sites, which drain into the skin surface (sinuses)	<i>Staphylococcus aureus</i>
Infections of sweat gland		
Hidradenitis	Chronic infection of obstructed apocrine (sweat) glands in the axilla, genital, or perianal areas with intermittent discharge (often foul smelling pus)	<i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , anaerobic streptococci, and <i>Bacteroides</i> spp.
Infections of sebaceous gland		
Sebaceous cyst	Generally sebaceous glands empty into hair follicles and ducts. If these portals get blocked, form sebaceous cysts that may become secondarily infected and cause staphylococcal abscesses	<i>Staphylococcus aureus</i>
Infections of nail		
Onychomycosis	Fungal infection of the nail Characterized by discolored, thick, fragile or cracked nails	Tinea unguium (dermatophyte) <i>Candida albicans</i>
Green nail	Nail separated from nail bed, becomes green with foul odor	<i>Pseudomonas aeruginosa</i>

- ❖ Infection of sebaceous gland
- ❖ Infection of sweat gland: e.g. hidradenitis
- ❖ Infection of nail: e.g. onychomycosis.

Infections Associated with Subcutaneous Tissue

Infections of the subcutaneous tissues may manifest as abscesses, ulcers, or boils. In many instances, infections of the epidermis and dermis extend deeper and become

subcutaneous infections. For example, cellulitis may extend to the subcutaneous tissues. The organisms isolated from the subcutaneous abscesses depend on the site of infection.

- ❖ ***Staphylococcus aureus*** remains the most common etiologic agent of subcutaneous abscess
- ❖ **Anaerobes** are commonly isolated from abscesses of the perineal, inguinal, and buttock areas; whereas

non-perineal infection is commonly caused by mixed infection containing facultative organisms

- ❖ **Meleney's ulcer** (also called as progressive synergistic gangrene): It is a slowly progressive chronic infection of the subcutaneous tissue that usually begins as an ulcer following trauma or surgery, and may lead to subcutaneous necrosis
 - There occurs true polymicrobial infection, in which microaerophilic streptococci grow synergistically with *S. aureus* and also other facultative or anaerobic organisms
 - Specimen should be taken from the advancing outer edge of the lesion (not from the central portion of the wound) for better recovery of microaerophilic streptococci.
- ❖ **Anaerobic cellulitis:** It is associated with the production of large amount of gas by organisms that may be present in the subcutaneous tissue. This type of infection is most often located in the extremities and is particularly common in diabetics.

Infection of Fascia (Necrotizing Fasciitis)

Necrotizing fasciitis is a serious life-threatening condition which involves infection of the fascia and often with the overlying soft tissue (Chapter 52).

- ❖ As no barrier exists to prevent the spread of infection at the fascial level, so fasciitis may extend widely and rapidly to involve larger areas of the body in a short amount of time
- ❖ **Agents:** Most common organisms associated with necrotizing fasciitis are group A streptococci, *S. aureus* or anaerobic bacteria, especially *Bacteroides* and *Clostridium* species.

Fournier's gangrene: It is a rare and often fulminant necrotizing fasciitis of the perineum and genital region frequently due to a synergistic polymicrobial infections, comprising of mixed aerobic and anaerobic bacteria.

Wound Infections

1. Postoperative (Surgical Site) Infections

Surgical site infections (SSI) are among the most common healthcare associated infections (HAIs) and occur after nearly 3% of all surgical procedures (Chapter 22).

- ❖ The source of infection in SSI includes the **patient's own flora** present on the skin (*S. aureus*, the most common organism causing SSI) or mucosa (*E. coli* or anaerobes for gastrointestinal mucosa) or **environmental source** (gram-negative bacilli such as *Pseudomonas* and *Acinetobacter*)
- ❖ SSIs can cause significant morbidity and mortality as well as economic burden if left untreated.

2. Bite Wound Infections

Bites and scratches from animals and humans allow the inoculation of microorganisms present in saliva to breach

the skin's protective barrier into deeper, susceptible host tissues. Most common bites are from dogs and cats.

- ❖ This leads to various infections ranging from cellulitis at the site of bite to systemic infections such as bacteremia or even encephalitis (rabies)
- ❖ Common infections transmitted by the dog bite include *Pasteurella*, and *Capnocytophaga* (Chapter 81) and rabies (Chapter 74).

3. Burn Wound Infections

Thermal burn injury may cause massive destruction of the skin and its appendages as well as impairment of the immune system (both humoral and cellular immune systems). Infectious complications are the major causes of morbidity and mortality in serious burn injuries. Loss of the cutaneous barrier facilitates entry of the patient's own endogenous (skin) flora and organisms from the hospital environment into a burn wound.

Pathogenesis

Initially, the burn wound is colonized with gram-positive bacteria (e.g. streptococci and staphylococci) from the surrounding tissue, reaching to the maximum load beneath the burn wound on day 4 after the burn injury.

- ❖ An eschar is formed over the burn wound, which is usually avascular because of the damaged blood vessels caused by the burn injury
- ❖ The avascularity of the eschar, along with the impairment of local immune responses favor further bacterial colonization and proliferation
- ❖ By day 7, the wound is colonized with other microbes from the gastrointestinal and upper respiratory flora or also from the hospital environment. Most common organisms implicated being *P. aeruginosa*, *Acinetobacter*, *Escherichia coli*, *Klebsiella*, *S. aureus* and fungi such as *Candida*, *Aspergillus* species, and the agents of mucormycosis and viruses such as herpes simplex virus.

Clinical Presentations

Burn wound infections may present in four forms—(i) burn wound impetigo, (ii) surgical site wound infection (purulent infection of the excised burn wound site and/graft site), (iii) cellulitis (when burn wound extended to surrounding healthy tissue), (iv) invasive infection in unexcised burn wounds. Green discoloration of the wound points towards *P. aeruginosa* infection.

Laboratory Diagnosis

Although burn wound infection is diagnosed clinically, laboratory has a role to identify the causative organism(s) so as to guide antimicrobial therapy.

- ❖ **Appropriate specimens** include purulent wound exudates, tissue biopsy specimen and blood cultures. Surface specimens should be collected with a moistened sterile swab using a minimal amount of pressure

- ❖ Sometimes a **quantitative culture** of a tissue biopsy specimen is performed to assess the severity of infection or to identify the most prevalent organism in a polymicrobial infection. Result of $\geq 10^5$ CFU/g of tissue is indicative of a potentially serious infection.

TREATMENT

Burn wound infections

The successful burn wound management comprises of the following components.

- ❑ Early surgical excision of the burned tissue
- ❑ Extensive debridement of the necrotic tissue
- ❑ Grafting of skin or skin substitutes from autograft or compatible allogenic graft
- ❑ Topical antimicrobial agents—silver sulfadiazine cream, mafenide acetate cream, silver nitrate cream, and nanocrystalline silver dressings
- ❑ Systemic antibiotics may be useful if sign of systemic involvement is present. However, prophylactic systemic antibiotics have no role in the management of burn wounds and in fact, can lead to subsequent colonization with resistant bacteria.

4. Sinus Tract and Fistula Infections

Sinus tract: A deep-seated infection (e.g. osteomyelitis) may occasionally develop a channel—called a sinus tract to the skin surface, which helps in draining the fluid and pus onto the skin.

- ❖ The organisms frequently involved in sinus tract formation with an underlying osteomyelitis include *S. aureus* (most common), gram-negative bacilli and anaerobes
- ❖ Chronic draining sinuses may also be found in patients with tuberculosis and nontuberculous mycobacteria infection, *Nocardia* infection, actinomycosis and infections associated with implanted foreign bodies
- ❖ Curetting or biopsy specimens from the debrided, cleansed sinus tract should be collected for culture.

Fistulas: These are abnormal channels connecting epithelial surfaces, either between two internal organs or between an organ and the skin.

- ❖ Examples include perirectal fistulas from the small bowel to the skin associated with Crohn's disease or chronic intra-abdominal infection
- ❖ When the bowel is involved, the associated underlying cause may be tuberculosis, actinomycosis, or malignancy. Biopsy is the specimen of choice. Specimen collection is a challenge due to the presence of indigenous bowel flora.

Skin Manifestations of Systemic Infections

Cutaneous manifestations of systemic infections, may be important clues for the clinician. For example, meningococemia may be associated with petechiae (tiny red hemorrhagic spots) on the skin. Scraping of petechiae may demonstrate the presence of gram-negative diplococci. Various organisms involved in systemic infections capable of producing cutaneous lesions are:

- ❖ Cocci such as meningococci, streptococci, *S. aureus*, and gonococci
- ❖ Gram-negative bacilli such as *Pseudomonas*, *B. pseudomallei*, *Vibrio vulnificus* and *Salmonella* Typhi (rose spots)
- ❖ *M. tuberculosis* (lupus vulgaris) and *M. leprae*
- ❖ Rickettsiae, *Bartonella* and *Streptobacillus moniliformis*
- ❖ Viral hemorrhagic fever and exanthematous viral diseases
- ❖ Fungi: *Candida*, *Cryptococcus*, *Histoplasma*, *Blastomyces*, and *Coccidioides*.

Diabetic Foot Ulcer

Patients with diabetes mellitus have a high risk of developing infections, especially in their lower extremities. If not promptly managed can lead to amputations and greatly increased mortality (Fig. 50.2).

- ❖ **Pathogenesis:** The excess blood glucose can result in impaired microvascular circulation and peripheral motor neuropathy
 - Subsequently any skin damaging injury or surgery greatly increases the risk of contracting infections
 - The foot ulcer of a diabetic patient does not heal as quickly as that of a healthy individual.
- ❖ **Laboratory diagnosis:** The following specimens may be subjected to culture—(1) aspirated fluid or pus taken from a deep pocket within the wound or (2) debrided infected tissue. Wound swab from the ulcers is not an ideal specimen
- ❖ **Microbiology:** The most common bacteria isolated include *S. aureus*, group B streptococci, members of the Enterobacteriaceae, *P. aeruginosa* and anaerobes (e.g. *Bacteroides fragilis*). The majority of severe infections are usually polymicrobial.

Decubitus Ulcer

Elderly or chronically ill, bedridden patients are prone to develop decubitus ulcer (also called as bed sore or pressure sore); the most common site being near the anus or on the lower extremities. Most frequent organisms infect the decubitus ulcer are gastrointestinal flora (e.g. *Bacteroides*



Fig. 50.2: Diabetic foot.

Source: Wikipedia (with permission).

fragilis, or nosocomial pathogens (e.g. *S. aureus* and *P. aeruginosa*).

Lymphadenitis and Lymphangitis

Lymphadenitis: It is the inflammation or enlargement of lymph nodes. Lymph nodes are small, ovoid nodules distributed in clusters along the course of lymphatic vessels located throughout the body. Their primary function is to filter out organisms and abnormal cells in lymph fluid.

- ❖ **Etiology:** Lymph node enlargement is a common feature in a variety of diseases which include infections, immunologic disorders, diseases of lymphoid or reticuloendothelial tissues, and malignancies
- ❖ **Infections:** Lymphadenopathy may be seen in various bacterial, viral, parasitic or fungal infections (Table 50.4). They represent a benign response to localized or systemic infection and may serve as a focal point for subsequent clinical investigation
- ❖ Lymphadenitis may affect a single node or a group of nodes (regional lymphadenopathy); unilateral or bilateral; soft or hard/indurated; painful or painless. The onset may be acute, subacute, or chronic.

Lymphangitis: It is defined as an inflammation of the lymphatic vessels that occurs as a result of infection at a site distal to the vessel. Etiological agents include:

- ❖ Group A beta-hemolytic streptococci (most common cause) and *Staphylococcus aureus*
- ❖ Gram-negative bacilli, and fungi: May cause cellulitis and resultant lymphangitis in immunocompromised hosts
- ❖ *Pasteurella multocida*: Associated with dog and cat bites; can cause cellulitis and lymphangitis
- ❖ *Aeromonas hydrophila*: Can contaminate wounds that occur in freshwater
- ❖ Lymphatic filariasis due to *Wuchereria bancrofti* and *Brugia malayi*: They are major cause of acute lymphangitis worldwide; signs and symptoms of lymphangitis caused by *W. bancrofti* are indistinguishable from those of bacterial lymphangitis
- ❖ Nodular lymphangitis commonly follows superficial inoculation with one of the following organisms:
 - *Sporothrix schenckii*
 - *Nocardia brasiliensis*
 - *Mycobacterium marinum*
 - *Leishmania panamensis* and *L. guyanensis*
 - *Francisella tularensis*.

In addition, individuals with diabetes, immunodeficiency, chronic steroid use, or other systemic illnesses have increased risk of developing serious or rapidly spreading lymphangitis.

MUSCULOSKELETAL INFECTIONS

Musculoskeletal infections include infections associated with muscle (myositis, myonecrosis) and infections associated with bone (osteomyelitis), joint infections (septic arthritis) and bone marrow.

Table 50.4: Infectious agents causing lymphadenitis.

Bacterial causes	
<i>Bartonella henselae</i> (cat scratch disease)	Single-node at scratch site; discrete, mobile, nontender
<i>Francisella tularensis</i> (tularemia)	Cervical, mediastinal, or generalized; tender
Atypical mycobacteria	Cervical, submandibular, submental (unilateral)
<i>Mycobacterium tuberculosis</i>	Mediastinal, mesenteric, anterior cervical (tender, discrete, firm, mobile); hematogenous spread
<i>Salmonella</i>	Generalized
<i>S. aureus</i> adenitis and <i>S. pyogenes</i> pharyngitis	Cervical, submandibular; unilateral, firm, tender
<i>Yersinia pestis</i> (plague)	Axillary, inguinal, femoral, cervical; extremely tender
<i>Yersinia enterocolitica</i>	Cervical or abdominal
Viral causes	
Cytomegalovirus	Generalized
Epstein-Barr virus (infectious mononucleosis)	Anterior cervical, mediastinal, bilateral; discrete, firm, nontender
Parvovirus and rubella virus	Posterior auricular, occipital, posterior cervical
Parasitic causes	
<i>Toxoplasma gondii</i>	Generalized, often nontender
Fungal causes	
<i>Coccidioides</i> and <i>Histoplasma</i>	Mediastinal
Seborrheic dermatitis	Occipital, postauricular

Infections of Muscle

Infectious myositis (inflammation of skeletal muscle) can be caused by a variety of organisms.

- ❖ **Bacterial myositis:** It present in several forms, caused by various organisms
 - *S. aureus* myositis: It is the most common cause of acute bacterial myositis, resulting from hematogenous spread
 - Pyomyositis: It refers to focal collections of suppuration in muscle, most often caused by *S. aureus*
 - Psoas abscess: It is a retroperitoneal collection of pus in the iliopsoas muscle compartment; most commonly caused by *S. aureus*. Other agents include streptococci, *E. coli* and *M. tuberculosis*
 - Streptococcal necrotizing myositis due to *S. pyogenes* or *S. agalactiae*
 - Clostridial myositis and gas gangrene: It is an edematous myonecrosis, caused by *Clostridium perfringens* (most common) or other clostridia species. It follows severe penetrating injuries that result in interruption of the blood supply and introduction of soil into wounds (Chapter 53)
 - Synergistic nonclostridial anaerobic myonecrosis

- Others: *Aeromonas*, *Bacteroides*, *Burkholderia pseudomallei*, *Vibrio vulnificus*, etc.
- ❖ **Viral myositis:** Viruses implicated in the pathogenesis of myositis include HIV, human T lymphotropic virus (HTLV), influenza, dengue, Coxsackieviruses, and echoviruses
- ❖ **Parasitic myositis:** Parasitic infections such as Chagas' disease, cysticercosis, trichinellosis are associated with myositis
- ❖ **Fungal myositis:** It usually occurs in an immunocompromised host. Causative agents are *Candida* species (commonest), *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides* species, or *Aspergillus* species.

Infections of Skeletal System

1. Osteomyelitis

Osteomyelitis is inflammation of the bone caused by an infecting organism.

- ❖ **Pathogenesis:** Organisms may spread to bones either due to: (1) direct invasion, secondary to events such as trauma, surgery, joint infection, presence of foreign bodies, prostheses or (2) from hematogenous spread. Once established, infections in bone may progress toward chronicity, particularly if blood supply is insufficient in the affected area
- ❖ **Agents:** *S. aureus* is the most common cause of acute osteomyelitis, followed by streptococci, and gram-negative bacilli—mainly *E. coli* and *P. aeruginosa* (in IV drug users)
 - Subacute osteomyelitis is typically caused by *M. tuberculosis* or *Brucella*
 - Children with sickle cell disease are at an increased risk for *Salmonella* osteomyelitis; sternum being the common site.
- ❖ **Site:** Vertebrae are the most common site of hematogenous osteomyelitis, but infection may also occur in the long bones, pelvic bones, and clavicle
- ❖ **Clinical manifestations:** Patients present with fever, local pain, swelling, or redness, point tenderness and difficulty in movement
- ❖ **Diagnosis:** A small piece of infected bone (from the most soft and necrotic area) may be obtained for culture. Small bits of bone can be ground with sterile broth to form a suspension for bacteriological culture
 - Blood cultures may be useful in case of hematogenous spread of infection
 - Bone biopsies may be sent for histopathologic examination and culture
 - Radiology: X-ray finding includes periosteal thickening. CT or MRI scan may reveal the extent and location of osteomyelitis.
- ❖ **Treatment:** Early institution of pathogen-directed appropriate antibiotic therapy is the mainstay of treatment. Surgery (extensive debridement of necrotic tissue) is indicated if the patient has not responded

to antibiotics or there is evidence of a persistent soft tissue abscess or concomitant joint infection is suspected.

2. Infectious Arthritis

Infectious arthritis refers to inflammation of the joint space, due to microbial infection.

Pathogenesis

Infection of the joint usually occurs secondary to hematogenous spread of organism, or as a direct extension of infection from the bone. It may also occur following injection of corticosteroids into joints or after insertion of prosthetic material (e.g. total hip replacement).

- ❖ It usually involves single joint (monoarticular), but hematogenous spread may lead to polyarticular infection
- ❖ In bacterial arthritis, the knees and hips are the most commonly affected joints.

Etiology

- ❖ **Bacterial arthritis:** Overall, *S. aureus* (commonest agent) or *Streptococcus pyogenes* are the most common etiologic agents of septic arthritis, accounting for approximately 70-80% of infections
 - *N. gonorrhoeae* is isolated most frequently in young adults and *H. influenzae* in children < 2 years of age
 - *P. aeruginosa* and *E. coli* are frequently isolated in IV drug users
 - Among anaerobic bacteria, *Bacteroides* may be recovered
 - Rare causes include Lyme arthritis (*Borrelia burgdorferi*) and rat bite fever due to *Streptobacillus moniliformis*
 - Chronic monoarticular arthritis is frequently due to mycobacteria, *Nocardia*, and fungi.
- ❖ **Viral arthritis:** Viruses are an important cause of infectious arthritis
 - Arboviruses such as Chikungunya (Chapter 34) and Zika virus (Chapter 79)
 - Chronic hepatitis B and C infection; due to immunocomplex deposition (Chapter 48)
 - Parvovirus B19 (Chapter 56).
- ❖ **Parasitic arthritis:** May be seen occasionally with *Dracunculus medinensis* or *Echinococcus granulosus* infection
- ❖ **Prosthetic joint infections:** Majority of infections occur during or immediately after surgery; caused by *S. aureus*, *S. pyogenes*, and enteric bacilli. Some infections may occur >1 year after surgery; caused by less virulent organisms such as coagulase-negative staphylococci or diphtheroids
- ❖ **Reactive polyarthritis:** It is seen in ~1% of non-gonococcal urethritis and 2% of enteric infections, particularly those due to *Yersinia enterocolitica*, *Shigella flexneri*, *Campylobacter jejuni*, and *Salmonella* species

- Only a minority of these patients have the other findings of classic reactive arthritis, including urethritis, conjunctivitis, uveitis, oral ulcers, and rash
- Most patients are male, have genetic predisposition; have been linked to the HLA-B27.

Diagnosis

Synovial fluid may be aspirated and sent for Gram staining and culture. Specimen can be directly inoculated into blood culture bottles for better recovery. The use of mycobacteria and fungal media must also be considered.

3. Infections of Bone Marrow

Infections where bone marrow involvement is common includes brucellosis, enteric fever, histoplasmosis, blastomycosis, tuberculosis, and leishmaniasis.

- ❖ Bone marrow culture is performed for *Brucella*, *Salmonella* (*S. Typhi* and *S. Paratyphi*) and fungi
- ❖ Amastigote forms of *Leishmania* can be visualized in smears or sections made from bone marrow material
- ❖ In HIV patients, certain opportunistic pathogens may cause disseminated infections infecting the bone marrow; e.g. cytomegalovirus, *Cryptococcus* and *Mycobacterium avium* complex.

EXPECTED QUESTIONS

I. Write short notes on:

1. 72-year-old male diabetic patient admitted to the hospital with complaints of swelling in the arm with pus discharge. On physical examination, the local area was found to be red, warm and tender. The swelling has a feel of fluid filled when pressed. Pus was aspirated and was sent to microbiology laboratory. What is the clinical diagnosis, list its etiological agents?
2. In what ways are infections in the subcutaneous tissues usually manifested?
3. What are the various ways the burn-wound infections can occur?
4. Why are patients with diabetes mellitus prone to infections?

CHAPTER PREVIEW

■ *Staphylococcus aureus* Infections

■ Coagulase-negative Staphylococcal Infections

INTRODUCTION

Gram-positive cocci are classified into two families—Micrococcaceae and Streptococcaceae, differentiated by the catalase test. Micrococcaceae are catalase positive, gram-positive cocci arranged in tetrads or clusters; whereas Streptococcaceae are catalase negative gram-positive cocci, arranged in pairs or chains.

Family Micrococcaceae comprises of genera—*Micrococcus* and *Staphylococcus*.

- ❖ *Micrococcus* species are skin commensals, usually not associated with human infections. They are 1–1.8 μm size, arranged in tetrads
- ❖ *Staphylococcus* species are arranged in clusters
 - *S. aureus* is the most pathogenic species; it produces an enzyme coagulase which forms the basis of coagulase test
 - Whereas, other species do not produce coagulase and are called as coagulase-negative staphylococci (CoNS). They are rarely pathogenic to man; may cause infections in immunocompromised patients
 - *S. epidermidis* is the most common CoNS infecting man. Others include—*S. saprophyticus*, *S. lugdunensis*, *S. schleiferi*, *S. haemolyticus* and *S. warneri*.

History

Staphylococcus was named after the Greek word, *Staphyle* means ‘a bunch of grapes’ and *kokkos* means ‘berry shaped’; by Sir Alexander Ogston (1880). Rosenbach (1884) named the species *S. aureus*—based on the characteristic golden yellow pigmentation of its colonies.

STAPHYLOCOCCUS AUREUS INFECTIONS

Staphylococcus aureus is catalase positive, coagulase positive, facultative anaerobe, non-motile, non-sporing and occasionally capsulated.

- ❖ They are spherical cocci, about 1 μm in diameter, arranged in grape-like clusters. This arrangement is due to cell-division in *S. aureus*; which occurs in multiple planes with daughter cells remain attached together

- ❖ It produces golden yellow pigmentation on nutrient agar and β -hemolytic colonies on blood agar
- ❖ *S. aureus* is the most virulent species among staphylococci; produces infections which range from localized pyogenic infections to life-threatening systemic infections in man
- ❖ Its importance as human pathogen is greatly enhanced especially in hospital environment because of its ability to develop drug resistance.

Virulence Factors

S. aureus possesses an array of virulence factors as listed in Table 51.1.

Cell Wall Associated Factors

Like most gram-positive bacteria, the cell wall of *Staphylococcus* consists of a thick peptidoglycan layer and teichoic acid. *S. aureus* has additional factors in the cell wall, such as protein A and clumping factor.

- ❖ **Peptidoglycan:** Similar to other gram-positive bacteria, the peptidoglycan layer of *Staphylococcus* is thicker

Table 51.1: Virulence factors of *Staphylococcus aureus*.

Cell wall associated factors
Peptidoglycan
Teichoic acid
Cell surface adhesins, e.g. clumping factor
Protein A
Toxins
Membrane active toxins
• Hemolysins— α , β , γ , δ
• Leukocidin (or panton valentine toxin)
Epidermolytic toxin (exfoliative toxin)
Enterotoxins
Toxic shock syndrome toxin
Extracellular enzymes
Coagulase
Heat stable thermonuclease
Deoxyribonuclease
Staphylokinase (fibrinolysin)
Others—hyaluronidase, lipase, and protease

- It confers rigidity to the cell wall and maintains the shape
- It induces inflammatory response and also has endotoxin-like activity.
- ❖ **Teichoic acid:** It is made up of ribitol phosphate polymers, helps in adhesion of cocci to mucosal surfaces and inhibits opsonization
- ❖ **Cell surface adhesins** are as follows:
 - Clumping factor/bound coagulase—it is a fibrinogen binding adhesin; responsible for slide coagulase reaction
 - Fibronectin binding adhesin
 - Collagen-binding adhesin.
- ❖ **Protein A (SpA):** It is a 42 kDa polypeptide, encoded by *spa* gene. It is present in 90–99% of human *S. aureus* strains (especially the Cowan I strains)
 - Protein A has many **biological properties**, such as anti-complementary, chemotactic, mitogenic, inhibition of opsonization and induction of platelet damage
 - **Mediates coagglutination reaction:** Protein A binds to Fc region of any IgG antibody, leaving Fab region free which binds to the corresponding antigen present in clinical samples. This test is obsolete now.
- ❖ **Microcapsule:** Some strains of *S. aureus* have polysaccharide microcapsule, which inhibits phagocytosis by neutrophils. The capsular polysaccharides are zwitterionic, i.e. they have both negative and positive charges, which is a feature that is critical for abscess formation.
- ❖ **It comprises of two proteins:** ET-A (chromosomal, heat stable) and ET-B (plasmid coded, heat labile)
- ❖ It often occurs in newborns and children, more often than adults
- ❖ Illness may vary from localized tender blisters and bullae formation to exfoliation and separation of outer epidermal layer leaving denuded underlying skin (the latter is called as **Nikolsky's sign**)
- ❖ The mucous membranes are usually spared
- ❖ Severe form in a newborn is called as Ritter's syndrome; characterized by fever, lethargy, and irritability with poor feeding
- ❖ Milder forms—pemphigus neonatorum and bullous impetigo.

Enterotoxin

Enterotoxin is expressed by nearly 50% of *S. aureus* strains and is responsible for staphylococcal food poisoning (Chapter 40, for detail).

- ❖ It is a **preformed toxin** (secreted in food before consumption) so that it can act rapidly. As a result, the **incubation period** is short (1–6 hours)
- ❖ **Serotyping:** Enterotoxins can be typed into 15 serotypes (A–E, G–P); all act as superantigens
 - Type A is most common to cause food poisoning
 - Serotype-F does not cause food poisoning; but causes toxic shock syndrome.

Toxic Shock Syndrome Toxin (TSST)

This toxin is responsible for toxic shock syndrome (TSS). It has two subtypes—TSST-1 and TSST-2; both act as superantigens.

- ❖ TSST-1 is actually a staphylococcal enterotoxin. Enterotoxin F (formerly called pyrogenic exotoxin C) is the most common type of TSST-1; rarely enterotoxin-B or C may also be associated
- ❖ **Risk factors:** Initially, toxic shock syndrome was reported from women using highly absorbent vaginal tampons during menstruation. Subsequently, TSS has been reported from both men and non-menstruating women as a complication of staphylococcal abscesses, osteomyelitis and post-surgical, traumatic or burn wound infections
- ❖ **Pathogenesis:** TSST-1 gets absorbed into circulation from the tampons; then being a superantigen it stimulates the T-cells non-specifically (by binding to V β region of T-cell receptor) causing excessive production of cytokines (cytokine storm), which leads to a potentially fatal multisystem disease. (Both TSST and enterotoxin are examples of superantigens, described in detail in Chapter 10)
- ❖ **Clinical features:** Patients present with fever, hypotension, mucosal (conjunctival) hyperemia, vomiting, diarrhea, confusion, myalgia, abdominal pain and erythematous rashes which desquamate later. Subsequently, there is rapid involvement of the other

Toxins

Membrane Active Toxins

Hemolysins

S. aureus produces four distinct hemolysins— α , β , γ and δ hemolysins. They are membrane damaging toxins, act on red blood cells (RBCs) leading to hemolysis. They exhibit various actions such as dermonecrotic, cytotoxic, neurotoxic and leukocidal activities.

Leukocidins/Panton Valentine Toxin

It is also called as Panton Valentine (PV) toxin; named after its discoverers.

- ❖ It acts synergistically with γ -hemolysin to damage leukocytes, RBCs and macrophages
- ❖ **Synergohymenotropic toxins:** γ -hemolysin and PV toxin are called as synergohymenotropic toxins. Because they are not active individually, but in combination, they are capable of producing hemolytic and leukocidal activity
- ❖ PV toxin is expressed on MRSA (methicillin-resistant *Staphylococcus aureus*) strains, which are associated with the community acquired infections.

Epidermolytic/Exfoliative Toxin (ET)

This toxin is responsible for staphylococcal scalded skin syndrome (SSSS).

organs such as liver, kidneys, lungs, gastrointestinal tract (GIT) and/or central nervous system (CNS)

❖ **Case definition** of TSS includes:

- *Clinical criteria:* (i) fever, (ii) rash, (iii) desquamation, (iv) hypotension, (v) multiorgan (≥ 3) involvement plus
- *Laboratory criteria:* Negative blood culture for other pathogens and lack of evidence of other causes of infections.

❖ Anti-TSST antibodies usually appear in the convalescent stage, they are protective in nature. TSS is more severe if anti-TSST antibodies fail to appear

❖ **Diagnosis:** Detection of TSST can be done by latex agglutination test and enzyme immunoassay. PCR-based assays are available for detection of TSST genes 1 and 2. Other findings may include altered liver/kidney function tests and low platelet count.

TREATMENT

Toxic shock syndrome

- ❑ As toxin causes capillary leak; aggressive parenteral fluid replacement and vasopressor to reverse hypotension should be initiated at the earliest
- ❑ Examine for and remove any colonized foreign body, e.g. vaginal tampon
- ❑ Clindamycin is the preferred drug for TSS (as it reduces the toxin synthesis). It is given along with anti-staphylococcal penicillin (e.g. cloxacillin) for MSSA or vancomycin for MRSA
- ❑ For clindamycin resistant cases: Linezolid can be used for toxin suppression. In such case, addition of vancomycin is not required.

Extracellular Enzymes

Coagulase

The unique feature of *S. aureus* is that, it secretes coagulase enzyme—the principle virulence factor. In host when it comes in contact with blood, brings about clotting or coagulation of blood by activating prothrombin, which in turn converts fibrinogen to fibrin.

- ❖ The fibrin clot surrounding the bacteria may protect it from phagocytosis and other defense mechanisms
- ❖ This property also serves as the basis of tube coagulase test—the most important test used for the identification of *S. aureus*.

Other Enzymes

- ❖ Heat stable thermonucleases and DNase (deoxyribonuclease) are the enzymes that are specific to *S. aureus*; not produced by any other staphylococcal species
- ❖ Staphylokinase (fibrinolysin) breaks down fibrin clots and may facilitate the spread of infection
- ❖ Hyaluronidase breaks down the connective tissue network
- ❖ Lipases and phospholipases breakdown the lipids.

Pathogenesis

Pathogenesis of *S. aureus* involves the following steps:

❖ **Colonization:** *S. aureus* colonizes on various body surfaces, such as anterior nares, oropharynx, axilla and perineal skin

❖ **Introduction into the tissue:** Organisms are introduced into the tissues as a result of minor abrasions or instrumentation. Then they adhere to the tissue surfaces; which is mediated by various adhesins, e.g. clumping factor and collagen-binding adhesion

❖ **Invasion:** *S. aureus* can invade into the tissues by elaborating enzymes, such as serine proteases, hyaluronidases, thermonucleases and lipases. These enzymes facilitate bacterial survival and local spread across tissue surfaces (e.g. lipases helps survival in lipid-rich areas like hair follicles)

❖ **Evasion of host defense mechanisms:** *S. aureus* exhibits various immune evasion mechanisms, such as:

- Anti-phagocytic activity mediated by microcapsule and protein A
- Inhibition of leukocyte migration (by chemotaxis inhibitory protein of staphylococci)
- Intracellular survival inside the endothelial cells (by formation of small colony variants).

❖ **Metastatic spread:** Finally, *S. aureus* spreads to various distant sites by hematogenous spread.

Clinical Manifestations

Staphylococcus aureus is a pluripotent pathogen, causing various diseases through both toxin-mediated and non-toxin-mediated mechanisms. It is responsible for both healthcare- and community-associated infections that range from relatively minor skin and soft tissue infections to life-threatening systemic infections (Table 51.2).

Epidemiology

Staphylococcus aureus is a part of normal human flora. About 20–40% of healthy population are carriers of *S. aureus*, colonizing the organism persistently or transiently.

❖ **Most common site(s)** of colonization are anterior nares and oropharynx; followed by skin (abraded), vagina, axilla, and perineum. These colonization sites serve as a reservoir for future infections

❖ The rate of colonization is higher among insulin-dependent diabetics, HIV-infected patients, patients undergoing hemodialysis, injection drug users, and individuals with skin damage

❖ Overall, *S. aureus* is a leading cause of healthcare-associated infections. In hospitals, the healthcare professionals are the potential carriers of *S. aureus*. Hospital strains are often multidrug resistant, spread to patients either from hospital staff/other patients/environment or also from patient's own endogenous flora.

Table 51.2: Clinical spectrum of *Staphylococcus aureus* infections.**Skin and soft tissue infections**

S. aureus is one of the most common cause of various skin and soft tissue infections such as:

- **Folliculitis:** Infection of the hair follicle, characterized by a pus point surrounded by induration and erythema (Fig. 51.1A)
- **Furuncle (boil):** Painful pustular lesion in hairy, moist regions due to infection of the hair follicle that extends to become true abscess
- **Carbuncle:** Severe, painful lesion in the lower neck region, extending to the deeper subcutaneous tissue
- **Abscess:** Collection of pus, appears painful and swollen; surrounded by erythema (Fig. 51.1B)
- Mastitis and breast abscess (in nursing mothers)
- **Impetigo:** It mainly occurs in children, usually appears as red sores on the face, that bursts and develops into **honey-colored crusts**
- Surgical site wound infections (most common cause)
- Cellulitis (inflammation of skin and subcutaneous tissue)—usually develops around a wound or an ulcer
- **Hidradenitis suppurativa:** A recurrent follicular infection in areas rich in apocrine glands, such as the axilla
- **Botryomycosis:** It is mycetoma-like condition, characterized by subcutaneous swelling, sinuses, and discharge containing granules (Chapter 58)

Note:

- The pustular lesions of *S. aureus* infection usually heals quickly when the pus is drained. The fibrinous wall around the abscess tend to prevent spread of the organisms and therefore, care should be taken not to break the fibrinous wall by manipulation or trauma
- Predisposing factors to *S. aureus* cutaneous infections are—chronic skin conditions (e.g. eczema), skin damage (trauma, injections) or poor personal hygiene

Musculoskeletal infections

S. aureus is the most common cause of various conditions such as:

- Septic arthritis (most commonly involved joints are knees, shoulders, hips, and phalanges)
- Osteomyelitis (most commonly affected site in children is long bones and in adults is vertebrae)
- **Pyomyositis** (skeletal muscle infection): Mainly seen in tropical climates, but also occurs in HIV-infected patients
- **Abscess:** Psoas abscess and epidural abscess

Respiratory tract infections

- Ventilator-associated pneumonia in adults
- Septic pulmonary emboli
- Postviral pneumonia (e.g. influenza)
- Empyema and pneumothorax
- Pneumatocele (shaggy, thin-walled cavities in lungs) in neonates: *S. aureus* is the most common cause

Bacteremia and its complications

- Sepsis, septic shock
- Central line associated blood stream infection (CLABSI): *S. aureus* and CoNS are the leading causative agents
- Metastatic foci of infection involving kidney, joints, bone and lung
- Infective endocarditis:
 - Native-valve endocarditis (left-sided)—*S. aureus* is the most common cause
 - Prosthetic-valve endocarditis
 - Intravenous drug use associated endocarditis (right sided)—*S. aureus* is the most common cause
 - Nosocomial endocarditis—associated with increased use of central line

UTI (Urinary tract infection)

- Staphylococcal UTI and pyelonephritis usually occur secondary to bacteremia
- Rarely UTI is seen following instrumentation and insertion of catheter or implants

Toxin-mediated illnesses

S. aureus causes the following toxin mediated diseases (as described earlier):

- Toxic shock syndrome
- Food poisoning
- Staphylococcal scalded-skin syndrome

Infections associated with CA-MRSA (Community associated methicillin-resistant *Staphylococcus aureus*)

Skin and soft tissues are the most common sites for colonization of CA-MRSA strains; about 5–10% of strains are invasive and can be life-threatening. Examples include:

- Necrotizing pneumonia
- Sepsis with Waterhouse–Friderichsen syndrome or purpura fulminans (*S. aureus* is rare cause; most commonly caused by meningococcus).
- Necrotizing fasciitis (*S. aureus* and *Streptococcus pyogenes* are the common causative agents)



Figs 51.1A and B: A. Staphylococcal folliculitis; B. Staphylococcal abscess (ruptured).

Source: Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

LABORATORY DIAGNOSIS

S. aureus infections

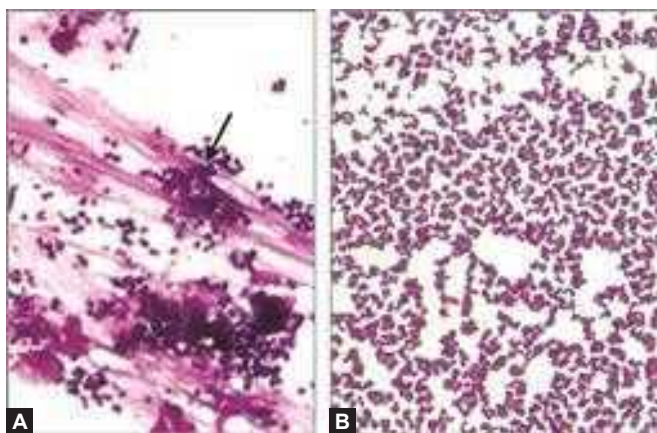
- ❑ **Direct smear microscopy:** Gram-positive cocci in clusters and pus cells
- ❑ **Culture**
 - Nutrient agar—golden yellow pigmented colonies
 - Blood agar—colonies with narrow zone of β -hemolysis
 - Selective media—such as mannitol salt agar, salt milk agar and Ludlam's medium
- ❑ **Culture smear microscopy:** Gram-positive cocci in clusters
- ❑ **Biochemical identification:**
 - Catalase test—positive
 - Coagulase test (slide and tube)—positive
 - Protein A detection
- ❑ **Identification by automated systems** such as VITEK and MALDI-TOF
- ❑ **Antimicrobial susceptibility testing**

Laboratory Diagnosis

Sample Collection

It depends on the nature of the lesion.

- ❖ Common specimens are pus, wound swab, sputum, midstream urine and blood



Figs 51.2A and B: A. Direct smear: arrow showing gram-positive cocci in clusters with pus cells; B. Culture smear showing gram-positive cocci in clusters.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ For blood culture, automated blood culture bottles are preferred
- ❖ For food poisoning: Feces, vomitus and toxin-contaminated food are collected
- ❖ For carriers, nasal swabs are recommended.

Direct Smear Microscopy

Gram staining of pus or wound swab reveals pus cells with gram-positive cocci in clusters (Fig. 51.2A). However, direct microscopy is of no value when *S. aureus* is a part of normal flora in the sample (e.g. sputum or feces).

Culture

Specimens are inoculated onto various media and incubated overnight at 37°C aerobically. The colony morphology is observed as follows:

- ❖ **Nutrient agar:** Colonies are 1–3 mm in size, circular, smooth, convex, opaque and easily emulsifiable. Most strains produce golden yellow non-diffusible **pigments** (made up of β carotene) (Fig. 51.3A)
- ❖ **Nutrient agar slope:** It produces golden yellow colonies of confluent growth, looks like oil paint appearance
- ❖ **Blood agar:** Colonies are similar to that on nutrient agar, in addition surrounded by a narrow zone of β -hemolysis (best observed in sheep blood agar) (Fig. 51.3B)
- ❖ **MacConkey agar:** Small pink colonies are produced due to lactose fermentation
- ❖ **Liquid medium (e.g. peptone water):** It produces uniform turbidity
- ❖ **Selective media:** They are useful when staphylococci are expected to be scanty or outnumbered by other bacteria in the sample (e.g. swabs from carriers, feces). Salt is added to the media, as it is inhibitory to other bacteria but not to staphylococci. Examples include:
 - Mannitol salt agar (nutrient agar with 7.5% NaCl)
 - Others: Salt milk agar and Ludlam's medium.

Culture Smear Microscopy

Gram staining from the colonies shows gram-positive cocci (1 μ m), arranged in clusters (Fig. 51.2B). Hanging drop reveals non-motile cocci.

Biochemical Tests for Identification

Catalase Test

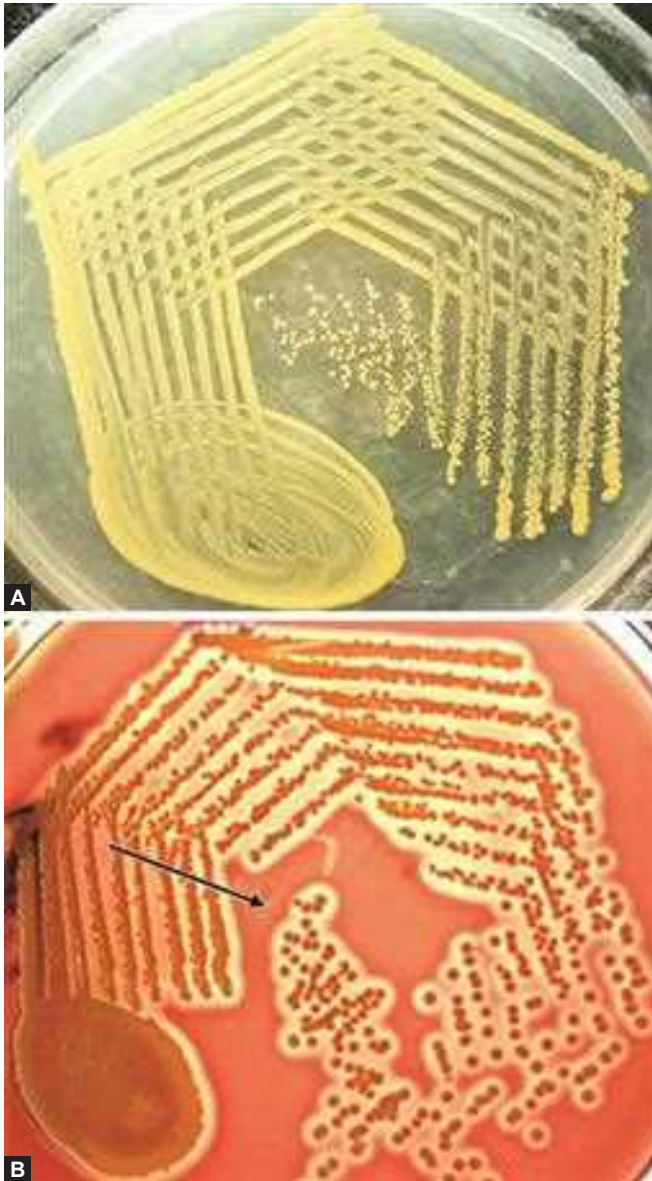
Staphylococci are catalase positive, which differentiates them from streptococci (catalase negative).

Tests to Differentiate *S. aureus* from CoNS

S. aureus can be differentiated from CoNS (coagulase-negative staphylococci) by various tests such as coagulase test and detection of protein A.

Coagulase Test

It is the most commonly performed biochemical reaction for identification of *S. aureus*.



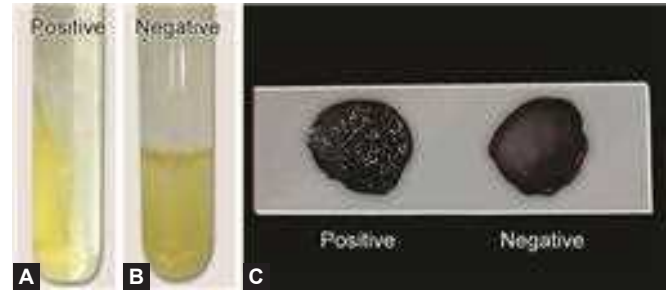
Figs 51.3A and B: Colonies of *S. aureus*: **A.** Nutrient agar—shows golden-yellow pigmented colonies; **B.** Blood agar—arrow shows narrow zone of beta-hemolysis surrounding the colonies.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Tube Coagulase Test

It detects free coagulase secreted by *S. aureus*.

- ❖ **Procedure:** Colony of *S. aureus* is emulsified in plasma in a test tube and incubated at 37°C for 4 hours
- ❖ **Positive test** is indicated by formation of a clot that does not flow when the test tube is tilted (Fig. 51.4A)
- ❖ The **negative tubes** (no clot formation) should be incubated overnight and re-examined as some strains may produce a delayed clot (Fig. 51.4B)
- ❖ This test is different from slide coagulase test, which is mediated by clumping factor.



Figs 51.4A to C: Coagulase test: **A.** Tube coagulase test (positive); **B.** Tube coagulase test (negative); **C.** Slide showing coagulase test.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Slide Coagulase Test

It detects clumping factor (i.e. bound coagulase).

- ❖ **Procedure:** A colony of *S. aureus* is emulsified with a drop of normal saline on a slide to form a milky white suspension. Then a loopful of plasma is added and mixed properly
- ❖ **Positive result:** It is indicated by formation of coarse clumps (Fig. 51.4C); whereas it remains as milky-white suspension if test is negative.

Protein A detection

Protein A is present on cell wall of *S. aureus*, which can be detected by latex agglutination test, which differentiates it from CoNS. It is a rapid test, result can be obtained within 5 minutes.

Automation for Identification

Automated systems such as VITEK and MALDI-TOF can also be used for rapid and precise identification.

Typing of *S. aureus*

Typing of *S. aureus* to subspecies level is done for epidemiological purpose to trace the source of infection. It is especially useful in outbreaks such as food poisoning affecting a larger community. Typing methods include both:

- ❖ Phenotypic methods such as bacteriophage typing and antibiogram typing
- ❖ Genotypic methods such as PCR-RFLP (restricted fragment length polymorphism), ribotyping, PFGE (pulse field gel electrophoresis) and sequence based typing.

Bacteriophage Typing

Phage typing involves differentiating strains of *S. aureus* into subspecies level based on their susceptibility to bacteriophages (pattern method of phage typing).

- ❖ **Phage type 80/81** is most commonly associated with outbreaks in hospitals. It is known as epidemic strain of *S. aureus*
- ❖ With the advent of molecular typing methods, phage typing has become obsolete nowadays.

Antimicrobial Susceptibility Test

As *S. aureus* develops resistance to antibiotics readily, drugs should be prescribed according to the antimicrobial susceptibility test. It is performed by disk diffusion method (on Mueller–Hinton agar) or by automated MIC detection method by microbroth dilution (e.g. VITEK).

Drug Resistance in *S. aureus* (Resistance to β -lactam Antibiotics)

Staphylococcus aureus shows resistance to β -lactam antibiotics in various way.

Production of β -lactamase Enzyme

β -lactamase or penicillinase enzymes cleave the β -lactam rings, and there by organisms producing these enzymes develop resistance to β -lactam antibiotics.

- ❖ This resistance is **plasmid coded**, can be transferred between *S. aureus* strains by **transduction**
- ❖ It is produced by >90% of strains of *S. aureus*
- ❖ This resistance can be overcome by addition of β -lactamase inhibitors such as clavulanic acid or sulbactam.

By Alteration of Penicillin-binding Protein (PBP)

It is shown by MRSA strains of *S. aureus*.

TREATMENT

S. aureus infections

Since *S. aureus* rapidly develops drug resistance, antibiotics should be cautiously chosen.

Parenteral therapy for serious infections

Sensitive to penicillin	DOC: Penicillin G
Methicillin sensitive <i>S. aureus</i> (MSSA)	DOC: Anti-staphylococcal penicillins such as nafcillin and cloxacillin <i>Note:</i> Vancomycin is inferior in terms of efficacy against MSSA, when compared to anti-staphylococcal penicillins; hence should not be prescribed for MSSA
Methicillin resistant <i>S. aureus</i> (MRSA)	DOC: Vancomycin (15–20 mg/kg bd) Alternative drugs: Discussed under MRSA treatment box

Empirical therapy (MRSA status not yet known): Vancomycin with/without an aminoglycoside (vancomycin is indicated only if MRSA risk is high or condition is serious, e.g. cardiac implant).

Oral therapy for skin and soft tissue infections

Sensitive to methicillin	<input type="checkbox"/> Dicloxacillin <input type="checkbox"/> Cephalexin/cefazolin
Resistant to methicillin (MRSA)	Clindamycin Alternative drugs: Cotrimoxazole, doxycycline, Linezolid

Abbreviations: DOC, drug of choice; bd, twice a day.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin resistance in *S. aureus* is mediated by a chromosomally coded gene called ***mecA* gene**, which alters penicillin-binding protein (PBP) present on *S. aureus* cell membrane to PBP2a.

- ❖ PBP is an essential protein needed for cell wall synthesis of bacteria. β -lactam drugs bind and inhibit this protein, thereby inhibiting the cell wall synthesis
- ❖ The altered PBP2a of MRSA strains has less affinity for β -lactam antibiotics; hence, MRSA strains are resistant to all β -lactam antibiotics
- ❖ **Risk factors:** Outbreaks of MRSA occur both in hospital and community settings. Risk factors common to these outbreaks include poor hygienic conditions, close contact, contaminated material, and individuals with damaged skin
- ❖ **Prevalence:** MRSA infection rate has been increasing over last few decades, though it varies from place to place
 - **World:** MRSA rates are higher (>50%) in America, some Asian and European countries and Malta. Countries with lowest MRSA rates are Netherlands and Scandinavia (<1%)
 - **In India,** the MRSA rate varies with years and place. In 2019, it was reported as 42% (by ICMR).

Types of MRSA

MRSA are either community or hospital associated (Table 51.3).

Detection of MRSA

- ❖ Antimicrobial susceptibility test using cefoxitin disk or oxacillin (MIC based method): These antibiotics are used as a surrogate marker for the identification of MRSA
- ❖ PCR detecting *mecA* gene
- ❖ Latex agglutination test detecting PBP2a.

TREATMENT

MRSA infections

- Vancomycin is the drug of choice for MRSA
- Alternative drugs include—teicoplanin, linezolid, daptomycin, telavancin and quinupristin/dalfopristin

Contd...

Table 51.3: Types of MRSA.

Community-associated MRSA (CA-MRSA)	Hospital-associated MRSA (HA-MRSA)
These strains express <i>mecA</i> gene subtype IV, V, VI	These strains express <i>mecA</i> gene subtype I, II, III
They are usually more virulent and express several toxins such as Panton Valentine (PV) toxin	They are multidrug resistant (but their virulence is relatively low)
They cause invasive skin and soft tissue infections such as necrotizing fasciitis (see Table 51.2)	They cause perioperative wound infections in hospitals and nosocomial outbreaks (hospital staff are the major carries)

Note: CA-MRSA and HA-MRSA terminologies are losing their relevance; as many CA-MRSA strains have been isolated in hospitals and vice versa.

Contd...

TREATMENT**MRSA infections**

- Linezolid is useful for skin and soft-tissue infection
- Daptomycin is indicated for endocarditis and complicated skin infections, not effective for pneumonia
- ❑ However, even drugs such as tetracycline, erythromycin or cotrimoxazole may also be effective in nonlife-threatening infections
- ❑ Antimicrobial susceptibility testing is necessary before an alternative drug is used

All β -lactam drugs should be avoided. However, 5th generation cephalosporins, such as ceftobiprole, ceftaroline and ceftolozane have shown some activity against MRSA.

Resistance to Vancomycin (VRSA and VISA)

Erroneous and overuse of vancomycin has led to the emergence of resistance to vancomycin. It may be of low grade resistance, known as VISA (vancomycin intermediate *S. aureus*) or high-grade resistance, known as VRSA (vancomycin-resistant *S. aureus*).

- ❖ The MIC (minimum inhibitory concentration) of vancomycin to VISA and VRSA isolates are 4–8 and >16 $\mu\text{g}/\text{mL}$ respectively. The current guidelines recommend consideration of alternative drugs if vancomycin MIC is >1 $\mu\text{g}/\text{mL}$, as treatment failure has been frequently observed beyond this MIC level
- ❖ VRSA is very rare. In India, it is reported from few places such as Hyderabad, Kolkata and Lucknow. However, VISA is more frequently reported than VRSA
- ❖ **Mechanisms:** VRSA is mediated by *van A* gene; whereas VISA is due to increase in cell wall thickness of *S. aureus*. The *van A* gene is believed to be acquired from a vancomycin-resistant strain of *Enterococcus faecalis* by horizontal conjugal transfer
- ❖ **Fitness cost:** Acquisition of a *van* gene is often associated with compensatory mutations in the genes responsible for survival which results in a reduced fitness of *S. aureus*. In contrast, 'fitness cost phenomenon' is not commonly observed in MRSA. This explains why VRSA is seen very rarely (<0.1%), whereas MRSA prevalence is so common (30–40%)
- ❖ **Treatment** of VRSA should be based on antimicrobial susceptibility report. Linezolid, telavancin, daptomycin and quinupristin/dalfopristin are the effective drugs.

Control Measures

Prevention of spread of *S. aureus* infections in hospitals involves:

- ❖ **Screening of MRSA carriers** among hospital staff should be done when there is an outbreak. Mannitol oxacillin agar is the preferred medium for this purpose

- ❖ **Treatment of carriers** is done by use of topical 2% mupirocin (for nasal carriers) and chlorhexidine body bath (for skin carriers)
- ❖ **Stoppage of antibiotic misuse** in hospitals
- ❖ Ensure proper infection control measures such as **hand hygiene** (most efficient way to prevent hospital spread), isolation of the patients and all other measures of contact precautions (described in detail in Chapter 21).

COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

Most of the coagulase-negative staphylococci (CoNS) are harmless commensals and less virulent than *S. aureus*; however, recently their role as pathogen is increasingly being reported.

Staphylococcus epidermidis

It is the most common CoNS (75–80%), isolated from clinical samples. It is present as normal flora on the skin, oropharynx and vagina; however, its pathogenic role is greatly enhanced in presence of prosthetic-devices.

- ❖ **Pathogenesis:** *S. epidermidis* involves a two-step process:
 1. **Initial adhesion to the prosthetic device:** The surface adhesins of the organism bind to host serum or tissue constituents, such as fibrinogen or fibronectin, coated on the implanted prosthetic surfaces
 2. **Colonization:** *S. epidermidis* can produce the extracellular polysaccharide material (glycocalyx or slime) that facilitates formation of a protective **biofilm** on the device surfaces. Biofilm appears to act as a barrier, protecting bacteria from host defense mechanisms as well as from antibiotics.
- ❖ **Manifestation:** *S. epidermidis* is the most common cause of prosthetic-device related infections, such as endocarditis with insertion of valvular prosthesis and ventricular shunt infections. It is also a common cause of stitch abscess
- ❖ It is coagulase negative, but positive for phosphatase test.

Staphylococcus saprophyticus

It causes urinary tract infection (UTI) in sexually active young women (Chapter 76).

Staphylococcus lugdunensis and Staphylococcus schleiferi

Recently, these organisms have been associated with more serious infections such as native-valve endocarditis and osteomyelitis. Their enhanced pathogenesis may be due to expression of virulence factors such as clumping factor (therefore, exhibit positive slide coagulase test) and lipase, which are usually absent in other CoNS.

EXPECTED QUESTIONS

I. Write essay on:

1. A 55-year-old male was admitted to the hospital with complaints of severe pain in the lateral aspect of his left calf and small amount of pus discharge from the ingrown hair. On physical examination, the local area was found to be red, warm and tender. Pus was aspirated and was subjected to Gram stain (showed gram-positive cocci in clusters), culture on blood agar (showed golden yellow pigmented beta-hemolytic colonies).
 - a. What is the clinical diagnosis and its causative organism?
 - b. List the infections caused by this organism.
 - c. List the virulence factors of this organism.
 - d. Briefly discuss the laboratory diagnosis.

II. Write short notes on:

1. Toxic shock syndrome.
2. MRSA (Methicillin-resistant *Staphylococcus aureus*).

III. Multiple Choice Questions (MCQs):

1. Scalded skin syndrome is mediated by:

- a. Hemolysin
- b. Coagulase
- c. Enterotoxin
- d. Epidermolytic toxin

2. All of the above can be given for the treatment of MRSA, except:

- a. Meropenem
- b. Vancomycin
- c. Cotrimoxazole
- d. Linezolid

3. Which is the least preferred antimicrobial for the treatment of methicillin-sensitive *S. aureus* (MSSA)?

- a. Dicloxacillin
- b. Cephalexin
- c. Cefazolin
- d. Vancomycin

4. Synergohymentropic toxins includes:

- a. α -hemolysin and panton valentine toxin

- b. β -hemolysin and panton valentine toxin
- c. γ -hemolysin and panton valentine toxin
- d. α -hemolysin and γ -hemolysin

5. *S. aureus* is differentiated from CoNS by all, except:

- a. Coagulase test
- b. DNase test
- c. Catalase test
- d. Protein A detection

6. Which sugar fermentation test differentiates *S. aureus* from CoNS?

- a. Glucose
- b. Sucrose
- c. Lactose
- d. Mannitol

7. All the following beta-lactam drugs can be given for the treatment of MRSA, except:

- a. Ceftaroline
- b. Ceftobiprole
- c. Piperacillin-tazobactam
- d. Ceftolozane

8. About MRSA all are true, except:

- a. In India, the MRSA prevalence in hospital is around 30–40%
- b. MRSA rates are higher in India than in America
- c. Mediated by *MecA* gene
- d. Cefoxitin disk is superior to oxacillin for detection

9. About VRSA all are true, except:

- a. VRSA is mediated due to Van gene
- b. VISA is due to increased cell wall thickening
- c. VRSA is more common than VISA
- d. Fitness cost phenomena is seen in VRSA

10. Community-associated MRSA (CA-MRSA) differs from hospital-associated MRSA by all, except:

- a. These strains express *mecA* gene subtype IV, V, VI
- b. Express more Pantone Valentine (PV) toxin
- c. They cause more invasive skin and soft tissue infection
- d. Multidrug resistant

11. *Staphylococcus epidermidis*, all are true, except:

- a. Accounts for 75% of CoNS
- b. Phosphatase negative
- c. Produces biofilm
- d. Causes stitch abscesses

Answers

1. d 2. a 3. d 4. c 5. c 6. d 7. c 8. b 9. c 10. d 11. b

CHAPTER PREVIEW

■ *Streptococcus pyogenes* Infections

■ Other β -hemolytic Streptococcal Infections

INTRODUCTION

Family Streptococcaceae are catalase negative gram-positive cocci, arranged in pairs or chains (due to single plane of division). *Streptococcus*, *Enterococcus* and pneumococcus are the important members of this family. However, according to the molecular structure, *Enterococcus* is now reclassified under separate family Enterococcaceae.

Streptococci are part of normal flora. However, some are important human pathogens, such as *Streptococcus pyogenes* causing pyogenic infections, *S. agalactiae* causing meningitis in newborn and *S. pneumoniae* causing pneumonia and meningitis in all age groups.

History

Billroth coined the term 'streptococci' (*streptos* meaning twisted or coiled), Ogston differentiated them from staphylococci and Rosenbach coined the species *S. pyogenes*; as it causes pyogenic infection.

Classification

Streptococci can be classified into—obligate anaerobes (e.g. *Peptostreptococcus*, described in Chapter 53) and aerobes and facultative anaerobes group. The latter can be further classified based on the hemolysis produced on 5% sheep blood agar into α , β and γ -hemolytic streptococci (Fig. 52.1).

- ❖ **α -hemolysis:** It is due to partial lysis of red blood cells (RBCs), producing a small (1-2 mm) zone of greenish discoloration surrounding the colonies. It is observed with viridans streptococci and pneumococci
- ❖ **β -hemolysis:** It is due to complete lysis of RBCs and zone of lysis is wide (2-4 mm). It is observed with *S. pyogenes* and other β -hemolytic streptococci
- ❖ **γ -hemolysis:** It is a misnomer, there is no hemolysis surrounding the colonies, hence no change in color, e.g. *Enterococcus*.

Lancefield grouping: The β -hemolytic streptococci were further classified by **Rebecca Lancefield** (1933) based on

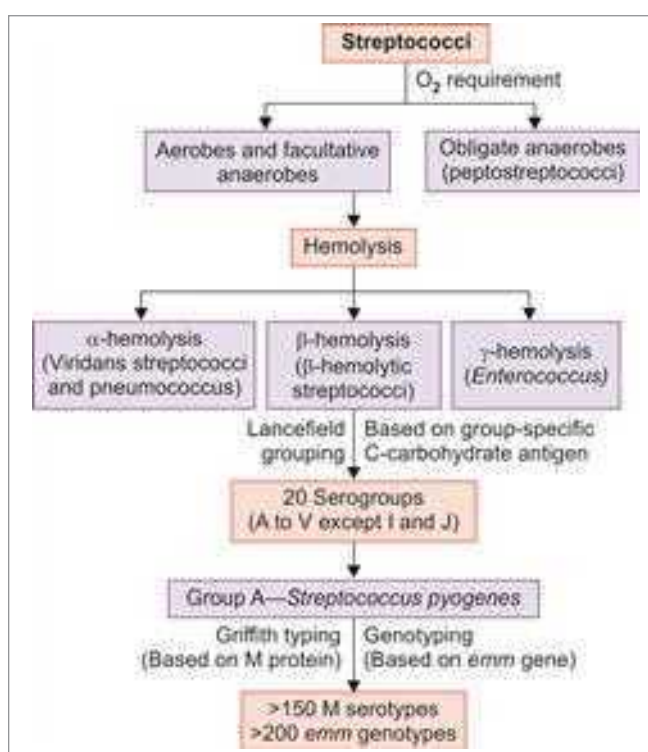


Fig. 52.1: Classification of family Streptococcaceae.

C-carbohydrate antigen present in the cell wall into 20 serological groups named as group A-V (except I and J).

Griffith typing: Majority of streptococci causing human infections belong to group A (*S. pyogenes*), which can be further classified into more than 150 serotypes based on M protein present in their cell wall.

Genotyping: Based on *emm* gene (gene encoding M protein); group A streptococci can be typed into >200 genotypes.

STREPTOCOCCUS PYOGENES INFECTIONS

S. pyogenes is the only species under Lancefield's group A *Streptococcus* (GAS). It is associated with a variety of

suppurative infections and can also trigger post-infectious nonsuppurative complications such as acute rheumatic fever and acute glomerulonephritis.

Virulence Factors and Pathogenicity

Virulence factors of *S. pyogenes* can be categorized into cell wall antigens, toxins and enzymes.

Cell Wall Antigens

Cell wall of streptococci is primarily composed of peptidoglycan layers, in which a variety of substances are embedded such as carbohydrates, teichoic acids, lipoproteins, and surface protein antigens.

- ❖ **Lipoteichoic acid:** Helps in adhesion to pharyngeal epithelial cells, and to other host cells and proteins such as fibronectin
- ❖ **C-carbohydrate antigens:** They are group-specific antigens, forms the basis of Lancefield grouping
- ❖ **M protein:** It is the principle virulence factor of group A *Streptococcus*. For details, refer the highlight box
- ❖ **Other cell wall proteins** such as:
 - Fimbriae and **F factor** (fibronectin binding protein)- help in adhesion
 - M protein-associated protein (**opacity factor**)- associated with skin infections.
- ❖ **Capsule:** Some strains of group A *Streptococcus* are capsulated, made up of hyaluronic acid
 - These strains produce mucoid colonies
 - Capsule is antiphagocytic, but not antigenic
 - It helps group A streptococci to colonize the pharynx by binding to CD44, a hyaluronic acid-binding protein expressed on epithelial cells.

M protein

It is the principle virulence factor of group A *Streptococcus*.

- It inhibits complement mediated opsonisation by phagocytes
- Antibody to M protein is protective in nature and promotes phagocytosis
- It complexes with fibrinogen that consequently bind to β_2 integrins of neutrophils, leading to release of inflammatory mediators which induce vascular leakage; causing streptococcal toxic shock syndrome
- Some M protein types are cross-reactive to human myocardial antigens and play an important role in the pathogenesis of rheumatic fever.

Toxins

Hemolysins

β -hemolytic streptococci such as group A, C and G produce two hemolysins—streptolysin-O and streptolysin-S (Table 52.1). They cause RBC membrane lysis, that leads to complete β -hemolysis surrounding the colonies. They can damage the membranes of neutrophils and platelets.

Table 52.1: Differences between streptolysin-O and streptolysin-S.

Streptolysin (SL-O)	Streptolysin (SL-S)
Oxygen labile (hence named as streptolysin-O) Heat labile	Oxygen stable Serum soluble (hence named as streptolysin-S)
Strongly antigenic	Not antigenic
Antistreptolysin-O antibodies (ASO) are raised in most of the streptococcal infections and are used as a standard marker for retrospective diagnosis of streptococcal infections (except in glomerulonephritis and pyoderma where ASO titer is low)	Not useful for serological diagnosis of streptococcal infections

Streptococcal Pyrogenic Exotoxin (SPE)

It is so named because it induces fever (pyrogenic). It is responsible for the pathogenesis of certain streptococcal infections such as scarlet fever, necrotizing fasciitis and toxic shock syndrome.

- ❖ It can be typed into three well-described antigenic distinct subtypes—SPE-A, B and C
- ❖ SPE-A and C are bacteriophage coded; whereas SPE-B is chromosomally mediated
- ❖ SPE-A and C are superantigens; like staphylococcal toxin (TSST-1), they also act as T cell mitogens which induce a massive release of cytokines causing fever, shock and tissue damage.

Enzymes

Streptokinase (Fibrinolysin)

It activates plasminogen to plasmin, thus breaks down the fibrin barrier around the infected site, thereby facilitating the spread of infection.

- ❖ Antibodies to streptokinase can be used for retrospective diagnosis of streptococcal infection
- ❖ **Therapeutic use:** Being fibrinolytic, this toxin can be used in the treatment of myocardial infarction and other thromboembolic disorders.

Streptodornase (DNase)

It breaks down the DNA, thus helps in liquefying the thick pus (containing large amount of DNA derived from nuclei of necrotic cells) and may be responsible for the serous nature of streptococcal exudates.

- ❖ **Therapeutic use:** Preparation containing streptodornase and streptokinase can be used to liquefy the thick exudates in empyema cases
- ❖ **Subtypes:** Streptodornase has four distinct subtypes DNase-A, B, C, and D; of which type-B is most antigenic
- ❖ **Diagnostic use:** Anti-DNase B antibodies can be used for retrospective diagnosis of infection, particularly the skin infections (pyoderma) and acute glomerulonephritis where ASO titer is low.

Other Enzymes

- ❖ **Hyaluronidase (spreading factor):** It breaks down the hyaluronic acid present in tissues, thus helps in the spread of infection along the intercellular space
- ❖ **Others** include NADase (nicotinamide adenine dinucleotidase), serine protease, C5a peptidase, neuraminidase, N-acetylglucosaminidase, esterase and phosphatase.

Clinical Manifestations

Group A *Streptococcus* (GAS) produces both suppurative and non-suppurative manifestations (Table 52.2). Throat is the primary site of invasion by GAS. Infection occurs through respiratory droplets.

Suppurative Infections

Respiratory Infections

Streptococcus pyogenes is the most common bacterial cause of **pharyngitis (sore throat)** in children; and accounts for 5 to 15% of all sore throats in adults. Infection occurs through respiratory droplets. It can rarely cause other respiratory infections such as scarlet fever (pharyngitis and rashes), pneumonia or empyema. Refer Chapter 60 for detail.

Superficial Skin and Soft Tissue Infections

Impetigo (Pyoderma)

It is a superficial infection of the skin, caused primarily by group A *Streptococcus* and occasionally by other species of streptococci or *S. aureus*.

- ❖ Risk factors include young children, warmer months, tropical climates, poor hygiene, colonization by group A *Streptococcus* and minor trauma
- ❖ Most common sites involved are face (nose and mouth) and legs



Figs 52.2A and B: Streptococcal skin infections: **A.** Impetigo; **B.** Erysipelas on malar area of face (peau d'orange skin).

Source: A. Wikipedia/Åsa Thörn, B. Public Health Image Library, Atlanta, ID# 2874/ Dr Thomas F Sellers, Emory University/Centers for Disease Control and Prevention (CDC) (with permission).

- ❖ Individual lesions begin as red papules, which evolve quickly into vesicles and then pustular lesions that break down and coalesce to form characteristic thin papery **honeycomb-like crusts** (Fig. 52.2A). Lesions are painless and not associated with fever.

Cellulitis

It involves the skin and subcutaneous tissues.

Erysipelas: It is a form of cellulitis, characterized by a tender, bright red, swollen and indurated *peau d'orange* texture of involved skin (due to involvement of the superficial lymphatics) along with fever and chills. Superficial blebs or bullae may form later.

- ❖ Most common sites are malar area of the face and the lower extremities (Fig. 52.2B)
- ❖ Recurrences are common, occur after many years, involving the same site.

Deep Soft Tissue Infections

Necrotizing Fasciitis

It is also known as **hemolytic streptococcal gangrene**. It involves the superficial and/or deep fascia invading the muscles (Fig. 52.3).

- ❖ **Source:** The source of the infection may be of two types:
 1. Traumatized skin: Most commonly caused by group A *Streptococcus* alone or along with *S. aureus*
 2. Gastrointestinal tract breach: It occurs due to abdominal surgery releasing the bowel flora. It is polymicrobial, involving anaerobic flora and gram-negative bacilli like *E. coli*.
- ❖ GAS is the most common cause, accounting for nearly 60% of total cases of necrotizing fasciitis. Common serotypes include M types 1 and 3 which produce streptococcal pyrogenic exotoxins
- ❖ The onset is acute and rapid, and is marked by severe pain with minimal erythema at the site of involvement. Patients present with malaise, fever, chills, and a toxic appearance in contrast to cellulitis, where the skin appears more abnormal, but tenderness is mild

Table 52.2: Suppurative and non-suppurative manifestations of *Streptococcus pyogenes*.

Suppurative	Non-suppurative
Respiratory infections (Chapter 60): <ul style="list-style-type: none"> • Pharyngitis/sore throat • Scarlet fever • Others (rare): Pneumonia, empyema, quinsy, sinusitis and otitis media 	Acute rheumatic fever Acute glomerulonephritis
Skin and soft tissue infections (Superficial): <ul style="list-style-type: none"> • Impetigo (pyoderma) • Cellulitis and erysipelas 	Guttate psoriasis Reactive arthritis
Deep soft tissue infections: <ul style="list-style-type: none"> • Necrotizing fasciitis • Streptococcal myositis 	PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections)
Bacteremia leading to endocarditis, osteomyelitis, septic arthritis, meningitis, etc.	
Toxic shock syndrome	
Puerperal sepsis (rare)	



Fig. 52.3: Necrotizing fasciitis of leg.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ Later on (over several hours), disease tends to be more severe. Skin becomes dusky or develops mottled erythema and anesthetized (due to infarction of the cutaneous nerves induced by spreading inflammatory process) with extensive necrosis of subcutaneous tissue, fascia and muscle (hence, GAS is also called as **flesh eating bacteria**).

Bacteremia

Streptococcal bacteremia occurs secondary to necrotizing fasciitis, rarely with pharyngitis or cellulitis or pneumonia. It leads to variety of focal infections including endocarditis, meningitis, septic arthritis, osteomyelitis, peritonitis, visceral abscesses and toxic shock syndrome.

Toxic Shock Syndrome (TSS)

Group A *Streptococcus* producing pyrogenic exotoxins may cause TSS secondary to soft tissue infection such as necrotizing fasciitis.

- ❖ In contrast to patients with staphylococcal TSS, the majority with streptococcal TSS are bacteremic
- ❖ The case definition of TSS includes: (i) isolation of *S. pyogenes* plus, (ii) hypotension plus, (iii) multiorgan (≥ 2) involvement.

Puerperal Sepsis

Being colonizer of female vagina, streptococci are often associated with infectious complications of childbirth, usually endometritis and associated bacteremia. Group B streptococci and anaerobic streptococci are more common to cause puerperal sepsis than GAS.

Non-suppurative Complications

Streptococcal antigens show molecular mimicry with human antigens (Table 52.3). Due to antigenic cross reactivity, antibodies produced against previous streptococcal infections cross react with the human tissue to produce lesions. This accounts for a number of non-suppurative complications such as:

Table 52.3: Antigenic cross reactivity between streptococcal antigens and the corresponding human antigens.

Streptococcal antigen	Human antigen
Cell wall M protein (of serotypes M1, M5, M6, and M19)	Myocardium (tropomyosin and myosin)
Cell wall C carbohydrate	Cardiac valves
Cytoplasmic membrane	Glomerular vascular intima
Peptidoglycan	Skin antigens
Hyaluronic acid	Synovial fluid

- ❖ Acute rheumatic fever
- ❖ Post-streptococcal glomerulonephritis (PSGN)
- ❖ Guttate psoriasis
- ❖ Reactive arthritis
- ❖ Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus pyogenes* (PANDAS).

Acute Rheumatic Fever

Acute rheumatic fever (ARF) occurs in people previously infected with streptococcal (group A) sore throat, as a result of an autoimmune reaction. It is a multisystem disease, causing cardiac (valvular damage), rheumatologic and neurologic manifestations. Patients have elevated streptococcal anti-streptolysin O antibodies (Chapter 28).

Post-streptococcal Glomerulonephritis (PSGN)

It typically occurs in children following streptococcal pyoderma (or rarely pharyngitis); characterized by lodging of antigen-antibody complexes on the glomerular basement membrane followed by complement activation. Patients have elevated streptococcal anti-DNase B antibodies. It is discussed in Chapter 76.

Epidemiology

Humans are the natural reservoir for group A *Streptococcus*. It is highly communicable, affecting all age groups. Disease in neonates is uncommon, due to protective maternal antibody. Pharyngitis is more common in children of 3–15 years of age. Outbreaks occur commonly in areas with close contacts, such as schools and military barracks, etc.

LABORATORY DIAGNOSIS *S. pyogenes* infections

- ❑ **Specimen collection and transport:** Depends on the site of the infection
- ❑ **Direct smear microscopy:** Pus cells with gram-positive cocci in short chains
- ❑ **Culture:**
 - Blood agar: Pinpoint colony with a wide zone of β -hemolysis
 - Selective media: Crystal violet blood agar
- ❑ **Culture smear:** Gram-positive cocci in short chains
- ❑ **Identification:**
 - Biochemical identification: It is catalase negative, bacitracin sensitive, CAMP test negative
 - Automated methods such as MALDI-TOF and VITEK

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L LABORATORY DIAGNOSIS*S. pyogenes* infections

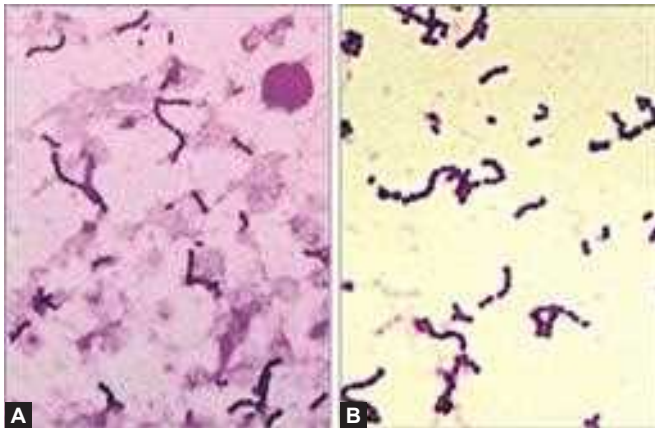
- ❑ **Typing:**
 - Lancefield grouping: Shows group A *Streptococcus*
 - Typing of group A *Streptococcus*: Griffith and *emm* typing
- ❑ **Serology:** ASO antibodies and anti-DNase B antibodies
- ❑ **Antimicrobial susceptibility testing.**

Laboratory Diagnosis of GAS Infections**Specimen Collection and Transport**

It depends on the site of the lesion. Common specimens are pus swab, exudates and blood. For blood culture, blood is inoculated into blood culture bottles. In streptococcal TSS, blood cultures are usually positive. In case of pharyngitis, throat swabs are useful specimen (Chapter 60, for detail).

Direct Smear Microscopy

Gram staining of pus or wound swab reveals pus cells with gram-positive cocci (0.5–1 μm) in chains (Fig. 52.4A).



Figs 52.4A and B: Streptococci: **A.** In Gram stained smear of pus; **B.** In culture smear showing gram-positive cocci in chains.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

However, direct microscopy is not useful when *S. pyogenes* is a part of normal flora in the sample (e.g. throat swab).

Culture

The specimens are inoculated onto various media and incubated overnight at 37°C aerobically in presence of 5–10% CO_2 . *S. pyogenes* is fastidious, does not grow on MacConkey agar and basal media like nutrient agar or peptone water broth. It grows only in media enriched with blood, serum or carbohydrate.

- ❖ **Blood agar:** Colonies are small 0.5–1 mm, pinpoint, with a wide zone of β -hemolysis (Fig. 52.5A)
- ❖ **Selective media** such as crystal violet blood agar can be used, which selectively allows the growth of *S. pyogenes*.

Culture Smear Microscopy

Gram stained smear from the colonies show gram-positive spherical cocci (0.5–1 μm), arranged in chains (Fig. 52.4B). Hanging drop reveals non-motile cocci.

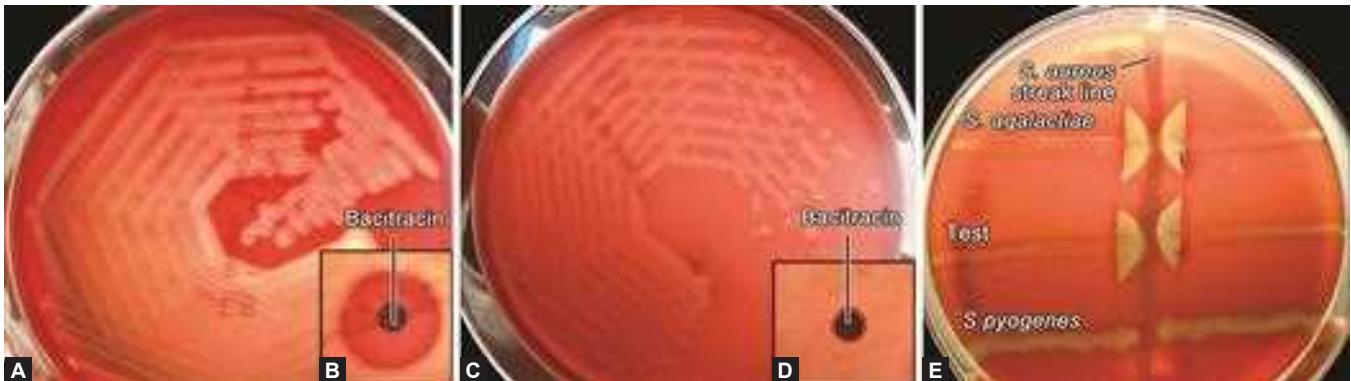
Biochemical Tests for Identification

- ❖ **Catalase test:** Streptococci are catalase negative. This test differentiates them from staphylococci which are catalase positive
- ❖ **Bacitracin sensitivity testing:** Group A *Streptococcus* is sensitive to bacitracin 0.04 U disk (any zone of inhibition around the disk is considered as positive test), while most of other β -hemolytic streptococci are resistant. Hence, it can be used as a rapid diagnostic test for GAS (Fig. 52.5B).

Various tests to differentiate GAS from group B β -hemolytic streptococci are tabulated in Table 52.4.

Automation for Identification

Automated systems such as VITEK and MALDI-TOF can also be used for rapid and precise identification.



Figs 52.5A and B: *Streptococcus pyogenes*: **A.** Growth on blood agar with wide zone of beta-hemolysis around the pin point colonies; **B.** Bacitracin sensitive.

Figs 52.5C to E: *Streptococcus agalactiae*: **C.** Growth on blood agar with wide zone of beta-hemolysis around the colonies; **D.** Bacitracin resistant; **E.** CAMP test positive.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Table 52.4: Differences between *Streptococcus pyogenes* and *S. agalactiae*.

Characters	<i>S. pyogenes</i>	<i>S. agalactiae</i>
Lancefield grouping	Group A	Group B
Bacitracin sensitivity test	Sensitive	Resistant
CAMP test*	Negative	Positive
β-hemolytic colonies	0.5–1 mm, pin point	Mucoid, slightly larger (2 mm)

*CAMP: Christie, Atkins, and Munch-Peterson test.

Lancefield Grouping

The biochemical identification of Group A *Streptococcus* can be further confirmed by Lancefield grouping. Lancefield grouping is extremely useful in epidemiological studies. Here, the β-hemolytic streptococci are grouped serologically based on C-carbohydrate antigen. Test involves extraction of C-carbohydrate antigen followed by testing with group specific antisera.

Typing of Group A Streptococci

Group A *Streptococcus* can further be typed based on two methods; phenotypic method, i.e. serological (Griffith typing) and genotypic method (*emm* typing).

Serology

In rheumatic fever and poststreptococcal glomerulonephritis (PSGN), a retrospective diagnosis of streptococcal infection may be established by detecting antibodies in patient's serum.

- ❖ **ASO (Anti-streptolysin O) antibodies:** ASO titer is elevated (>200 IU/mL) in most of the streptococcal infections except pyoderma and PSGN. It is detected by latex agglutination test and nephelometry method
- ❖ **Anti-DNase-B antibodies:** Titer more than 300–350 IU/mL is diagnostic of PSGN and pyoderma.

Antimicrobial Susceptibility Test (AST)

AST is performed by disk diffusion method (on Mueller-Hinton agar with 5% sheep blood) or by automated MIC detection method by microbroth dilution (e.g. VITEK).

TREATMENT

S. pyogenes infections

Penicillin is the drug of choice for pharyngeal infections as well as for suppurative infections. Resistance to penicillin is not reported yet.

- ❑ However, failure to penicillin treatment may occur due to: (i) noncompliance, if discontinued before 10 days of full course of oral penicillin V, (ii) β-lactamases produced by normal throat flora such as *Moraxella*
- ❑ Macrolide, such as erythromycin is given to patients allergic to penicillin. However, resistance to macrolides is common
- ❑ Treatment shortens the clinical syndrome, at least modestly, reduces transmission to others, decreases the risk of

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TREATMENT

S. pyogenes infections

suppurative and non-suppurative sequelae such as ARF. However, treatment does not reduce the risk of PSGN. Treatment of streptococcal SSTIs is outlined in Table 52.5.

OTHER β-HEMOLYTIC STREPTOCOCCAL INFECTIONS

Group B Streptococci (*S. agalactiae*)

Pathogenesis and Clinical Manifestations

Approximately 5–40% of women are vaginal or rectal carriers of group B *Streptococcus* (GBS). Hence, the GBS infection is common in neonates and in pregnancy.

- ❖ Group B *Streptococcus* has been recognized as a major cause of neonatal sepsis and meningitis (Chapter 71)
- ❖ Infections in pregnancy can lead to peripartum fever, endometritis and puerperal sepsis (Chapter 77)
- ❖ Infections in adults generally involve elderly or people with underlying chronic illness, such as diabetes mellitus or malignancy. Common infections are cellulitis and soft tissue infections (including infected diabetic skin ulcers), UTI, pneumonia and endocarditis
- ❖ Group B *Streptococcus* has a capsular polysaccharide which can be typed into ten serotypes.

Laboratory Diagnosis

Isolation of GBS from blood culture and CSF culture are the mainstay of diagnosis. GBS is catalase negative like all streptococci, but exhibits the following biochemical properties that differentiate it from group A *Streptococcus* (Table 52.4).

- ❖ **Colonies:** The colonies of GBS are β-hemolytic, similar to that of *S. pyogenes*; however they are mucoid and slightly larger (2 mm) (see Fig. 52.5C)
- ❖ **CAMP positive:** CAMP factor (named after the discoverers—Christie, Atkins-Munch-Petersen) is a phospholipase produced by GBS that enhances the

Table 52.5: Treatment of *S. pyogenes* skin and soft-tissue infections.

Conditions	Treatment recommended
Impetigo and PSGN	Benzathine penicillin G, IM single dose; or Oral penicillin V for 10 days
Erysipelas/cellulitis	Mild: Procaine penicillin Severe: Penicillin G
Necrotizing fasciitis	Surgical debridement (most crucial) + Penicillin G + Clindamycin
Streptococcal toxic shock syndrome	Penicillin G + Clindamycin + Intravenous immunoglobulin (against streptococcal pyrogenic exotoxin)

Abbreviations: IM, intramuscular; PSGN, poststreptococcal glomerulonephritis.

hemolysis of *S. aureus*. When GBS is streaked on blood agar plate perpendicular to *S. aureus*, an enhanced arrowhead-shaped hemolysis is produced at their junction, pointing towards *S. aureus* streak line (see Fig. 52.5E)

- ❖ Bacitracin resistant (see Fig. 52.5D)
- ❖ Species identification can be confirmed by Lancefield serogrouping and automations such as MALDI-TOF

TREATMENT

S. agalactiae infections

Penicillin/ampicillin plus gentamicin are the drug of choice for all GBS infections.

Group C and G Streptococci

Group C and G streptococci comprise of the following species.

- ❖ ***S. dysgalactiae* subsp. *equisimilis***: It carries both group C and G antigens. This is the most common species to infect humans, causes pharyngitis, cellulitis and soft tissue infections, pneumonia, bacteremia, endocarditis, and septic arthritis, UTI, meningitis and puerperal sepsis
- ❖ ***S. equi***: It carries group C antigen; has two subspecies equi and zooepidemicus
 - Both cause infections animals, especially horses and cattle; rarely cause human infections through consumption of unpasteurized milk or contact with infected animals
 - Spectrum of disease include pneumonia, bacteremia, endocarditis, meningitis and septic arthritis.
- ❖ **Diagnosis**: They produce β -hemolytic colonies on blood agar of >0.5 mm size. Species identification is made by Lancefield serogrouping and automations such as MALDI-TOF
- ❖ **Treatment**: Penicillin is the drug of choice.

Group F Streptococci

They are also called **minute streptococci**. They grow poorly on blood agar, occasionally cause suppurative infection.

Streptococcus MG is an α -hemolytic strain belonging to this group. Demonstration of antibodies to *Streptococcus* MG in the patient's sera has been used for diagnosis of primary atypical pneumonia (caused by *Mycoplasma pneumoniae*).

Group D Streptococci

Group D streptococci comprise of enterococci (fecal streptococci, described below) and non-enterococci (*S. gallolyticus* and *S. equinus*). Both groups possess the common group D lipoteichoic acid antigen and give positive bile esculin hydrolysis test; but differ in many properties. Compared to *Enterococcus*, non-enterococcal Group D are:

- ❖ More susceptible to antibiotics such as penicillin
- ❖ Do not grow in presence of 6.5% salt
- ❖ Less pathogenic to humans
- ❖ Not a part of human intestinal flora.

S. gallolyticus (formerly *S. bovis*) and *S. infantarius* are commensal of intestine of animals. In humans, they occasionally cause endocarditis associated with colorectal cancer or polyps. Penicillin is the drug of choice.

ENTEROCOCCUS AND OTHER STREPTOCOCCI

Enterococcus and other streptococci principally cause infections of various body sites; therefore, they are discussed under the respective infective syndromes.

- ❖ ***Enterococcus***: It is non or γ -hemolytic, found as normal flora of human intestine; causes widespread infection including UTI (Chapter 76), meningitis, intra-abdominal infection and endocarditis
- ❖ **Viridans streptococci**: They are α -hemolytic, found as normal flora in oral cavity; may occasionally cause dental caries and endocarditis (subacute bacterial endocarditis) (Chapter 28)
- ❖ ***Streptococcus pneumoniae***: It is α -hemolytic, primarily causes pneumonia (Chapter 61), spreads further to cause various invasive diseases such as bacteremia and meningitis. It can also cause local infections such as otitis media and sinusitis.

EXPECTED QUESTIONS

I. Write essay on:

1. Chinu, a 3-year-old girl from Mangaluru presented with tender, bright red, subcutaneous swelling on malar area of the face with indurated peau d'orange texture of involved skin along with fever and chills. A clinical diagnosis of cellulitis was made. The culture of the aspirated pus revealed beta-hemolytic pin point colonies.
 - a. What is the most likely etiologic agent?
 - b. Describe the virulence factors and the other clinical manifestations produced by the etiological agent?
 - c. Briefly discuss the laboratory diagnosis of this clinical condition.

Answers

1. a 2. b 3. b

II. Write short note on:

1. Necrotizing fasciitis.

III. Multiple Choice Questions (MCQs):

1. **Serotyping of *Streptococcus pyogenes* is based on which of the following protein?**
 - a. M protein
 - b. T protein
 - c. R protein
 - d. Carbohydrate antigen
2. ***Streptococcus pyogenes* can be differentiated from *S. agalactiae* by testing susceptibility to:**
 - a. Optochin
 - b. Bacitracin
 - c. Polymyxin
 - d. Novobiocin
3. **CAMP test is useful in identification of:**
 - a. *S. pyogenes*
 - b. *S. agalactiae*
 - c. *S. pneumoniae*
 - d. Viridans streptococci

Gas Gangrene and Infections due to Non-sporing Anaerobes

CHAPTER 53

CHAPTER PREVIEW

- Clostridial Infections
- *Clostridium perfringens* Infections
- Non-sporing Anaerobic Infections

INTRODUCTION

Anaerobic bacteria do not have cytochrome system for oxygen metabolism and hence are unable to neutralize toxic oxygen metabolites. Therefore, they either cannot grow in presence of oxygen (**obligate anaerobes**) or do not utilize oxygen but tolerate its presence (**aerotolerant anaerobes**). Anaerobes need special requirements to grow in culture such as:

- ❖ **Anaerobic condition:** This can be achieved by various methods such as (Chapter 3.3):
 - McIntosh and Fildes's anaerobic jar
 - GasPak system
 - Anoxomat system
 - Anaerobic glove box workstation
 - Pre-reduced anaerobically sterilized (PRAS) media.
- ❖ **Medium with low redox potential:** This can be achieved by adding to the media with reducing substances such as unsaturated fatty acid, ascorbic acid, glutathione, cysteine, glucose, sulfites and metallic iron.

Obligate anaerobes can be grouped into spore bearing (e.g. *Clostridium*) and non-sporing anaerobes (described later in this chapter).

Clinical Presentations of Anaerobic Infections

Anaerobic infections are associated with various clinical clues, such as:

- ❖ Infections adjacent to mucosal surfaces that bear anaerobic flora
- ❖ Predisposing factors such as ischemia, tumor, penetrating trauma, foreign body, or perforated viscus
- ❖ Spreading gangrene involving skin, subcutaneous tissue, fascia, and muscle
- ❖ Foul smelling putrid pus
- ❖ Abscess formation
- ❖ Septic thrombophlebitis
- ❖ Toxemia and fever not marked
- ❖ Failure to respond to antibiotics that do not have significant anaerobic activity
- ❖ Organisms are seen under Gram stain, but fail to grow in routine aerobic culture

- ❖ **Special features** may be observed such as:

- Gas in specimen (gas gangrene)
- Black pigment that fluoresce (*Prevotella melaninogenica*)
- Sulfur granules (*Actinomyces*).

Laboratory Diagnosis of Anaerobic Infections

Specimens

All clinical specimens for anaerobic culture must be handled meticulously as brief exposure to oxygen may kill obligate anaerobes and result in failure to isolate them in the laboratory.

- ❖ Accepted specimens: Tissue bits, necrotic materials, aspirated body fluids or pus in syringes
- ❖ Unacceptable specimens: All swabs, sputum or voided urine
- ❖ Specimens should be immediately put into RCM broth or other anaerobic transport media and brought to the laboratory as soon as possible.

Robertson's cooked meat (RCM) broth

It is the most commonly used anaerobic media. It contains chopped meat particles (beef heart), which provide glutathione and unsaturated fatty acids, which take up oxygen and create lower redox potential and thus permit the growth of obligate anaerobes (Fig. 53.1A).

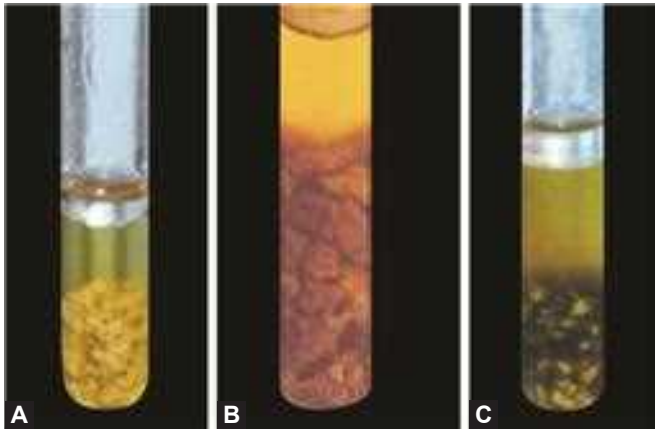
Microscopy

All clinical specimens from suspected anaerobic infections should be Gram stained and examined for the characteristic morphology.

Cultural Identification

Samples should be processed immediately under anaerobic condition which can be created by various methods as described earlier.

- ❖ **Culture:** Various culture media can be used for the isolation of anaerobes, such as:
 - Anaerobic blood agar and Neomycin blood agar
 - Egg yolk agar and Phenylethyl agar (PEA)



Figs 53.1A to C: Robertson cooked meat broth: **A.** Uninoculated; **B.** Pink and turbid (*C. perfringens*); **C.** Black and turbid (*C. tetani*).

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- BHIS agar: Brain–heart infusion agar added with supplements, such as vitamin K and hemin
- *Bacteroides bile esculin* agar (BBE agar).
- ❖ **Identification of anaerobes** is based on:
 - Biochemical tests
 - Susceptibility to antibiotic disks
 - Gas liquid chromatography
 - Automated systems such as MALDI-TOF.

TREATMENT

Anaerobic infections

Common antibiotics given for anaerobic infections are:

- Metronidazole plus penicillin- DOC for odontogenic infections
- Carbapenems (e.g. meropenem)
- β -lactam/ β -lactamase inhibitor combination (amoxicillin-clavulanate, piperacillin-tazobactam)
- Clindamycin (in case of penicillin allergy)

Choice of antibiotics depends on the site of infection, severity of infection, type of anaerobe involved and susceptibility to antibiotics. Antimicrobial resistance in anaerobic bacteria is an increasing problem.

CLOSTRIDIAL INFECTIONS

Clostridia are gram-positive bacilli, having bulging spores (in contrast to the genus *Bacillus* which has nonbulging spores), and encompass more than 60 species.

- ❖ Clostridia are saprophytes found in soil, fresh water, marine water, decaying vegetation, animal matter and sewage; thus play a major role in recycling of organic matter

- ❖ They are also harbored in intestine of vertebrates and invertebrates including human beings
- ❖ However, few members may cause a variety of infections in humans such as:

- *C. perfringens*: Causes gas gangrene, an edematous myonecrosis like condition
- *C. tetani*: Causes tetanus; mediated by a neurotoxin (tetanus toxin) which causes skeletal muscle spasm and autonomic nervous system disturbance (Chapter 72)
- *C. botulinum*: Produces a powerful neurotoxin called botulinum toxin that causes **botulism**, characterized by flaccid paralysis of muscles. It presents in three clinical forms—food poisoning, wound infection and infant botulism (Chapter 40)
- *C. difficile*: Causes pseudomembranous colitis in hospitalized patients who have a history of prolonged intake of broad spectrum antibiotics (Chapter 43).
- ❖ **Spore:** In clostridia, the spores (Fig. 53.2) are wider than the vegetative bacteria giving rise to swollen or spindle-shaped appearance (*Clostridium* is named from the word 'Kloster' meaning spindle). Spore formation occurs in unfavorable conditions. Most of the clostridia bear a subterminal spores (e.g. *C. perfringens*) except:
 - *C. bifermentans*: Produces central and oval spore
 - *C. tetani*: Produces spherical and terminal spore (drum stick appearance)
 - *C. tertium*: Produces oval and terminal spore (tennis racket appearance).

- ❖ **In RCM broth:** Clostridia grow well in RCM broth, producing turbidity in the medium which may be of two types (Figs 53.1B and C):

1. Proteolytic clostridia turn the meat black and produce foul odor, e.g. *C. tetani*, *C. botulinum* A, B and F
2. Saccharolytic species turn the meat pink, e.g. *C. perfringens*, *C. difficile* and *C. botulinum* C, D and E.

CLOSTRIDIUM PERFRINGENS INFECTIONS

C. perfringens (previously, *C. welchii*) is a commensal in the large intestine of human beings and animals. It is also found as saprophyte in soil, dust and air.

- ❖ It is capsulated, non-motile, gram-positive bacillus
- ❖ It bears subterminal bulging spores; but does not produce spores in tissues or in culture media (especially the gas gangrene strains)
- ❖ It is invasive as well as toxigenic.



Fig. 53.2: Types of spores of various *Clostridia* species.

Virulence Factors

The virulence factors produced by *C. perfringens* can be grouped into different types.

- ❖ **Four major toxins:** Alpha (α), beta (β), epsilon (ϵ), and iota (ι)
- ❖ Eight minor toxins: Gamma (γ), delta (δ), lambda (λ), kappa (κ), theta (θ), eta (η), mu (μ), and nu (ν)
- ❖ They also produce heat labile enterotoxin
- ❖ Soluble substances are produced such as neuraminidase, histamine, bursting factor (produce muscle lesions) and circulating factor (inhibit phagocytosis).

Clinical Manifestations

C. perfringens infections are mostly polymicrobial involving other clostridia species. Various manifestations include:

Clostridial Wound Infections

MacLennan has classified them as follows:

- ❖ **Simple wound contamination:** It involves the wound surface contamination, without invasion of underlying tissue, as occurs in absence of devitalized tissue
- ❖ **Anaerobic cellulitis:** It involves the fascial plane with minimal toxin release, without muscle invasion
- ❖ **Anaerobic myositis (gas gangrene):** Muscle invasion occurs, which leads to gas in the muscle compartment with abundant toxin release (described later).

Clostridial Enteric Infections

- ❖ **Food poisoning:** It is caused by *C. perfringens* type A enterotoxin (coded by *cpe* gene)
 - It occurs following consumption of improperly cooked contaminated meat. Spores being heat resistant survive and germinate later when the food is cooled
 - Infective dose: About 10^8 viable vegetative bacilli producing enterotoxin are required to initiate the infection
 - Enterotoxin acts by forming pores in the intestinal mucosal membrane
 - Diagnosis: By detection of enterotoxin in feces by enzyme immunoassay.
- ❖ **Enteritis necroticans** (gas gangrene of the bowel): It is a life-threatening condition characterized by ischemic necrosis of the jejunum and gas in the tissue plane
 - It is also known as **pigbel** in Papua New Guinea and **darmbrand** in Germany
 - It is caused by *C. perfringens* type C strains, producing β toxin.
- ❖ **Necrotizing enterocolitis:** It resembles enteritis necroticans but is associated with *C. perfringens* type A and has been found in North America
- ❖ **Gangrenous appendicitis.**

Other Clostridial Infections

- ❖ **Bacteremia:** *C. perfringens* followed by *C. tertium* and *C. septicum* are commonly associated with bacteremia

- ❖ **Skin and soft-tissue infections:** *C. perfringens*, *C. histolyticum*, *C. septicum*, *C. novyi*, and *C. sordellii* cause necrotizing infections of the skin and soft tissues
- ❖ Infection of the endometrium leading to toxic shock syndrome—can be associated with *C. sordellii*
- ❖ Meningitis and brain abscess
- ❖ Panophthalmitis (due to *C. sordellii* or *C. perfringens*).

Gas Gangrene

Definition (Oakley, 1954)

Gas gangrene is defined as a rapidly spreading, edematous myonecrosis, occurring in association with severely crushed wounds contaminated with pathogenic clostridia, particularly with *C. perfringens*. Previously, the disease was called **malignant edema** or **clostridial myonecrosis**.

Etiological Agents

Gas gangrene is always polymicrobial and is caused by many clostridial species.

- ❖ **Established agents:** *C. perfringens* (most common, 60% of the total cases) and *C. novyi* and *C. septicum* (20–40%)
- ❖ **Probable agents:** They are less commonly implicated, e.g. *C. histolyticum*, *C. sporogenes*, *C. fallax*, *C. bifementans*, *C. sordellii*, *C. aerofoetidum*, and *C. tertium*.

Pathogenesis

The development of gas gangrene requires:

- ❖ **Anaerobic environment:** Crushing injuries of muscles such as road traffic accidents (causing laceration of large or medium-sized arteries), open fractures of long bones or foreign bodies (bullet injuries) or devitalized tissues lead to interruption in the blood supply and tissue ischemia. Anoxic muscles start utilizing pyruvate anaerobically to produce lactic acid
- ❖ **Contamination of wound** with clostridial spores present in the soil (during war or road traffic accident) or clothes
- ❖ Rarely, spontaneous **non-traumatic gas gangrene** occurs via hematogenous seeding of normal muscle with bowel clostridia, as occurs in people with gastrointestinal pathologies (e.g. colonic malignancy).

Virulence Factors Mediating Gas Gangrene

Toxins produced by *C. perfringens*

Once introduced *C. perfringens* proliferates locally and elaborates exotoxins, chiefly α -toxin and θ -toxin.

- ❑ **α -toxin** is the principle virulence factor. It has both phospholipase C and sphingomyelinase activities
 - It activates the platelet adhesion molecule **GpIIb/IIIa** and neutrophil receptors **CD11b/CD18**, leading to formation of aggregates of platelets and neutrophils in the blood vessels causing occlusion
 - α -toxin directly suppresses myocardial contractility leading to reduction in the cardiac output and results in profound hypotension

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- ❑ **θ-toxin** causes marked vasodilation by activating mediators (e.g. prostacyclin, platelet-activating factor).

Toxins produced by other Clostridia

- ❑ *C. septicum* produces four main toxins: α -toxin (lethal, hemolytic, necrotizing activity), β -toxin [deoxyribonuclease (DNase)], γ -toxin (hyaluronidase), and septicolysin, protease and neuraminidase
- ❑ *C. novyi* has four subtypes (A–D). Type-A produces bacteriophage coded alpha-toxin, which commonly causes gas gangrene.

Clinical Manifestations of Gas Gangrene

The **incubation period** is variable. Depending upon the nature of injury, the amount of wound contamination and the type of clostridial species involved, the incubation period varies. For example:

- ❖ 10–48 hours for *C. perfringens*
- ❖ 2–3 days for *C. septicum*
- ❖ 5–6 days for *C. novyi*.

Various manifestations include:

- ❖ Sudden onset of excruciating pain at the affected site
- ❖ Rapid development of a foul-smelling thin serosanguineous discharge
- ❖ Gas bubbles (**crepitus**) in the muscle planes (Fig. 53.3)
- ❖ Brawny edema and induration
- ❖ Such gangrenous tissues later may become liquefied and sloughed off
- ❖ Shock and organ failure develop later
- ❖ Associated with higher mortality rate (50%).

LABORATORY DIAGNOSIS

Gas gangrene

- ❑ **Specimen:** Necrotic tissues, muscle fragments and exudates from deeper parts of the wound
- ❑ **Direct microscopy:** Thick, stubby, boxcar-shaped gram-positive bacilli without spore are suggestive of *C. perfringens*
- ❑ **Culture:**
 - Media: Robertson cooked meat broth, egg yolk agar, etc.
 - Incubation: Anaerobically (by GasPak or Anoxomat, etc.)
- ❑ **Identification of *C. perfringens***
 - Target hemolysis (double zone hemolysis)
 - Nagler's reaction: Opalescence surrounding the streak line on egg yolk agar
 - Reverse CAMP test: Positive
 - Heat tolerance test: Positive
 - In litmus milk: Produces stormy clot reaction.

Laboratory Diagnosis of Gas Gangrene

Based on the clinical diagnosis of gas gangrene, treatment should be started as early as possible. Laboratory diagnosis has role only for (1) confirmation of the clinical diagnosis, (2) species identification.



Fig. 53.3: Gas gangrene of the right leg showing swelling and discoloration of the right thigh with bullae, and palpable crepitus.

Source: Wikipedia/Cases/Engelbert Schröpfer, Stephan Rauthe and Thomas Meyer (with permission).

Specimen

Ideal specimens are necrotic tissues, muscle fragments and exudates from deeper part of the wound, where the infection appears to be more active.

- ❖ Blood culture may be positive for *C. perfringens* and *C. septicum*. However, *C. perfringens* bacteremia can occur even in the absence of gas gangrene
- ❖ Swabs rubbed over the wound surface or soaked in exudates are not satisfactory
- ❖ Specimens should be put into Robertson's cooked meat broth and transported immediately to the laboratory.

Direct Microscopy

Gram stained films provide clues about the species of clostridia present. Absence of neutrophils in the infected tissues is a characteristic feature.

- ❖ Thick, stubby, boxcar-shaped, gram-positive bacilli without spore—suggestive of *C. perfringens* (Fig. 53.4)

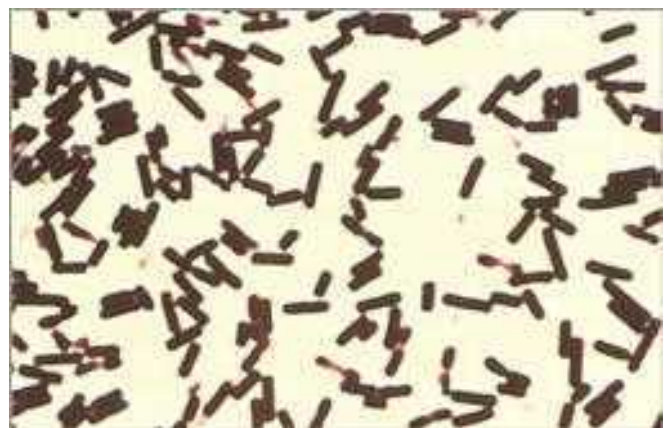


Fig. 53.4: Gram stained smear of *Clostridium perfringens*.

Source: Public Health Image Library/ID# 11196, Don Stalons/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ Spore bearing gram-positive bacilli suggest other clostridia species
 - Citron bodies (boat or leaf-shaped pleomorphic irregularly stained bacilli with spores)—suggest *C. septicum*
 - Large rods with oval sub-terminal spores—suggest *C. novyi*.

Identification

C. perfringens can be further identified by the following properties. Culture plates should be incubated anaerobically at 37°C for 2 days.

- ❖ **Target hemolysis** (double zone hemolysis, Fig. 53.5A): On blood agar, *C. perfringens* produce an inner narrow zone of complete hemolysis (due to θ -toxin), surrounded by a much wider zone of incomplete hemolysis (due to the alpha toxin)
- ❖ **Nagler's reaction:** *C. perfringens* produces an opalescence surrounding the streak line on egg yolk agar or media containing 20% human serum (due to lecithinase activity of α -toxin). Opalescence can be inhibited by incorporating anti- α -toxin to the medium (Fig. 53.5B). The test is also positive for *C. bifermentans*, *C. baratti* and *C. sordellii* (all produce α -toxin)
- ❖ **Reverse CAMP test:** *C. perfringens* is streaked over the center of blood agar plate and *Streptococcus agalactiae* is streaked perpendicular to it. Presence of enhanced zone of hemolysis (arrow-shaped) pointing towards *C. perfringens* indicates the test is positive (Fig. 53.5C)
- ❖ **Heat tolerance:** *C. perfringens* can grow when RCM broth is incubated at 45°C for 4–6 hours. This differentiates it from other organisms in the specimen
- ❖ In litmus milk, *C. perfringens* produces “**stormy clot reaction**” due to fermentation of lactose producing acid and vigorous gas.

Automated methods such as MALDI-TOF is currently the method of choice of rapid and accurate identification of various clostridia species.

TREATMENT

Gas gangrene

- ❑ **Early surgical debridement** is the most crucial step in the management of gas gangrene. All devitalized tissues should be widely resected so as to remove conditions that produce anaerobic environment. Closure of wounds should be delayed for 5–6 days until the sites are free from infection
- ❑ **Antibiotics:** Combination of penicillin and clindamycin is recommended for 10–14 days
- ❑ **Hyperbaric oxygen:** It may kill the obligate anaerobic clostridia such as *C. perfringens*; however, it has no effect on aerotolerant clostridia (*C. septicum*)
- ❑ **Passive immunization** with anti- α -toxin antiserum.

NON-SPORING ANAEROBIC INFECTIONS

Medically important non-sporing anaerobes can be classified into gram-positive and gram-negative groups (Table 53.1).

Beside the list, there are several other anaerobes that occur in soil and water and may be of industrial and agricultural importance.

Anaerobes as normal flora

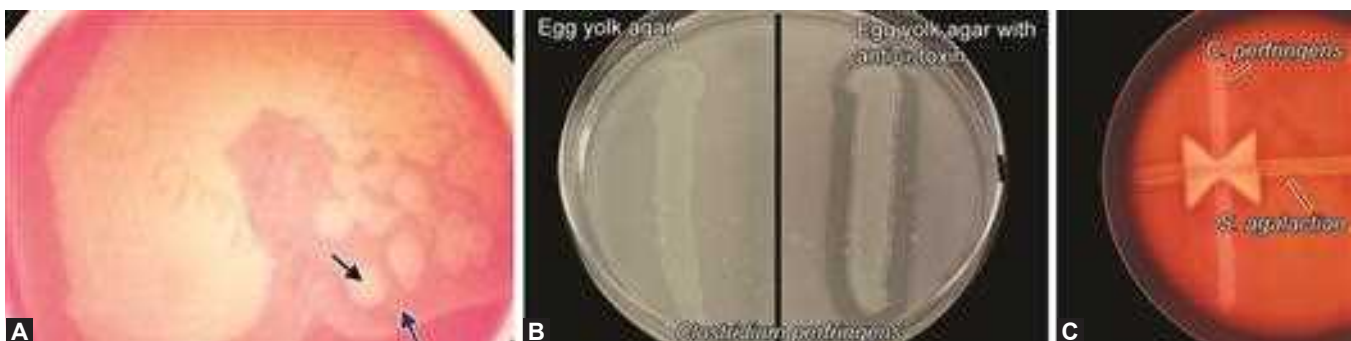
Non-sporing anaerobes are often a part of normal flora of mouth, GIT, skin and genital tract of man. Common examples include:

- ❑ **Oral cavity:** Anaerobic cocci, *Actinomyces*, *Fusobacterium*, *Bifidobacterium*, *Prevotella* and Spirochetes

Contd...

Table 53.1: Classification of non-sporing anaerobes

Gram-positive cocci	Gram-negative cocci
<ul style="list-style-type: none"> • <i>Peptostreptococcus</i> • <i>Peptococcus</i> 	<ul style="list-style-type: none"> • <i>Veillonella</i>
Gram-positive bacilli	Gram-negative bacilli
<ul style="list-style-type: none"> • <i>Bifidobacterium</i> • <i>Eubacterium</i> • <i>Propionibacterium</i> • <i>Lactobacillus</i> • <i>Actinomyces</i> • <i>Mobiluncus</i> 	<ul style="list-style-type: none"> • <i>Bacteroides</i> • <i>Prevotella</i> • <i>Porphyromonas</i> • <i>Fusobacterium</i> • <i>Leptotrichia</i>
	Spirochetes (Chapter 77, 32)
	<ul style="list-style-type: none"> • <i>Treponema</i>, <i>Borrelia</i>



Figs 53.5A to C: A. Target hemolysis of *C. perfringens*—zone of incomplete hemolysis (blue arrow) and zone of complete hemolysis (black arrow); B. Nagler's reaction; C. Reverse CAMP test.

Source: A and B. Department of Microbiology, JIPMER, Puducherry; C. Dr Padmaja A Shenoy, Department of Microbiology, Kasturba Medical College, Manipal, Karnataka (with permission).

Contd...

- ❑ **Stomach:** *Lactobacillus*
- ❑ **Intestine:** Anaerobic cocci, *Bacteroides fragilis*, *Fusobacterium*, *Bifidobacterium* and *Prevotella*
- ❑ **Vagina:** Anaerobic cocci, *Lactobacillus*, *Prevotella* and *Bifidobacterium*
- ❑ **Skin:** *Propionibacterium*.

Beneficial Role

Being a part of normal flora, they modulate various physiological functions of human beings.

- ❑ They compete with the pathogenic bacteria through depletion of nutrients and production of enzymes and toxic metabolites
- ❑ They modulate host's intestinal innate immune response
- ❑ *Bacteroides* ferment carbohydrates and produce volatile fatty acids that are used by the host as an energy source
- ❑ They are responsible for the production of vitamin K and bile acids in the intestine
- ❑ Polysaccharide A of *Bacteroides fragilis* influences the normal development and function of immune system and protects against inflammatory bowel disease
- ❑ Lactobacilli maintain the vaginal acidic pH which prevent colonization of pathogens.

CLINICAL PRESENTATIONS

Anaerobic infections occur when the harmonious relationship between the host and the bacteria is disrupted. Disruption of skin and mucosal barrier by surgery, trauma, tumor, ischemia, or necrosis allows the penetration of many anaerobes, resulting in various infections.

Anaerobic Cocci

The anaerobic cocci occur as normal flora of skin, mouth, intestine and vagina.

- ❖ ***Peptococcus* and *Peptostreptococcus*** are gram-positive cocci arranged in pair or chain. They are normal flora of skin, mouth, intestine and vagina. However, they are recovered from various clinical infections such as puerperal sepsis, skin and soft tissue infections and brain abscess
- ❖ ***Veillonellae*** are small gram-negative cocci, occurring in pairs or short chains. They are usually nonpathogenic. Rarely, they can cause infections like meningitis, endocarditis, and osteomyelitis.

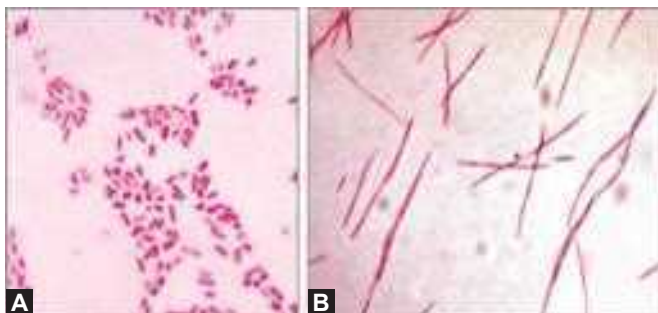
Gram-positive Non-sporing Anaerobic Bacilli

- ❖ ***Bifidobacterium* species:** They are non-motile, pleomorphic bacilli that frequently exhibit branching. The name is derived from their typical appearance (bifid Y-shaped cells). They occur as beneficial normal flora in the mouth and gut and are mostly nonpathogenic; often used as a part of probiotics
- ❖ ***Eubacterium* species:** They are commensals in mouth and intestine. They are rarely pathogenic (periodontitis)

- ❖ ***Propionibacterium* species:** They are related to corynebacteria and are usually labelled as anaerobic diphtheroids. They are skin commensals
 - *P. acnes* is the most common species, which is a common contaminant in blood and CSF. Taxonomically, it is re-classified as *Cutibacterium acne*
 - It can cause acne vulgaris and folliculitis. It can also cause prosthetic hip joint and prosthetic heart valve infections.
- ❖ ***Lactobacillus* species:** They are non-motile gram-positive bacilli that frequently show bipolar and barred staining. They are part of normal flora of mouth, gut and vagina. It is often used as a part of probiotics
 - **In stomach:** Lactobacilli in the stomach (e.g. *L. acidophilus*) synthesize vitamins, such as biotin, vitamin B12 and vitamin K, which are useful to man
 - **In the oral cavity:** Lactobacilli form acid by the fermentation of sucrose and other dietary carbohydrates which dissolve the mineral components of enamel and dentine, resulting in dental caries
 - **In vagina:** *Lactobacillus* species in adult vagina (known as Doderlein's bacilli) produce lactic acid that maintains the acidic pH of the adult vagina protecting from various infections. In prepubertal and postmenopausal vagina, lactobacilli are scanty which predispose to many infections.
- ❖ ***Actinomyces:*** They are branching filamentous anaerobic gram-positive bacilli (described in Chapter 55)
- ❖ ***Mobiluncus* species:** They are motile, curved, anaerobic gram-variable bacilli, isolated from the vagina in cases of *bacterial vaginosis*, along with *Gardnerella vaginalis*.

Gram-negative Non-sporing Anaerobic Bacilli

- ❖ ***Bacteroides fragilis:*** It is the most common commensal in the human intestine. At the same time, it is also probably the most frequent anaerobe isolated from the clinical specimens
 - They are non-sporing, non-motile, obligate anaerobes, very pleomorphic, appearing as slender rods or coccobacillary forms (Fig. 53.6A)
 - Virulence factors include—(i) Capsular polysaccharide, (ii) lipopolysaccharide, (iii) enterotoxin
 - It causes peritonitis and intra-abdominal abscess following bowel injury (most common manifestation, Chapter 47) and pelvic inflammatory disease (PID)
 - It is also implicated in brain abscesses, chronic suppurative otitis media, bacteremia, endocarditis and in empyema producing foul smelling pus
 - Enterotoxigenic strains can cause diarrhea.
- ❖ ***Prevotella:*** It has many species; grouped into:
 - Pigmented (e.g. *P. melaninogenica*):
 - ♦ Produces black or brown colored colonies
 - ♦ Colonies produce characteristic red fluorescence when exposed to ultraviolet light



Figs 53.6A and B: Gram stained smear of: **A.** *Bacteroides fragilis*; **B.** *Fusobacterium* species.

Source: A. Public Health Image Library, ID# 3084/Dr VR Dowell, Jr./Centers for Disease Control and Prevention (CDC), Atlanta; B. Microbes Wiki/Jhoman (with permission).

- ♦ It has been isolated from lung or liver abscess, mastoiditis, pelvic and genitourinary infections, and lesions of intestine and mouth.
- Nonpigmented: Examples include *P. denticola* and *P. buccalis*; which can cause periodontal disease and aspiration pneumonitis.
- ❖ **Porphyromonas:** It differs from *Bacteroides* in being asaccharolytic and pigmented
 - *P. gingivalis* is responsible for periodontal disease

- *P. endodontalis* causes dental root canal infections.
- ❖ **Fusobacterium species:** They are long, thin spindle-shaped bacilli with pointed ends (Fig. 53.6B)
 - *F. nucleatum* is a normal inhabitant of the mouth and can cause oral infection, pleuropulmonary sepsis, CNS infection (e.g. brain abscess) and septic arthritis
 - *F. necrophorum* is agent of Lemierre's syndrome (a form of thrombophlebitis).
- ❖ **Leptotrichia buccalis** (formerly called *Fusobacterium fusiforme*): They are long, thin spindle-shaped bacilli with pointed ends
 - They are part of the normal oral flora
 - They are implicated in an acute necrotizing gingivostomatitis known as **Vincent's angina**.

Vincent's angina

It is an acute necrotizing ulcerative gingivostomatitis, caused by oral anaerobes such as *Prevotella*, and *Fusobacterium* and spirochetes such as *Borrelia vincentii* (Chapter 59).

The clinical presentations, laboratory diagnosis and treatment of non-sporing anaerobic infections are similar to that of other anaerobes described at the beginning of this chapter.

EXPECTED QUESTIONS

I. Write essay on:

1. Rajesh, a 23-year-old male was admitted 5 days after a crush injury to his right leg following a road traffic accident. He had been treated by a local village quack. On examination, the wound which was bandaged with a soiled gauze, appeared to be heavily contaminated with soil, the local muscles appeared to have been crushed, there was edema and pain at the site and crepitus was felt on palpation.
 - a. What is the clinical condition? List the etiological agents responsible for this condition.
 - b. Describe in detail the pathogenesis of this condition.
 - c. Describe in detail the laboratory diagnosis of this condition.

II. Write short notes on:

1. Laboratory diagnosis of gas gangrene.
2. Non-sporing anaerobes.

Answers

1. d 2. a 3. c

III. Multiple Choice Questions (MCQs):

1. **Characteristic of anaerobic bacteria is:**
 - a. Foul smelling discharge
 - b. Fail to grow in aerobic media
 - c. Gas in tissue
 - d. All of the above
2. **Principle toxin responsible for gas gangrene is:**
 - a. Alpha toxin
 - b. Theta toxin
 - c. Beta toxin
 - d. Delta toxin
3. **The agent of Lemierre's syndrome is:**
 - a. *Leptotrichia buccalis*
 - b. *Fusobacterium fusiforme*
 - c. *Fusobacterium necrophorum*
 - d. *Fusobacterium nucleatum*

CHAPTER PREVIEW

- Clinical Manifestations
- Immune Response
- Complications
- Epidemiology
- Laboratory Diagnosis
- Treatment of Leprosy

INTRODUCTION

Mycobacterium leprae is the causative agent of leprosy; a disease of antiquity, having been recognized since long time such as:

- ❖ Vedic times in India (described as Kushta Roga in Sushruta Samhita, 600 BC)
- ❖ Biblical times in the Middle East
- ❖ Hippocrates, 460 BC.

The credit of discovery of lepra bacilli goes to **GH Armauer Hansen** (1873) in Norway. Although, *M. leprae* was the first bacterial pathogen of humans to be described, still it remains one of the least understood organisms probably because it is not cultivable. However, **Shepard** (1960) had done a breakthrough by multiplying the lepra bacilli in the footpads of mice kept at a low temperature (20°C).

Social stigma: Leprosy was once believed to be highly contagious disease.

- ❖ Due to fear, ignorance, superstitious beliefs and characteristic deformities and disfigurement produced in the patients, leprosy remained as a social stigma over many years
- ❖ Patients were considered as ‘unclean’ and socially out casted
- ❖ Today, with early diagnosis and effective treatment, patients can lead productive life in the community and the deformities can largely be prevented.

CLINICAL MANIFESTATIONS

Leprosy is a chronic granulomatous disease of humans, primarily involving cooler parts of the body (skin, peripheral nerves, upper respiratory tract, eyes, and testes, etc.), but are capable of affecting any tissue or organs causing bony deformities and disfigurements in untreated cases.

Incubation period: Leprosy has a long incubation period, an average of 5–7 years (vary between 2 and 40 years)

- ❖ This can be attributed to the longer generation time of lepra bacilli of **12–13 days** as compared to 14 hours

for tubercle bacillus and about 20 minutes for coliform bacilli

- ❖ Lepromatous cases have longer incubation period than tuberculoid cases.

Classification of Leprosy

Leprosy can be classified into various categories based on clinical, bacteriological, immunological and histological status of the patients. There are three classification schemes (Table 54.1). Following initiation of treatment or alteration of host immunity, the leprosy category of patients changes from one type to another type. There is another clinical classification, described below.

Clinical Classification

Based on the number of skin lesions, presence of nerve involvement and identification of bacilli on slit-skin smear, leprosy can be classified into two categories. This classification is used by the leprosy control program for the treatment of patients (described later).

- ❖ **Paucibacillary (PB) leprosy:** A case of leprosy which fulfills all the criteria—(i) 1 to 5 skin lesions, (ii) no nerve involvement, and (iii) slit-skin smear negative for lepra bacilli
- ❖ **Multibacillary (MB) leprosy:** A case of leprosy which fulfills any one of the criteria—(i) >5 skin lesions; or (ii) nerve involvement (neuritis); or (iii) slit-skin smear positive for lepra bacilli.

Table 54.1: Classifications of leprosy.

Ridley-Jopling classification (1966)	Madrid classification (1953)	Indian classification* (1981)
Lepromatous leprosy (LL)	Lepromatous type	Lepromatous type
Borderline Lepromatous leprosy (BL)	Borderline	Borderline
Borderline leprosy (BB)	Indeterminate type	Indeterminate type
Borderline tuberculoid leprosy (BT)	Tuberculoid type	Pure neuritic type
Tuberculoid leprosy (TT)	—	Tuberculoid type

*By Leprosy Association of India.

Characters	Lepromatous leprosy (LL)	Tuberculoid leprosy (TT)
Bacillary load	Multibacillary	Paucibacillary
Bacteriological index	4–6+	0–1+
Skin lesions	Many, symmetrical Margin is irregular Lesions appear as: <ul style="list-style-type: none"> • Multiple nodules (lepromata) • Plaques and xanthoma-like papules Leonine facies and eyebrow alopecia	One or few, asymmetrical Margin is sharp Lesions appear as: Hypopigmented, annular macules with elevated borders Tendency towards central clearing
Nerve lesion	Nerve lesions appear late Hypoesthesia is a late sign Variable nerve palsies	Early anesthetic skin lesion, Enlarged thickened nerves, Nerve abscess seen (common in BT)
CMI	Low	Normal
Lepromin test	Negative	Positive
Humoral immunity	Exaggerated	Normal
Macrophages	Foamy type (lipid-laden)	Epithelioid type
Langhans giant cells	Not seen	Found

Abbreviations: BT, borderline tuberculoid leprosy; CMI, cell-mediated immunity.

Leprosy is a bipolar disease. Under any classification scheme mentioned in Table 54.1, lepromatous and tuberculoid cases are the two extreme poles of the disease (Table 54.2).

Lepromatous Leprosy (LL)

It is seen when the host resistance is low to lepra bacilli.

- ❖ **Multibacillary disease:** Large number of acid-fast bacilli are found in large clumps (globi) inside the macrophages (lepra cells) (see Fig. 54.3)
- ❖ **Skin lesions** are many, symmetrical with irregular margin. Lesions appear as superficial nodular lesions (Fig. 54.1A) (lepromata) or plaques or xanthoma-like papules. Lesions on face produce **leonine facies** appearance. Loss of eye brow/lashes may occur late
- ❖ **Nerve lesions:** The nerve involvement occurs very late in LL patients
- ❖ **Host immunity:**
 - The CMI of the LL patient is grossly impaired. As a result the lepromin test and lymphocyte transformation test become negative
 - In contrast, the humoral immune response is exaggerated in LL patients (described later).
- ❖ **Spread:** Bacilli invade nasal mucosa and are shed in large numbers in nasal secretions. LL patients are most infectious. Later on, reticuloendothelial system, eyes,



Figs 54.1A and B: A. Nodular lesions of lepromatous leprosy; B. Hypopigmented skin lesions of tuberculoid leprosy (arrow showing).

Source: Public Health Image Library, A. ID# 15508/Dr Andre J Lebrun, B. ID# 15503/ Arthur E Kaye, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

testes, kidneys and bones are also involved. Eventually, any organ can be involved except CNS and lungs. Bacteremia is common

- ❖ **Prognosis:** LL patients have a poor prognosis.

Tuberculoid Leprosy (TT)

It is seen in patients showing a high degree of resistance to lepra bacilli.

- ❖ **Paucibacillary disease:** Bacilli are scanty in the lesions
- ❖ **Skin lesions** are few, asymmetric and sharply demarcated, consisting of hypopigmented, annular macules (anesthetic patches) with elevated borders (Fig. 54.1B)
- ❖ **Nerve lesion:** Nerve involvement occurs early. Nerves are often enlarged and thickened. Nerve abscess may be seen (common in TT and BT). Pronounced nerve damage may lead to deformities, particularly in the hands and feet
 - Most common nerves involved are **ulnar** nerve followed by post-auricular nerve
 - Medial popliteal nerve is never involved.
- ❖ **Host immunity:**
 - CMI is adequate and the lepromin test is positive
 - Humoral immune response is also normal.
- ❖ **Least infectious:** TT patients are least infectious among all types
- ❖ **Prognosis:** TT patients have a good prognosis among all the types.

Other Categories of Leprosy

- ❖ **Borderline type:** It is seen in patients possessing characteristics in between tuberculoid and lepromatous types. They may shift to either TT or LL type, depending on chemotherapy or alterations in the host resistance
- ❖ **Indeterminate type:** This denotes those early unstable cases with one or two hypopigmented macules and definite sensory impairment. Lesions are bacteriologically negative
- ❖ **Pure neuritic type:** These patients develop neural involvement without any skin lesion. Cases are bacteriologically negative.

IMMUNE RESPONSE

Immune response to the lepra bacilli is the most important factor that determines the outcome of the infection.

- ❖ **Innate immunity:** People show a high degree of innate immunity to lepra bacilli so that only a minority of those infected develop clinical disease
- ❖ **Cell-mediated immune response:** *M. leprae* being obligate intracellular, CMI plays a vital role in the control of the disease. The category of leprosy develops is determined by the CMI status of the individual
 - People with low CMI usually develop LL type of lesions
 - People with intact CMI develop TT type lesions.
- ❖ **Humoral immune response:** Antibodies have a minor role in disease control as *M. leprae* is intracellular. In LL patients, there occurs an exaggerated T_H2 response, which releases cytokines that cause polyclonal B cell activation producing high titer of antibodies—both specific and nonspecific antibodies.

COMPLICATIONS

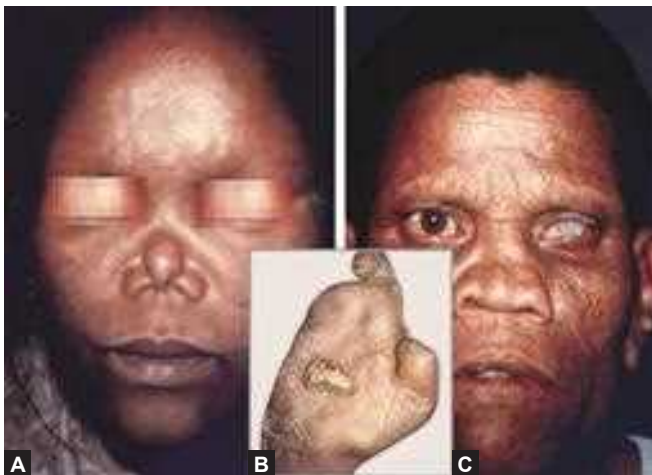
Complications in leprosy patients may be of two types—deformities and allergic response (called **lepra reactions**).

Deformities

About 25% of untreated cases develop deformities in due course of time which may arise due to—(1) nerve injury leading to muscle weakness or paralysis, or (2) disease process (facial deformities or loss of eyebrow), or (3) infection or injury (ulcers).

Common deformities include (Figs 54.2A to C):

- ❖ **Face:** Leonine facies, sagging face, loss of eyebrow/eyelashes, saddle nose and corneal opacity and ulcers
- ❖ **Hands:** Claw hand and wrist drop



Figs 54.2A to C: Deformities seen in untreated lepromatous leprosy: **A.** Saddle nose deformity; **B.** Bony deformity; **C.** Corneal opacity.

Source: Public Health Image Library, ID#, A. 15456, B. 15459, C. 15494/ Dr Andre J Lebrun, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Feet:** Foot drop, clawing of toes, inversion of foot, and plantar ulcers.

Lepra Reactions

Though leprosy runs as a chronic disease, several allergic type of acute exacerbations occur throughout its course, called lepra reactions (two types, I and II); described in detail in Table 54.3.

EPIDEMIOLOGY

- ❖ **Source of infection:** Multibacillary (LL and BL) cases are the most important sources of infection. Asymptomatic cases can also have a role in transmission. Tuberculoid leprosy cases do not transmit infection efficiently
- ❖ **Mode of transmission:** *M. leprae* has multiple routes of transmission. Portal of entry is either nose or skin
 - Nasal droplet infection (aerosols containing *M. leprae*) is the most common mode. A sneeze from an untreated LL patient may contain $>10^{10}$ lepra bacilli
 - Contact transmission (skin):
 - ♦ Direct contact from person to person
 - ♦ Indirect contact with infected soil, fomites such as clothes and linens.
 - Direct dermal inoculation during tattooing.
- ❖ **Communicability:** Leprosy is not highly communicable. Intimate and prolonged contact is necessary for

Table 54.3: Lepra reactions.

Characters	Lepra reaction type I	Lepra reaction type II*
Hyper-sensitivity	Type IV (delayed hypersensitivity)	Type III (immune complex-mediated)
Seen with	Borderline leprosy	Lepromatous variety (BL, LL)
Manifests as	Inflammation of previous lesions, new skin lesions and neuritis	Crops of painful erythematous papules which become nodular called ENL
Progresses as	If occurs before treatment—progresses towards LL (down grading reaction) If occurs after treatment—progresses towards TT (reversal reaction)	It usually occurs following the start of chemotherapy
T-helper response	T_H1 predominates leads to \uparrow IFN- γ and IL-2	T_H2 predominates—leads to \uparrow IL-6, IL-8. TNF- α plays a central role
Other organs	Usually not affected	Eyes, testes and kidney are affected
Treatment	Glucocorticoid	Glucocorticoid, thalidomide, clofazimine and antipyretics

Note: *Type II lepra reaction occurs following the institution of chemotherapy. Antibiotics kill the bacilli releasing the antigens which combine with circulating antibodies and the immune complexes formed are deposited in skin and various parts of the body.

Abbreviations: ENL, erythema nodosum leprosum; IFN, interferon; IL, interleukin.

transmission. Only about 5% of spouses living with leprosy patients develop disease. The disease is more likely if contact occurs during childhood

- ❖ **Environmental factors** that promote infection include people of rural areas, moist soil, humidity and overcrowding. Males are affected twice commonly than females.

Leprosy Elimination

In 1992, the WHO launched a campaign to eliminate leprosy as a public health problem by year 2000. The goal was kept as <1 case per 10,000 population.

- ❖ **India:** With the intense effort from National Leprosy Eradication Program (NLEP), India has achieved the elimination status by **December 2005**; although many Southeast Asian countries yet to achieve this
- ❖ **Global Leprosy Strategy (2016-20):** Launched by WHO in 2016, which aims at reducing disability among children and avoiding social discrimination in all countries by 2020.

Geographical Distribution

Once leprosy was worldwide in distribution, but now, it is almost exclusively confined to the developing nations of Asia, Africa, Latin America, and Pacific.

- ❖ **World:** In 2018, the new leprosy cases registered globally were 2,08,619 with a prevalence of 0.2 per 10,000 population
- ❖ **India:** Though the disease burden has declined considerably to below elimination cut-off level, still India accounts for maximum number of leprosy cases globally
 - As of 31st March 2018, total of 90,709 cases are on record in India, with a prevalence rate of 0.67 per 10,000 population. The new cases detected in year April 2017 to March 2018 were 1,26,164
 - Five states/UTs reported prevalence above elimination cut-off of >1/10,000 population as on 31st March 2018—highest prevalence from Dadra and Nagar Haveli, followed by Chhattisgarh, Odisha and Bihar.

LABORATORY DIAGNOSIS

1. Smear Microscopy

Smear microscopy is done to demonstrate the acid-fast bacilli in the lesions.

Specimen Collection

Total six samples are collected; four from skin (forehead, cheek, chin and buttock), one from ear lobe and nasal mucosa by nasal blow/scraping.

- ❖ **Slit skin smear** is the technique followed to collect the skin and ear lobe specimens
 - The **edge of the lesion** is the preferred site. Lesion is cleaned with spirit, then is pinched up tight to minimize bleeding

- A 5 mm long incision is made with a scalpel, deep enough to get into the infiltrated layers
- After wiping off blood or lymph that may have exuded, the scalpel blade is rotated transversely to scrape the sides and base of the incision to obtain a tissue pulp from below the epidermis which is smeared uniformly over an area of 8 mm diameter on a slide.
- ❖ **Nasal specimens:** (1) **Nasal blow:** Early morning mucus material is collected by blowing the patient's nose on a clean cellophane sheet; or (2) **Nasal scraping:** By using a mucosal scraper to scrape the nasal septum sufficiently to remove a piece of mucous membrane, which is transferred onto a slide and teased out into a uniform smear. Nasal scrapings are not recommended as a routine
- ❖ **Biopsy** from the thickened nerves and nodular lesions maybe necessary in some cases.

Appearance

M. leprae is less acid-fast compared to tubercle bacilli, therefore the smears are stained by Ziehl-Neelsen technique by using 5% sulfuric acid for decolorization. Under oil immersion objective, red acid-fast bacilli are seen, arranged singly or in groups (cigar like bundles), bound together by lipid-like substance, the glia to form **globi**. The globi are present inside the foamy macrophages called **Virchow's lepra cells or foamy cells** (Fig. 54.3).

- ❖ **Live bacilli** will be uniformly stained with parallel sides and round ends and length is five times the width
- ❖ **Dead bacilli** are less uniformly stained and have fragmented and granular appearance.

Grading of the Smear

The smears are graded, based on the number of bacilli per oil immersion field (OIF) as follows:

- 1–10 bacilli in 100 OIF = 1+
- 1–10 bacilli in 10 OIF = 2+

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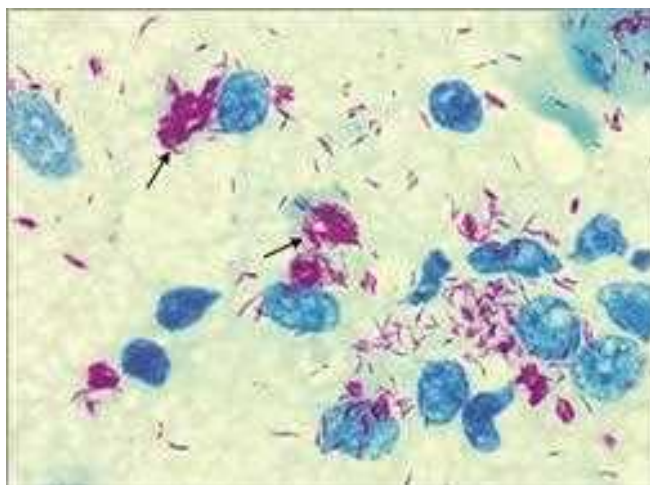


Fig. 54.3: Acid-fast stained slit skin smear showing numerous *Mycobacterium leprae* singly or in globi (arrows).

Source: Dr Isabella Princess, Apollo Hospital, Chennai (with permission).

Contd...

- 1–10 bacilli per OIF = 3+
- 10–100 bacilli per OIF = 4+
- 100–1000 bacilli per OIF = 5+
- >1000 bacilli or bacilli in clumps and globi in each OIF = 6+

Bacteriological index (BI): It is based on the total number of bacilli (live and dead) seen per oil immersion field. The bacteriological index (BI) is calculated by totalling the number of pluses scored in all the smears and divided by the number of smears examined.

Morphological index (MI): It is expressed as the percentage of uniformly stained bacilli out of the total number of bacilli counted. MI is a better marker to monitor the treatment response. Following clinical improvement by treatment, the MI should fall down, whereas the BI may remain the same.

SFG percentage (solid, fragmented granular rod percentage): Since the percentage of solid, fragmented granular rods are recorded separately, this method gives better picture of bacterial morphology and is a more sensitive indicator of monitoring the treatment response than MI.

2. Mouse Foot Pad Cultivation

M. leprae is not cultivable either in artificial culture media or in tissue culture; hence, it does not follow the Koch's postulates. The only certain way to cultivate *M. leprae* is by inoculating the specimens into foot pad of mice and keeping at 20°C for 6–9 months. Other animals such as nine banded armadillo (*Dasypus novemcinctus*) can also be used.

- ❖ **Advantages:** (1) it is 10 times more sensitive than microscopy, (2) useful in detecting drug resistance, (3) evaluating the potency of drugs, (4) detects viability of the bacilli
- ❖ **Disadvantages:** (1) time-consuming (6–9 months), and (2) ethical issues regarding use of animals.

3. Antibody Detection

- ❖ **FLA-ABS** (Fluorescent leprosy antibody absorption test): It is widely used to identify subclinical cases. It detects *M. leprae* specific antibodies irrespective of duration and stage of the disease. It claims to be 92% sensitive and 100% specific
- ❖ **ELISA** detecting IgM antibodies to PGL-1 (phenolic glycolipid-1) antigen of *M. leprae* are found in 95% of patients with untreated LL patients and the titre decreases with effective therapy. However, its sensitivity is low (60%) in TT patients.

4. Test for Detecting CMI (Lepromin Test)

Lepromin test is discovered by Mitsuda (1919). It demonstrates the delayed hypersensitivity reaction against the lepra antigen. It also indicates an intact host's CMI. However, it is not used for diagnosis of active infection. It is useful in classifying lesions of leprosy and also used as a prognostic indicator.

- ❖ **Procedure:** Lepromin antigen is injected intradermally to forearm and reading is taken at two occasions
- ❖ **At 48hr (Early or Fernandez reaction):** Induration (>10 mm) is produced at the site of inoculation which corresponds to DTH reaction to lepra antigen and indicates past exposure to lepra bacilli
- ❖ **At 21 days (Late or Mitsuda reaction):** A nodule of >5 mm size is formed at the site of inoculation which subsequently ulcerates
 - If positive, indicates that the patient's CMI is intact (which usually occurs in TT cases) and good prognosis
 - If negative, indicates absence of CMI (which usually occurs in LL cases) and poor prognosis.

T TREATMENT

Leprosy

Because of risk of development of drug resistance to single drug, WHO recommends multidrug therapy (MDT) for treatment of leprosy.

- Recommended drugs: Dapsone, rifampicin and clofazimine
- Alternate drugs: Ethionamide, quinolones (ofloxacin), minocycline and clarithromycin.

WHO Regimen (2018)

- **3-drug regimen:** WHO recommends a 3-drug regimen of rifampicin, dapsone and clofazimine for all leprosy patients.
 - Dapsone (100 mg) is given daily, self-administered
 - Rifampicin (600 mg) is given once a month under supervision
 - Clofazimine (300 mg) is given once a month under supervision, and by 50 mg daily, self-administered
 - **Duration of treatment**—6 months for paucibacillary leprosy and 12 months for multibacillary leprosy
 - **Follow-up** is conducted annually for 2 years for paucibacillary leprosy and for five years for multibacillary leprosy.
- Note:* Earlier to 2018, clofazimine was given only for multibacillary leprosy. Many countries are yet to adopt the new WHO regimen.

PREVENTION OF LEPROSY

Active case finding and effective treatment of cases is the most important measure to control leprosy.

- ❖ **BCG vaccine:** There is no effective vaccine available so far. Trials were done using BCG vaccine alone or in combination with killed lepra bacilli, ICRC bacillus
- ❖ **MIP vaccine:** A killed leprosy vaccine has been recently developed in India in 2018, using *Mycobacterium indicus pranii* (MIP). Trials have shown that if the vaccine is given to people in close contact, cases can be brought down by 60% in three years
- ❖ **Chemoprophylaxis:** Dapsone may be effective when given to high-risk household contacts of tuberculoid patients, but not for lepromatous patients; hence not recommended
- ❖ Hospitalized patients need not be isolated as transmission requires prolonged contact.

EXPECTED QUESTIONS

I. Write short notes on:

1. Laboratory diagnosis of leprosy.
2. Lepromatous leprosy.
3. Epidemiology of leprosy.
4. Treatment of leprosy.

Miscellaneous Bacterial Infections of Skin and Soft Tissues

CHAPTER 55

CHAPTER PREVIEW

- Anthrax
- Actinomycetes Infections
- Non-venereal Treponematoses
- Other Bacterial SSTIs

This chapter covers various bacterial skin and soft tissue infections (SSTIs) such as anthrax, actinomycosis, nocardiosis, non-venereal treponematoses and others.

ANTHRAX

Anthrax is a zoonotic disease, caused by a spore-forming obligate aerobe, *Bacillus anthracis*. It mainly affects animals. Humans acquire the infection through contact with an infected animal or by inhaling spores. It has also gained importance recently because of its ability to be used as a biological weapon.

Historical Importance

Considerable historical interest is attached to anthrax bacillus due to the following reasons:

- ❖ It was the first pathogenic bacterium seen under microscope (by Pollender, 1849)
- ❖ It was the first bacterium to be isolated in pure culture by Robert Koch (1876) and the Koch's postulates were made, based on *B. anthracis*
- ❖ Anthrax vaccine was the first live attenuated bacterial vaccine prepared (by Louis Pasteur, 1881).

Virulence Factors and Pathogenesis

Pathogenesis of anthrax is due to two important virulence factors—anthrax toxin and capsule.

Anthrax Toxin

It is a tripartite toxin, consisting of three fragments.

1. **Edema factor:** It is the active fragment; acts as adenylyl cyclase and increases host cell cAMP (cyclic adenosine monophosphate). It is responsible for edema and other manifestations seen in anthrax
2. **Protective factor:** It is the binding fragment that binds to the host cell receptors and facilitates the entry of other fragments into the host cells
3. **Lethal factor:** It causes cell death; acts by cleaving host cell MAPK (mitogen-activated protein kinases).

These fragments are not toxic individually, but in combination, they produce local edema and generalized shock. Toxin synthesis is controlled by a plasmid (pX01). Loss of plasmid makes the strain avirulent. This was probably the basis of original anthrax vaccine prepared by Pasteur.

Anthrax Capsule

B. anthracis has a polypeptide capsule, made up of polyglutamate (in contrast to the polysaccharide capsule present in most of the other capsulated bacteria).

- ❖ Capsule is plasmid (pX02) coded
- ❖ It inhibits complement mediated phagocytosis.

Clinical Manifestations

Animal Anthrax

Anthrax is primarily a zoonotic disease. Herbivorous animals such as cattle, sheep and less often horses and pigs are affected more commonly than the carnivorous animals.

- ❖ Infection occurs in susceptible animals by ingestion of the spores present in the soil. Direct spread from animal to animal is rare
- ❖ Anthrax in animals presents as a fatal septicemia; however, localized cutaneous lesions may be produced rarely
- ❖ Infected animals discharge large number of bacilli from the mouth, nose and rectum. These bacilli sporulate in soil and remain as the source of infection for man.

Human Anthrax

Transmission

Human beings acquire infection by:

- ❖ Cutaneous mode—by spores entering through the abraded skin; seen in people with occupational exposure to animals (most common mode)
- ❖ Inhalation of spores
- ❖ Ingestion of carcasses of animals dying of anthrax containing spores (manifested as bloody diarrhea).

Table 55.1: Differences between cutaneous anthrax and pulmonary anthrax.		
Features	Cutaneous anthrax	Pulmonary anthrax (Chapter 61)
Other name	Hide porter's disease (As it commonly occurs in dock workers carrying loads of hides and skins on their bare backs)	Wool sorter's disease (As it is seen in workers of wool factory, who acquire infection by inhalation of dust from infected wool)
Transmission	Cutaneous exposure to spores (enter through abraded skin)	Inhalation of spores
Characterized by	Malignant pustule (Fig. 55.1) The lesion begins as a papule that evolves into a painless vesicle followed by the development of a coal-black, necrotic eschar surrounded by non-pitting indurated edema <ul style="list-style-type: none"> • The name anthrax, which means coal, comes from the black color of the eschar • However, it is a non-malignant condition 	Hemorrhagic pneumonia Bacilli spread by lymphatics or blood, leading to— <ul style="list-style-type: none"> • Bacteremia • Hemorrhagic mediastinitis • Hemorrhagic meningitis
Occupational exposures	Dock worker, butcher, abattoir and farmer	Workers of wool factory
Occurrence	Most common (95%)	Rare
Prognosis	Self-limiting, rarely becomes fatal if untreated	Fatal
Bioterrorism	Rarely associated with bioterrorism	Most common form of anthrax to be associated with bioterrorism



Fig. 55.1: Malignant pustule.

Source: Public Health Image Library, ID# 1934/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Clinical Types

There are mainly three types of human anthrax.

1. Cutaneous anthrax (described in Table 55.1)
2. Pulmonary anthrax (Table 55.1 and Chapter 61)
3. Intestinal anthrax: It is rare; occurs due to ingestion of spores contaminated with meat of animals dying of anthrax. It is highly fatal and manifests as bloody diarrhea.

Agent of Bioterrorism

B. anthracis is one of the most common agent of bioterrorism. It has been widely used in various biological warfare, such as outbreaks in Sverdlovsk in 1979 and in United States in 2001 (Annexure 3).

- ❖ Pulmonary anthrax is the most common form to cause bioterrorism outbreaks
- ❖ Transmission occurs via inhalation of anthrax spores from contaminated animal products

- ❖ Though it has a high fatality, with prompt initiation of antibiotic therapy, survival is possible.

Epidemiology

Incidence of human anthrax is highest in Africa, and Central and Southern Asia. Human anthrax cases may be of two types:

1. Non-industrial cases: These result from agricultural exposure to animals
2. Industrial cases: These result from infected animal products such as hides, hair, bristles and wools.

LABORATORY DIAGNOSIS

Anthrax

- ❑ **Specimen:** Pus, sputum, blood, CSF
- ❑ **Direct demonstration**
 - Gram staining: Gram-positive, large rectangular bacilli
 - McFadyean's reaction: Shows amorphous purple capsule surrounding blue bacilli (polychrome methylene blue stain)
 - Direct IF: Detects capsular antigen
 - Ascoli's thermo precipitation test
- ❑ **Culture**
 - Nutrient agar: Medusa head appearance colonies
 - Blood agar: Dry wrinkled, nonhemolytic colonies
 - Gelatin stab agar: Inverted fir tree appearance growth
 - Selective media:
 - ◆ Solid medium with penicillin: String of pearl appearance
 - ◆ PLET medium.
- ❑ **Culture smear:** Gram-positive rods with bamboo stick appearance
- ❑ **Antibodies detection** by ELISA
- ❑ **Molecular diagnosis:** PCR using using *BA pX01/02* primers.

Laboratory Diagnosis

There is a high-risk of laboratory acquired infection of anthrax, hence utmost precautions should be taken and specimens should be processed in appropriate biological safety cabinets.

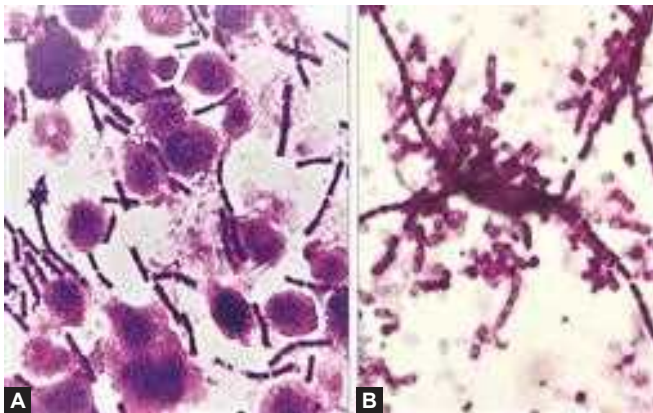
Specimen Collection

The specimens should be collected before starting antibiotic treatment. The useful specimens are:

- ❖ Pus, swab or tissue from the malignant pustule
- ❖ Sputum in pulmonary anthrax
- ❖ Blood (in septicemia)
- ❖ CSF (in hemorrhagic meningitis)
- ❖ Gastric aspirate, feces or food (in intestinal anthrax)
- ❖ Ear lobes from dead animals.

Direct Demonstration

- ❖ **Gram staining:** Reveals gram-positive, large rectangular rods (3–10 μm \times 1–1.6 μm). Spores are usually not seen in clinical samples (Fig. 55.2A)
- ❖ **McFadyean's reaction:** Polypeptide capsule can be demonstrated by staining with Gurr's polychrome



Figs 55.2A and B: Gram stain of *B. anthracis*: **A.** Direct smear—shows gram-positive, large rectangular bacilli and pus cells; **B.** Culture smear—shows gram-positive bacilli with non-bulging spores (bamboo stick appearance).

Source: A. Public Health Image Library/ID#: 1811, Centers for Disease Control and Prevention (CDC), Atlanta (with permission); B. Department of Microbiology, JIPMER, Puducherry (with permission).

methylene blue stain for 30 seconds. Capsule appears as amorphous purple material surrounding blue bacilli (Fig. 55.3A). This is used for presumptive diagnosis of anthrax in animal

- ❖ **Direct immunofluorescence test (direct-IF):** It detects capsular and cell wall polypeptide antigens by using fluorescent tagged monoclonal antibodies. It is used for confirmation of the diagnosis during bioterrorism outbreaks
- ❖ **Ascoli's thermoprecipitation test:** It is a ring precipitation test, performed when specimens are received in the putrid form.

Culture

Bacillus anthracis is aerobic, non-fastidious, grows in ordinary media and has a wide temperature range (12–45°C) of growth.

Colony morphology of *B. anthracis* after 24 hours incubation of plates is as follows:

- ❖ **Nutrient agar:** Colonies are 2–3 mm in size, irregular, round, opaque, grayish white with a frosted glass appearance
 - Medusa head appearance:** When colonies are viewed under low power microscope, the edge of the colony which is composed of long interlacing chains of bacilli, appears as locks of matted hair (Fig. 55.3B)
- ❖ **Blood agar:** It produces dry wrinkled, non-hemolytic colonies (Fig. 55.3C)
- ❖ **Gelatin stab agar:** Growth occurs as **inverted fir tree appearance** (due to liquefaction of gelatin which occurs maximum at the surface, and then slows down towards the bottom)
- ❖ **Selective media:**
 - Solid medium with penicillin: Colony smear shows bacilli arranged in string of pearl appearance
 - PLET medium: It consists of polymyxin, lysozyme, EDTA and thallos acetate added in heart infusion agar.



Figs 55.3A to C: **A.** McFadyean's reaction—amorphous purple capsule surrounding blue bacilli (polychrome methylene blue stain); **B.** Medusa head colonies of *Bacillus anthracis* on nutrient agar (10x magnification); **C.** Non-hemolytic dry wrinkled colonies of *Bacillus anthracis* on blood agar.

Source: A. Department of Pathobiology, University of Guelph, Canada; B. Dr J Glenn Songer, Iowa State University, USA; C. Public Health Image Library/ID#: 1897/Dr Larry Stauffer, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Culture Smear

- ❖ **Gram staining:** Reveals **bamboo stick appearance**, i.e. long chain of gram-positive bacilli with non-bulging spores (appear as empty space) (Fig. 55.2B). Spores of *Bacillus* should be differentiated from that of bulging spores of another gram-positive bacillus, *Clostridium*
- ❖ **Spores:** They can be demonstrated by using special stains, such as hot malachite green (Ashby's method) or modified acid-fast staining using 0.25% sulfuric acid (spores are acid-fast).

Molecular Diagnosis

PCR with specific primers can be used for further confirmation.

- ❖ PCR using *BA pX01* primer targeting gene coding for protective antigen
- ❖ PCR *BA pX02* primers targeting capsular gene.

TREATMENT

Anthrax

Anthrax can be successfully treated if the disease is promptly recognized and appropriate therapy is initiated early.

- ❑ **Cutaneous anthrax:** Amoxicillin, ciprofloxacin or doxycycline for 7-10 days
- ❑ **Pulmonary/intestinal anthrax:** Treatment comprises of antibiotic regimen along with anthrax immunoglobulin
- ❑ **Post-exposure prophylaxis:** Following exposure, ciprofloxacin or doxycycline is given for 60 days along with anthrax vaccine (BioThrax, 3 doses).

Prevention

The general control measures include:

- ❖ Disposal of animal carcasses by burning or by deep burial in lime pits
- ❖ Decontamination (usually by autoclaving) of animal products
- ❖ Protective clothing and gloves for handling potentially infectious materials.

Live Attenuated, Non-capsulated Spore Vaccine

It is commercially available as Stern vaccine, which is extensively used in animals, remains protective for 1 year following single injection. However, it is not safe for human use.

Adsorbed (Alum Precipitated) Toxoid Vaccine

It is prepared from the protective antigen. Antibodies formed against protective antigen neutralize it and thus prevent attachment of toxin to the host cell. It is safe and effective for human use (e.g. BioThrax). It is indicated for pre-exposure and post-exposure prophylaxis (Table 55.2).

Other Bacillus Species of Human Importance

Anthraxoid Bacilli

Bacillus species other than the anthrax bacillus, are collectively called **anthracoid bacilli**.

Table 55.2: Adsorbed anthrax vaccine (BioThrax).

Indication	Route	Dosing schedule
Pre-exposure prophylaxis for persons at high-risk of exposure	Intramuscular (0.5 mL/dose)	Primary series: 0,1, and 6 months Boosters: at 6 and 12 month after primary series and then yearly
Post-exposure prophylaxis following exposure to suspected or confirmed case	Subcutaneous (0.5 mL/dose)	0, 2, and 4 weeks post-exposure combined with antimicrobial therapy

- ❖ Except *B. cereus*, most of them are non-pathogenic and are common contaminants in laboratory cultures
- ❖ They have a general resemblance to anthrax bacilli such as producing dry wrinkled colonies and in smear they appear as chains of spore-bearing gram-positive bacilli
- ❖ However, they differ from anthrax bacilli in many ways such as they are motile, and produce hemolytic colonies on blood agar, etc.

Bacillus cereus

It is a normal habitant of soil, also widely isolated from food items such as vegetables, milk, cereals, spices, meat and poultry. It mainly causes food poisoning (Chapter 40); can also cause ocular and various other systemic infections.

Bacillus thuringiensis

It is closely related to *B. cereus* and may occasionally produce food poisoning. It is also used as larvicidal agent for mosquito control.

Bacillus (Spores) Used as Sterilization Control

Bacillus species are used as biological indicators to check the efficacy of sterilization process. For example;

- ❖ *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) is used for autoclave, hydrogen peroxide gas plasma sterilizer and liquid acetic acid sterilizer
- ❖ *Bacillus atrophaeus* is used for ethylene oxide sterilizer and dry heat sterilizer.

ACTINOMYCETES INFECTIONS

Actinomycetes are diverse group of gram-positive bacilli arranged in chains or branching filaments. Though they are true bacteria, but similar to fungi, they form a mycelial network of branching filaments. Most of them are soil saprophytes or normal human commensals. Important genera include:

- ❖ *Actinomyces*: They are anaerobe and non-acid-fast; produce a clinical condition called actinomycosis
- ❖ *Nocardia*: They are aerobe and acid-fast; cause actinomycetoma and pulmonary infection

- ❖ *Actinomadura*: They are aerobe and non-acid fast; cause actinomycetoma
- ❖ *Streptomyces*: They are aerobe and non-acid-fast; rarely cause actinomycetoma in man. They also remain as an important source of antibiotics such as streptomycin
- ❖ Thermophilic actinomycetes such as *Micropolyspora* and *Thermoactinomyces* can cause hypersensitivity pneumonitis (farmer's lung and bagassosis).

Actinomycosis

Actinomyces are soil saprophytes and commensals of oral cavity. In humans, they cause actinomycosis. *A. israelii* is the most common species infecting man.

Pathogenesis

Actinomycosis is a chronic suppurative and granulomatous infection characterized by multiple abscesses with formation of sinuses, discharge containing granules and on later stage; fibrosis and tissue destruction.

- ❖ The name refers to ray-like appearance of the organism in the granules (*Actinomyces*, meaning ray fungus)
- ❖ **Mode of infection:** As *Actinomyces* are commensals of oral cavity, the infection is mostly **endogenous** and may result from trauma, e.g. dental extraction
- ❖ The bacteria bridge the mucosal or epithelial surface of the mouth, grow in an anaerobic niche, induce a mixed inflammatory response, and form painless indurated swelling with sinuses which may drain pus containing granules to the skin surface
- ❖ The infection may spread to the neighboring organs including the bones and induce tissue destruction
- ❖ Often, the hard indurated swellings (described as 'wooden'), are mistaken as malignant tumors.

Clinical Manifestations (Actinomycosis)

Oral-cervicofacial actinomycosis: This is the most common form, usually presents as a painless, slow-

growing, hard mass with cutaneous fistulas, a condition commonly known as **lumpy jaw** (Fig. 55.4A).

Other forms are rare such as:

- ❖ **Thoracic actinomycosis;** may present as cavitory lesions and pleural effusion
- ❖ **Abdominal form:** It occurs due to spillage of intestinal flora secondary to bowel surgery or other conditions of bowel such as appendicitis
- ❖ **Pelvic form:** It occurs following intrauterine contraceptive devices (IUCDs) insertion
- ❖ Brain abscesses
- ❖ Bone destruction and soft tissue infections
- ❖ **Disseminated form:** It may occur due to hematogenous spread. Lungs and liver are the common sites; where multiple nodules are formed
- ❖ **Dental caries and periodontal diseases:** Mainly caused by *A. naeslundii* and *A. odontolyticus*.

Laboratory Diagnosis

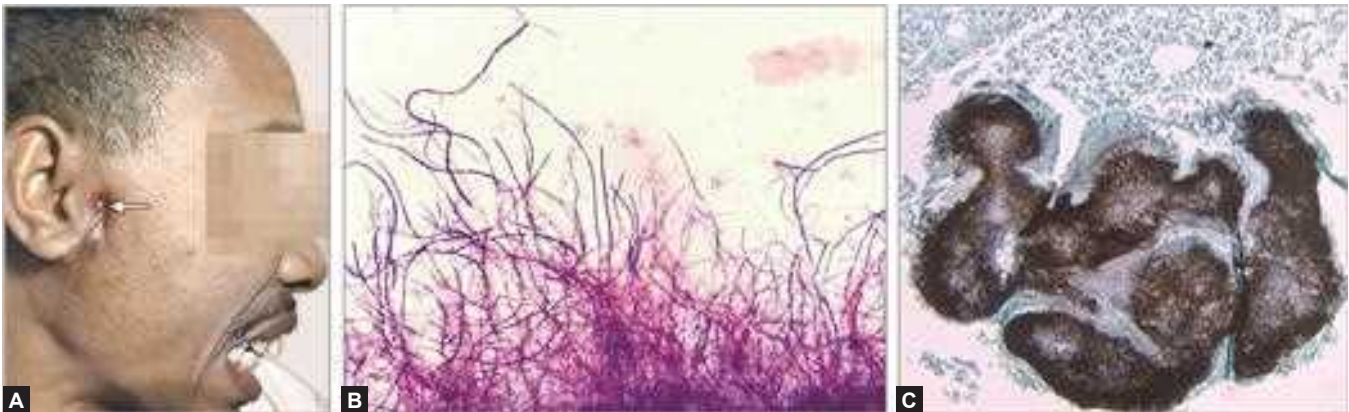
Specimen

Based on the affected site, the specimens collected include discharge from the sinuses or fistula, rarely sputum, bronchial washings, and cervicovaginal secretions.

Direct Microscopy

Pus discharge is thoroughly washed in saline in a test tube and the sediment is collected that contains gritty, white or yellowish **sulfur granules**, of <5 mm in size. Granules are crushed between two slides and smears are made. Because actinomycetes are part of normal oral and genital-tract flora, their isolation in the absence of sulfur granules specimens is of less significance.

- ❖ **Gram-staining (Brown-Brenn modification):** It shows a central mass of gram-positive branching, filamentous bacilli, radiating peripherally with hyaline, club-shaped ends (Fig. 55.4B)



Figs 55.4A to C: A. Actinomycosis (painless, slow-growing, hard mass with cutaneous fistula) (arrow showing); B. Gram-positive filamentous bacilli; C. Gomori's stained smear showing sun-ray appearance.

Source: A. Public Health Image Library, ID# 2856, CDC, Atlanta; B. Dr Isabella Princess, Apollo Hospitals, Chennai; C. ID# 10601/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ Granules of actinomycosis are hard and not emulsifiable which differentiates them from granules produced in other conditions
- ❖ **Histopathological staining** such as hematoxylin-eosin and Gomori's stained tissue sections may reveal granules composed of eosinophilic clubs surrounding basophilic filaments and inflammatory cells such as neutrophils and foamy macrophages (sun-rays appearance) (Fig. 55.4C).

Culture

Pus containing sulfur granules are washed and cultured anaerobically at 37°C on media such as:

- ❖ **Thioglycollate broth:** Growth of *A. israelii* resembles **fluffy balls** at the bottom of the tube, this can be differentiated from other species (*A. bovis* produces uniform turbidity)
- ❖ **Brain heart infusion (BHI) agar:** It forms small **spidery colonies** at 48 hours which become enlarged and heaped up in 10 days.

Species Identification

It is done when the culture isolate is subjected to:

- ❖ Biochemical reactions
- ❖ Automated methods such as MALDI-TOF
- ❖ Molecular methods, such as PCR detecting 16S-rRNA gene are also available for speciation.

TREATMENT

Actinomycosis

- ❑ Recommended regimen include IV ampicillin or IV penicillin G for 4–6 weeks followed by oral penicillin V or amoxicillin for 6–12 months. Lesser duration may cause relapse
- ❑ Doxycycline, ceftriaxone or clindamycin can be given to people with penicillin allergy
- ❑ Surgical removal of the affected tissues may be required for extensive lesions.

Nocardiosis

Nocardia species (named after Edmond Nocard, 1898) are gram-positive branching filamentous bacilli similar to *Actinomyces*; however, they differ from the latter by being aerobic and acid-fast (Table 55.3). They are environmental saprophytes found in soil and vegetations. Though more than 100 species have been identified, only few (nine) species are associated with human disease. *N. asteroides* (so named due to its star-shaped colonies) and *N. brasiliensis* are the most common pathogens.

Pathology and Pathogenesis

Nocardiosis occurs worldwide, more common among adult males. Soil is the natural habitat of *Nocardia*. Infection is acquired from soil either by:

- ❖ **Inhalation of fragmented bacterial filaments:** Leads to development of pulmonary nocardiosis that may disseminate later

Table 55.3: Differences between *Actinomyces* and *Nocardia*.

Features	<i>Actinomyces</i>	<i>Nocardia</i>
Acid-fastness	Nonacid-fast	Partially acid-fast
Oxygen requirement	Anaerobe	Obligate aerobe
Habitat	<ul style="list-style-type: none"> • Found as oral flora • Infections occur endogenously 	<ul style="list-style-type: none"> • Usual habitat is soil • Infections occur exogenously
Risk factors	Disease occurs in immunocompetent host also	Usually affects people with low immunity
Clinical forms	Cervicofacial, abdominal and others	Pulmonary, CNS forms, Actinomycetoma
Granules	Sulfur granules are hard and not emulsifiable, consist of branching filamentous bacilli and surrounded by clubs (sun-ray appearance)	<ul style="list-style-type: none"> • Granules are soft and lobulated and also show sun-ray appearance • Commonly found in mycetoma, rare in other conditions
Culture	<ul style="list-style-type: none"> • Spidery molar teeth colony in solid media • Fluffy ball at bottom of the liquid medium 	Colonies are creamy, wrinkled and pink Isolation is done in: <ul style="list-style-type: none"> • Selective media • Paraffin bait technique • LJ medium
Treatment	Penicillin	Cotrimoxazole

Abbreviation: LJ; Lowenstein–Jensen medium.

- ❖ **Transcutaneous inoculation of the bacteria:** Leads to various cutaneous and subcutaneous manifestations (e.g. mycetoma)
- ❖ Person-to-person spread is not known.

Risk Factors

Cell-mediated immunity plays an important role in controlling the disease. Hence nocardiae act as opportunistic pathogen, tend to occur frequently in immunocompromised conditions including AIDS, corticosteroid treatment, organ transplantation and tuberculosis.

Clinical Manifestations

Pulmonary Nocardiosis

Lobar pneumonia is the most common form of pulmonary nocardiosis, characterized by subacute onset of cough with thick, purulent sputum. It may rarely spread directly to adjacent tissues, leading to pericarditis, mediastinitis, laryngitis, tracheitis and bronchitis.

Extrapulmonary (Disseminated) Nocardiosis

In half of the pulmonary nocardiosis cases, dissemination occurs via blood. It typically presents as subacute abscess. **Brain abscess** is the most common form followed by abscesses of skin, kidneys, bone and muscle.

Actinomycetoma

Actionomycetoma is a chronic granulomatous condition of bacterial etiology, characterized by subcutaneous nodular

swelling, multiple sinuses and discharge containing granules. This condition can also be caused by fungi, known as eumycetoma (described in Chapter 58).

Laboratory Diagnosis

Specimen

Depending on the site affected, various specimens collected such as sputum, pus from abscess and granules.

Granules present in discharge are collected in sterile gauze or loop by pressing the sinuses from the periphery to express them out (as in case of actinomycetoma).

Direct Microscopy

- ❖ **Gram staining (Brown–Brenn modification):** Reveals gram-positive branching and filamentous bacilli of width 0.5–1 µm
- ❖ **Modified acid-fast staining** using 1% sulfuric acid as decolorizer (Kinyoun method): Nocardiae are partially acid-fast and appear as branching and filamentous red colored acid-fast bacilli (Fig. 55.5)
- ❖ Granules are washed several times in saline, crushed between two slides and observed under microscope. Granules are 0.5–2 mm sized microcolonies composed of branching filamentous bacilli
- ❖ **Histopathology (H and E stain) of the granules:** Shows multilobulated with sun-ray appearance.

Culture

Nocardiae are obligate aerobes that grow on various media such as brain heart infusion agar and Sabouraud dextrose agar (SDA) when incubated at 37°C for 2 days to 2 weeks. Colonies are creamy, wrinkled, pigmented (orange or pink colored) and adhere firmly to the medium. Some colonies possess abundant aerial growth and have a cotton wool ball appearance.

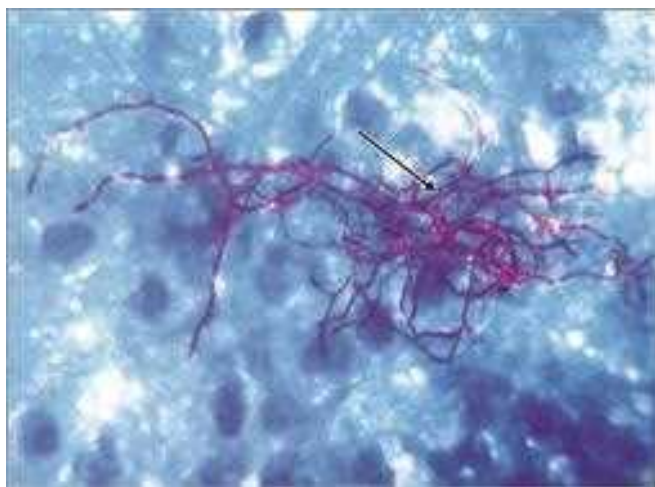


Fig. 55.5: Acid-fast filamentous branching bacilli of *Nocardia* (modified acid-fast stain).

Source: Dr Suchitra Mishra, Department of Microbiology, HiTech Medical College, Rourkela, Odisha (with permission).

Recovery of *Nocardia* from the samples containing *Actinomadura* and *Streptomyces* can be done by:

- ❖ **Using selective media:**
 - Buffered yeast extract containing polymyxin and vancomycin
 - Sabouraud dextrose agar with chloramphenicol.
- ❖ **Paraffin bait technique:** Media using paraffin as the sole carbon source have been shown to be effective for isolation of nocardiae from soil and clinical samples
- ❖ **Lowenstein–Jensen medium:** Produces moist glabrous colonies (differentiates from mycobacteria).

Identification

Species identification is made by appropriate biochemical tests or automated methods such as MALDI-TOF.

TREATMENT

Nocardiosis

- ❑ **Cotrimoxazole** are the drug of choice for all forms of nocardiosis
- ❑ For severe disease such as brain abscess or pneumonia, cotrimoxazole plus imipenem is recommended
- ❑ **Duration of treatment**
 - For severe disease: 6–12 months (for intact host defense) or 1 year (for deficient host defense)
 - For lymphadenitis, skin abscess: 2 months
 - For actinomycetoma: Until 6–12 months after cure
 - For keratitis: Until 2 months after cure
- ❑ Aspiration or drainage of the abscesses should be carried out to limit the spread of infection.

Actinomadura Infections

Actinomadura is the most frequent cause of actinomycetoma, significantly outnumbering the cases caused by *Nocardia*.

- ❖ *A. madurae* and *A. pelletieri* are important species
- ❖ Granules are usually white to yellow except in case of *A. pelletieri* that produces red colored granules
- ❖ Microscopy of the specimens containing granules reveals branching filamentous bacilli
- ❖ Colonies have a **molar tooth** appearance after 48 hours in culture with sparse aerial growth
- ❖ Most isolates are susceptible to amikacin and imipenem.

NON-VENEREAL TREPONEMATOSES

Endemic or non-venerale treponematoses are caused by three close relatives of *T. pallidum*, out of which the first two are considered as subspecies of *T. pallidum*:

- ❖ *T. pertenue* (causes yaws)
- ❖ *T. endemicum* (causes endemic syphilis)
- ❖ *T. carateum* (causes pinta).

They differ from *T. pallidum* (Chapter 77), being transmitted by non-sexual mode (by direct contact) and produce non-genital cutaneous manifestations.

Yaws

Yaws (also known as pian, framboesia, or bouba) is an endemic disease caused by *T. pallidum* subspecies *pertenue*.

- ❖ **Epidemiology:** Yaws is endemic in the tropical areas of Africa, South-east Asia, and Central America. The number of cases were 59,307 cases in 2016 from eight endemic countries world wide. There was a recent outbreak in Cameroon in 2017
 - In India, cases were found from tribal hilly areas of Odisha, Chhattisgarh, Assam, Andhra Pradesh and Madhya Pradesh
 - However, India actively participated in yaws eradication programme in 1996 and has reported no new cases since 2003. India has achieved the yaws free status in the year 2016 (as declared by WHO).
- ❖ **Transmission** is by direct skin-to-skin contact (abraded or lacerated skin)
- ❖ **Clinical manifestation:** Incubation period is about 3–4 weeks. The majority of cases are children. It is clinically characterized by:
 - Primary lesion (“mother yaw”): Extragenital papule on extremities that enlarges in moist warm weather to become papillomatous or raspberry-like (thus the name ‘framboesia’)
 - Regional lymphadenopathy may be developed
 - Secondary eruptions are more generalized
 - Skin lesions may take several forms such as macular, papular, or papillomatous. Painful lesions on the feet result in a crab-like gait (“crab yaws”)
 - Periostitis may result in nocturnal bone pain
 - **Late yaws** occurs in 10% of untreated persons, and is manifested by destructive lesions (gumma) of skin, bone, and joints. Destruction of the nose, maxilla, palate and pharynx may be developed, termed as **gangosa**
 - Relapses are common during the first 5 years.

Yaws eradication

In 2012, WHO initiated the Yaws Eradication Strategy, also referred to as “the Morges strategy”, aiming for global eradication by 2020.

- Criteria for Eradication include (i) absence of new indigenous case for three years, and (ii) absence of evidence of transmission for 3 years, measured with sero-surveys (RPR test) among children
- Mass treatment of azithromycin to at least 90% of the targeted at-risk population is carried out.

TREATMENT

Yaws

- Azithromycin (single oral dose) is the preferred choice for treatment (WHO). Patients should be examined 4 weeks after treatment for clinical recovery and for detection of azithromycin resistance
- Benzathine penicillin (single intramuscular dose) is reserved for patients who clinically fail on azithromycin, or are allergic to azithromycin.

Endemic Syphilis

Endemic Syphilis (also called by local names: *bejel*, *siti*, *dichuchwa*, *njovera*, *skerljevo*) is caused by *T. pallidum* subspecies *endemicum*.

- ❖ **Epidemiology:** It is endemic in arid areas of Asia (Syria, Saudi, Iraq, and Iran), Africa (Ghana, Mali, Niger, and Senegal) and Australia, but not in Americas
- ❖ **Transmission:** Bejel is transmitted by direct contact, by kissing or by sharing the drinking and eating utensils.

Clinical Manifestations

- ❖ **Early manifestations:** It starts as oral papule which progresses to mucosal patches on the oral mucosa and mucocutaneous lesions resembling the condylomata lata of secondary syphilis. Periostitis and regional lymphadenopathy are common
- ❖ **Late manifestations:** It occur in the form of destructive gummas, osteitis, and gangosa which are more common than in late yaws.

Pinta

Pinta (also known as *mal del pinto*, *carate*, *azul*, *purupuru*) is the most benign of all treponemal infections, caused by *T. carateum*.

- ❖ **Epidemiology:** Pinta is limited to Central America and northern South America, where it is found rarely and only in remote villages
- ❖ **Transmission** is by direct skin-to-skin contact
- ❖ **Clinical manifestations:** Pinta is characterized by marked changes in skin color without causing destructive lesions. It is manifested as:
 - **Pruritic papules:** They occur as primary lesions on the extremities or face
 - **Pintides** are disseminated secondary lesions, characterized by deeply pigmented, pruritic lesions. They are infectious and may persist for years
 - **Dyschromic macules** are the late pigmented lesions which may contain treponemes.

Diagnosis of Non-venereal Treponematoses

Diagnosis is based on the clinical manifestations, dark ground microscopy and serological tests.

- ❖ As they are virtually indistinguishable from *T. pallidum* antigenically, the serological tests used for syphilis can also be used for the diagnosis of non-venereal treponematoses
- ❖ Till date, there is no test available that can differentiate between various treponemes.

TREATMENT

Pinta and Endemic syphilis

Both patients and their contacts can be treated by benzathine penicillin.

OTHER BACTERIAL SSTIs

Meningococcal Rashes

A non-blanching rash (petechial or purpuric) develops in more than 80% of the *Neisseria meningitidis* infections, distributed mainly on the trunk and extremities. In fulminant meningococemia (called Waterhouse-



Fig. 55.6: Child with gangrene of lower extremities due to meningococemia.

Source: Public Health Image Library, ID# /1335/Mr Gust/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Friderichsen syndrome), the rashes progress to large purpuric types (called purpura fulminans). Rarely, gangrene of extremities may be developed in extreme cases (Fig. 55.6). *N. meningitidis* principally causes outbreaks of meningitis (Chapter 71).

Cutaneous Diphtheria

Corynebacterium diphtheriae mainly causes toxin-mediated exudative pharyngitis with pseudomembrane formed over tonsil (Chapter 60). It may occasionally produce skin lesions; caused by organism itself (not toxin-mediated). Hence it is possible that, the skin lesions may also be caused by nontoxicogenic strains.

- ❖ It presents as punched-out ulcerative lesions with necrosis, or rarely pseudomembrane formation; most commonly occurs on the extremities (Fig. 55.7)
- ❖ There is an increasing trend of cutaneous diphtheria in the recent years, especially in vaccinated children; because antitoxins present in vaccinated people cannot prevent the disease.

Tuberculous Skin Lesions

M. tuberculosis can cause several SSTIs.

- ❖ **Scrofuloderma:** It is a tuberculous skin lesion caused by the involvement of the skin by direct extension, usually from the underlying tuberculous lymphadenitis



Fig. 55.7: Cutaneous diphtheria.

Source: Public Health Image Library, ID# /1941, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Lupus vulgaris:** Apple jelly nodules are formed over the face, usually seen in females.

SSTI due to Nontuberculous Mycobacteria (NTM)

Cutaneous lesions are produced by NTM species (Chapter 63).

- ❖ ***M. marinum*:** It is a photochromogen, produces pigments only in presence of light. It is acquired from water sources (fish tanks, swimming pool) and enters through minor trauma. Various lesions produced are:
 - It typically causes papules or ulcers known as **swimming pool granuloma or fish tank granuloma**
 - Tender nodules: Spread in a sporotrichoid pattern similar to *Sporothrix schenckii*.
- ❖ ***M. ulcerans*:** It is a waterborne skin pathogen, found mainly in the tropics of Africa, Central South America and South-east Asia
 - It is the agent of **Buruli ulcer** (named after Buruli district of Uganda, where a large outbreak had occurred)
 - Lesions are typically painless ulcers and nodules that become necrotic later
 - It can also cause osteomyelitis and limb deformities
 - Exotoxin: It produces mycolactone toxin which may be involved in the pathogenesis of the disease.
- ❖ *M. fortuitum* and *M. chelonae*: They cause injection site abscess
- ❖ Other NTM species that cause SSTI include *M. avium-intracellulare* and *M. abscessus*.

Erysipeloid

Erysipelothrix rhusiopathiae is a gram-positive bacillus that causes **erysipeloid**; characterized by violaceous swelling with severe pain, but no pus; which differentiates it from staphylococcal and streptococcal erysipelas where pus is seen at the infection site. Most common site is fingers (called “seal finger” and “whale finger”).

- ❖ **Transmission:** It usually infect animal handlers (abattoir workers, butchers) and fishermen; transmitted by direct inoculation from animals or animal products
- ❖ **Treatment:** Penicillin G is the drug of choice for severe infections. It is intrinsically resistant to vancomycin.

Enterobacteriaceae

Several members of family Enterobacteriaceae such as *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Providencia* and *Morganella* can cause various skin and soft tissue infections.

- ❖ The infections range from cellulitis, abscess, burn wound infection, etc.
- ❖ Most of these agents principally cause infections of other systems and therefore discussed elsewhere.

Non-fermenters

Various non-fermenting gram-negative bacilli such as *Pseudomonas*, *Acinetobacter*, *Burkholderia*, etc. are associated with skin and soft-tissue infections.

SSTI due to *Pseudomonas aeruginosa*

Pseudomonas aeruginosa can cause various SSTIs, bone and joint infections (Chapter 65).

- ❖ **Burns wound infection:** *Pseudomonas* is the most common organism to infect the burn wounds
- ❖ **Ecthyma gangrenosum:** It is an acute necrotizing condition resulting from bacteremia, occurs commonly in patients with febrile neutropenia and AIDS
- ❖ **Dermatitis** (folliculitis and other papular or vesicular lesions): It causes outbreaks in spas and swimming pools
- ❖ **Toe-web infections** (in the tropics)
- ❖ **Green nail syndrome:** It is a 'paronychia' (inflammation of the tissues adjacent to the nail with green pus formation). It results from prolonged submersion of the hands in water
- ❖ Cellulitis (characterized by **blue green pus**)
- ❖ Osteomyelitis and septic arthritis.

Melioidosis

It is a multisystem disease, caused by *Burkholderia pseudomallei*. In early stage, it presents as an acute, localized infection, characterized by localized nodule, fever, general muscle aches, which may eventually progress rapidly to infect the bloodstream (Chapter 65).

Erythema Migrans (in Lyme Disease)

It is caused by *Borrelia burgdorferi*; transmitted by tick bite (Chapter 32). In its early localized stage, an annular maculopapular lesion may develop at the site of the tick bite called erythema migrans, commonly involving thigh, groin, and axilla (Fig. 55.8). It may be absent in 20% of the cases.

SSTIs Caused by Rickettsiae

Various rickettsial infections produce rashes; distributed in different body sites, depending up on the disease type (Chapter 31).



Fig. 55.8: Erythema migrans: Annular bull's eye pattern rash.

Source: Public Health Image Library, Centers for Disease Control and Prevention (CDC), Atlanta, ID# 9874/J. Gathany (with permission).

- ❖ **Epidemic typhus:** Rashes are seen all over the body except palms and soles
 - ❖ **Endemic typhus:** Rashes are seen over the trunk and face
 - ❖ **Rocky Mountain spotted fever:** Rashes appear typically on extremities (wrist and ankles) and trunk. Initially they are maculopapular, later become hemorrhagic (Fig. 55.9)
 - ❖ **Rickettsial pox:** Here, characteristic vesicular rashes are seen similar to chickenpox.
- Various other cutaneous manifestations in rickettsial and related infections include:
- ❖ **Eschar** (painless black crusted lesions surrounded by an erythematous halo) may be present at the site of vector bite. It is typically observed in mite-transmitted rickettsial infections such as rickettsial pox and scrub typhus
 - ❖ **Verruga peruana:** It is a late manifestation of *Bartonella bacilliformis* infection, characterized by cutaneous vascular lesions
 - ❖ **Cat-scratch disease:** Caused by *Bartonella henselae*, characterized by painless papule at the site of cat scratch.

Vibrio vulnificus Cutaneous Infection

Apart from sepsis, *V. vulnificus* can also cause primary wound infection (*Vulnificus* is Latin word for "wound maker") (Chapter 42).

- ❖ Characterized by painful erythematous swelling or cellulitis or even vesicular, bullous or necrotic lesions
- ❖ It generally affects people without underlying disease, in contrast to sepsis, which is usually observed in patients with underlying disease such as liver disease, iron overload or immunosuppression.

Wound infections can also be produced by *Vibrio parahaemolyticus*.

Other skin manifestations seen in bacterial infections include:

- ❖ **Rose spot (rashes)** are seen in enteric fever, caused by *Salmonella* Typhi (Chapter 30)



Fig. 55.9: Characteristic rash seen in Rocky Mountain spotted fever.

Source: Public Health Image Library, ID#:14489/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **In acute rheumatic fever:** Two important cutaneous manifestations may be seen (Chapter 28)
 - **Subcutaneous nodules:** Occur as painless, small, mobile lumps beneath the skin overlying bony prominences, particularly of the hands, feet, and elbows
 - **Erythema marginatum:** They are pink macular rashes that undergo appearing and disappearing before the examiner's eyes.
- ❖ **Genital ulcers:** Various bacterial agents such as *Treponema pallidum*, *Haemophilus ducreyi*, *Chlamydia*, *Klebsiella granulomatis* can cause genital ulcers; discussed in Chapter 77
- ❖ **Aeromonas:** Various species such as *A. hydrophila*, *A. caviae* and *A. veronii* are associated with musculoskeletal and wound infections (Chapter 42).

EXPECTED QUESTIONS

I. Write essay on:

1. A farmer presented to the outpatient department with history of papulovesicular lesion over the neck region, which later on developed into a coal-black, necrotic wound for the past 3 to 4 days. After being examined, the doctor sent a tissue from that necrotic area to the laboratory. Gram staining revealed gram-positive rod shaped bacilli arranged in chains.
 - a. What is the clinical diagnosis and causative agent?
 - b. Describe pathogenesis and various forms of clinical presentation of this infection.
 - c. Mention the laboratory investigations to confirm the diagnosis.

II. Write short notes on:

1. Yaws.
2. Nocardiosis.
3. Erythema migrans.

III Multiple Choice Questions (MCQs):

1. **Gram-stain morphology of *Bacillus anthracis* is:**
 - a. Tennis racket appearance
 - b. Drum stick appearance

- c. Bamboo stick appearance
- d. Spectacle glass appearance

2. "Malignant pustule" is a term used for:

- a. An infected malignant melanoma
- b. A carbuncle
- c. A rapidly spreading rodent ulcer
- d. Anthrax of the skin

3. In erysipeloid, the route of infection is:

- a. Direct inoculation
- b. Ingestion
- c. Inhalation
- d. None of the above

4. Which of the following actinomycete is acid-fast?

- a. *Streptomyces*
- b. *Actinomadura*
- c. *Nocardia*
- d. *Actinomyces*

5. Bejel is caused by:

- a. *Treponema pertenuae*
- b. *Treponema endemicum*
- c. *Treponema pallidum*
- d. *Treponema carateum*

Answers

1. c 2. d 3. a 4. c 5. b

Viral Exanthems and Other Cutaneous Viral Infections

CHAPTER 56

CHAPTER PREVIEW

- Herpesvirus Infections
 - Herpes Simplex Virus Infections
 - Varicella-Zoster Virus Infections
- Parvovirus Infections
- Human Papilloma Virus Infections
- Pox Virus Infections
- Measles
- Rubella
- Hand-foot-and-mouth Disease

An exanthem is an eruption or rash on the skin, that may be associated with fever or other systemic symptoms. Majority of exanthems have an infectious etiology; most commonly by viruses. Exanthems may also be seen due to drug reactions.

The viruses that can cause exanthematous and other types of skin lesions include (Table 56.1):

- ❖ **Herpesviruses:** Herpes simplex virus, varicella-zoster virus, rarely Epstein-Barr virus (secondary to ampicillin therapy) and HHV-6 and HHV-7 infection
- ❖ **DNA viruses** other than herpesviruses such as: smallpox virus, parvovirus and human papilloma viruses (HPV)
- ❖ **RNA viruses** such as: Measles, Rubella and Coxsackie viruses. Exanthematous rashes are also a common feature of viral hemorrhagic fever, discussed in Chapter 34.

Table 56.1: Viral exanthems and other cutaneous viral infections.	
Viruses	Viral exanthems/other skin lesions
Herpesviruses	
Herpes simplex virus	Vesicular lesions
Varicella-zoster virus	Chickenpox and zoster
Epstein-Barr virus	Following ampicillin therapy
Human herpesvirus-6	Roseola infantum (exanthem subitum or sixth disease)
Other DNA viruses	
Poxviruses	Smallpox Molluscum contagiosum*
Parvovirus	Erythema infectiosum (fifth disease) Papular-purpuric gloves and socks syndrome*
Human papilloma virus (HPV)	Warts* : Common warts, flat warts, plantar warts, anogenital warts (condyloma acuminatum)
RNA viruses	
Measles virus	Rashes, Koplik's spots
Rubella virus	Rashes
Coxsackie viruses	Hand-foot-and-mouth disease
Agents of viral hemorrhagic fever	Dengue, Ebola and others (Chapter 34)

*These are not exanthematous lesions.

HERPESVIRUS INFECTIONS

GENERAL PROPERTIES

Herpesviridae comprises of a group of viruses that possess a unique property of establishing latent or persistent infections in their hosts and later on undergoing periodic reactivation.

Morphology

Herpesviruses are large (150–200 nm size), spherical in shape with icosahedral symmetry.

- ❖ **Nucleocapsid:** They possess a linear dsDNA, surrounded by a capsid comprising of capsomeres (Fig. 56.1)
- ❖ **Envelope:** The nucleocapsid is surrounded by a lipid envelope into which glycoprotein spikes are inserted; which help in viral entry by binding to the specific host cell receptors
- ❖ **Tegument:** Between the capsid and envelope, there is an amorphous, sometimes asymmetric structure present called tegument

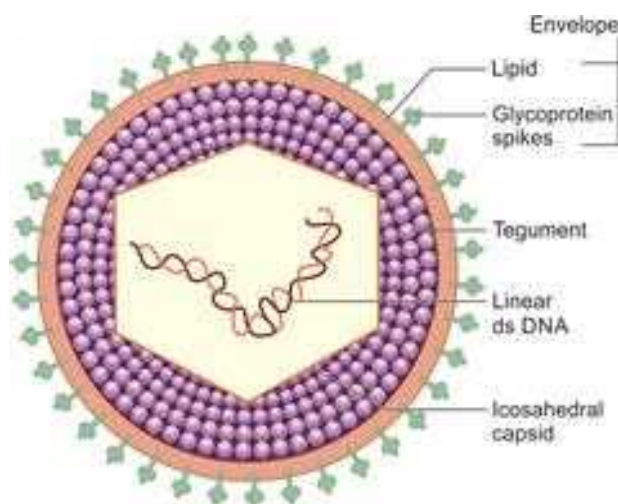


Fig. 56.1: Herpes simplex virus (schematic diagram).

Subfamily (“-herpesvirinae”)	Duration of replication and cytopathology	Site of latency	Genus	Species	
				Official name	Common name
Alpha	Short (12–18 hours) Cytolytic	Neurons	<i>Simplexvirus</i>	Human herpesvirus 1	Herpes simplex virus type 1
				Human herpesvirus 2	Herpes simplex virus type 2
			<i>Varicellovirus</i>	Human herpesvirus 3	Varicella-zoster virus
Beta	Long (>24 hours) Cytomegalic	Glands, kidneys	<i>Cytomegalovirus</i>	Human herpesvirus 5	Cytomegalovirus
				Long (>24 hours) Lymphoproliferative	Lymphoid tissues (T cells)
	Human herpesvirus 7	Human herpesvirus 7			
Gamma	Variable Lymphoproliferative	Lymphoid tissues (B cells)	<i>Lymphocryptovirus</i>	Human herpesvirus 4	Epstein-Barr virus
			<i>Rhadinovirus</i>	Human herpesvirus 8	Kaposi’s sarcoma-associated herpesvirus

❖ **Replication** of herpesviruses takes place in the host cell nucleus and is similar to replication of any other dsDNA virus as described in Chapter 4. The only difference is that the linear dsDNA of herpesviruses becomes circular inside the host cell and then replicates by **rolling circle mechanism**.

Classification

Family Herpesviridae comprises of three subfamilies (α , β and γ)—classified based on the site of latency, duration of growth cycle and type of cytopathology they produce (Table 56.2).

- ❖ Each subfamily in turn has one or more genera and each genus consists of a few species
- ❖ For general use, the common names are still popular, which are also followed in this textbook
- ❖ There are about 100 herpesviruses infecting different animals, out of which only eight are human herpesviruses which infect exclusively man (Table 56.2).

HERPES SIMPLEX VIRUS INFECTIONS

Herpes simplex viruses belong to α -subfamily of Herpesviridae.

- ❖ They are extremely widespread and exhibit a broad host range; can infect many types of cells and different animals. However, the human herpesviruses infect exclusively man
- ❖ They replicate fast (12–18 hours cycle), spread fast and are cytolytic
- ❖ They can cause a spectrum of diseases, involving skin, mucosa and various organs
- ❖ They undergo **latency in nerve cells**; reactivate later causing recurrent lesions.

Herpes simplex viruses (HSV) are of two distinct types: HSV-1 and HSV-2. They differ from each other in many aspects (Table 56.3).

Pathogenesis

Primary Infection

- ❖ **Transmission** occurs through abraded skin or mucosa from any site, but more commonly by:

Table 56.3: Differences between HSV-1 and HSV-2.

Properties	Herpes simplex virus 1	Herpes simplex virus 2
Common modes of transmission	Direct contact with mucosa or abraded skin	Sexual mode or vertical mode
Latency in	Trigeminal ganglia	Sacral ganglia
Age affected	Young children	Young adults
Common manifestations	<ul style="list-style-type: none"> • Oral-facial mucosal lesions • Encephalitis and meningitis • Ocular lesions • Skin lesions— above the waist 	<ul style="list-style-type: none"> • Genital lesions • Skin lesions— below the waist • Neonatal herpes
Neurovirulence	Less	More
Drug resistance	Less	More
Antigenic homology	HSV-1 and 2 show >80% antigenic homology	
DNA homology	HSV-1 and 2 show >50% homology in the genomic sequence	

- **HSV-1:** Oropharyngeal contact with infected saliva or direct skin contact
- **HSV-2:** Sexual contact or rarely vertical mode (from mother to fetus).
- ❖ **Site of infection:** HSV replicates at the local site of infection and produces lesions anywhere, but more commonly in:
 - HSV-1 lesions are confined to areas above the waist (most common site—around mouth)
 - HSV-2 produces lesions below the waist (most common site—genital area).
- ❖ **Spread via nerve:** Virus then invades the local nerve endings and is transported by retrograde axonal flow to the dorsal root ganglia, where it replicates further, and then undergoes latency
- ❖ Primary HSV infections are usually mild; in fact, most are asymptomatic
- ❖ However in immunocompromised hosts, viremia occurs that leads to widespread organ involvement and systemic manifestations.

Latent Infection

- ❖ HSV has a tendency to undergo latency in neurons:
 - HSV-1: undergoes latency in trigeminal ganglia
 - HSV-2: undergoes latency in sacral ganglia.
- ❖ HSV does not replicate in latent stage and also cannot be isolated during latency.

Recurrent Infections

Reactivation of the latent virus can occur following various provocative stimuli, such as fever, axonal injury, physical or emotional stress, and exposure to ultraviolet light.

- ❖ **Via the axonal spread**, virus goes back to the peripheral site and further replicates in skin or mucosa producing secondary lesions
- ❖ **Recurrent infections** are less extensive and less severe because of presence of pre-existing host immunity that limits the local viral replication
- ❖ Recurrent infections are usually asymptomatic; but the virus continues to shed in the secretions.

Clinical Manifestations

Both HSV-1 and 2 have been isolated from nearly all mucocutaneous sites and viscera; however, in general, oral-facial infections are common with HSV-1, whereas HSV-2 frequently causes genital infections and intrauterine infections. The incubation period ranges from 1 to 26 days (median, 6–8 days).

Oral-facial Mucosal Lesions

Oral-facial mucosal lesions are the most common manifestation of HSV infections (Figs 56.2A and B).

- ❖ Most common affected site is buccal mucosa
- ❖ Most frequent primary lesions are gingivostomatitis and pharyngitis
- ❖ Most frequent recurrent lesion is herpes labialis (painful vesicles near lips) (Fig. 56.2A)
- ❖ Other lesions produced are—ulcerative stomatitis, tonsillitis and vesicular lesions on the eyelids (Fig. 56.2B)
- ❖ Many cases are asymptomatic but can predispose to secondary bacterial infection.



Figs 56.2A and B: A. Vesicular lesions on lips and tongue due to HSV-1 infection; B. Periocular vesicular lesions due to HSV-1 infection.

Source: Public Health Image Library, A. ID# 12616 (Robert E Sumpter), B. ID# 6492 (Dr KL Hermann)/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Cutaneous Lesions

HSV usually infects through abraded skin and causes various cutaneous lesions.

- ❖ **Herpetic whitlow:** Lesions present on the fingers of dentists and hospital personnel
- ❖ **Febrile blisters** (herpes febrilis): Fever due to any other cause can provoke HSV to cause recurrent blisters
- ❖ **Herpes gladiatorum:** Mucocutaneous lesions present on the body of wrestlers
- ❖ Skin lesions are often severe on underlying eczema or burns which permit extensive local viral replication and spread
- ❖ **Eczema herpeticum:** Caused by HSV-1 in patients with chronic eczema. Similar lesions are also produced by vaccinia virus or coxsackievirus A16 infection; these conditions together are designated as Kaposi's varicelliform eruptions
- ❖ **Erythema multiforme:** HSV is commonly associated with this condition. Herpes antigens have been detected in the immune complexes found in serum or skin biopsies.

Other Infective Syndromes of HSV

- ❖ **CNS infections:** HSV causes various neurological manifestations such as encephalitis, meningitis, Bell's palsy (due to facial nerve involvement) etc. (Chapters 73 and 74)
- ❖ **Ocular manifestations:** HSV causes various ocular manifestations such as keratoconjunctivitis, corneal ulcer and blindness. Detail is discussed in Chapter 78
- ❖ **Genital lesions:** HSV-2 is more common than HSV-1 to produce genital lesions; described as bilateral, painful, multiple, tiny vesicular ulcers (Chapter 77)
- ❖ **Visceral and disseminated herpes:** HSV viremia results in disseminated infection; involving multiple organs
 - **Risk factors:** Immunocompromised patients, underlying malnutrition or AIDS, pregnant women and transplant recipients are at a higher risk of disseminated infection
 - **Common manifestations** include: Pneumonitis, tracheobronchitis and hepatitis.
- ❖ **Neonatal herpes:** Transmission is more common during birth than in utero. Neonates are almost always symptomatic and present with either local lesions or disseminated infection or CNS infections (Chapter 79).

Epidemiology

Herpes simplex viruses are worldwide in distribution. No animal reservoirs or vectors are involved with the human viruses. HSV-1 and 2 differ in their epidemiological pattern.

Epidemiological Pattern of HSV-1

- ❖ **Transmission:** HSV-1 infection is more common and is transmitted by contact with infected secretions (saliva)

- ❖ Primary infection occurs early in life and is either asymptomatic or remains confined to oropharyngeal disease
- ❖ **Age:** Children are commonly affected
- ❖ **Adults:** Antibodies develop in 70–90% of adults, but they fail to eliminate the virus from the body. Most adults become carriers throughout the life, occasionally get transient recurrent attacks.

Epidemiological Pattern of HSV-2

- ❖ HSV-2 is transmitted by sexual or vertical routes
- ❖ Primary infection occurs in adult life. Antibodies develop only in 20% of people particularly among black women than men and whites
- ❖ HSV-2 tends to recur more often than HSV-1, irrespective of the site of infection.

LABORATORY DIAGNOSIS

HSV infections

- ❑ **Cytopathology** (Tzanck preparation) by Wright's or Giemsa stain—detects inclusion bodies (Lipschultz body) and formation of multinucleated giant cells
- ❑ **Virus isolation by:**
 - Conventional cell lines—used to demonstrate diffuse rounding and ballooning of cell lines
 - Shell vial culture—detects antigens in cell line by IF.
- ❑ **Viral antigen detection** in specimen by direct IF
- ❑ **HSV DNA detection** by PCR and real-time PCR (detecting glycoprotein B and UL 30 genes)
- ❑ **Antibody detection** by ELISA or other formats—detecting antibodies to glycoprotein G.

Laboratory Diagnosis

The sensitivity of all the methods to diagnose HSV infection depends on the type of specimen, as well as the type of infection. The sensitivity is more for vesicular lesions and primary infection than for ulcerative lesions and recurrent infections.

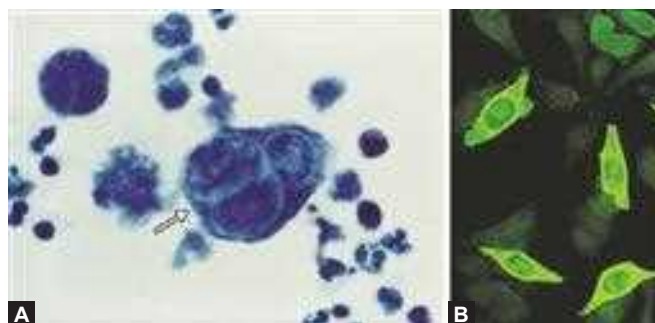
Cytopathology

Scrapings obtained from the base of the lesion can be stained with Wright's or Giemsa (Tzanck preparation), or Papanicolaou stain. Sensitivity of staining is low (<30% for mucosal swabs). It cannot differentiate between HSV-1, HSV-2, and varicella-zoster virus; as all of them produce similar but characteristic cytopathological changes such as:

- ❖ Production of Cowdry type A intranuclear inclusion bodies (Lipschultz body)
- ❖ Formation of multinucleated giant cells with faceted nuclei and ground glass chromatin (Tzanck cells) (Fig. 56.3A)
- ❖ Ballooning of infected cells, margination of chromatin.

Virus Isolation

It remains the most definitive tool for HSV diagnosis. McCoy cell lines are preferred for isolation of HSV. Viral growth can be detected in 2–4 days by:



Figs 56.3A and B: **A.** Tzanck smear of a tissue scraping showing multinucleated giant cell (Tzanck cell) in the center (arrow showing); **B.** Indirect IF for HSV1/2 antibody detection.

Source: A. Public Health Image Library, ID# 14428/Centers for Disease Control and Prevention (CDC), Atlanta; B. Euroimmun (with permission).

- ❖ **Characteristic cytopathic effect:** Diffuse rounding and ballooning of the infected cells
- ❖ Viral antigen detection by neutralization test or immunofluorescence staining with specific antiserum
- ❖ Shell vial technique can be followed to decrease the detection time to <24 hours.

Viral Antigen Detection by Immunofluorescence

Viral antigen detection (targeting cell surface glycoprotein antigens) by direct IF is also a sensitive and specific assay. It can differentiate HSV-1 from HSV-2.

HSV DNA Detection

- ❖ Polymerase chain reaction (PCR) is the most sensitive test for detecting HSV infections and can be used to differentiate between HSV-1 and HSV-2
- ❖ Real-time PCR has been developed targeting genes such as glycoprotein B and UL 30; which are more sensitive; can quantitate the viral load in specimens (prognostic marker of HSV encephalitis) and also can differentiate between HSV-1 and -2
- ❖ The BioFire FilmArray is an automated nested multiplex PCR. Its Meningitis/Encephalitis (ME) panel can detect simultaneously 14 microbial pathogens causing CNS infections, including HSV-1 and HSV-2.

Antibody Detection

Antibodies appear in 4–7 days after the infection and peak in 2–4 weeks. IgM appears first and is replaced by IgG, which persists for life.

- ❖ Most available tests usually detect IgG or total antibodies, hence cannot differentiate between recent and past infections. Seroconversion or a rise in titer is more meaningful
- ❖ Serologic assays (e.g. ELISA) based on the type-specific antigens such as glycoprotein G antigens (gG1 and gG2) can differentiate between HSV-1 and HSV-2. Western blot is more accurate, with 98% sensitivity and specificity
- ❖ Both ELISA and indirect IF formats are available (Fig. 56.3B).

TREATMENT

HSV infections

Several specific antiviral drugs are effective for HSV infections. Acyclovir is the drug of choice. It acts by inhibiting viral DNA polymerase.

- ❑ **For mucocutaneous infections:** Acyclovir, famciclovir and valacyclovir have been the mainstay of treatment
- ❑ **Ocular infection:** Topical idoxuridine, trifluorothymidine, topical vidarabine, and cidofovir are used
- ❑ **For HSV encephalitis and neonatal herpes,** acyclovir is the treatment of choice.

Acyclovir resistance has been reported among few HSV strains which have altered substrate specificity for phosphorylating acyclovir. Resistance is more common in HSV-2 and among immunocompromised patients. Foscarnet is the drug of choice to treat such cases.

Prevention

General measures can be taken such as:

- ❖ Use of condom to prevent genital herpes
- ❖ Neonatal herpes can be prevented by prior administration of acyclovir to mothers during third trimester of pregnancy or delivery by elective cesarean section
- ❖ No vaccine is currently licensed. Several vaccine trials are going on, such as recombinant HSV-2 glycoprotein vaccine.

Infection control measures: Patients with mucocutaneous herpes in hospitals, should be kept on contact precautions until lesions are dry and crusted (Chapter 21).

VARICELLA-ZOSTER VIRUS INFECTIONS

Varicella-zoster virus (VZV) produces vesicular eruptions (rashes) on the skin and mucous membranes in the form of two clinical entities:

1. **Chickenpox:** It is characterized by generalized diffuse bilateral vesicular rashes which occur following primary infection, usually affecting children (Fig. 56.4A)
2. **Zoster or shingles:** It occurs following reactivation of latent VZV, present in the trigeminal ganglia that occurs mainly in adult life. Vesicular rashes are unilateral and segmental (confined to the skin innervated by a single sensory ganglion) (Figs 56.4B and C).

Chickenpox

Pathogenesis

VZV enters through the upper respiratory mucosa or the conjunctiva by aerosol (most common) and contact transmission. From the local site, it spills over to blood and then is carried through the infected mononuclear cells to target sites such as:

- ❖ Skin (produces rashes)
- ❖ Respiratory tract (shed in respiratory secretions)
- ❖ Neurons (undergoes latency).

Clinical Manifestations

- ❖ Incubation period is about 10–21 days (2–3 weeks)
- ❖ **Rashes** are the main manifestation of chickenpox
 - Rashes are vesicular (Fig. 56.4A)
 - Centripetal in distribution: Usually start on the face and trunk, spread rapidly to involve flexor surfaces; sparing distal part of the limbs
 - Bilateral and diffuse in distribution
 - Rashes appear in multiple crops: Lesions in various stages of evolution, such as maculopapules, vesicles, pustules, and scabs can be found in one area at the same time
 - Fever appears with each crop of rashes.
- ❖ Chickenpox is a disease of childhood



Figs 56.4A to C: A. Rashes of Chickenpox; B and C. Segmental distribution of rashes of Zoster.

Source: A. Public Health Image Library, ID#/2882/JD Millar/ Centers for Disease Control and Prevention (CDC), Atlanta (with permission); B. CW Leung, Department of Pediatrics and Adolescent Medicine, Princess Margaret Hospital, Hong Kong (with permission); C. Recommendations of the Advisory Committee on Immunization Practices [ACIP]. MMWR Morb Mortal Wkly Rep 2008;57[RR-5]:1 (with permission).

- ❖ When occurs in adults, it is more severe with bullous and hemorrhagic rashes leaving behind pitted scars on skin after recovery.

Complications

Complications are more common in adults and in immunocompromised individuals.

- ❖ **Most common infectious complication is:** Secondary bacterial infections of the skin
- ❖ **Most common extracutaneous complication is:** CNS involvement (cerebellar ataxia, encephalitis and aseptic meningitis), usually occurs in children
- ❖ **Most serious complication is:** Varicella pneumonia, which develops more commonly in adults (up to 20% of cases) than in children and is particularly severe in pregnant women
- ❖ **Reye's syndrome** can occur secondary to VZV infection. It is characterized by fatty degeneration of liver following salicylate (aspirin) intake
- ❖ **Other complications are:** Myocarditis, nephritis, corneal lesion and arthritis
- ❖ **Chickenpox in pregnancy:** Chickenpox in pregnancy can affect both mother and the fetus.
 - Mothers are at high-risk of developing varicella pneumonia
 - Fetus is at higher risk of developing **congenital varicella syndrome** (in early pregnancy); characterized by cicatricial skin lesions and limb hypoplasia (Chapter 79, for detail).

Epidemiology

Chickenpox is a highly contagious disease.

- ❖ It is primarily a disease of the temperate regions; occurs throughout the year with prevalence of 13–16/1,000 people per year
- ❖ **Age:** Common in children between 1 to 14 years of age
- ❖ **Period of infectivity:** Child is infectious from 2 days before the onset of rash to 5 days thereafter, until the vesicles are crusted
- ❖ One attack gives lifelong immunity
- ❖ **Reservoir:** Humans are the only known reservoir host
- ❖ **Source of infection:** Patients are the only source, there are no carriers
- ❖ Secondary attack rate is about 70–90%.

Zoster or Shingles or Zona

Zoster usually occurs due to reactivation of latent VZV in old age (>60 years of age), in immunocompromised individuals or occasionally in healthy adults.

- ❖ It usually starts with severe pain in the area of skin or mucosa supplied by one or more groups of sensory nerves and ganglia
- ❖ **Rashes:** They are unilateral and segmental, confined to the area of skin supplied by the affected nerves (Figs 56.4B and C)

- ❖ **Most common nerve involved** is ophthalmic branch of trigeminal nerve. Head, neck and trunk are the most common affected sites.

Complications of Zoster

- ❖ **Post-herpetic neuralgia** (pain at the local site lasting for months): It is the most common complication in elderly patients
- ❖ **Zoster ophthalmicus:** Unilateral painful crops of skin rashes surrounding the eye
- ❖ **Ramsay Hunt syndrome** develops when geniculate ganglion of facial nerve is involved. It is characterized by triad of ipsilateral facial paralysis, ear pain, and vesicles on the face, tympanic membrane and external auditory meatus
- ❖ **Visceral disease**, especially pneumonia can occur which is the most common cause of death (<1%) in zoster patients
- ❖ Recurrent or chronic zoster is common with HIV-infected patients.

Laboratory Diagnosis (VZV)

Laboratory diagnosis of VZV infection is as follows:

- ❖ **Specimen collection:** Common specimens include vesicular lesions, scabs and maculopapular lesions
- ❖ **Cytopathology:** Giemsa staining of the scrapings from the ulcer base (Tzanck smear) reveals cytopathological changes similar to that of HSV infection, such as formation of multinucleated giant cells
- ❖ **Virus isolation:** Virus isolation in various cell lines can also produce HSV-like cytopathic effects such as diffuse rounding and ballooning of infected cells
- ❖ **VZV-specific methods:**
 - Specific antigen detection by direct immunofluorescence staining
 - Specific IgM and IgG antibody detection by ELISA
 - PCR detecting VZV-specific genes
 - The BioFire Meningitis/Encephalitis (ME) Panel tests simultaneously 14 microbial pathogens causing CNS infections including VZV.

TREATMENT

VZV infections

Acyclovir, famciclovir or valacyclovir are the agents of choice. It can prevent the complications of chickenpox and can also halt the progression of zoster in adults, but cannot prevent post-herpetic neuralgia.

Vaccine

- Live attenuated vaccine using **Oka strain** of VZV is available.
- ❖ It is given to children after 1 year of age; 2 doses, first dose is given at 12–15 months and second at 4–6 years
- ❖ In seronegative adults; 2 doses given at 1-month gap
- ❖ As this vaccine is not being given as a part of national immunization schedule, the countrywide vaccine coverage is inadequate leading to frequent outbreaks

- ❖ Transmission of the vaccine virus can occasionally cause mild rashes in the recipient
- ❖ **Protectivity:** The vaccine is >80% effective in preventing chickenpox in children but less so in adults (70%). However, it is 95% effective in preventing severe disease.

VZIG (Varicella-zoster Immunoglobulin)

- ❖ It is useful for **post-exposure prophylaxis**; given within 96 hours (preferably within 72 hours) of exposure. As adults are at higher risk of varicella-related deaths, VZIG is recommended for adults, especially those who are at greater risk for complications—immunocompromised persons such as HIV, pregnancy, etc.
- ❖ It is also indicated for **neonates born to mothers** suffering from chickenpox if the onset of chickenpox in mother is between <5 days before delivery till 48 hours after delivery. VZIG is not indicated for the neonate if the mother has zoster.

Infection Control Measures

Patients infected with VZV should be kept in isolation. Airborne precautions (e.g. negative air-flow rooms) plus contact precautions must be followed until lesions are dry and crusted (Chapter 21). For localized zoster in an immunocompetent host, contact precaution alone can be followed.

OTHER HERPESVIRUS INFECTIONS

Other herpesviruses include:

- ❖ **Epstein-Barr virus:** EBV causes infectious mononucleosis (Chapter 68) and various malignancies (Chapter 80). It can produce rashes following ampicillin therapy
- ❖ **Cytomegalovirus:** CMV causes congenital infection (Chapter 79); also produces various infections in transplant recipients
- ❖ **Human herpesvirus 6:** It infects the T cells by binding to CD46 receptor. It has two variants 6A and 6B
 - Transmission is through infected oral secretions
 - **Sixth disease:** In children, HHV-6 (usually the 6B variant) causes sixth disease, also called as *exanthem subitum* or *roseola infantum*. It is characterized by high grade fever and skin rashes
 - In older age groups, HHV-6 has been associated with mononucleosis-like syndrome.
- ❖ **Human herpesvirus 7:** HHV-7 also shows tropism for T cells, transmitted by oral secretions, mainly in children
 - It shares 30–50% DNA homology with HHV-6
 - It has been associated with fever, seizures, respiratory or gastrointestinal symptoms, and pityriasis rosea.
- ❖ **Human herpesvirus 8:** It causes a malignancy called Kaposi sarcoma in HIV-infected individuals (Chapter 80).

OTHER DNA VIRUSES CAUSING CUTANEOUS INFECTIONS

PARVOVIRUS INFECTIONS

Morphology

Parvoviruses are the simplest animal viruses infecting humans, responsible for a common childhood exanthema called as **erythema infectiosum (fifth disease)**.

- ❖ They are the smallest viruses (18–26 nm size)
- ❖ Non-enveloped with icosahedral symmetry
- ❖ They are the only DNA viruses to possess single-stranded DNA
- ❖ Parvovirus B19 is the most common parvovirus, pathogenic to man
- ❖ They depend upon the host cell enzymes for replication.

Pathogenesis

Parvovirus B19 exclusively infects humans.

- ❖ **Transmission:** By the respiratory route (most common), followed by blood transfusion and transplacental route
- ❖ **Infects precursors of RBCs:** It has a special tropism for erythroid progenitor cells present in the adult bone marrow and fetal liver as it binds to blood group P antigen as receptors; which are present on the RBC surface
- ❖ This results in destruction of RBCs and inhibition of erythropoiesis.

Clinical Manifestations

- ❖ **Erythema infectiosum (or fifth disease):** In children, it produces rashes on the face with characteristic **slapped cheek appearance** (Fig. 56.5). Adult women present with symmetrical polyarthropathy which usually involves the hand joints and knee
- ❖ **Transient aplastic crisis:** It can occur in infected patients with underlying hemolytic anemia, resulting in severe acute anemia



Fig. 56.5: Fifth disease or rashes with slapped cheek appearance.

Source: Wikipedia/Andrew Kerr (with permission).

- ❖ **Pure red cell aplasia:** It can occur in those with underlying immunosuppression due to persistent B19 infections, resulting in chronic anemia
- ❖ **Non-immune hydrops fetalis** can occur in fetus, which results in fatal anemia and fetal death. Transplacental transmission occurs in 30% of cases and maximum risk is in the second trimester
- ❖ **Papular-purpuric gloves and socks syndrome:** It presents as rapidly progressive, painful, pruritic, and symmetric swelling and erythema of the distal hands and feet, usually in the spring and summer; which may be confused with acute meningococemia.

Laboratory Diagnosis

- ❖ **Molecular methods:** The most sensitive assay for diagnosis is PCR, which detects viral DNA (e.g. genes coding for VP1 and VP2) from serum, tissue or respiratory secretions. Real time PCR is used for quantification of viral load in blood, during acute infections
- ❖ **Antibody detection:** ELISA has been available detecting antibodies against VP1 and VP2 antigens. IgM appears early, indicates recent infection and remains elevated for 2–3 months
- ❖ **Antigen detection:** Immunohistochemistry has been used to detect viral antigens in fetal tissues and bone marrow.

TREATMENT

Parvovirus infections

- ❑ No antiviral drug is available
- ❑ Symptomatic treatment is given
- ❑ Immunoglobulins containing neutralizing antibodies to human parvovirus are available commercially.

HUMAN PAPILLOMAVIRUS INFECTIONS

Human papillomavirus (HPV) is a DNA virus, belongs Papillomaviridae family. It has selective tropism for epithelium of skin and mucous membranes. It has >100 serotypes, which produce an array of infections ranging from benign warts, to malignant neoplasia of cervix (Chapter 80).

Benign warts: They are small, hard, rough growth on the skin (Fig. 56.6). They are of following types:

- ❖ **Common skin warts** (verruca vulgaris) and flat warts (verruca plana) are common in children (seen with serotypes 2, 4, 27, 57)
- ❖ **Plantar warts** (verruca plantaris)–benign lesion, widely prevalent among adolescents (seen with serotype 1)
- ❖ **Anogenital warts** (condyloma acuminatum): It is a sexually transmitted infection, seen among adults and is associated with HPV serotypes 6 and 11 (Chapter 77).

POXVIRUS INFECTIONS

Morphology

Poxviruses are the largest (400 nm in length × 230 nm in diameter) among all the viruses, large enough to be seen under light microscope.



Fig. 56.6: Plantar warts.

Source: Wikipedia (with permission).

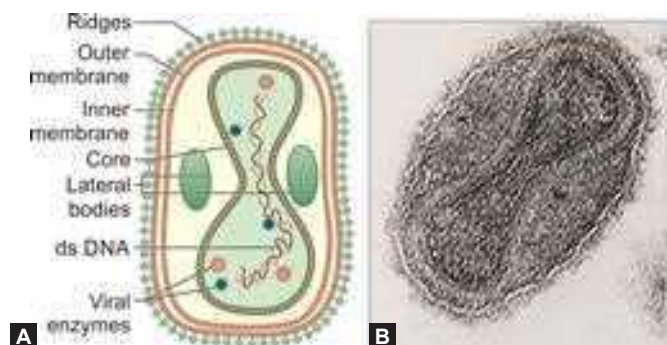
- ❖ Most complex viruses; their structure does not fit into either icosahedral or helical symmetry
- ❖ Brick-shaped or ellipsoid
- ❖ **Envelope:** Externally, there is an envelope, made up of two lipoprotein membranes (outer and inner) with ridges arising from the outer membrane. The envelope encloses a core and two structures of unknown function called lateral bodies (Figs 56.7A and B)
- ❖ Core or the nucleocapsid is biconcave dumbbell shaped
- ❖ Possess single linear dsDNA. It is the only DNA virus that replicates in the cytoplasm.

The following are the poxviruses of human importance.

- ❖ **Variola:** It was the causative agent of **smallpox**, the first disease to be eradicated from the world
- ❖ **Vaccinia:** It was used as a vaccine for smallpox
- ❖ **Molluscum contagiosum virus:** It causes warty lesions called as molluscum contagiosum.

Smallpox

Smallpox was the first infectious disease to be eradicated from the world. It was characterized by highly contagious severe exanthema (rashes). The agents of smallpox were variola major and variola minor.



Figs 56.7A and B: A. Smallpox virus (schematic diagram); B. Smallpox virus (electron micrograph).

Source: B. ID# 1849 Dr Fred Murphy, Sylvia Whitfield/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Smallpox Time Line

- ❖ Last natural case of variola major was seen in a Bangladeshi woman in Assam in May 1975
- ❖ Last natural case of variola minor was seen in Merca, Somalia, 26th October 1977
- ❖ **Eradication** was declared by WHO nearly after three years of the last case, i.e. on 8th May 1980
- ❖ **Laboratory spread:** There was a small outbreak in Birmingham (1978), due to accidental spread of the virus from the virus laboratory, following which stocks from most laboratories have been destroyed
- ❖ **Maintenance:** Currently, only two laboratories hold stocks of smallpox virus
 - CDC (Centers for Disease Control and Prevention) Atlanta (USA)
 - Center for Research on Virology and Biotechnology, Koltsova (Russia).
- ❖ **Agent of bioterrorism:** As vaccination was stopped following eradication, people borne after 1980 are not immunized. Hence, smallpox virus can be a potential agent of bioterrorism.

Reasons that Made Eradication Successful

- ❑ Variola was an exclusively human pathogen, no animal reservoir
- ❑ Source: Patients were the only source, there were no carriers
- ❑ Case detection was easy-due to characteristic appearance of rashes (Table 56.4)
- ❑ Subclinical cases were not transmitting the disease
- ❑ Global smallpox eradication program was launched in 1967 by WHO (World Health Organization). With a strong international cooperation and intense effort; disease was wiped out nearly after 10 years
- ❑ Highly effective live vaccinia vaccine
 - Freeze dried form was used (↑stability)
 - Multiple puncture technique was followed to administer the vaccine by using a bifurcated needle, which was found to be simple, effective and economical.

Clinical Manifestations

The portal of entry of the virus was via the mucous membranes of the upper respiratory tract (aerosol transmission).

Table 56.4: Differences between smallpox and chickenpox	
Smallpox	Chickenpox
Incubation period: 12 days (7–17 days)	Incubation period: 15 days (10–21 days)
Site of rash: Palm, sole and extensor surface	Site of rash: Axilla and flexor surface
Rash: Deep seated and appear in single stage, evolution is slow, centrifugal distribution	Rash: Superficial and pleomorphic (appear in crops) evolution is rapid, dew drop rashes, centripetal distribution
Fever subsides with appearance of rash	Fever rises with each crop of rash

- ❖ After an incubation period of 12 days (7–17 days), the patient developed the rashes
- ❖ **Description of rashes:** Smallpox rashes were unique in appearance and could easily be differentiated from that of chickenpox (Table 56.4)
 - Rashes were deep seated and all rashes in an area appeared in one stage, evolution was slow (Fig. 56.8)
 - Centrifugal distribution-palm and sole and extensor surface were affected first
 - Fever subsided with appearance of rash.

Laboratory Diagnosis

- ❖ **Direct detection in scrapings from rashes:**
 - Intracytoplasmic inclusion bodies (Paschen bodies)
 - Electron microscopy: Brick-shaped appearance with biconcave DNA core.
- ❖ **Egg inoculation:** Characteristic pock formation is seen on the chorioallantoic membrane (CAM) of a chick embryo.

TREATMENT

Smallpox

Cases used to be treated in the past with:

- ❑ Vaccinia immunoglobulins
- ❑ Antiviral drugs such as methisazone, cidofovir or tecovirimat.

Vaccination

- ❖ **Live vaccinia vaccine** was highly effective.
 - It was given as a single dose, at 1–2 years of age
 - As un-attenuated live virus was used, adverse reactions were common; such as mild vaccinia-induced rashes.
- ❖ **Cowpox vaccine** discovered by Edward Jenner (the father of vaccination) was in use before vaccinia vaccine was available
- ❖ **Variolation** was the first attempt of providing artificial immunity against smallpox. It was in use even before cowpox vaccine was available (17th–18th century). Healthy people were inoculated with the skin scraping of a smallpox patient.



Fig. 56.8: Smallpox rashes over the face.

Source: Public Health Image Library, ID# 3/Centers for Disease Control and Prevention (CDC), Atlanta/Cheryl Tyron (with permission).

Vaccinia

Vaccinia virus cross-reacts with variola and the antibodies produced against vaccinia are protective for variola. The antigenic cross reactivity was so much that vaccinia was able to eradicate variola globally.

However, vaccinia differs from variola in many ways:

- ❖ It is non-pathogenic to humans or produces milder skin lesions
- ❖ Produces an inclusion body called Guarnieri body (variola produces Paschen body)
- ❖ On CAM, vaccinia virus produces larger and hemorrhagic and necrotic pock lesions than variola.

Molluscum Contagiosum

Molluscum contagiosum virus is an obligate human poxvirus that produces characteristic skin lesions.

Clinical Manifestations

- ❖ **Lesions:** It produces dome-shaped, pink pearly wart-like lesions (2–5 mm size), umbilicated, with a dimple at the center (Fig. 56.9A). Lesions are found singly or in clusters, anywhere on the body except on the palms and soles. Genital lesions are seen in adults
- ❖ **Transmission:**
 - Children are commonly affected, acquire infection by direct and indirect contact (e.g. by barbers, common use of towels, swimming pools)
 - Rarely sexual transmission has been reported in young adults.
- ❖ **Self-limiting:** Lesions disappear in 3–4 months. There are no systemic complications
- ❖ **In HIV-infected patients:** Disease is more generalized, severe and persistent.

Laboratory Diagnosis

- ❖ **Molluscum bodies** are the intracytoplasmic eosinophilic inclusions seen in skin scrapings stained with histopathological stains (Fig. 56.9B)
- ❖ Electron microscopy and PCR can be used for confirmation
- ❖ **Not cultivable:** It cannot be propagated in tissue culture, embryonated egg or in animals.

TREATMENT

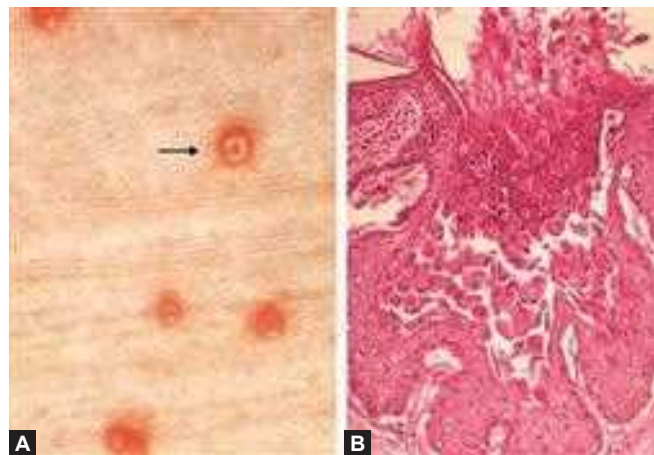
Molluscum contagiosum

Surgical removal of the lesions by ablation (by cryotherapy or laser therapy) is the mainstay of treatment. Cidofovir has shown to have some efficacy. As this virus does not cross-react with any other poxviruses, smallpox vaccine is not protective.

Other Poxviruses of Human Importance

They are zoonotic, mainly infect various animals. Human infection is rare.

- ❖ **Monkeypox:** Can produce vesicular rash similar to smallpox but milder. Infection can be prevented by smallpox vaccination



Figs 56.9A and B: A. Molluscum contagiosum lesions on skin; B. Histopathology of skin showing molluscum bodies.

Source: A. CDC/ L Sperling, MD, Walter Reed Army Medical Center; B. Public Health Image Library, ID# 860/Centers for Disease Control and Prevention (CDC), Atlanta/Dr Edwin P Ewing, Jr (with permission).

- ❖ **Orf virus:** Produce localized skin lesions, called as contagious pustular dermatitis or mouth sore
- ❖ **Pseudocowpox (Paravaccinia):** Can rarely infect milk handlers to produce nodular skin lesions called milker's nodule
- ❖ **Cowpox and buffalopox:** Can produce pox-like lesions and mild systemic illness.

RNA VIRUSES CAUSING CUTANEOUS INFECTIONS

MEASLES

Measles is an acute, highly contagious childhood disease, characterized by fever and respiratory symptoms, followed by typical maculopapular rash (see Fig. 56.10B). The causative agent, measles virus is a RNA virus, belongs to Paramyxoviridae family (Chapter 66).

Pathogenesis

- ❖ **Transmission:** Measles is transmitted via the respiratory route either by—
 - Droplets inhalation over short distances (common)
 - Small-particle aerosols that remain suspended especially in schools, hospitals, and enclosed public places in the air for longer period (less common).
- ❖ **Spread:** The virus multiplies locally in the respiratory tract; then spreads to the regional lymph nodes → enters into the bloodstream through infected monocytes (primary viremia) → further multiplies in reticuloendothelial system → spills over to blood (secondary viremia) → disseminates to various sites
- ❖ **Target sites:** The virus is predominantly seeded in the epithelial surfaces of the body, including the skin, respiratory tract, and conjunctiva.



Figs 56.10A to C: **A.** Koplik spot in buccal mucosa (measles) (arrow showing); **B.** Measles rashes (on face); **C.** Multinucleated giant cell of measles infected cell lines (arrow showing).

Source: A. Public Health Image Library, ID# 6111; B. ID# 17980; C. ID# 859/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Clinical Manifestations

Incubation period is about 10 days which may be shorter in infants and longer (up to 3 weeks) in adults. Disease can be divided into three stages.

1. Prodromal Stage

This stage lasts for 4 days (i.e. from 10th to 14th day of infection) and is characterized by manifestations such as:

- ❖ **Fever** is the first manifestation, occurs on day 1 (i.e. on 10th day of infection)
- ❖ **Koplik's spots** are pathognomonic of measles, appear after two days following fever (i.e. on 12th day of infection) and are characterized by:
 - White to bluish spot surrounded by an erythema
 - Appear first on buccal mucosa near second lower molars (Fig. 56.10A)
 - Rapidly spread to involve the entire buccal mucosa and then fade with the onset of rash.
- ❖ **Non-specific symptoms** may be present such as cough, coryza, nasal discharge, redness of eye, diarrhea or vomiting.

2. Eruptive Stage

Maculopapular dusky red rashes appear after 4 days of fever (i.e. on 14th day of infection).

- ❖ Rashes typically appear first **behind the ears** → then spread to face, arm, trunk and legs → then fade in the same order after 4 days of onset (Fig. 56.10B)
- ❖ Rashes are typically absent in HIV infected people.

Fever (10th day) → Koplik's spot (12th day) → rash (14th day)

3. Post-measles Stage

It is characterized by weight loss and weakness. There may be failure to recover and gradual deterioration into chronic illness.

Complications

Children of <5 years, adults of >20 years, pregnant women, and immunocompromised hosts are at higher risk of complications.

Secondary Bacterial Infections

Following measles, there is profound immune suppression and fall of cell-mediated immunity which in turn predisposes to various secondary bacterial infections.

- ❖ Otitis media and bronchopneumonia are most common
- ❖ Recurrence of fever or failure of fever to subside with the rash
- ❖ Worsening of underlying tuberculosis with a false negative Mantoux test.

Complications Due to Measles Virus Itself

- ❖ Giant-cell pneumonitis (Hecht's pneumonia) in immunocompromised children, and in HIV infected people
- ❖ Acute laryngotracheobronchitis (croup)
- ❖ Diarrhea, leads to malnutrition including vitamin A deficiency.

Central Nervous System Complications

CNS complications are rare, but most severe (Chapter 74).

- ❖ Subacute sclerosing panencephalitis (SSPE) is the most important CNS complication
- ❖ Others include post-measles encephalomyelitis and measles inclusion body encephalitis.

LABORATORY DIAGNOSIS

Measles

- ❑ **Specimen:** Nasopharyngeal swab
- ❑ **Antigen detection:** By using anti-nucleoprotein antibodies
- ❑ **Virus isolation:** By inoculation of specimen into monkey or human kidney cell line, produces CPE as multinucleated giant cells (Warthin-Finkeldey cells)
- ❑ **Antibody detection in serum:** Against nucleoprotein antigen by ELISA
- ❑ **Reverse-transcription PCR:** detects viral RNA.

Laboratory Diagnosis

Specimens

Nasopharyngeal swab, conjunctival swab, blood, respiratory secretions, and urine are the ideal specimens. Synthetic swabs are recommended.

Antigen Detection

Measles antigens in the infected cells can be detected directly by using anti-nucleoprotein antibodies (direct immunofluorescence test).

Virus Isolation

- ❖ **Cell lines:** Monkey or human kidney cells are optimal cell lines used for isolation of measles virus
- ❖ Cytopathic effect may be observed after 7–10 days of inoculation into cell lines characterized by multinucleated giant cells (**Warthin-Finkeldey cells**) containing both intranuclear and intracytoplasmic inclusion bodies (see Fig. 56.10C)
- ❖ Shell vial culture is recommended for early detection within 2–3 days.

Antibody Detection

- ❖ Detection of measles-specific IgM antibody in serum or oral fluid or four-fold rise of IgG antibody titer between acute and convalescent-phase sera is taken as significant
- ❖ Demonstration of raised titers of anti-measles antibody in the CSF is diagnostic of SSPE
- ❖ ELISA is the most recommended test that uses recombinant measles nucleoprotein (NP) antigen.

Reverse-transcription PCR

RT-PCR is available targeting measles specific RNA such as N gene(nucleoprotein) in clinical specimen.

- ❖ It is extremely sensitive and specific
- ❖ It may also permit characterization of measles virus genotypes for molecular epidemiologic studies
- ❖ RNA can be detected in specimens up to 10–14 days post rashes, in contrast to virus isolation, which often becomes negative after 3 days of rash.

Measles genotypes

There are 8 clades of measles which are further grouped into 23 recognized genotypes (WHO)
Genotype D8 followed by B3 and D4 are the commonly reported globally as well as from India.

TREATMENT

Measles

- ❑ There is no specific antiviral therapy available for measles
- ❑ Treatment is symptomatic and consists of general supportive measures
- ❑ Vitamin A has been effective in reducing the morbidity and mortality due to measles.

Prevention

General Preventive Measures

Airborne precaution such as isolation in negative pressure room, use of PPEs such as N95 respirator, etc. must be followed while handling measles cases (Chapter 21 for detail).

Measles Vaccine

Live attenuated vaccine is available for measles.

- ❖ **Strains:** The following vaccine strains are used currently
 - Schwartz strain (currently serves as the standard in much of the world)
 - Edmonston-Zagreb strain
 - Moraten strain.
- ❖ Vaccine is prepared in chick embryo cell line
- ❖ **Reconstitution:** Vaccine is available in lyophilized form and it has to be reconstituted with distilled water and then should be used within 4 hours
- ❖ Vaccine is thermolabile, hence, it must be stored at -20°C
- ❖ One dose (0.5 mL) containing more than 1000 infective viral units is administered subcutaneously
- ❖ **Combined vaccines:** Measles vaccine is available in combined form with rubella (MR vaccine), with mumps and rubella vaccine (MMR vaccine) and with varicella (MMR-V vaccine)
- ❖ **Indication:** Under national immunization schedule of India, measles-rubella (MR) vaccine is given at 9 completed months to 12 months along with vitamin A supplements and second dose of MR vaccine at 16–24 months
- ❖ **Contraindications** for MMR vaccine are: Severe life-threatening allergic reaction to MMR vaccine, advanced immune deficiency state (e.g., transplant, chemotherapy, advanced HIV), pregnancy, active tuberculosis, or has received another live vaccine within the past 30 days
- ❖ **Side effects include:**
 - Mild measles like illness may develop in 15–20% of vaccines. There is no spread of the vaccine virus in the community
 - Toxic shock syndrome (due to contamination of vial with *S. aureus* toxins).
- ❖ **Contacts:** Susceptible contacts over 9–12 months may be protected against measles if the measles vaccine is given within 3 days of exposure. This is because incubation period of measles induced by the vaccine strain is about 7 days, compared to 10 days for the naturally occurring measles
 - Measles immunoglobulin (Ig) can also be given within 3 days, at a WHO recommended dose of 0.25 mg/kg of body weight
 - However, both vaccine and Ig should not be given together. At least 8–12 weeks of gap must be maintained.

Epidemiology

Measles is endemic throughout the world with epidemics which recur regularly every 2–3 years, typically in late winter and early spring.

- ❖ **Source:** Cases are the only source of infection. Carriers are not known to occur. In-apparent or sub-clinical infections are rare

- ❖ **Reservoir:** Humans are the only reservoir of infection. There is no animal reservoir
- ❖ **Infective material:** Virus is shed in the secretions of nose, throat and respiratory tract of cases of measles, especially during the prodromal stage and early stage of rash
- ❖ **Period of communicability:** Patients are infectious from four days before to four days after the onset of rash. Patients are highly contagious, isolation is recommended from the onset of prodromal stage until third day of rash
- ❖ **Secondary attack rate** is very high (>90%)
- ❖ **Age:** Measles is a childhood disease
 - Children (6 months to 3 years) are the most susceptible group in developing countries
 - Older children (>5 years) are commonly affected in developed countries or in vaccinated population.
- ❖ **Immunity:** No age is immune if there is no previous immunity
 - There is single serotype hence one attack (vaccine or infection) gives lifelong immunity
 - Infants are protected up to 6 months due to pre-existing maternal antibodies.
- ❖ **Epidemic** of measles occurs if proportion of susceptible children exceeds 40%. Though disease burden has much decreased after the vaccine is made available, measles is still a leading cause of death of young children in many developing countries
- ❖ **World:** The measles cases have dramatically reduced in recent days due to widespread vaccination
 - In 2018, there were 3,59,921 confirmed cases of measles worldwide (with 1.4 lakh deaths); compared to >40 lakh cases in 1980)
 - Congo reported the maximum cases in 2018, followed by Ukraine and Pakistan.
- ❖ **India:** The burden of measles is substantially reduced due to widespread vaccination. In 2018, around 19,474 cases were reported from India; compared to >1 lakh cases in 1980.

Measles elimination

Measles is amenable to eradication. With the efficient and widespread immunization program, it is possible to eradicate measles from the world.

WHO has introduced 'The Strategic Plan for achieving and maintaining **Measles and Rubella elimination** in WHO South-East Asia Region: 2020–2024'. The following objectives are set to achieve this target:

1. ≥95% coverage with two doses of MR vaccine
2. Develop and sustain a case-based surveillance system
3. Develop and maintain an accredited measles and rubella laboratory network
4. Strengthen support and linkages to achieve the above three strategic objectives.

Measles is said to be eliminated if the confirmed cases are <1 per million population. The American region and some of the Western Pacific Region (Australia, Hong Kong, Japan, Korea, New Zealand, Sri Lanka, etc.) have recently achieved the measles elimination status.

RUBELLA

Rubella virus produces a childhood exanthema similar to that of measles. Therefore, rubella is also known as **German measles**. However, unlike measles, it is highly teratogenic; can cause congenital rubella syndrome.

- ❖ Rubella belongs to Togaviridae family, and is the only member under genus *Rubivirus*
- ❖ It contains ssRNA, surrounded by a capsid (C) protein and an envelope
- ❖ Its envelope contains a lipid layer from which two types of spike-like glycoproteins (E1 and E2) are projected
- ❖ There is only one serotype and humans are its only known reservoirs
- ❖ **Clinical forms:** Rubella may present in two clinical forms—postnatal infection and congenital infection.

Postnatal Rubella

Postnatal rubella may occur during neonatal age, childhood, and adult life.

Transmission

Rubella virus spreads from person-to-person by respiratory droplets via upper respiratory mucosa.

Spread

Rubella virus replicates locally in the nasopharynx, and then spreads to the lymph nodes. Subsequently, viremia develops after 7–9 days, and lasts until 14th day by which time both antibody and rashes appear almost simultaneously suggesting an immunologic basis for the appearance of rash.

Clinical Manifestations

- ❖ Incubation period is about 14 days (range, 12–23 days)
- ❖ Subclinical infection may be seen in 20–50% of cases
- ❖ Rashes are often the first manifestations in children, but in older children and adults, 1 to 5-day prodrome often precedes the rash, which includes low-grade fever, malaise, and upper respiratory symptoms
- ❖ **Rashes** are generalized and maculopapular in nature, start on the face, extend to trunk and extremities, and disappear in 3 days (Fig. 56.11A)
- ❖ **Lymphadenopathy** (occipital and postauricular) is the most striking feature
- ❖ **Forchheimer spots** may be seen in some cases. They are pin-head sized petechiae; develop on the soft palate and uvula; usually start with the onset of rash.

Complications

Arthralgia and arthritis are common in adults, particularly in women. Thrombocytopenia and encephalitis are rarely encountered.

Laboratory Diagnosis

- ❖ **Isolation of virus:** Nasopharyngeal or throat swabs taken 6 days before and after the onset of rash



Figs 56.11A and B: A. Child with rubella rash; B. Cataract seen in congenital rubella infection (arrows showing).

Source: Public Health Image Library, A. ID# 10146; B. ID#4284/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- Ideal cell lines: Monkey or rabbit origin cell lines may be used
- It can also be identified more rapidly in cell lines by shell vial technique.
- ❖ **Serology (Antibody detection):**
 - ELISA is the preferred method for rubella diagnosis. It detects both IgM and IgG separately. Various antigens employed are whole virus lysate or recombinant E1/E2 antigens
 - Results need to be confirmed by **IgG avidity test** to differentiate active infection from the past infection or vaccination.
- ❖ **Molecular test:** RT-PCR is available for detecting rubella specific RNA (nucleoprotein N gene) in clinical specimens.

Congenital Rubella Syndrome

The most serious consequence of rubella virus infection is congenital rubella syndrome. Rubella is highly teratogenic; affects ear (deafness), eyes (cataract, Fig. 56.11B), and heart (patent ductus arteriosus). The severity is maximum in first trimester. Detail is discussed in Chapter 79.

Epidemiology

- ❖ **Source:** The cases are the only source of infection. There is no known carrier state
- ❖ **Duration of protection:** Once infected, the person acquires lifelong immunity. In India, still 40% females of reproductive age group are susceptible to rubella infection
- ❖ **Period of communicability** of rubella is about 1 week before to 1 week after the appearance of rash
- ❖ **Transmission** occurs via—airborne droplet, transplacental and rarely via contact, and sexual modes
- ❖ **Occurrence:** Rubella occurs worldwide and throughout the year with a peak in the spring. Epidemics occur every 6–8 years, with explosive pandemics every 20–25 years
- ❖ **The largest rubella epidemic** (postnatal) occurred globally in 1962–1965. However; with increasing vaccine use, the epidemics are encountered less nowadays

- ❖ **World:** In 2018, 26,006 confirmed rubella cases were reported globally. Nigeria followed by China accounted for maximum cases
- ❖ **India:** In 2018, India reported 2,328 number of rubella cases. There was an outbreak of rubella reported from Rajasthan in 2014
- ❖ **Genotype:** Based on E1 protein coding region, Rubella has been typed into 13 genotypes; four of which are commonly circulating in world: 1E, 1G, 1J, and 2B. Genotype 2B is the predominant type in the World and also from India.

TREATMENT

Rubella

Rubella is a mild, self-limited illness and no specific treatment is available.

Prevention

General Preventive Measures

Airborne precaution must be followed while handling rubella cases (refer Chapter 21 for detail).

Rubella Vaccine

RA 27/3 is a live attenuated vaccine for rubella, prepared from human diploid fibroblast cell line.

- ❖ It is available singly or in combination with vaccines of mumps and measles (MMR vaccine)
- ❖ **Schedule:** Single dose (0.5 mL) of vaccine is administered subcutaneously
- ❖ Following vaccination, seroconversion occurs in 90% of recipients and immunity persists for 14–16 years or probably lifelong
- ❖ **Indication:** In India, rubella vaccine is indicated to all women of reproductive age (first priority group) followed by all children (1–14 years). Under national immunization schedule, rubella vaccine is given along with measles (MR vaccine) at 9–12 months of age and second dose at 16–24 months in selected states
- ❖ **Precautions:**
 - Vaccine is contraindicated in pregnancy
 - As it is teratogenic, pregnancy should be avoided at least for 4 weeks (28 days) following vaccination
 - However, if a woman conceives <4 weeks following vaccination: wait and watch policy should be followed. No immediate termination of pregnancy is required
 - Infants below 1 year should not be vaccinated due to possible interference from persisting maternal antibody.

HAND-FOOT-AND-MOUTH (HFMD) DISEASE

HFMD disease usually affects children; is characterized by ulcerations on oral and pharyngeal mucosa and vesicular rashes on the palms and soles, which heal without crusting (Figs 56.12A to C). Fever and sore throat with flu like symptoms are the other manifestations.



Figs 56.12A to C: Vesicular eruptions in Hand-foot-and-mouth (HFMD) disease: **A.** Hand; **B.** Foot; **C.** Mouth.

Source: A and B. Centers for Disease Control and Prevention (*with permission*); C. Wikipedia, Mr Midgley (*with permission*).

- ❖ **Agents:** HFMD is mainly caused by Coxsackieviruses, rarely by other enteroviruses
 - Coxsackievirus A16 is the most common cause
 - Coxsackievirus A6 can cause HFMD with more severe manifestation
 - Enterovirus 71 also has been associated with cases in East and Southeast Asia.
- ❖ **Transmission:** The virus can spread to others through an infected person's nose and throat secretions, such as saliva, sputum, or nasal mucus, fluid from blisters or scabs and also feces. Transmission occurs through contact (direct or indirect) and droplets.

EXPECTED QUESTIONS

I. Write essay on:

1. A 7-year-old boy had developed multiple painful vesicles over the lips and buccal mucosa. His parents revealed that two children of his school had a similar presentation few days back. Scrapings taken from the lesion demonstrated presence of multinucleated giant cell (Tzanck cell).
 - a. What is the most probable diagnosis?
 - b. List the other agents causing this type of infection.
 - c. How is this infection diagnosed in the laboratory?

II. Write short notes on:

1. A child presents with vesicular rashes, which appeared first on the face and trunk, spread rapidly to involve flexor surfaces; sparing distal part of the limbs. Rashes are bilateral and diffuse in distribution, appear in multiple crops. Fever appears with each crop of rashes. What is the clinical diagnosis? Discuss about the prevention of this disease.
2. A child presents with rashes, starts behind the ears and then spread over body. On examination, bluish white spots were seen in buccal mucosa. What is the clinical diagnosis. Discuss about the prevention of this disease.

III. Multiple Choice Questions (MCQs):

1. All of the following are clinical manifestations of Parvovirus B19 infection, *except*:
 - a. Erythema infectiosum

- b. Transient aplastic crisis
- c. Condyloma acuminata
- d. Hydrops fetalis

2. The correct sequence of manifestation seen in measles is:

- a. Fever → Koplik's spot → rash
- b. Koplik's spot → fever → rash
- c. Rash → fever → Koplik's spot
- d. Rash → Koplik's spot → fever

3. Subacute sclerosing panencephalitis (SSPE) is a complication following which viral infection?

- a. Mumps
- b. Measles
- c. Rubella
- d. Influenza

4. Which virus-vaccine strain combination is not correct?

- a. Mumps—Jeryl-Lynn strain
- b. Measles—Edmonston-Zagreb strain
- c. Rubella—Schwarz strain
- d. Chickenpox—Oka strain

5. Following rubella vaccination, pregnancy should be avoided for at least how many months?

- a. 1 month
- b. 2 months
- c. 3 months
- d. 6 months

Answers

1. c 2. a 3. b 4. c 5. a

Parasitic Infections of Skin, Soft Tissue and Musculoskeletal System

CHAPTER 57

CHAPTER PREVIEW

- Cutaneous Leishmaniasis
- Cutaneous Filariasis
- Dracunculiasis
- Trichinellosis
- Cutaneous Larva Migrans
- Parasitic Agents that Rarely Cause SSTI

A number of parasites can cause skin and soft tissue infections (SSTIs) and musculoskeletal infections (MSIs) (Table 57.1). The major cutaneous parasites are discussed first, followed by the agents that rarely cause SSTI-MSI.

CUTANEOUS LEISHMANIASIS

Apart from visceral leishmaniasis (VL), the *Leishmania* species can produce various cutaneous and mucocutaneous manifestations—associated with several old world and new world species of *Leishmania*.

❖ **CL:** Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis with a global annual incidence of 6-10 lakh new cases. About 95% of CL cases occur in the America, the Mediterranean basin, the Middle East and Central Asia

Table 57.1: Parasitic infections of skin, soft tissue and musculoskeletal system.

Protozoan infections	Cestode infections
<ul style="list-style-type: none"> • Cutaneous leishmaniasis* • Cutaneous amoebiasis • <i>Acanthamoeba</i> skin lesions • Trypanosoma chancre and chagoma • Sarcocystosis 	<ul style="list-style-type: none"> • Muscular cysticercosis • Sparganosis (<i>Spirometra</i>)
	Trematode infections
	<ul style="list-style-type: none"> • Cercarial dermatitis • Cutaneous paragonimiasis
	Nematode infections
	<ul style="list-style-type: none"> • Cutaneous filariasis* • Dracunculiasis* • Trichinellosis* • Cutaneous larva migrans*

*Include the major parasitic infections of skin, soft tissue and muscle.

❖ **MCL:** Over 90% of mucocutaneous leishmaniasis (MCL) cases occur in Bolivia, Brazil, Ethiopia and Peru.

Note: Post kala-azar dermal leishmaniasis (PKDL), another cutaneous manifestation, that occurs few months to years following VL (Chapter 36).

Old World Cutaneous Leishmaniasis (CL)

Old world CL is caused by *Leishmania tropica* complex, which in turn comprises of three species—*L. tropica*, *L. aethiops* and *L. major* (Table 57.2). They vary from each other in their geographical distribution, reservoir (human or animal) and species of sandfly vector involved in transmission and the type of cutaneous lesions produced.

Life Cycle

The life cycle of the *L. tropica* complex is same as *L. donovani* (refer Chapter 36, Figure 36.2), except that:

- ❖ **Vector:** They are transmitted by sandfly, but different species of *Phlebotomus*
- ❖ **Habitat:** In humans, the amastigote forms reside in reticuloendothelial cells of skin (they do not migrate to viscera).

Clinical Features

L. tropica complex produces various cutaneous manifestations.

❖ **Cutaneous leishmaniasis:** This condition is also known as “**Oriental sore**”, Delhi boil, Aleppo boil and Baghdad Button, etc.

- It occurs on face and hands, begins as painless papule, becomes nodular and finally it ulcerates

Table 57.2: Various agents of cutaneous leishmaniasis.

Species	Geographical distribution	Clinical syndrome	Vector (Sandfly)	Reservoir	Transmission
<i>L. L. tropica</i> (Oriental sore)	Western India, North Africa, and Middle East	CL, LR	<i>Phlebotomus sergenti</i>	Humans	Anthroponotic (human)
<i>L. L. aethiops</i>	Ethiopia, Uganda, and Kenya	CL, DCL	<i>P. longipes</i>	Hyraxes	Zoonotic
<i>L. L. major</i>	Middle East, India, China, Africa, Central and Western Asia	CL	<i>P. papatasi</i>	Rodents	Zoonotic

Abbreviations: CL, cutaneous leishmaniasis; LR, leishmania recidivans; DCL, diffuse cutaneous leishmaniasis; L.L., *Leishmania Leishmania*.



Figs 57.1A and B: Clinical features of: **A.** Cutaneous leishmaniasis; **B.** Leishmaniasis recidivans.

Source: A. World Health Organization, "Manual on visceral leishmaniasis control" (with permission); B. Global Skin Atlas/Image Number 2268/Nameer Al-Sudany (with permission).

- Lesions usually heal spontaneously or sometimes leaves behind disfigurement or scar (Fig. 57.1A).
- ❖ **Leishmaniasis recidivans (LR):** It is a granulomatous response producing new lesions that occur years after healing of primary sore due to *L. tropica*. It is characterized by scaly, erythematous papules and nodules, develop in the center or periphery of a previously healed sore. It is primarily seen in Iran and Iraq (Fig. 57.1B)
- ❖ **Diffuse cutaneous leishmaniasis (DCL):** It is caused by *L. aethiopica*. It is characterized by the lack of a CMI response to the parasite, resulting in widespread cutaneous lesions
 - It is rare, about 100 cases have been reported so far, mainly from Kenya and Ethiopia
 - Lesions may be confused with that of lepromatous leprosy (LL)
 - Patients usually have low CMI and therefore the Montenegro skin test is negative
 - It responds poorly to treatment; disease may last long.

Laboratory Diagnosis of CL

- ❖ **Microscopy:** Amastigotes can be demonstrated from punch biopsies taken from the edge of the active lesion and touch preparation (impression smear) stained with Giemsa
- ❖ **Culture:** Aspiration from the ulcers can be cultured in NNN medium and Schneider's Drosophila medium for the isolation of promastigote forms

- ❖ **Montenegro test:** Positive leishmanin skin test indicates delayed hypersensitivity reaction to the parasite. However, it is negative in diffuse CL.

TREATMENT

Cutaneous Leishmaniasis (Old World)

Local therapy is usually recommended. Options available are (i) paromomycin (15%) or methyl benzethonium (12%) ointment for 20 days, (ii) intralesional antimonials, (iii) cryotherapy, and (iv) thermotherapy.

Systemic therapy: It is required only for disfiguring or scarring lesions. Various options available are:

- *L. major*—(i) fluconazole for 6 weeks, (ii) pentavalent antimonials with or without pentoxifylline for 10–20 days
- *L. tropica*—pentavalent antimonials for 10–20 days. In LR cases, it is combined with oral allopurinol
- *L. aethiopica*—pentavalent antimonials plus paramomycin for 60 days or longer.

New World Cutaneous Leishmaniasis

New World cutaneous leishmaniasis is mainly caused by:

- ❖ *Leishmania Leishmania (L.L.) mexicana* complex (Table 57.3)
- ❖ *Leishmania Viannia (L.V.) braziliensis* complex (Table 57.3)
- ❖ *Leishmania Leishmania (L.L.) chagasi*: It causes atypical CL and American VL (only new world species to cause VL). It occurs in rural areas of Central and South American region (Chapter 36, Table 36.1).

Their morphology and life cycle (refer Chapter 36, Figure 36.2), are identical to that of *L. donovani*, except:

- ❖ Geographical distribution is restricted to central and south America
- ❖ **Vector:** *Lutzomyia* species
- ❖ **Reservoir of infection:** Dogs, foxes (zoonotic)
- ❖ The amastigote forms in humans reside in reticuloendothelial cells of skin and mucous membrane (do not invade viscera).

Leishmania mexicana Complex

L. mexicana complex can cause CL and DCL, depending upon the species involved (Table 57.3).

<i>Leishmania Leishmania mexicana</i> complex			<i>Leishmania Viannia braziliensis</i> complex		
Species	Geographical distribution	Clinical syndrome	Species	Geographical distribution	Clinical syndrome
<i>L. L. mexicana</i>	Central America and northern parts of South America (the Amazon basin)	CL (chiclero ulcer) DCL	<i>L. V. braziliensis</i>	Brazil	CL, MCL (espundia), LR (rare)
<i>L. L. amazonensis</i>		CL, DCL and VL (rare)	<i>L. V. panamensis</i>	Panama, Colombia	CL, MCL
<i>L. L. venezuelensis</i>		CL	<i>L.V. guyanensis</i>	Guyana	CL (forest yaws), MCL
<i>L. L. garnhami</i>		CL, DCL	<i>L. V. peruviana</i>	Peru	CL (Uta), MCL
<i>L. L. pifanoi</i>					
Reservoir: Forest rodents, marsupial and humans			Reservoir: Dogs, foxes, forest rodents and humans		
Vector: <i>Lutzomyia</i> species and Transmission: Zoonotic (for both complexes)					

Abbreviations: CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; LR, leishmaniasis recidivans; *L.L.*, *Leishmania Leishmania*; *L.V.*, *Leishmania Viannia*.

Chiclero ulcer

L. mexicana causes a specific form of CL called as **chiclero ulcer** (or bay sore); characterized by persistent ulcerations in pinna. It is seen in Central America among workers living in forests harvesting chicle plants to collect chewing gum latex.

Leishmania viannia braziliensis Complex

They cause CL similar to oriental sore but they are more severe. In addition, they also cause MCL (Table 57.3).

Espundia (mucocutaneous leishmaniasis or MCL)

L. braziliensis infects mucous membrane of the nose, oral cavity, pharynx or larynx; clinically presents months to years after the CL.

- ❑ Ulcerative lesions are formed with erosion of the soft tissue and the cartilages leading to loss of lips, soft part of nose and soft palate (Fig. 57.2).
- ❑ Gradually, the nasal septum may be destroyed, resulting in nasal collapse with hypertrophy of upper lip and nose leading to development of “**tapir nose**”.

The cutaneous lesions of *L.V. guyanensis* and *L.V. peruviana* are known as forest yaws (pian bois) and uta respectively.

Laboratory diagnosis of new world CL is similar to as discussed for old world CL.

TREATMENT**Cutaneous Leishmaniasis (New World)**

In contrast to Old World CL, systemic therapy is recommended for New World CL, as the lesions are more chronic, multiple and have tendency for mucosal involvement. **Local therapy** can be given in addition; agents used for treatment are same as used for Old World CL.

The regimen for **systemic therapy** depends upon the causative agent.

- ❑ *L. mexicana*: Ketoconazole or miltefosine for 28 days
- ❑ *L. guyanensis* and *L. panamensis*: Pentamidine or pentavalent antimonials or miltefosine for 28 days
- ❑ *L. braziliensis*: Pentavalent antimonials for 20 days; or amphotericin B
- ❑ *L. amazonensis*, *L. peruviana* and *L. venezuelensis*: Pentavalent antimonials for 20 days
- ❑ Relapse treatment: Amphotericin B or pentavalent antimonials plus topical imiquimod is recommended

For MCL (all species): (i) pentavalent antimonials with or without oral pentoxifylline for 30 days, (ii) amphotericin B, (iii) in country like Bolivia, miltefosine is the agent of choice.

Preventive and control measures for leishmaniasis are discussed in Chapter 36.

CUTANEOUS FILARIASIS

Filarial nematodes such as *Wuchereria* and *Brugia* cause lymphatic filariasis (Chapter 37). There are many other filarial nematodes which reside in skin and subcutaneous tissue producing several cutaneous manifestations—*Loa loa*, *Onchocerca volvulus*, and *Mansonella* species.



Fig. 57.2: Mucocutaneous leishmaniasis or espundia.

Source: Calvopina et al. BMC Infectious Diseases 2006 (with permission).

Loa loa

Loa loa (also called as African eye worm), cause infection of subcutaneous tissue and eyes. Infection is restricted to West and Central Africa.

Life Cycle

Life cycle is similar to that of *W. bancrofti* except for the vector, which is female *Chrysops* species (deer flies, or tabanid flies).

- ❖ Following mosquito bite (during day time), the L₃ larvae are transmitted to man and transform into adult worms which migrate to subcutaneous tissues and eyes
- ❖ Microfilariae released from gravid female worms migrate to the blood and exhibit a diurnal periodicity.

Clinical Features

It produces lesions in subcutaneous tissue and eyes.

- ❖ **Calabar swellings:** This is the most common form of loiasis, also called as **fugitive swelling**. It is a subcutaneous swelling developing on the extremities (knee or wrist)
- ❖ **Ocular manifestations** such as conjunctival granuloma, edema of the eye lid leading to proptosis (bulging)
- ❖ **Complications:** Meningoencephalitis is the most severe complication, occurs in DEC treated patients with higher microfilaremia. Nephropathy and cardiomyopathy are the other rare complications noted.

Laboratory Diagnosis

Peripheral blood smear examination reveals the characteristic microfilariae.

- ❖ As the microfilaria shows diurnal periodicity, blood is collected in day time between 10 am to 3 pm
- ❖ They are sheathed, measure 275 (250–300) μm long and bear a column of nuclei extending till the tail tip
- ❖ Adult worms can be isolated from the eye or biopsy of the subcutaneous swelling.

Other methods include—(1) **molecular method** such as nested PCR-based assays for the detection of specific DNA, and (2) **antibody detection:** Lateral flow assay (ICT)

is recently developed which detects antibodies to LI-SXP-1 antigen.

TREATMENT

Loiasis

- ❑ **Diethylcarbamazine (DEC)** is the drug of choice—multiple courses are necessary to resolve loiasis completely
- ❑ **Glucocorticoids:** It is required in heavy microfilaremia, to reduce inflammatory reactions against microfilariae and thereby preventing neurological complications
- ❑ **Albendazole or ivermectin** is effective in reducing microfilarial loads, but ivermectin is contraindicated in heavily infected patients with loiasis
- ❑ **Surgical removal** of the adult worms is rarely required if they migrate through the bridge of the nose or through the conjunctiva.

Onchocerca volvulus

Onchocerca volvulus is the causative agent of “**river blindness**” in man. It is endemic in West Africa and also in South and Central America.

Life Cycle

Life cycle is similar to that of *W. bancrofti*, except the vector is *Simulium* (black flies).

- ❖ Following the bite of black flies, the L₃ larvae are transmitted to man and transform into adult worms which migrate to subcutaneous tissues and eyes
- ❖ Gravid female worms release microfilariae, which migrate to the blood and are infective to the black flies.

Clinical Features

Patients are asymptomatic when the worm load is less. However, heavy infections can result in various manifestations

- ❖ **Skin (Dermatitis):** Intense pruritus and generalized papular rashes are the most common manifestations
 - **Leopard skin:** Skin become hypo to hyperpigmented giving rise to leopard skin appearance
 - **Sowda:** It is a chronic hyperreactive form of dermatitis, occurs in a subset of individuals from West Africa.
- ❖ **Onchocercoma:** It is characterized by formation of subcutaneous nodules that are firm, nontender, containing the coiled adult worms and rarely microfilariae. In African patients, nodules are common over the trunk, while in patients from America, they tend to develop on the head, neck and shoulders
- ❖ **Ocular lesions** include bilateral blindness (river blindness), conjunctivitis with photophobia, keratitis (refer Chapter 78)
- ❖ **Lymphadenopathy** in the inguinal and femoral areas is commonly noted. The enlarged nodes may hang down (“**hanging groin**”) and may predispose to hernia.

Laboratory Diagnosis

Detection of the Parasite

Detection of microfilariae in a skin snip smear is the gold standard method for diagnosis of onchocerciasis.

Microfilariae are found either in the skin (90%) or in the nodules (10%).

- ❖ **Skin snips technique:** Skin over iliac crests is lifted by a needle and a small piece (1 to 3 mm) is excised with a sterile scalpel blade
- ❖ **Microfilariae:** They measure 254 µm long, pointed tail tip without any nuclei. They are unsheathed and nonperiodic
- ❖ **Adult worms** may be detected from the biopsy of the subcutaneous nodules but it is less sensitive.

Other Methods

- ❖ **Serology:** Cocktail of recombinant antigens of *O. volvulus* can be used to detect specific antibodies which show better specificity and do not cross react with other nematodes. However, it cannot differentiate the current from the past infection
- ❖ **Molecular methods:** PCR detecting onchocercal DNA in skin snips or even from skin scrapings is available
- ❖ **Mazzotti skin test:** Topical application of DEC on the skin leads to local reaction (erythema and itching) to the dead worm. It is also called as DEC patch test.

TREATMENT

Onchocerciasis

Ivermectin is the drug of choice for onchocerciasis.

- ❑ It is active against the microfilariae but not against the adult worms
- ❑ It is given orally in a single dose of 150 µg/kg, either yearly or biannually
- ❑ It is contraindicated in areas of Africa co-endemic for *O. volvulus* and *L. loa*.

Surgical excision is recommended when nodules are located on the head.

Mansonella species

Mansonella species are named after Patric Manson. They rarely infect humans and are either nonpathogenic or asymptomatic in most of the individuals.

Mansonella perstans

It is found mainly in the Central Africa and in Central and South America.

- ❖ **Transmission:** It is transmitted by *Culicoides* (midges)
- ❖ Adult worms reside in serous cavities, mesentery and perirenal tissues. Microfilariae circulate in the blood without periodicity
- ❖ **Clinical features:** Usually nonpathogenic, but occasionally, it can cause manifestations like angioedema, urticaria, pruritus and **calbar-like swelling** similar to that of *Loa loa*. It also produces acute periorbital inflammation; known as **bung-eye** or **bulge-eye**
- ❖ **Laboratory diagnosis:** Microfilariae in peripheral blood are nonperiodic, nonsheathed, measures 195 µm long with a straight tail and blunt end. Body nuclei are extended till the tail tip
- ❖ **Treatment:** DEC or albendazole are found to be effective.

Mansonella streptocerca

M. streptocerca is found mainly in tropical forest area western and central sub-Saharan Africa.

- ❖ **Transmission:** It is transmitted by the biting midges (*Culicoides grahamsi*)
- ❖ **Clinical feature:** Many infected individuals are asymptomatic, although some people may develop inguinal lymphadenopathy, pruritus, dermatitis with hypopigmented macule similar to leprosy except that there is no sensory loss
- ❖ **Laboratory diagnosis:** The diagnosis is made by detection of the characteristic microfilariae in skin snips. It is nonperiodic, nonsheathed, measures 210 µm with a curved tail (looks like **Shepherd's crook**). Nuclei are extended till the blunt tail tip
- ❖ **Treatment:** DEC is effective for streptocerciasis.

Mansonella ozzardi

M. ozzardi is found mainly in Central and South America and transmitted by *Culicoides* (midges).

- ❖ **Clinical features:** Most infections are asymptomatic, but occasionally cause lymphadenopathy, urticaria, pruritus, pulmonary symptoms, arthralgia and keratitis
- ❖ **Diagnosis:** Microfilariae can be detected in peripheral blood; which are nonperiodic, nonsheathed, measures 200 µm long with a fine attenuated hooked and pointed tail tip without any nuclei
- ❖ **Treatment:** Ivermectin is effective.

Dirofilaria species

Dirofilaria species are parasites of lower animals. Humans are unusual hosts. Hence, the parasite undergoes an incomplete development in humans either in the lungs, eyes and or subcutaneous tissue, producing various lesions. Man acquires infection by the bite of mosquito (*Aedes*, *Culex*, *Anopheles*, or *Mansonia*) containing L_3 filariform larvae.

Prevention of Cutaneous Filariasis

Vector control is useful in highly endemic areas. Insecticide spraying can be carried to destroy the breeding sites. In addition, for onchocerciasis mass administration of ivermectin every 6–12 months is being used to interrupt the transmission in endemic areas.

DRACUNCULIASIS

It is a parasitic infection by the somatic nematode, *Dracunculus medinensis*. It is also called as **Guinea worm disease** or **dracunculiasis**, characterized by cutaneous blisters.

Epidemiology

Dracunculiasis is a crippling parasitic disease on the verge of eradication, currently endemic to only to 3-4 countries

in Sub-Saharan Africa. About 187 countries including India have been already declared free of transmission.

- ❖ In 1986, an estimated 3.5 million new cases occurred globally. After the initiation of control program, there was a dramatic fall of cases
- ❖ In 2019, only 54 human cases reported in 2019, mainly from Chad, few from other African countries such as South Sudan, Angola and Cameroon.

Life Cycle (Fig. 57.3)

The life cycle involves two hosts—definitive host (man) and intermediate host (*Cyclops*).

- ❖ **Mode of transmission:** Man gets infection by drinking water from stagnant pools containing minute fresh water crustaceans (*Cyclops*) infected with third stage L_3 larvae (infective form)
- ❖ **In man:** Cyclops are digested in stomach releasing the L_3 larvae
 - The larvae penetrate small intestine and migrate to the thoracic muscle, develop to form adult worms. The female worms migrate throughout the body, ultimately reach the skin, particularly over the ankles, feet, and lower legs
 - When skin comes in contact with water, the female worm induces a local blister that eventually ruptures, releasing large numbers of L_1 larvae into the water.
- ❖ **In Cyclops:** The motile free-swimming L_1 larvae infect *Cyclops*. They molt twice to form L_3 larvae which are infective to man over a period of 2 weeks.

Pathogenesis and Clinical Features

Signs and symptoms appear approximately 1 year after the infection; when gravid adult female worm emerges near the surface of the skin.

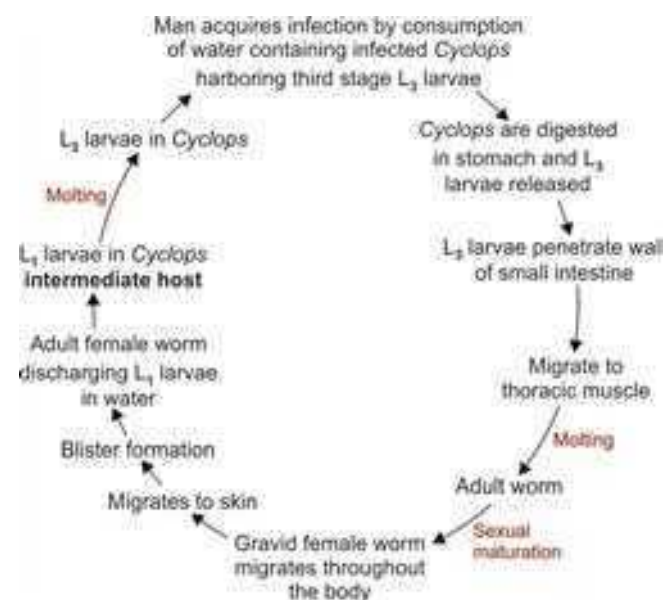


Fig. 57.3: Life cycle of *Dracunculus medinensis*.



Figs 57.4A and B: Dracunculiasis: **A.** Blister formed; **B.** Adult female worm of *Dracunculus medinensis* emerging from the blister.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ Characterized by painful blister from which the female worm emerges, accompanied by local erythema, fever, nausea and pruritus (Fig. 57.4A)
- ❖ The most common sites—lower leg, ankle and foot
- ❖ Secondary bacterial infections may occur at the blister site.

Laboratory Diagnosis

Dracunculiasis is diagnosed by:

- ❖ **Detection of adult worm:** This is possible when the gravid female worms appear in the blisters (Fig. 57.4B). The calcified adult worms from the deeper tissue can be detected by X-ray
- ❖ **Detection of L₁ larvae:** When the leg with ulcer is placed in a container with cold water, a large number of motile larvae are discharged which can be examined under the microscope
- ❖ **Antibody detection:** Antibodies to *D. medinensis* can be detected by ELISA
- ❖ Peripheral blood *Eosinophilia*.

TREATMENT

Dracunculiasis

- ❑ **Worm removal:** Worms are slowly and gently extracted over a period of 15–20 days using a small stick and wounding out daily with small traction. Heavy pressure should be avoided because breaking the worm can lead to allergic reactions and secondary bacterial infection.
- ❑ **Symptomatic treatment:** Includes application of wet compresses to the affected skin, administration of analgesics and prevention of secondary bacterial infection by the use of topical antibiotics. There are no anti-helminthic drugs known to be effective against *D. medinensis*.

Reasons for Eradication from India

The national Guinea worm eradication program was launched in 1984 with technical assistance from World Health Organization (WHO).

Simple and cost-effective measures were taken to eradicate the disease such as:

- ❖ **Provision of safe drinking water:** Filtration of drinking water, installing hand pumps and pipes
- ❖ **Cyclops control:** Killing copepods in sources of drinking water by application of abate (temephos) larvicide

- ❖ Provision of clean drinking water from boreholes or wells (was a major source of infection)
- ❖ Health education about boiling or filtering of drinking water
- ❖ Treatment of cases.

TRICHINELLOSIS

Trichinella spiralis causes **trichinellosis** (or trichinosis), which is a zoonotic infection acquired from domestic pigs or other carnivores.

Epidemiology

Human trichinellosis is widely prevalent in the pork eating countries (more in temperate zone than tropics) like Europe and America. It is very rare in India, except for the outbreak in 2010, which occurred in Uttarakhand affecting 18 people eating roasted wild boar meat called kachmoli.

Morphology

Like other somatic nematodes, it has an adult worm (1.5–3 mm long), and four stages of larvae. There is no egg stage. L₁ larva is the infective form, which forms cysts in muscles.

Life Cycle (Fig. 57.5)

The life cycle involves one host— usual host is pig (or other animals); man is an accidental host and acts as dead end.

- ❖ **Mode of transmission:** By ingestion of raw or uncooked pork containing infective form L₁ larvae. It is estimated that 100–300 larvae are required to initiate the infection
- ❖ **Intestinal phase:** Ingested L₁ larvae are released from pig meat in the intestine and then develop into adult worms. Being viviparous, adult worms directly lay larvae (L₁) after fertilization
- ❖ **Migration phase:** The L₁ larvae penetrate the intestine and carried to skeletal muscles via intestinal lymphatics and mesenteric venous circulation
- ❖ **Encystment phase:** L₁ larvae become encysted inside the skeletal muscle cells

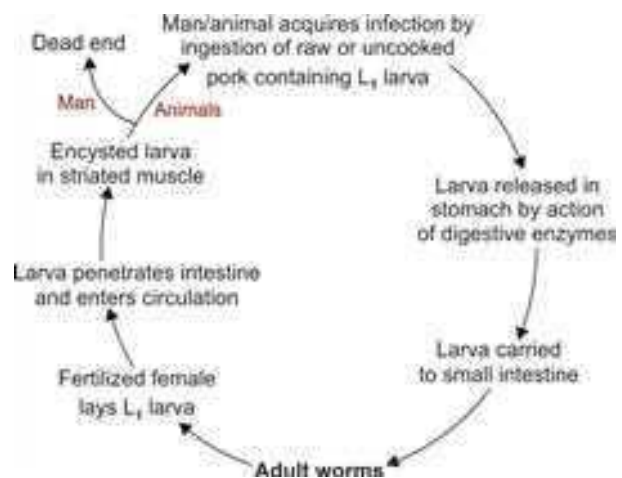


Fig. 57.5: Life cycle of *Trichinella spiralis*.



Fig. 57.6: Larva of *Trichinella* liberated from bear meat (microscopic view).

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

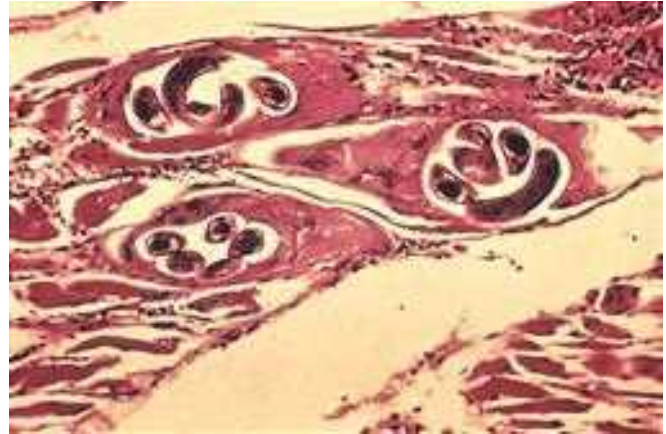


Fig. 57.7: *Trichinella* cysts in the human muscle biopsy (hematoxylin and eosin stain).

Source: Public Health Image Library, ID# 5234/ Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- The cyst measures $400 \times 260 \mu\text{m}$ in size. The larva remains coiled; hence, the species name is given as “*spiralis*” (Fig. 57.6)
- The muscle cells are modified to form **nurse cells**, which provide nourishment and contain the parasite for years
- The cyst undergoes fibrosis and calcification after weeks to years
- Only skeletal muscle cells are infected, encystment does not occur in cardiac and smooth muscles.

Pathogenicity and Clinical Features

Clinical symptoms of trichinellosis depend on the phase of parasitic invasion.

- ❖ **Intestinal stage:** Most of the infections are asymptomatic; however, heavy load of parasite can provoke watery diarrhea (most common feature) during the first week after infection
- ❖ **Larval migration:** Symptoms appear in the second week after infection
 - Periorbital and facial edema is common
 - Hemorrhages in the subconjunctiva, retina and nail beds (“splinter” hemorrhages)
 - Maculopapular rash
 - Migration to heart, CNS and lungs may occur, but rare.
- ❖ **Muscle encystment:** Occurs 2–3 weeks after infection. Common symptoms are myositis with myalgia, muscle edema, and weakness. Extraocular muscles are the most common to be involved, followed by biceps, muscles of the jaw, neck, lower back, and diaphragm.

Laboratory Diagnosis

Demonstration of Larvae

Definite diagnosis by the demonstration of larvae in muscle biopsy; obtained from gastrocnemius, deltoid, and biceps.

- ❖ **Direct slide technique:** The fresh muscle tissue should be compressed between glass slides and examined under low power microscope
- ❖ **Histopathologic examination:** Performed either on fresh muscle tissues directly or after artificial digestion of muscle mass by pepsin (Fig. 57.7).

Antibody Detection

ELISA is available detecting parasite specific IgG antibody against excretory secretory antigen of muscle larvae. It confirms the diagnosis but cannot differentiate past and present infection. It also cross reacts with other nematodes.

Coproantigen Detection

A modified double sandwich ELISA has been developed using polyclonal antibodies raised in rabbit to detect larval somatic antigens in stool. It is detectable from first day of infection up to third week.

Bachman Intradermal Test

Intradermal injection of Bachman antigen (prepared from *Trichinella* larvae) causes an immediate induration and erythema. It becomes positive in 2–3 weeks of infection and persists for life, hence cannot differentiate past infection present infection.

Other Tests

- ❖ **Blood eosinophilia:** Elevated in more than 90% symptomatic patients
- ❖ **Elevated muscle enzymes:** Elevated serum creatine phosphokinase
- ❖ **X-ray** to detect the calcified muscle cyst.

TREATMENT

Trichinellosis

- ❑ **Mild infection:** Symptomatic treatment is required with bed rest, antipyretics, and analgesics

Contd...

Contd...

TREATMENT**Trichinellosis**

- ❑ **Moderate infection:** Mebendazole and albendazole are active against enteric stages of the parasite, but their efficacy against encysted larvae has not been conclusively demonstrated
- ❑ **Severe infection:** Glucocorticoid is added which is beneficial for severe myositis and myocarditis.

Prevention

Trichinellosis can be prevented by the following measures.

- ❖ Direct inspection of pork and microscopic examination of small tissue samples of pig diaphragm before commercial use
- ❖ Maintenance of strict standards for freezing, cooking, and curing of pork and pork products is necessary
- ❖ All parts of pork muscle tissue must be heated to >58.3°C. Microwaving might not kill the parasite
- ❖ Sanitary disposal of dead animals.

CUTANEOUS LARVA MIGRANS

Cutaneous larva migrans refers to the skin lesions produced by nematodes of lower animals, when they accidentally infect man.

Arrested Life Cycle and Pathogenesis

Lower animals such as felines act as natural hosts. Humans are abnormal accidental host. Filariform larva (L₃) are the infective form present in soil, transmitted by skin penetration.

- ❖ **Agents:** CLM is mainly caused by the nonhuman hookworm species (that usually infect lower animals): *A. brasiliensis*, *A. caninum* and *A. ceylanicum*. Rarely CLM may also be caused by:
 - Human nematodes such as *Strongyloides stercoralis*, *Ancylostoma duodenale* and *Necator americanus*
 - Other nematodes of lower animals such as *Gnathostoma spinigerum*.
- ❖ **In lower animals:** These nematodes when infect lower animals, the larvae migrate to various organs (e.g. intestine) where they develop into adult worms, which mate to lay eggs (e.g. in feces) and the cycle continues
- ❖ **In humans:** The larvae of these lower animal nematodes when accidentally infect man, they are not able to complete their normal development (because humans are the unusual host for them) and their life cycle gets arrested. The larvae wander around aimlessly in skin and subcutaneous tissue, producing a condition called as **cutaneous larva migrans (CLM)** or **creeping eruption**.

Note: Another form of larva migrans occurs called as 'visceral larva migrans'; where the life cycle gets arrested after the larvae migrate to various viscera. It is discussed in Chapter 49.

Clinical Features

Larvae wander in the superficial layers of the skin of feet, legs, thigh, buttock and back, produce linear tracks and provoke allergic reaction in previously sensitized patients that leads to:

- ❖ **Ground itch:** Pruritic maculopapular dermatitis and rashes (ground itch) at the site of skin penetration of hookworm larvae
- ❖ **Larva currens:** Migrating *Strongyloides* larvae produce the pathognomonic serpiginous urticarial rash called as **larva currens** near the legs.

Laboratory Diagnosis

Diagnosis is made mainly by clinical feature (presence of the linear tracks) and history of exposure. Larvae are usually not detected in skin biopsy.

- ❖ Recently, PCR has been developed for detection of larval DNA in human tissues
- ❖ Elevated eosinophilia may be seen in peripheral blood or sputum. Charcot-Leyden crystals in sputum may be seen.

TREATMENT**Cutaneous larva migrans**

- ❑ Oral and topical thiabendazole is effective
- ❑ Freezing the advancing end of creeping eruption in ethyl chloride is useful.

PARASITIC AGENTS THAT RARELY CAUSE SSTI

There are a number of parasitic agents that rarely cause SSTI.

- ❖ **Cutaneous amoebiasis:** Also called as amoebiasis cutis. It is a virulent form of amoebiasis, presents as perianal skin ulcers as extension of amoebic colitis; may also be seen following anal intercourse. It may also present as skin lesion on abdominal wall due to migration of trophozoites secondary to a ruptured amoebic liver abscess (Chapter 45, for detail)
- ❖ **Acanthamoeba skin lesions:** In HIV patients with CD4 T cell <200 /μL, *Acanthamoeba* in addition to granulomatous encephalitis, produces various cutaneous infections (nasal ulcers, skin ulcers and abscesses), and musculoskeletal abscesses
- ❖ **Trypanosoma chancre:** It is a self-limited inflammatory lesion produced in early stage of *Trypanosoma brucei* infection; may appear a week after the bite of an infected tsetse fly and lasts for 1–2 weeks (Chapter 36, for detail)
- ❖ **Chagoma:** It is seen in early stage of Chagas' disease, caused by *Trypanosoma cruzi*. An erythematous subcutaneous nodule is formed at the site of deposition of reduviid bug's feces. It is painful, commonly occurs on face and may take 2–3 months to resolve (Chapter 36)
- ❖ **Muscular sarcocystosis:** *Sarcocystis* is a zoonotic parasite; *S. hominis* (infects cattle) and *S. suihominis* (infects pigs). Human infection is extremely rare; nearly

200 cases are reported so far, including 11 cases from India

■ **Host:** Dogs and cats are definitive host; whereas cattle and pigs (and accidentally man) serve as intermediate hosts.

Note: Occasionally, man acts as definitive host; which leads to intestinal sarcocystosis (Chapter 45)

■ **Life cycle:** Infection is transmitted to man by ingestion of food or water contaminated with oocysts, which are carried in blood vessels, invade muscle cells where they develop into sarcocysts

■ **Clinical features:** Characterized by the deposition of cysts in the muscle, leading to muscle pain, weakness and myositis. Deposition in cardiac muscle can lead to myocarditis and pericarditis (rarely)

■ **Diagnosis:** Histological examination of muscle biopsy can be done to demonstrate the sarcocysts in cardiac and skeletal muscle. They measure 100–325 µm in size, contain numerous PAS positive bright red bradyzoites measuring 7–16 µm in size

■ **Treatment:** No specific treatment is available. Infection is generally self-limited.

❖ **Cysticercosis:** It is caused by *Taenia solium*, characterized by deposition of encysted larvae (cysticercus cellulosae) in various organs; most commonly in the brain (producing neurocysticercosis, Chapter 75) followed

by other sites such as subcutaneous tissue, muscle and eyes

■ Subcutaneous cysticercosis: Can present as palpable nodules

■ Muscular cysticercosis: Manifests as muscular pain, weakness or pseudohypertrophy.

❖ **Sparganosis:** *Spirometra* is a cestode infecting dogs and cats. Human infection is extremely rare, results in deposition of its larva (called sparganum) in various tissues such as subcutaneous tissues, muscles, eyes and visceral organs like brain and lymphatics

❖ **Cercarial dermatitis:** In *Schistosoma* infection, after 2 or 3 days of skin penetration of the infective form (cercaria larvae), an itchy maculopapular rash develops on the affected areas of the skin. It is also called as **swimmer's itch** (Chapter 46, for detail)

❖ **Cutaneous paragonimiasis:** It is observed mainly in *Paragonimus skrjabini* infection (Chapter 69, for detail)

■ **Migratory subcutaneous nodules** may be seen in 20–60% of *P. skrjabini* and 10% of *P. westermani* infected patients. They form tender nodules which vary from few millimeters to 10 cm

■ **Larva migrans:** In China, *P. skrjabini* does not develop to the adult worm stage, but migrates to various places such as skin, brain and liver causing trematode larva migrans.

EXPECTED QUESTIONS

I. Write short notes on:

1. Cutaneous leishmaniasis.
2. Guinea worm infection.
3. Cutaneous larva migrans.

II. Multiple Choice Questions (MCQs):

1. Vector for leishmaniasis:

- a. Sandfly
- b. Reduviid bugs
- c. Tsetse fly
- d. *Anopheles* mosquito

2. New World leishmaniasis is caused by:

- a. *Leishmania donovani*
- b. *Leishmania braziliensis*
- c. *Leishmania tropica*
- d. *Leishmania major*

3. Oriental sore is caused by:

- a. *Leishmania mexicana*
- b. *Leishmania braziliensis*
- c. *Leishmania tropica*
- d. *Leishmania chagasi*

4. Chiclero's ulcer is caused by:

- a. *Leishmania mexicana*
- b. *Leishmania braziliensis*
- c. *Leishmania peruviana*
- d. *Leishmania chagasi*

5. Causative agent of Calabar swelling is:

- a. *Dracunculus medinensis*
- b. *Wuchereria bancrofti*
- c. *Brugia malayi*
- d. *Loa loa*

Answers

1. a 2. b 3. c 4. a 5. d

Fungal Infections of Skin, Soft Tissue and Musculoskeletal System

CHAPTER 58

CHAPTER PREVIEW

- Superficial Mycoses
- Subcutaneous Mycoses
- Opportunistic Mycoses of Skin and Soft Tissue

INTRODUCTION

Fungal infections of skin and soft tissue can be classified into:

- ❖ **Superficial mycoses:** These are fungal infections involving the skin, hair, nail and mucosa. Examples include—tinea versicolor, tinea nigra, piedra and dermatophytosis
- ❖ **Subcutaneous mycoses:** These are the mycotic infections of the skin, subcutaneous tissue and sometimes bone, resulting from inoculation of saprophytic fungi of soil or decaying matter. They are mainly confined to the tropics and subtropics. Examples include—mycetoma, sporotrichosis, chromoblastomycosis, rhinosporidiosis and microsporidiosis
- ❖ **Opportunistic mycoses of skin and soft tissue:** They can act as human pathogen in presence of opportunities such as low immunity (e.g. HIV-infected individuals). Examples include:
 - *Penicillium marneffe* infection
 - Candidiasis (cutaneous and mucocutaneous)
 - Cutaneous mucormycosis
 - Cutaneous aspergillosis
 - Cutaneous cryptococcosis.

SUPERFICIAL MYCOSES

Tinea Versicolor

Tinea versicolor (or pityriasis versicolor) is a chronic recurrent condition involving the superficial layer (stratum corneum) of skin, caused by a lipophilic fungus *Malassezia furfur*.

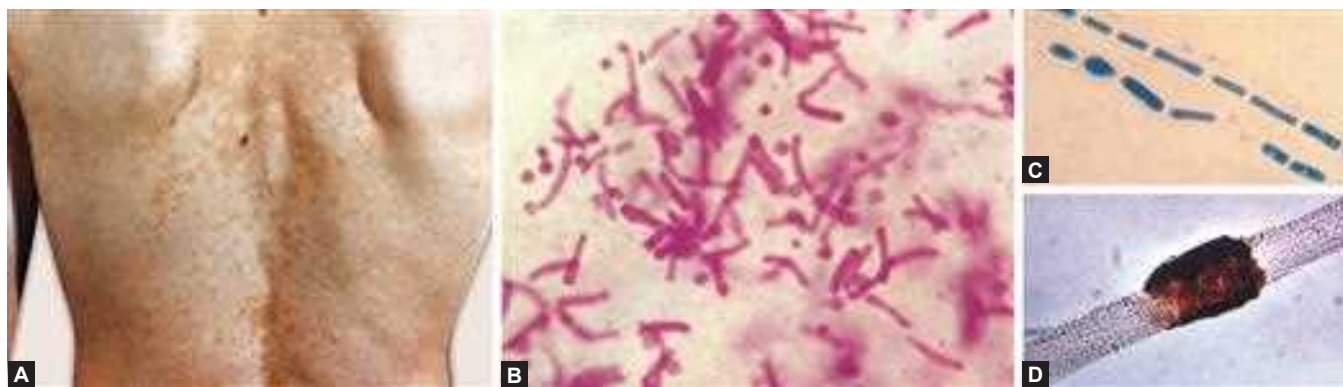
Clinical Manifestations

It is characterized by flat-round scaly patches of hypo- to hyperpigmentation of skin (Fig. 58.1A).

- ❖ Lesions are non-inflammatory and non-pruritic (or rarely pruritic)
- ❖ Lesions can be mistaken for vitiligo, but the latter is not scaly
- ❖ Areas rich in sebaceous glands are commonly involved such as neck, chest, or upper arms
- ❖ Disease is more common in moist humid areas.

Other manifestations caused by *Malassezia furfur* include:

- ❖ **Seborrheic dermatitis:** It manifests as erythematous pruritic scaly lesions called **dandruff in adults** and cradle



Figs 58.1A to D: **A.** Tinea versicolor (hypopigmented patches); **B.** *Malassezia furfur* (yeast cells and hyphae with spaghetti and meatballs appearance); **C and D.** Piedra: **C.** Arthrospores of *Trichosporon beigelii* (white piedra); **D.** Black nodule on hair shaft (black piedra).

Source: Public Health Image Library/Dr. Lucille KG A. ID# 12534; B. ID# 2916; C. ID# 3936 and D. ID#:3937/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

cap in babies. It can be severe in patients with advanced AIDS

- ❖ Atopic dermatitis
- ❖ Folliculitis (hair follicle infection)
- ❖ Disseminated infection can occur rarely.

Laboratory Diagnosis

Diagnosis of tinea versicolor is largely made clinically. The laboratory diagnostic methods are as follows:

- ❖ **Direct microscopy:** Skin scrapings are examined microscopically after treating with 10% KOH. Mixture of budding yeasts and short septate hyphae are seen, described as **spaghetti and meatballs** appearance (Fig. 58.1B)
- ❖ **Culture:** *Malassezia furfur* being lipophilic, SDA with olive oil overlay is the ideal media for culture. Typical 'fried egg' colonies appear after incubating for 5–7 days at 32–35°C
- ❖ **Urease test:** It gives a positive urease test
- ❖ **Wood's lamp examination:** Under Wood's lamp, the scaly lesions show golden yellow fluorescence.

TREATMENT

Tinea versicolor

Topical lotions like selenium sulfide shampoo, ketoconazole shampoo or cream, terbinafine cream should be used for 2 weeks.

Tinea Nigra

It is characterized by painless, black, non-scaly patches present on palm and sole; more commonly in females. It is caused by *Hortaea werneckii*. It is a black-colored yeast like fungus.

Piedra

Piedra is characterized by nodule formation on hair shaft, which may be either black or white in color.

White Piedra

Here, white nodules are formed on the hair shaft, which are less firmly attached.

- ❖ **Agent:** *Trichosporon beigeli*
- ❖ **Identifying feature:** *T. beigeli* is an urease positive, yeast like fungus; produces creamy white colonies, containing hyaline septate hyphae intervening with rectangular **arthrospores** (Fig. 58.1C).

Black Piedra

It is characterized by formation of black nodules, which are firmly attached to the hair shaft (Fig. 58.1D).

- ❖ **Agent:** *Piedraia hortae*
- ❖ **Identifying feature:** It is a phaeoid fungus; produces reddish brown colonies; containing dark brown thick septate hyphae with ascus containing ascospores.

Dermatophytoses

Dermatophytoses (or tinea or ringworm) is the most common superficial mycoses affecting skin, hair and nail; caused by a group of related fungi (called **dermatophytes**)

Table 58.1: Classification of dermatophytes based on their usual habitat.

Habitat	<i>Trichophyton</i>	<i>Microsporum</i>	<i>Epidermophyton</i>
Anthropophilic	<i>T. rubrum</i> <i>T. mentagrophytes</i> <i>T. schoenleinii</i> <i>T. tonsurans</i> <i>T. violaceum</i>	<i>M. audouinii</i>	<i>E. floccosum</i>
Zoophilic	<i>T. equinum</i> <i>T. verrucosum</i>	<i>M. canis</i> <i>M. equinum</i>	
Geophilic	<i>T. ajelloi</i>	<i>M. gypseum</i>	<i>E. stockdaleae</i>

that are capable of infecting keratinized tissues. These include:

- ❖ **Trichophyton species:** Infect skin, hair and nail
- ❖ **Microsporum species:** Infect skin and hair
- ❖ **Epidermophyton species:** Infect skin and nail.

Depending on the usual habitat (humans, animals, or soil), dermatophytes are classified as follows (Table 58.1):

- ❖ **Anthropophilic:** These are the fungal agents exclusively infect humans
- ❖ **Zoophilic:** They infect animals as well as birds
- ❖ **Geophilic:** These fungal species are frequently isolated from soil.

Pathogenesis

Dermatophyte infection is acquired by direct contact with soil, animals or humans infected with fungal spores. Then the spores are carried to different areas due to scratching of the inoculated site. Predisposing factors include moist humid skin and tight ill-fitting underclothing.

- ❖ **Skin:** Dermatophytes grow in a centrifugal pattern in the stratum corneum; leading to the formation of characteristic well-demarcated **annular-** or **ring-shaped** pruritic scaly skin lesions with central clearing and raised edges. Scaling, erythema, and rarely blister formation may occur
- ❖ **Nails:** They invade the nails through the lateral or superficial nail plates and then spread throughout the nails
- ❖ **Hair shafts:** Dermatophytes can invade within the hair shaft or may be found surrounding it. Hair becomes brittle and areas of alopecia may appear. A deep and persistent suppurative folliculitis may be produced; called as **Majocchi granuloma**
- ❖ Lesions are not produced by the tissue invasion by the fungi per se; but in response to the host's inflammatory reaction elicited by the fungal antigens
- ❖ Males are more commonly infected than females as progesterone is inhibitory to dermatophyte growth
- ❖ Severity depends on the infecting fungi, immune status of the host and the site of lesion
 - Anthropophilic dermatophytes are the most common dermatophytes infecting humans. They cause relatively mild and chronic lesions but respond poorly to treatment

- In contrast, geophilic and zoophilic species, being less adapted to human hosts, produce more acute inflammatory response and severe infections; but they tend to resolve more quickly.

Clinical Types

Depending on the site of involvement, various clinical types of dermatophytic or tinea or ring worm infections are produced (Table 58.2). Incubation period is about 1–2 weeks.

Dermatophytid or Id Reaction

Occasionally, hypersensitivity to dermatophyte antigens may occur, which leads to appearance of secondary eruption in sensitized patients because of circulation of allergenic products. However, these lesions are distinct from the primary ringworm lesions as they occur distal to primary site and fungal culture often turns negative.

Table 58.2: Clinical types of dermatophytoses.	
Clinical types	Area involved
Tinea capitis (infection of the scalp)	Scaly patches are produced on scalp, in which hair shafts are broken off right above the skin. It is of various types
1 Kerion	It is a painful inflammatory reaction, producing boggy lesions on scalp Agent: <i>Trichophyton verrucosum</i>
2 Favus	Cup like crust (scutula) is formed around the infected hair follicle with minimal hair shaft involvement (Fig. 58.2A) Agent: <i>Trichophyton schoenleinii</i>
3 Ectothrix	Arthrospores are formed on the surface of hair shaft (Fig. 58.3A) Agents: <i>M. audouinii</i> , <i>M. canis</i> , and <i>T. mentagrophytes</i>
4 Endothrix	Arthrospore are formed within the hair completely filling the hair shaft; thus, can result in alopecia (Fig. 58.3B) Agents: <i>T. tonsurans</i> and <i>T. violaceum</i>
Tinea corporis	Infection of the non-hairy skin of the body (trunk and limbs) (Fig. 58.2D)
Tinea pedis (athlete foot)	Infection of the web space between the toes, which then spreads to the sole in a "moccasin" pattern (Fig. 58.2C)
Tinea cruris (or jock itch)	Infection of the groin area
Tinea barbae	Infection of the beard and moustache area of face
Tinea faciei	Infection of the non-bearded area of face (Fig. 58.2B)
Tinea imbricata	Concentric lesions of the skin Agent: <i>T. concentricum</i>
Tinea unguium (nail plate infection)	Infection of nail beds Agents: <i>T. mentagrophytes</i> and <i>E. floccosum</i>
Tinea manuum	Infection of the palmar aspect of hands



Figs 58.2A to D: Ring worm infections (Tinea): **A.** Tinea capitis (favus); **B.** Tinea faciei; **C.** Tinea pedis; **D.** Tinea corporis.

Source: Public Health Image Library: A. ID#: 2936; B. ID#: 4807; C. ID#: 2939; D. ID#: 2938 Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Laboratory Diagnosis

Woods Lamp Examination

Certain dermatophytes fluoresce when the infected lesions are viewed under Wood's lamp. It is usually positive for various *Microsporum* species and *Trichophyton schoenleinii*. Other dermatophytes do not fluoresce under Wood's lamp. Fluorescence is due to the presence of pteridine pigment in cell wall.

Specimen Collection

Skin scrapings, hair plucks (broken or scaly ones) and nail clippings are obtained from the active margin of the lesions and are kept in folded black paper. Hair should be plucked, but not cut.

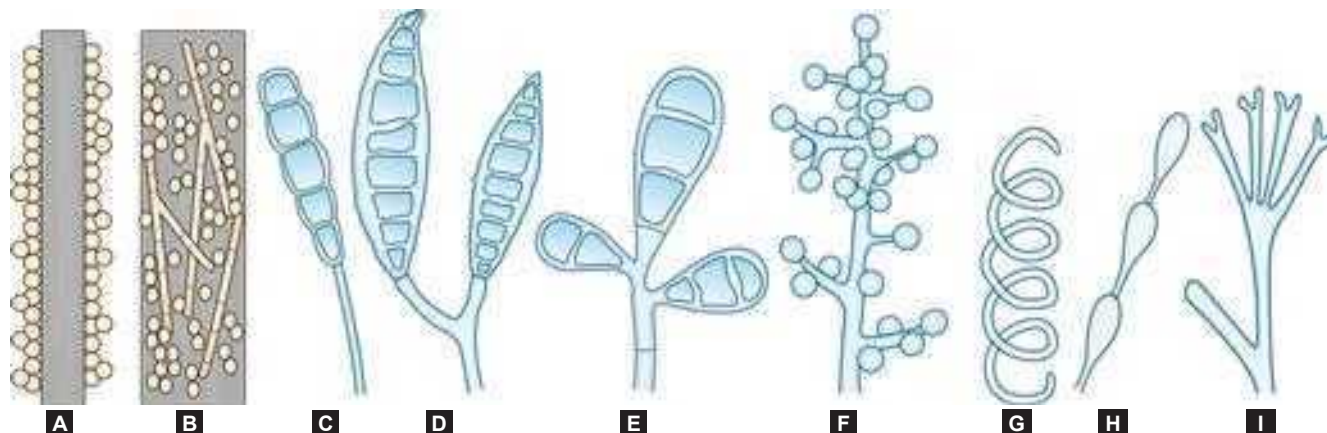
Direct Examination

The specimen is mounted in KOH (10% for skin scrapings or hair, 20–40% for nail clippings) or calcofluor white stain and is examined for the presence of thin septate hyaline hyphae with arthroconidia (see Chapter 6, Figs 6.2A and B). When hair is involved, the arthroconidia may be found on the surface of the hair shaft (ectothrix) or within the shaft (endothrix) (Figs 58.3A and B).

Culture

Specimens should be inoculated onto SDA containing cycloheximide and incubated at 26–28°C for 4 weeks. Potato dextrose agar is used to stimulate the sporulation. Identification is made by:

- ❖ **Macroscopic appearance** of the colonies such as—rate of growth, texture, pigmentation, colony topography



Figs 58.3A to I: **A.** Ectothrix; **B.** Endothrix infection of hair shaft by dermatophytes; **C to E.** Macroconidia of various dermatophyte species: **C.** *Trichophyton mentagrophytes*; **D.** *Microsporum canis*; **E.** *Epidermophyton floccosum*; **F.** Microconidia of *Trichophyton mentagrophytes*; **G to I.** Special types of hyphae seen in dermatophytes: **G.** Spiral hypha; **H.** Racquet hypha; **I.** Favic chandelier.

❖ **Microscopic appearance:** The colonies are teased and LPCB mount is made to demonstrate the hyphae and spores (or conidia):

- **Conidia:** Two types of spores or conidia are observed such as small unicellular **microconidia**, and large septate **macroconidia**; both are used for identification of species (Table 58.3 and Figs 58.3C to F)
- **Special hyphae:** Dermatophytes possess thin septate hyaline hyphae; some species have specialized hyphae such as spiral hyphae, racquet hyphae and favic chandeliers (Figs 58.3G to I).

Identification features (macroscopic and microscopic) of commonly encountered dermatophyte species is given in Table 58.4.

Table 58.3: Distribution of conidia of dermatophytes.

Dermatophytes*	Macroconidia	Microconidia
<i>Trichophyton</i>	Rare, thin walled, smooth, pencil shaped	Abundant
<i>Microsporum</i>	Numerous, thick walled, rough, spindle shaped	Rare
<i>Epidermophyton</i>	Numerous, smooth walled, club shaped	Absent

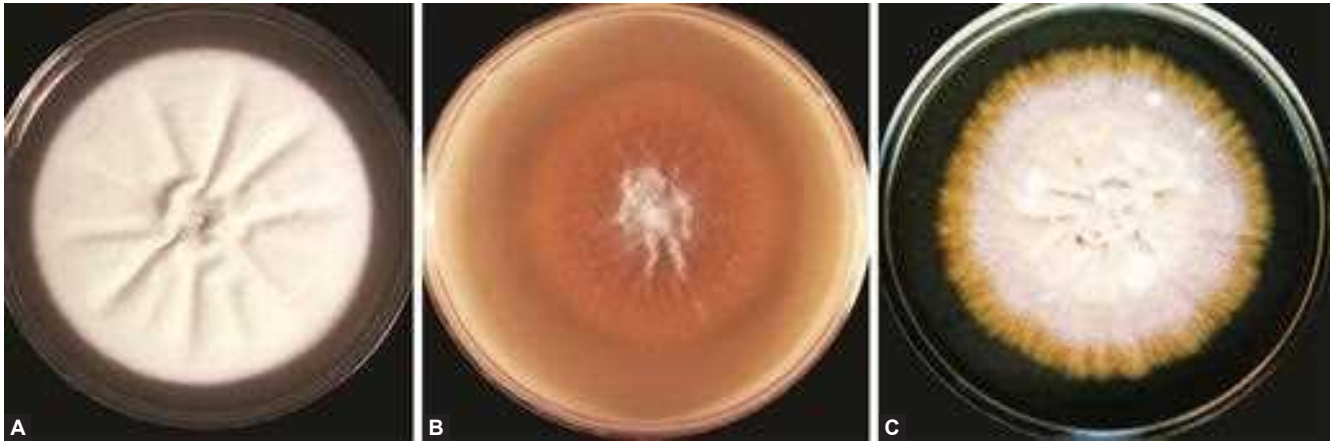
*Appearance of microconidia and macroconidia may vary depending on the dermatophyte species.

Other Methods of Diagnosis

Apart from culture, there are several other methods available for identification of dermatophytes such as:

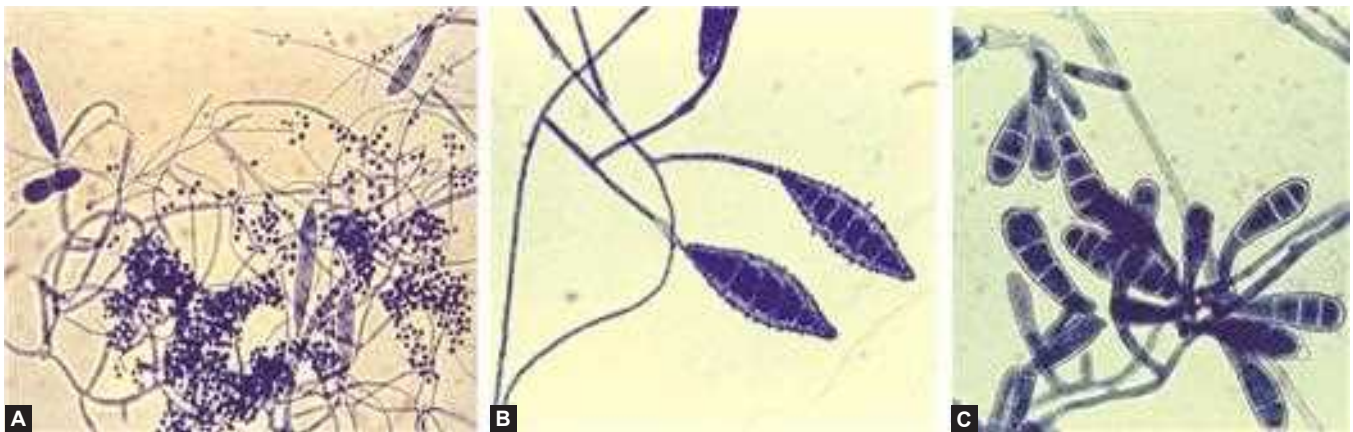
Table 58.4: Identification features of commonly encountered dermatophyte species.

Dermatophytes	Macroscopic appearance	Microscopic appearance
<i>T. rubrum</i>	Velvety, red pigment on reverse	Microconidia—tear drop shaped, plenty Macroconidia—few, long, pencil shaped
<i>T. mentagrophytes</i> (Figs 58.4A, 58.5A)	White to tan Powdery Pigment variable	Microconidia—numerous, round to pyriform Macroconidia—cigar shaped Spiral hyphae seen
<i>T. schoenleinii</i>	Smooth, waxy	Microconidia and macroconidia—rare or absent Chlamydo spores seen Hyphae—hyphal swelling and favic chandelier seen
<i>T. violaceum</i>	Slow growing, waxy Violet pigment on reverse	Microconidia and macroconidia—rare or absent Distorted hyphae seen Chlamydo spores seen
<i>M. audouinii</i>	Slow growing, velvety, brownish	Thick-walled chlamydo spores seen Macroconidia and microconidia—rare
<i>M. gypseum</i>	Buff colored, powdery	Macroconidia—abundant, thick walled, spiny, spindle shaped, 4–6 septa, rounded ends Microconidia—rare
<i>M. canis</i> (Figs 58.4B, 58.5B)	Cottony, orange pigment on reverse	Macroconidia—abundant, thick walled, spiny, spindle shaped, up to 15 septa, pointed ends Microconidia—rare
<i>E. floccosum</i> (Figs 58.4C, 58.5C)	Powdery, folded, yellowish green	Macroconidia club or clavate shaped in clusters, 4–6 septa Microconidia—absent



Figs 58.4A to C: Macroscopic (colony) appearance of various dermatophytes on SDA: **A.** *Trichophyton mentagrophytes*; **B.** *Microsporum canis*; **C.** *Epidermophyton floccosum*.

Source: Public Health Image Library/ A. ID#: 14717; B. ID#: 15474; C. ID#: 2937/Dr Lucille KG/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Figs 58.5A to C: Microscopic appearance of various dermatophyte species (LPCB mount): **A.** *Trichophyton mentagrophytes*; **B.** *Microsporum canis*; **C.** *Epidermophyton floccosum*.

Source: Public Health Image Library/ A. ID#: 15105; B. ID#: 15472; C. ID#: 14588, Centers for Disease Control and prevention (CDC), Atlanta (with permission).

- ❖ **Hair perforation test:** It is positive for *Trichophyton mentagrophytes* and *Microsporum canis*. The test is performed by inoculating a colony into a petri dish containing water, yeast extract, and hair. Fungi pierce the hair producing wedge-shaped perforations
- ❖ **Urease test:** *Trichophyton mentagrophytes* is urease positive
- ❖ **Dermatophyte test medium and Dermatophyte identification medium:** They are used for presumptive identification. These tests are based on color change in the medium due to production of alkali metabolites
- ❖ **Molecular methods:** PCR can be used to detect species specific genes (e.g. chitin synthase gene)
- ❖ **Skin test:** It is done for detecting hypersensitivity to dermatophyte antigen (trichophytin).

TREATMENT

Dermatophytoses

- ❑ **Oral terbinafine or itraconazole** are the drugs of choice for treatment of dermatophytosis. Duration of treatment depends

Contd...

Contd...

TREATMENT

Dermatophytoses

on the affected site (1–2 weeks for skin lesions, 6 weeks for hair infection, 3 months for onychomycosis). They can be given as pulse therapy

- ❑ Alternative: Oral griseofulvin and ketoconazole may be given. Some resistance has been reported to griseofulvin
- ❑ Topical lotion such as whitfield ointment or tolnaftate can be applied.

SUBCUTANEOUS MYCOSES

The agents of subcutaneous mycoses usually inhabit the soil. They enter the skin by traumatic inoculation with contaminated material and tend to produce the granulomatous lesions in the subcutaneous tissue.

Mycetoma

Mycetoma is a chronic, slowly progressive granulomatous infection of the skin and subcutaneous tissues.

- ❖ Clinically, it is manifested as a triad of swelling, discharging sinuses and presence of granules in the discharge
- ❖ Mycetoma is also known as **Maduramycosis** or **Madura foot**, as it was first described in Madurai, South India, by John Gill (1842).

Types of Mycetoma and Causative Agents

Mycetoma can be of two types. It can be caused by either fungal agents (eumycetoma) or bacterial agents (actinomycetoma). They differ from each other by various properties like color of granules/grains and in clinical manifestations, etc. (Tables 58.5 and 58.6).

There is a third category called **botryomycosis** which refers to a mycetoma like condition caused by some bacteria such as *Staphylococcus aureus*.

Pathogenesis

The causative agents enter the skin or subcutaneous tissue from the contaminated soil, usually by the accidental trauma such as thorn prick or splinter injury. Then the disease evolves slowly; initially micro-abscesses are formed by the polymorphs, replaced later by chronic granulomatous tissue in skin and subcutaneous tissues.

Table 58.5: Agents of mycetoma and types of grains they produce.

Eumycetoma	Actinomycetoma (Chapter 55)
<p>Black granules:</p> <ul style="list-style-type: none"> • <i>Madurella mycetomatis</i> • <i>Madurella grisea</i>* • <i>Exophiala jeanselmei</i> • <i>Curvularia</i> species <p>White granules:</p> <ul style="list-style-type: none"> • <i>Pseudallescheria boydii</i> • <i>Aspergillus nidulans</i> • <i>Acremonium</i> species • <i>Fusarium</i> species 	<p>White to yellow granules:</p> <ul style="list-style-type: none"> • <i>Nocardia</i> species • <i>Streptomyces somaliensis</i> • <i>Actinomadura madurae</i>: It is the most frequent cause, significantly outnumbering the cases caused by <i>Nocardia</i> <p>Pink to red granules: <i>Actinomadura pelletieri</i></p>

*Recently renamed as *Trematosphaeria grisea*.

Table 58.6: Clinical manifestations of eumycetoma and actinomycetoma.

Clinical manifestations	Eumycetoma	Actinomycetoma
Tumor	Single, well-defined margins	Multiple tumor masses with ill-defined margins
Sinuses	Appear late, few in number	Appear early, numerous with raised inflamed opening
Discharge	Serous	Purulent
Grains	Black/white	White/ red
Bone	Osteosclerotic lesions	Osteolytic lesions
Grains contain	Fungal hyphae (> 2 µm)	Filamentous bacteria (< 2 µm)

Clinical Manifestations

Hallmark of mycetoma is presence of clinical triad consisting of (Fig. 58.6):

- ❖ Tumor like swelling, i.e. tumefaction
- ❖ Discharging sinuses
- ❖ Discharge oozing from sinuses containing granules.

Eumycetoma and actinomycetoma vary clinically (Table 58.6). Feet are the most common site affected, although any site can be involved. There may be involvement of underlying fasciae and bones, producing osteolytic or osteosclerotic bony lesions. Lesions are usually painless.

Epidemiology

Mycetoma is endemic in Africa, India, the Central and South America, and has a non-uniform distribution.

- ❖ Overall, actinomycetoma is more common (60%) than eumycetoma (40%) globally, whereas eumycetoma is more common in Africa
- ❖ However, within a country, the distribution may vary in different regions
- ❖ A meta-analysis done in 2013 showed that most of the cases are reported globally from Mexico, Sudan and India
- ❖ In India, Rajasthan reports the maximum cases of mycetoma per year followed by Tamil Nadu and West Bengal. Actinomycetoma predominates in India (65%), except in Rajasthan where eumycetoma is more common.

Laboratory Diagnosis

Specimen Collection

The lesions should be cleaned with antiseptics and the grains should be collected on sterile gauze by pressing the sinuses from periphery or by using a loop.

Direct Examination

Granules are thoroughly washed in sterile saline; crushed between the slides and examined.



Fig. 58.6: Mycetoma of foot.

Source: Public Health Image Library/ ID#: 14816/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Macroscopic appearance of granules** such as color, size, shape, texture may provide important clue to identify the etiological agent
- ❖ **If eumycetoma is suspected:** Grains are subjected to KOH mount, which reveals hyphae of 2–6 μm width along with chlamydospores at margin
- ❖ **If actinomycetoma is suspected:** Grains are subjected to Gram staining which reveals filamentous gram-positive bacilli (0.5–1 μm wide). Modified acid-fast stain is performed if *Nocardia* is suspected, as it is partially acid-fast
- ❖ **Histopathological staining of the granules:**
 - Eumycetoma: Reveals granulomatous reaction with palisade arrangement of hyphae in the cement substance (Fig. 58.7A)
 - Actinomycetoma: Shows granulomatous reaction with filamentous bacteria at the margin (Fig. 58.7B).

Culture

Granules obtained from deep biopsies are the best specimen for culture as they contain live organisms. Both fungal (e.g. SDA) and bacteriological media (such as Lowenstein-Jensen media) should be included in the panel.

- ❖ Identification of the eumycetoma agents is usually carried out by observation of the growth rate, colony morphology, production of conidia and their sugar assimilation patterns
- ❖ Agents of actinomycetoma can be identified by their growth rate, colony morphology, urease test, acid fastness and decomposition of media containing casein, tyrosine, xanthine, etc.

TREATMENT

Mycetoma

Treatment of mycetoma consists of surgical removal of the lesion followed by use of:

- Antifungal agents for eumycetoma (itraconazole or amphotericin B for 8–24 months) or
- Antibiotics for actinomycetoma such as Welsh regimen (amikacin plus cotrimoxazole).

Sporotrichosis

Sporotrichosis or Rose Gardner's disease is presented as subcutaneous noduloulcerative lesions; caused by a thermally dimorphic fungus, *Sporothrix schenckii*.

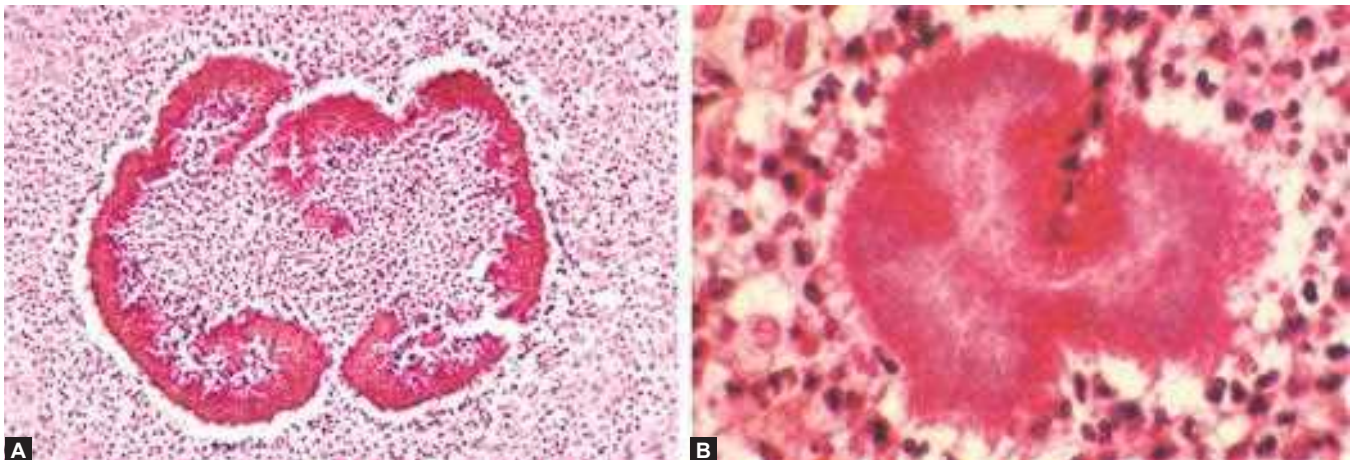
Pathogenesis

Spores of *S. schenckii* are introduced into skin following minor trauma caused by thorn prick or splinter injury. Enzymes secreted by the fungus, such as serine proteinase and aspartic proteinase help in local invasion. *S. schenckii* has a typical tendency to spread along the lymphatics.

Clinical Manifestations

Sporotrichosis is a chronic subcutaneous pyogranulomatous disease. Incubation period is about 3 weeks. Various clinical types have been observed.

- ❖ **Lymphocutaneous type:** It is the most common type (80%) and is characterized by:
 - Noduloulcerative lesions (painless) occur along the lymphatics; called as sporotrichoid pattern of spread, which is also seen in other conditions (see highlight box below)
 - Lymph nodes become enlarged, suppurative, indurated and have cord like feeling on palpation.
- ❖ **Other clinical types** are rare such as:
 - Osteoarticular type: It is seen among alcoholics
 - Pulmonary type: It occurs following spore inhalation, seen in people with underlying chronic obstructive pulmonary disease (COPD)
 - Disseminated sporotrichosis: It occurs in immunocompromised patients (such as AIDS)
 - Fixed cutaneous type: Single nodule is found, that is less progressive and does not spread by lymphatics. It is more common in endemic areas such as Mexico where people show strong immunity against the fungi.



Figs 58.7A and B: A. Eumycetoma (black grain and cement like substance); B. Actinomycetoma caused by *Nocardia brasiliensis* (hematoxylin-eosin staining).

Source: Public Health Image Library/ A. ID#: 4331; B. ID#: 15055/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Sporotrichoid lymphocutaneous infection

It is a syndrome characterized by the development of superficial cutaneous lesions that progress along the dermal and subcutaneous lymphatics

- ❑ **Common causes are:** *Sporothrix schenckii*, *Nocardia brasiliensis*, *Mycobacterium marinum* or *Leishmania brasiliensis*
- ❑ **Rare causes are:** Coccidioidomycosis, cryptococcosis, blastomycosis, histoplasmosis, anthrax, melioidosis, lepromatous leprosy, lupus vulgaris, *Francisella tularensis* and cowpox virus.

Epidemiology

Sporotrichosis is prevalent in tropical countries with high humidity.

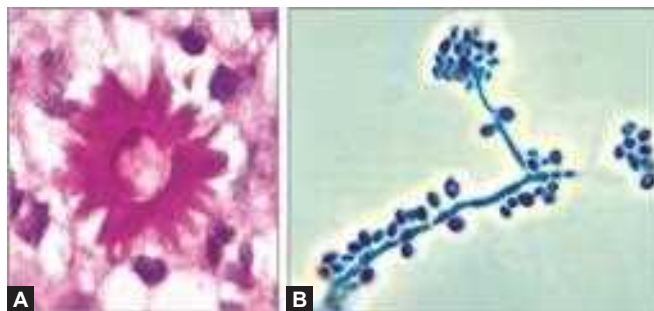
- ❖ **World:** It has been reported frequently from Central South America, South Africa and India
- ❖ **In India,** sporotrichosis is prevalent in sub-Himalayan hilly areas of northeast states ranging from Himachal Pradesh to Assam. Other endemic foci are northern Karnataka and southern Maharashtra
- ❖ **Source:** *S. schenckii* has been isolated from decaying vegetations (such as wood, bark, leaves), and soil
- ❖ **Risk factors** include people walking bare foot, certain occupations such as farmers and gardeners.

Laboratory Diagnosis

- ❖ **Direct microscopy:** Specimens such as pus, aspirate from nodules, curettage or swabbing from ulcers are subjected to KOH mount or calcofluor staining which demonstrate elongated yeast cells of 3–5 µm in diameter. But the sensitivity is very low
- ❖ **Histopathological staining** (e.g. hematoxylin and eosin) of tissue sections reveals cigar-shaped asteroid bodies

Asteroid body consists of a central basophilic yeast cell surrounded by radiating extensions of eosinophilic mass, composed of antigen-antibody complexes (Fig. 58.8A). Such eosinophilic halo is described as **Splendore-Hoeppli phenomenon**; which is also observed in zygomycosis, candidiasis, aspergillosis and blastomycosis.

- ❖ **Culture:** It is the most definitive tool for diagnosis. Specimens are inoculated onto SDA and blood agar in duplicate and incubated at 25°C and 37°C simultaneously, because *S. schenckii* is a dimorphic fungus
 - **At 25°C:** It produces mycelial form, consisting of slender delicate hyphae with conidia arranged in **flower-like pattern** (Fig. 58.8B)
 - **At 37°C:** It produces yeast form, characterized by moist creamy white colonies which turn brown black in 10–14 days.
- ❖ **Serology:** Latex agglutination test detects serum antibodies in patients with extracutaneous form of the disease, but is not always diagnostic
- ❖ **Skin test:** It may demonstrate delayed type of hypersensitivity reaction against sporotrichin antigen.



Figs 58.8A and B: *Sporothrix schenckii*: **A.** Yeast form (asteroid body); **B.** Mold form showing thin septate hyphae with flower-like sporulation.

Source: A. Dr Manoj Singh and Dr M Ramam, AIIMS, New Delhi; B. Public Health Image Library/Dr Libero Ajello B. ID#: 4208/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

TREATMENT**Sporotrichosis**

Itraconazole is the drug of choice for all forms of sporotrichosis; except for disseminated form where amphotericin B is recommended. Treatment is given until 2–4 weeks after the lesions resolve.

Chromoblastomycosis

Chromoblastomycosis refers to slow growing chronic subcutaneous lesions caused by group of dematiaceous or phaeoid fungi (i.e. darkly pigmented fungi) that produce a characteristic morphology called **sclerotic body**.

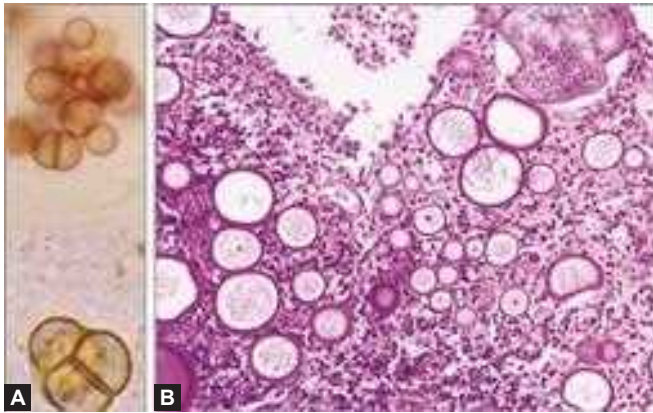
- ❖ **Agents** of chromoblastomycosis include:
 - *Fonsecaea pedrosoi* and *F. compacta*
 - *Phialophora verrucosa*
 - *Cladosporium carrionii*
 - *Rhinocladiella aquaspersa*.
- ❖ **Lesions** are typically slow growing and polymorphic, such as verrucose (most common type), crusted, ulcerative and nodular or tumor like
- ❖ Most commonly seen in tropical or subtropical climates, often in rural areas
- ❖ **Sclerotic bodies:** Histopathological appearance of these fungi is characterized by formation of brown thick walled round cells (5–12 µm size) with multiple internal transverse septa. They are also called **Medlar bodies** or **muriform cells** or golden-brown septate “copper pennies” (Fig. 58.9A).

TREATMENT**Chromoblastomycosis**

It consists of surgical removal (cryosurgery or laser therapy) of the lesion followed by antifungals (itraconazole).

Phaeohyphomycosis

Phaeohyphomycosis refers to chronic subcutaneous lesions, caused by dematiaceous or phaeoid fungi other than that are described in chromoblastomycosis (i.e. they do not produce sclerotic bodies). They exist in mycelial form. Agents include:



Figs 58.9A and B: A. Sclerotic body; B. *Rhinosporidium seeberi*—spherules containing sporangia filled with endospores (H and E stain).

Source: A. Claudio Guedes Salgado, Associate Professor, Dermato-Immunology Laboratory, Institute of Biological Sciences, Pará Federal University, Brazil; B. Public Health Image Library/Dr Martin Hicklin/ID#: 3107/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ *Alternaria* species
- ❖ *Bipolaris* species
- ❖ *Curvularia* species
- ❖ *Exophiala jeanselmei*
- ❖ *Cladophialophora bantiana*: It is neurotropic, produces brain abscess, frontal lobe being the most common site affected (Chapter 75).

Rhinosporidiosis

Rhinosporidiosis is a chronic granulomatous disease, characterized by **large friable polyps in the nose** (most common site), conjunctiva and occasionally in ears, larynx, bronchus and genitalia.

- ❖ **Agent:** It is caused by *Rhinosporidium seeberi*. Its taxonomic status is controversial. Previously classified under fungi, now, it is considered to be an aquatic protistan parasite
- ❖ **Source:** Stagnant water is the main source of infection. Spores are inhaled while taking bath in contaminated ponds and rivers
- ❖ **Distribution:** Rhinosporidiosis is common in tropical countries, especially in Sri Lanka and India (Tamil Nadu, Kerala, Odisha and Andhra Pradesh)
- ❖ **Diagnosis** is made by histopathology of the polyps that demonstrates **spherules** (large sporangia up to 350 μm size, that contain numerous endospores, each 6–9 μm in size) (Fig. 58.9B). It is stained better with mucicarmine stain. *R. seeberi* has not been cultivated yet.

TREATMENT

Rhinosporidiosis

Radical surgery with cauterization is the mainstay of treatment. Dapsone has been found to be effective. Recurrence is common.

Microsporidial Myositis

Certain microsporidia species (earlier considered parasites) such as *Pleistophora*, *Trachipleistophora*, *Brachiola*, and

Anncaliia can cause myositis, and may present as myalgia (Chapter 45, for detail).

OPPORTUNISTIC MYCOSES OF SKIN AND SOFT TISSUE

Penicillium marneffe Infection

Penicillium marneffe is a thermally dimorphic fungus (yeast at 37°C and mold at 25°C), that causes opportunistic infection in HIV-infected patients. It is recently renamed as *Talaromyces marneffe*. Other *Penicillium* species are discussed in Chapter 69.

Epidemiology

P. marneffe is endemic in Southeast Asian countries including Thailand, Vietnam and India (Manipur).

Pathogenesis

P. marneffe is found mostly in rural areas where the bamboo rats are prevalent, which are the reservoirs of infection; however, there is no direct rat to man transmission.

- ❖ Immunocompromised hosts (e.g. patients with advanced AIDS) are at higher risk
- ❖ Transmission is by inhalation of conidia from the environment
- ❖ Mold to yeast conversion occurs in the lungs and then the yeast form spreads via blood to the reticuloendothelial system.

Clinical Manifestations

P. marneffe produces two types of clinical manifestations.

1. **Systemic infection:** The manifestations are similar to that of disseminated histoplasmosis such as fever, weight loss, dyspnea, lymphadenopathy and hepatosplenomegaly
2. **Skin lesions:** Warty lesions mimicking that of molluscum contagiosum are seen.

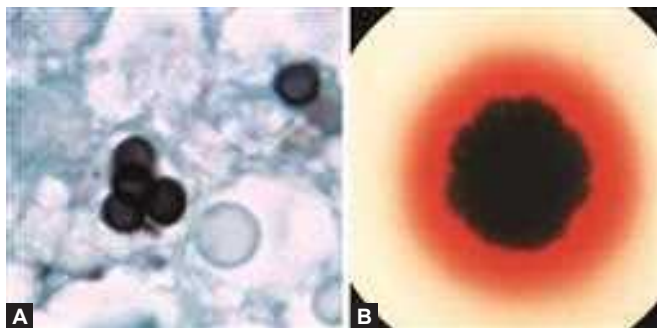
Laboratory Diagnosis

- ❖ **Histopathological staining** of tissue sections, skin scrapings or blood smear shows **oval or elliptical yeast cells with central septation**, which indicates that these cells divide by transverse fission rather than budding (Fig. 58.10A)
- ❖ **Culture:** *P. marneffe* being dimorphic; produces yeast like colonies at 37°C and mold form at 25°C
- ❖ The mold form has a characteristic **brick red pigment** (Fig. 58.10B), but the microscopic appearance of mold form is similar to other *Penicillium* species.

TREATMENT

Penicilliosis

AIDS patients with severe penicilliosis are treated with amphotericin B till the condition improves followed by maintenance therapy with itraconazole for 12 weeks. In mild penicilliosis, itraconazole is recommended for 12 weeks.



Figs 58.10A and B: *Penicillium marneffe*: **A.** Methenamine silver staining shows yeast cells with central septations; **B.** Red pigmented colony (mold form).

Source: Public Health Image Library/Dr Libero Ajello, A. ID#: 11959; B. ID#: 11967/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Mucocutaneous Candidiasis

Candida species (Chapter 38) are the most common fungal agent to cause lesions of skin and mucosa.

Mucosal Candidiasis

The various mucosal manifestations include:

- ❖ Oropharyngeal candidiasis (oral thrush): It presents as white, adherent, painless patches in the mouth (Fig. 58.11A)
- ❖ Vulvovaginitis: It is characterized by pruritus, pain, and vaginal discharge that is usually thin, but may become whitish curd like in severe cases
- ❖ Balanitis and balanoposthitis (occurring in uncircumcised males)
- ❖ Esophageal candidiasis
- ❖ Angular stomatitis and denture stomatitis
- ❖ **Chronic mucocutaneous candidiasis:** It is seen in infants and children with deficient CMI (T cell defect)
 - Lesions are produced involving hair, nail, skin, and mucous membrane; which are usually resistant to treatment
 - It is associated with other endocrine abnormalities.

Cutaneous Candidiasis

The following cutaneous manifestations are produced in candidiasis:

- ❖ **Intertrigo:** It is characterized by erythema and pustules in the skin folds; associated with tight fitting undergarments and sweating
- ❖ **Paronychia** (involving nail-skin interface) and onychomycosis (fungal infection of nail) (Fig. 58.11B)
- ❖ **Diaper candidiasis:** Pustular rashes, associated with use of diapers in infants
- ❖ Perianal candidiasis
- ❖ **Erosio interdigitalis blastomycetica:** It is an infection affecting the web spaces of hands or toes
- ❖ Generalized disseminated cutaneous candidiasis, seen in infants.



Figs 58.11A and B: Candidiasis: **A.** Oral thrush; **B.** Onychomycosis.

Source: Public Health Image Library/A. ID#: 1217; B. Mr Gust, ID#: 15669/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Allergic Candidiasis

Various allergic manifestations seen in candidiasis include:

- ❖ **Candidid:** This is an allergic reaction to the metabolites of *Candida*, characterized by vesicular lesions in the web space of hands and other areas, similar to that of dermatophytid reaction (both conditions are together called 'id' reaction)
- ❖ **Other allergic reactions** include: gastritis, irritable bowel syndrome and eczema.

Candida Arthritis, Osteomyelitis, and Myositis

Musculoskeletal infections can rarely occur following candidemia and disseminated candidiasis.

- ❖ Common sites of involvement are knee, vertebral column (adults), ribs and leg bones (< 20 yr) and sternum (after cardiac surgery)
- ❖ Usually asymptomatic; history reveals underlying risk factors for disseminated candidiasis. On examination, reveals pain localized over the affected site.

Laboratory Diagnosis

Depending on the site of infection, various specimens can be collected such as whitish mucosal patches, skin scrapings and nail clippings.

- ❖ **Direct microscopy:** Gram-positive oval budding yeast cells with pseudohyphae
- ❖ **Culture on SDA** (Sabouraud dextrose agar): Produces creamy white and pasty colony
- ❖ **Tests for species identification (Chapter 38):**
 - Germ tube test (positive for *C. albicans*)
 - Dalmau plate culture
 - CHROMagar
 - Molecular methods such as PCR.

TREATMENT

Candidiasis

- ❑ Cutaneous candidiasis or oral thrush: The drug of choice is topical azole (clotrimazole or miconazole) or nystatin cream
- ❑ Esophageal and vulvovaginal candidiasis: The drug of choice is oral fluconazole or caspofungin.

Cutaneous Mucormycosis

Agents of mucormycosis such as *Rhizopus*, *Mucor* can occasionally cause cutaneous lesions (Chapter 69).

- ❖ The agents may enter by external implantation (soil exposure from trauma or penetrating injury) or from hematogenous dissemination
- ❖ It can be highly invasive, penetrating into muscle, fascia, and even bone (e.g. **necrotizing fasciitis**). The prognosis is extremely poor, especially in presence of a hematogenous setting
- ❖ **Treatment:** Prompt, aggressive surgical debridement and early institution of appropriate antifungal is crucial.

Cutaneous Aspergillosis

Cutaneous aspergillosis may result secondary to disseminated aspergillosis or due to direct invasion. The latter is more common in neutropenic patients at the site of IV catheter insertion and in burn patients, following trauma, or after surgery (especially *Aspergillus flavus*). It appears as an erythematous area that progresses to a necrotic eschar (Chapter 69).

Cutaneous Cryptococcosis

Cryptococcus neoformans usually causes meningitis in HIV-infected individuals (Chapter 75). Skin lesions can occur secondary to disseminated cryptococcosis and can be highly variable, including papules, plaques, purpura, vesicles, tumor-like lesions, and rashes.

EXPECTED QUESTIONS

I. Write essay on:

1. A 21-year-old male had developed characteristic well-demarcated annular or ring-shaped pruritic scaly skin lesions with central clearing and raised edges. Culture of the skin scraping done on Sabouraud's dextrose agar reveals velvety colonies with red pigment on the reverse. Microscopy of the culture isolate reveals plenty of tear drop-shaped microconidia and few, long, pencil-shaped macroconidia.
 - a. What is the clinical diagnosis and what is the most likely etiological agent?
 - b. Describe the various clinical manifestations produced by this organism.
 - c. Add a note on the laboratory diagnosis of this condition.
2. Classify various types fungal diseases. Describe the etiology, clinical manifestations and laboratory diagnosis of mycetoma.

II. Write short notes on:

1. A patient coming from Himachal Pradesh, presents with multiple skin lesions. Microscopy reveals cigar-shaped yeast cells and asteroid bodies. Microscopy of culture shows 'flower like' pattern.

Identify the etiological agent and discuss the clinical manifestations and laboratory diagnosis?

2. *Penicillium marneffe* infection.

III. Multiple Choice Questions (MCQs):

1. Organism that does not affect nail:

- a. *Trichophyton*
- b. *Epidermophyton*
- c. *Microsporum*
- d. *Candida albicans*

2. Germ tube test is diagnostic for:

- a. *Candida glabrata*
- b. *Candida albicans*
- c. *Cryptococcus*
- d. *Coccidioides immitis*

3. Asteroid bodies is observed in:

- a. Sporotrichosis
- b. Histoplasmosis
- c. Candidiasis
- d. Chromoblastomycosis

4. Chromoblastomycosis is caused by:

- a. *Epidermophyton*
- b. *Histoplasma*
- c. *Fonsecaea*
- d. *Penicillium*

5. Tinea versicolor is caused by:

- a. *Candida albicans*
- b. *Trichophyton rubrum*
- c. *Trichophyton violaceum*
- d. *Malassezia furfur*

6. Spherules are seen in:

- a. Rhinosporidiosis
- b. Chromoblastomycosis
- c. Mucormycosis
- d. Aspergillosis

Answers

1. c 2. b 3. a 4. c 5. d 6. a

Respiratory Tract Infections

SECTION

8

SECTION OUTLINE

59. Infective Syndromes of Respiratory Tract
60. Bacterial Pharyngitis: *Streptococcus pyogenes* Pharyngitis, and Diphtheria
61. Bacterial Lobar Pneumonia: Pneumococcal Pneumonia, *Haemophilus influenzae* Pneumonia and Others
62. Bacterial Atypical (Interstitial) Pneumonia: *Mycoplasma*, *Chlamydia* and *Legionella*
63. Tuberculosis and Nontuberculous Mycobacteria Infections
64. Pertussis (*Bordetella pertussis*)
65. Infection due to Non-fermenting Gram-negative Bacilli
66. Myxovirus Infections of Respiratory Tract: Influenza, Parainfluenza, Mumps, Respiratory Syncytial Virus and Others
67. Coronavirus Infections Including Covid-19
68. Miscellaneous Viral Infections of Respiratory Tract: Rhinovirus, Adenovirus and Infectious Mononucleosis (Epstein-Barr Virus)
69. Parasitic and Fungal Infections of Respiratory Tract
 - Parasitic Infections: Paragonimiasis and Others
 - Fungal Infections: Zygomycosis, Aspergillosis, Pneumocystosis and Others



Cartridge-based Nucleic Acid Amplification Test (CBNAAT)



Revolutionized the TB diagnostics and treatment

- Turnaround time of 2hr
- Also detects rifampicin resistance
- Best for extrapulmonary specimen

Infective Syndromes of Respiratory Tract

CHAPTER 59

CHAPTER PREVIEW

- Upper Respiratory Tract Infections
- Oral Cavity Infections
- Lower Respiratory Tract Infections

INTRODUCTION

The respiratory tract is divided into two segments, the upper and the lower respiratory tract.

- ❖ The upper respiratory tract (URT) includes the nasal cavity, paranasal sinuses, pharynx (throat), epiglottis, and larynx
- ❖ The lower respiratory tract (LRT) comprises of trachea, bronchi, which are divided into bronchioles and lungs, with the surrounding pleura.

Normal Commensals

There are a number of bacteria that reside in upper respiratory tract (oral cavity and nasopharynx) as indigenous flora. Some of these organisms are potential pathogens elsewhere in the body and may invade the respiratory tract under certain circumstances—previous damage by a viral infection, low host immunity, or physical damage to the respiratory epithelium (e.g. from smoking).

Respiratory Commensals

Common examples of respiratory commensals are:

- ❑ Streptococci (α and nonhemolytic)
- ❑ *Neisseria* (non-pathogenic species, other than *N. gonorrhoeae* and *N. meningitidis*)
- ❑ Diphtheroids
- ❑ *Moraxella catarrhalis*
- ❑ Coagulase negative staphylococci such as *S. epidermidis*
- ❑ Anaerobes: *Prevotella*, *Peptostreptococcus*, *Fusobacterium*
- ❑ Major pathogens of other body sites may colonize in the nasopharynx less frequently, such as *Haemophilus*, meningococcus, pneumococcus and *S. aureus*
- ❑ In hospitalized patients on the ventilator, the organisms present in the hospital environment, such as gram-negative rods *Acinetobacter*, *Pseudomonas*, *Burkholderia* and Enterobacteriaceae may colonize the URT
- ❑ Yeasts such as *Candida albicans*.

Defence Mechanisms of Respiratory Tract

Respiratory tract serves as portal of entry for a number of pathogenic organisms. Encounters between

microorganisms and the respiratory tract occur several times a day. However, establishment of infection after such contact tends to be the exception rather than the rule. This is due to the defense mechanisms provided by the respiratory tract which prevent the establishment of infection.

- ❖ **Nasal cavity:** Nasal hairs, convoluted passages, and the nasal turbinates capture larger inhaled particles before they reach the lower respiratory tract
- ❖ **Secretory products:** Secretory IgA and nonspecific antibacterial substances (lysozyme) in respiratory secretion
- ❖ **Tracheobronchial tree:** The branching architecture of the tracheobronchial tree traps particles on the airway lining
- ❖ **Mucociliary clearance:** Cilia and mucous lining of the trachea helps in clearing or killing the potential pathogens
- ❖ **Reflexes** such as coughing, sneezing, and gag reflex offer critical protection from aspiration
- ❖ **Normal flora** of URT prevents colonization by pathogenic organisms in the URT by several mechanisms such as—competing for space, nutrients and through production of bacteriocins and other metabolic products that are toxic to the invading organism (Chapter 7)
- ❖ **Alveolar macrophages:** After escaping the above described defense mechanisms, the pathogens of URT can establish infection locally; whereas the pathogens of LRT can gain access into the lungs. In such a case, the establishment of infection can be prevented by alveolar macrophages, which ingest and subsequently destroy the organisms.

UPPER RESPIRATORY TRACT INFECTIONS

The various URT infections (URTI) and the common microbial pathogens implicated are listed in Table 59.1. The classification of microbial infections into URTI and LRTI is artificial, as many URTI pathogens can occasionally infect lungs such as *B. pertussis*, influenza, coronaviruses (e.g. COVID-19), etc.

Table 59.1: Upper and lower respiratory tract infections and the major pathogens implicated.

Upper respiratory tract infections	
Pharyngitis and tonsillitis	Viral: Influenza, coronavirus Bacterial: <i>S. pyogenes</i> , <i>C. diphtheriae</i>
Laryngitis	Influenza and parainfluenza viruses
Acute laryngotracheo-bronchitis (croup)	Parainfluenza virus
Epiglottitis	<i>Haemophilus influenzae</i> b
Parapharyngeal infections	
Peritonsillar abscess (quinsy)	<i>S. pyogenes</i> , <i>S. aureus</i> and anaerobes
Ludwig's angina	Polymicrobial and anaerobic
Infections of nasal cavity and sinuses	
Rhinitis	Rhinoviruses and others
Atrophic rhinitis (ozena)	<i>Klebsiella ozaenae</i>
Rhinoscleroma	<i>Klebsiella rhinoscleromatis</i>
Sinusitis	<i>S. pneumoniae</i> , <i>H. influenzae</i>
Infections of the oral cavity	
Stomatitis	Herpes simplex virus
Oral thrush	<i>Candida albicans</i>
Dental, periodontal infections	Anaerobes and viridans streptococci
Salivary gland infections	Mumps and others
Vincent's angina	<i>Borrelia vincentii</i> and <i>Fusobacterium</i>
Lower respiratory tract infections	
Bronchitis	Respiratory viruses
Bronchiolitis	Respiratory syncytial virus
Whooping cough (Pertussis)	<i>Bordetella pertussis</i>
Pneumonia	
Lobar pneumonia	<i>Pneumococcus</i> , <i>H. influenzae</i> , <i>S. aureus</i> , <i>K. pneumoniae</i> , and other gram-negative bacilli
Atypical pneumonia	<i>Mycoplasma</i> , <i>Chlamydia</i> , viruses
Pulmonary tuberculosis	<i>Mycobacterium tuberculosis</i>
Rare causes of LRTI	
Fungal pneumonia	<i>Pneumocystis jirovecii</i> and others
Parasitic lung disease	<i>Paragonimus</i> , <i>Ascaris</i> and others
Lung abscess	Primary: Anaerobes Secondary: Gram-negative bacilli
Pleural effusion and empyema	Bacterial (secondary to pneumonia), tubercular and viral

Note: The classification of microbial infections into URTI and LRTI is artificial; many pathogens causing URTI can occasionally infect lungs and cause LRTI; such as *B. pertussis*, influenza virus, coronavirus, etc. At the same time, many pathogens causing LRTI such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* may occasionally cause URTI like pharyngitis.

Pharyngitis and Tonsillitis

Pharyngitis (or sore throat) refers to the inflammation of the pharynx, is one of the most common upper respiratory tract infection; affecting both children and adults. In children, most often it presents with tonsillitis (inflamed tonsils).

Etiologic Agents

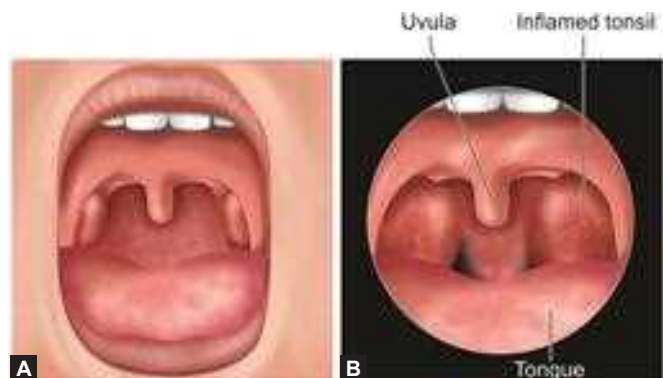
Pharyngitis has varied etiology; the most frequent organisms involved are:

- ❖ **Bacterial agents:** *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, other β -hemolytic *Streptococcus* (group C and G) are major agents. Other rare causes include:
 - *Arcanobacterium hemolyticum*
 - *Fusobacterium necrophorum*
 - *Mycoplasma pneumoniae* (usually associated with cough)
 - *Neisseria gonorrhoeae* (due to orogenital sexual contact).
- ❖ **Viral agents:** Respiratory viruses account for majority of pharyngitis cases. Viral pharyngitis should be suspected if concurrent manifestations such as conjunctivitis, coryza, cough, skin rash, vesicles on pharynx, etc. are associated. Etiological agents include:
 - Influenza and parainfluenza viruses
 - Coronaviruses including the virus causing COVID-19
 - Epstein-Barr virus (causes infectious mononucleosis)
 - Enteroviruses: Coxsackie A virus (vesicular pharyngitis or herpangina), echoviruses, enterovirus 71
 - **Less common agents:** Adenovirus, rhinovirus, herpes simplex virus or HIV (as acute retroviral syndrome).

Clinical Manifestations

Throat (pharyngeal) pain is the earliest symptom, recognized when the person complains of difficulty in swallowing or drinking. Pharynx becomes erythematous (red) and swollen. Depending on the causative microorganism, different tissue response may be observed.

- ❖ Enlarged and inflamed tonsil, a consistent finding in children (Figs 59.1A and B)
- ❖ Inflammatory exudate—fluid with protein, inflammatory cells, and cellular debris; seen in bacterial infection such as *S. pyogenes*
- ❖ Membrane over the tonsils can be seen in diphtheria (pseudomembrane); rarely in infectious mononucleosis



Figs 59.1A and B: A. Healthy throat; B. Sore throat, showing inflamed pharynx with enlarged and inflamed tonsils and uvula.

- ❖ Vesicles (small blisters), seen in herpes
- ❖ Mucosal ulceration, or enlarged nasopharyngeal swollen lymph nodes; seen in infectious mononucleosis.

Pathogenesis

Pathogenic mechanisms differ and depend on the organism causing the pharyngitis; either toxin-mediated (*C. diphtheriae*) or through liberation of several virulence factors (*S. pyogenes*) or by direct invasion (*Arcanobacterium*)

Agents of bacterial pharyngitis are discussed in Chapter 60 and agents of viral pharyngitis are discussed in Chapter 66 (influenza), 67 (coronaviruses) and 68 (other viral agents).

Laryngitis

Acute laryngitis (inflammation of the larynx) has an abrupt onset and is usually self-limited. The patient presents with hoarseness and lowering or deepening of the voice (Fig. 59.2).

- ❖ **Viral agents:** Acute laryngitis is almost exclusively (>90%) associated with viral infections such as influenza and parainfluenza viruses, rhinoviruses, adenoviruses, coronaviruses, and human metapneumovirus
- ❖ **Bacterial agents** may rarely cause laryngitis; presence of exudate or membrane on the laryngeal mucosa—streptococcal infection, mononucleosis, or diphtheria should be suspected
- ❖ **Treatment:** Only symptomatic treatment is required; antimicrobial therapy is not indicated.

Chronic laryngitis: Occasionally, symptoms last for >3 weeks. It is most often caused by vocal abuse; less frequently caused by infections.

Acute Laryngotracheobronchitis (Croup)

Croup is a potentially more serious disease as the infection extends downward from the larynx to involve the trachea or even the bronchi.

- ❖ **Agents:** Parainfluenza viruses are the major etiologic agents. Other causes include influenza viruses, respiratory syncytial virus, adenoviruses, rhinoviruses, and enteroviruses; and a bacterial agent such as *Mycoplasma pneumoniae*
- ❖ **Age:** It commonly occurs in young children (< 3 years age)

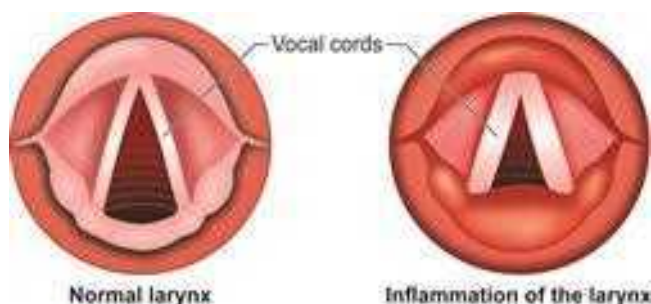


Fig. 59.2: Normal and inflamed larynx.

- ❖ **Manifestation:** Illness is characterized by variable fever, inspiratory stridor (produced by turbulent airflow through a partially obstructed larynx), hoarseness, and a *harsh barking nonproductive cough*. In young infants, severe respiratory distress and fever are common symptoms
- ❖ **Treatment:** Only symptomatic treatment is required; antimicrobial therapy is not indicated.

Epiglottitis

Epiglottitis is an infection of the epiglottis and other soft tissues above the vocal cords. It can lead to significant edema and inflammation.

- ❖ **Agents:** In contrast to laryngitis which is primarily caused by viruses, epiglottitis is usually associated with bacterial infections. *Haemophilus influenzae* type b is the primary cause of epiglottitis; although its incidence is greatly reduced because of widespread vaccination. Other commonly implicated organisms include *S. pyogenes*, pneumococcus and *S. aureus*
- ❖ **Age:** Children (2-6 years of age) are commonly infected
- ❖ **Manifestations:** Children typically present with fever, difficulty in swallowing because of pain, drooling, and respiratory obstruction with inspiratory stridor. It can be life-threatening if patient's airway gets obstructed
- ❖ **Diagnosis:** Bacteriologic culture of the epiglottis is contraindicated because swabbing of the epiglottis may lead to respiratory obstruction. Blood cultures may be performed as *H. influenzae* bacteremia usually occurs in children with epiglottitis
- ❖ **Treatment:** Cefotaxime alone, or ceftriaxone + vancomycin are the primary antibiotics given. Tracheostomy may be needed in case of respiratory obstruction.

Parapharyngeal Infections

1. Peritonsillar Abscess (or Quinsy)

It is deep neck infection that occurs as a complication of tonsillitis. It usually affects children (> 5 years age) and young adults.

- ❖ Unless promptly managed, it can spread to adjacent tissues, as well as erode into the carotid artery to cause an acute hemorrhage
- ❖ **Organisms:** The common organisms implicated are *S. pyogenes*, *S. aureus*, viridans streptococci and nonsporing anaerobe such as *Fusobacterium necrophorum*
- ❖ **Treatment:** Includes surgical drainage plus antibiotic such as piperacillin-tazobactam to cover the common etiological agents.

2. Ludwig's Angina

Ludwig's angina is a form of **diffuse cellulitis of the mandibular space** (bilateral) present in the floor of the mouth; subsequently may spread to sublingual and submental spaces. It was first described by the German physician, von Ludwig in 1836.

- ❖ **Source:** This infection most commonly arises from an adjacent **dental infection**; typically from an infected second or third molar tooth
- ❖ **Etiology:** Often polymicrobial and anaerobic; some common organisms isolated include viridans streptococci, staphylococci, *Peptostreptococcus*, *Prevotella*, *Porphyromonas* and *Fusobacterium*
- ❖ **Manifestations:** It is aggressive, rapidly spreading cellulitis, without lymphadenopathy, with potential for airway obstruction
 - Presents with bilateral lower facial swelling around the mandible and upper neck
 - Elevation of the floor of mouth may be seen due to sublingual space involvement
 - Posterior displacement of the tongue may occur, creating the potential for a compromised airway
 - Other symptoms may include painful neck swelling, tooth pain, dysphagia, shortness of breath, fever, and general malaise.
- ❖ **Treatment:** Comprises of—(i) sufficient airway management, (ii) early and aggressive antibiotic therapy, (iii) incision and drainage of localized abscesses if any, and (iv) adequate nutrition and hydration support.

Infections of Nasal Cavity and Sinuses

Rhinitis

Rhinitis (also called as common cold) is an inflammation of the nasal mucous membrane. It clinically presents as running nose, sneezing, inflammatory edema of the nasal mucosa, and watery eyes with variable fever. Rhinitis is typically associated with viral infections.

- ❖ **Viral agents** (20–25%): Rhinoviruses are the most common cause. Others include coronaviruses, influenza viruses, adenoviruses, parainfluenza, respiratory syncytial virus and enteroviruses
- ❖ **Bacterial agents** (10–15%) associated with rhinitis include *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Streptococcus pyogenes*.

Atrophic Rhinitis (or Ozena)

It is caused by *Klebsiella pneumoniae* subspecies *ozaenae*, characterized by chronic foul smelling mucopurulent nasal discharge; affecting elderly people. It is biochemically inactive organism. Ciprofloxacin or levofloxacin is given for 2 months.

Rhinoscleroma

It is caused by *Klebsiella pneumoniae* subspecies *rhinoscleromatis*.

- ❖ It is a rare form of chronic, granulomatous hypertrophy of the nose. It presents as tumor-like growth, may cause prolonged nasal obstruction. Infection may spread to the sinuses and occasionally the pharynx and larynx
- ❖ It is prevalent in Southeast Europe, India and in Central America

- ❖ **Treatment:** Ciprofloxacin or levofloxacin is given for 2–3 months.

Sinusitis

Sinusitis is characterized by inflammation of the lining of the paranasal sinuses.

Etiology

- ❖ **Acute bacterial sinusitis:** The common pathogens causing sinusitis are *S. pneumoniae* (most common, 33%), *H. influenzae* (32%), and *Moraxella catarrhalis* (9%); less commonly, *S. aureus*, anaerobes and *S. pyogenes* are implicated. *S. aureus* is a common pathogen in sphenoid sinusitis
- ❖ **Viral sinusitis:** Viruses account for 15% of sinusitis. The common URT viruses such as rhinovirus, influenza, parainfluenza and coronaviruses are the primary viral pathogens in acute sinusitis
- ❖ **Fungal sinusitis:** Allergic fungal sinusitis, though rare may be seen in infection with fungi such as *Aspergillus*, *Alternaria*, *Bipolaris* and *Curvularia*.

Clinical Manifestations

Common features are facial pressure and frontal headache, loss of smell, nasal congestion and postnasal drip. Additional findings depend upon the etiology.

- ❖ **For bacterial sinusitis:** Fever, maxillary toothache, purulent nasal discharge and unilateral facial pain. Symptoms typically last for >10 days
- ❖ **Viral sinusitis:** Improves by 7–10 days
- ❖ **For allergic rhinosinusitis:** Postnasal drip, sneezing, itchy eyes, headache, tearing, red eyes, etc.

Diagnosis

Microbiological diagnosis is very difficult. Diagnosis is often made clinically, in adjunction with radiological features.

TREATMENT	Sinusitis
Treatment in most cases is empiric, targeting the common bacterial pathogens.	
<ul style="list-style-type: none"> □ Amoxicillin ± clavulanate is the first line treatment, given for 5–7 days. Alternative drugs include clindamycin or levofloxacin □ Sinusitis are common cause of overuse of antibiotics <ul style="list-style-type: none"> ➢ Antibiotics are indicated only when specific symptoms are present for bacterial sinusitis such as fever or purulent nasal discharge ➢ When sinus symptoms are suggestive of viral or allergic; antibiotics must be avoided and wait and watch policy should be followed. 	

Laboratory Diagnosis of URTI

Specimen Collection and Transport

- ❖ **Throat swab** (oropharyngeal swab) containing fibrous exudates is the ideal specimen for pharyngitis. It should be collected by vigorous rubbing of sterile swab over the posterior pharynx and both the tonsillar pillars. Two

swabs may be collected, one for direct smear and the other for culture

- ❖ **Specimens** other than throat swabs include:
 - In suspected diphtheria, a portion of pseudo-membrane may be obtained
 - Nasopharyngeal swab is preferred for *B. pertussis* or viruses like influenza or coronavirus. It is collected by inserting flexible swab through nose into posterior nasopharynx and then rotating for 5 seconds.
- ❖ **Types of swabs:** Dacron, or Rayon swabs are suitable for collecting most URT microorganisms. Flocked swabs are preferable. Cotton swabs can be used for *S. pyogenes*, but not suitable for viruses and *B. pertussis*
- ❖ **Transport:** For isolation of most URT bacterial pathogens, swabs should be processed within 4 hours. However for molecular diagnosis (bacteria or viruses), the specimens can be stored at 4°C and can be processed late. Special transport media such as Amies or Stuart's media (charcoal based) may be preferred for *B. pertussis*.

Diagnostic Methods

- ❖ **Direct smear:** Gram staining is not useful for most of the URTI. Albert stain may be performed when diphtheria is suspected
Note: If oral thrush or Vincent's angina is suspected, Gram staining can be performed.
- ❖ **Culture:** Specimens may be inoculated onto blood agar and chocolate agar. Additional media may be used such as potassium tellurite agar (for *C. diphtheriae*), Regan-low media (for *B. pertussis*)
- ❖ **Molecular diagnosis:** This is the gold standard method for detection for respiratory viruses such as influenza or coronaviruses and others
 - Real-time PCR is available for targeting genes specific for SARS CoV-2, influenza A H1N1 and other respiratory viruses
 - BioFire FilmArray, an automated multiplex PCR is commercially available. Its respiratory panel can simultaneously detect 22 most common pathogens involved in URTI (17 viruses and 3 bacteria) in a turnaround time of 1 hour. It has an overall sensitivity and specificity of 95% and 99% respectively.
- ❖ **Antigen detection tests** may be useful for detection of certain URT pathogens
 - Rapid antigen detection test for detection of Group A carbohydrate antigen of *S. pyogenes* in throat swabs
 - Direct fluorescent antibody (DFA) staining for detection of *B. pertussis* in nasopharyngeal secretions
 - Recently a rapid chromatographic immunoassay has been developed for detection of SARS CoV-2 from nasopharyngeal swabs.
- ❖ **Antibody detection** in serum is less useful for URT pathogens. They only provide retrospective evidence of infection and are used for seroepidemiological purpose.

ORAL CAVITY INFECTIONS

Infections of the Oral Mucosa

Stomatitis

Stomatitis is an inflammation of the mucous membranes of the oral cavity. It is caused by herpes simplex virus (HSV); characterized by multiple painful tiny vesicular lesions on the oral mucosa and in oropharynx. Recurrences are common, but the lesions are less severe. HSV infection of the oral cavity is more common in immunosuppressed patients.

Oral Thrush (Oral Candidiasis)

Candida spp. can also invade the oral mucosa, especially in immunosuppressed patients (e.g. HIV). It is characterized by whitish patches of exudate (the area of inflammation) which are observed on the buccal mucosa (Chapter 58, Fig. 58.11A). It may extend to the tongue, oropharynx or esophagus; especially in HIV-infected individuals.

Dental and Periodontal Infections

Dental infections include infections of the tooth or its supporting periodontal structures.

- ❖ Dental infections most commonly occur when the bacteria invade the pulp and spread to surrounding tissues. This can occur due to trauma, dental procedures, or dental caries (cavity formation in the tooth due to destruction of its mineralized tissues; caused by viridans streptococci)
- ❖ Periodontal infections mostly result from poor or ineffective dental hygiene leading to plaque formation and subsequent inflammation of tissues around the teeth.

Etiologic Agents

Common organisms implicated in dental infections are primarily the anaerobic bacteria and viridans streptococci found in the oral cavity.

- ❖ Anaerobic bacteria: They account for up to 50% of dental infections, which include:
 - Anaerobic cocci (*Peptostreptococcus* and *Veillonella*)
 - Anaerobic gram-negative bacilli such as *Prevotella*, *Porphyromonas*, *Bacteroides fragilis*, and *Fusobacterium*.
- ❖ Viridans streptococci: *Streptococcus anginosus* group are usually found in 20 to 30% of dental infections.

Common Dental Infections

Common dental and periodontal infections include:

- ❖ **Root canal infections**, with or without periapical abscess
- ❖ **Dental (periapical) abscess:** It is common in children; occurs secondary to dental caries
 - Dental caries erode the protective layers of the tooth (i.e. enamel) which allows bacteria to invade the pulp, producing pulpitis

- Pulpitis can progress to necrosis, with bacterial invasion of the alveolar bone, causing an abscess.
- ❖ **Periodontal abscess:** It involves the supporting structures of the teeth (periodontal ligaments, alveolar bone). This is the most common dental abscess in adults, but may also occur in children
- ❖ **Spread:** Dental infections have a potential of spreading to the surrounding tissues such as gingivitis (gums), orofacial infections, osteomyelitis of the jaw, perimandibular space infections, etc.

Salivary Gland Infections

Parotitis (inflammation of the parotid gland) can be caused by both bacteria and viruses.

- ❖ **Mumps** is traditionally the most common virus to cause parotitis (Chapter 66); although the cases are significantly reduced since the advent of childhood vaccination. Influenza virus and enteroviruses may also cause parotitis
- ❖ **Acute suppurative parotitis:** It is seen among severely sick malnourished, or elderly patients
 - Characterized by painful, tender swelling of the parotid gland; purulent drainage may be evident at the opening of the duct (of the gland) in the mouth
 - *S. aureus* is the major pathogen but occasionally Enterobacteriaceae, other gram-negative bacilli, and oral anaerobes may also be implicated.
- ❖ A **chronic bacterial parotitis** has been described involving *Staphylococcus aureus*.

Vincent's Angina

It is an acute necrotizing ulcerative gingivitis, also called as Trench mouth or Vincent's stomatitis.

- ❖ **Clinical manifestations:** It presents as gingivitis with sudden onset of painful bleeding gums, foul breath, and a bad taste
 - The gingival mucosa becomes ulcerated and may be covered by gray "**pseudomembrane**," resembling diphtheria, but peels off easily
 - Patients may become systemically ill, mortality is high if not treated.
- ❖ **Agents:** Organisms such as *Prevotella*, *Borrelia vincentii* and *Fusobacterium* species have been implicated. They are normal flora in the mouth
- ❖ **Diagnosis:** Demonstration of spirochetes and fusiform bacilli in stained smears of exudate from the lesion remains the mainstay of diagnosis
- ❖ **Treatment** consists of debridement and antibiotics (metronidazole plus penicillin).

LOWER RESPIRATORY TRACT INFECTIONS

Various lower respiratory tract infections (LRTI) include bronchitis, bronchiolitis, whooping cough, pneumonia, tuberculosis, fungal and parasitic lung disease, lung abscess, pleural effusion, and empyema (Table 59.1).

Bronchitis

Acute bronchitis is defined as self-limited inflammation of the bronchial tree due to infections. Most infections occur during the winter.

- ❖ **Etiology:** Most commonly caused by respiratory viruses (20–50%) such as RSV, influenza and coronavirus. Bacterial causes include *Mycoplasma pneumoniae* (5%), *Chlamydophila pneumoniae* (5%) and *Bordetella pertussis*
- ❖ **Manifestations:** Acute bronchitis presents with persisting cough, variable fever, and sputum production. The sputum is often clear at the onset but may become purulent as the illness persists.

Chronic bronchitis: It is clinically defined as cough with expectoration for at least 3 months/ year during a period of 2 consecutive years. It usually affects adults.

- ❖ In contrast to acute bronchitis (which typically has an infectious etiology) in chronic bronchitis, there occurs chronic irritation of bronchial mucosa due to factors such as cigarette smoking
- ❖ **Smokers with underlying COPD** (chronic obstructive pulmonary disease) may suffer from *acute bacterial exacerbation of chronic bronchitis*—characterized by increased dyspnea, increased sputum viscosity/purulence, and increased sputum volume.

TREATMENT

Bronchitis

- ❑ **Acute bronchitis:** Antibiotics are not effective and are not indicated except when bacterial cause is suspected (e.g. pertussis). Symptomatic treatment is the mainstay of treatment such as antitussive ± inhaled bronchodilators.
- ❑ **Chronic bronchitis:** Antibiotic therapy is recommended only if presented with acute bacterial exacerbations. Amoxicillin or azithromycin is given for mild cases, whereas for hospitalized patients with severe disease, anti-pseudomonas coverage is also given such as levofloxacin, cefepime or piperacillin-tazobactam.

Bronchiolitis

Bronchiolitis (inflammation of bronchioles) is almost always caused by respiratory viruses. It usually affects infants and young children; and most commonly during winter.

- ❖ **Agents:** Respiratory syncytial virus (RSV) is the most important etiology; others include influenza virus, human metapneumovirus, parainfluenza viruses, adenovirus, rhinovirus and coronavirus
- ❖ **Manifestations:** Characterized by respiratory distress with expiratory wheeze, tachypnea, nasal flaring, retractions, and irritability.

TREATMENT

Bronchiolitis

Mainstay of therapy is symptomatic management such as hydration, oxygen supplement and suctioning to clear the airway. Antibiotics should not be used for bronchiolitis unless there is clear evidence of a secondary bacterial infection.

Whooping Cough (Pertussis)

It is a respiratory disease, caused by a gram-negative bacterium *Bordetella pertussis*; clinically characterized by paroxysmal cough. Each paroxysm consists of bursts of 5–10 repetitive violent spasmodic coughs, often within a single expiration which ends with an audible sound or whoop (details described in Chapter 64).

Pneumonia

Pneumonia refers to infection of the pulmonary parenchyma; which can be classified into: (1) community acquired—patients acquire infection in the community, (2) hospital acquired—patients acquire infection in the healthcare facility.

Community-acquired Pneumonia (CAP)

The etiology of CAP is different than that of HAP (Table 59.2); which vary depending upon the comorbidities present (Table 59.3).

- ❖ *Streptococcus pneumoniae* followed by *Mycoplasma pneumoniae* are the most common agents of CAP
- ❖ Others include *H. influenzae*, *Chlamydomphila pneumoniae* and viral pneumonia.

Pathogenesis

Pathogenesis of CAP depends upon two factors: (i) microbial pathogens that gain access to the lungs, and (ii) host's response to those pathogens.

Microbial pathogens gain access to the lungs

1. Microorganisms gain access to the lower respiratory tract in several ways—
 - By aspiration from the oropharynx (most common): It occurs frequently during sleep (especially in the elderly), or when consciousness is lowered

Table 59.2: Agents of community-acquired and healthcare-associated pneumonia.	
Community-acquired pneumonia (CAP) ¹	Healthcare-acquired pneumonia (HAP) ^{1,2}
Bacterial pneumonia: Includes <i>Streptococcus pneumoniae</i> , <i>Mycoplasma pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Chlamydomphila pneumoniae</i> Viral pneumonia: Includes influenza, coronaviruses (including COVID-19), respiratory syncytial virus, parainfluenza, adenoviruses	Multidrug resistant (MDR) pathogens found in the hospital environment <ul style="list-style-type: none"> • <i>Pseudomonas</i> • <i>Acinetobacter</i> • <i>Escherichia coli</i> • <i>Klebsiella pneumoniae</i> • MRSA (methicillin resistant <i>S. aureus</i>)

Note:

¹The **distinction between CAP and HAP is vague**; many individuals presenting to outpatient department with pneumonia have been found to be infected with the MDR-pathogens. Factors responsible for this phenomenon include the widespread use of antibiotics in the community, earlier discharge of patients from hospitals to their homes, increased use of outpatient IV antibiotic therapy.

²VAP (ventilator-associated pneumonia) accounts for majority of HAP.

Table 59.3: Co-morbidities of community-acquired pneumonia and possible etiological agents.

Factors	Possible etiological agents
Alcoholism	Pneumococcus, anaerobes, coliforms
COPD and/or smoking	<i>Haemophilus influenzae</i> , <i>Moraxella catarrhalis</i> , pneumococcus, <i>Legionella</i>
Post CVA-aspiration	Oral flora, pneumococcus
Post-obstruction of bronchi	Pneumococcus, anaerobes
Post-influenza	Pneumococcus, <i>S. aureus</i>
Neutropenia, low immunity	<i>P. aeruginosa</i>
Injection drug use	<i>Staphylococcus aureus</i>
Structural lung disease (e.g. bronchiectasis)	<i>P. aeruginosa</i> , <i>Burkholderia cepacia</i> , <i>S. aureus</i>
Decreased consciousness	Oral anaerobes, gram-negative enteric bacteria

Abbreviations: CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease.

- Inhalation of pathogens through infected droplets
 - Via hematogenous spread, e.g. from tricuspid endocarditis (rare)
 - Contiguous extension from an infected pleural or mediastinal space (rare)
2. Microorganisms escape the defense mechanisms of the host's respiratory tract (as described at the beginning of this chapter, e.g. mucociliary clearance).

Host immune response

Once the microorganisms reach lungs, they are engulfed by the resident alveolar macrophages and subsequently are either killed or eliminated by the mucociliary escalator, or through the lymphatics.

- ❖ Only when the capacity of the alveolar macrophages to ingest or kill the microorganisms exceeds, clinical pneumonia manifests
- ❖ In that situation, the inflammatory response is initiated by the alveolar macrophages rather than preventing the proliferation of microorganisms, triggers the clinical syndrome of pneumonia. They release a cascade of cytokines, which act as inflammatory mediators causing host tissue damage (**cytokine storm**)
 - Interleukin (IL)-1 and tumor necrosis factor (TNF) induce fever
 - Chemokines, such as IL-8 and granulocyte colony-stimulating factor, stimulate the release of neutrophils.
- ❖ Subsequently the inflammatory mediators create an alveolar capillary leak, as seen in the acute respiratory distress syndrome (ARDS). The capillary leak results in a radiographic infiltrate, rales are detectable on auscultation, and hypoxemia results from alveolar filling.

Hospital-acquired Pneumonia (HAP)

Hospitalized patients are at increased risk of developing pneumonia; majority of which are attributed to the presence of ventilator.

- ❖ The normal respiratory flora of hospitalized patient gets quickly replaced by multidrug resistant gram-negative organisms present in the hospital environment, such as *Pseudomonas*, *Acinetobacter*, etc.
- ❖ Endotracheal intubation damages the respiratory epithelium, and thus helps oropharyngeal bacteria to gain access directly into the lower respiratory tract.

Ventilator-associated pneumonia is a major healthcare associated infection, has been discussed in detail in Chapter 22.

Clinical Manifestations of Pneumonia

Based on area of lungs involved, and the type of cough produced, pneumonia can be traditionally grouped into typical and atypical pneumonia.

Lobar (typical) pneumonia: It refers to infection of lung parenchyma (alveoli). It is characterized by consolidation radiologically, which gives a dull note on percussion and productive cough with purulent sputum (Fig. 59.3). It is mostly caused by pyogenic organisms such as:

- ❖ *Pneumococcus* (most common agent)
- ❖ *Haemophilus influenzae*
- ❖ *Staphylococcus aureus*
- ❖ Gram-negative bacilli such *Klebsiella pneumoniae*.

Interstitial or atypical pneumonia: It refers to infection of interstitial space of lungs (Fig. 59.4). Cough is characteristically non-productive. Radiologically, it presents as patchy reticulonodular opacities (chest X-ray) and ground glass opacities (CT scan). It is mostly caused by organisms such as:

- ❖ *Chlamydomphila pneumoniae*
- ❖ *Mycoplasma pneumoniae*
- ❖ Viral agents causing pneumonia
- ❖ Fungal agents causing pneumonia
- ❖ *Legionella* species.

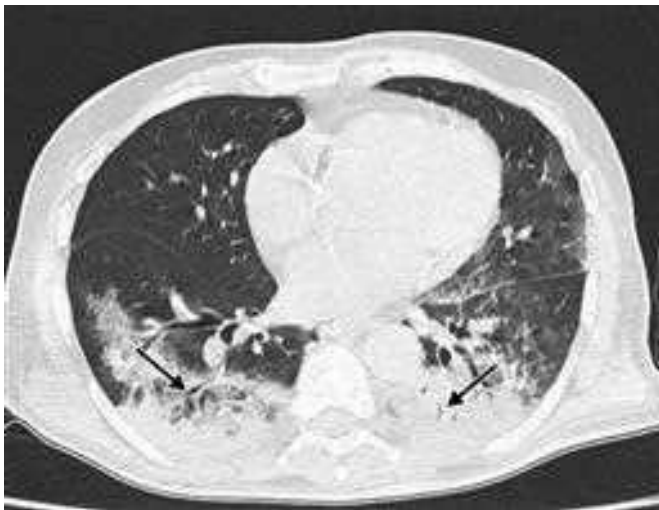


Fig. 59.3: CT scan depicting lobar pneumonia, with consolidation in both the lower lobes.

Source: Dr Sunitha V, Department of Radiology, JIPMER, Puducherry (with permission).

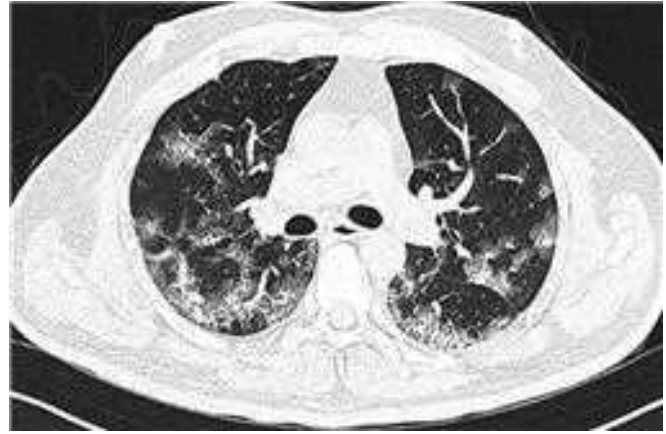


Fig. 59.4: A case of COVID-19 pneumonia with CT scan lungs showing ground-glass opacity seen in both lungs, predominantly subpleural in distribution. Ground-glass opacity is a feature of interstitial pneumonia.

Source: Dr Sunitha V, Department of Radiology, JIPMER, Puducherry (with permission).

However, there is considerable overlapping. The typical agents can be atypical in presentation and vice-versa.

Clinical Features

Clinical features which may be found in both types of pneumonia include:

- ❖ Fever, with chills and/or sweats, tachycardia
- ❖ Increased respiratory rate (tachypnea), with increased use of accessory muscles of respiration
- ❖ Dyspnea (shortness of breath)
- ❖ If the pleura is involved, the patient may experience pleuritic chest pain
- ❖ Gastrointestinal symptoms: may be seen up to 20% of patients such as nausea, vomiting, and/or diarrhea
- ❖ Other symptoms may include fatigue, headache, myalgia, and arthralgia
- ❖ Severely ill patients may develop septic shock and multi-organ failure.

TREATMENT

Community-acquired pneumonia

Empiric regimen differs for inpatients and outpatients. Hospitalization in CAP is determined based on CURB-65 scoring system (Table 59.4).

The prognosis of CAP can be predicted by a scoring system called CURB-65. If CURB-65 score is >1, prognosis is poor and requires hospitalization.

CAP, hospitalized (if CURB65 score >1):

- ❑ IV ceftriaxone plus azithromycin *or*
- ❑ IV levofloxacin (not recommended for severe CAP)
- ❑ Add vancomycin if CA-MRSA is suspected
- ❑ Add piperacillin-tazobactam, if *Pseudomonas* is suspected

CAP, outpatient (if CURB-65 score ≤1):

For outpatients, empirical regimen is dependent on presence upon comorbidities.

- ❑ If no comorbidity present: Oral amoxicillin or doxycycline is given

Contd...

Table 59.4: Prediction of prognosis in community-acquired pneumonia (CAP).

The prognosis of CAP can be predicted by a scoring system called CURB-65.

- **C** (confusion of new onset) = 1 point
 - **U** (blood urea nitrogen >19 mg/dL) = 1 point
 - **R** (respiratory rate >30 min) = 1 point
 - **B** (BP <90/60) = 1 point
 - 65 (Age ≥65 years) = 1 point
- Higher the score, greater is the mortality
- If the score ≤1, outpatient therapy is indicated
 - If the score >1, patient should be hospitalized

Contd...

TREATMENT Community-acquired pneumonia

- If comorbidity present (e.g. chronic heart, lung, liver or renal diseases, diabetes, alcohol use disorder, neoplastic disease, asplenia):
 - Oral amoxicillin-clavulanate + Azithromycin *or*
 - Oral levofloxacin.

Duration of treatment: 5-7 days (guided by clinical stability such as afebrile >48h, normalization of vital signs).

Rare Etiological Agents of Pneumonia

Fungal Pneumonia

Fungal pneumonia is uncommon, but occurs more commonly in individuals with weakened immune systems due to HIV/AIDS, immunosuppressive drugs, etc. (Chapter 69).

- ❖ It is most often caused by *Pneumocystis jirovecii* (causes pneumocystis pneumonia or PCP) or due to *Aspergillus*
- ❖ Fungal agents causing systemic mycoses; include *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*
- ❖ Travel history gives an important clue about the disease; histoplasmosis is common in the Mississippi river basin and coccidioidomycosis is common in the Southwestern USA.

Rare Bacterial Agents Causing Pneumonia

Bacterial agents that rarely cause pneumonia include

- ❖ *Burkholderia pseudomallei*: Causes melioidosis
- ❖ *Coxiella burnetii*: Causes Q fever
- ❖ *Chlamydophila psittaci*: Patients gives history of exposure to birds
- ❖ *Chlamydia trachomatis* serotype D-K: Causes infant pneumonia
- ❖ *Actinomyces* and *Nocardia*
- ❖ *Bacillus anthracis*: Causes pulmonary anthrax or Wool Sorter's disease
- ❖ *Yersinia pestis*: Causes pneumonic plague.

Parasitic Lung Disease

Parasitic agents that infect lungs (Chapter 69) include:

- ❖ *Paragonimus westermani*: Causes endemic hemoptysis

- ❖ *Ascaris lumbricoides*: Causes Loeffler's pneumonia
- ❖ Filarial nematodes: Causes hypersensitivity in lungs, called tropical pulmonary eosinophilia (TPE)
- ❖ Parasites that infect elsewhere, rarely infect lungs such as *E. histolytica*, *Cryptosporidium* and *Echinococcus*, etc.

Tuberculosis

Tuberculosis (TB), one of the greatest killer of mankind, is an infectious disease caused by an acid-fast bacterium *Mycobacterium tuberculosis* (discussed in Chapter 63).

- ❖ It spreads from an infected person by aerosol transmission
- ❖ Although one-quarter of the world's population has been infected, most infections lead to latent tuberculosis—i.e. they do not develop the disease and are not capable of transmission
- ❖ About 5–15% of latent infections (especially who are immunocompromised) progress to active disease which, if left untreated, kills about half of those affected
- ❖ Every year about 100 lakh cases of TB occur worldwide with nearly 15 lakh deaths
- ❖ TB generally affects the lungs (pulmonary TB), but can also affect other parts of the body (extrapulmonary TB)
- ❖ The classical symptoms of active **pulmonary TB** are chronic cough with blood-containing mucus, fever, night sweats, and weight loss; which last long (for months)
- ❖ Early diagnosis and institution of appropriate anti-tubercular therapy is the key to successful outcome.

Lung Abscess

Lung abscess represents necrosis and cavitation of the lung following microbial infection. It usually presents as a single cavity of >2 cm size.

Etiology

Lung abscess can present in two forms: primary or secondary.

- ❖ **Primary lung abscess:** It accounts for ~80% of cases. It generally results from aspiration, mainly caused by anaerobic bacteria (or microaerophilic streptococci) present in the oral cavity. It usually occurs in the absence of an underlying pulmonary or systemic condition
- ❖ **Secondary lung abscess** arises in the setting of an underlying condition, such as a bronchial foreign body or tumor or immunocompromised condition such as HIV. Common organisms implicated are *S. aureus*, gram-negative rods (e.g. *Pseudomonas*, Enterobacteriaceae), *Nocardia*, *Legionella* and fungi such as *Aspergillus*, Mucorales, *Cryptococcus*, and *Pneumocystis jirovecii*.

Clinical Manifestations

Initially, lung abscess presents similar to that of pneumonia, with fever, productive cough, and chest pain.

- ❖ In anaerobic etiology, progression is slow and indolent; with features of night sweats, fatigue, and occasionally foul-smelling sputum (in case of putrid lung abscess)
- ❖ In cases with non-anaerobic etiology such as *S. aureus*, the course is more fulminant with rapid progression.

TREATMENT

Lung abscess

- ❑ In primary lung abscesses, clindamycin is the first line antibiotic of choice
- ❑ In secondary lung abscesses, antibiotic coverage should be pathogen-directed, based on culture susceptibility report. A prolonged course is often required until resolution of the abscess.

Pleural Effusion and Empyema

Pleural effusion refers to the presence of an excess quantity of fluid in the pleural space; an area which lies between the lung and the chest wall. There are various causes of pleural effusion; infection, malignancy, heart failure, cirrhosis and pulmonary embolism, etc. Our discussion will be limited to infection-related pleural effusion; which may occur secondary to bacterial pneumonia, tuberculosis or viral infection.

- ❖ **Parapneumonic pleural effusion:** It occurs secondary to bacterial pneumonia (more common), lung abscess, or bronchiectasis. When pleural effusion becomes grossly purulent, it is called as *empyema*
 - Patients present with an acute febrile illness consisting of chest pain, sputum production, and dull note observed on percussion
 - Presence of excess pleural fluid can be demonstrated with radiograph, or CT scan. If free fluid separates the lung from the chest wall by >10 mm, a therapeutic thoracentesis (drainage of pleural effusion) should be performed.
- ❖ **Tuberculous pleuritis:** It usually occurs in primary TB and is primarily due to a hypersensitivity reaction to tuberculous protein in the pleural space
 - Patients present with fever, weight loss, dyspnea, and/or pleuritic chest pain
 - Elevated TB markers in the pleural fluid such as adenosine deaminase or interferon.
- ❖ **Viral pleural effusion:** Pleural effusion secondary to viral infections is less common, usually gets undiagnosed. These effusions resolve spontaneously with no long-term residua.

Laboratory Diagnosis of LRTI

Specimen Collection

Important specimens for LRTI include sputum, induced sputum, tracheal aspirate, bronchoalveolar lavage (BAL), protected specimen brush (PSB), lung aspirate (collected by transtracheal aspiration) and pleural fluid (collected by thoracentesis)

Microscopy

- ❖ **Gram staining** of the sputum or other specimens is done for two purposes
 1. *Sputum quality:* If pus cells are >25 and epithelial cells are <10 per low power field, such samples are regarded as good quality sputum, where the chance of recovery of the pathogen is more
 2. *Presumptive identification:* Gram stain gives a preliminary clue about the etiological agent based on their morphology; for example—
 - ♦ Gram-positive cocci, pair, lanceolate shaped—suggestive of pneumococcus
 - ♦ Pleomorphic gram-negative coccobacilli—suggestive of *Haemophilus influenzae*.
- ❖ **Acid fast staining** of sputum by Ziehl-Neelsen technique is performed to demonstrate the acid-fast bacilli, e.g. *M. tuberculosis*
- ❖ **GMS stain** (Gomori methenamine silver stain) is used to demonstrate *Pneumocystis jirovecii*.

Culture

Specimens should be collected before antibiotic therapy for better yield of organisms.

- ❖ **Sputum culture** is a standard culture method for pneumonia. Only the good quality sputum specimens as revealed by Gram stain should be subjected to culture
 - Specimens are inoculated onto blood agar, chocolate agar and MacConkey agar and incubated overnight
 - The yield of positive cultures from sputum samples is variable ($\leq 50\%$).
- ❖ **Blood culture** may also be performed in addition especially in hospitalized patients, although its yield is low (~5–14% of CAP)
- ❖ **For lung abscess:** When a primary lung abscess is suspected, lung aspirate is subjected for anaerobic culture. Sputum is not a suitable specimen for anaerobic culture. However for secondary lung abscess, sputum and blood cultures can be performed
- ❖ **For *M. tuberculosis*:** Specimens should be inoculated onto LJ medium or automated MGIT (Mycobacteria growth indicator tube) culture
- ❖ **For fungal pathogen isolation:** Sabouraud dextrose agar is used.

Serology (Antibody Detection)

Antibody detection tests can be used for diagnosis of atypical pneumonia pathogens such as *Mycoplasma*, *Chlamydia*, *Coxiella burnetii* and viruses.

- ❖ A fourfold rise in specific IgM antibody titer between acute- and convalescent-phase serum samples is generally considered diagnostics
- ❖ They are less popular in recent days because of the time required to obtain a final result for the convalescent-phase sample.

The various antibody detection methods available are:

- ❖ **Mycoplasma:** Cold agglutination test, and ELISA formats are available
- ❖ **Chlamydial antibodies:** Micro-immunofluorescence test can be performed
- ❖ **Q fever (*Coxiella burnetii*):** Indirect immunofluorescence assay (IFA) is available
- ❖ **Viral infections:** Antibody detection methods are available for influenza or COVID-19. However, antibodies usually appear late, therefore not of much use in clinical diagnosis, although useful for estimating seroprevalence.

Antigen Detection Tests

Various antigen detection methods available for lower respiratory tract pathogens include:

- ❖ **Immunochromatographic test (ICT)** is available for detection of pneumococcal antigens in urine
- ❖ **Enzyme immunoassay** is available to detect *L. pneumophila* serogroup 1 specific soluble antigens in urine
- ❖ **Direct fluorescent antibody tests** are available for influenza virus and respiratory syncytial virus, and *B. pertussis*, but are poorly sensitive and technically challenging.

Molecular Test

Molecular methods are emerging as the most promising tool for diagnosis of LRTI.

- ❖ **Multiplex PCR assays** are available where multiple primers targeting the genes specific for the common suspected agents of LRTI are used
- ❖ **BioFire FilmArray** is an automated multiplex PCR commercially available
 - Its pneumonia panel can simultaneously detect 33 pneumonia pathogens; including both bacterial and viral agents from sputum or BAL specimen with a turnaround time of 1 hour
 - For common bacterial pathogens which are usually the colonizers in the respiratory tract; it gives semi-quantitative results (genome copies/mL), therefore helps in differentiating colonization and disease.
- ❖ **Real time PCR** is the gold standard diagnostic method for detection of the agents of viral pneumonia such as influenza and SARS-CoV-2
- ❖ **Automated Real time PCR** such as GeneXpert and Truenat can be used for identification of *M. tuberculosis* in sputum. In addition, it also detects rifampicin resistance.

EXPECTED QUESTIONS

I. Write essay on:

1. A 16-month-old boy was admitted with fever, lethargy, productive cough with purulent sputum and shortness of breath. On examination, dull note on percussion and consolidation on auscultation were noted. Sputum and blood specimens were obtained, and sent for bacteriological culture.
 - a. What is the clinical diagnosis and the etiological agents?

- b. Discuss the pathogenesis, clinical presentation, laboratory diagnosis and treatment of this clinical condition.

II. Write short notes on:

1. Acute laryngotracheobronchitis (Croup).
2. Vincent's angina.
3. Pleural effusion.

CHAPTER PREVIEW

- Streptococcal Pharyngitis
- Diphtheria
- Rare Causes of Bacterial Pharyngitis

INTRODUCTION

Pharyngitis (or sore throat) is one of the most common upper respiratory tract infections (URTI). Viral pharyngitis accounts for the vast majority of cases, and is usually self-limited. Bacteria are also important etiologic agents of pharyngitis, require specific antibiotic treatment; if not given, can lead to serious complications and sequelae. The common etiological agents of bacterial pharyngitis include:

- ❖ *Streptococcus pyogenes* (most common)
- ❖ *Corynebacterium diphtheriae*
- ❖ Other rare causes include:
 - Other β -hemolytic streptococci (group C and G)
 - *Arcanobacterium hemolyticum*
 - *Fusobacterium necrophorum*
 - *Mycoplasma pneumoniae*
 - *Neisseria gonorrhoeae*.

STREPTOCOCCAL PHARYNGITIS

Streptococcus pyogenes (or group A *Streptococcus*) is the most common bacterial cause of pharyngitis in children and accounts for 5 to 15% of all sore throats in adults. Infection occurs through respiratory droplets. In addition, it causes skin and soft tissue infections (Chapter 52).

Clinical Manifestations

Streptococcal sore throat presents as either localized (tonsillitis) or diffuse (pharyngitis).

- ❖ **Symptoms:** Manifests as erythema and swelling of pharyngeal mucosa with purulent exudate formation (Fig. 60.1)
- ❖ **Younger children (<3 years)** present with a syndrome of fever, malaise, and lymphadenopathy without exudative pharyngitis
- ❖ **Suppurative complications:** May occur due to—(1) direct extension of infection from the pharynx to deeper tissues causing peritonsillar abscess (or quinsy) and retropharyngeal abscess, (2) very rarely downward spread to the respiratory tract causing pneumonia or empyema, (3) hematogenous spread leading to bacteremia and meningitis



Fig. 60.1: Streptococcal sore throat, with enlarged tonsils covered with white fibrinous exudate.

Source: James Heilman, Wikipedia (with permission).

- ❖ **Non-suppurative complications** may develop 2–3 weeks following streptococcal sore throat, mediated by autoimmune mechanism
 - **ARF:** Acute rheumatic fever (ARF) is the most common non-suppurative complication developed following streptococcal pharyngitis; involves cardiac valves, joints and other sites (Chapter 28)
 - **PSGN:** Post streptococcal glomerulonephritis (PSGN) is another sequela that usually develops following streptococcal pyoderma, but can rarely occur following streptococcal sore throat (Chapter 76).
- ❖ **Differential diagnosis** of streptococcal sore throat include:
 - *Diphtheria:* It can be differentiated from streptococcal pharyngitis by presence of membrane over the tonsil
 - *Viral pharyngitis:* It is differentiated from streptococcal pharyngitis by presence of concomitant rhinorrhea, oral ulcers, cough and/or hoarseness.
- ❖ **Scarlet fever:** It is mediated by streptococcal pyrogenic exotoxins (e.g. SPE-A, B, and C)

- It is characterized by pharyngitis and rashes (with sandpaper feel), strawberry tongue (enlarged papillae on a coated tongue). Rashes in the skin folds are called Pastia's lines
- Scarlet fever has become less common now, although strains producing SPE continue to be prevalent in the community. Reasons are not clear.

Laboratory Diagnosis

Culture

Throat (oropharyngeal) swabs should be collected by vigorous rubbing of a sterile cotton swab over both tonsillar pillars. Two swabs may be collected, one for direct smear and the other for culture.

- ❖ **Culture:** On blood agar, *S. pyogenes* forms small pinpoint colonies of 0.5–1 mm size with a wide zone of β -hemolysis (Chapter 52, Fig. 52.5A)
- ❖ **Culture smear:** Gram stained smear from the colonies show gram-positive spherical cocci (0.5–1 μm), arranged in short chains (Chapter 52, Fig. 52.4B)
- ❖ **Identification:** *S. pyogenes* is identified by conventional biochemical tests (catalase negative and susceptible to bacitracin) or by automated identification systems such as MALDI-TOF or VITEK.

Rapid Antigen Detection Test (RADT)

RADT is available commercially, which detects Group A carbohydrate antigens from throat swab by latex agglutination or enzyme immunoassay for the diagnosis of pharyngitis.

- ❖ As it is highly specific (>95%), a positive result can be relied upon for definitive diagnosis and eliminates the need for throat culture
- ❖ However, the sensitivity of this test is variable (55–90%), therefore a negative result should always be confirmed by throat culture.

ASO Titer

Anti-streptolysin O (ASO) antibodies appear late; therefore, a retrospective diagnosis of streptococcal infection may be

established by detecting ASO antibodies in patient's serum. This may be useful for establishing rheumatic fever.

Molecular Test

Commercial PCR assay is available for the detection of *S. pyogenes* in throat swab. It is a rapid point of care test (turnaround time of 15 minutes), claims to be more sensitive than antigen detection test and therefore if it is negative, eliminates the need for culture.

TREATMENT

Streptococcal pharyngitis

- ❑ **For pharyngitis:** Benzathine penicillin G, IM single dose or oral penicillin V for 10 days is recommended
- ❑ **For recurrent pharyngitis:** Clindamycin or amoxicillin-clavulanate is recommended
- ❑ **For concomitant pneumonia and empyema:** Penicillin G + drainage of empyema is recommended.

DIPHTHERIA

Introduction

Diphtheria is a highly infectious childhood disease caused by the bacterium *Corynebacterium diphtheriae*, which primarily infects the throat and produces toxin (diphtheria toxin) that causes an exudative pharyngitis and membranous tonsillitis. If not promptly treated, it can be life-threatening.

- ❖ The genus *Corynebacterium* is a gram-positive, non-capsulated, non-sporing, non-motile bacillus. It is irregularly stained and frequently shows **club-shaped swellings** (Fig. 60.2A) (Greek word *koryne*, meaning club)
- ❖ It has several species; the most important pathogenic being *C. diphtheriae*. The other *Corynebacterium* species (called as diphtheroids) are usually found as commensals of throat and skin in man, can occasionally cause infections
- ❖ *Corynebacterium diphtheriae* typically shows two characteristic features, which differentiates it from other *Corynebacterium* species



Figs 60.2A to C: *Corynebacterium diphtheriae*: **A.** Club-shaped bacilli in methylene blue-stained smear; **B.** Gram-stained smear shows V- or L-shaped bacilli with cuneiform arrangement; **C.** Albert's stain shows dark blue metachromatic granules at the ends of the green bacilli (schematic).

Source: Public Health Image Library, A. ID# /7323/PB. Smith; B. ID# /1943, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

1. **Chinese letter or cuneiform arrangement:** They appear as V- or L-shaped in smear, because the bacterial cells divide and daughter cells tend to lie at acute angles to each other. This type of cell division is called **snapping type of division** (Fig. 60.2B)
2. **Metachromatic granules:** They are present at ends or poles of the bacilli.

Metachromatic Granules

Also called polar bodies or Babes–Ernst bodies or volutin granules).

- ❑ They are storage granules of the organism, composed of polymetaphosphates
- ❑ Granules are stained strongly gram-positive compared to remaining part of the bacilli. The granules take up bluish purple metachromatic color when stained with Loeffler's methylene blue
- ❑ However, they are better stained with special stains, such as Albert's, Neisser's and Ponder's stain (Fig. 60.2C)
- ❑ Granules are well developed on enriched media, such as blood agar or Loeffler's serum slope
- ❑ Volutin granules can also be possessed by other organisms such as—by *Corynebacterium xerosis* and *Gardnerella vaginalis*.

History

Diphtheria is an ancient disease, known since the time of Hippocrates.

- ❖ Diphtheria was first recognized by Pierre Bretonneau (1826). He used the term diphthérite (Greek word *diphtheros*—meaning leather like) to describe the characteristic manifestation, i.e. leathery pseudomembrane formation over the tonsil
- ❖ *C. diphtheriae* was first observed by Klebs (1883) and first cultivated by Loeffler (1884); hence, it is known as **Klebs-Loeffler bacillus**.

Virulence Factors (Diphtheria Toxin)

Diphtheria toxin (DT) is the primary virulence factor responsible for diphtheria.

- ❖ Toxin is a polypeptide chain, comprises of two fragments—A (active) and B (binding)
- ❖ Fragment B binds to the host cell receptors (such as epidermal growth factor) and helps in entry of fragment A
- ❖ Fragment A gets internalized into the cell and then acts by the mechanism given below.

Mechanism of Diphtheria Toxin (DT)

- ❑ Fragment A is the active fragment, which causes ADP ribosylation of elongation factor 2 (EF-2) → leads to inhibition of EF-2 → leads to inhibition of translation step of protein synthesis
- ❑ Exotoxin A of *Pseudomonas* has a similar mechanism like that of DT.

Factors Regulating Toxin Production

The production of diphtheria toxin is dependent on various factors.

- ❖ **Phage coded:** DT is coded by a bacteriophage called β -corynephage, carrying *tox* gene. *C. diphtheriae* remains toxigenic as long as the phages are present inside the bacilli (lysogenic conversion)
- ❖ **Iron concentration:** The toxin production depends on the optimum concentration of iron (0.1 mg per liter)
- ❖ **Other species:** DT is also produced by *C. ulcerans* and *C. pseudotuberculosis*.

Toxoid is used for Vaccination

Diphtheria toxin is antigenic and antitoxins are protective in nature. However, as it is virulent, it cannot be given directly for vaccination.

- ❖ Toxin can be converted to toxoid, which is used for vaccination. Toxoid is a form of toxin, where the virulence is lost, retaining its antigenicity
- ❖ Toxoid formation is promoted by formalin, acidic pH and prolonged storage
- ❖ **Park William 8** strain of *C. diphtheriae* is used as a source of toxin for the preparation of vaccine
- ❖ **LF unit:** DT is expressed as Limit of flocculation (Lf) unit. 1 Lf unit is the amount of toxin which flocculates most rapidly with one unit of antitoxin.

Pathogenicity and Clinical Manifestations

Pathogenesis of diphtheria is toxin mediated.

- ❖ Diphtheria is toxemia but never a bacteremia
- ❖ Bacilli are noninvasive, present only at local site (pharynx), secrete the toxin which spreads via bloodstream to various organs
- ❖ It is the toxin which is responsible for all types of manifestations including local (respiratory) and systemic complications (except the skin lesions, which is caused due to the organism, not toxin).

Respiratory Diphtheria

This is the most common form of diphtheria. Tonsil and pharynx (faucial diphtheria) are the most common sites followed by nose and larynx. Incubation period is about 3–4 days.

- ❖ **Faucial diphtheria:** Diphtheria toxin elicits an inflammatory response, that leads to necrosis of the epithelium and exudate formation
 - This leads to formation of mucosal ulcers, lined by a tough leathery greyish white **pseudomembrane coat**; composed of an inner band of fibrin surrounded by neutrophils, RBCs and bacteria (Fig. 60.3A)
 - It is so named as it is adherent to the mucosal base and bleeds on removal, in contrast to the true membrane which can be easily separated.



Figs 60.3A and B: **A.** Pseudomembrane covering the tonsils classically seen in diphtheria; **B.** Bull neck appearance (arrow showing).

Source: A. Department of Microbiology, JIPMER, Puducherry; B. Public Health Image Library/ID#5325, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Extension of pseudomembrane:** In severe cases, it may extend into the larynx and bronchial airways, which may result in fatal airway obstruction leading to asphyxia. This mandates immediate tracheostomy
- ❖ **Bull-neck appearance:** It is characterized by massive tonsillar swelling and neck edema. Patients present with foul breath, thick speech, and stridor (noisy breathing) (Fig. 60.3B).

Cutaneous Diphtheria

It presents as punched-out ulcerative lesions with necrosis, or rarely pseudomembrane formation; most commonly occurs on the extremities.

- ❖ Cutaneous diphtheria is due to the organism itself and is not toxin-mediated. Hence, it is possible that, the skin lesions may also be caused by non-toxigenic strains
- ❖ There is increasing trend of cutaneous diphtheria nowadays, especially in vaccinated children; because antitoxins present in vaccinated people cannot prevent the disease.

Systemic Complications

Polyneuropathy and myocarditis are the late toxic manifestations of diphtheria, occurring after weeks of infection. Other complications of diphtheria include pneumonia, renal failure, encephalitis, cerebral infarction, and pulmonary embolism.

- ❖ **Neurologic manifestations:** It is a toxin mediated non-inflammatory demyelinating disorder; presented with:
 - Cranial nerve involvement
 - Peripheral neuropathy
 - Ciliary paralysis.
- ❖ **Myocarditis:** It is typically associated with arrhythmias and dilated cardiomyopathy.

LABORATORY DIAGNOSIS

Diphtheria

Isolation of the *Corynebacterium diphtheriae*

- ❑ **Specimen:** Throat swab and a portion of pseudomembrane
- ❑ **Direct smear**
 - Gram stain: Club shaped gram-positive bacilli with Chinese letter arrangement
 - Albert's stain: Green bacilli with bluish black metachromatic granules
- ❑ **Culture media**
 - Enriched medium: Blood agar, chocolate agar and Loeffler's serum slope
 - Selective medium: Potassium tellurite agar and Tinsdale medium, produces black colonies
- ❑ **Identification**
 - **Biochemical tests** such as sugar fermentation tests using Hiss's serum sugar media
 - **Automated identification systems** such as MALDI-TOF or VITEK

Diphtheria toxin demonstration

- ❑ In vivo tests (Guinea pig inoculation): Subcutaneous and intracutaneous tests
- ❑ In vitro tests:
 - Elek's gel precipitation test
 - Detection of *tox* gene-by PCR
 - Detection of toxin-by ELISA or ICT
 - Cytotoxicity on cell lines.

Laboratory Diagnosis

The diagnosis of diphtheria is based on the clinical signs and symptoms plus laboratory confirmation.

- ❖ Because of the risk of respiratory obstruction, specific treatment should be instituted immediately on clinical suspicion without waiting for laboratory reports
- ❖ Laboratory diagnosis is necessary only for:
 - Confirmation of clinical diagnosis
 - Initiating the control measures
 - Epidemiological purposes.

Laboratory diagnosis consists of isolation of the bacilli and toxin demonstration.

Isolation of Diphtheria Bacilli

Specimen

Useful specimens include: (1) throat swab (one or two) containing fibrinous exudates, (2) a portion of pseudomembrane, (3) nose or skin specimens (if infected).

Direct Smear Microscopy

- ❖ **Gram stain:** *C. diphtheriae* appear as irregularly stained club-shaped gram-positive bacilli of 3–6 μm length, typically arranged in **Chinese letter** or **cuneiform** arrangement (V- or L-shaped). It is difficult to differentiate them from other commensal coryneforms found in the respiratory tract (see Fig. 60.2B)
- ❖ **Albert's stain:** It is more specific for *C. diphtheriae*; they appear as green bacilli with bluish black metachromatic

granules at the poles. Details of Albert's staining procedure is given in Chapter 3.3 (see Fig. 60.2C).

Culture Media

Enriched Medium

C. diphtheriae is fastidious, aerobic and facultative anaerobe; does not grow on ordinary medium. It grows best in enriched medium such as blood agar, chocolate agar and Loeffler's serum slope. Plates are incubated at 37°C aerobically.

- ❖ **Blood agar:** Colonies are small circular, white and sometimes hemolytic (mitis biotype)
- ❖ **Loeffler's serum slope:** Colonies appear as small, circular, glistening, and white with a yellow tinge in 6–8 hours (Fig. 60.4A)
 - **Advantages:** (1) Growth can be detected as early as 6–8 hours. (2) Best medium for metachromatic granules production
 - **Disadvantages:** Being an enriched medium, if incubated beyond 6–8 hours, it supports growth of other throat commensals also.

Selective Medium

Selective media are best for isolation of *C. diphtheriae* from cases as well as from carriers, as the normal flora will be inhibited.

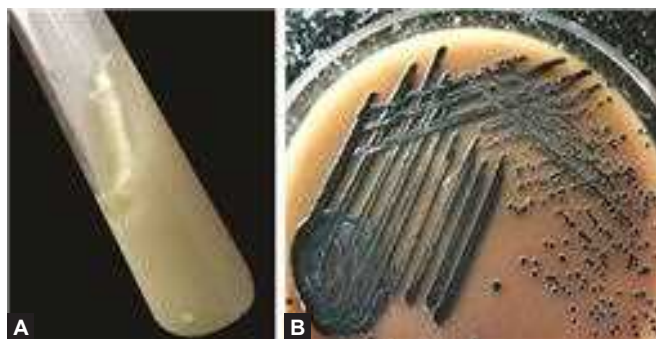
Potassium tellurite agar (PTA): *C. diphtheriae* reduces tellurite to metallic tellurium which gets incorporated into the colonies giving them black color (Fig. 60.4B). *C. ulcerans* and *C. pseudotuberculosis* can also grow on PTA producing black colored colonies.

- ❖ **Advantage:** Throat commensals are inhibited
- ❖ **Disadvantage:** Colonies appear only after 48 hours of incubation.

Identification

Species identification of *C. diphtheriae* is made by:

- ❖ **Biochemical tests** such as—(i) Sugar fermentation test using Hiss's serum sugar media, (ii) Pyrazinamidase test, and (iii) Urease test. The latter two tests are negative for *C. diphtheriae*



Figs 60.4A and B: A. Loeffler's serum slope; B. Potassium tellurite agar shows black colonies.

Source: A. Department of Microbiology, JIPMER, Puducherry; B. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ **Automated identification systems** such as MALDI-TOF or VITEK.

Toxin Demonstration

As the pathogenesis is due to diphtheria toxin, mere isolation of bacilli does not complete the diagnosis. Toxin demonstration should be done following isolation, which can be performed by in vivo and in vitro methods.

In Vivo Tests (Animal Inoculation)

In vivo toxin demonstration can be done by inoculation of culture broth into **guinea pig**. With the advent of other techniques, this method is rarely followed nowadays.

In Vitro Test

- ❖ **Elek's gel precipitation test:** This is a type of immunodiffusion in gel described by Elek (1949)
 - The strain isolated is streaked onto a media containing a filter paper soaked with antitoxin
 - If the strain is toxigenic, it liberates the toxin which diffuses in the agar and meets with the antitoxin to produce an arrow-shaped precipitation band
 - This test can also be used to know the relatedness between the strains isolated during an outbreak. The precipitate bands of outbreak isolates (streaked adjacent) when meet with each other, three patterns may be observed (Fig. 60.5)
 1. Cross-over with each other—indicates unrelated strain

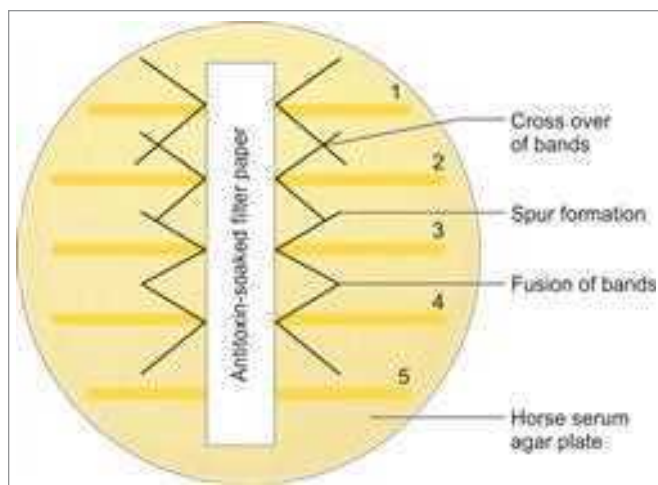


Fig. 60.5: Elek's gel precipitation test.

- **Isolates 1 to 4** are toxigenic strains (as precipitation bands are formed due to binding of toxin produced by the strains with antitoxin released by the filter paper)
- **Isolates 1 and 2:** The precipitation bands crossed over, indicates the toxins are not-identical and therefore strains are unrelated
- **Isolate 2 and 3:** There is partial fusion of precipitation bands, indicates the toxins are partially identical and therefore strains are partially related to each other
- **Isolates 3 and 4:** The precipitation bands fused with each other, indicates the toxins are identical to each other and therefore strains are completely related
- **Isolate 5** is non-toxigenic strain (no precipitation band is formed).

2. Spur formation—indicates partially related strain
3. Fused with each other—indicates identical strain.

❖ **Other in vitro tests** include:

- Detection of *Tox* gene by PCR
- Detection of diphtheria toxin by ELISA or immunochromatographic test (ICT)
- Cytotoxicity produced on cell lines.

Typing of *C. diphtheriae*

Typing methods are useful for epidemiological studies, to know the relatedness between the isolates. Biotyping was in use in the past, based on which there are four biotypes of *C. diphtheriae*—*gravis*, *intermedius*, *mitis* and *belfanti*. They vary in virulence and toxin production; *gravis* being 100% toxigenic and more virulent.

Epidemiology

Worldwide, there is declining trend of diphtheria cases in most countries including India, due to widespread vaccination coverage.

- ❖ **Source of infection:** Carriers (95%) are more common source of infection than cases (5%)
- ❖ **Carriers:** They may be temporary (persist for a month) or chronic (persist for a year or more). Nasal carriers are more dangerous due to frequent shedding than throat carriers. Incidence of carrier rate varies from 0.1% to 5%
- ❖ **Transmission** is via the respiratory droplets (coughing/sneezing), or rarely by contact with infected skin lesions
- ❖ **Reservoir:** Humans are the only reservoir
- ❖ **Age:** Diphtheria is common in children aged 1–5 years. With wide spread immunization, a shift in the age has been observed from preschool to school age. Newborns are usually protected due to maternal antibodies
- ❖ **Situation in world:** Due to wide spread immunization, cases were drastically declined by >95% over last 3 decades. However, there is global resurgence of diphtheria in recent years; 16,648 cases were reported in 2018
- ❖ **Russian epidemic:** An epidemic of adult diphtheria occurred in Russia during 1990–97 with > 1.1 lakh cases and 3,000 deaths. It was controlled by improved vaccination of children and adults
- ❖ **Situation in India:** India still accounts for the highest burden (60–70%) of diphtheria cases in the world (8,788 cases were reported in 2018)
- ❖ **Resurgence of diphtheria** in recent years is a public health problem in India
 - In 2019, outbreaks have been reported from Tamil Nadu, Kerala and Karnataka and few other states
 - Majority (>70%) of cases are from children 5–10 years or more; which explains low coverage of diphtheria vaccine especially the booster doses, as the primary cause of its resurgence
 - Waning immunity in adults may be a minor cause which contributes to adult diphtheria cases.

TREATMENT

Diphtheria

Treatment should be started immediately on clinical suspicion of diphtheria.

- ❑ **Antidiphtheritic serum or ADS (antitoxin):** Passive immunization with antidiphtheritic horse serum is the treatment of choice as it neutralizes the toxin.
 - A test dose should be given to check for hypersensitivity
 - It is given either IM or IV and the dose depends on stage of illness:
 - ◆ Early stage (< 48 hours): 20,000–40,000 units
 - ◆ If pharyngeal membranes present: 40,000–60,000 units
 - ◆ Late stage (> 3 days, with bull neck): 80,000–120,000 units.
- Human antitoxin therapy is under development.
- ❑ **Antibiotics:** Penicillin or erythromycin is the drug of choice. Antibiotic plays a minor role as it is of no use once the toxin is secreted. However, antibiotics are useful:
 - If given early (<6 h of infection), before the toxin release
 - Prevent further release of toxin by killing the bacilli
 - Treatment of cutaneous diphtheria
 - Treatment of carriers: Drug of choice is erythromycin.

Note: Disease is typically no longer contagious after 48h of therapy. Elimination of the organism should be documented by two consecutive negative cultures, after the therapy is completed.

Prophylaxis

Infection Control Measures

Patient should be placed in isolation room and all the steps of droplet precaution should be followed for the prevention of transmission of *C. diphtheriae* in hospitals (refer Chapter 21).

Post-exposure Prophylaxis

For close contacts (e.g. household), booster dose of diphtheria vaccine + penicillin G (single dose) or erythromycin (7–10 days) is recommended.

Vaccination

Diphtheria toxoid is used for vaccination as it induces antitoxin production in the body. A protective titer of more than 0.01 Unit/mL of antitoxin can prevent all forms of diphtheria. However, vaccine is not effective for:

- ❖ Prevention of cutaneous diphtheria
- ❖ Elimination of carrier state.

Types of Vaccine

- ❖ **Single vaccine:** Diphtheria toxoid (DT) is prepared by incubating toxin with formalin and then the toxoid is adsorbed on to alum. Alum acts as adjuvant and increases the immunogenicity of toxoid
- ❖ **Combined vaccine:** Various vaccines available are:
 - **DPT:** Contains DT (diphtheria toxoid), **Pertussis** (whole cell) and **TT** (tetanus toxoid). **Pertussis** component acts as **adjuvant** and increases the immunogenicity of DT and TT
 - **DaPT:** Contains DT, TT and acellular pertussis (aP)

- **Td:** It contains tetanus toxoid and adult dose (2 Lf) of diphtheria toxoid
- **Pentavalent vaccine:** DPT can also be given along with hepatitis B and *Haemophilus influenzae* type b.

Administration of Diphtheria Vaccine

- ❖ **Schedule:** Under National Immunization Schedule (NIS) of India 2020 (Chapter 20, Table 20.4):
 - **Children:** Total seven doses are given; three doses of pentavalent vaccine at 6, 10 and 14 weeks of birth, followed by two booster doses of DPT at 16–24 months and 5 years and another two booster doses of Td at 10 years and 16 years
 - **Pregnant woman** also should receive two doses of Td at one month interval
 - The introduction of Td boosters in place of TT at 10 and 16 years and also in pregnancy is an attempt made to reduce the resurgence of diphtheria in adults.
- ❖ **Site:** DPT is given deep intramuscularly (IM) at anterolateral aspect of thigh, (gluteal region is not preferred as fat may inhibit DPT absorption)
- ❖ **Thiomersal** (0.01%) is used as preservative
- ❖ **Storage:** DPT should be kept at 2–8°C; if accidentally frozen then it has to be discarded
- ❖ **Dose:** The usual dose (given to children) of diphtheria toxoid contains 25 Lf units, whereas the adult dose contains 2 Lf units

Two commercial preparations of DPT are available. One dose (0.5 mL) of vaccine contains:

- ❑ **Glaxo:** 25 Lf (DT), 5 Lf (TT), 20,000 million (pertussis killed bacilli)
- ❑ **Kasauli:** 30 Lf (DT), 10 Lf (TT), 32,000 million (pertussis killed bacilli).

- ❖ **Protective titer:** Following vaccination, an antitoxin titer of ≥ 0.01 IU/mL is said to be protective
- ❖ **Adult immunization:** As adult diphtheria cases are increasingly being reported, adult immunization by Td vaccine has been recommended
 - Adults >18 years, who have completed primary vaccination: Td booster dose is indicated every 10 years till 65 years
 - Adults >18 years who have not completed primary vaccination: 3 doses of Td given at 0, 1 month, and 1 year.

Adverse Reactions following DPT Administration

- ❖ **Mild:** Fever and local reaction (swelling and indurations) are observed commonly
- ❖ **Severe:** Whole cell killed vaccine of *B. pertussis* is encephalitogenic. It is associated with neurological complications, which is more common in children > 7 years age
 - In contrast, acellular pertussis is not associated with neurological complications

- Therefore, for children >7 years age who have not received primary vaccination, DaPT comprising acellular pertussis vaccine is recommended.

❖ Absolute contraindication to DPT include:

- Hypersensitivity to previous dose
- Progressive neurological disorder.

Schick Test

It is a toxin-antitoxin neutralization test, was used long back, to test the susceptibility of individual to diphtheria before starting immunization. It is obsolete now.

Diphtheroids

Diphtheroids or coryneforms are the nondiphtherial corynebacteria, that usually exist as normal commensals in the throat, skin, conjunctiva and other areas. However, they have been associated with invasive disease, particularly in immunocompromised patients. They can be differentiated from *C. diphtheriae* by many features such as:

- ❖ Stains more uniformly than *C. diphtheriae*
- ❖ Palisade arrangement: Arranged in parallel rows rather than cuneiform pattern (Fig. 60.6)
- ❖ Absence of metachromatic granules (except *C. xerosis*).

Coryneforms that are rarely pathogenic to man are:

- ❖ **Clinically resembling diphtheria:** *C. ulcerans* and *C. pseudotuberculosis* produce diphtheria toxin and cause localized ulcerations in throat, clinically resembling diphtheria
 - *C. ulcerans* causes infections in cows. Human infections may occur through cow's milk
 - *C. pseudotuberculosis* (Preisz–Nocard bacillus) causes pseudotuberculosis in sheep and suppurative lymphadenitis in horses. Human infection is very rare.
- ❖ ***C. minutissimum*:** It causes a localized infection of skin (axilla and groin), called as 'erythrasma'. On Wood's lamp examination, erythrasma lesions emit coral red color

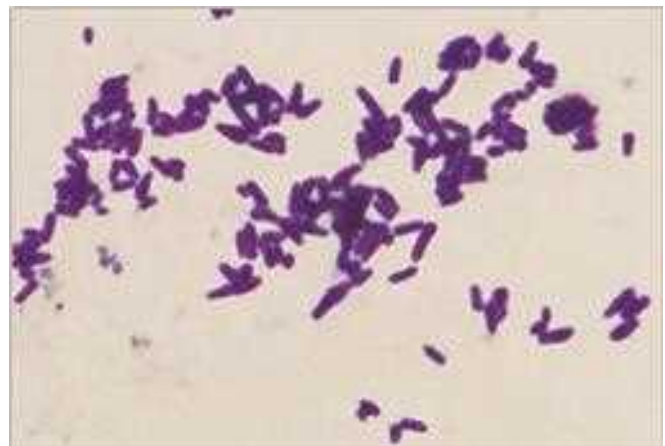


Fig. 60.6: Diphtheroids—Palisade arrangement of gram-positive bacilli.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ ***C. jeikeium***: It is lipophilic species, colonizes skin of hospitalized patients. It can cause bacteremia, endocarditis and meningitis, especially in immunocompromised hosts. It is usually multidrug resistant, responds only to vancomycin
 - ❖ ***C. urealyticum***: It is skin commensal, rarely causes urinary tract infection (pyelonephritis) and alkaline encrusted cystitis (struvite stones in alkaline urine) in immunocompromised and renal transplant recipients
 - ❖ ***C. pseudodiphtheriticum***: It is a known commensal in throat. However, in immunocompromised patients, it can cause exudative pharyngitis (may mimic respiratory diphtheria) and endocarditis
 - ❖ ***C. parvum*** is frequently used as an immunomodulator.
- Diagnosis:** Nucleic acid amplification test (NAAT) of throat swab targeting *N. gonorrhoeae* is the test of choice. However, it may be false positive owing to the presence of commensal *Neisseria* species in the oropharynx, and therefore should always be confirmed by culture
- Treatment:** Ceftriaxone + azithromycin is the regimen of choice.
- Other rare causes of bacterial pharyngitis include:**
- ❖ Other β -hemolytic streptococci (group C and G)
 - ❖ *Arcanobacterium hemolyticum*: It was formerly placed under *Corynebacterium*. It can cause pharyngitis and skin ulcers. It is β -hemolytic, produces a positive reverse CAMP test. Azithromycin is given for treatment
 - ❖ *Fusobacterium necrophorum*: It is an anaerobic gram-negative spindle-shaped bacillus, rarely causes pharyngitis (Chapter 53)
 - ❖ *Mycoplasma pneumoniae*: It usually causes atypical pneumonia; rarely can also be associated with pharyngitis (Chapter 62).

Pharyngitis can be a prime manifestation of various other sexually-transmitted infections—herpes, HIV/AIDS, rarely secondary syphilis.

RARE CAUSES OF BACTERIAL PHARYNGITIS

Gonococcal Pharyngitis

Neisseria gonorrhoeae is a sexually-transmitted bacterium, commonly causes urethritis; rarely can cause pharyngitis following transmission by orogenital sexual contact (Chapter 77).

EXPECTED QUESTIONS

I. Write essay on:

1. A child aged 7 years with high grade fever, toxic, pain in the throat, inability to swallow was brought to the casualty. On examination, a white patch was found on the fauces, which started bleeding when touched. No history of immunization is available.
 - a. What is the clinical diagnosis?
 - b. Name the etiological agent causing this clinical condition.
 - c. Write in detail pathogenesis and laboratory diagnosis of this condition.
 - d. Discuss the management of this condition.

II. Write short notes on:

1. DPT vaccines.
2. Streptococcal pharyngitis.

III. Multiple Choice Questions (MCQs):

1. **Production of early metachromatic granules can be seen best in which of the following media:**
 - a. Nutrient agar
 - b. Chocolate agar

- c. Loeffler's serum slope
- d. Potassium tellurite agar

2. Which of the following site is most commonly affected by *C. diphtheriae*?

- a. Skin
- b. Conjunctiva
- c. Faucial
- d. Kidney

3. Metachromatic granules of *Corynebacterium diphtheriae* can be stained by all of the following special stains, except:

- a. Neisser's stain
- b. Ziehl-Neelsen stain
- c. Albert's stain
- d. Ponder's stain

4. Diphtheria toxin is produced by all, except:

- a. *C. diphtheriae*
- b. *C. ulcerans*
- c. *C. pseudotuberculosis*
- d. *C. xerosis*

5. Scarlet fever is caused by:

- a. *Streptococcus pyogenes*
- b. *Corynebacterium diphtheriae*
- c. *Streptococcus* group C and G
- d. *Arcanobacterium hemolyticum*

Answers

1. c 2. c 3. b 4. d 5. a

CHAPTER PREVIEW

- Pneumococcal Pneumonia
- Staphylococcal Pneumonia
- Rare Causes of Bacterial Pneumonia
- *Haemophilus influenzae* Pneumonia
- Gram-negative Bacilli Pneumonia

INTRODUCTION

Based on area of lungs involved, and type of cough produced, bacterial pneumonia is traditionally classified into two groups; typical (lobar) and atypical or (interstitial) pneumonia.

- ❖ **Lobar or typical pneumonia:** It involves infection of the lung parenchyma and its alveoli. It is characterized by consolidation (gives a dull note on percussion) and productive cough with purulent sputum. It is mostly caused by pyogenic organisms such as:
 - *Streptococcus pneumoniae*
 - *Haemophilus influenzae*
 - *Staphylococcus aureus*
 - Gram-negative bacilli.
- ❖ **Interstitial or atypical pneumonia** occurs in the interstitial space of lungs. Cough is characteristically non-productive. It is mostly caused by bacteria such as *Mycoplasma*, *Chlamydia*, *Legionella* species, etc. These agents discussed in Chapter 62.

However, there is considerable overlapping. The typical agents can be atypical in presentation and vice-versa.

Clinical features which may be found in both types of pneumonia include:

- ❖ Fever, with chills and/or sweats, tachycardia
- ❖ Increased respiratory rate (tachypnea), with increased use of accessory muscles of respiration
- ❖ Dyspnea (shortness of breath)
- ❖ If the pleura is involved, the patient may experience pleuritic chest pain
- ❖ Gastrointestinal symptoms: May be seen up to 20% of patients such as nausea, vomiting, and/or diarrhea
- ❖ Other symptoms may include fatigue, headache, myalgia, and arthralgia
- ❖ Severely ill patients may develop septic shock and multiorgan failure.

In lieu of epidemiological point of view, pneumonia can be classified into: (1) community-acquired—patients acquire the infection in the community (Chapter 59), (2) healthcare associated—patients acquire the infection in the hospital setting (Chapter 22).

This chapter will focus mainly on the typical bacterial agents causing lobar pneumonia.

PNEUMOCOCCAL PNEUMONIA

Streptococcus pneumoniae (commonly referred to as pneumococcus) is the leading cause of lobar pneumonia, otitis media in children and meningitis in all ages. They are α -hemolytic and may present as commensals in human upper respiratory tract. They differ from α -hemolytic viridans streptococci in many ways such as their shape (lanceolate-shaped diplococci), bile solubility, optochin sensitivity and presence of a polysaccharide capsule (Table 61.1).

Virulence Factors and Pathogenesis

S. pneumoniae possesses a number of virulence factors.

- ❖ **Capsular polysaccharide:** It is the principal virulence factor, protects the cocci from phagocytosis. Typing of pneumococci is based on capsular polysaccharide structure. Till date, there are 98 serotypes reported
- ❖ **C-carbohydrate antigen** (C-polysaccharide): This antigen precipitates with an acute phase reactant protein called **C-reactive protein (CRP)**. CRP is not an antibody to pneumococcal C-antigen. It is a marker of acute inflammation, raised in many acute inflammatory conditions

Table 61.1: Differences between *Streptococcus pneumoniae* and Viridans streptococci.

Features	<i>S. pneumoniae</i>	Viridans streptococci
Arrangement	Gram-positive cocci in pairs	Gram-positive cocci in long chains
Morphology	Lanceolate shaped	Round/oval
Capsule	Present	Absent
On blood agar	Draughtsman or carrom coin colony	Minute colony
Bile solubility	Soluble in bile	Insoluble in bile
Inulin	Fermented	Not fermented
Optochin	Sensitive	Resistant

- ❖ **Pneumolysin:** It is a membrane damaging toxin, which inhibits neutrophil chemotaxis and phagocytosis, similar to streptolysin-O
- ❖ **Autolysin:** It is an amidase enzyme that cleaves its own peptidoglycan leading to autolysis of cells. This property is responsible for the characteristic bile solubility and draughtsman appearance of pneumococcal colonies. Release of cell wall fragments leads to a self-perpetuating inflammatory response that contributes to the pathogenesis
- ❖ **Other virulence factors:** Include pneumococcal surface proteins (PspA, PspC), IgA protease, neuraminidase and pneumococcal surface adhesin A (PsaA).

Clinical Manifestations

Pneumococci first colonize the human nasopharynx, which usually occurs at an early age. From the nasopharynx, the bacteria spread either via the bloodstream to distant sites (e.g. brain, joint, bones and peritoneal cavity) or spread locally to cause otitis media or pneumonia.

Various manifestations include:

- ❖ **Lobar pneumonia:** *S. pneumoniae* is the most common cause of lobar (alveolar) pneumonia. Though starts as noninvasive illness due to contiguous spread from the nasopharynx, it soon becomes bacteremic and invasive. Patients present with productive purulent cough, fever and chest pain. Important signs are dullness on percussion due to consolidation and crackles on auscultation
- ❖ **Empyema and parapneumonic effusion** may occur as complications of pneumococcal pneumonia
- ❖ **Invasive pneumococcal disease (IPD):** Defined as an infection confirmed by isolation of pneumococci from a normally sterile site. Various examples include:
 - Bloodstream infection
 - Pyogenic meningitis: *S. pneumoniae* is the leading cause of meningitis in all ages (Chapter 71)
 - Other invasive manifestations: *S. pneumoniae* can cause osteomyelitis, septic arthritis, endocarditis, pericarditis, primary peritonitis; rarely, brain abscess and hemolytic-uremic syndrome.
- ❖ **Noninvasive manifestations:** Pneumococci can cause various noninvasive infections such as otitis media (most common) and sinusitis.

Epidemiology

- ❖ **Source** of infection is human upper respiratory tract of carriers (less often patients)
- ❖ **Carrier rate:** Up to the age of 5 years, 70–90% of children harbor *S. pneumoniae* in the nasopharynx. In adults, the colonization occurs less frequently (nearly 40%)
- ❖ **Mode of transmission** is by inhalation of contaminated droplet nuclei
- ❖ Infection usually leads to colonization and carrier state. Disease results only when the host resistance is lowered due to presence of associated risk factors.

Risk Factors

- ❖ **Children (<2 years):** Children are at higher risk to develop pneumococcal infection because of their inability to produce adequate antibodies against the capsular antigen; owing to the immature immune system
- ❖ **Splenectomy, sickle cell disease and other hemoglobinopathies:** As spleen is the site of destruction of capsulated bacteria, the conditions where the opsonization and clearance of circulating bacteria by the spleen is hampered, there is increased risk of pneumococcal infection
- ❖ **Underlying comorbid diseases:** Such as chronic lung, heart, kidney and liver disease, cochlear implants, diabetes mellitus and immunosuppression (e.g. HIV)
- ❖ **Viral URTI:** Underlying viral upper respiratory tract infections (e.g. influenza)
- ❖ **Nature of infecting serotypes:** Serotypes vary in their virulence, geographical distribution and age affected
 - **Most common:** 6 and 19F are reported universally as major serotypes. In India serotypes 1, 6, 19A and 19F are commonly reported
 - **Age:** In children, seven serotypes (1, 5, 6A, 6B, 14, 19F and 23F) account for nearly 60% of IPD cases in most part of the world. In contrast, the serotypes causing IPD vary widely among adults which may be attributed to the wide variation in the vaccination status in adults.

LABORATORY DIAGNOSIS Pneumococcal infections

- ❑ **Specimen collection:** Sputum, CSF, pleural fluid
- ❑ **Direct smear microscopy:** Reveals pus cells and lanceolate-shaped gram-positive diplococci, surrounded by a clear halo (due to capsule)
- ❑ **Capsular antigen detection in CSF:** By latex agglutination
- ❑ **C-antigen detection** in urine and CSF by ICT
- ❑ **Culture**
 - Blood agar: It forms draughtsman or carrom coin shaped colonies
 - Chocolate agar: It produces greenish discoloration (bleaching effect)
- ❑ **Culture smear:** Reveals lanceolate-shaped gram-positive diplococci
- ❑ **Identification:**
 - Biochemical identification: It is bile soluble, optochin sensitive, inulin fermenter
 - Automation methods such as MALDI-TOF and VITEK
- ❑ **Serotyping:** By Quellung reaction or latex agglutination test
- ❑ **Molecular methods:** Such as multiplex PCR
- ❑ **Non-specific findings:** ↑ acute phase reactant proteins, e.g. C-reactive protein, procalcitonin
- ❑ **Antimicrobial susceptibility testing.**

Laboratory Diagnosis of Pneumococcal Infections

General laboratory diagnosis of various pneumococcal infections is discussed below.

Specimen Collection

Depending on the site of infection, specimens such as sputum, cerebrospinal fluid (CSF), pleural fluid and other sterile body fluids are collected. Automated blood culture is useful for invasive infection. Other specimens include aspiration of sinus material and ear discharge/aspirate.

Direct Smear Microscopy

Direct microscopy of smears made from specimens show numerous pus cells and **lanceolate or flame-shaped** gram-positive cocci (1 μm) in pairs, surrounded by a clear halo (due to capsule). Direct microscopy is extremely useful especially for meningitis, as empirical treatment (antibiotics) can be started early (Fig. 61.1).

Antigen Detection

- ❖ **Capsular antigens:** Detection of capsular antigens in CSF is more sensitive than microscopy. It is done by latex agglutination test using latex beads coated with anticapsular antibodies
- ❖ **C-antigens:** Detection of C-polysaccharide antigen in urine by immunochromatographic test (ICT) is useful for diagnosis of pneumonia; however it gives positive results even for nasopharyngeal carriers. Therefore, it is not useful in children where the carriage rate is high. This ICT is also available for antigen detection from CSF in meningitis cases.

Culture

S. pneumoniae is fastidious, does not grow in basal media like nutrient agar or nutrient broth. Specimens are inoculated onto enriched media, such as blood agar, and chocolate agar and incubated for 24 hours at 37°C in presence of 5–10% of CO₂.

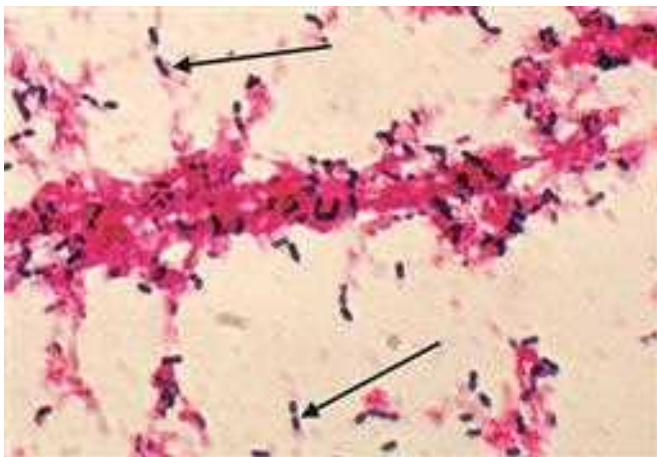


Fig. 61.1: Pneumococci in gram-stained smear of sputum [lanceolate shaped gram-positive cocci in pair surrounded by clear halo (capsule)].

Source: Public Health Image Library, ID#/2896/Dr Mike Miller/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Blood agar:** Colonies are initially small dome shaped, surrounded by green zone of α -hemolysis. Colonies on prolonged incubation, undergo autolysis, and therefore have a central depression with an elevated rim; giving rise to **draughtsman-shaped** or **carrom coin-shaped** appearance (Fig. 61.2A)
- ❖ **Chocolate agar:** It produces greenish discoloration (described as bleaching effect) (Fig. 61.2C).

Culture Smear

Gram stained smear of the colonies reveals lanceolate or flame-shaped gram-positive cocci (1 μm) in pairs. Motility testing by hanging drop shows non-motile cocci.

Identification

Pneumococci are catalase negative and can be differentiated from viridans streptococci (which are also α -hemolytic, found as oral commensals in sputum specimens) in various ways (Table 61.1).

- ❖ **Bile solubility:** Pneumococci are soluble in bile (sodium deoxycholate) due to their enhanced autolytic activity in presence of bile. Viridans streptococci are insoluble in bile (Fig. 61.2D)
- ❖ **Optochin sensitivity:** Pneumococci are sensitive to optochin disk and produce wider zone of inhibition (14 mm or more) (Fig. 61.2B). Viridans streptococci are resistant to optochin
- ❖ **Inulin fermentation:** Pneumococci can ferment inulin to form acid, but not viridans streptococci
- ❖ **Automated methods** such as MALDI-TOF and VITEK can also be used for early and accurate identification. This is very useful in early institution of pathogen-directed therapy.

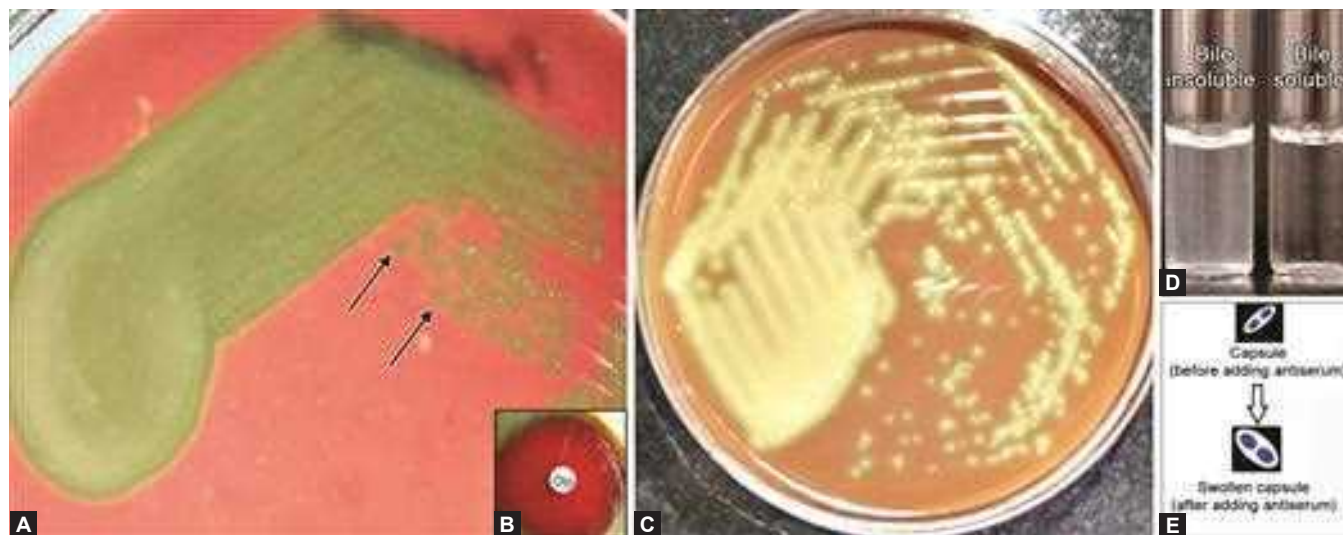
Typing of *S. pneumoniae*

- ❖ **Quellung or Neufeld reaction:** This test was routinely done in the past at bedside, directly from sputum samples from pneumonia cases. When specimen is treated with type-specific antiserum, along with methylene blue dye; capsule becomes swollen, sharply delineated and refractile (Fig. 61.2E)
- ❖ Currently, serotyping is done by latex agglutination test using type specific antisera.

Molecular Methods

Molecular methods such as PCR are highly sensitive, more useful when organism load is scanty (e.g. CSF), detect earlier than culture and also help in serotype identification.

- ❖ **Real time PCR** is even more sensitive, specific, takes less time and is quantitative
- ❖ **Multiplex PCR** (e.g. BioFire FilmArray, bioMérieux) and Multiplex real time PCR can be used for simultaneous detection of common agents of lobar pneumonia from sputum or bronchoalveolar lavage



Figs 61.2A to E: Properties of pneumococci: **A.** α -hemolytic draughtsman-shaped colonies on blood agar; **B.** Sensitive to optochin; **C.** Bleaching effect on chocolate agar; **D.** Bile solubility test (left—viridans streptococci, not soluble in bile; right—pneumococcus, soluble in bile); **E.** Quellung reaction.

Source: A. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; B to D. Department of Microbiology, JIPMER, Puducherry (with permission).

- ❖ **Common genes** targeted include: *lytA* (autolysin gene), *ply* (pneumolysin) and *psaA* (pneumococcus surface antigen A).

Nonspecific Findings

- ❖ Elevated acute phase reactant proteins such as C-reactive protein, procalcitonin
- ❖ Leukocytosis
- ❖ Chest X-ray shows infiltrates and lobar consolidation. (In children—distinctly spherical consolidation is seen in upper part of the lower lobe, called round pneumonia).

Antimicrobial Susceptibility Test (AST)

AST is necessary for institution of appropriate antibiotic treatment. It can be performed by disk diffusion test on Mueller Hinton blood agar or by automated MIC detection method by microbroth dilution (e.g. VITEK). The latter is the preferred method as disk diffusion break points of several antibiotics (e.g. penicillin, ceftriaxone) are not available for pneumococcus.

TREATMENT

Pneumococcal infections

The treatment regimen for pneumococci varies depending upon the type of infective syndrome.

- ❑ **Pneumonia:** Oral therapy with amoxicillin for five days remains the standard treatment among outpatients. Alternative drugs include IV penicillin or ceftriaxone, oral quinolone (levofloxacin or moxifloxacin), clindamycin or azithromycin
- ❑ **Meningitis:** As mortality is very high (~ 20%), treatment should be initiated as early as possible. Ceftriaxone/cefotaxime \pm vancomycin is given for 10-14 days

Contd...

TREATMENT

Pneumococcal infections

- ❑ **Other invasive infections:** For stable children, penicillin, cefotaxime or ceftriaxone can be instituted. In critically ill children, the treatment should be same as that of meningitis
- ❑ **Otitis media:** Oral amoxicillin for 7-10 days is the drug of choice.

Drug Resistance in Pneumococcus

In about 30% of IPD, pneumococcus shows resistance to at least one antibiotic or more.

Penicillin resistance in pneumococci has been reported increasingly nowadays. This resistance is due to alteration of penicillin-binding protein (PBP) to PBP2b. This altered PBP2b has low affinity for β -lactam drugs. The gene coding for altered PBP is acquired by transformation and horizontal transfer of DNA from related streptococcal species.

Multidrug resistant (MDR) *S. pneumoniae* (non-susceptible to ≥ 3 antibiotic classes) is increasingly reported. Resistance to penicillin, tetracycline, erythromycin, sulfonamides and clindamycin have been shown to coexist.

- ❖ **Serotype 19A** is the most common serotype to exhibit multidrug resistance
- ❖ Some serotypes can undergo **capsule switching** (change from one serotype to another), which may contribute to the development of antibiotic resistance.

Prevention and Vaccination

Measures to prevent pneumococcal disease include vaccination, treatment of underlying diseases (that

Contd...

	PPSV23	PCV13
Name	23-valent pneumococcal polysaccharide vaccine	Pneumococcal conjugate vaccine
Brand	Pneumovax 23	Prevnar13
Serotypes included	Contains 23 serotypes of <i>S. pneumoniae</i> 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F	Contains 13 serotypes of <i>S. pneumoniae</i> 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, & 23F. Conjugated with diphtheria toxoid
Coverage	Covers 70–80% of invasive serotypes in adults	Covers only 30–40% of invasive serotypes in adults, however it covers most of the serotypes infecting children
Immunogenicity	Less immunogenic: Capsular antigen being T cell independent antigen, is less immunogenic to children, hence not given to children <2 years age	More immunogenic: The protein conjugate acts as adjuvant and increases the immunogenicity of capsular antigen. Hence, PCV is effective against children <2 years age
Duration	Provides short-term immunity (3–5 years)	Provides longer immunity
Herd	Promotes herd immunity	Does not promote herd immunity
Effect on carriers	Does not provide mucosal immunity, hence no effect on carriers	Provides mucosal immunity; hence, it can eradicate carriage from nasopharynx
Cost	Less expensive	More expensive
Indication	Adults ≥65 years Adults 19–64 years age with underlying risk factors	Indications in adults same as PPSV23 Children (as primary immunization)

increase the risk of pneumococcal disease), infection control measures (droplet precautions, refer Chapter 21), and prevention of antibiotic overuse.

Pneumococcal Vaccines

There are two vaccines available for pneumococcus: (i) 23-valent pneumococcal polysaccharide vaccine (PPSV23), and (ii) pneumococcal conjugate vaccine (PCV13). The differences is given in Table 61.2.

Schedule: The type of vaccine recommended and its schedule depends up on the age group.

- ❖ **After birth:** PCV13 is recommended, three primary doses at 6th, 10th and 14th week of age and booster at 15 months
- ❖ **Age ≥65 years:** Either PPSV23 only can be given or PCV13 followed by PPSV23 can be given ≥1 year gap
- ❖ **Age 19–64 years:** Pneumococcal vaccine is recommended only in presence of underlying risk factors. Depending upon the type of risk factors, either PPSV23 alone is given or PCV13 followed by PPSV23 is given. The risk factors are:

- **Immunocompetent persons:** With alcoholism and smoking, chronic heart, liver and lung disease, diabetes mellitus, cochlear implants and CSF leaks
- **Splenic dysfunction:** Congenital or acquired asplenia, sickle cell disease/other hemoglobinopathies
- **Immunocompromised persons:** Chronic renal failure, congenital or acquired immunodeficiencies, HIV infection, malignancy and solid organ transplant.

HAEMOPHILUS INFLUENZAE PNEUMONIA

Introduction

Haemophilus species are oxidase positive, capsulated pleomorphic gram-negative bacilli that require special growth factors present in blood, such as factor X and V (*Haemo* means blood, *philus* means loving). The important species are:

- ❖ *H. influenzae*: It is the most pathogenic species, which causes pneumonia and meningitis in children
- ❖ Other species encountered are as follows:
 - *H. ducreyi*: It causes a sexually transmitted disease called **chancroid**, which presents as genital ulcer (described in Chapter 77)
 - *H. aegyptius*: It causes conjunctivitis and rashes (Chapter 78)
 - *H. haemolyticus* and *H. parahaemolyticus* produce hemolysis on blood agar. They are also commensals in throat or mouth
 - *H. aphrophilus* and *H. paraphrophilus* (renamed as *Aggregatibacter aphrophilus* and *A. paraphrophilus*): Associated with infective endocarditis (Chapter 28)
 - *H. parainfluenzae*: It can cause infective endocarditis (Chapter 28).

Subsequent discussion is confined to *H. influenzae*. Other species are discussed under the respective systems which they principally infect.

History

H. influenzae is also called **Pfeiffer's bacillus** as it was discovered by Pfeiffer (1892). The species name was coined wrongly, thinking that it would cause human influenza which is actually a viral disease caused by influenza virus.

Virulence Factors

Various virulence factors of *H. influenzae* are:

- ❖ Capsular polysaccharide is the most important virulence factor, acts by inhibiting phagocytosis
- ❖ **Other virulence factors** include—(i) bacterial endotoxin, (ii) outer member proteins, (iii) IgA1 proteases, and (iv) pili and other adhesion proteins.

Serotyping

Based on the capsular polysaccharide of *H. influenzae*, it can be typed into six serotypes (a to f). However, some strains lack capsule and are referred to as nontypeable strains.

- ❖ ***H. influenzae* serotype b (Hib)** is the most virulent among all types and accounts for most of the invasive infections
- ❖ **Hib capsule** has a unique chemical structure, made up of polyribosyl ribitol phosphate (PRP) antigen. It is strongly immunogenic, induces IgG, IgM and IgA antibodies which are bactericidal, opsonic and protective. Hence, PRP antigen is used for vaccination
- ❖ Next to Hib, **nontypeable strains** are commonly isolated clinically. Other capsular serotypes are very rarely isolated.

Clinical Manifestations

H. influenzae Type b (Hib)

H. influenzae type b is the most common and most invasive serotype of *H. influenzae*. It causes systemic disease by invasion and hematogenous spread from the respiratory tract to distant sites such as the meninges, bones, and joints.

- ❖ **Central nervous system infections:** It can cause pyogenic meningitis and subdural effusion. Mortality rate is high if untreated (Chapter 71)
- ❖ **Epiglottitis:** It is a cellulitis of the epiglottis and supraglottic tissues. It is life threatening as it can lead to acute airway obstruction. It typically affects older children (2–7 years old) and rarely adults
- ❖ **Community acquired bacterial pneumonia:** Hib causes pneumonia in infants, which is clinically similar to other types of bacterial pneumonia except that, pleural involvement is more common in Hib infection
- ❖ **Parameningeal focus** of infection is often present in adults (such as sinusitis and otitis), from which the infection may spread to meninges
- ❖ **Less common** invasive conditions include:
 - Cellulitis of neck and head region
 - Osteomyelitis, septic arthritis
 - Pericarditis
 - Empyema and bronchiectasis
 - Orbital cellulitis, endophthalmitis
 - Urinary tract infection
 - Bacteremia without an identifiable focus.

Nontypeable *H. influenzae*

Next to Hib, nontypeable strains are the most common group encountered clinically. They cause disease by local invasion of mucosal surfaces. The clinical manifestations include:

- ❖ Childhood otitis media (most common cause)
- ❖ Exacerbations of chronic obstructive pulmonary disease (COPD): They are the most common bacterial cause for this condition
- ❖ Community-acquired bacterial pneumonia in adults with underlying COPD or AIDS
- ❖ Puerperal sepsis and neonatal bacteremia: These infections can be caused by nontypeable strains that usually colonize the female genital tract

Table 61.3: Differences between type b and nontypeable *Haemophilus* strains.

Features	Type b strains	Nontypeable strains
Capsule	Made up of poly ribosyl ribitol phosphate (PRP)	Noncapsulated
Manifestations	Invasive—meningitis, epiglottitis, pneumonia, bacteremia	Noninvasive—otitis media (in children) and pneumonia (in adults)
Commonly affect	Children	Adults
Spread	Hematogenous spread	Contiguous spread
Vaccine	Hib vaccine is available	Not available

- ❖ Sinusitis in adults and children
 - ❖ Rarely they cause invasive infections, especially in countries where Hib vaccines are used widely.
- The differences between Hib and nontypeable *Haemophilus* strains are tabulated in Table 61.3.

Epidemiology

- ❖ **Host:** *H. influenzae* is exclusively human pathogen
- ❖ **Mode of transmission** is through respiratory route; either by droplet inhalation or contact with infected secretions or fomites
- ❖ **Age:** Invasive *H. influenzae* infection is common in children < 5 years of age and also adults >65 years
- ❖ **Risk factors** such as household contact, day care centre, asplenia, socioeconomic conditions, and genetic differences
- ❖ **Incidence:** The incidence of invasive Hib infection is drastically reduced due to the widespread use of Hib vaccine and now nontypeable *H. influenzae* strains cause the majority of invasive disease among all age groups.

LABORATORY DIAGNOSIS

H. influenzae infections

- ❑ **Specimens:** Sputum, blood, CSF
 - Processed immediately, should never be refrigerated
- ❑ **Direct examination:**
 - Pleomorphic gram-negative coccobacilli
 - Capsule detection: By Quellung reaction
 - Antigen detection: By latex agglutination test, direct-IF or ELISA
- ❑ **Culture:**
 - Blood agar with *S. aureus* streak line shows satellitism
 - Others: Chocolate agar, Fildes agar and Levinthal's agar
- ❑ **Identification:**
 - Biochemical tests such as disk test for X and V factor
 - Automated systems such as MALDI-TOF or VITEK
- ❑ **Typing methods** such as biotyping and serotyping (using specific antisera)
- ❑ **Molecular method:** Multiplex PCR (BioFire FilmArray), detecting common agents of pyogenic meningitis in CSF
- ❑ **Antimicrobial susceptibility testing**

Laboratory Diagnosis of *H. influenzae* Infections

Specimen Collection and Transport

Depending upon the site of infection, various specimens may be collected such as CSF, blood, sputum, pus, aspirates from joints, middle ears or sinuses

- ❖ As *H. influenzae* is highly sensitive to low temperature, the specimens for culture **should never be refrigerated**
- ❖ The specimens should be transported to the laboratory without any delay and processed immediately.

Direct Detection

- ❖ **Gram staining** of CSF and other specimen shows pleomorphic gram-negative coccobacilli (Fig. 61.4A)
- ❖ **Capsule detection** (Quellung reaction): Capsular swelling occurs when a drop of CSF is mixed with type b antiserum and methylene blue and observed under the microscope
- ❖ **Antigen detection:** The type b capsular antigen can be detected in CSF, urine or other body fluids by—(1) latex agglutination test using latex particles coated with antibody to type b antigen or (2) direct-IF test or (3) ELISA.

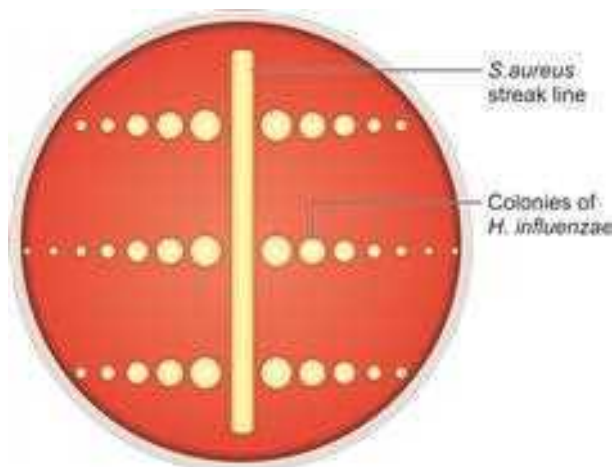


Fig. 61.3: Satellitism of *Haemophilus influenzae* (schematic diagram).

Culture

H. influenzae is highly fastidious, requires two accessory growth factors (factor X and V) in blood for their growth.

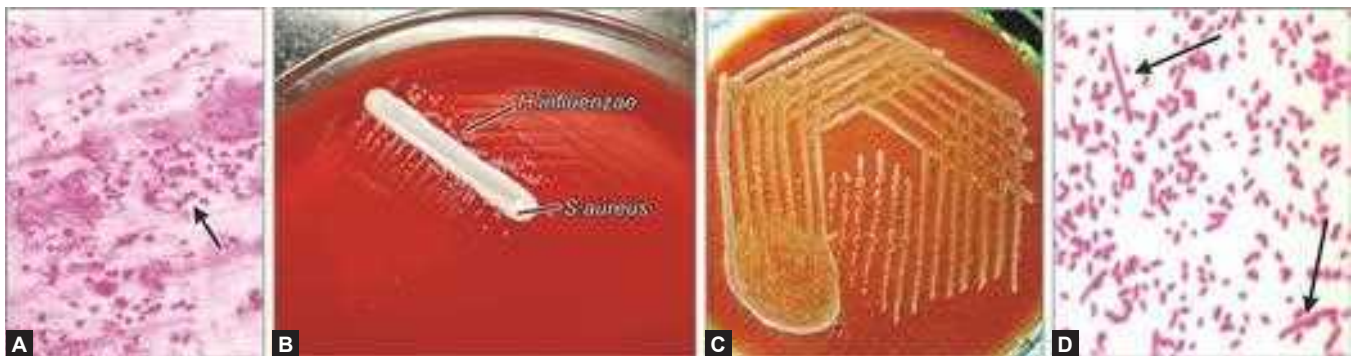
- ❖ Factor X is a hemin, present freely in blood
- ❖ Factor V is nicotinamide adenine dinucleotide (NAD), which is present inside RBCs. It is also produced by some bacteria, such as *Staphylococcus aureus*.
The growth of *H. influenzae* may vary in different media depending upon the availability of X and V factors.
- ❖ **No growth on basal media:** Nutrient agar and peptone water lack X and V factors, therefore they do not support the growth of *H. influenzae*
- ❖ **Growth is scanty on blood agar:** It is because only factor X is available freely in blood agar and factor V is largely intracellular, present only inside the RBCs. It is available in very minute quantities freely in the medium
- ❖ **Grows well on chocolate agar:** While preparing chocolate agar, blood is poured into molten agar at 75°C which lyses RBCs releasing excess of factor V. Hence, it supports the growth of *H. influenzae* (Fig. 61.4C)
- ❖ **Grows well on blood agar with *S. aureus* streak line:** Colonies of *H. influenzae* grow adjacent to *S. aureus* streak line—a phenomenon called as satellitism

Satellitism

H. influenzae can grow on blood agar if the source of V factor is provided (Figs 61.3 and 61.4B)

- ❑ When *S. aureus* is streaked across a blood agar plate perpendicular to the *H. influenzae* streak line, factor V is released from *S. aureus*
- ❑ Therefore, it forms larger colonies adjacent to *S. aureus* streak line and size of the colonies decreases gradually away from the *S. aureus* streak line
- ❑ This phenomenon is called satellitism, a property that is routinely employed for the isolation of *H. influenzae*.

- ❖ **Special media** such as Fildes agar and Levinthal's agar; containing factor X and V
- ❖ **Haemophilus selective medium** containing bacitracin and sucrose, is useful for sputum specimen



Figs 61.4A to D: **A.** Gram-stained smear showing pleomorphic gram-negative bacilli; **B.** Satellitism of *H. influenzae* around *S. aureus* streak line; **C.** Colonies of *H. influenzae* on chocolate agar; **D.** Colony smear showing pleomorphic gram-negative bacilli.

Source: A. Department of Microbiology JIPMER, Puducherry; B to D. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ It is largely aerobic and growth is poor anaerobically. Culture plates are incubated at 37°C in candle jar. Growth is enhanced by 5–10% CO₂.

Culture Smear and Motility Testing

Gram staining of culture isolates reveals pleomorphic gram-negative bacilli (Fig. 61.4D) and hanging drop reveals nonmotile bacilli.

Biochemical Tests

- ❖ Catalase positive and oxidase positive
- ❖ **Disk test for X and V requirement:** *Haemophilus* species vary in their X and V requirement. This property can be exploited for speciation by inoculating the isolate onto medium lacking X and V factors and then placing the X, V, and combined XV disks on the medium for demonstrating the growth requirements (Fig. 61.5)
 - Growth surrounding X and XV disks only: Produced by species that require X factor, but not V factor; examples include *H. ducreyi* and *A. aphrophilus*
 - Growth surrounding V and XV disks only: Produced by species that require X factor, but not V factor; examples include *H. parainfluenzae*, *H. parahaemolyticus* and *A. paraphrophilus*
 - Growth surrounding combined XV disk only: Produced by species that require both X and V factors; examples include: *H. influenzae*, *H. aegyptius* and *H. haemolyticus* (Fig. 61.5).

Typing Methods

- ❖ **Biotyping:** Strains of *H. influenzae* is typed based on biochemical properties into eight biotypes (I–VIII)
 - Most of the clinical isolates belong to type I, II and III
 - Majority of invasive type b strains belong to biotype I



Fig. 61.5: *H. influenzae* showing growth around combined XV disk only, but not around X and V disks.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- ❖ **Serotyping:** It is carried out by using type-specific antisera.

Molecular Method

- ❖ **Multiplex PCR:** It is useful in simultaneous detection of common agents of pyogenic meningitis such as pneumococcus, meningococcus, *Listeria* and *H. influenzae* (targeting conserved capsular gene *bexA*)
- ❖ **BioFire FilmArray:** It is an automated multiplex PCR; the respiratory panel can simultaneously detect 33 pathogens including *H. influenzae*.

Antimicrobial Susceptibility Testing (AST)

AST is performed by disk diffusion method (on *Haemophilus* test medium or chocolate agar) or by automated MIC detection method by microbroth dilution (e.g. VITEK).

TREATMENT

H. influenzae infections

- ❑ For invasive infections due to *H. influenzae* type b, cephalosporins such as ceftriaxone, cefotaxime (for 1–2 weeks) are the drug of choice. Alternatively ampicillin plus chloramphenicol may be used
- ❑ Nontypeable strains of *H. influenzae* are often resistant to β-lactams [due to β-lactamase production (20–35% of strains) or rarely by expressing altered penicillin binding protein-3]. Those strains are usually susceptible to quinolones (levofloxacin) and macrolides (azithromycin).

Prophylaxis

Hib Conjugate Vaccine

The polyribosyl ribitol phosphate (PRP) capsular antigen of *H. influenzae* type b is used for vaccination.

- ❖ As capsular antigens are poorly immunogenic to children, they are conjugated with adjuvants such as diphtheria toxoid, tetanus toxoid and *N. meningitidis* outer membrane proteins
- ❖ In addition to eliciting protective antibody, this vaccine can also reduce the rate of pharyngeal colonization with Hib
- ❖ **Schedule:** Under national immunization program, Hib vaccine is given in combination with DPT, hepatitis B (pentavalent vaccine) at 6, 10 and 14 weeks of birth. It is administered in IM route, at anterolateral side of mid-thigh
- ❖ **Protection:** More than 95% of infants develop protective antibody levels after receiving a primary series of 2 or 3 doses
- ❖ The widespread use of conjugate vaccines has dramatically reduced the incidence of Hib disease in the developed countries.

Chemoprophylaxis

Oral rifampin is the drug of choice. It is indicated to:

- ❖ Household contacts or
- ❖ Health care workers (if two or more cases occur in the hospital within 60 days).

STAPHYLOCOCCAL PNEUMONIA

Staphylococcus aureus causes pneumonia in selected clinical settings.

- ❖ **Infant pneumonia:** *S. aureus* is a leading cause of pneumonia in newborns and infants; presents with dyspnea, fever, and respiratory failure
 - Chest X-ray reveals characteristic **pneumatocele** (shaggy, thin-walled cavities)
 - Complications such as pneumothorax and empyema may be developed subsequently.
- ❖ **VAP in adults:** *S. aureus* can cause pneumonia in hospitalized patients who are on mechanical ventilation, called ventilator-associated pneumonia (VAP). Patients who are nasal carriers are at increased risk to develop VAP
- ❖ **Post-viral CAP:** Community-acquired pneumonia due to *S. aureus* often follow viral infections—most commonly influenza
- ❖ **CA-MRSA:** Community-associated MRSA strains can be more virulent and can cause necrotizing pneumonia. *Staphylococcus aureus* mainly causes skin and soft tissue infections, discussed in Chapter 51.

GRAM-NEGATIVE BACILLI PNEUMONIA

Gram-negative bacilli are increasingly associated to cause lobar pneumonia, particularly VAP in the healthcare facility. Most of these agents are multidrug resistant (MDR) pathogens found in the hospital environment.

- ❖ **Non-fermenters** such as *Pseudomonas aeruginosa*, *Acinetobacter*, *Burkholderia cepacia*. They are the major cause of VAP, discussed in Chapter 65
- ❖ **Enterobacteriaceae:** Various members of the family Enterobacteriaceae can cause pneumonia such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* species and *Serratia marcescens*. *E. coli* causes various other infections such as UTI, diarrhea, etc., therefore discussed elsewhere. Other agents are discussed here.

Enterobacteriaceae

1. *Klebsiella pneumoniae*

Klebsiella are usually found as commensals in human intestine and as saprophytes in soil.

- ❖ It has three species—*K. pneumoniae*, *K. oxytoca*, and *K. granulomatis*
- ❖ *K. pneumoniae* further comprises of three subspecies—*pneumoniae*, *ozaenae* and *rhinoscleromatis*. ***K. pneumoniae* subspecies *pneumoniae*** is the most pathogenic among all.
- ❖ It is responsible for severe lobar pneumonia, urinary tract infections, meningitis (neonates), septicemia and pyogenic infections such as abscesses and wound infections
- ❖ It frequently colonizes the oropharynx of the hospitalized patients and is a common cause of nosocomial infections. Most of the hospital strains are multidrug resistant
- ❖ Pneumonia tends to be destructive with production of thick, mucoid, brick red sputum. Some time, the sputum has a thin and currant jelly-like appearance
- ❖ Some strains can rarely cause diarrhea and have been shown to produce an *E. coli* like heat stable enterotoxin.

Hypervirulent Strains of *K. pneumoniae* (hvKp)

It is a strain of *K. pneumoniae*, which is recently emerged as a pathogen of global concern.

- ❑ **Hypervirulence:** hvKp is more virulent than classical *K. pneumoniae*; possesses several virulence factors such as siderophore, increased capsule production, etc. Two capsular serotypes are there K1 (more common) and K2
- ❑ **Manifestations:** hvKp is capable of causing various community-acquired infections such as:
 - Pyogenic liver abscess (most common)
 - Metastasize from liver to distant sites, such as eye, lung and CNS
 - It can also cause primary extrahepatic infections including bacteremia, pneumonia and soft tissue infections
- ❑ **Identification:** hvKp strains are hypermucoid— a viscous string is formed when a loop is used to stretch the colony on an agar plate (Fig. 61.6C).



Figs 61.6A to C: **A.** Direct smear (sputum) of *Klebsiella pneumoniae*; showing pus cells with gram-negative bacilli with clear halo (capsule) (arrows showing), **B.** *Klebsiella* on MacConkey agar (Mucoid dome-shaped pink-colored lactose-fermenting colonies); **C.** Hypervirulent *Klebsiella* showing string test positive.

Source: A. Department of Microbiology, JIPMER, Puducherry (with permission); B and C. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Laboratory Diagnosis

Klebsiella belongs to the family Enterobacteriaceae. Similar to *E. coli*, *Klebsiella* species are also lactose fermenters; however, they differ in being non-motile and capsulated (possess capsular polysaccharide).

Specimens collected depend upon the site of infection such as sputum, endotracheal aspirate, urine, blood, exudate specimen.

K. pneumoniae shows the following properties:

- ❖ **Gram staining:** It is short, plump, straight capsulated gram-negative rods (Fig. 61.6A)
- ❖ **Culture:** On MacConkey agar, it produces large dome-shaped mucoid (due to capsule) sticky, pink color, lactose fermenting colonies (Fig. 61.6B)
- ❖ **Identification:** Identification of *K. pneumoniae* from colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as described below
 - It is catalase positive and oxidase negative
 - **ICUT tests:** Indole test (negative), citrate test (positive), urease test (positive) and TSI (triple sugar iron agar) test shows acid/acid, gas present, H₂S absent.

TREATMENT

Klebsiella infections

Treatment of *Klebsiella* is global challenge owing to marked drug resistance, which is much higher than that of *E. coli*.

- In healthcare facilities with high prevalence of MDR *K. pneumoniae*, empirical treatment should be started with higher spectrum antimicrobial. The therapy can be tailored based on the susceptibility report
- Carbapenems, amikacin or β-lactam/β-lactamase inhibitor combinations (BL/BLIs) such as piperacillin-tazobactam or cefoperazone-sulbactam are usually the agents of choice for hospital acquired MDR infections. Polymyxins or tigecycline are the next line antimicrobials, used for carbapenem resistant isolates.

Resistance in *Klebsiella pneumoniae*

- It is intrinsically resistant to ampicillin and ticarcillin
- **MDR:** Majority of *Klebsiella* strains acquired in the hospitals are multi-drug resistant (MDR); resistant to cephalosporins, quinolones, co-trimoxazole, etc. However, if found susceptible, then these agents may be preferred.
- **ESBL:** In India (2019), 40–90% of strains of *K. pneumoniae* are ESBL (extended spectrum β-lactamases) producers
- **Carbapenem resistance:** In India, carbapenem resistance in *K. pneumoniae* is reported to be around 28–56%; out of which 37% are colistin resistant. Carbapenem resistance is mainly due to production of carbapenamases such as:
 - New Delhi β-lactamase (NDM)
 - *K. pneumoniae* carbapenamase (KPC).

Other *Klebsiella* species cause infection of other sites and therefore discussed elsewhere.

- ❖ *K. pneumoniae* subspecies *ozaenae* and *rhinoscleromatis* are pathogens of nasal cavity and have been discussed in Chapter 59

- ❖ The infections produced by *K. oxytoca* are similar to that of *K. pneumoniae* subspecies *pneumoniae*

- ❖ *Klebsiella granulomatis*: It is the agent of a sexually transmitted disease called granuloma inguinale or donovanosis. It is described in detail in Chapter 77.

2. *Enterobacter* species

Enterobacter species have become increasingly important nosocomial pathogens.

- ❖ *E. aerogenes* and *E. cloacae* are the most commonly isolated species from the clinical specimens. *E. aerogenes* has been recently renamed as *Klebsiella aerogenes*
- ❖ They are widely distributed in water, sewage, soil and feces of healthy persons
- ❖ They are opportunistic pathogens, implicated in infected wounds, urinary and respiratory tract infections (pneumonia) and occasionally can cause septicemia and meningitis
- ❖ *E. sakazaki* (recently named as *Cronobacter sakazakii*) can cause bacteremia and meningitis in neonates and is associated with consumption of powdered milk
- ❖ **Identification** is made either by automated systems such as MALDI-TOF or VITEK; or by conventional biochemical tests
- ❖ Most of the *Enterobacter* isolates are multi-drug resistant; and the guideline for treatment is the same as that for *E. coli* and *Klebsiella*
- ❖ *Enterobacter* is intrinsically resistant to amoxicillin-clavulanate and ampicillin-sulbactam.

3. *Serratia marcescens*

Serratia marcescens is the medically most important species. Human infection with other species is rare. It is categorized into two groups.

Pigmented Group

They produce characteristically a red non-diffusible pigment called **prodigiosin** (Fig. 61.7).

They are saprophytes found in water, soil and food. They may grow in sputum after collection and make the sputum red (due to pigment production). This condition is known as 'pseudohemoptysis'.

Non-pigmented Group

Non-pigmented group of *S. marcescens* is being increasingly reported in various **healthcare-associated infections**, such as lobar pneumonia, meningitis, endocarditis, septicemia, urinary, and wound infections.

TREATMENT

Serratia infections

The hospital strains are often multiple drug resistant (produce AmpC β-lactamases). The guideline for treatment is same as that for *E. coli* and *Klebsiella* except that, it is intrinsically resistant to a number of antibiotics such as ampicillin, first and second generation cephalosporins, amoxicillin-clavulanate, ampicillin-sulbactam, nitrofurantoin and polymyxins.

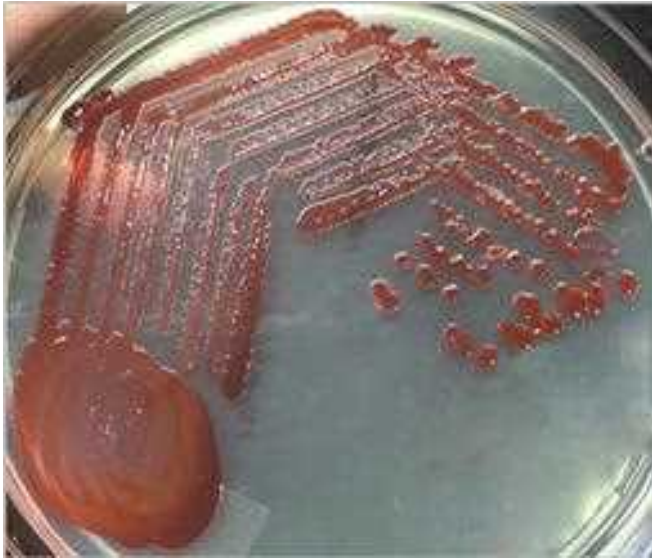


Fig. 61.7: *Serratia marcescens* (red-colored colonies).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

RARE CAUSES OF BACTERIAL PNEUMONIA

Pulmonary Anthrax

Pulmonary anthrax, also called as Wool Sorter's disease is caused by *Bacillus anthracis*. It is a rare form of anthrax. Major form of anthrax is cutaneous anthrax, discussed in Chapter 55.

- ❖ **Transmission** occurs via inhalation of spores during exposure to contaminated animal products
- ❖ Also called as **Wool sorter's disease**, as it is seen in workers of a wool factory who acquire the infection by inhalation of dust from infected wool
- ❖ **Bioterrorism:** Pulmonary anthrax is the most common form of anthrax to be associated with bioterrorism
- ❖ **Incubation period** is of 1–7 days, but up to 6 weeks or longer is reported
- ❖ **Hemorrhagic pneumonia:** Presents initially with prodromal stage of fever, myalgia and lethargy, followed by abrupt onset of hemorrhagic pneumonia leading to respiratory failure
- ❖ **Spread:** Can spread by lymphatics or blood, leading to—severe sepsis, hemorrhagic mediastinitis and hemorrhagic meningitis
- ❖ **Chest X-ray** shows mediastinal widening and/or pleural effusions
- ❖ **Prognosis** is usually poor, often fatal.

TREATMENT

Pulmonary anthrax

Treatment comprises of antibiotic regimen along with anthrax immunoglobulin.

- ❑ **Antibiotic regimen** comprises of induction and maintenance therapy

Contd...

Contd...

TREATMENT

Pulmonary anthrax

- **Induction therapy:**
 - ◆ If meningitis is suspected: Ciprofloxacin + meropenem + linezolid for 2–3 weeks
 - ◆ If meningitis is excluded: Ciprofloxacin + clindamycin or linezolid for 2 weeks
- **Maintenance therapy:** Oral ciprofloxacin or doxycycline is continued to complete 60 days course
- ❑ **Anthrax immunoglobulin:** Raxibacumab and Obiltoximab are monoclonal antibodies that neutralizes anthrax toxin (protective antigen).

Pneumonic Plague

It is caused by a zoonotic pathogen *Yersinia pestis*.

Human plague occurs in three clinical forms—(1) bubonic (most common form), (2) pneumonic, and (3) septicemic.

- ❖ **Transmission:** In contrast to bubonic plague (transmitted by rat flea); pneumonic plague results from inhalation of bacilli in droplets expelled from another person or an animal with plague pneumonia
- ❖ **Incubation period** is shorter than bubonic plague, about 1–3 days
- ❖ **Manifestations:** The onset is sudden and is characterized by fever, headache and respiratory symptoms—productive cough or hemoptysis, dyspnea, and chest pain
- ❖ **Prognosis:** Though pneumonic plague is rare (<1%), it is highly infectious and highly fatal
- ❖ **Agent of bioterrorism:** Aerosolized *Y. pestis* is a possible source of bioterrorism attack, especially in non-endemic regions
- ❖ **Treatment:** Gentamicin or streptomycin are given for treatment.

Plague is discussed in detail in Chapter 81.

Other bacterial agents that can occasionally cause pneumonia include:

- ❖ **Nocardia:** Lobar pneumonia is the most common pulmonary form, characterized by subacute onset of cough with thick, purulent sputum. It may rarely spread directly to adjacent tissues, leading to pericarditis, mediastinitis, laryngitis, tracheitis and bronchitis (Chapter 55)
- ❖ **Actinomyces:** It usually causes skin and soft tissue infection (Chapter 55). Pulmonary actinomycosis is rare, results from hematogenous spread. It is characterized by formation of multiple nodules in the lungs
- ❖ **Streptococcus pyogenes:** It is an upper respiratory tract pathogen (Chapters 52 and 60), can occasionally cause pneumonia in previously healthy individuals. The common manifestations are chest pain (due to pleural effusion), fever, chills, and dyspnea. Cough is present, but less prominent

- ❖ ***Streptococcus agalactiae***: Infants with early-onset disease due to *S. agalactiae* are bacteremic, one-third to one-half develop pneumonia and/or respiratory distress syndrome. *S. agalactiae* can also cause pneumonia in adults, although less common (Chapter 52)
- ❖ ***Corynebacterium diphtheriae***: It is an upper respiratory tract pathogen; pneumonia is a rare complication (Chapter 60)
- ❖ **Nontyphoidal salmonellae**: They can cause lobar pneumonia, and its complications such as lung abscess and empyema. The majority of cases occur in patients with lung cancer, structural lung disease, sickle cell disease, or glucocorticoid use (Chapter 41)
- ❖ ***Aeromonas hydrophila*** is also a known etiological agent of lobar pneumonia (Chapter 42)
- ❖ ***Rhodococcus equi***: It is gram-positive coccobacilli, is associated with necrotizing pneumonia and granulomatous infection, particularly in immunocompromised individuals
- ❖ ***Moraxella catarrhalis***: It is a harmless commensal of upper respiratory tract and genital tract
 - **Pathogenesis**: It causes opportunistic lower respiratory tract infections, especially in adults with chronic obstructive airway disease
 - It has also been isolated in cases of otitis media, less commonly in meningitis, endocarditis and sinusitis
 - **Morphology**: Gram-negative diplococci, 0.6–1 µm oval with flattened adjacent sides
 - **Culture**: It grows on basal medium like nutrient agar. Identification is confirmed by automated systems such as MALDI-TOF or by various biochemical reactions
 - Some strains of *M. catarrhalis* secrete beta-lactamases which destroy penicillin that makes β-lactam antibiotics ineffective to meningococci and other penicillin-sensitive bacteria of the respiratory tract
 - *Moraxella* has another species, *M. lacunata*, which causes angular conjunctivitis (Chapter 78).

EXPECTED QUESTIONS

I. Write essay on:

1. Alisha, A 4-year-old girl from Bhubaneswar was brought to the emergency room by her parents due to an acute onset of fever, productive cough and dyspnea for past two days. Physical examination revealed dull note on percussion. Direct examination of the sputum revealed plenty of pus cells and gram-positive, lanceolate-shaped diplococci surrounded by a halo.
 - a. What is your clinical diagnosis of this condition and the most likely etiologic agent?
 - b. How will you confirm the etiological agent in the laboratory?
 - c. Describe the virulence factors and pathogenesis of the etiological agent.
 - d. How will you manage this clinical condition?

II. Write short notes on:

1. Pneumococcal vaccines.
2. Pulmonary anthrax.
3. Hypervirulent *Klebsiella*.
4. 2-year-orphan boy presented with fever, cough and dyspnea for past 4 days. Sputum culture revealed colonies near *S. aureus* streak line on a blood agar. What is the etiological diagnosis and mention how will you prevent this condition.

III. Multiple Choice Questions (MCQs):

1. **Which of the following agent of meningitis can grow on chocolate agar but not on blood agar?**
 - a. *Neisseria meningitidis*
 - b. *Haemophilus influenzae*
 - c. *Moraxella catarrhalis*
 - d. *Escherichia coli*
2. ***Haemophilus influenzae* grows on all of the following media, except:**
 - a. Chocolate agar
 - b. Fildes' agar
 - c. Nutrient agar
 - d. Blood agar with *S. aureus* streak line
3. **Polyribosyl ribitol phosphate (PRP) antigen is present in the capsule of *H. influenzae*:**
 - a. Serotype a
 - b. Serotype b
 - c. Serotype c
 - d. Serotype d
4. **Next to Hib, which strains are the most common group of *H. influenzae* encountered clinically?**
 - a. Serotype a strains
 - b. Serotype c strains
 - c. Serotype d strains
 - d. Nontypeable strains
5. **Wool Sorter's disease is caused by:**
 - a. *Bacillus anthracis*
 - b. *Staphylococcus aureus*
 - c. *Yersinia pestis*
 - d. *Rhodococcus equi*

Answers

1. b 2. c 3. b 4. d 5. a

Bacterial Atypical (Interstitial) Pneumonia

CHAPTER 62

CHAPTER PREVIEW

- *Mycoplasma pneumoniae*
- Chlamydiae pneumonia
- Legionellosis
- Other Bacterial Atypical Pneumonia

INTRODUCTION

Atypical (or interstitial) pneumonia refers to infection occurring in the interstitial space of lungs. Cough is characteristically non-productive. The common agents include:

- ❖ **Respiratory viruses** such as influenza viruses (Chapter 66), corona viruses (Chapter 67) respiratory syncytial virus (Chapter 66) and others such as Epstein-Barr virus and adenoviruses (Chapter 68)
- ❖ **Bacterial agents:** The bacterial agents causing atypical pneumonia are:
 - *Mycoplasma pneumoniae*: It is also called as “walking pneumonia” (as symptoms tend to be milder than others agents causing pneumonia)
 - *Chlamydiae*: *Chlamydophila pneumoniae*, *C. psittaci*, *Chlamydia trachomatis* serotypes D to K
 - *Legionella* species: Causes Legionnaires’ disease
 - Less common bacterial agents: *Coxiella burnetii* (Q fever), *Francisella tularensis* (pulmonary tularemia), and *Orientia tsutsugamushi* (scrub typhus).

It is important to differentiate atypical pneumonia from typical lobar pneumonia (described in Chapters 59 and 61) as the antibiotics used for their treatment are different. However, there is considerable overlap; many typical agents can be atypical in their presentation and vice-versa.

This chapter will mainly focus on the bacterial agents causing atypical pneumonia.

MYCOPLASMA PNEUMONIA

Mycoplasma pneumoniae is one of the common bacterial agent causing atypical pneumonia; the disease is described as primary atypical pneumonia.

General Properties

Mycoplasmas are the smallest microbes capable of free-living in the environment and self-replicating on artificial culture media. They have the following characteristics as mentioned below:

- ❖ They resemble the viruses in certain properties such as:
 - Size: They are very small, 150–350 nm in size

- They are filterable by bacterial filters.
- ❖ They differ from viruses as:
 - They are **free living** in the environment
 - They can grow on artificial cell-free culture media.
- ❖ They **lack a rigid cell wall**, which is replaced by a triple-layered cell membrane containing sterol. Therefore, they are completely resistant to antibiotics acting on cell wall such as β -lactams
- ❖ **Pleomorphic:** They are highly pleomorphic, exist in coccoid, bacillary or filamentous or even in helical forms (*Spiroplasma*)
- ❖ They are poorly gram-negative, better stained by Giemsa stain
- ❖ They reproduce by binary fission and budding
- ❖ They are non-sporing and non-flagellated, usually non-motile. However, **gliding motility** is described in some species which is due to their specialized tip structures
- ❖ **Contaminants of cell cultures:** Mycoplasmas are common contaminants of continuous cell lines, thus interfere with the growth of viruses in cell cultures
- ❖ **L-form:** As mycoplasmas lack cell wall permanently, it has been suggested that mycoplasmas may represent stable L-forms of bacteria; but genetic, antigenic and biochemical properties do not support this hypothesis.

History

- ❖ The name ‘*Mycoplasma*’ is derived from *Myco* meaning fungus-like, forming branching filaments and *plasma*, denoting their plasticity of shape
- ❖ They were previously called as **pleuropneumonia-like organisms (PPLO) and Eaton’s agent** (after the Monroe Eaton, who first isolated).

Classification

- ❖ Family Mycoplasmataceae comprises of two genera— (1) *Mycoplasma*, and (2) *Ureaplasma*. The pathogenic species infecting humans are:
 - *Mycoplasma pneumoniae*: Causes atypical pneumonia; discussed in detail below

- Other species cause urogenital infections (Chapter 77), such as:
 - ◆ *Mycoplasma hominis*
 - ◆ *Mycoplasma genitalium*
 - ◆ *Ureaplasma urealyticum*
 - ◆ *Ureaplasma parvum*.

Pathogenesis

Attachment of *M. pneumoniae* to the respiratory mucosa is the most important step in pathogenesis, which is mediated by its membrane bound adhesion proteins (e.g. cytoadhesin P1 protein). Following which, it induces injury to host respiratory tissue.

Epidemiology

Mycoplasma pneumoniae infection occurs worldwide.

- ❖ **Transmission:** *M. pneumoniae* is transmitted by respiratory droplets
- ❖ **Facilitating factors:** The transmission is favored by close contacts as in families, military bases, boarding schools, and summer camps
- ❖ Infections tend to be endemic, with periodic epidemics every 4–7 years
- ❖ Incubation period is about 2–4 weeks.

Clinical Manifestations

M. pneumoniae produces various infections; which are as follows:

Upper Respiratory Tract Infections (URTI)

URTI manifests as pharyngitis, tracheobronchitis or rarely as otitis media. It is acute in onset and is 20 times more common than pneumonia.

Pneumonia

M. pneumoniae causes “atypical” community acquired interstitial pneumonia similar to pneumonia caused by other agents, such as *Chlamydomphila pneumoniae*, *Legionella pneumophila* and viral pneumonia.

- ❖ This is also referred to as Eaton agent pneumonia or primary atypical pneumonia or walking pneumonia (as symptoms tend to be milder than pneumonia due to other agents)
- ❖ Pneumonia develops in 3–13% of infected individuals; its onset is usually gradual
- ❖ It is characterized by wheeze or rales, dry cough and peribronchial pneumonia with thickened bronchial markings and streaks of interstitial infiltration on chest X-ray
- ❖ Pneumonia usually is mild and self-limited, but can progress into severe disease with acute respiratory distress syndrome (ARDS).

Extrapulmonary Manifestations

They are rare, occur either as a result of active *Mycoplasma* infection (e.g. septic arthritis) or due to postinfectious

autoimmune phenomena (e.g. Guillain–Barré syndrome). Various manifestations include:

- ❖ **Neurologic:** Meningoencephalitis, encephalitis, Guillain–Barré syndrome and aseptic meningitis
- ❖ **Dermatologic:** Skin rashes including erythema multiforme major (Stevens–Johnson syndrome)
- ❖ **Cardiac:** Myocarditis, pericarditis
- ❖ **Rheumatologic:** Reactive arthritis
- ❖ **Hematologic:** Anemia and hypercoagulopathy.

LABORATORY DIAGNOSIS *Mycoplasma pneumoniae*

- ❑ **Specimen:** Throat swabs, nasopharyngeal aspirates bronchial brushing, BAL and lung biopsies
- ❑ **Culture:**
 - Solid medium containing PPLO agar: Produces fried egg appearance colonies
 - Liquid medium containing PPLO broth: Produces turbidity and a color change
- ❑ **Identification:** Accurate species identification from colonies is made by biochemical tests or by automated identification systems such as MALDI-TOF
- ❑ **Antigenic detection:** By Direct IF, antigen capture ELISA
- ❑ **Antibody detection in serum:**
 - Specific: Indirect-IF, latex agglutination assays and ELISA using protein P1 antigens
 - Nonspecific: Cold agglutination test and *Streptococcus* MG test
- ❑ **Molecular methods:** Detects 16S rRNA and P1 adhesin gene.

Laboratory Diagnosis

Specimen Collection and Transport

Ideal specimens are throat swabs, nasopharyngeal aspirates, bronchial brushing, bronchoalveolar lavages (BAL) and lung biopsies.

- ❖ Specimens must be placed immediately into the following transport media to avoid drying:
 - **Standard *Mycoplasma* fluid medium** containing fetal bovine serum, gelatine and penicillin
 - **Viral transport medium**, added with ampicillin and cefotaxime.
- ❖ Transportation should be immediate. If delay is expected, then specimens should be stored at 4°C for 48 hours and beyond that at –70°C.

Culture

Specimens are inoculated in culture media (such as PPLO agar or PPLO broth) and incubated at 37°C for 5–7 days or sometimes even up to 1–3 weeks.

- ❖ **In liquid medium** (containing PPLO broth): *M. pneumoniae* growth is detected by turbidity and a color change
- ❖ **In solid medium** (containing PPLO agar): The colonies appear very small (200–500 µm size), embedded on agar surface described as **fried egg appearance** colonies (Fig. 62.1)
- ❖ The colonies can be examined by hand lens or use of special stain such as Dienes’ staining (nonspecific stain that imparts color to *Mycoplasma* colonies on agar)

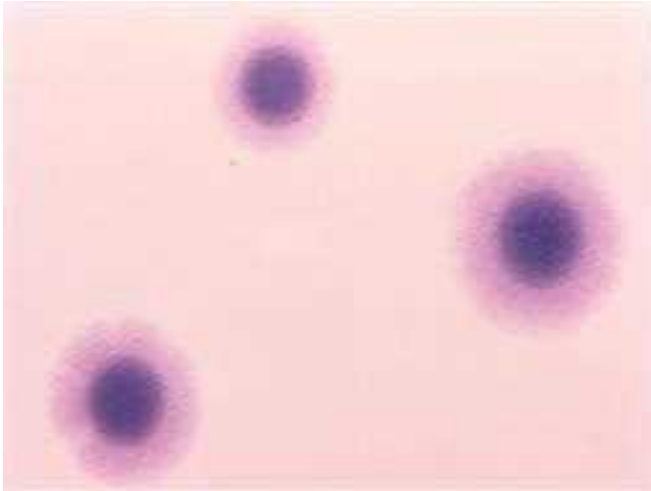


Fig. 62.1: *Mycoplasma* colonies (typical fried egg appearance).

Source: Public Health Image Library, ID# 11024/Dr E Arum; Dr N Jacobs/ Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ Culture of respiratory specimens has a sensitivity of $\leq 60\%$ and specificity of 100%.

Identification

Accurate species identification from colonies is made either by conventional biochemical test or by automated identification systems such as MALDI-TOF.

Antigenic Detection

- ❖ Direct immunofluorescence test: Detects *Mycoplasma* antigens directly in the clinical specimens
- ❖ Capture ELISA assay is available using monoclonal antibodies against P1 adhesion antigen.

Antibody Detection in Serum

Specific Antibody Detection Tests

Antibodies specific to *M. pneumoniae* protein and glycolipid antigens can be detected after about 1 week of illness, and peak at 3–6 weeks and then decline gradually. Serological assays have a sensitivity and specificity of 55–100%.

IgM antibodies are frequently elevated in children with acute infection whereas in adults, IgA-antibody detection remains the method of choice for the diagnosis of acute infection.

Various specific antibody detection tests are as follows:

- ❖ Immunofluorescence assays
- ❖ Latex agglutination assays
- ❖ ELISA using protein P1 antigens.

Nonspecific Antibody Detection Tests

Heterophile agglutination tests such as **Cold agglutination test** and ***Streptococcus MG* tests** were in use in the past; now obsolete as they are neither specific nor sensitive (positive only in 30–50% of cases).

Molecular Methods

- ❖ **PCR** targeting *M. pneumoniae* specific 16S rRNA gene and P1 adhesion gene is available. PCR has a sensitivity of 65–90% and specificity of 90–100%
- ❖ **Multiplex PCR** has been developed detecting the common agents of atypical pneumonia—*M. pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*
- ❖ **Real-time PCR:** It is useful for quantitative detection of *M. pneumoniae*
- ❖ **BioFire FilmArray:** It is an automated multiplex PCR, the respiratory panel can simultaneously detect 22 pathogens including *M. pneumoniae*.

TREATMENT

Mycoplasma pneumoniae

- ❑ Macrolides are drug of choice (oral azithromycin, 500 mg on day 1, then 250 mg on days 2 to 5)
- ❑ Alternative drugs are as follows:
 - Doxycycline
 - Respiratory fluoroquinolones such as levofloxacin, moxifloxacin and gemifloxacin (not ciprofloxacin).

CHLAMYDIAE PNEUMONIA

There are three *Chlamydia* species (Chapter 77)—*C. pneumoniae*, *C. psittaci*, *C. trachomatis*; all capable of causing atypical (interstitial) pneumonia.

Psittacosis (*Chlamydia psittaci*)

C. psittaci is a pathogen of parrots and other psittacine birds causing psittacosis.

- ❖ **Reservoirs:** Pet birds (parrots, parakeets, macaws, and cockatiels) and poultry (turkeys and ducks) act as natural reservoir of infection and are involved in transmission of infection to humans
- ❖ **Mode of transmission:** *C. psittaci* can be transmitted to humans by inhalation of aerosols from avian nasal discharges and from infectious avian fecal or feather dust
- ❖ **Clinical manifestations:** Incubation period ranges from 5–19 days. It can present as—(1) Respiratory manifestation (most common form), varies from a mild influenza-like syndrome to an interstitial pneumonia, (2) rarely septicemia and typhoid-like syndrome
- ❖ **Treatment:** Tetracycline is the drug of choice, given 250 mg four times a day for at least 3 weeks to avoid relapse. Erythromycin (500 mg four times a day, per oral) is given alternatively.

Chlamydia pneumoniae

C. pneumoniae is an exclusively human pathogen. It is transmitted from person to person by inhalational route. It causes various manifestations.

- ❖ **Atypical pneumonia:** *C. pneumoniae* is a common cause of atypical (interstitial) pneumonia accounting for 10% of cases of community-acquired pneumonia
- ❖ **Upper respiratory tract involvement** is frequent, such as pharyngitis and sinusitis

- ❖ **Atherosclerosis:** There is a strong evidence of association between *C. pneumoniae* and atherosclerosis of coronary and other arteries
- ❖ **Asthma and COPD:** *C. pneumoniae* may cause exacerbations of bronchial asthma and COPD (chronic obstructive pulmonary disease)
- ❖ **Treatment:** Tetracycline or erythromycin (500 mg four times a day) is recommended for 10–14 days.

Chlamydia trachomatis (Infant Pneumonia)

C. trachomatis serotypes D to K can cause infant pneumonia.

- ❖ It is an interstitial pneumonia that develops within 3 weeks to 3 months of birth
- ❖ Infection spreads from conjunctiva to pharynx via the nasolacrimal duct
- ❖ Infection via the eustachian tube may cause otitis media
- ❖ **Treatment:** Erythromycin is given orally at a dosage of 50 mg/kg per day in four divided doses, for 2 weeks.

C. trachomatis mainly causes urethritis and ocular infections, discussed in Chapters 77 and 78. General laboratory diagnosis chlamydial infection is discussed in detail in Chapter 77.

LEGIONELLOSIS

Legionellae are fastidious, pleomorphic gram-negative, short rods, associated with interstitial pneumonia, known as—Legionnaires' disease.

History

Legionella was first recognized in 1976 when an outbreak of pneumonia took place at a Philadelphia hotel during an American Legion convention.

Classification

- ❖ Out of several species infecting man, *L. pneumophila* is the most important species, associated with 80–90% of human infections. It consists of 15 serogroups. Majority of cases are associated with **serogroup 1** followed by 4 and 6
- ❖ Other species are rarely associated with human infection particularly in immunocompromised state, such as *L. micdadei* (Pittsburgh pneumonia agent), *L. wadsworthii* and *L. longbeachae*.

Epidemiology

- ❖ **Reservoir:** *Legionella* inhabits on aquatic bodies which could be either:
 - Natural water sources, such as rivers, streams or even inside amoeba
 - Artificial aquatic sources, such as air conditioners, water coolers, shower head, large plumbing system and decorative fountains
 - *L. longbeachae* has been isolated from natural soil and commercial potting soil
 - There is no animal reservoir
 - There is no carrier stage.

- ❖ **Transmission:** Multiple routes have been proposed
 - Aspiration (predominant mode): It occurs either via oropharyngeal colonization or directly via drinking of contaminated water
 - Aerosols from contaminated air conditioners, nebulizers, and humidifiers
 - Direct instillation into the lungs during respiratory tract manipulations
 - There is no man-to-man transmission.
- ❖ **Predisposing factors for *Legionella* infections include:**
 - Smoking, alcoholism or chronic lung disease (like chronic obstructive pulmonary disease or emphysema)—impair mucociliary clearance
 - Advanced age
 - Immunosuppression-transplantation, HIV infection, steroid therapy
 - Prior hospitalization
 - Patients with nasogastric tubes or those undergoing surgery with general anesthesia promotes aspiration.

Pathogenesis

Following entry, legionellae reach the lungs where they are engulfed by alveolar macrophages by a mechanism called as **coiling phagocytosis**. They invade and grow within the alveolar macrophages by inhibiting phagosome-lysosome fusion. Being intracellular organism, cellular immunity is responsible for the recovery.

Clinical Manifestations

Pontiac Fever

It is an acute, flu-like illness characterized by malaise, fever, and headache. Incubation period is about 24–48 hours. It is self-limiting, never develops into pneumonia.

Legionnaires' Disease (Pneumonia)

It is an interstitial atypical pneumonia with incubation period about 2–10 days.

- ❖ It is characterized by non-productive cough (with or without blood tinged), dyspnea, chest pain, high fever and diarrhea
- ❖ Chest X-ray shows pulmonary infiltrates
- ❖ *Legionella* is among the leading causes of pneumonia both in the community and hospital settings.

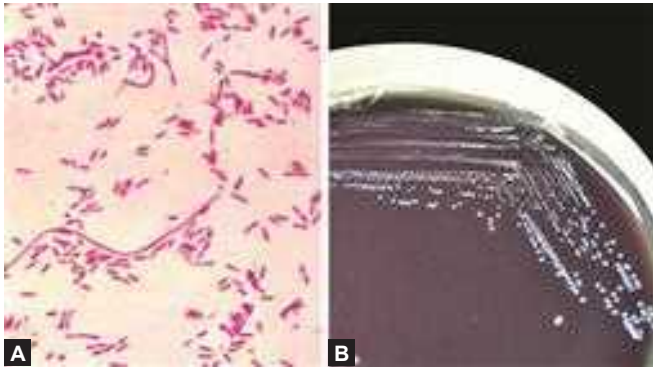
Extrapulmonary Legionellosis

Usually it results from blood-borne dissemination from the lung.

- ❖ The most common extrapulmonary site is heart (myocarditis, pericarditis and prosthetic valve endocarditis)
- ❖ Other manifestations include sinusitis, peritonitis, pyelonephritis, skin and soft tissue infection.

Laboratory Diagnosis

Useful specimens for Legionnaires' disease include sputum, bronchoalveolar lavage fluid, bronchial wash and pleural fluid.



Figs 62.2A and B: *Legionella*: **A.** Gram-staining; **B.** Growth on BCYE agar.

Source: Public Health Image Library, A. ID#: 15328/ Dr Gilda Jones; B. ID#: 11766/ Megan Mathias and J. Todd Parker/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

❖ Direct microscopy:

- **Gram stain** reveals numerous neutrophils but no organisms (as legionellae are poorly stained, often missed or sometimes appear as faint pleomorphic gram-negative rods or coccobacilli) (Fig. 62.2A)
- Silver impregnation and Giemsa stains can be used
- Direct immunofluorescence test using monoclonal or polyclonal sera is more specific but sensitivity is poor than culture. It is more useful in advanced stage of disease
- Acid-fast staining: *L. micdadei* is weakly acid fast.
- ❖ **Culture:** Culture is highly sensitive (80–90%) and specific (100%) and provides definite diagnosis
 - **Buffered charcoal, yeast extract (BCYE) agar:** Legionellae are highly fastidious and grow on complex media, such as **BCYE agar** after 3–5 days of incubation at 37°C in 5% CO₂ (Fig. 62.2B)
 - **Identification:** Species identification of *Legionella* from colonies is made either by conventional biochemical tests or by automated identification systems such as MALDI-TOF.
- ❖ **Antibody detection:** Primarily, serology is used for epidemiologic purpose (sero-prevalence estimation)
 - Indirect immunofluorescent antibody test and enzyme immunoassays are available
 - Antibodies usually appear late after 12 weeks; a single titer of 1:256 gives presumptive evidence of Legionnaires' disease

- Cross-reactivity has been observed with other *Legionella* species.
- ❖ **Urinary antigen test:** Enzyme immunoassays are available to detect *L. pneumophila* serogroup 1 specific soluble antigens in urine. Advantages include as follows:
 - It is rapid, cheaper, easy to perform
 - It is highly sensitive (70 to 90%), and specific (95–100%)
 - Antigen in urine is detectable shortly after the onset of symptoms and disappears over 10 months
 - The test is not affected by prior antibiotic administration.
- ❖ **Molecular methods:** BioFire FilmArray; automated multiplex PCR is available. Its respiratory panel can simultaneously detect 22 pathogens including *L. pneumophila*.

T TREATMENT

Legionnaires' disease

Macrolides (especially azithromycin) and the respiratory quinolones (e.g. levofloxacin) are now the antibiotics of choice.

Water Surveillance for Legionella

- ❖ **Indication:** Routine water surveillance for *Legionella* is not recommended; but should be performed annually or more often for certain high-risk settings, such as transplant units
- ❖ **Procedure:** Large volume of water (1–10 L) are collected and subjected to membrane filtration, followed by culture on BCYE media.

OTHER BACTERIAL ATYPICAL PNEUMONIA

- ❖ **Coxiella burnetii:** It causes Q fever, transmitted by inhalational mode. Interstitial pneumonia is a common presentation in acute Q fever (Chapter 31)
- ❖ **Rickettsial infections:** Interstitial pneumonia may be a rare complication seen in various rickettsial infections such as scrub typhus, epidemic typhus, endemic typhus and Rocky Mountain spotted fever. It occurs in severe cases, due to vascular injury (Chapter 31)
- ❖ **Francisella tularensis** is the causative agent of 'tularemia' primarily a zoonotic disease (Chapter 81). **Pulmonary tularemia** can result from aerosol inhalation (laboratory workers) or can spread to the lungs following bacteremia. Patients present with atypical pneumonia.

EXPECTED QUESTIONS

I. Write short notes on:

1. *Mycoplasma pneumoniae*.
2. Psittacosis.
3. Legionnaires' disease.

II. Multiple Choice Questions (MCQs):

1. The agent of primary atypical pneumonia is:
 - a. *Legionella pneumophila*

- b. *Klebsiella pneumoniae*
- c. *Mycoplasma pneumoniae*
- d. *Streptococcus pneumoniae*

2. Which of the following is associated with pathogenesis of atherosclerosis?

- a. *M. pneumoniae*
- b. *C. psittaci*
- c. *C. pneumoniae*
- d. *Legionella*

Answers

1. c 2. c

Tuberculosis and Nontuberculous Mycobacteria Infections

CHAPTER 63

CHAPTER PREVIEW

■ Tuberculosis

■ Nontuberculous Mycobacteria Infections

INTRODUCTION

Mycobacteria comprise of several species which produce various infections ranging from pulmonary, extrapulmonary, and cutaneous infections. They exhibit the following common properties:

- ❖ **Acid fastness:** The unique property of Mycobacteria is 'acid-fast', i.e. they resist decolorization by dilute mineral acids
 - Acid fastness is due to—(1) presence of high content of mycolic acids in the cell wall, and (2) integrity of the cell wall
 - This property is used as an important identification feature in the diagnostic laboratories.
- ❖ They are non-motile, non-sporing, non-capsulated, weakly gram-positive, straight or slightly curved rod-shaped bacteria, which are obligate aerobes
- ❖ They sometimes show **branching filamentous forms** resembling fungal mycelium (*myces* meaning fungus, reflecting the mold-like pellicle formation on liquid media).

History

Robert Koch (1882) isolated the tubercle bacillus and proved its causative role in tuberculosis as it satisfies the Koch's postulates.

Classification

Mycobacteria can be classified into:

- ❖ ***M. tuberculosis* complex:** It is responsible for tuberculosis (TB) in man
- ❖ ***M. leprae* (Hansen's bacilli):** It causes leprosy, characterized by anesthetic skin patches and lesions of peripheral nerves. If not treated, can lead to deformities affecting eyes, nose, hands and feet (Chapter 54)
- ❖ **Nontuberculous mycobacteria (NTM):** These are diverse group of mycobacteria. They are either saprophytic in nature, e.g. *M. goodii* (from tap water); or may be found as commensal (*M. smegmatis* in urine). Some of them can cause opportunistic human infections, e.g. *M. kansasii*.

TUBERCULOSIS

Tuberculosis, caused by *M. tuberculosis* complex is one of the oldest disease of mankind, and is a major cause of death worldwide. It usually affects the lungs, although other organs are also involved.

***M. tuberculosis* complex includes:**

- ❖ ***M. tuberculosis* :** It is the most common species to cause TB in man
- ❖ ***M. bovis* (bovine tubercle bacillus)**
- ❖ **Other members** are less common human pathogens, such as *M. africanum*, *M. microti*, *M. caprae*, *M. pinnipedii*, *M. canetti*, *M. suricattae*, *M. orygis*, *M. mungi* and the recently described dassie bacillus and chimpanzee bacillus.

These species are so closely related to each other by antigenic and molecular analysis that, they are regarded by many authors as variants of a single species. However, they can be distinguished from each other by certain properties.

Antigenic Structure

Antigens of *M. tuberculosis* are mainly of two types:

1. **Cell wall (insoluble) antigens:** The cell wall consists of several distinct layers (Fig. 63.1):
 - **Peptidoglycan layer:** It maintains the shape and rigidity of the cell
 - **Arabinogalactan layer:** It is a major structural component of the mycobacterial cell wall
 - **Mycolic acid layer:** It is the principal constituent, made up of long chain fatty acids attached to arabinogalactan. It confers very low permeability to the cell wall and is responsible for **acid fastness** and also reduces the entry of most antibiotics
 - **Outermost layer:** It consists of lipids (dimycocerosates and acylglycerols), glycolipids and mycosides (phenolic glycolipids)
 - **Proteins** (e.g. porins, transport proteins): They are found throughout the various layers
 - **Plasma membrane:** This layer is present beneath the cell wall, into which various proteins, phosphatidylinositol mannosides, and **lipoarabino-**

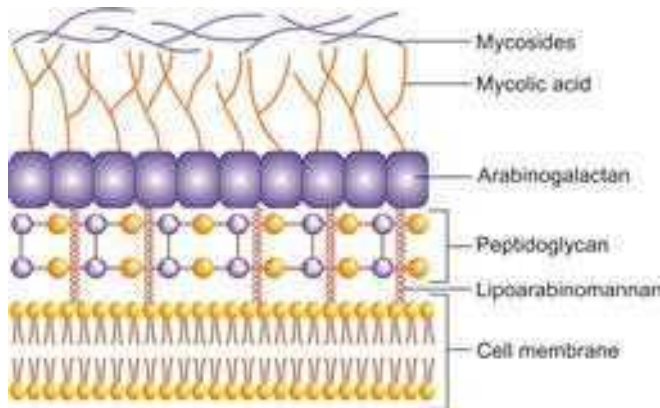


Fig. 63.1: Cell wall of *M. tuberculosis*.

mannan (LAM) are inserted. LAM is an important antigen, which facilitates the survival of tubercle bacilli within the macrophages. It is also used as a target antigen for the TB diagnosis.

2. **Cytoplasmic (soluble) antigens:** These include antigen 5, antigen 6, antigen 60; and are used in the serodiagnosis of tuberculosis.

Pathogenesis

Source of Infection

The source of infection of *M. tuberculosis* complex may be— (1) human (e.g. cases of pulmonary tuberculosis), (2) bovine source (e.g. consumption of unpasteurized infected milk).

Mode of Transmission

Air-borne: *M. tuberculosis* is mainly transmitted by inhalation of **aerosols**, generated while coughing, sneezing, or speaking of infected patients. They are tiny dry droplet nuclei (<5 μm size), which may remain suspended in the air for several hours and are easily inhaled. There may be as many as 3,000 infectious nuclei per cough.

Other modes of transmission are rare, such as:

- ❖ **Inoculation:** Transmission of infection through direct skin contact with an infected person is uncommon
- ❖ **Ingestion:** Swallowing of sputum (in infants) or consumption of unpasteurized (infected) milk.

Risk Factors

The risk factors favoring the transmission of infection include:

- ❖ Sputum positive patients (sputum showing acid-fast tubercle bacilli in microscopy) transmit more efficiently than sputum negative patients
- ❖ **Bacillary load:** At least 10^4 bacilli/mL in sputum is required for an effective transmission
 - Adult patients with cavitory lesions in lung have more bacillary load in sputum (10^5 – 10^7 AFB/mL), therefore transmit more efficiently
 - Culture-negative pulmonary TB and extrapulmonary TB patients are essentially noninfectious.
- ❖ Overcrowding in poorly ventilated rooms.

Following infection, not all, but only a minor proportion of people develop progressive disease. They usually have the following endogenous risk factors such as:

- ❖ **Low cell-mediated immunity:** For example, HIV-infected people
- ❖ **Other comorbid conditions** such as: Post-silicosis, post-transplantation (renal, cardiac), jejunioileal bypass, gastrectomy, chronic renal failure/hemodialysis, diabetes, IV drug abuse, smoking, etc.
- ❖ **Age:** Late adolescence and early adulthood periods are more prone. Elderly people are at increased risk due to waning immunity and underlying comorbidity
- ❖ **Sex:** Risk is higher in women at 25–34 years of age, while at older ages, men have a greater risk.

Sequence of Pathogenic Events

The sequence of pathogenic events that take place are as follows:

- ❖ **Droplet nuclei** containing tubercle bacilli from infectious patients are inhaled. Majority are trapped in the upper airways and expelled out by the ciliary action of the mucosal cells; only a fraction (usually <10%) of small droplets reach the alveoli
- ❖ **Adhesion to macrophages:** Mycobacterial surface lipoarabinomannan (LAM) binds to complement receptors and mannose receptors present on the surface of macrophages. This leads to internalization of bacilli
- ❖ **Phagocytosis by macrophages:** It is enhanced by complement (C3b) mediated opsonization of bacilli
- ❖ **Survival inside the macrophages:** This is due to bacterial cell wall LAM which **impairs phagosome-lysosome fusion** by inhibiting an increase in intracellular Ca^{2+} and phosphatidylinositol 3-phosphate
- ❖ If the bacilli are successful in arresting phagolysosome fusion, then they happily replicate inside the macrophage. The macrophage eventually ruptures and releases its bacillary contents which infect other uninfected phagocytes and infection cycle continues.

Host Immune Response

Cell-mediated Immune Response

Host's cell-mediated immune response to tubercle bacilli is critical to contain the infection.

- ❑ Macrophages present the mycobacterial antigens to T_H (T helper) cells and activate them into T_{H1} and T_{H2} subsets. T_{H1} cells release cytokines such as IL-2 and IFN- γ , which activate monocytes and macrophages
- ❑ Thus, activation of T_{H1} cells leads to the development of two host responses: A macrophage-activating response and a tissue-damaging response. The balance between the two determines the outcome of the infection, as follows:

1. Macrophage-activating response:

Majority of individuals show resistance to infection and are able to contain the bacilli.

- IFN- γ activates the resting alveolar macrophages into activated macrophages which are capable of killing and digesting the tubercle bacilli

Contd...

Contd...

- These activated macrophages aggregate around the center of the lesion and form characteristic granuloma called tubercles.

Tubercles: Tubercles are the essential pathological findings in tuberculosis. Formation of tubercle is a favorable sign. They are primarily of two types:

- i. **Hard tubercles:** Tubercles are initially hard, composed of a central zone containing activated macrophages (epithelioid and giant cells) and a peripheral zone of lymphocytes and fibroblasts
- ii. **Soft tubercles:** Later, the central part of the lesion undergoes caseous necrosis, and it contains necrotic material resembling soft cheese. Growth of *M. tuberculosis* is inhibited within this necrotic environment because of low oxygen tension and low pH. Eventually, the lesion heals and calcifies. The viable bacilli may remain dormant within the macrophages or within the necrotic material for many years without causing further tissue destruction.

2. Tissue-damaging response:

In a minority of cases, especially those associated with risk factors (as mentioned above), the macrophage-activating response is weak and the bacilli are more virulent.

- Here the mycobacterial growth can be inhibited only by an intensified **delayed hypersensitivity reaction (DTH)** which leads to lung tissue destruction
- The caseous necrosis becomes liquefied, containing large numbers of bacilli which further spread by three ways:
 1. Direct draining into the airways, and then get discharged into the environment (while coughing and talking).
 2. Lymphatic spread and there by reseeding into the same or opposite lung → then disseminate to other organs.
 3. Hematogenous spread to various organs. Specially among young children with immature immunity, hematogenous spread may lead to fatal miliary TB or tuberculous meningitis.

Humoral Immune Response

T_H2 cells derived cytokines such as IL-4, IL-5 activate B cells to produce antibodies.

- *M. tuberculosis* being obligate intracellular organism, humoral immunity plays a minor role
- However, the anti-LAM antibodies play a role in preventing dissemination of tuberculosis in children.

Clinical Manifestations

Tuberculosis (TB) is classified as pulmonary and extrapulmonary forms.

Pulmonary Tuberculosis (PTB)

Pulmonary tuberculosis (PTB) accounts for 60–90% of all cases of tuberculosis (TB). It can be further categorized into primary or postprimary (secondary) types (Table 63.1).

Extrapulmonary Tuberculosis (EPTB)

EPTB results from hematogenous dissemination of tubercle bacilli to various organs. Though EPTB constitutes

about 10–40% of all cases of TB, in HIV-positive patients, the frequency is much higher accounting up to two-thirds of all cases of tuberculosis.

Virtually all organ systems may be affected however, the sites commonly involved (in order of frequency) are:

- ❖ **Tuberculous lymphadenitis:** It is the most common form (mainly in children), accounting for 35% of all EPTB cases. The most common sites are posterior cervical and supraclavicular lymph nodes. It presents as painless swelling in the neck region without warmth or color change
- ❖ **Pleural tuberculosis:** It accounts for 20% of all EPTB cases. It presents as pleural effusion. Tuberculous empyema is a less common complication that develops due to rupture of a cavity into pleural space with spillage of tubercle bacilli, which may form a bronchopleural fistula
- ❖ **Tuberculosis of the upper airways:** Involving larynx, pharynx, and epiglottis. Hoarseness, dysphonia, and chronic productive cough are the common clinical presentation
- ❖ **Genitourinary tuberculosis:**
 - Renal tuberculosis (Chapter 76)
 - Genital tuberculosis: In female patients, fallopian tubes and the endometrium are commonly involved causing infertility. In males, epididymis is the most common site (Chapters 77).
- ❖ **Skeletal tuberculosis:** Weight-bearing joints, such as spine (Pott's disease or tuberculous spondylitis is most common), hips and knees are commonly affected.
 - With advanced disease, collapse of vertebral bodies results in kyphosis (gibbus) and a paravertebral 'cold' abscess may also form
 - Cold abscess may penetrate the chest wall to present as soft tissue mass; or may penetrate inguinal ligaments and present as psoas abscess.
- ❖ **Tuberculosis of CNS:** It occurs commonly in children. Tuberculous meningitis and tuberculoma are the common forms (Chapter 71)
- ❖ **Gastrointestinal tuberculosis:** Terminal ileum and cecum are the most common sites involved. The route of spread may be due to swallowing of sputum with direct seeding, hematogenous spread, or ingestion of cow's milk infected with *M. bovis* (in developing countries **TB peritonitis** occurs either by direct spread, ruptured lymph nodes or hematogenous seeding.)
- ❖ **Tuberculous pericarditis:** It occurs as a direct extension from adjacent lymph nodes or following hematogenous spread. It occurs in elderly people, in countries with low TB prevalence
- ❖ **Tuberculous skin lesions (Chapter 55):**
 - **Scrofuloderma:** It is a skin condition caused by tuberculous involvement of the skin by direct extension, usually from underlying tuberculous lymphadenitis
 - **Lupus vulgaris:** Apple jelly nodules are formed over the face in females.

Table 63.1: Comparison of primary and secondary pulmonary tuberculosis.		
Features	Primary pulmonary tuberculosis	Postprimary (adult-type)/secondary pulmonary tuberculosis
Results due to	Initial exogenous infection with tubercle bacilli	<ul style="list-style-type: none"> Exogenous reinfection Endogenous—reactivation of the latent primary lesion
Age group affected	Children	Adults
Parts of the lungs commonly affected	Subpleural lesion affecting, middle and lower lung lobes	Apical and posterior segments of the upper lobes (areas of high oxygen tension)
Lesions formed at the initial sites	Fibrotic nodular lesions are formed (Ghon focus)	Hematogenous seedling in the apex of lungs called Simon's focus Reactivated Simon focus with central caseation (Assmann focus)
Lymph node	Ghon focus with associated hilar lymphadenopathy is common (called primary complex)	Lymph node involvement is unusual
Clinical features	It may be asymptomatic or may present with fever, productive cough (with or without hemoptysis) and occasionally pleuritic chest pain, night sweating, weight loss	Lesions undergoing necrosis and tissue destruction, leading to cavity formation (Fig. 63.2) Symptoms are similar, but more pronounced
Fate	In the majority of cases: <ul style="list-style-type: none"> Lesions heal spontaneously Primary complex becomes calcified (Ranke complex) Rarely, in children with impaired immunity, progressive primary TB (develops and spreads by local invasion and by lymphatics)	<i>In majority of cases:</i> The necrotic material breaks into the airways, leading to: <ul style="list-style-type: none"> Bronchogenic spread to the same or opposite lung forms satellite lesions, which coalesce producing caseating pneumonia Expectoration of bacteria-laden sputum Hematogenous spread leading to seedling of bacilli in various parts of the body and granuloma formation. Rarely heals spontaneously



Fig. 63.2: CT scan of the lungs showing cavitation in the left upper lobe—suggestive of pulmonary tuberculosis.

Source: Dr Sunitha V, Department of Radiology, JIPMER, Puducherry (with permission).

- ❖ **Miliary or disseminated tuberculosis:** Hematogenous spread of tubercle bacilli results in the formation of yellowish 1–2 mm size granulomatous lesions resembling **millet seeds** (thus termed as miliary) in various organs. It is more common in HIV-infected people
- ❖ **Post-TB aspergillosis:** Chronic pulmonary aspergillosis may develop due to colonization of *Aspergillus fumigatus* in the residual TB cavities
 - It may manifest as simple aspergilloma (fungal ball) or chronic cavitary aspergillosis
 - It presents as respiratory impairment, hemoptysis and weight loss.

HIV-associated Tuberculosis

Tuberculosis is one of the most common opportunistic diseases among HIV-infected persons due to low

CMI. Worldwide, TB occurs in 70–80% of HIV-infected individuals, EPTB being more common than PTB—common presentations are lymphatic, disseminated, pleural and meningitis.

Epidemiology

About a quarter of the current world population is infected asymptotically with *M. tuberculosis*, of which 5–10% develop the clinical disease during their lifetime.

- ❖ **World:** The WHO has estimated 10 million new cases of TB occurred in 2018 with a global incidence of 130 new cases per one lakh population per year
 - **Deaths due to TB** was estimated to be 12 Lakh in HIV-negative and 2.5 lakh in HIV-coinfected people in 2018; which was much less compared to deaths (around 17 lakh) in the year 2000. Over 95% of TB deaths occurred in low and middle-income countries
 - **WHO regions:** The highest burden of TB was from South-East Asia (44%), followed by Africa (24%) and the Western Pacific (18%)
 - **Countries:** Eight countries accounted for two-third of the total TB burden, with **India** having the largest share (27% of total TB cases and 33% of total TB deaths), followed by China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and South Africa. In highly endemic area, each sputum AFB-positive patient may spread the infection up to 20 contacts due to a delay in making the diagnosis.
- ❖ **India:** In 2018, about 27 lakh cases occurred India; with highest burden from Uttar Pradesh (20% of total TB cases) followed by Maharashtra. In contrast, Delhi followed by Chandigarh and Puducherry accounted for the highest number of cases per Lakh population

- ❖ TB is one of the top 10 causes of death worldwide and the leading cause among infectious diseases, ranking above HIV/AIDS and COVID-19 in 2020.

LABORATORY DIAGNOSIS

Tuberculosis

Diagnosis of active tuberculosis

Specimen collection

- ❑ In pulmonary TB: Sputum (2 specimens—spot and early morning), gastric aspirate (in children)
- ❑ In EPTB: Specimens vary depending on the site involved

Digestion, decontamination and concentration of specimen:

- ❑ Modified Petroff's method (4% NaOH)
- ❑ NALC (N-acetyl-L-cysteine) + 2% NaOH

Direct microscopy by acid-fast staining:

- ❑ Ziehl-Neelsen (ZN) technique—long slender, beaded, less uniformly stained red color acid-fast bacilli
- ❑ Kinyoun's cold acid-fast staining
- ❑ Fluorescent (auramine) staining—it is more sensitive and smears can be screened more rapidly than ZN stain

Conventional culture media—take 6–8 weeks

- ❑ Lowenstein Jensen (LJ) medium—shows rough, tough and buff colored colonies in 6–8 weeks

Automated culture methods—take 3–4 weeks

- ❑ **MGIT system:** Detects growth as well as resistance to antitubercular drugs (ATDs)

Culture identification

- ❑ Automated identification—by MALDI-TOF
- ❑ MPT 64 antigen detection—by ICT

Molecular methods

- ❑ PCR detecting *IS6110* gene
- ❑ **CBNAAT (GeneXpert) and Truenat**—for identification and detection of resistance to rifampicin; has a turnaround time 2 hours
- ❑ **Line probe assay (e.g. Genotype TB)**—for identification and detection of resistance to 1st and 2nd line ATDs; has a turnaround time of 2–3 days.

Diagnosis of latent tuberculosis

By tuberculin skin test (e.g. Mantoux test) and interferon gamma release assay (IGRA).

Laboratory Diagnosis

Laboratory diagnosis of active tuberculosis can be established by various methods described below. The diagnosis of latent tuberculosis is explained later in the description.

Specimen Collection

In PTB, *two sputum* samples are recommended—**spot sample** (collected on the same day under supervision) and **early morning sample** (collected on the next day). Alternatively 2 spot samples at least one hour apart can be collected.

- ❖ **Sputum collection booths** should be located away from other people, outside in an open well ventilated space; as air dilutes the aerosols generated during coughs

- ❖ **Early morning sputum specimen** should be collected in empty stomach, after rinsing the mouth well; so as to remove any food remnants, as they interfere with smear examination

- ❖ **Inhale deeply:** Patient should be advised to inhale deeply (2–3 times) and cough out deep from the chest during exhalation and then to spit the sputum into the wide mouthed screw capped container

- ❖ **Quality:** Sputum should be at least 3–5 mL in quantity; thick and purulent (yellowish mucus). Salivary specimens that appear watery should be rejected.

The extrapulmonary specimens vary depending on the site involved, which can be divided into two categories (Table 63.2).

Digestion, Decontamination and Concentration

Sputum and specimens from non-sterile sites subjected to smear microscopy and culture need prior treatment for digestion (to liquefy the thick pus cells and homogenization), decontamination (to inhibit the normal flora) and concentration (to increase the yield). However, this step is not required for molecular methods and also for processing of extrapulmonary specimens collected aseptically from sterile sites. Commonly used methods are:

- ❖ **Modified Petroff's method (4% NaOH):** Sputum is mixed with 4% sodium hydroxide, centrifuged and the

Table 63.2: Extrapulmonary specimens.

Sterile site specimens collected aseptically

Optimum specimens	CSF, pericardial fluid, synovial fluid and ascitic fluid
Suboptimal specimens (organism load is less)	Pleural fluid (20–50 mL is collected and centrifuged) Blood (indicated only for disseminated TB and co-infected with HIV)

Specimens containing normal flora

Swabs	Considered suboptimal specimen. The only recommended swabs are: <ul style="list-style-type: none"> • <i>Laryngeal swabs:</i> Collected early morning in empty stomach or • Swab from discharging sinus
Urine	Three early morning specimens collected (500 mL/ specimen, centrifuged) on different days as TB bacilli in urine are shed intermittently
Stool	For disseminated TB in HIV infected patients and infants
Other respiratory specimens	Bronchial secretions (2–5 mL) Bronchoalveolar lavage (20–50 mL) Transbronchial and other biopsies (collected in sterile normal saline)
Gastric lavage	Recommended for children (tend to swallow sputum), or ICU patients (aspiration) Early morning lavage should be collected and processed early (<4 hours)

Note: Samples for culture should never be collected in formalin. If histopathological examination is required, two samples should be collected.

sediment is neutralized with phosphate buffer saline. This method is recommended for LJ culture

- ❖ **NALC (N-acetyl-L-cysteine) + 2% NaOH:** This is superior to Petroff's method for isolation. NALC liquefies the sputum and NaOH kills the normal flora. This method is more compatible with automated culture systems.

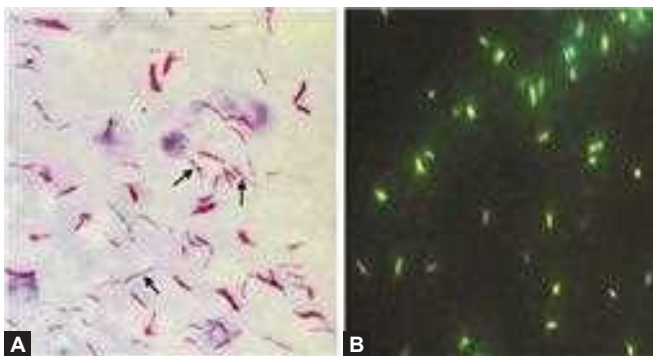
Direct Microscopy by Acid-fast Staining

Ziehl-Neelsen (ZN) Technique (Hot Method)

Smears are prepared from thick mucopurulent part of sputum or with the sediment obtained after concentration. Optimum thickness of the smear can be assessed by placing the smear on printed matter. The print should be just readable through the smear. Then the smear is stained by acid-fast stain (for procedure, refer 'acid-fast staining' in Chapter 3.3).

❖ Interpretation

- **Negative result:** At least 100 oil immersion fields should be examined for 10–15 minutes before giving a negative report
- **Positive result:** *M. tuberculosis* appears as long slender, beaded, less uniformly stained red colored acid-fast bacilli (AFB) (Fig. 63.3A).
- ❖ **Presumptive diagnosis:** Microscopy provides only presumptive diagnosis. If typical beaded appearance is seen, then it should be reported as 'acid-fast bacilli resembling *M. tuberculosis* are seen by smear microscopy by ZN stain'
- ❖ **Advantages:** Smear microscopy is rapid, easy to perform at peripheral laboratories and is cheaper
- ❖ **Disadvantages:** (i) Smear microscopy is less sensitive than culture, (ii) low sensitivity with a detection limit of 10,000 bacilli/mL of sputum, (iii) it cannot determine the viability of bacilli
 - It is difficult to differentiate *M. tuberculosis* from saprophytic mycobacteria present in tap water or even as commensal in clinical samples such as gastric aspirate, and urine



Figs 63.3A and B: **A.** ZN staining of sputum smear showing long, slender and beaded red colored acid-fast bacilli; **B.** Auramine phenol staining of sputum smear—Tubercle bacilli appear bright brilliant green against the dark background.

Source: A and B. Department of Microbiology, JIPMER, Puducherry (with permission).

Table 63.3: RNTCP guidelines for grading of ZN stained sputum smears.

No. of AFB seen	OIF to be screened	Grading	Result
No AFB in 100 OIF	100	0	Negative
1–9/ 100 OIF	100	Scanty*	Positive
10–99/100 OIF	100	1+	Positive
1–10/ OIF	50	2+	Positive
>10/ OIF	20	3+	Positive

Abbreviations: AFB, acid-fast bacilli; OIF, oil immersion fields; ZN, Ziehl-Neelsen.

*Record the actual no. of bacilli seen in 100 fields-e.g. "Scanty 8".

- **Acid alcohol** (3% hydrochloric acid+ 95% ethyl alcohol) can be used to differentiate *M. tuberculosis* (acid and alcohol-fast) from *M. smegmatis* (only acid-fast, but not alcohol-fast) in urine sample.

RNTCP Guidelines for Grading of Sputum Smear

Revised National Tuberculosis Control Programme (RNTCP) of India has given guidelines for grading of **ZN stained sputum smears** (Table 63.3).

RNTCP grading is useful for:

- ❑ Monitoring the treatment response of the patients
- ❑ Assessing the severity of disease
- ❑ Assessing the infectiousness of the patient: Higher the grade more is the infectiousness. Smear negative patients (<10,000 bacilli/mL of sputum) are less infectious. However, grading of the sputum smear depends upon the quality of sputum collected.

Kinyoun's Cold Acid-fast Staining

It differs from ZN stain in that—(i) heating is not required, (ii) phenol concentration in carbol fuchsin is increased, and (iii) duration of carbol fuchsin staining is more.

Fluorescence Staining

It is a fluorescent staining technique, uses auramine-phenol solution (for 7–10 min) as primary stain, 0.5% acid alcohol (for 2 min, twice) as decolorizer and 0.1% potassium permanganate (for 30 sec) as counter stain. Then the slide is examined under fluorescent LED (light-emitting diode) microscope.

- ❖ The bacilli appear brilliant yellow against the dark background (Fig. 63.3B)
- ❖ Smears are screened by using 20× or 25× objective, hence can be screened faster (2 min for 100 fields)
- ❖ It is more sensitive than ZN staining and has been the recommended screening method by RNTCP
- ❖ However, artifacts may confound with the interpretation. Hence the reading should be taken by an expert.

Culture Methods

Culture is traditionally considered as the gold standard method of diagnosis of TB. It offers several advantages:

- ❖ It is more sensitive than microscopy with the detection limit of 10–100 viable bacilli
- ❖ Indicates viability: TB bacilli growing on culture indicates that they are viable

- ❖ Drug susceptibility testing can be performed.

RNTCP recommended culture media include both conventional solid media (Lowenstein-Jensen medium) and automated liquid culture, such as Mycobacteria Growth Indicator Tube (MGIT).

Conventional Solid Media (Lowenstein-Jensen Medium)

Lowenstein-Jensen (LJ) medium has been the most widely used and recommended by RNTCP.

- ❖ It is composed of coagulated hen's eggs, mineral salt solution, asparagine and malachite green (as a selective agent)
- ❖ Inoculated media are incubated for a prolonged duration of 6–8 weeks. This is because of the slow-growing nature of tubercle bacilli (long generation time of 10–15 hours)
- ❖ **Colonies:** *M. tuberculosis* produces typical rough, tough and buff-colored colonies (Fig. 63.4A). In contrast, *M. bovis* produces smooth, moist and white colored colonies that break up easily when touched.

Conventional liquid media are Kirchner's medium and Middlebrook 7H9 medium. They are not routinely used.

Automated Liquid Culture

Automated culture systems monitor the growth continuously and offer a faster turnaround time compared to conventional culture.

- ❖ Positive growth (99%) gets detected within 3–4 weeks. However, the negative result is reported after 6 weeks of incubation
- ❖ They use liquid broth such as Middlebrook 7H9 medium supplemented with enriched growth media and antibiotic mixture (to inhibit other organisms).

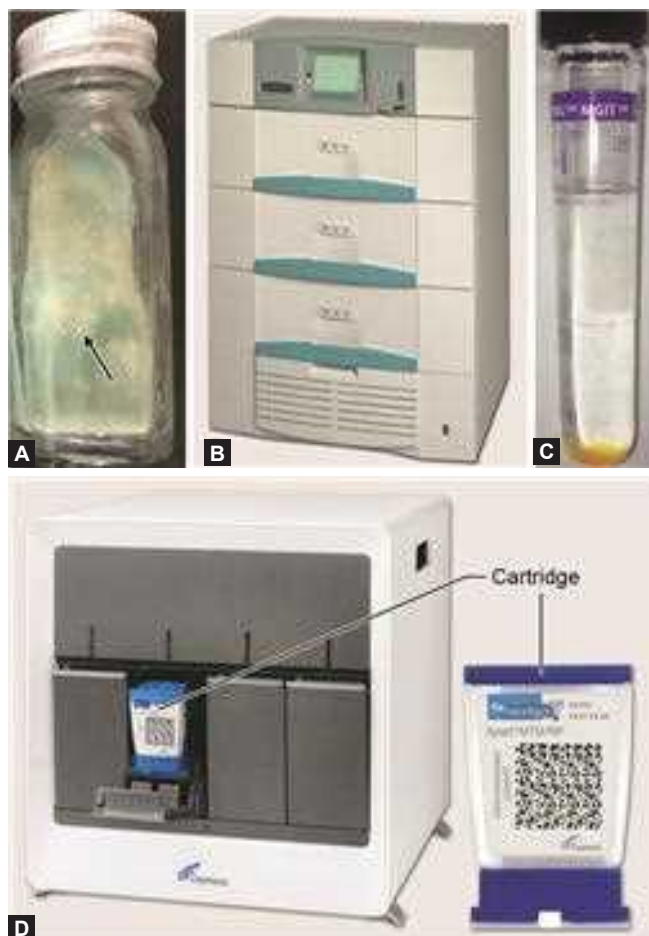
Various automated systems available are:

- ❖ **BACTEC MGIT (Mycobacteria growth indicator tube):** This is the automated system endorsed by WHO and RNTCP (Figs 63.4B and C)
 - **Uses:** (i) It detects growth of mycobacteria, and (ii) also performs the drug susceptibility testing against first-line and second-line antitubercular drugs
 - **Principle:** It uses an oxygen sensitive fluorescent compound, dissolved in the broth. Initially, the large amount of dissolved oxygen in the medium quenches emissions from the fluorescent compound. Later, actively respiring microorganisms consume the oxygen; the quenching effect is lost which allows the fluorescence to be detected.
- ❖ Other automated systems include BacT/ALERT systems.

Culture Identification

The colonies grown on LJ media and the broth from a positively flagged automated culture bottle are first subjected to acid-fast stain. If found AFB positive, then further tests are done for species identification.

- ❖ **MPT 64 antigen** detection by rapid immunochromatographic test: MPT64, a 28 Da antigen is specific for



Figs 63.4A to D: Culture media/culture systems for *M. tuberculosis*: **A.** Lowenstein-Jensen medium (arrow showing rough, tough and buff-colored colonies); **B.** BACTEC MGIT; **C.** MGIT liquid culture medium; **D.** GeneXpert system with cartridge.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

M. tuberculosis complex (*M. tuberculosis*, *M. bovis* and *M. africanum*) and negative for NTM (nontuberculous mycobacteria)

- ❖ **Automated identification system** such as MALDI-TOF
- ❖ **Biochemical tests** such as niacin test and **Rabbit pathogenicity tests** were in use in the past; now obsolete.

Serology

Serological methods (both antigen and antibody detection methods) are not recommended because of low sensitivity; cross-reactivity with other mycobacteria and variable antibody response against different epitopes. WHO has banned the use of serological tests.

Molecular Methods

Molecular methods are extremely useful as:

- ❖ They take less time than culture
- ❖ They are more sensitive than culture. This is very much useful for extrapulmonary samples that are usually paucibacillary

- ❖ They can also detect the genes coding for drug resistance
- ❖ Used for epidemiological typing of strains.

There are several molecular methods available as described below.

Polymerase Chain Reaction (PCR)

Nested PCR targeting *IS6110* gene was the most common molecular test used earlier. Other genes which were targeted by using PCR—*MPT64* gene, *65 KDa* and *38 KDa* genes.

Automated Real Time PCR

With the advent of automated real time PCR systems, the diagnosis of TB has been completely revolutionized. In addition, these systems can be used for diagnosis of other diseases such as COVID-19. Methods available are:

1. Cartridge-based nucleic acid amplification test (CBNAAT): e.g. GeneXpert
2. Chip-based real-time PCR: e.g. Truenat.

GeneXpert (CBNAAT)

GeneXpert (Cepheid's) is the CBNAAT system endorsed by WHO and is used in India under RNTCP (see Fig. 63.4D).

- ❖ **Rapid:** It has the lowest turnaround time (2 hours) among all the diagnostic methods currently available for TB
- ❖ **Principle:** It is based on real-time PCR technique; simultaneously detects: (i) MTB complex DNA, and (ii) rifampicin resistance (mutations of the *rpoB* gene). It uses five probes targeting various sequences of *rpoB* gene
- ❖ **EPTB:** WHO recommends GeneXpert as the initial test for diagnosis of EPTB; especially for CSF, lymph nodes and other tissue specimens. As the bacilli are more in number in the pleural wall; the ideal specimen for pleural TB is a pleural biopsy; not pleural fluid
- ❖ **Diagnostic utility:** The detection limit of GeneXpert is about 131 bacilli/mL of specimen. Compared to culture, the sensitivity and specificity are as follows:
 - For detection of TB bacilli: It is 88% sensitive and 99% specific
 - For detection of rifampicin resistance: It is 95% sensitive and 98% specific.
- ❖ **Disadvantages:** (i) Very expensive, (ii) cannot further speciate MTB complex
- ❖ **Next generation GeneXpert (Xpert Ultra):** It was endorsed by WHO in 2017
 - It contains two additional molecular targets to detect TB (*IS6110*, *IS1081*)
 - It is more sensitive and specific than GeneXpert, with a detection limit of 16 bacilli/mL.

Chip Based NAAT (Truenat)

Truenat is a chip-based real-time PCR system, validated recently by Indian Council of Medical Research (ICMR), in 2017; also endorsed by WHO in 2019. It is used in India under RNTCP. It has been developed by the Indian firm Molbio Diagnostics.

- ❖ **Advantages:** It is an automated battery operated device; can be used at level of primary health center where GeneXpert cannot be used as it needs uninterrupted power supply and air conditioning. The turnaround time is around one hour

- ❖ **Disadvantages:** (i) Very expensive, (ii) cannot further speciate MTB complex, and (iii) it tests only limited samples (1-4) at a time.

Line Probe Assay (LPA)

Line probe assay involves probe-based detection of amplified DNA in the specimen.

- ❖ **Uses:** LPA in TB diagnostics has the following uses:
 - Identification of MTB complex
 - Detection of resistance to first-line antitubercular drugs (ATDs)
 - Speciation of MTB complex and NTM
 - Detection of resistance to second line ATDs.
- ❖ **Limitation:** LPA can be performed only on positive cultures or smear positive clinical specimens. It is not recommended for smear negative specimens as the sensitivity is low
- ❖ LPA is useful particularly in isoniazid mono-resistant cases of TB, which are not diagnosed by GeneXpert.

RNTCP Diagnostic Algorithm

RNTCP recommended diagnostic algorithm should be followed for the diagnosis and further management of tuberculosis.

- ❖ **For pulmonary TB in adult** (Flowchart 63.1): First sputum smear examination is performed
 - If smear positive—diagnosis is confirmed
 - If smear negative, but chest X-ray is suggestive or in presence of high clinical suspicion—CBNAAT is performed to confirm the diagnosis.
- ❖ **For pediatric pulmonary TB:** CBNAAT is directly performed
- ❖ **For EPTB:** EPTB specimens are paucibacillary, therefore CBNAAT is directly performed. If not available, liquid culture (MGIT) is performed.

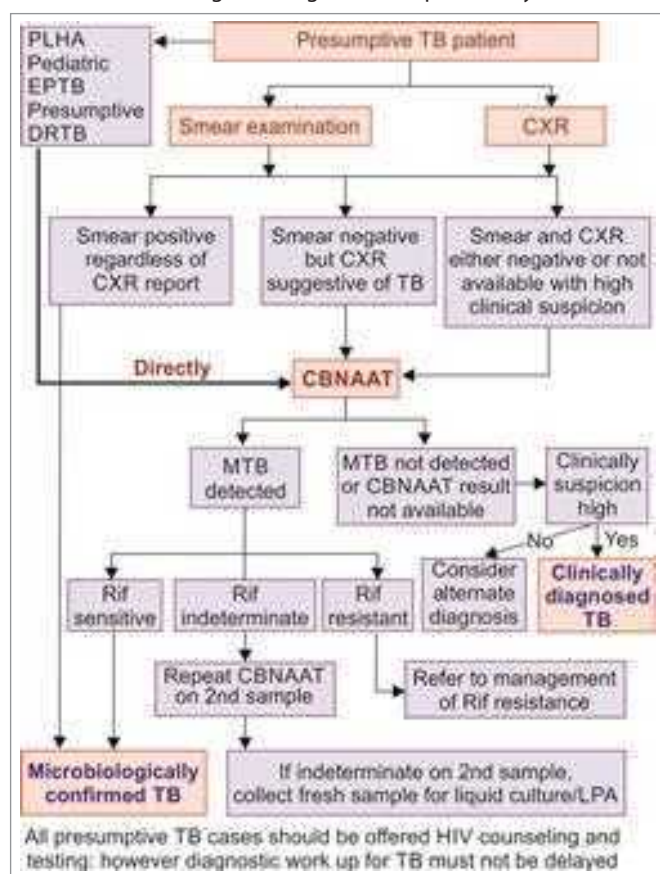
Diagnosis of Latent Tuberculosis

Latent tuberculosis is diagnosed by demonstration of delayed or type IV hypersensitivity reaction against the tubercle bacilli antigens. Two methods are available, (1) tuberculin skin test, (2) IFN- γ release assay.

Tuberculin Skin Test (TST)

Traditionally, the tuberculin skin test has been in use for diagnosis of latent TB for >100 years. It was discovered by Von Pirquet in 1907.

- ❖ **Antigens used:** WHO recommends a preparation of purified protein derivative, known as PPD-23 for performing TST
- ❖ **Dosage:** It is expressed in tuberculin unit (TU). One TU is equal to 0.00002 mg of PPD

Flowchart 63.1: Diagnostic algorithm for pulmonary tuberculosis.

Source: Adapted from RNTCP guideline 2016.

Note: **Presumptive pulmonary TB** refers to a person with any of the following—(i) cough >2 weeks, (ii) fever >2 weeks, (iii) significant weight loss, (iv) hemoptysis, (v) any abnormalities in the chest X-ray.

Abbreviations: Rif, Rifampicin; CBNAAT, cartridge-based nucleic acid amplification test; PLHA, people living with HIV/AIDS; DRTB, drug resistant tuberculosis; CXR, chest X-ray; LPA, line probe assay.

- ❖ **Procedure:** Mantoux test is the most commonly employed method. 0.1 mL of PPD containing 1 TU is injected intradermally into flexor surface of forearm
- ❖ **Reading:** It is taken after 48–72 hours. At the site of inoculation, an induration surrounded by erythema is produced. If the width of the induration is:
 - ≥10 mm: Positive (tuberculin reactors)
 - 6–9 mm: Equivocal/doubtful reaction
 - <5 mm: Negative reaction.
- ❖ **Interpretation of result:**
 - **Adults:** Positive TST in adults only indicates present or past exposure with tubercle bacilli but does not confirm the presence of the active stage of the disease. Hence, it is only used as an epidemiological marker
 - ♦ Prevalence of tuberculosis is calculated by counting all tuberculin reactors in a community
 - ♦ Incidence of tuberculosis is calculated by counting new converters to TST in a community.
 - **Children:** In children, a positive test indicates active infection and used as a **diagnostic marker**.

- ❖ **False-positive:** The test becomes positive after
 - BCG vaccination (after 8–14 weeks)
 - Nontuberculous mycobacteria infection.
- ❖ **False-negative:** The test may become negative in various conditions such as—early or advanced TB, miliary TB, decreased immunity (HIV-infected people).

Interferon Gamma Release Assay (IGRA)

This uses highly specific *M. tuberculosis* antigens such as CFP10 (culture filtrate protein) and ESAT6 (early secreted antigenic target-6); both coded by RD1 genes.

- ❖ **Procedure:** In contrast to TST, it is an in vitro test. Sensitized T lymphocytes collected from suspected individuals, are exposed to ESAT-6/CFP-10 antigens, which lead to release of high level of IFN- γ from the T lymphocytes. An ELISA format is available commercially (QuantiFERON-TB Gold assay)
- ❖ **Advantage:** It is highly specific; there are no false-positive results.

DST (Drug Susceptibility Testing)

Several methods of DST (drug susceptibility testing) are available which can be grouped into:

Phenotypic Methods

- ❖ **MGIT** (used for 1st and 2nd line drugs): Resistance is determined by growth of TB bacilli in drug containing tube as compared to the control tube (drug free) within 4–21 days of incubation
- ❖ **Proportion method** (used for 1st and 2nd line drugs): An isolate is considered resistant to a given drug when growth of 1% or more is observed in the drug containing LJ medium compared to the control LJ medium without drug after 42 days of incubation.

Genotypic Methods

- ❖ **GeneXpert:** It is used for detection of resistance to rifampicin, targeting five different sequences of *rpoB* gene. Turnaround time is <2 hours
- ❖ **Line probe assay (LPA):** It detects resistance to both first-line (FL) and second-line (SL) drugs; with a turnaround time of 2–3 days.

U-DST

Universal-Drug Susceptibility Testing (U-DST) refers to testing all TB patients for resistance to at least rifampicin (by performing CBNAAT). U-DST program has been rolled out across India since January 2018.

TREATMENT

Tuberculosis

Anti-tubercular drugs (ATDs)

ATDs are enlisted in Table 63.4; classified into two groups.

1. First-line drugs: Used for the treatment of drug susceptible-TB (DS-TB)

Contd...

Contd...

TREATMENT Tuberculosis

2. Second-line drugs: Used for the treatment of drug resistant-TB (DR-TB)

Treatment of tuberculosis aims to:

- Interrupt transmission by rendering patients non-infectious
- Prevent morbidity and death by curing patients
- Prevent the emergence of drug resistance
- Prevent relapse.

To achieve the aims, the following strategies are followed:

- **Multidrug therapy:** Combination of more than one drug for rapid and effective killing of tubercle bacilli
- **Short course chemotherapy** lasting for 6 months (or longer for DR-TB)
- **Two phase chemotherapy:** The short course is divided into—
 - *Intensive phase:* Aims at aggressive treatment with multiple ATDs that rapidly kill the bacilli making the smear negative, followed by:
 - *Continuation phase:* Aims at killing the remaining dormant bacilli and prevents relapse
- **DOTS strategy** (Directly Observed Treatment, Short course): It is recommended by RNTCP and WHO. Here, the strategies used are:
 - The entire treatment course is supervised to improve the patient's compliance
 - Treatment response is also monitored by sputum smear microscopy at the end of each phase.
- **Universal-DST:** It refers to performing drug-susceptibility testing (DST) for all TB-patients—first performing CBNAAT to determine rifampicin susceptibility; followed by line probe assay (LPA) (Flowchart 63.2)
 - *If found as rifampicin-sensitive,* then line LPA is performed for other first-line ATDs (e.g. isoniazid)
 - *If found as rifampicin-resistant,* then LPA is performed for second-line agents such as fluoroquinolones (FQs) and second-line injectable (SLI) agents.

Treatment regimens

The treatment should be planned only after the result of DST are available. The treatment regimens are of various types, depending upon the resistance pattern (Table 63.5).

- **Standard regimen for DS-TB:** It is a six-month course; comprises of two phases.
 - *Intensive phase,* with four drugs (HRZE) for two months; followed by *continuation phase,* with three drugs (HRE) for four months
 - *FDC:* All drugs must be given in fixed dose combination (FDC) tablets as per appropriate weight bands (Table 63.6)
 - *Daily-oral regimen:* The FDC tablets should be taken orally, once in a day.
- **Regimens for DR-TB:** The treatment for DR-TB is complex, consists of use of higher numbers of second-line agents, given for longer duration. The composition of the regimen (Table 63.5) depends upon the type of DR-TB (discussed subsequently).
- **Follow-up of treatment:** Patients should be followed up at scheduled intervals for the assessment of improvement in clinical and laboratory parameters.
 - *Clinical follow-up:* Should be carried out at least monthly during treatment

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TREATMENT Tuberculosis

- *Follow-up laboratory investigation:* For PTB cases, sputum smear examination is done at the end of intensive phase. At the end of treatment, sputum smear plus culture is done for every patient.
- *Long term follow-up* is carried out up to 2 years of completion of treatment
- *Follow-up for MDR TB:* Sputum smear and culture are performed every month during intensive phase and every three months during continuation phase.

Newer Anti-tubercular Drugs

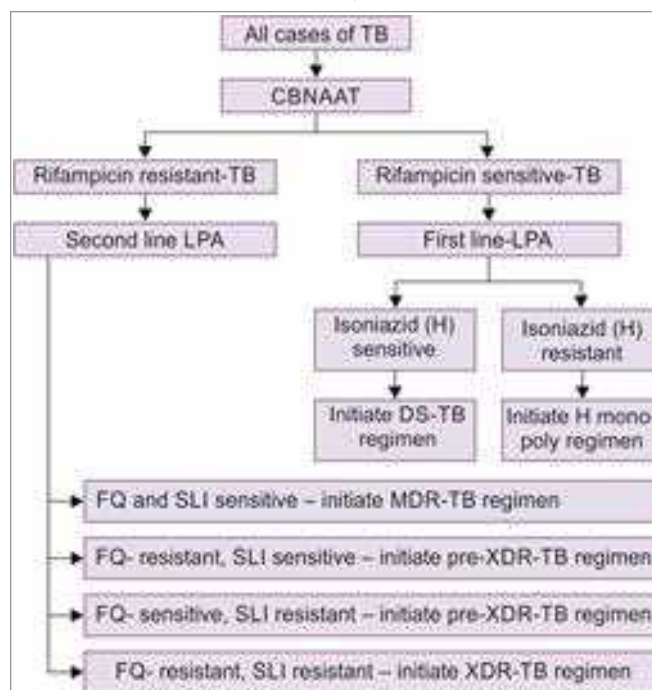
Bedaquiline and delamanid are only two drugs developed specifically for the treatment of TB in the last 40 years.

Bedaquiline

Bedaquiline is a new second line ATD of diarylquinoline class, approved for use as second line ATD since 2015. It is considered as miracle drug for the treatment of XDR-TB.

- ❖ It acts by inhibiting mycobacterial ATP synthase
- ❖ It is strongly mycobactericidal; has no cross resistance with other ATDs
- ❖ Showed significant benefit in improving the time to culture conversion in MDR-TB patients
- ❖ **Indication:** It is recommended for MDR-TB or XDR-TB cases which are resistant to fluoroquinolones and/or second-line injectable drugs

Flowchart 63.2: Treatment regimens based on drug-susceptibility testing.



Note: For details on various treatment regimens, refer Table 63.5.

Abbreviations: FQ, fluoroquinolones; SLI, second-line injectable agents; CBNAAT, cartridge based nucleic acid amplification test; LPA, line probe assay; MDR-TB, multidrug-resistant TB; XDR-TB, extensively drug-resistant TB.

ATDs for DS-TB	ATDs for DR-TB (Second-line agents)	
First-line Agents • Isoniazid (H) • Rifampicin (R) • Pyrazinamide (Z) • Ethambutol (E)	Group A: Fluoroquinolones • Levofloxacin (Lfx) • Moxifloxacin (Mfx) • Gatifloxacin (Gfx)	Group D: Add-on agents (not part of the core MDR-TB regimen) <i>Group D1: First-line agents</i> • Pyrazinamide (Z) • Ethambutol (E) • High-dose isoniazid (Hh) <i>Group D2: New agents</i> • Bedaquiline (Bdq) • Delamanid (Dlm) <i>Group D3: Other agents</i> • p-aminosalicylic acid (PAS) • Imipenem-cilastatin (Ipm /CIs) • Meropenem (Mpm) • Amoxicillin-clavulanate (Amx-Clv) • Thioacetazone (T)
	Group B: Second-line injectable agents • Amikacin (Am) • Capreomycin (Cm) • Kanamycin (Km) • Streptomycin (S)	
	Group C: Other second-line agents • Ethionamide/Prothionamide (Eto/Pto) • Cycloserine/Terizidone (Cs/Trd) • Linezolid (Lzd) • Clofazimine (Cfz)	

Type	Regimen name	Intensive phase ¹	Continuation phase
DS-TB	No resistance	(2) H R E Z	(4) H R E
H mono/poly	H monopoly DR-TB regimen	(3-6) Lfx Km R E Z	(6) Lfx R E Z
MDR-TB or RR-TB	Shorter MDR-TB regimen ⁴	(4-6) Mfx ^h Km Eto Cfz Z Hh E	(5) Mfx ^h Cfz Z E
	Conventional MDR-TB regimen ⁵	(6-9) Lfx Km Eto Cs Z E	(18) Lfx Eto Cs E
Pre-XDR-TB ²	Regimen for MDR/RR + R/FQ class	(6-9) Km Eto Cs Z Lzd Cfz + (6) Bdq	(18) Eto Cs Lzd Cfz
	Regimen for MDR/RR+ R/ SLI class	(6-9) Lfx Cm ⁷ Eto Cs Z Lzd Cfz + (6) Bdq	(18) Lfx Eto Cs Lzd
XDR-TB	Regimen for MDR/RR-TB plus R/FQ and SLI	(6-12) Mfx ^h Cm ⁷ Eto Cs Lzd Cfz Z E	(18) Mfx ^h Eto Cs Lzd Cfz E

Note:

¹If the intensive phase is prolonged, the injectable agent is only given three times a week in the extended intensive phase.

²**Pre-XDR-TB:** Resistance to either fluoroquinolones or second-line injectable agents, but not both. RNTCP does not use this terminology.

³Mfx^h refers to high dose of moxifloxacin.

⁴**Shorter MDR-TB regimen** is recommended for pulmonary TB, pleural TB and TB lymph node.

⁵**Conventional MDR-TB regimen** is recommended for extrapulmonary TB (except pleural TB and TB lymph node) and in pregnancy.

⁶The duration of treatment is given in bracket () under each regimen.

⁷If kanamycin is found resistant, then add capreomycin to the regimen if found susceptible.

Abbreviations: DS-TB, drug sensitive TB; H mono/poly, mono-resistance or poly-resistance to isoniazid; MDR-TB; multidrug resistant TB; RR-TB, rifampicin resistant TB; XDR-TB, extensively drug-resistant TB; R/FQ, resistant to fluoroquinolones; R/SLI, resistant to second-line injectable agents; H, isoniazid; R, rifampicin; E, ethambutol; Z, pyrazinamide; Lfx, levofloxacin; Km, kanamycin; Mfx, moxifloxacin; Eto, ethionamide; Cfz, clofazimine; Hh, high-dose isoniazid; Cs, cycloserine; Lzd, linezolid; Bdq, bedaquiline; Cm, capreomycin.

Weight category	Number of tablets (FDCs)	
	Intensive phase (HRZE)	Continuation phase (HRE)
25–39 kg	2 tablets	2 tablets
40–54 kg	3 tablets	3 tablets
55–69 kg	4 tablets	4 tablets
≥70 kg	5 tablets	5 tablets

Note: Fixed dose combination (FDC) tablet:

- Each FDC tablet of intensive phase contains- isoniazid (75 mg), rifampicin (150 mg), pyrazinamide (400 mg), and ethambutol (275 mg)
- Each FDC tablet of continuation phase contains- isoniazid (75 mg), rifampicin (150 mg), and ethambutol (275 mg)

- ❖ It is contraindicated in pregnancy and age <18 years
- ❖ **Regimen:** Bedaquiline containing regimen should contain four other second-line ATDs depending upon the sensitivity.

Delamanid

Delamanid is a new ATD introduced in 2018; derived from nitroimidazole class.

- ❖ **Mechanism of action:** It acts by inhibiting mycolic acid synthesis of bacterial cell wall
- ❖ **Indication:** It is approved for use in the treatment of DR-TB (MDR, XDR or mixed pattern DR-TB) in a combination regimen
- ❖ **Exclusion criteria:** Delamanid should not be used for the following: (i) Children under 6 years, (ii) pregnant and breastfeeding women, (iii) patients with prolonged QT interval, and (iv) if hypersensitivity develops
- ❖ **Current status in India:** Seven states have been identified as initial sites for the introduction of delamanid under the RNTCP—Punjab, Chandigarh, Rajasthan, Karnataka, Odisha, Kerala and Lakshadweep.

Resistance to Antitubercular Drugs

Drug resistance is the most worrisome aspect of management of tuberculosis. Development of drug resistance may occur due to:

- ❖ **Primary or pretreatment drug resistance:** It develops in a strain infecting a patient who has not previously been treated. It mostly occurs due to infection of an individual by a drug resistant strain. Primary resistance accounts for minority of cases
- ❖ **Acquired resistance** (secondary or post treatment): It develops when the infective strain is initially sensitive, becomes resistant later. It is usually due to inappropriate or inadequate treatment. This is much more common than primary resistance.

Mechanism of Drug Resistance

Mechanism of resistance in tubercle bacilli is due to point mutation in the genome of *M. tuberculosis* which occurs at a rate of once in 10^8 cell divisions (Table 63.7).

Rationale of Using Multidrug Therapy

The most worrisome aspect of chemotherapy is development of drug resistance especially when monotherapy is used.

- ❖ This can be effectively checked by multiple drug therapy
- ❖ Incidence of resistance to one drug is independent of that to another. Hence, the probability of a strain to be resistant to two drugs is much lower, than when these drugs are used independently.

Failure to adhere to the multidrug regimen is the most important reason for development of resistance, which may be due to:

- ❖ Prolonged duration of regimen
- ❖ Poor compliance of the patient
- ❖ Development of toxicity to the drugs
- ❖ Improper supervision and follow-up.

Resistance to Antitubercular Drugs (ATDs)

Mono resistance

Defined as resistance to one first-line ATD only.

Poly resistance

Defined as resistance to >1 first-line ATD other than both isoniazid and rifampicin.

Rifampicin resistance (RR)

Defined as rifampicin resistance with or without resistance to other ATDs. It includes any resistance to rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR.

Multidrug-resistant tuberculosis (MDR-TB)

- ❑ **Definition:** MDR-TB is defined as resistance to both isoniazid and rifampicin with or without resistance to other first-line ATDs
- ❑ **Epidemiology:**
 - **World:** In 2018, 3.4% of new and 18% of previously treated cases are MDR/RR-TB; with highest proportions in countries of the former Soviet Union.

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Table 63.7: Drug-resistant genes present in *M. tuberculosis*.

Drugs	Drug-resistant genes
Isoniazid	Enoyl ACP reductase (<i>inhA</i>) Catalase-peroxidase (<i>katG</i>) Alkyl hydroperoxide reductase (<i>AhpC</i>)
Rifampicin	RNA polymerase subunit B (<i>rpoB</i>)
Pyrazinamide	Pyrazinamidase (<i>pncA</i>)
Ethambutol	Ribosomal protein subunit 12 (<i>rpsL</i>)
Streptomycin	Ribosomal protein subunit 12 (<i>rpsL</i>) 16s ribosomal RNA (<i>rrs</i>) Aminoglycoside phosphotransferase gene (<i>strA</i>)
Fluoroquinolones	DNA gyrase (<i>gyr A</i> and <i>B</i>)

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- **India:** In 2018, MDR-TB rate in India was 6.19% in all TB patients (2.8% of all new TB cases and 11.6% of retreatment cases).
- ❑ **Treatment of MDR-TB:** The RNTCP regimen comprises of an intensive phase of 4–9 months with 6–7 second-line agents, followed by continuation phase of 5–18 months with 4 second-line agents (Table 63.5)
- ❑ **DOTS-Plus program** is initiated by RNTCP in the year 2000, to cover the diagnosis and treatment of MDR-TB cases.

Extensively drug-resistant tuberculosis (XDR-TB)

- ❑ **Definition:** They are MDR-TB cases which are also resistant to:
 - Fluoroquinolones (levofloxacin/moxifloxacin/gatifloxacin), and
 - At least one second-line injectable agents (amikacin/capreomycin/kanamycin).
- ❑ **Epidemiology: In India (2018),** XDR-TB accounted for 1.3% in all MDR-TB patients (2.3% in new and 0.91% in previously treated patients)
- ❑ **Treatment** of XDR-TB is extremely difficult. XDR-TB has a very rapidly progressing clinical course with high mortality. The RNTCP regimen is even more prolonged with use of higher number of second-line agents (Table 63.5).

Pre-XDR-TB

It refers to resistance to either fluoroquinolones (FQs) or second line injectable (SLI) agents, but not both. In 2018 (India), among MDR-TB cases, 21.8% were resistant to any FQs and 3.6% were resistant to any SLI agent. The RNTCP regimen given for treatment of Pre-XDR-TB is depicted in Table 63.5.

Global and National TB Programmes

The End TB Strategy (WHO)

In 2016, the WHO has launched “The End TB Strategy by 2035” in order to end the global tuberculosis epidemic. It has the following objectives:

- ❖ About 95% reduction by 2035 in number of TB deaths compared with 2015
- ❖ About 90% reduction by 2035 in TB incidence rate compared with 2015
- ❖ Zero percent TB-affected families facing catastrophic costs due to TB by 2035.

Every year, **March 24th** is observed as 'World Tuberculosis Day' globally. The theme of World Tuberculosis Day 2020— "It's time to End TB"

Revised National Tuberculosis Control Programme (RNTCP)

The Government of India has launched this health program in 1992, in collaboration with WHO and World Bank. In 2020, RNTCP has been renamed as National Tuberculosis Elimination Programme (NTEP).

The main strategies of RNTCP are:

- ❖ Detecting >70% of estimated cases by quality sputum microscopy
- ❖ Cure rate not less than 85%
- ❖ Involvement of NGOs (Non-Government Organizations)
- ❖ Implementing DOTS (Directly Observed Treatment, Short course): A community-based treatment and care of TB patients under supervision
- ❖ Implementing DOTS Plus: For detection and treatment of MDR-TB.

Structural Organization

From centre to periphery, the TB laboratories under government of India are designated as follows:

Central TB division → National TB reference laboratory → State TB division → Intermediate TB reference laboratory → Tuberculosis unit → District microscopy center (DMC)
The four national TB reference laboratories are:

1. National TB Institute, Bengaluru
2. National Institute of TB and Respiratory Disease, New Delhi
3. National Institute of Research in Tuberculosis, Chennai
4. JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra.

National Strategic Plan, India (2020–2025)

In parallel to WHO, Government of India has initiated National Strategic Plan (2020–2025), aiming at elimination of TB epidemic with a vision of TB-Free India with zero deaths, disease and poverty due to tuberculosis by 2025.

Nikshay: It is a web-portal introduced since 2018 for surveillance of TB. Government of India has made it mandate for all health care providers (both government and private sectors) to notify all new TB cases through this web.

99DOTS: It is named so as a very high success rate (of about 99%) is expected by this remote in-built techno-supervision. Is a low-cost, mobile phone-based technology available since 2016 that enables real-time remote monitoring of daily intake of treatment in TB/HIV co-infected patients.

Vaccine Prophylaxis Against Tuberculosis

Bacillus Calmette-Guérin Vaccine (BCG)

BCG vaccine was developed by Calmette and Guerin (1921).

- ❖ **BCG strain:** In India, WHO recommended *Danish 1331* strain of BCG is used. It is prepared in Central BCG laboratory, Guindy, Chennai

- ❖ **Reconstitution of BCG:** BCG is available in lyophilized form, should be reconstituted before administration. This is done by using normal saline as diluent. Distilled water is never used as it is irritant. Once reconstituted; it has to be administered within 1 hour

- ❖ **Administration of BCG:** 0.1 mL (0.1 mg TU) of BCG vaccine is administered above the insertion of left deltoid by intradermal route, using a 26 gauge tuberculin syringe

- ❖ **Phenomena after BCG:** If BCG is properly injected intradermally, then the following phenomena develop at the inoculation site:

- After 2–3 weeks: Papule develops
- 5–6 weeks: Shallow ulcer develops, which is covered with crust
- 6–12 weeks: Permanent tiny round scar (4–8 mm diameter) is formed
- 8–14 weeks: Mantoux test becomes positive.

If overdose is given: The lesion or scar becomes larger and irregular in size.

- ❖ **Protection:**

- Efficacy: Many trials have shown that BCG has a variable efficacy of 0–80%
- Duration of immunity lasts only for 15–20 years
- Though BCG may not protect from the risk of tuberculosis infection, it surely gives protection to infants and young children against the development of complications such as tuberculous meningitis and disseminated tuberculosis.

- ❖ **Complications following BCG:**

- Most common complications include ulceration at the vaccination site and regional lymphadenitis
- Rarely, keloid or lupus lesion, and osteomyelitis may develop
- Very rarely, non-fatal meningitis, progressive tuberculosis and disseminated BCG infection ("BCGitis") are reported in people with low immunity.

- ❖ **Indications of BCG**

- Direct BCG: BCG is directly given to the newborn soon after birth. This strategy is followed by most of the developing countries including India. If not given at birth it can be given later, maximum up to 2 years
- Indirect BCG: BCG is given after performing tuberculin skin test.

- ❖ **Contraindications to BCG include:**

- HIV-positive child
- Child born to AFB positive mother
- Child with low immunity
- Generalized eczema
- Pregnancy.

- ❖ **Other uses of BCG are:**

- BCG induces non-specific stimulation of the immune system; thus provides some protection against certain diseases such as leprosy and leukemia

- BCG has been tried as an adjunctive therapy in malignancies, such as bladder carcinoma (Onco TICE strain of BCG).

VPM1002

It is a recombinant BCG vaccine, under phase II trial (2017). It is prepared by replacing the urease C encoding gene from *Listeria monocytogenes* which improves its immunogenicity (promotes phagolysosome fusion).

Chemoprophylaxis

Treatment of selected high-risk tuberculin reactors (i.e. people with latent tuberculosis) aims at preventing active disease. Isoniazid or ethambutol for six months have been tried. However, chemoprophylaxis has several shortcomings such as—(1) it is expensive, (2) risk of developing tuberculosis is minimal in tuberculin reactors, and (3) side effects of the drugs.

Hence, INH preventive therapy (IPT) can be restricted to limited indications such as:

- ❖ Adults with HIV who are unlikely to have active TB
- ❖ Children with HIV who have no TB symptoms and who are unlikely to have active TB
- ❖ All children with HIV who have successfully completed treatment for TB.

NONTUBERCULOUS MYCOBACTERIA INFECTIONS

Nontuberculous mycobacteria (NTM) were formerly called atypical mycobacteria or mycobacteria other than tubercle bacilli (MOTT).

NTM are diverse group of mycobacteria that are isolated from birds, animals, and from environmental sources, such as soil and water. They are opportunistic pathogens, occasionally associated with human infection. Man-to-man transmission is not known.

Saprophytic mycobacteria are isolated from soil, water and other environmental sources. They do not cause any disease in humans and are distinct from NTM. Examples include *M. phlei* (from grass), *M. smegmatis* (from smegma, a common contaminant in urine).

Classification of NTM

Nontuberculous mycobacteria (NTM) have been classified (Table 63.8) into four groups by Runyon (1959), based on pigment production and rate of growth.

1. Photochromogens

They produce pigments only when the colonies are exposed to light. This group contains the following pathogens:

- ❖ ***M. marinum***: It is acquired from water sources (fish tanks, swimming) and enters through minor trauma. It typically causes papules or ulcers known as **swimming pool granuloma** or fish tank granuloma (Chapter 55)

Table 63.8: Runyon's classification of nontuberculous mycobacteria.

Runyon group	Property	Species
I. Photochromogens	Produce pigments only in light	<i>M. marinum</i> , <i>M. asiaticum</i> , <i>M. simiae</i> , <i>M. kansasii</i> , <i>M. genavense</i>
II. Scotochromogens	Produce pigments both in dark and light	<i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. gordonae</i> <i>M. celatum</i> , <i>M. flavescens</i>
III. Non-photochromogens	Do not produce pigments	<i>M. avium-intracellulare</i> complex (MAC), <i>M. xenopi</i> , <i>M. ulcerans</i> , <i>M. paratuberculosis</i> , <i>M. malmoense</i>
IV. Rapid growers	Grow within one week	<i>M. chelonae</i> , <i>M. fortuitum</i> , <i>M. smegmatis</i> , <i>M. abscessus</i>

- ❖ ***M. asiaticum***: It is rarely associated with pulmonary disease and bursitis
- ❖ ***M. simiae***: It was originally isolated from monkeys. It is principally isolated from pulmonary lesions
- ❖ ***M. kansasii***: It causes chronic pulmonary disease resembling tuberculosis
- ❖ ***M. genavense***: It grows very slowly and rarely causes infection in patients with advanced HIV.

2. Scotochromogens

They produce pigments (yellow, orange or red) even when cultures are incubated in dark, but intensity of color may increase on exposure to light.

- ❖ ***M. scrofulaceum***: It causes scrofula (cervical lymphadenitis) in children
- ❖ ***M. gordonae***: It is often found as commensal in tap water and is a common contaminant of clinical specimens. It is rarely isolated from pulmonary specimens; however its pathogenic potential is doubtful
- ❖ ***M. szulgai***: It behaves as a scotochromogen at 37°C and photochromogen at 25°C. It may occasionally cause pulmonary disease and bursitis
- ❖ ***M. celatum***: It is a rare cause of pulmonary infection.

3. Nonphotochromogens

They do not produce any pigments. Examples include:

- ❖ ***M. avium-intracellulare* complex (MAC)**: They comprise of two related organisms—*M. avium* (Battey bacillus, isolated from birds) and *M. intracellulare*
 - They are opportunistic pathogens, especially in HIV-infected people with low CD4 T cell count (<50/μL)
 - MAC can cause various manifestations: Lymphadenitis, respiratory infection and disseminated disease.
- ❖ ***M. xenopi***: It has been isolated from hospital water supplies, and associated with nosocomial outbreaks. It is found as a commensal, but rarely causes pulmonary disease especially in HIV-infected people

- ❖ ***M. ulcerans***: It typically produces painless ulcers and nodules (called as **Buruli ulcer**), found mainly in the tropics. It can also cause osteomyelitis and limb deformities (Chapter 55)
- ❖ ***M. malmoense***: It can cause pulmonary disease and rarely lymphadenitis
- ❖ ***M. paratuberculosis*** (Johne's bacillus): It mainly causes disease in cattle. It is associated with the pathogenesis of Crohn's disease, but this link has not been proved yet.

4. Rapid Growers

This group of NTM grow in culture within 1 week of incubation. Examples include:

- ❖ ***M. fortuitum*** and ***M. chelonae***: They cause post-trauma injection abscess and catheter-related infections
- ❖ ***M. abscessus***: It can cause pulmonary infection.

The clinical manifestations of NTM are tabulated in Table 63.9.

Laboratory Diagnosis

- ❖ **Specimens**: Sputum, lymph node aspirate, pus or exudate, biopsy from skin lesions are the usual specimens, depending on the type of infection
- ❖ **Microscopy by ZN staining**: Shows red acid-fast bacilli which needs to be differentiated from *M. tuberculosis*
- ❖ **Culture on LJ media**: Several species of NTM grow well on LJ medium, however a few grow sparsely
- ❖ **Pigment production**: LJ media are incubated in dark and light separately for distinguishing between photochromogens and scotochromogens
- ❖ **Identification**: Species of NTM can be differentiated from *M. tuberculosis* complex by:
 - Negative for MPT64 antigen by ICT: Suggestive of NTM infection differentiating it from *M. tuberculosis*

Table 63.9: Clinical manifestations of nontuberculous mycobacteria (NTM).

Disease	Organisms
Pulmonary infection	<i>M. avium-intracellulare</i> (MAC) <i>M. kansasii</i> , <i>M. xenopi</i> , <i>M. malmoense</i> , <i>M. szulgai</i> , <i>M. abscessus</i>
Lymph node infection	<i>M. avium-intracellulare</i> (MAC) <i>M. scrofulaceum</i> —causes scrofula <i>M. malmoense</i>
Cutaneous infection (Discussed in Chapter 55)	<i>M. marinum</i> —causes swimming pool or fish tank granuloma <i>M. ulcerans</i> —causes Buruli ulcer <i>M. abscessus</i> <i>M. fortuitum</i> and <i>M. chelonae</i> —cause injection abscess <i>M. avium-intracellulare</i> (MAC)
Disseminated infection	<i>M. avium-intracellulare</i> (MAC) <i>M. kansasii</i>

- Biochemical tests; which are less commonly used now
- Newer methods: MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) and molecular methods such as PCR (preferred methods for species identification of NTM).

TREATMENT Nontuberculous mycobacteria infections

Just as in tuberculosis, NTM infections are treated with multidrug therapy and are associated with the emergence of drug resistance and relapse.

- *M. avium-intracellulare* complex (MAC), *M. kansasii* and *M. marinum* infections often require multidrug therapy with macrolide (clarithromycin or azithromycin), ethambutol, and a rifamycin (rifampin or rifabutin)
- NTM are resistant to most of the first and second-line anti-tubercular drugs.

EXPECTED QUESTIONS

I. Write essay on:

1. Rajesh, a 28-year-old male, was admitted to the hospital with complaints of low-grade fever, loss of weight and appetite and chronic cough with expectoration for past 6 months. Sputum examination revealed long, slender and beaded acid-fast bacilli.
 - a. What is your provisional diagnosis?
 - b. Describe the pathogenesis of this condition.
 - c. Mention the laboratory diagnosis in detail.
 - d. Mention briefly about drug resistance that can occur in this etiological agent.

II. Write short notes on:

1. BCG vaccine.
2. MDR-TB.
3. Nontuberculous mycobacteria infections

III. Multiple Choice Questions (MCQs):

1. A positive tuberculin skin test is indicated by an area of induration of:
 - a. <5 mm in diameter
 - b. 6–9 mm in diameter
 - c. No induration
 - d. ≥10 mm in diameter
2. How much bacillary load in sputum is required for an effective transmission of *M. tuberculosis*?
 - a. 10 bacilli/mL
 - b. 100 bacilli/mL
 - c. 1000 bacilli/mL
 - d. 10000 bacilli/mL
3. Survival of *M. tuberculosis* inside the macrophages is due to:
 - a. Inhibition of entry into the host cell
 - b. Inhibition of entry into the phagosome
 - c. Inhibition of phagosome-lysosome fusion
 - d. Inhibits degradation by lysosomal enzymes

Answers

1. d 2. d 3. c

Pertussis (*Bordetella pertussis*)

CHAPTER PREVIEW

- Virulence Factors
- Clinical Manifestations
- Epidemiology
- Laboratory Diagnosis
- Treatment
- Prevention

Bordetella is highly fastidious, very small, gram-negative coccobacillus, described first by Bordet and Gengou in 1906. It is a non-fermenter, belongs to family Alcaligenaceae. It comprises of several species.

- ❖ *Bordetella pertussis*: It causes whooping cough in children, a highly contagious vaccine preventable bacterial disease, characterized by paroxysmal cough ending in a high-pitched inspiratory sound described as “whoop”
- ❖ *B. parapertussis*: It causes a milder form of whooping cough
- ❖ *B. bronchiseptica*: It is a pathogen of domestic animals that causes kennel cough in dogs, atrophic rhinitis and pneumonia in pigs, and pneumonia in cats. Rarely, respiratory infections in humans have been reported.

PERTUSSIS

B. pertussis causes a violent paroxysmal productive cough in children called **whooping cough** or **pertussis**.

Virulence Factors

B. pertussis produces a wide array of toxins and biologically active products that are important in its pathogenesis and in immunity. Most of these virulence factors are under the control of a single genetic locus that regulates their production.

- ❖ **Tracheal cytotoxin**: It is a part of cell wall peptidoglycan, which causes damage to the cilia of respiratory epithelial cells
- ❖ **Pertussis toxin (PT)**: It is the most important virulence factor, and is expressed only by *B. pertussis*
 - PT is similar to cholera toxin in its structure and mechanism
 - It causes activation of adenylyl cyclase, leading to ↑ concentrations of cAMP; which is responsible for producing a variety of biologic effects seen in whooping cough.
- ❖ **Adhesins**: They play a role in bacterial attachment. Examples include:

- Filamentous hemagglutinin (FHA)
- Pertactin, an outer-membrane protein
- Fimbriae or pili or agglutinogens.

Other virulence factors include: Adenylyl cyclase toxin, dermonecrotic toxin and bacterial endotoxin.

Pathogenesis

Pertussis is primarily a toxin-mediated disease. The bacteria attach to the ciliated epithelial cells of nasopharynx, multiply by producing variety of toxins to cause local mucosal damage and thereby impairs host defense mechanism.

Clinical Manifestations

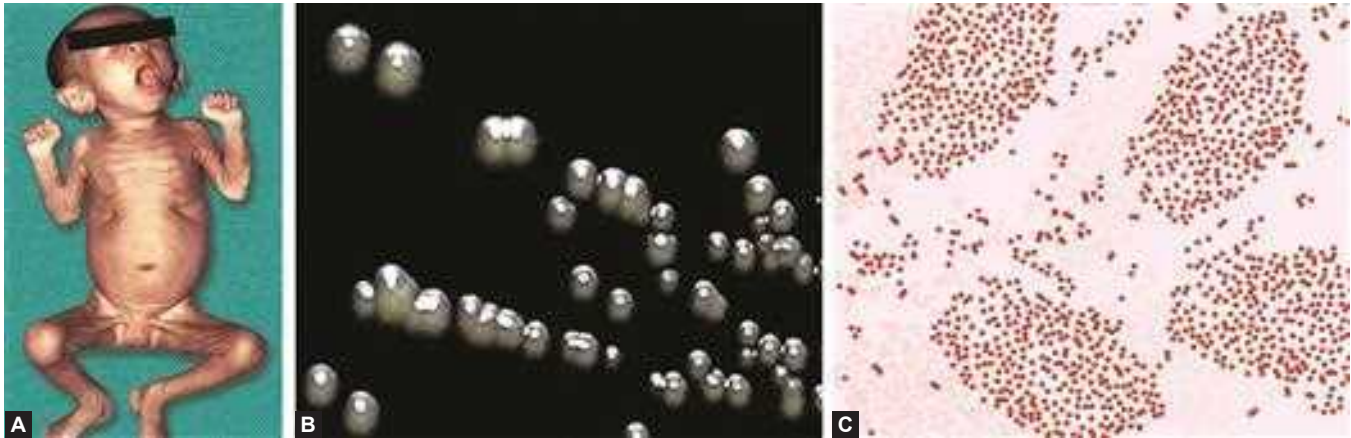
The clinical course of whooping cough (or pertussis or 100 days fever) passes through three stages following an incubation period of 7–10 days.

- ❖ **Catarrhal phase**: It lasts for 1–2 weeks, and is characterized by common cold like nonspecific symptoms, such as coryza, lacrimation, mild cough, low-grade fever and malaise. It is highly infectious stage. In this stage, both smear and cultures are likely to be positive
- ❖ **Paroxysmal phase**: This stage lasts for 1–6 weeks. Patients are less infectious; smear and culture may become negative. It is characterized by specific symptoms, such as whooping cough and post-tussive vomiting (see the below box)
- ❖ **Convalescent stage**: This stage lasts for 1–3 months. It occurs following the paroxysmal stage, during which the frequency and severity of coughing gradually decreases. Antibodies may appear in serum.

Whooping Cough

Each paroxysm consists of bursts of 5–10 repetitive violent spasmodic coughs, often within a single expiration which ends with an audible sound or whoop. Whoop occurs due to rapid inspiration against a closed glottis at the end of the paroxysm (Fig. 64.1A).

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Figs 64.1A to C: A. Female infant suffering from pertussis; B. Colonies of *Bordetella pertussis* on Regan-Lowe agar (mercury drops appearance); C. Gram-stained smear (schematic) of *Bordetella pertussis* (thumb print appearance).

Source: A. Public Health Image Library, ID# 6379/Centers for Disease Control and Prevention (CDC), Atlanta; B. American Type Culture Collection (ATCC)® 9797 (with permission).

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- ❑ Paroxysms may be precipitated by noise, eating or physical contact. In between the paroxysms, patient may appear to be normal
- ❑ The frequency of paroxysms varies widely, from several per hour to 5–10 per day
- ❑ Episodes are often worse at night
- ❑ During a spasm, there may be visible neck vein distension, bulging of eyes, tongue protrusion and cyanosis
- ❑ Weight loss may be seen, but fever is uncommon.

Complications

Complications are more common among infants than among older children or adults.

- ❖ **Pressure effects** during the violent spasms of coughing results in subconjunctival hemorrhage, hernias, pneumothorax, rib fracture and petechiae on the face and body
- ❖ **Pneumonia** may develop especially in old age, due to secondary infection due to encapsulated bacteria. If occurs in infants, it is usually due to *B. pertussis*
- ❖ **Neurological complications**, such as convulsions, encephalopathy and coma may also occur.

Differential Diagnosis

Whooping cough like symptoms may be seen with:

- ❑ *Mycoplasma pneumoniae*
- ❑ *Chlamydomphila pneumoniae*
- ❑ Adenovirus
- ❑ Influenza virus
- ❑ Rhinovirus

Epidemiology

Whooping cough is a highly communicable disease, with high attack rates of 80–100%; which reduces to 20% in immunized populations.

- ❖ **Host:** It is exclusively human disease and there is no animal reservoir
- ❖ **Source:** Early cases (catarrhal stage) are the main source of infection. Even though previously it was believed that there is no carrier state, recent molecular studies proved that a transient carrier state in the nasopharynx occurs among children following the disease, which may contribute to the spread of the infection. However, there is no evidence of a long-term carrier state
- ❖ **Age:** Whooping cough is predominantly a disease of pre-school children below 5 years. As the maternal antibodies are not protective, infants remain the most vulnerable group, accounting for highest morbidity and mortality
- ❖ **A shift of median age:** Pertussis has shifted from infants to older children and adolescence in countries with high vaccination coverage. This indicates that pertussis immunizations or natural infection do not provide lifelong immunity
- ❖ **Mode of transmission:** It is via inhalation of droplets (by coughing or sneezing or even talking) or rarely through direct contact
- ❖ **Recent outbreaks:** Several outbreaks have been reported recently; which includes the Washington epidemic in 2012 and California epidemic in 2014
- ❖ **Worldwide,** the incidence of pertussis is declining. WHO estimated around 1,51,074 cases of pertussis in 2018; compared to >20 Lakh cases in 1980. Most cases occurred in unvaccinated children; although the global vaccine coverage was 86% in 2018
- ❖ **India:** There is a marked decline of the disease after launch of the vaccine under universal immunization programme in India. In 2016, pertussis cases reported from India were 37,274
- ❖ There is no cross protection to *B. parapertussis* infection.

LABORATORY DIAGNOSIS

Pertussis

- ❑ **Specimen:** Nasopharyngeal secretions, collected by alginate swabs
- ❑ **Direct smear:** Gram-negative coccobacilli and pus cells
- ❑ **Culture:**
 - Regan-Lowe medium and Bordet-Gengou agar
 - Produces mercury drops or bisected pearls colony
- ❑ **Culture smear:** Reveals small, ovoid gram-negative coccobacilli arranged in 'thumb print' appearance
- ❑ **Identification:** By automated methods such as MALDI-TOF or VITEK
- ❑ **Detection of serum antibodies:** By enzyme immunoassays
- ❑ **PCR:** Detecting IS481 and PT promoter region genes
- ❑ **Typing of *B. pertussis*:** By serotyping and genotyping.

Laboratory Diagnosis

- ❖ **Specimen collection:** Nasopharyngeal secretions are the best specimens which may be obtained by— (i) nasopharyngeal aspiration (best method), or (ii) pernasal swab. Throat and/or sputum specimens are unacceptable
 - **Type of swabs used:** For culture, alginate swabs are the best followed by dacron swabs. However, for PCR, only dacron or rayon swabs are recommended
 - ◆ Cotton swabs are not satisfactory as fatty acids present in cotton may inhibit the growth of the bacilli. However charcoal-impregnated cotton swabs (Stuart's) may be useful
 - ◆ It is recommended to collect **six swabs**, at 1–2 days intervals to achieve maximum yield.
 - Cough plate method and post-nasal swabs used before are no longer recommended.
- ❖ **Transport:** Specimens should be processed immediately. If delay is expected, then suitable charcoal-based medium (Amies, Regan-Lowe, Jones-Kendrick transport media) can be used
- ❖ **Direct detection:** *B. pertussis* may be directly detected from nasopharyngeal secretions by **direct immunofluorescence test** using fluorescein labelled polyclonal or monoclonal antibodies. Because of poor sensitivity (18–75%) and specificity (65–99%), it is not widely used
- ❖ **Culture:** Nasopharyngeal culture remains the gold standard method of diagnosis
 - *B. pertussis* is a strict aerobe, grows best at 35–37°C. It is fastidious, requires special complex media for primary isolation, such as—(i) Regan and Lowe medium, (ii) Bordet-Gengou glycerine-potato-blood agar
 - Colonies are greyish white, convex with a shiny surface appear after 3–5 days, described as **mercury drops or bisected pearls appearance** (Fig. 64.1B)
 - Culture remains positive only during the first three weeks of infection (i.e. mainly in catarrhal stage) where the symptoms are nonspecific
 - Culture becomes negative within 5 days of starting antibiotics.
- ❖ **Culture smear:** Gram-staining of culture reveals small, ovoid coccobacilli, tend to arrange in loose clumps, with clear spaces in between giving a **thumb print** appearance (Fig. 64.1C)
- ❖ **Identification** from colonies can be done by automated methods such as MALDI-TOF or VITEK
- ❖ **Detection of serum antibodies:** Enzyme immunoassays (EIAs) using purified antigens of *B. pertussis*, such as pertussis toxin, FHA and pertactin are the methods of choice
 - Demonstration of a rise of IgG antibodies in paired sera or detection IgA or IgM antibodies provides definite diagnosis
 - However, antibodies are also elevated in immunized people.
- ❖ **Molecular methods:** PCR remains positive in first four weeks of onset of symptoms. It is being increasingly used in many laboratories replacing culture, because of increased sensitivity, specificity and quicker results. The most common targeted genes are IS481 and the pertussis toxin promoter region genes
- ❖ **Other findings:** Lymphocytosis is common among young children but not among adolescents.

TREATMENT

Pertussis

As pertussis is mainly toxin mediated, antibiotics are less useful once the infection is established. However, they play a vital role to eliminate the bacteria from nasopharynx.

- ❑ Macrolides are the drugs of choice (azithromycin for 5 days or erythromycin for 7–14 days)
- ❑ Cotrimoxazole is recommended as an alternative in macrolide resistance or allergy.

Isolation in a quiet environment may inhibit the stimulation of paroxysms. Cough suppressants are not much effective.

Prevention

Chemoprophylaxis

Erythromycin is widely recommended as chemoprophylaxis for household contacts of pertussis cases.

Vaccine

Whole-cell Pertussis Vaccines

It is prepared by heating followed by chemical inactivation and purification of whole *B. pertussis* bacilli.

- ❖ Efficacy is good, average being 85%
- ❖ **DPT vaccine:** In India and many other countries, whole cell (WC) pertussis vaccine is given under national immunization programme, along with diphtheria toxoid and tetanus toxoid (DPT)
 - Three doses of pentavalent vaccine (DPT, hepatitis B and *H. influenzae*) are given at 6, 10 and 14 weeks, followed by two boosters of DPT at 1½ years and 5 years.
 - *Pertussis* component acts as an adjuvant and increases immunogenicity of DT and TT. For detail of DPT vaccine, refer Chapter 60.

- ❖ **Adverse effects:** WC vaccine is associated with the following adverse effects, such as:
 - Common: Fever, injection-site pain, erythema, swelling, and irritability
 - Uncommon: *Bordetella pertussis* is encephalitogenic. It is associated with neurological complications (encephalitis, prolonged convulsion) and **hypotonic hyporesponsive syndrome**. The estimated risk is 1:1,70,000 doses administered.
- ❖ **Contraindication:** Because of the adverse effects, the WC vaccine is contraindicated in—
 - Children more than 5–6 years age
 - Any associated progressive neurological conditions

- Children with strong family history of epilepsy
- Hypersensitivity to previous dose.

Acellular Pertussis Vaccine

It is composed of pertussis toxoid and 2 or more other bacterial components such as FHA, pertactin or fimbriae.

- ❖ Though the efficacy is same as WC vaccine, it is associated with fewer side effects as compared with the latter and can be safely given after 5–6 years
- ❖ It is available as DaPT (along with diphtheria and tetanus toxoid). It is recommended for older children who have missed their primary doses of DPT.

EXPECTED QUESTIONS

I. Write short notes on:

1. Virulence factors of *Bordetella pertussis*.
2. Laboratory diagnosis of pertussis.
3. Vaccination against pertussis.

II. Multiple Choice Questions (MCQs):

1. **Mercury drop appearance colony of *B. pertussis* is seen on which of the following culture media?**
 - a. Blood agar
 - b. Chocolate agar

- c. Regan-Lowe agar
- d. Nutrient agar

2. Pertussis toxin is produced by:

- a. *B. pertussis*
- b. *B. parapertussis*
- c. *B. avium*
- d. *B. bronchiseptica*

3. Which is the highly infective stage in whooping cough?

- a. Catarrhal stage
- b. Paroxysmal stage
- c. Convalescent stage
- d. All of the above

Answers

1. c 2. a 3. a

Infections due to Non-fermenting Gram-negative Bacilli

CHAPTER 65

CHAPTER PREVIEW

- *Pseudomonas* Infections
- *Burkholderia* Infections
- *Acinetobacter* Infections
- *Stenotrophomonas* Infections
- *Elizabethkingia* Infections

INTRODUCTION

Non-fermenting gram-negative bacilli (NF-GNB) do not ferment any sugars, but they utilize the sugars oxidatively.

- ❖ Most of the NF-GNB exist as environmental commensals in hospitals that inhabit in moist environments, detergents and IV fluids. They are resistant to multiple antibiotics. However, they cause various infections in hospitalized patients, of which respiratory infections are noteworthy. Examples include:
 - *Pseudomonas aeruginosa*
 - *Burkholderia cepacia*
 - *Acinetobacter baumannii*
 - *Stenotrophomonas maltophilia*
 - *Elizabethkingia meningosepticum*
- ❖ However, there are some non-fermenters which are principally community associated pathogens. Classical example is *Burkholderia pseudomallei*, which causes infections of various systems, of which the notable are respiratory, skin and soft tissue, and bloodstream infections.

PSEUDOMONAS INFECTIONS

Pseudomonas is an oxidase positive, pigment producing, non-fermenting gram-negative bacilli. It is a major pathogen responsible for most of the hospital acquired infections and also of importance in patients with cystic fibrosis

Virulence Factors and Pathogenesis

The pathogenesis of *Pseudomonas* is greatly attributed to its ability to develop widespread resistance to multiple antibiotics and disinfectants, and also by producing a number of virulence factors.

- ❖ **Colonization:** The first event to initiate the infection is to adhere and colonize the host surface. Various factors help in adhesion, such as pili or fimbriae (the organ of attachment) and polar flagellum (mediates chemotactic motility to reach the host's surface)

- ❖ **Toxin-mediated immune evasion and tissue injury:** *Pseudomonas aeruginosa* produces probably the largest number of toxins and enzymes among the gram-negative bacteria. These can be grouped into:

- **Nondiffusible toxins** (e.g. exotoxins S, U, T, and Y): Colonized *Pseudomonas* injects these toxins into the host cells, which allow the bacteria to evade the phagocytic cells and induce tissue injury by their cytotoxic activity
- **Diffusible toxins:** For example, exotoxin A, proteases, phospholipases, hemolysins, elastases, pyocyanin, etc. They can act freely and mediate tissue injury.

Exotoxin A: This is the most important virulence factor of *P. aeruginosa*. It inhibits protein synthesis by inhibiting elongation factor-2 (mechanism of action is similar to diphtheria toxin).

- ❖ **Host's inflammatory response:** Host elicits inflammatory responses as a defense mechanism against various components of the bacilli, such as endotoxin and flagellin, mediated through the Toll-like receptors. However, florid and stronger inflammatory responses can lead to tissue injury and septic shock
- ❖ **Pigment production:** *Pseudomonas* produces a number of pigments which diffuse freely into the surroundings, inhibit other bacteria and mediate tissue injury
 - Pyocyanin: It is a blue-green pigment, produced only by *P. aeruginosa*
 - Fluorescein (or pyoverdin): It gives greenish-yellow color to the colony produced by most of the species
 - Pyorubin (imparts red color)
 - Pyomelanin (imparts brown-black color).
- ❖ **Alginate coat:** Mucoid strains of *Pseudomonas* have a slime layer or alginate layer which facilitates biofilm formation, thus helps in adhesion to host cells and purulent mucus. Such strains can cause infections in patients with cystic fibrosis
- ❖ **Capsule:** Many strains of *Pseudomonas* possess polysaccharide capsule, which prevents the bacteria from phagocytosis

- ❖ **Multidrug resistance:** *Pseudomonas* is known to possess genes coding for resistance to several antimicrobial agents; thereby helping the bacilli to survive under antibiotic pressure especially in the hospital environment. **Biofilm formation** is another mechanism by which it prevents the entry of antibiotics into the bacterial cell
- ❖ **Multi-disinfectant resistance:** It helps the bacilli to grow in presence of various disinfectants; thus, spreading the infection in the hospitals
- ❖ **Wide temperature range:** *Pseudomonas* survives in extremes of temperatures (5–45°C), which allows the bacilli to be ubiquitous.

Clinical Manifestations

Pseudomonas aeruginosa is notorious to cause infections at almost all sites; most common being lungs, skin and soft tissues. Most of the infections are encountered in hospitalized patients who get colonized with the organisms either from the heavily contaminated hospital environment or from the hospital staff (through contaminated hands). Colonized patients develop the disease in the presence of underlying risk factors such as burn wounds, patients with immunosuppression and post surgeries. The manifestations are as follows.

- ❖ **Healthcare-associated infections** such as—(i) ventilator associated pneumonia (VAP), (ii) central-line associated bloodstream infection (CLABSI), (iii) catheter-associated urinary tract infection (CAUTI), (iv) surgical site infection (SSI)
- ❖ **Chronic respiratory tract infections:** It occurs in patients with underlying conditions that cause airway damage such as cystic fibrosis, or bronchiectasis. These infections are usually caused by mucoid strains of *Pseudomonas*
- ❖ **Bacteremia** leading to sepsis and septic shock
- ❖ **Infective endocarditis (native valves):** It occurs among IV drug abusers
- ❖ **Ear infections:** The infections are either mild, such as **Swimmer's ear** (among children) or serious necrotizing form designated as **malignant otitis externa** (in elderly diabetic patients)
- ❖ **Eye infections** such as corneal ulcers (in contact lens wearers) and endophthalmitis secondary to bacteremia
- ❖ **Shanghai fever:** It is a mild febrile illness resembling typhoid fever
- ❖ **Skin and soft tissue infections** such as burns wound infection, ecthyma gangrenosum, green nail syndrome and cellulitis with blue-green pus (Chapter 55).
- ❖ **Other infections**
 - Bone and joint infections such as osteomyelitis and septic arthritis
 - Meningitis (in postoperative or post-traumatic patients).

LABORATORY DIAGNOSIS

Pseudomonas infections

- ❑ **Sample collection:** Pus, wound swab, urine, etc.
- ❑ **Direct smear:** Gram-negative bacilli, and pus cells
- ❑ **Culture:**
 - Nutrient agar: Opaque, irregular colonies with metallic sheen (iridescence) and blue green diffusible pigments
 - Blood agar: β -hemolytic grey moist colonies
 - MacConkey agar: NLF colonies
 - Selective media: e.g. cetrimide agar
- ❑ **Culture smear and motility:** Motile, gram-negative bacilli
- ❑ **Identification:**
 - Catalase positive and oxidase positive
 - ICUT tests: Indole (-), Citrate (+), Urease (-), TSI:K/K, gas (-), H_2S (-)
 - Automated identification such as MALDI-TOF or VITEK
- ❑ **Antimicrobial susceptibility testing**

Laboratory Diagnosis

Specimen

Various specimens such as pus, wound swab, urine, sputum, blood or CSF are collected, depending upon the site infected.

Direct Smear

Gram staining of the specimen shows plenty of pus cells and slender gram-negative bacilli and occasionally capsulated.

Culture

Pseudomonas is non-fastidious, can grow in ordinary media. It is an obligate aerobe, hence specimens after being inoculated onto various media, should be incubated aerobically, at 37°C for 24 hours.

- ❖ **Peptone water:** *Pseudomonas* forms uniform turbidity with a **surface pellicle** formation, due to more oxygen tension at surfaces
- ❖ **Nutrient agar:** It produces large, opaque, irregular colonies with a metallic sheen (described as **iridescence**) (Fig. 65.1)
 - **Pigments:** Most strains produce diffusible pigments which are either blue-green (pyocyanin), or yellow green (pyoverdin). Some strains are non-pigmented
 - Mostly colonies of *Pseudomonas* have a characteristic sweet ether or alcohol-like **fruity odor**.
- ❖ **Blood agar:** It produces β -hemolytic gray moist colonies on blood agar
- ❖ **MacConkey agar:** It produces pale non-lactose fermenting colonies
- ❖ Selective media such as **cetrimide agar** can be used to isolate the organism from mixed growth in purulent specimens.

Culture Smear and Motility

Culture smear shows gram-negative bacilli. They are actively motile with single polar flagellum (by hanging drop test).



Fig. 65.1: *Pseudomonas aeruginosa* showing: Nutrient agar with large irregular colonies with metallic sheen and green color pigmentation.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Identification

Identification of *Pseudomonas* from colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as described below.

- ❖ Oxidase and catalase positive
- ❖ Non-fermenter: Does not ferment any sugars, but utilize sugars oxidatively
- ❖ **ICUT test:**
 - Indole test is negative
 - Citrate test: positive
 - Urease test: negative
 - Triple sugar iron (TSI) test: The test shows alkaline slant/alkaline butt (no change), with no gas and no H₂S.

Antimicrobial Susceptibility Testing (AST)

AST is essential to administer appropriate antibiotics. It is performed by disk diffusion method (on Mueller-Hinton agar) or by automated MIC detection method by microbroth dilution (e.g. VITEK)

Typing Methods

P. aeruginosa is an important cause of healthcare-associated infections, it is essential to type the isolates beyond the species level, to find out the relatedness between the isolates. This is useful during outbreaks, to trace the source of infection. For epidemiological studies, various typing methods are used such as—(i) bacteriocin (pyocin) typing, (ii) antibiogram typing, (iii) serotyping, (iv) molecular typing methods, such as pulse-field gel electrophoresis (PFGE) and sequence-based typing method.

TREATMENT

Pseudomonas infections

Pseudomonas species exhibit higher degree of resistance to most of the antibiotics. Only limited antimicrobial agents have antipseudomonal action, such as:

- ❑ **Penicillins:** Piperacillin, mezlocillin, ticarcillin
- ❑ **Cephalosporins:** Ceftazidime, cefoperazone, ceftolozane, and cefepime
- ❑ **β-lactam/β-lactamase inhibitor** combinations (piperacillin-tazobactam and cefoperazone-sulbactam)
- ❑ **Carbapenems:** Imipenem, meropenem and doripenem
- ❑ **Monobactam:** Aztreonam
- ❑ **Aminoglycosides:** Tobramycin, gentamicin, amikacin
- ❑ **Quinolones:** Ciprofloxacin, levofloxacin
- ❑ **Polymyxins:** Polymyxin B, colistin (in nebulized form for pneumonia)

Note: *Pseudomonas aeruginosa* is intrinsically resistant to ceftriaxone, amoxicillin-clavulanate, ampicillin-sulbactam, ertapenem, tetracyclines, tigecycline, cotrimoxazole and chloramphenicol. Therefore, these drugs should not be used in the therapy.

Drug Resistance

Pseudomonas possesses a number of drug resistant plasmids which confer resistance to several antibiotics. Many strains are producers of β-lactamases, such as ESBL (extended spectrum β-lactamases), carbapenemases (e.g. New Delhi metallo-β-lactamase, NDM), and AmpC β-lactamases.

Preventive Measures

Infection control measures (contact precaution) such as hand hygiene are crucial to limit the spread of the infection (see Chapter 21).

BURKHOLDERIA INFECTIONS

Burkholderia species are also oxidase positive non-fermenters similar to *Pseudomonas*; however, they differ from the latter in being:

- ❖ Bipolar stained (safety pin appearance)
- ❖ Resistant to polymyxin B.

Melioidosis (*Burkholderia pseudomallei*)

B. pseudomallei is the causative agent of melioidosis.

- ❖ **Habitat:** *B. pseudomallei* is a saprophyte of soil and water and have large number of animal reservoirs. Melioidosis also occurs in rats, rabbits and guinea pigs
- ❖ **Mode of transmission:** Humans and animals are infected by various routes such as inoculation, inhalation, aspiration or ingestion. Man to man transmission is very rare
- ❖ **Virulence factors:** *B. pseudomallei* is perhaps the most virulent among the non-fermenters. Several virulence factors are described such as polysaccharide capsule, lipopolysaccharide, toxins, enzymes and proteins (such as hemolysin, lipases and proteases)

- ❖ **Risk factors:** Melioidosis occurs mostly when underlying risk factors co-exist such as diabetes, renal failure in adults and traumatic inoculation in children, weather (rainy season) and occupation (rice farmers)
- ❖ **Incubation period** may range from 2 days to many years. Some cases may have long latency; presented long time after the exposure; hence melioidosis is also known as ‘**Vietnam time-bomb disease**’
- ❖ **Clinical feature:** Melioidosis can present with an array of manifestations (hence called as ‘**great mimicker**’); grouped into four types of clinical presentations:
 - **Acute, localized infection:** Presents as localized nodule, fever, general muscle aches, and may progress rapidly to infect the bloodstream
 - **Sub-acute (Pulmonary) infection:** Ranges from mild bronchitis to severe tuberculosis-like pneumonia with cellulitis and lymphangitis
 - **Acute bloodstream infection:** Seen in patients with underlying illness such as HIV, renal failure and diabetes and presents as septicemia (septic shock) with metastatic pus-filled skin lesions and disorientation
 - **Chronic suppurative infection** forming abscesses: Involves various organs such as joints, viscera, lymph nodes, skin, brain, liver, lung, bones, and spleen.
- ❖ **Bioterrorism:** *B. pseudomallei* can be used as a potential agent of biological warfare
- ❖ **Geographical distribution:** It is estimated that around 1.65 lakh new cases of melioidosis occur worldwide every year with mortality as high as 50%
 - **World:** Melioidosis has been endemic in Thailand, Australia, Singapore, Indian subcontinent and other Southeast Asian countries
 - **India:** Melioidosis has been reported mainly from South India such as Tamil Nadu, Karnataka, Puducherry and Kerala.
- ❖ **Laboratory diagnosis:** Depending on the site of infection, various specimens are collected such as sputum, purulent discharge from lesion, aspirated pus, etc.

- **Direct microscopy:** They are gram-negative bacilli that typically exhibit a **bipolar or safety pin** appearance (Fig. 65.2A), which is better appreciated when stained with methylene blue
- **Culture:** *B. pseudomallei* is an obligate aerobe, grows on various media, e.g. nutrient agar, blood agar and MacConkey agar. Colonies are typically rough and corrugated, similar to the colonies of *Pseudomonas stutzeri* (Fig. 65.2B). **Ashdown’s medium** is used as a selective medium, where it produces wrinkled purple colonies (Fig. 65.2C)
- **Identification:** Species identification is made by conventional biochemical tests (e.g. resistance to polymyxin B) or by automated methods such as VITEK. MALDI-TOF is not yet validated for identifying *B. pseudomallei*
- **Latex agglutination test** with specific antisera can be used to identify them from cultures.

T TREATMENT

Melioidosis

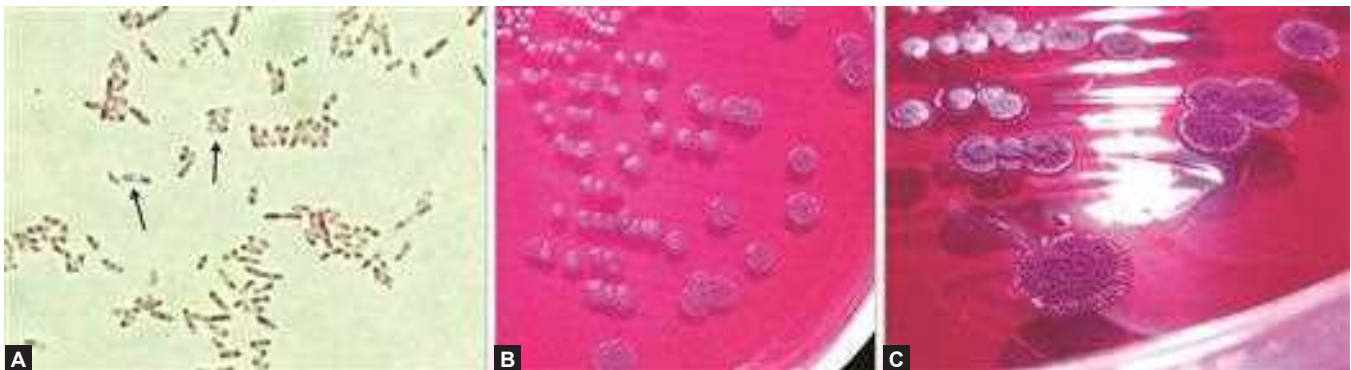
Treatment of melioidosis consists of:

- ❑ **Intensive phase** (2 weeks): Ceftazidime or a carbapenem is given followed by;
- ❑ **Maintenance phase** (12 weeks): Oral cotrimoxazole is given to eradicate the bacilli and to prevent relapse. Doxycycline or amoxicillin-clavulanate are the alternatives.
- ❑ *B. pseudomallei* is intrinsically resistant to many antibiotics including penicillin, first and second-generation cephalosporins, macrolides, rifamycins, colistin and aminoglycosides.
- ❑ **Post-exposure prophylaxis:** After exposure (particularly following a laboratory accident); combined treatment of cotrimoxazole with doxycycline is recommended.

Note: *B. pseudomallei* are intrinsically resistant to penicillin, ertapenem, polymyxins and fosfomycin. Therefore, these drugs should not be used in therapy.

Burkholderia mallei Infections

B. mallei is a pathogen of horses; where it causes **glanders** (nasal discharge and ulcers in the nasal septum) and **farcy** (skin lesions and lymph node involvement).



Figs 65.2A to C: *Burkholderia pseudomallei*: **A.** Gram stained smear-arrows showing gram-negative bacilli with safety pin or bipolar appearance; **B.** Rough and corrugated colonies on MacConkey agar; **C.** Wrinkled purple colonies on Ashdown’s medium.

Source: Dr Isabella Princess, Apollo Hospitals, Chennai (with permission).

- ❖ **Transmission:** Unlike other species, *B. mallei* is not an environmental organism. It is strictly zoonotic, transmitted from horses to man either by direct inoculation or inhalation
- ❖ **Human infection** is characterized by:
 - Local skin nodules and lymphadenitis (if transmitted by inoculation)
 - Pneumonia, ulceration of the trachea and sepsis (if transmitted by inhalation).
- ❖ **Laboratory diagnosis:** It is similar to that of *B. pseudomallei*. However, *B. mallei* differs from *B. pseudomallei* in being non-motile and oxidase negative
- ❖ **Treatment:** It is same as that of *B. pseudomallei*.

Burkholderia cepacia Complex Infections

B. cepacia complex comprises of 18 closely related genomic species and currently it is the most commonly encountered *Burkholderia* species.

- ❖ It is an environmental organism that inhabits moist environments, detergents and IV fluids
- ❖ It has been recognized as a plant pathogen causing onion rot (cepia, Latin for onion)
- ❖ **Virulence factors:** It possesses multiple virulence factors, such as:
 - Cable pilus: A type of fimbriae which is capable of binding to lung mucus
 - Elastase
 - LPS of *B. cepacia* is among the most potent of all bacteria; stimulates inflammatory response in the lungs.
- ❖ **Various clinical manifestations** include:
 - **Cepacia syndrome:** It is characterized by a rapidly fatal respiratory infection and septicemia in patients with cystic fibrosis
 - **Nosocomial pathogen:** It is resistant to multiple antibiotics, hence has been emerging as an important nosocomial pathogen in ICUs causing pneumonia, wound infections, etc.
- ❖ **Laboratory diagnosis:** Clinical and environmental specimens can be inoculated on selective media. Optimum growth occurs at 30°C. Biochemical reactions or automated methods such as MALDI-TOF or VITEK can be carried out to differentiate the genomospecies.
- ❖ **Prevention:** Implementation of infection control measures is crucial to prevent nosocomial spread of infection (refer contact precaution, Chapter 21).

TREATMENT

Burkholderia cepacia infections

B. cepacia is intrinsically resistant to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, ertapenem, polymyxins and fosfomycin. Therefore, these drugs should not be used in the treatment regimen.

The effective drugs are:

- Cotrimoxazole, meropenem, and doxycycline are the most effective agents
- Levofloxacin, ceftazidime-avibactam, ceftolozane-tazobactam are the alternatives.

ACINETOBACTER INFECTIONS

Acinetobacter are saprophytic bacilli, present in the environment (soil, water and phytosphere). However, during the last two decades, it has gained increasing attention as a nosocomial pathogen.

- ❖ **Genomospecies:** DNA hybridization studies have shown that *Acinetobacter* can be grouped into several genomospecies. *A. baumannii* is the most pathogenic species. Most other species such as *A. calcoaceticus* and *A. lwoffii* are rarely pathogenic to man
- ❖ **Sources:** Hospital environment is heavily contaminated with these organisms. They are commensals in skin, oral cavity and intestine. The carriage rate is much higher in the health care setting than community
- ❖ **Promote colonization:** Unhygienic practices in hospitals (contaminated hands of staff) and warm hospital environment (summer) promote colonization. Patients with underlying diseases or immunosuppression are predisposed to invasion and pathogenesis
- ❖ **Pathogenesis:** It is not fully understood
 - **Multidrug resistance:** Its ability to develop drug resistance rapidly to almost all available antibiotics makes it dangerous in hospital settings
 - **Various virulence factors** are also attributed to the pathogenesis such as:
 - ♦ Outer membrane protein A (OmpA): It mediates adhesion, invasion and cytotoxicity through mitochondrial damage
 - ♦ LPS: It induces inflammatory responses that leads to tissue injury
 - ♦ Ability to form biofilm.
- ❖ **Clinical manifestations:** *A. baumannii* causes widespread hospital infections such as:
 - Ventilator associated pneumonia
 - Central line associated bloodstream infection
 - Post-neurosurgical meningitis
 - Catheter-associated UTI
 - Wound and soft tissue infections
 - Infections in burn patients.
- ❖ **Epidemiology:** Several hospital outbreaks due to *Acinetobacter* have been reported throughout the world
- ❖ **Laboratory diagnosis:** It is an obligate aerobe, grows well on ordinary medium. Specimens can be inoculated onto blood agar (non-hemolytic colonies) and MacConkey agar (lactose non-fermenting colonies with faint pink tint) (Fig. 65.3). Important identification features are:
 - **Gram staining:** They are gram-negative coccobacilli
 - Oxidase negative and catalase positive
 - Non-fermenter of sugars and non-motile
 - **ICUT tests:** Indole test (negative), citrate test (positive), urease test (negative) and TSI (triple sugar iron agar) test shows alkaline slant/alkaline butt with no gas and no H₂S
 - Species identification can also be made by automated identification systems such as MALDI-TOF or VITEK.



Fig. 65.3: Lactose non-fermenting colonies (with faint pink tint) of *Acinetobacter* on MacConkey agar.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

- **Antimicrobial susceptibility testing** is performed by disk diffusion method (on Mueller–Hinton agar) or by automated MIC detection method by microbroth dilution (e.g. VITEK).
- ❖ **Prevention:** Infection control measures such as improved hand hygiene are essential to prevent nosocomial infections due to *Acinetobacter* (refer contact precaution, Chapter 21).

TREATMENT

Acinetobacter infections

Acinetobacter is notorious to develop resistance to multiple drugs including β -lactams, aminoglycosides and quinolones.

- β -lactam resistance can be attributed to production of β -lactamases such as metallo β -lactamases (MBL), AmpC β -lactamases and OXA-type β -lactamases. Choice of antibiotics should always be guided by susceptibility reports
- Most of the hospital acquired isolates are resistant to carbapenems
- Common antibiotics indicated are β -lactam/ β -lactamase inhibitor combinations (e.g. piperacillin-tazobactam), tigecycline and colistin.

Note: *Acinetobacter* is intrinsically resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, ertapenem, aztreonam, chloramphenicol and fosfomycin. Therefore, these drugs should not be used in therapy.

STENOTROPHOMONAS INFECTIONS

S. maltophilia is a saprophyte found in the rhizosphere (soil surrounding the plant roots).

- ❖ **Colonization:** The organism is acquired from the environment which is favored by:
 - Immunocompromised conditions
 - Patients on broad-spectrum antibiotics.
- ❖ **Clinical manifestations:** *S. maltophilia* can cause various hospital infections such as pneumonia in ventilated patients, bloodstream infections and ecthyma gangrenosum in neutropenic patients
- ❖ **Laboratory diagnosis:** Identification of *S. maltophilia* is made either by conventional biochemical tests or by automated identification systems such as MALDI-TOF or VITEK.

TREATMENT

Stenotrophomonas infections

S. maltophilia is intrinsically resistant to most antibiotics such as most of the beta-lactams including carbapenems, polymyxins, aminoglycosides and fosfomycin. The recommended antibiotics are cotrimoxazole, minocycline, and levofloxacin.

ELIZABETHKINGIA INFECTIONS

E. meningosepticum is the main species; formerly placed under the genus *Chryseobacterium* or *Flavobacterium*.

- ❖ **Manifestations:** It is saprophyte in soil, water and hospital environment. However, it causes nosocomial infections in patients with underlying immunosuppression such as:
 - Neonatal meningitis
 - Pneumonia, sepsis, endocarditis, bacteremia and soft tissue infections.
- ❖ **Laboratory diagnosis**
 - It is a non-motile, gram-negative bacillus
 - Identification of *E. meningosepticum* is made either by conventional biochemical tests or by automated identification systems such as MALDI-TOF or VITEK.
- ❖ **Treatment:** It is susceptible to fluoroquinolones and cotrimoxazole; however β -lactams should be given with caution as it produces β -lactamases.

Elizabethkingia anophelis is another species, which is recently emerged as a nosocomial pathogen causing sepsis, especially in the neonatal units.

EXPECTED QUESTIONS

I. Write short notes on:

1. Virulence factors of *Pseudomonas aeruginosa*.
2. Melioidosis.
3. Medically important nonfermenters.

II. Multiple Choice Questions (MCQs):

1. **Ecthyma gangrenosum is caused by:**
 - a. *Pseudomonas*
 - b. *Bordetella*
 - c. *Brucella*
 - d. *H. influenzae*

2. Drug used in *Pseudomonas* treatment:

- a. Cefixime
- b. Ceftazidime
- c. Ampicillin
- d. Cotrimoxazole

3. Which of the following drug is not active against *Stenotrophomonas maltophilia*?

- a. Cotrimoxazole
- b. Colistin
- c. Meropenem
- d. Levofloxacin

Answers

1. a 2. b 3. c

Myxovirus Infections of Respiratory Tract

CHAPTER 66

CHAPTER PREVIEW

- Orthomyxoviridae
 - Influenza Viruses
- Paramyxoviridae
 - Parainfluenza Viruses
- Mumps Virus
- Respiratory Syncytial Virus

Myxoviruses are a group of viruses that bind to mucin receptors on the surface of RBCs (*myxo* in Greek meaning 'mucin'); resulting in clumping of RBCs together to cause hemagglutination. Most of the myxoviruses are respiratory pathogens.

Myxoviruses are divided into two families—(1) Orthomyxoviridae and (2) Paramyxoviridae. Both differ from each other in various aspects; the most important difference is the presence of segmented RNA in Orthomyxoviridae family. Important human pathogens are:

- ❖ **Orthomyxoviridae:** Influenza viruses —cause upper respiratory tract infections (URTIs), rarely can cause pneumonia
- ❖ **Paramyxoviridae:** It includes several viruses
 - Parainfluenza virus: Mainly cause laryngotracheobronchitis and other URTIs
 - Mumps virus: Causes parotitis in children (salivary gland infection) and rarely complications such as meningitis
 - Measles virus: Cause exanthematous lesions, can lead to rare but serious complications of CNS (Chapter 56 and 74)
 - Respiratory syncytial virus: Causes acute bronchiolitis in infants
 - Metapneumovirus: Causes URTIs
 - Zoonotic paramyxoviruses such as Nipah and Hendra viruses: Mainly cause encephalitis (Chapter 74).

ORTHOMYXOVIRIDAE INFECTIONS

Influenza viruses are the members of Orthomyxoviridae family. They are one of the major causes of morbidity and mortality and have been responsible for several epidemics and pandemics of respiratory diseases in the last two centuries.

INFLUENZA

Influenza viruses consist of four genera—influenza A, B, C and D.

Morphology (Fig. 66.1)

Influenza viruses are spherical in shape, measure about 80–120 nm in size.

- ❖ **Helical symmetry:** It comprises of a helical nucleocapsid, surrounded by an envelope
- ❖ **Viral RNA** comprises of **multiple segments** of negative sense single stranded RNA. Each segment codes for a specific viral protein having a specific function
 - Influenza A and B contain **eight segments** of RNA
 - Influenza C and D contain seven segments of RNA. The segment coding for neuraminidase is absent.
- ❖ **Site of replication:** RNA replication occurs typically in the nucleus (in contrast to most other RNA viruses which replicate in the cytoplasm)
- ❖ **Viral proteins:** Influenza virus contains eight structural proteins (PB1, PB2, PA, NP, HA, NA, M1 and M2) and two non-structural proteins (NS1 and NS2)
 - **PB1, PB2, and PA** are the polymerase proteins responsible for RNA transcription and replication
 - **Nucleoprotein (NP)** is the major capsid protein, associated with viral RNA to form a ribonucleoprotein (RNP) or nucleocapsid with a helical symmetry

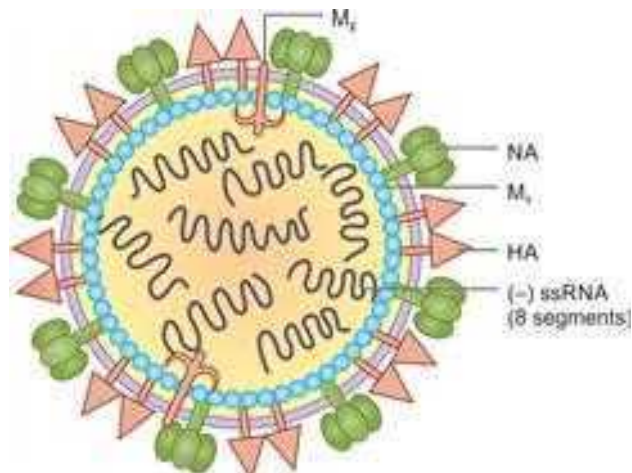


Fig. 66.1: Influenza virus (schematic diagram).

- **Matrix proteins: M1 protein** is the major viral protein. It forms a shell (protein layer) underneath the envelope. **M2 proteins** form ion channels in the envelope, help in transport of molecules
- **Non-structural proteins: NS1** is an interferon antagonist and inhibits pre-mRNA splicing. **NS2** helps in export of molecules across the nucleus
- **Hemagglutinin (HA) and neuraminidase (NA)** are the glycoproteins inserted into the lipid envelope.
- ❖ **Envelope:** It consists of a lipid envelope into which two types of glycoproteins are inserted.
 1. **Hemagglutinin (HA):** It is triangular-shaped peplomer. It binds to mucin or sialic acid receptors on the respiratory epithelial cells, thus facilitating viral entry
 2. **Neuraminidase (NA):** It is mushroom shaped, present fewer in number than HA. It is a sialidase enzyme that degrades the sialic acid receptors on the host cells
 - ◆ It facilitates release of virus particles from infected cell surfaces during budding process by preventing self-aggregation of virions to the host cells
 - ◆ NA also helps the virus to pass through the mucin layer in the respiratory tract to reach the target epithelial cells.

Antigenic Subtypes and Nomenclature

Based on RNP and M proteins, influenza viruses are divided into four genera: A, B, C and D.

Subtypes: Based on HA and NA antigens,

- ❖ Influenza A has distinct 18 H subtypes (H1 to H18) and 11 N subtypes (N1-N11)
 - Most of the subtypes infect animals and birds, but occasionally undergo genetic changes and infect humans to cause major epidemics and pandemics
 - For example, Six HA (H1, H2, H3, H5, H7 and H9) and two NA (N1 and N2) subtypes have been recovered from humans.
- ❖ **Influenza B viruses** are not classified into subtypes, but have diverged into lineages. Currently, circulating influenza type B viruses belong to either B/Yamagata or B/Victoria lineage
- ❖ **Influenza C virus** is detected less frequently and usually causes mild infections, thus does not present public health importance
- ❖ **Influenza D virus** primarily infects cattle and are not pathogenic to humans.

The standard nomenclature system for influenza virus:

Any influenza virus isolates should be designated based on the following information: Influenza virus type/ host (indicated only for non-human origin)/geographical origin/strain number/year of isolation/(HA NA subtype). For examples:

- ❖ **Human strain:** Influenza A/Hong Kong/03/1968 (H3N2)
- ❖ **Non-human strain:** Influenza A/swine/Iowa/15/ 1930 (H1N1).

Antigenic Variation

Antigenic variation is the unique property of influenza viruses, which is due to the result of antigenic changes occurring in HA and NA peplomers. It is of two types:

Antigenic Variations

1. Antigenic Drift

It is a minor change occurring due to **point mutations** in the HA/NA gene, resulting in alteration of amino acid sequence of the antigenic sites on HA/NA, such that virus can partially escape recognition by the host's immune system. The new variant must sustain two or more mutations to become epidemiologically significant.

- Seen in both influenza virus type—A and B
- Results in outbreaks and minor periodic epidemics
- Antigenic drift occurs more frequently, every 2–3 years.

2. Antigenic Shift

It is an abrupt, major drastic, discontinuous variation in the sequence of a viral surface protein (HA/NA), that occurs due to **genetic reassortment** between genomes of two or more influenza viruses infecting the same host cells; resulting in a new virus strain, unrelated antigenically to the predecessor strains. Thus, antibodies developed against previous strain (due to infection or vaccination) become ineffective.

- Occurs only in influenza A virus
- Results in pandemics and major epidemics, e.g. H1N1 pandemics of 2009
- Antigenic shift occurs less frequently every 10–20 years.

Pathogenesis

- ❖ **Transmission:** It is transmitted by (i) **inhalation of respiratory droplets** generated by coughing and sneezing. This mode can infect only those people who are within 1-meter distance, (ii) **via contact with surfaces or fomites** infected with respiratory droplets and then touching nose, eyes or mouth
- ❖ **Target cell entry:** Viral HA attaches to specific sialic acid receptors on the respiratory mucosa that leads to viral entry
- ❖ **Multiply locally:** Virus replicates in the infected cells and infectious daughter virions spread to the adjacent of respiratory epithelial cells over several hours
- ❖ **Spread:** Very rarely, virus spreads to the lower respiratory tract or spills over bloodstream to involve extra-pulmonary sites
- ❖ **Local damage:** Influenza virus infection causes cellular destruction and desquamation of superficial mucosa of the respiratory tract, which may predispose to secondary bacterial infection.

Host Immune Response

Humoral immunity: It is the predominant immunity that provides resistance against influenza infections. Immunity developed is both type and subtype-specific and long-lasting.

- ❖ Antibodies against HA and NA are protective in nature, and are subtype-specific
- ❖ Antibodies to HA prevent initiation of infection by inhibiting viral entry; whereas antibodies to NA decrease the severity of the disease and prevent the transmission of the virus to contacts
- ❖ Antibodies against other viral proteins are not protective
- ❖ Antibodies against the ribonucleoprotein are type-specific and are useful in typing viral isolates as influenza A or B or C
- ❖ All the three types of influenza viruses (i.e. A, B and C) are antigenically unrelated and there is no cross-protection
- ❖ Immunity may be incomplete following influenza infection; reinfection can occur with the same virus.

Components of both cell-mediated immunity (e.g. cytotoxic T cells) and innate immunity (NK cells, interferons) are also important in providing immunity against influenza infections.

Clinical Manifestations

Incubation Period

It is about 18–72 hours, which directly depends upon the inoculum size and the immune status of the host.

Uncomplicated Influenza (Flu Syndrome)

Majority of the individuals are either asymptomatic or develop minor upper respiratory symptoms such as chills, headache, and dry cough, followed by high-grade fever, myalgia and anorexia. It is a self-limiting condition, indistinguishable from the infections caused by other upper respiratory tract pathogens.

Complications

- ❖ **Pneumonia:** Secondary bacterial pneumonia is the most common complication to occur in patients infected with influenza virus. Common agents are staphylococci, pneumococci and *Haemophilus influenzae*. Primary influenza pneumonia is rare but leads to more severe complication
- ❖ **Other respiratory tract complications** include worsening of chronic obstructive pulmonary disease, exacerbation of chronic bronchitis, bronchiolitis, otitis media, parotitis and asthma
- ❖ **Extrapulmonary complications:** Myositis, rhabdomyolysis, myocarditis, encephalitis, post-influenza Guillian-Barre syndrome
- ❖ **Reye's syndrome:** It is fatty degeneration of liver with acute encephalopathy occurring in children and

adolescents (2 to 16 years of age) following aspirin or salicylate intake. Though the cause is unknown, this condition is often seen following influenza B, varicella-zoster and rarely influenza A viral infections.

Epidemiology

Influenza viruses cause seasonal flu epidemics worldwide almost every year, however they differ widely in severity and the extent of spread.

- ❖ **Incidence:** It is estimated that annually about 3–5 million cases of severe illness and 3–6 lakhs of deaths occur due to seasonal flu epidemics worldwide and is associated with significant economic impact
- ❖ **Global pandemics** of novel influenza A subtypes occur every 10–40 years, which can cause much higher mortality than seasonal flu
- ❖ **Seasonality:** Influenza outbreaks are common during winters. The most common seasonal flu strain varies from season to season and from place to place (e.g. H3N2 in Puducherry in 2018)
- ❖ **Annual attack rate:** About 5–10% in adults and 20–30% in children are infected annually
- ❖ **Epidemiological pattern:** It depends upon the nature of antigenic variation that occurs in the influenza types (as described earlier).

Risk Factors

Following risk factors are important determinants for patients going for complications following influenza.

- ❑ **Age:** Child of age < 2 years or age ≥ 65 years
- ❑ **Chronic diseases:** Chronic pulmonary, cardiac, renal, hematologic, metabolic, neurological, and neuro-developmental disorders
- ❑ **Immunosuppression** (including HIV/AIDS, use of long-term corticosteroids, post-transplant patients), diabetes mellitus
- ❑ **Other risk factors:** Extreme obesity, residing in nursing home, American Indians and Alaska Natives, and pregnancy. People with these risk factors are the first priority group for receiving influenza vaccination.

History of Influenza Outbreaks

Till now several influenza pandemics and major epidemics have occurred worldwide (Table 66.1).

- ❖ **Seroarcheology:** The outbreaks that occurred prior to influenza isolation (influenza isolated first in 1933 using ferrets) were detected later by retrospective serologic survey of individuals alive during those years
- ❖ **The severe most pandemic** (Spanish flu) recorded so far was the swine flu strain H1N1 in 1918–1919, where >50 million people died, mostly due to secondary bacterial pneumonia. This strain was not a reassortant, but believed to be derived entirely from an avian strain that had adapted to human conditions and pigs acted as a mixing vessel

Table 66.1: Major influenza outbreaks.

Years	Subtype	Extent of outbreak
1889–1890	H2N8	Severe pandemic
1900–1903	H3N8	Moderate epidemic
1918–1919	H1N1 ^a (HswN1) (Spanish flu)	Severe pandemic
1933–1935	H1N1 ^a (H0N1)	Mild epidemic
1946–1947	H1N1	Mild epidemic
1957–1958	H2N2 (Asian flu)	Severe pandemic
1968–1969	H3N2 (Hong Kong flu)	Moderate pandemic
1977–1978 ^b	H1N1 (Russian flu)	Mild pandemic
2009–2010	H1N1 pdm09	Pandemic

^aHemagglutinins formerly designated as Hsw and H0 are now classified as variants of H1.

^bFrom 1978 up to 2008–2009, viruses of the H1N1 and H3N2 subtypes circulated either in alternating years or concurrently.

- ❖ This was followed by series of several epidemics and pandemics as mentioned in Table 66.1

Sialic Acid Receptors

Sialic acid receptors found on the host cell surfaces are specific for HA antigens of influenza virus, which in turn determines the different host specificities of influenza virus.

- ❑ **Alpha-2–6 sialic acid receptors** are specific for human influenza strains and are found abundantly on human upper respiratory tract epithelium, but not on lower respiratory tract. This explains why most human flu strains cause mild upper respiratory tract infections but not pneumonia
- ❑ **Alpha-2–3 sialic acid receptors** are specific for avian influenza strains and are found abundantly on bird's intestinal epithelium
 - In humans, they are present in very few numbers on upper respiratory tract, and also on some epithelial cells in the lower tract
 - This explains why avian flu strains cannot easily infect humans and need close contact. However, once infected, they can infect the lower respiratory tract and cause pneumonia.

Why pigs are the most common mixing vessels?

- ❑ Both α -2–3 and α -2–6 sialic acid receptors are found on the same respiratory epithelial cells of pigs and swine flu strains have specificity for both the receptor types
- ❑ Hence pigs can be infected simultaneously by human, swine and avian strains, thus serving as a mixing vessel
- ❑ Reassortment between the segments of various strains can take place inside the same swine cell.

Avian Flu

Birds are the primary reservoir for influenza viruses.

- ❖ All influenza subtypes (18H types and 11N types) are found in birds and some of the subtypes can be transmitted to mammals (e.g. H1, H2, H3, H5, H7 and

H9 to humans; H1 and H3 to swine; and H3 and H7 to horses)

- ❖ Usually the avian flu strains are highly virulent as they possess **PB1F2** protein, which targets host mitochondria and induces apoptosis.

Avian Flu Infection in Birds

- ❖ Bird flu strains are highly lethal to chickens and turkeys (but avirulent to ducks) and are the major cause of economic loss in poultry causing severe mortality in chickens
- ❖ Unlike in mammals, avian flu multiplies in intestinal tracts of birds and shed through feces into water (avian flu is a water-borne disease in birds)
- ❖ The influenza viruses do not undergo antigenic variation in birds, because of the short life span of birds.

Avian Flu Infection in Humans

It is believed that, to date, all human pandemic strains have originated by reassortment between avian and human influenza viruses and the mixing has occurred in pigs.

A/H5N1 is the most common avian flu strain that has been endemic in the world for the past 15 years.

- ❖ **Origin:** It was first reported from Hong Kong in 1997 and has spread to various countries including India within few years
- ❖ **Transmission** to man occurs only from birds, and requires close respiratory contact
- ❖ **Less morbidity, but more mortality:** As there is no human to human transmission, morbidity is less. However, the avian flu strains are highly virulent (due to presence of PB1F2 protein) and mortality rate is >60%. Globally only 861 cases have been reported between 2003 to 2019, out of which about 455 people succumbed death (52%)
- ❖ **Clinical features:** H5N1 avian flu strains are associated with higher rates of pneumonia (>50%) and extrapulmonary manifestations such as diarrhea and CNS involvement.

Other avian flu strains infecting humans are:

- ❖ A/H7N7(Netherlands)
- ❖ A/H9N2 (Hong Kong)
- ❖ A/H7N9 (caused an outbreak in China, 2013).

Laboratory Diagnosis (Avian Flu)

Avian flu strains can be identified by real time reverse transcriptase PCR detecting specific HA and NA genes.

Influenza A (H1N1) pdm09

It has caused the most recent pandemic of influenza, emerged in California in March 2009 and then rapidly spread to the entire world including India over the next few months.WHO declared the pandemic on 11th June 2009.

Epidemiology

- ❖ **Origin:** H1N1 2009 flu originated by genetic reassortment of four strains (1 human strain + 2 swine strains + 1 avian strain) and the mixing had occurred in pigs (Fig. 66.2)
- ❖ Though people commonly use the word 'swine flu' to describe H1N1 2009 flu, but this is not the correct terminology as it is a **reassortant of four strains**
- ❖ **Transmission:** It can be transmitted from human to human, which has accounted for its rapid spread
- ❖ However, it is less virulent (as it lacks the *PB1 F2* protein)
- ❖ Therefore in contrast to H5N1, the H1N1 2009 flu has caused more morbidity but less mortality.

Clinical Features

- ❖ **Uncomplicated influenza:** Most of the cases present with mild upper respiratory tract illness and diarrhea
- ❖ **Complicated/severe influenza** can occur very rarely in high-risk groups, is characterized by features such as secondary bacterial pneumonia, dehydration, CNS involvement, and multiorgan failure.

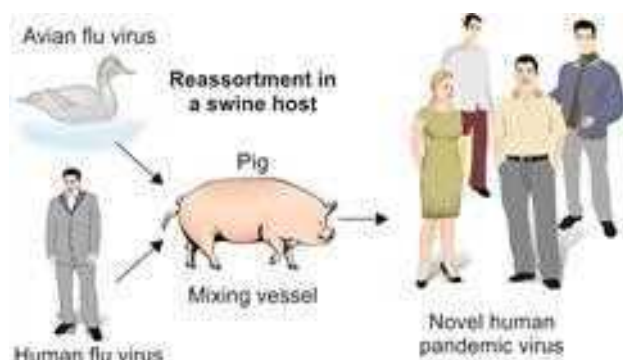


Fig. 66.2: Evolution of pandemic influenza virus.

Categorization of Seasonal Influenza A/H1N1

Ministry of Health and Family Welfare, Government of India has published the guideline on categorization of seasonal influenza A/H1N1 cases (Table 66.2). While screening the patients with influenza like illness, this guideline helps in taking decision on performing laboratory test, initiating antiviral treatment and putting the patient on home isolation or hospitalization.

Epidemiological Surveillance for Influenza

Influenza surveillance is routinely carried out globally and also at national level. This helps to monitor the changes in the circulating influenza strain and serves as a global alert mechanism for the emergence of pandemic influenza viruses.

- ❖ **GISRS:** Influenza surveillance has been conducted globally through Global Influenza Surveillance and Response System (GISRS) under World Health Organization (WHO)
- ❖ **IDSP** (Integrated Disease Surveillance Program) under NCDC (National Center for Disease Control) conducts Influenza (H1N1) surveillance in India
- ❖ **India (2019):** According to IDSP Report 2019—28,798 cases of H1N1 were reported with 1,218 deaths. Rajasthan reported the maximum cases, followed by Gujarat; whereas Maharashtra reported the maximum deaths. The mortality rate is around 4-5% every year
- ❖ **World:** According to WHO, 3-6 lakh deaths occur due to seasonal flu annually (4.0-8.8 per 1 lakh population). Global pandemics of novel influenza A subtypes occur every 10-40 years, which can cause much higher mortality than seasonal flu.

Table 66.2: Guideline on categorization of seasonal influenza A/H1N1 cases during screening for home isolation, testing, treatment and hospitalization (issued by Ministry of Health and Family Welfare, Government of India).

Category	Definition	Laboratory testing for H1N1*, treatment** and isolation
Category A	Influenza like illness (ILI) Mild fever plus cough/sore throat with or without bodyache, headache, diarrhea and vomiting	<i>Laboratory testing for H1N1</i> —not required <i>Treatment</i> —only symptomatic, antiviral drugs not required <i>Isolation</i> —confine patients at home, avoid contact with public and high-risk members in the family
Category B	Category A plus any one: i. High-grade fever and severe sore throat or ii. Presence of any of the risk factors (as described earlier in the highlight box, under epidemiology)	<i>Laboratory testing for H1N1</i> —not required <i>Treatment</i> —symptomatic treatment required. Antiviral drug (oseltamivir) may be required <i>Isolation</i> —confine patients at home, avoid contact with public and high-risk members in the family
Category C	Severe acute respiratory syndrome (SARI) Category B plus any one: i. Breathlessness, chest pain, fall in blood pressure, sputum mixed with blood, bluish discoloration of nails ii. Children with influenza-like illness who had a severe disease as manifested by the red flag signs (inability to feed well, convulsions, difficulty in breathing, etc.) iii. Worsening of underlying chronic conditions	<i>Laboratory testing for H1N1</i> —required <i>Immediate hospitalization</i> —required <i>Treatment</i> —start antiviral drug (oseltamivir) immediately without waiting for laboratory result <i>Isolation</i> —all components of droplet precaution to be followed (refer 'prevention of influenza' section)

* Real-time reverse transcriptase PCR is recommended to detect and quantify the specific HA and NA genes of H1N1.

** Oseltamivir (Tamiflu) tablet or Zanamivir (inhalational form).

LABORATORY DIAGNOSIS

Influenza

- ❑ **Specimen:** Nasopharyngeal swab, kept at 4°C
- ❑ **Isolation of virus:**
 - Inoculation in embryonated eggs and primary monkey kidney cell lines
 - Growth is detected by hemadsorption, hemagglutination test.
- ❑ **Viral antigens** detection by direct IF test
- ❑ **Molecular methods:** Simultaneously detects seasonal flu strains such as A/H1N1, A/H3N2, Influenza B
 - RT PCR: detects viral RNA
 - Real time-RT PCR: quantifies viral RNA
- ❑ **Antibody detection** by hemagglutination inhibition test, neutralization test and ELISA.

Laboratory Diagnosis

Specimen Collection

- ❖ **Ideal specimens** are nasopharyngeal swab or lavage fluid, nasal aspirate or to a less extent throat swab
- ❖ **Swabs** with a synthetic tip (e.g. polyester or Dacron swabs) are best for specimen collection (Fig. 66.3). Cotton or alginate swabs are unsatisfactory
- ❖ **Transport:** Swabs are immediately put inside the viral transport media, kept at 4°C during transport up to 4 days, thereafter at -70°C.

Isolation of Virus

Embryonated eggs (amniotic cavity) and primary monkey kidney cell lines have been the methods of choice for the isolation of influenza viruses in the past. The viral growth in cell line was detected by hemadsorption or hemagglutination test. Because of technical difficulty, isolation is not routinely performed for diagnostics purpose.

Direct Immunofluorescence Test

Viral antigens coated onto epithelial cells can be directly detected in nasal aspirates by using fluorescent tagged antibodies. This is rapid, but less sensitive than viral isolation.

Molecular Methods

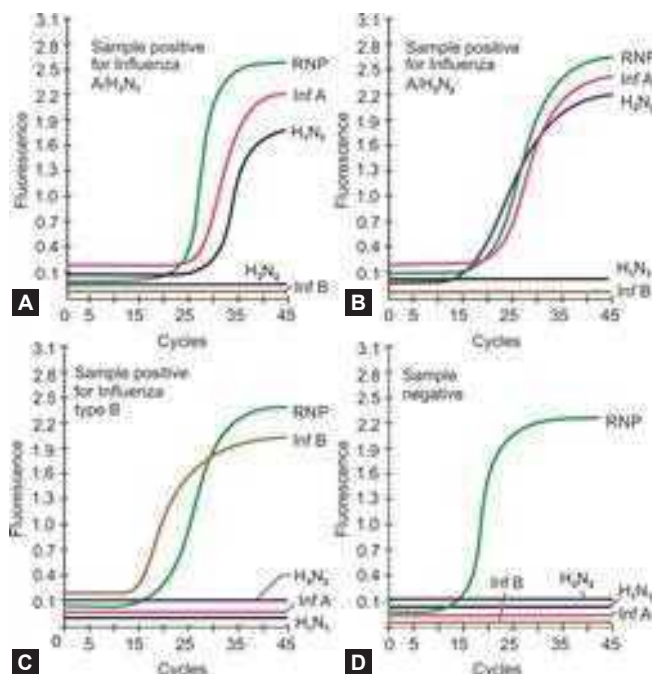
Molecular methods have revolutionized the diagnosis of influenza.

- ❖ **RT-PCR** (reverse transcriptase polymerase chain reaction): It is highly sensitive, specific and rapid



Fig. 66.3: Viral transport medium and swab.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).



Figs 66.4A to D: Real time RT-PCR showing the result (amplification curves) of specimen(s) tested positive for Influenza type: **A.** A/H₁N₁; **B.** A/H₃N₂; **C.** Type B; **D.** Negative (refer Table 66.3).

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Table 66.3: Real time RT-PCR for seasonal influenza types.

Simultaneously detects five genes	Specimens positive with influenza type			Influenza negative
	A/H1N1	A/H3N2	Type B	
Influenza A (matrix gene)	+	+	-	-
H1N1 (HA gene)	+	-	-	-
H3N2 (HA gene)	-	+	-	-
Influenza B (HA gene)	-	-	+	-
RNP (ribonucleoprotein)*	+	+	+	+

*RNP (ribonucleoprotein) is used as internal control in real time RT-PCR; if it is not detected, then the test is considered invalid.

(turnaround time of <1 day). It can also detect the specific type and subtype of influenza virus

- ❖ **Real-time RT-PCR:** It is currently the gold standard method for influenza diagnosis. It is quantitative, has higher sensitivity and specificity than RT-PCR with turnaround time of 2–3 hours. It simultaneously detects the three common seasonal flu strains (A/H1N1, A/H3N2 and type B). The result is expressed as the emission of fluorescence during the cycles as described in Figure 66.4 and Table 66.3
- ❖ **BioFire FilmArray Respiratory Panel (RP)** tests simultaneously 20 respiratory pathogens, including Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H1-2009 and Influenza B.

Antibody Detection (Serology)

Various assays are available to detect subtype specific serum antibodies by using specific influenza antigens. It

is mainly useful for sero-epidemiology purpose, not for clinical diagnosis; as antibodies may be present in normal individuals. The tests available are: ELISA, neutralization test, and previously used HAI (hemagglutination inhibition) test.

TREATMENT

Influenza

Specific antiviral therapy is available for influenza virus infection. Start therapy within 48 hours of onset of symptoms for influenza.

- ❑ **Neuraminidase inhibitors** (such as zanamivir, oseltamivir, and peramivir) can be administered for influenza A and influenza B infections
 - It is the drug of choice for A/H1N1 2009 flu, A/H5N1 avian flu and influenza-B
 - Dosage:
 - ◆ Oseltamivir (Tamiflu 75 mg tablets)
 - ◆ Zanamivir (10 mg, inhalational form).
 - Schedule:
 - ◆ For treatment—given twice a day for 5 days
 - ◆ For chemoprophylaxis—given once daily. Duration depends on the clinical setting; usually 7 days.
- ❑ **Matrix protein M2 inhibitor** such as amantadine and rimantadine can be given for some strains of influenza A infection. However, strains of A/H1N1 2009 flu and A/H5N1 avian flu and influenza B virus have developed resistance.

Prevention

General Preventive Measures

Measures of droplet precaution (Chapter 21) should be followed:

- ❖ **Strict hand hygiene**
- ❖ **Isolation room:** Patients should be kept in isolation room or cohorting to be followed
- ❖ **Containment of coughs and sneezes**
 - Respiratory hygiene and cough etiquette
 - Use of personal protective equipment (PPE) such as gloves, 3-ply masks, gown and goggles for a HCW. Patient should wear a mask.
- ❖ **Work restriction:** CDC recommends that people with influenza-like illness remain at home until at least 24 hours after they are free of fever (<100°F) without the use of fever-reducing medications.

Vaccine Prophylaxis

Vaccine Strains

Based on WHO recommendations, influenza vaccines are prepared every year.

- ❖ **Strains to be included** in the vaccine depend upon the strains isolated in the previous influenza seasons and strains that are anticipated to circulate in the upcoming season
- ❖ **Formulations:** Influenza vaccines are available in cocktail of either three strains or four strains
 - **Trivalent form:** Comprises of three strains: A/H1N1, A/H3N2 and influenza B strain
 - **Quadrivalent form:** Comprise of four strains: A/H1N1, A/H3N2 and two lineages of influenza B strain.

- ❖ **Composition:** The lineages of A/H1N1, A/H3N2 and influenza B strains included in vaccine change every year
 - The lineages included in vaccine are different for northern and southern hemispheres
 - WHO constantly reviews the vaccine composition and decides on which lineages to be included in vaccine every year.
- ❖ **Types:** Both injectable (inactivated) and nasal spray (live attenuated) vaccines are available.

Injectable Vaccines

Injectable vaccines are the most widely used vaccines in immunization programs.

- ❖ **Types:** There are three types of injectable vaccines
 1. **Inactivated influenza vaccine (IIV)**, e.g. Fluzone: It is prepared by growing the vaccine strains in embryonated chick eggs; then harvested, purified, inactivated and then standardized to contain 15 µg of HA/dose.
 2. **Cell culture-based inactivated influenza vaccine (ccIIV3)**; e.g. Flucelvax: Same as IIV, but prepared in cell lines such as Madin-Darby Canine Kidney (MDCK) cell line.
 3. **Recombinant influenza vaccine (RIV)**, e.g. FluBlok: Contains recombinant influenza HA antigens in trivalent/quadrivalent formulations. RIV does not contain any egg protein.
- ❖ **Schedule:** Single dose administered by intramuscular (IM) route; except for 6 months-8 years of age (2 doses are required ≥4 weeks apart)
- ❖ **Timing of vaccination:** Optimally before onset of influenza season, i.e. by end of October
- ❖ **Efficacy:** The vaccine efficacy varies from 25–67% (25% for H3N2, 42% against type B and 67% against H1N1). The efficacy is lower if vaccine virus does not match to currently circulating viruses in the locality. Immunity lasts for 6–12 months
- ❖ **Side effects:** Mild reactions can occur in 5% of cases such as redness at injection site, fever and aches. Serious side effects such as allergic reactions can occur very rarely
- ❖ **Indications:** Routine annual influenza vaccination is recommended for all persons aged ≥6 months who do not have contraindications. If not feasible, then high-risk groups should be given first priority for vaccination (high-risk group individuals have been listed in highlight box, under epidemiology earlier)
- ❖ **Contraindication:** IIV should not be administered to people who have history of severe allergic reaction to previous dose of vaccine. In patients with egg allergy, vaccine can be given, but under supervision
- ❖ **Travelers:** If traveling to an area of increased influenza activity; can consider vaccination, preferably ≥2 weeks before departure.

Live Attenuated Influenza Vaccine (LAIV)

This vaccine is generated by reassortment between currently circulating strains of influenza A and B virus with a cold-adapted attenuated master strain which is adapted to grow at 25–33°C.

- ❖ Such live attenuated strains can grow in upper respiratory tract (at 33°C) but not in lower respiratory tract (at 37°C); therefore they may cause mild flu like symptoms but never infect lower respiratory tract, hence never cause serious adverse effects
- ❖ It is a trivalent vaccine, administered by intranasal spray
- ❖ **Indication:** It can be given to all healthy persons of 2–49 years age (except in pregnancy), but is not given to high-risk groups.

Chemoprophylaxis

Antiviral drugs are not recommended for routine seasonal or pre-exposure prophylaxis. It is recommended only for post-exposure and during outbreak situations in hospitals.

- ❖ **Indications:** Following exposure to an influenza case, it is recommended to the following groups: (i) if not vaccinated or vaccinated recently (<2 weeks), (ii) HIV infected people
- ❖ **Duration:**
 - *Non-outbreak exposure* (e.g. in community): It should be started as soon as possible following exposure (within 48 hours) and continued for 7 days
 - *During outbreaks in hospitals* (for elderly persons, children and health care workers): Duration for a minimum of 2 weeks, and to be continued up to 1 week after the last known case was identified.
- ❖ **Antiviral drugs** recommended are:
 - Oseltamivir is the drug of choice. It is given as 75 mg orally, once a day for 7 days
 - Zanamivir: 10 mg (two 5-mg inhalations) once daily for 7 days.
- ❖ **Efficacy:** The efficacy of chemoprophylaxis is about 70–90% in preventing influenza.

PARAMYXOVIRIDAE INFECTIONS

Paramyxoviridae contains a group of viruses, which are transmitted via the respiratory route following which:

- ❖ They may cause localized respiratory infection in children (e.g. respiratory syncytial virus, metapneumoviruses and parainfluenza viruses) or;
- ❖ They may disseminate throughout the body to cause highly contagious diseases of childhood such as mumps (parotid enlargement) and measles (Chapter 56).

Paramyxoviruses resemble orthomyxoviruses in morphology, but they differ by the following properties (Fig. 66.5).

- ❖ They are larger (100–300 nm) in size and more pleomorphic
- ❖ Possess linear non-segmented RNA (compared to segmented RNA in influenza virus)

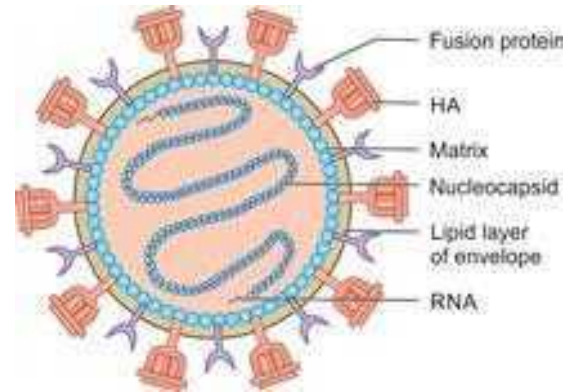


Fig. 66.5: Schematic diagram of paramyxoviruses (measles virus).

- ❖ Contain six structural proteins (compared to 8 in influenza virus)
- ❖ **HA and NA antigens:** The paramyxoviruses vary from each other in expression of HA and NA antigens
 - Parainfluenza and mumps possess both HA and NA antigens (similar to influenza virus)
 - Measles virus possess HA, but lack NA
 - RSV and metapneumoviruses lack both HA and NA.

Note: There are few zoonotic paramyxoviruses such as Nipah and Hendra viruses, which can occasionally infect humans (Chapter 74).

PARAINFLUENZA

Human parainfluenza viruses are one of the major causes of lower respiratory tract disease in young children. They have five serotypes:

- ❖ Types 1 and 3 belong to the genus *Respirovirus*
- ❖ Types 2, 4 a and 4b belong to the genus *Rubulavirus*.

Clinical Manifestations

- ❖ Transmission is by respiratory route (by direct salivary contact or by large-droplet aerosols)
- ❖ The incubation period appears to be 5–6 days
- ❖ Virus multiplies locally and causes various respiratory manifestations such as:
 - **Mild common cold syndrome** like rhinitis and pharyngitis are the most common presentation, seen with all serotypes
 - **Croup (laryngotracheobronchitis):**
 - ♦ Occurs in 2–3% of cases
 - ♦ Typically seen with type 1 and 2
 - ♦ Involves children (between 6 to 18 months of age).
 - **Pneumonia or bronchiolitis:**
 - ♦ Occurs very rarely
 - ♦ Seen especially with serotype 3
 - ♦ Involves infants below 6 months of age.
 - **Otitis media:** It is the most common complication of parainfluenza virus infection.
- ❖ Reinfections are common, but less severe. There is no cross protection between the serotypes.

Epidemiology

Parainfluenza viruses are worldwide in distribution.

- ❖ Type 3 is the most prevalent serotype. It exists as endemic throughout the year and annual epidemics occur during spring
- ❖ Types 1 and 2 infections are less common and seasonal, and tend to cause epidemics during the rainfall or winter, cyclically every alternate year
- ❖ Type 4a and 4b cause much milder illness and these serotypes are the most difficult to be isolated
- ❖ Parainfluenza viruses are important cause of outbreaks in pediatric wards, day care centers and in schools.

Laboratory Diagnosis

- ❖ **Antigen detection:** Viral antigens in the infected exfoliated epithelial cells of the nasopharynx can be detected by direct-IF test by using specific monoclonal antibodies. It is rapid, but less sensitive than viral isolation
- ❖ **Viral isolation:**
 - **Specimens** such as nasal washes, bronchoalveolar lavage fluid and lung tissue can be used
 - **Primary monkey kidney cell line** is most sensitive and alternatively, a continuous monkey kidney cell line-LLC-MK2 can be used
 - They produce little or no cytopathic effect
 - Viral growth can be detected by demonstration of antigen by direct-IF test
 - Shell vial technique is followed to enhance viral replication.
- ❖ **Serum antibodies** can be measured by neutralization test, HAI test or ELISA. Presence of IgM or four-fold rise of IgG titer is indicative of active infection
- ❖ **Reverse transcriptase PCR** assays are highly specific and sensitive but available only in limited settings
- ❖ **BioFire FilmArray respiratory panel (RP)** tests simultaneously 20 respiratory pathogens, including parainfluenza serotypes.

Animal Parainfluenza Viruses

Certain animal parainfluenza viruses are related to the human strains.

- ❖ Sendai virus of mice is a subtype of human parainfluenza virus type 1
- ❖ SV5, a common contaminant of primary monkey kidney cell lines, is related to parainfluenza virus type 2
- ❖ Shipping fever virus of cattle and sheep (SF4) is a subtype of parainfluenza virus type 3.

Avian Parainfluenza Viruses (Newcastle Disease Virus or NDV)

NDV (also called Ranikhet virus in India) produces pneumoencephalitis in young chickens and mild flu like illness in older birds.

Human infection is rare and occupational; characterized by mild self-limiting conjunctivitis that may occur in workers handling infected birds.

MUMPS

Mumps virus is the most common cause of parotid gland enlargement in children. In severe cases, it can also cause orchitis and aseptic meningitis.

Pathogenesis

- ❖ **Transmission** is through the respiratory route via droplets, saliva, and fomites
- ❖ **Primary replication** occurs in the nasal mucosa or upper respiratory mucosa → infects mononuclear cells and regional lymph nodes → spills over to bloodstream resulting in viremia → dissemination
- ❖ **Target sites:** Mumps virus has a special affinity for glandular epithelium. The classic sites include salivary glands, testes, pancreas, ovaries, mammary glands and central nervous system.

Clinical Manifestation

- ❖ **Incubation period** is about 19 days (range, 7–23 days)
- ❖ **Inapparent infection:** Up to half of the infected people are either asymptomatic or present with non-specific symptoms such as fever, myalgia and anorexia. This is more common in adults than in children
- ❖ **Bilateral parotitis:** Acute non-suppurative parotid gland enlargement is the most common specific manifestation, present in 70–90% of the cases (Fig. 66.6)
 - Rarely, parotitis may be unilateral
 - In some cases, other salivary glands may also be involved.



Fig. 66.6: Parotitis in a mumps virus-infected patient (arrow showing).

Source: Public Health Image Library, ID# 1861/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Epididymo-orchitis** is the next most common manifestation of mumps, developing in 15–30% of cases in postpubertal males. Orchitis is unilateral in most of the cases, hence infertility following mumps orchitis is rare
- ❖ **Aseptic meningitis** occurs in less than 10% of cases, with a male predominance. It is self-limiting condition except the deafness (due to cranial nerve palsy) which may be permanent
- ❖ **Oophoritis** occurs in about 5% of women
- ❖ **Pancreatitis** occurs in 4% of infections and may lead to diabetes
- ❖ **Atypical mumps:** Parotitis may be absent in 10% of cases and patients directly present with aseptic meningitis.

Epidemiology

Mumps is endemic worldwide, sporadic cases occurring throughout the year, with a peak in cases typically in winter and spring. Epidemics occur every 3–5 years; typically associated with unvaccinated people living in overcrowded areas.

- ❖ **Period of communicability:** Patients are infectious from 1 week before to 1 week after the onset of symptoms
 - The most contagious period is within 1–2 days before the onset of symptoms
 - Infective material: Mumps virus is shed in saliva, respiratory droplets, and urine.
- ❖ **Source:** Cases (both clinical and subclinical cases) are the source of infection
 - There is no carrier state
 - Subclinical cases (30–40% of all cases) are responsible for maintaining the cycle of infection.
- ❖ **Reservoir:** Humans are the only reservoir of infection
- ❖ **Incidence:** About 5 lakh cases of mumps occur every year globally (4,99,512 cases in 2019, WHO report). The number of cases have been reduced after the start of immunization
- ❖ **Age:** Children of **5–9 years** of age are most commonly affected; however, no age is exempt if there is no previous immunity. The disease tends to be more severe in adults
- ❖ **Immunity:** One attack (either by vaccine or infection) gives lifelong immunity
- ❖ The secondary attack rate is high (86%).

LABORATORY DIAGNOSIS

Mumps

- ❑ **Specimen:** Buccal or oral swab
- ❑ **Antigen detection** by direct IF test
- ❑ **Viral isolation:** By using primary monkey kidney cell lines or by shell vial technique
- ❑ **Serum antibodies** by ELISA, neutralization test, HAI test
- ❑ **RT PCR:** Detects viral RNA.

Laboratory Diagnosis

- ❖ **Specimens:** The buccal or oral swab specimens are the most ideal specimens, although mumps virus can also be detected in saliva, CSF, urine (shed up to two

weeks), seminal fluid and rarely blood. Massaging the parotid gland area for 30 seconds prior to swabbing is recommended

- ❖ **Direct viral antigen detection** can be done by using mumps-specific immunofluorescent staining (direct-IF) of clinical specimens
- ❖ **Virus isolation:** Monkey kidney cells are the preferred cell lines. Specimens should be inoculated immediately
 - Viral growth after 1–2 weeks can be detected by demonstration of cytopathic effect (cell rounding and giant cell formation) or hemadsorption
 - Shell vial technique is followed for rapid detection in 1–2 days.
- ❖ **Serum antibodies detection:** ELISA is the most widely used assay; which detects antibodies against mumps specific whole-virus antigen, nucleoprotein and hemagglutinin antigens. Mumps ELISA is highly specific, does not cross-react with parainfluenza antibodies
- ❖ **Reverse-transcription PCR** is available to detect mumps specific RNA such as N gene (nucleoprotein) in clinical specimen. It is highly sensitive and specific.

TREATMENT

Mumps

- ❑ There is no specific antiviral drug available. Treatment is mostly symptomatic
- ❑ Mumps immunoglobulin is available, but not effective, hence not recommended for treatment or post-exposure prophylaxis.

Prevention (Live Attenuated Vaccine)

- ❖ **Vaccine strain:** Live attenuated **Jeryl Lynn strain** is the recommended strain used worldwide
- ❖ Mumps vaccine is prepared in chick embryo cell line
- ❖ **Mumps vaccine is available as**
 - Trivalent MMR vaccine (live attenuated measles-mumps-rubella vaccine) or
 - Quadrivalent MMR-V vaccine (contains additional live attenuated varicella vaccine)
 - Monovalent mumps vaccine (not commonly used).
- ❖ **Schedule:** Two doses of MMR is given by subcutaneous route at 1 year (12–15 months) and 4–6 years (before starting of school)
- ❖ **Efficacy** is about 88% after the second dose. Neutralizing antibodies appear in 95% of the recipients. The duration of long-term immunity is unknown.

RESPIRATORY SYNCYTIAL VIRUS INFECTION

Respiratory syncytial virus (RSV) is a major respiratory pathogen of young children and is the most common cause of lower respiratory disease (bronchiolitis and pneumonia) in infants.

Pathogenesis

- ❖ **Transmission:** RSV is spread by (i) direct contact (contaminated fingers or fomites and by self-inoculation

onto the conjunctiva or anterior nares) or (ii) by large droplets inhalation

- ❖ **Spread:** RSV replicates locally in the epithelial cells of the nasopharynx and may spread to the lower respiratory tract to cause bronchiolitis and pneumonia.

Clinical Manifestations

RSV causes a wide spectrum of respiratory illnesses.

- ❖ **Incubation period** is about 3–5 days
- ❖ **Infants:** RSV is the most common cause of lower respiratory tract infection below 1 year of age, causing bronchiolitis, pneumonia, and tracheobronchitis in 25–40% of infected infants
- ❖ **Symptoms:** It begins with running nose, fever and accompanied by cough, wheezing and dyspnea
 - Chest X-ray shows peribronchial thickening, diffuse interstitial infiltration and occasionally lobar consolidation
 - Infection is severe in premature infants and underlying congenital cardiac disease, bronchopulmonary dysplasia, nephrotic syndrome, or immunosuppression.
- ❖ **Adults:** RSV produces influenza-like upper respiratory symptoms such as common cold, running nose, sore throat, and cough. Infections are common in overcrowded communities (military recruits)
- ❖ **Recurrent infection** is common in both children and adults, but is much milder (common cold).

Laboratory Diagnosis

Antigen Detection

Direct identification of viral antigens in clinical samples is rapid and sensitive. Two methods are commonly used; both use monoclonal antibodies specific for RSV.

- ❖ Direct immunofluorescence test detecting antigens on exfoliated cells or
- ❖ ELISA detecting antigens in nasopharyngeal secretions.

Virus Isolation

HeLa and HEP-2 are the most sensitive cell lines for RSV isolation. As RSV is extremely labile, freezing should be avoided and specimens should be processed immediately.

- ❖ A characteristic cytopathic effect, **syncytium formation** (multinucleated giant cell)—appears after 10 days. Hence, it is named as syncytial virus
- ❖ Sensitivity of virus isolation or antigen detection are excellent in children (80–95%), but low in adults.

Molecular Methods

Molecular methods provide definitive diagnosis for RSV detection

- ❖ **RT-PCR** amplifying viral RNA (such as *nucleoprotein N* gene) has shown higher sensitivity and specificity, particularly in adults
- ❖ **BioFire FilmArray respiratory panel (RP)** tests simultaneously 20 respiratory pathogens, including RSV.

Antibody Detection

Various formats such as immunofluorescence and ELISA are available for antibody detection.

- ❖ Serum antibodies are of less diagnostic importance; rather they are the markers of prevalence of infection (epidemiological significance).

Epidemiology

Infection with RSV occurs worldwide.

- ❖ **Seasonality:** Annual epidemics tend to occur following rainfall, in winter and spring and last up to 5 months. Infection is not seen in summer
- ❖ **Age:** RSV is a leading respiratory pathogen in children. Infants between ages of 6 weeks to 6 months of age are commonly affected, with peak incidence at 2 months
- ❖ **Prevalence:** About 70% of infants are infected by 1 year of age and almost all by 2 years of age.

TREATMENT

RSV infection

Unlike other respiratory viruses, specific antiviral drug is available for treatment of RSV.

- ❑ **Ribavirin** is the drug of choice
 - It is indicated for severe infections in infants. However, its beneficial effect in older children and adults is doubtful
 - It is administered as aerosols for 3–6 days. Oral ribavirin is not recommended.
- ❑ Supportive care is also needed such as removal of secretions, administration of oxygen, bronchodilators and ventilatory support
- ❑ Immunoglobulin with high titers of antibody to RSV was used in the past, but was found ineffective and hence it is not recommended currently.

HUMAN METAPNEUMOVIRUS

Human metapneumovirus was first reported in 2001, though the avian strains were prevalent since 1970s.

- ❖ They cause both upper and lower respiratory tract illnesses similar to those caused by RSV but less severe and tend to affect slightly older children
- ❖ It may be the second most common cause (next to RSV) of lower respiratory infection in young children
- ❖ They also cause respiratory disease in adults with underlying hematologic malignancies
- ❖ **Diagnosis:** RT-PCR is available to amplify the RNA extracted from respiratory specimens. Specific antigens in nasopharyngeal secretions can be detected by direct-IF test.

EXPECTED QUESTIONS

I. Write essay on:

1. In early 2018, a 62-year-old debilitated man from Maharashtra presented with symptoms of severe upper respiratory tract infection. He had a history of exposure to a patient having similar condition. Nasopharyngeal swab collected was sent to the reference laboratory for real time PCR which revealed that causative agent as influenza A/H1N1.
 - a. What is the mechanism of emergence of this particular strain of the virus?
 - b. Describe the pathogenesis, mode of transmission and laboratory diagnosis of the causative agent.
 - c. Add a note on the epidemiological impact of the recent 2018 epidemic in India produced by this causative agent.
 - d. What are the preventive measures available for this condition?
2. Describe the pathogenesis and laboratory diagnosis of measles virus infection.

II. Write short notes on:

1. Mumps.
2. H1N1 2009 pandemic flu.

III. Multiple Choice Questions (MCQs):

1. **Most common cause of secondary bacterial pneumonia in patients infected with influenza virus:**
 - a. *Staphylococcus*
 - b. *E.coli*
 - c. Pneumococci
 - d. *Haemophilus influenzae*
2. **Reye's syndrome is a complication seen after all the following viral infections, except:**
 - a. Influenza B
 - b. Varicella-zoster
 - c. Influenza A
 - d. Measles
3. **The trivalent vaccine for influenza includes all, except:**
 - a. A/H1N1
 - b. A/H5N1
 - c. A/H3N2
 - d. Influenza B strain
4. **Which of the following statement about mumps is not correct?**
 - a. Bilateral parotitis is the most common presentation
 - b. Other salivary glands are never involved
 - c. Atypical mumps presents as meningitis
 - d. Incubation period is about 19 days

5. Which of the following statements concerning antigenic drift in influenza viruses is correct?

- a. It results in major antigenic changes
- b. It is exhibited only by influenza A viruses
- c. It is due to frame-shift mutations in viral genes
- d. It occurs frequently than antigenic shift

6. Which of the following paramyxoviruses has a surface glycoprotein lacking hemagglutinin activity?

- a. Measles virus
- b. Mumps virus
- c. Parainfluenza virus type 1
- d. Respiratory syncytial virus

7. Which of the following statement is not correct, for the management of patient with category A influenza like illness?

- a. Laboratory testing for H1N1 is not required
- b. Treatment with oseltamivir is required
- c. Confine the patients at home
- d. Avoid contact with public and high-risk members in the family

8. About Global Influenza Surveillance and Response System (GISRS), which statement(s) is/are correct?

- a. It monitors the evolution of influenza viruses
- b. Provides recommendations in areas including laboratory diagnostics, vaccines and treatment
- c. Serves as a global alert mechanism for the emergence of influenza viruses with pandemic potential
- d. All of the above

9. According to CDC recommendation, the people with influenza-like illness remain at home until how much time after they are free of fever (<100°F) without the use of fever-reducing medications.

- a. At least 24 hours
- b. At least 48 hours
- c. At least 7 days
- d. At least 10 days

10. Chemoprophylaxis for influenza is recommended for all the following situations, except:

- a. Routine seasonal pre-exposure prophylaxis
- b. During outbreak situations in hospitals
- c. Following exposure to an influenza case if not vaccinated or vaccinated recently (<2 weeks)
- d. Following exposure to an influenza case if the individual is HIV infected

Answers

1. a 2. d 3. b 4. b 5. d 6. d 7. b 8. d 9. a 10. a

Coronavirus Infections Including COVID-19

CHAPTER 67

CHAPTER PREVIEW

- Severe Acute Respiratory Syndrome (SARS)
- Middle East Respiratory Syndrome (MERS)
- Coronavirus Disease (COVID)-2019

INTRODUCTION

Coronaviruses (CoV) cause respiratory tract infections in man; illness ranging from mild common cold to severe disease like pneumonia.

Morphology

Coronaviruses are enveloped; carrying petal or club-shaped or crown-like peplomer spikes giving appearance of solar corona (Fig. 67.1).

- ❖ They are large (120–160 nm) spherical viruses having a helical symmetry
- ❖ They possess linear, positive-sense ssRNA of 26 to 32 kbp size, largest among the non-segmented RNA viruses.

Classification

Coronaviridae family contains two subfamilies: Coronavirinae and Torovirinae. The former has been grouped into four genera— α , β , γ and δ . Most of them

infect animals except γ *Coronavirus* species, which are the pathogens of birds. Human infection is uncommon except few who have adapted to human conditions.

Human Coronaviruses

There are seven recognized coronaviruses that are known to cause human infections (Table 67.1); most of them belong to *Betacoronavirus* except the first two (229E and NL63) which belong to *Alphacoronavirus*.

They spread by droplet transmission (though coughing or sneezing) and also by close personal contact, such as touching infected persons or objects and then subsequently touching mouth, nose, or eyes.

SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

SARS-CoV had caused an explosive epidemic in China in 2003, known as severe acute respiratory syndrome.

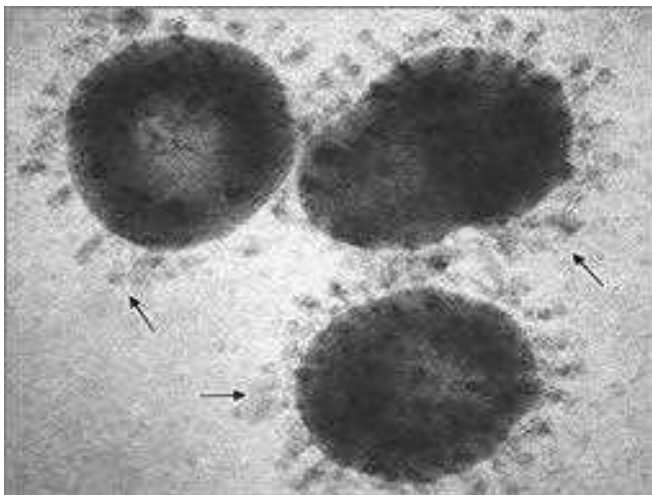


Fig. 67.1: Electron micrograph of coronavirus (petal or club-shaped peplomers) (arrows showing).

Source: ID#10270, Public Health Image Library, /Centers for Disease Control and Prevention (CDC), Atlanta/Dr Fred Murphy; Sylvia Whitfield (with permission).

Table 67.1: Human coronaviruses.

Coronaviruses that produce milder disease

Common coronaviruses that are widespread in distribution, affecting people of most part of the world and cause mild upper respiratory tract infections

1. Human coronavirus 229E
2. Human coronavirus NL63
3. Human coronavirus OC43
4. Human coronavirus HKU1

Coronaviruses that produce severe disease

Coronaviruses that caused explosive outbreaks of severe respiratory disease with higher mortality are:

5. **SARS-CoV** (Severe acute respiratory syndrome coronavirus): It has caused an explosive epidemic called '**SARS**' in China in 2003
6. **MERS-CoV** (Middle East respiratory syndrome coronavirus): It has caused an explosive epidemic '**MERS**' in Middle East in 2012
7. **SARS-CoV-2** (Severe acute respiratory syndrome coronavirus-2): It is the causative agent of an ongoing explosive pandemics affecting the whole world in 2019-20; called **COVID-19** (Coronavirus disease, 2019)

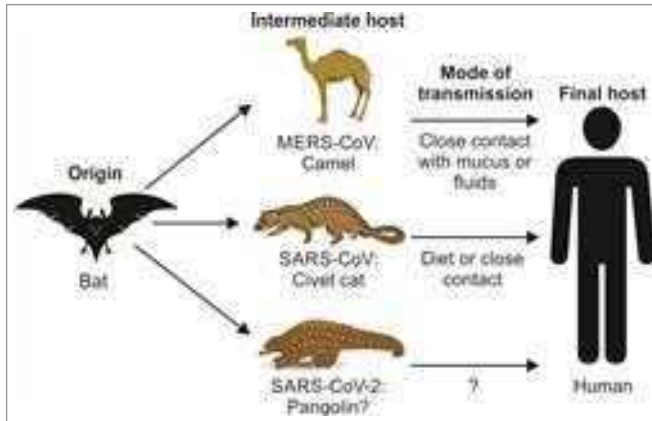


Fig. 67.2: Origin of MERS-CoV, SARS-CoV and SARS-CoV-2.

- ❖ **History:** SARS was first recognized in China in 2003 by WHO physician Dr Carlo Urbani. He diagnosed it in a businessman who had travelled from China, through Hong Kong, to Hanoi, Vietnam. The businessman and the doctor who first diagnosed SARS both died from the illness
- ❖ **Epidemiology:** During 2003 outbreak, the SARS virus, spread from Asia to various regions of the world causing nearly 8,098 cases in 29 countries, with over 774 deaths. However, India remained free from the infection. Since 2003, no case has been reported from anywhere in the world
- ❖ **Source:** SARS-CoV infection in humans is believed to be contracted from animals, including monkeys, Himalayan palm civets, raccoon dogs, cats, dogs, and rodents (Fig. 67.2)
- ❖ **Transmission:** SARS-CoV is primarily transmitted from person to person (droplet or contact); mainly during the second week of illness, which corresponds to the peak of virus excretion in respiratory secretions
- ❖ **Clinical manifestations** include severe lower respiratory tract infection; characterized by muscle pain, headache, sore throat and fever, followed by the onset of respiratory symptoms mainly cough, dyspnea and pneumonia. In some cases, it may progress to acute respiratory distress syndrome
- ❖ **Treatment:** There was no effective vaccine or drug available. Cases were managed only symptomatically
- ❖ **Infection control:** Implementation of appropriate infection control practices was the main reason behind bringing the global outbreak to an end.

MIDDLE EAST RESPIRATORY SYNDROME (MERS)

MERS-CoV can cause a severe form of lower respiratory illness with a mortality rate of 35%.

Epidemiology

MERS was first reported in Saudi Arabia in 2012.

- ❖ Between 2012–January 2020, about 2,519 laboratory-confirmed cases of MERS-CoV with 858 deaths (mortality 34.3%) have been reported to WHO from 27 different countries. India was not affected
- ❖ Saudi Arabia accounted for 84% (2,121 cases, including 788 related deaths) of the cases followed by other Middle East countries
- ❖ **Origin:** The origin of the virus is not fully understood but, according to the analysis of different virus genomes, it is believed that it might have originated in bats and was transmitted to camels sometime in the distant past (Fig. 67.2)
- ❖ **Source:** Dromedary camels are a major reservoir host for MERS-CoV and an animal source of MERS infection in humans
- ❖ **Transmission:** Both zoonotic and human to human transmission have been reported
 - **Zoonotic:** MERS-CoV can be transmitted through direct or indirect contact with infected dromedary camels
 - **Human-to-human:** It does not pass easily from person to person unless there is close contact, such as providing unprotected care to an infected person by family members and healthcare workers.
- ❖ **High-risk to acquire infection:** People at increased risk for MERS-CoV infection include:
 - Recent history of travel from the Arabian Peninsula within 14 days
 - Close contacts of a confirmed case of MERS
 - Healthcare workers not following recommended infection control precautions
 - People with exposure to infected camels
 - Elderly people are at higher risk of developing severe disease and complications including death.
- ❖ **High-risk for severe disease:** Elderly people with diabetes, renal failure, chronic lung disease, and immunocompromised persons are considered to be at high-risk of severe disease from MERS-CoV infection.

Clinical Manifestations

- ❖ Incubation period is about 2–14 days
- ❖ Typical MERS symptoms include fever, cough and shortness of breath
- ❖ Pneumonia is common, but not always present
- ❖ Gastrointestinal symptoms, including diarrhea, have also been reported
- ❖ Complications such as acute respiratory distress syndrome and kidney failure occur, especially in people with underlying comorbid conditions.

Laboratory Diagnosis

- ❖ **Detection of antibodies** in serum indicates past-exposure. ELISA is primarily used for screening of antibodies; which should be confirmed by immunofluorescence assay (IFA) and microneutralization assays

- ❖ **Molecular method:** Detection of specific MERS-CoV RNA by real-time RT-PCR in respiratory specimens indicates active infection. However, laboratory confirmation requires detection of at least two MERS-CoV specific genes such as *upE* and *ORF1b* present in the upstream of the *E* gene
- ❖ **Antigen detection:** Capture ELISA detecting nucleocapsid protein in nasopharyngeal aspirate is under evaluation.

Treatment and Prevention

No vaccine or specific treatment is currently available, however several vaccines and drug trials are under development. Treatment is supportive and based on the patient's clinical condition. General preventive measures include:

- ❖ Regular hand washing before and after touching animals (especially dromedary camels) and should avoid contact with sick animals
- ❖ Avoid consumption of raw or undercooked camel products, including milk and meat; should be consumed only after pasteurization, cooking or other heat treatment.

CORONAVIRUS DISEASE (COVID)-2019

Coronavirus disease-2019 (COVID-19) is an acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has caused an explosive catastrophic pandemic that affected almost all part of the world, and produced significant loss of lives and the worst financial crisis recorded ever, since World War II.

EPIDEMIOLOGY

History

SARS-CoV-2 originated from China and subsequently spread rapidly to affect rest the world over a period of 3-4 months—one of the fastest spreading infectious disease recorded in the history of mankind.

- ❖ **Wuhan:** It was first identified in December 2019 in Wuhan, China, which produced a large cluster of pneumonia cases— hence, the virus was initially called as the '*Wuhan Virus*'. Subsequently it was named as the 2019- novel coronavirus (2019-nCoV)
- ❖ **Origin:** In further studies, it was found that it's a β -*Coronavirus*, with highly identical genome (96% homology) to SARS-like bat coronavirus, pointing towards bat as the natural host (Fig. 67.2)
- ❖ **PHEIC:** As the cases continued to rise, the outbreak was declared as '*Public Health Emergency of International Concern (PHEIC)*' on 30th January 2020
- ❖ **Nomenclature:** On 11th February 2020, WHO announced the official name '*COVID-19*' for this new coronavirus disease and also renamed the virus as SARS-CoV-2 because its genome closely resembled to SARS-CoV.
- ❖ **Pandemic:** The disease spread rapidly in an explosive manner. On 11th March 2020, WHO declared it as a *global pandemic*. By that time, about 114 countries were affected with >118,000 cases and 4,291 deaths

- ❖ The explosive spread continued, affecting over 200 countries in the next couple of months.

Situation in World (As of March 2021)

As this explosive pandemic is still ongoing, the epidemiological data including the geographical distribution is expected to change over time. The information given in this chapter is as of March 2021.

- ❖ **Total cases:** By March 2021, over 11.5 crore cases were reported globally with > 25 lakh deaths
- ❖ **Daily count:** Every day, nearly 2 to 4 lakh cases are being reported with >9,000 deaths globally
- ❖ **Highest cases:** USA accounted for maximum cases (>2.9 crore), followed by India, Brazil and Russia
- ❖ **Highest cases (%):** In terms of total cases per 100 population, countries such as Czechia (11%), USA (8.8%), Israel (8.5%), Portugal (7.9%) and Bahrain (7%), were worst affected, compared to India (0.8%)
- ❖ **Mortality rate:** The severity of the disease varies among the countries and also across different time spans. Countries such as France, Italy, UK and Belgium reported a mortality rate of > 10%, in the early phase of the pandemic, which reduced subsequently. In contrast USA (1.8%) and India (1.4%) reported lower mortality rate during the pandemic. The average mortality rate in the world is estimated to be 2.2%
- ❖ **China:** Rapid rise of cases occurred in China at the beginning (over 80,000 cases by end of February 2020). Wuhan accounted for the majority (>80%) of cases in China. However, cases rapidly declined thereafter
- ❖ **Italy:** Italy was one of those countries which were worst hit in the early phase of this pandemic. The country was not prepared when the disease started. As a result, there was a rapid surge of cases with a mortality rate >10%
- ❖ **USA:** Accounts for highest number of cases (>3 crore) with a mortality rate of 1.8%
- ❖ **Growth curves:** The growth curves of COVID-19 cases vary among the countries. Different countries are at different stages of pandemic. In countries such as Italy, Spain and USA, the disease spread quickly reaching its peak early; whereas in other countries like India the disease had a slower rate of growth (initially)
- ❖ **Variants:** The increase number of cases in various parts of the world, in the later phase of the pandemic may be due to emergence of various variants of the SARS-CoV-2 virus, such as UK variant, Brazilian variant and South African variant
- ❖ **India** was one among those countries where the COVID-19 pandemic had a slower growth curve to reach its peak. India accounts for second highest number of cases (>1.1 crore) with a mortality rate of 1.4%.

MORPHOLOGY

The SARS-CoV-2 comprises of a nucleocapsid, surrounded by an envelope. It measures 120 nm in size; has a helical symmetry. It possesses 4 structural proteins (N, S, M and

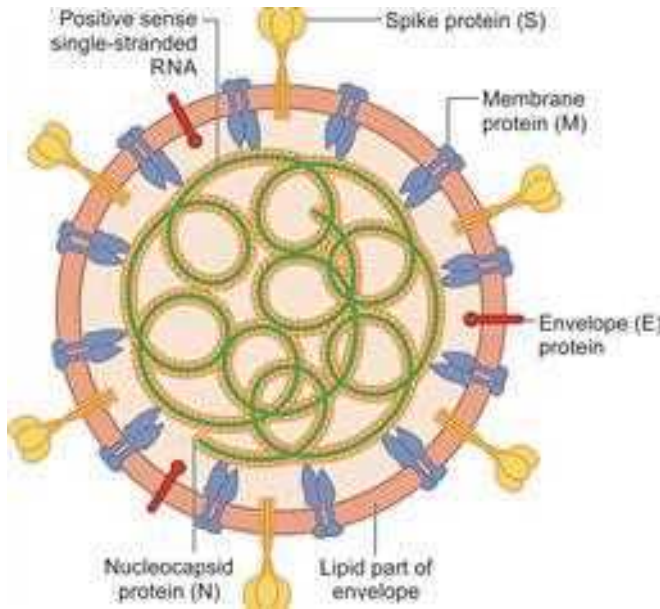


Fig. 67.3: Structure of SARS-CoV-2.

E), 16 nonstructural proteins and several other accessory proteins (Fig. 67.3).

- ❖ **Nucleocapsid** consists of a positive-sense single-stranded RNA (~30 kb genome size), surrounded by nucleocapsid protein (N)
- ❖ The **envelope** is lipoprotein in nature; the lipid part is host-derived into which a number of proteins are embedded such as:
 - **Spike protein (S):** Helps in the attachment to the host cells. Neutralizing antibodies are produced against S protein are protective in nature. It has two subunits.
 - ♦ S1 subunit possesses the receptor-binding domain (RBD), which binds to a specific receptor in the host cell surface
 - ♦ S2 subunit facilitates virus-cell membrane fusion
 - **Membrane glycoprotein (M):** It is the most abundant structural protein, gives the shape to the virus
 - **Envelope protein (E):** It is a transmembrane protein and with ion channel activity; found in small quantities.
- ❖ **Non-structural proteins:** They include several enzymes which help in replication of the virus, e.g. RNA-dependent RNA polymerase (RdRp), helicase, etc.

PATHOGENESIS

Transmission

COVID-19 virus is primarily transmitted via respiratory droplets and contact routes.

Droplet Transmission

Droplet transmission occurs when a person is in close contact (within 1 meter) with an infected person. Exposure to potentially infective respiratory droplets occurs, for

example, through coughing, sneezing or very close personal contact resulting in the inoculation of entry portals such as the mouth, nose or conjunctiva. Use of mask can prevent droplet transmission.

Contact Transmission

Transmission of the COVID-19 virus can occur directly by contact with infected people, or indirectly:

- ❖ By contact with the surfaces in the immediate environment or
- ❖ With objects used on or by the infected person (e.g. stethoscope or thermometer) or
- ❖ Through fomites (inanimate objects) in the immediate environment around the infected person such as infected clothes, utensils, furniture.

Following contact (direct or indirect), the virus can only be transmitted by touching the contaminated hand to a person's mouth, nose or conjunctiva. Frequent hand hygiene following potential contact exposure is crucial to prevent this type of transmission.

Aerosol Transmission

Aerosol transmission (spread of the infected droplet nuclei beyond one meter) is not documented yet, although active research is on-going in this regard. However, in specific settings in which aerosol-generating procedures are performed (e.g. endotracheal intubation), aerosol transmission of the COVID-19 virus may be possible (Chapter 21). Use of N95 respirator is important to prevent this type of transmission.

Pre-symptomatic Transmission

It is defined as the transmission of the COVID-19 virus from a person who is infected and shedding the virus but has not yet developed symptoms. This type of transmission has been observed in people **1-3 days** before the onset of their symptom, with the highest viral loads detected around the day of symptom onset, followed by a gradual decline over time.

Host Cell Entry

SARS-CoV-2 enters into the target host cells by binding of its spike glycoprotein (S) antigen with the host cell receptor, i.e. angiotensin converting enzyme-2 (ACE-2). This is also the receptor for SARS-CoV.

- ❖ **Spike protein cleavage:** For virus entry into a host cell, its S protein needs to be cleaved, which is mediated by host cell proteases; of which TMPRSS 2 (transmembrane protease serine 2) is important
- ❖ **Fusion:** Cleavage of S protein produces S1 subunit which binds to ACE-2 and S2 subunit which causes fusion of viral envelope with host cell membrane. Then follows the entry of the virus via endosomal pathway
- ❖ **ACE-2 receptors** are highly expressed on type-II alveolar cells in lungs and on the epithelial cells of oral mucosa; also found on cells of heart, kidney, endothelium and

intestine. Therefore, the patients develop extrapulmonary manifestations in addition to respiratory symptoms.

Note: The primary function of ACE-2 is that, it helps to reduce blood pressure and has anti-inflammatory action.

Development of ILI

ACE-2 receptors are highly expressed on the epithelial cells of oral mucosa. Therefore, at the initial stage, SARS-CoV-2 infects the pharyngeal epithelium, induces inflammation. This accounts for the influenza-like illness (ILI) which occurs at the beginning stage of most of the symptomatic cases.

Development of ARDS

The leading cause of mortality in patients with COVID-19 is hypoxemic respiratory failure which can result in acute respiratory distress syndrome (ARDS).

Reduced Surfactants

In lungs, ACE-2 receptors are highly expressed on type-II alveolar cells. These cells normally produce pulmonary surfactants which lower the alveolar surface tension. In COVID-19 patients the following events take place.

❖ Damage to the type-II alveolar cells leads to reduced production of pulmonary surfactants; as a result of which alveoli tend to collapse. The air-liquid-interphase is

perturbed which leads to fluid retention in the interstitial space

❖ To prevent collapse, the muscular movement of inspiration becomes hyperactive, which results in increased lung volume in the interstitial space. The "low pressure area" created in the interstitial space attracts liquid, which further contributes to edema in the lungs.

Cytokine Storm

The presence of SARS-CoV-2 in lung induces an uncontrolled generalized immune response. Several immune cells like neutrophils, T-lymphocytes, macrophages are recruited to the lungs (Fig. 67.4).

❖ **Acute cytokine influx:** These immune cells release pro-inflammatory cytokines—IL-2, IL-2R, IL-6, IL-7, IL-8, IL-10, G-CSF, IFN- γ , IP-10, MCP-1, MCP-3, TNF- α and others

❖ **Host injury:** The elevated cytokines leads to various consequences such as:

- Tissue damage and necrosis
- Further recruitment of leukocytes
- **Impaired gas exchange**, which leads to reduced blood oxygenation and tissue hypoxia
- **Endothelial damage** of pulmonary vasculature, leading to vasodilation, microvascular thrombosis and hemorrhage, and hypercoagulability

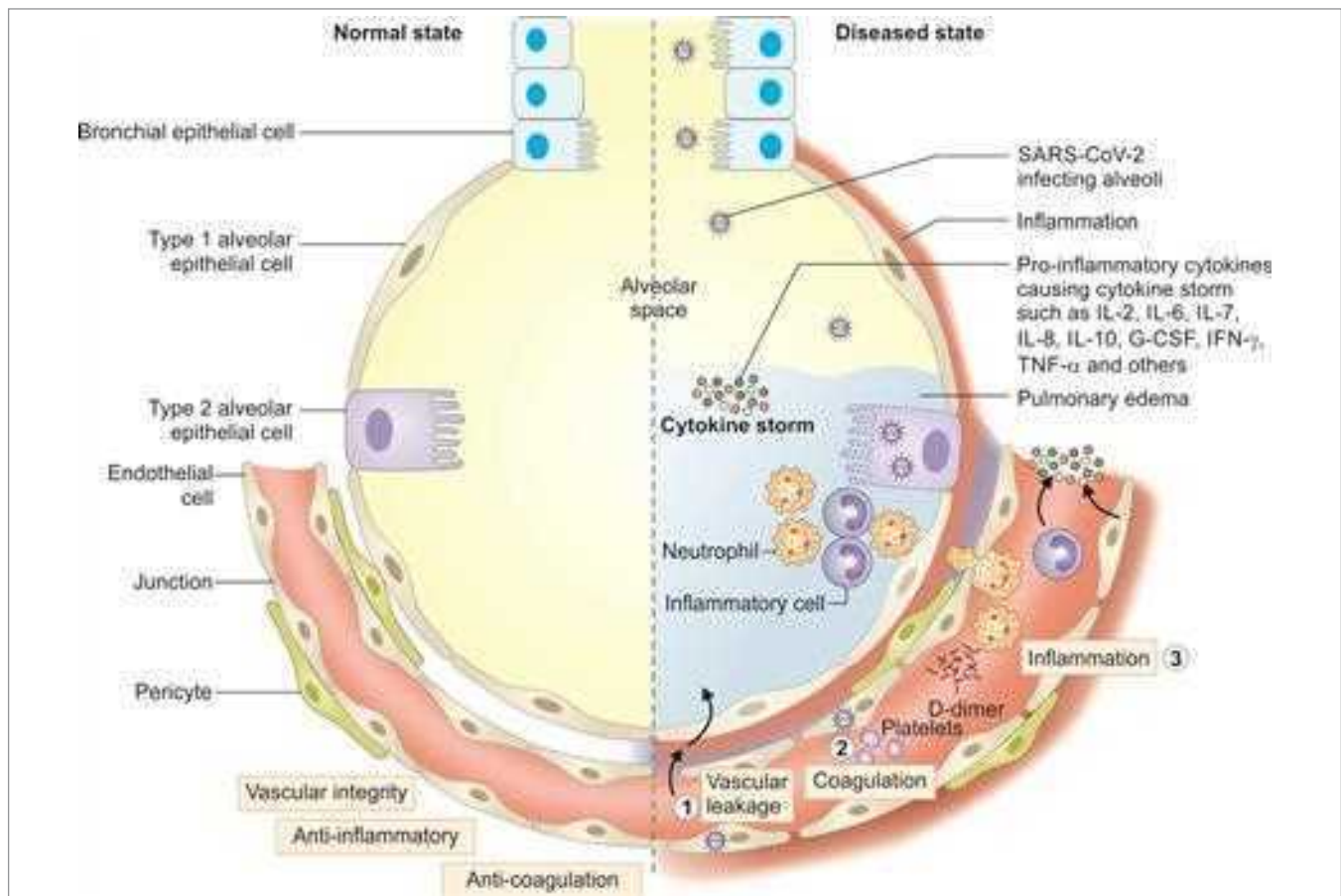


Fig. 67.4: Cytokine storm seen in COVID-19 patients.

- **Dilatation of blood vessels** underlying the alveoli: This allows passage of fluid from the blood vessels to lungs which leads to pulmonary edema. These infiltrates in the lungs appear as ‘**ground-glass**’ in chest imaging
- **Fibrosis:** In the later stage, there occurs recruitment of fibroblast, which causes lung fibrosis, and ultimately, leads to respiratory failure. This stage is called as *acute respiratory distress syndrome (ARDS)*, eventually leading to respiratory failure
- ❖ **Multiorgan failure:** Cytokines can also induce damage to other organs of the body such as heart, kidney, liver, etc. There occur several catastrophic events such as sepsis, septic shock and multiorgan failure including acute kidney injury and cardiac injury
- ❖ **Risk factors:** The major risk factors for severe disease are:
 - **Age** > 60 years (risk increases with age)
 - **Underlying comorbidities** such as diabetes, hypertension, cardiac disease, chronic obstructive lung disease, cerebrovascular disease, chronic kidney disease, immune-suppression and cancer.

CLINICAL MANIFESTATIONS

The incubation period for COVID-19 (time between infection and symptom onset) is on an average of 5-6 days, but can be as long as 14 days. COVID-19 patients may present with following signs and symptoms:

- ❖ **Common features:** Fever, cough with expectoration, fatigue, shortness of breath, myalgia, rhinorrhea, sore throat, diarrhea. Loss of smell or taste sensation may occasionally occur preceding the onset of respiratory symptoms
- ❖ **Atypical symptoms:** Particularly seen in older people and immune-suppressed patients—such as fatigue, reduced alertness, reduced mobility, diarrhea, loss of appetite, delirium, and absence of fever. Children might not develop fever or cough as frequently as adults.

Clinical Severity

Based on the clinical severity, the disease may be classified into the three clinical stages (Table 67.2).

LABORATORY DIAGNOSIS

COVID-19

- ❑ **Specimens:** Throat and nasal swabs
- ❑ **NAAT:** Nucleic acid amplification testing
 - *Formats:* Real time RT-PCR, automated formats (CBNAAT and Truenat)
 - *Gene targets:* Screening (*E, N, M* genes), confirmatory (*RdRp, N2* genes, etc.)
- ❑ **Antigen detection assay:** Point-of-care test; detects nucleocapsid protein antigen in nasopharyngeal swab
- ❑ **Antibody (IgG) detection assay:** Used for serosurveillance and survey in high-risk and vulnerable group; not for clinical diagnosis

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Table 67.2: Clinical severity of COVID-19 disease.

Clinical stage	Clinical presentations
Mild disease	
ILI ((influenza-like illness)	Patients with uncomplicated upper respiratory tract infection, may have mild symptoms such as fever, cough, sore throat, nasal congestion, malaise, headache Without evidence of breathlessness or hypoxia (normal saturation)
Moderate disease	
Pneumonia with no signs of severe disease	Dyspnea, fever and cough Hypoxia, SpO ₂ <94%, respiratory rate ≥ 24 per minute
Severe disease : Called as severe acute respiratory illness (SARI)	
Severe pneumonia	Clinical signs of pneumonia plus one of the following sign of severe respiratory distress: (i) Respiratory rate >30 /min or (ii) SpO ₂ <90%
Acute respiratory distress syndrome (ARDS)	Symptoms: Onset of new or worsening respiratory symptoms within one week Chest imaging: Shows bilateral opacities, not fully explained by effusions, lobar or lung collapse, or nodules Decreased PaO₂/FiO₂ (normal value ≈ 500): ARDS can be classified into—mild (<300), moderate (<200), and severe (<100); (when PEEP or CPAP is maintained at ≥5 cm H ₂ O)
Sepsis	Acute life-threatening multiorgan dysfunction: Clinically diagnosed by SOFA (sequential organ failure assessment) score (as described in Chapter 29)
Septic shock	Persisting hypotension despite volume resuscitation, requiring vasopressors to maintain mean arterial pressure ≥65 mm Hg and serum lactate level >18 mg/dL (as described in Chapter 29)

Abbreviations: PEEP, positive end-expiratory pressure; CPAP, continuous positive airway pressure; SpO₂, blood oxygen saturation level; PaO₂/FiO₂; ratio of arterial oxygen partial pressure (in mm Hg) to fractional inspired oxygen.

Contd...

LABORATORY DIAGNOSIS

COVID-19

- ❑ **Sequencing:** To determine mutations in the viral genome
- ❑ **Viral culture:** Used for research purpose
- ❑ **Nonspecific tests** include:
 - Radiology (chest CT scan): Ground-glass appearance
 - Biomarkers: IL-6, D-dimer

LABORATORY DIAGNOSIS

Laboratory diagnosis is necessary only in specific indications as per Government of India, such as:

- ❖ Patient with influenza-like illness (ILI) symptoms or severe acute respiratory infection (SARI)
- ❖ Asymptomatic direct and high-risk contacts of a confirmed case to be tested once between day 5 and day 10 of contact
- ❖ History of international travel in the last 14 days.

Specimen Collection and Transport

- ❖ **Preferred specimens:** Throat (i.e. oropharyngeal) and nasal swabs are the preferred specimens. Dacron or polyester flocked swabs are used, dipped in viral transport media (VTM) after collection
- ❖ **Alternative specimens include:** Nasopharyngeal swab, bronchoalveolar lavage (BAL) or endotracheal aspirate (in ventilated patients)
- ❖ **PPE:** Appropriate PPE should be used for specimen collection such as gloves, gown, N95 respirator and face shield
- ❖ **Specimen transport and packing:** Samples collected should be properly labelled, packed in three layers (triple packaging method) and transported to the laboratory maintaining an adequate cold chain
- ❖ **Storage:** Upon receipt, the specimens should be stored at appropriate temperature (4 °C for <5 days and -77 °C for >5 days)
- ❖ **Biosafety precaution:** Initial processing of the specimen (before inactivation) should take place in a biological safety cabinet (BSC). The laboratory should have the following biosafety facility
 - For NAAT: Require biosafety level-2 facility
 - For culture: Require biosafety level-3 facility
 - For point-of-care test (Antigen detection): Can be performed without employing a BSC.

Nucleic Acid Amplification Testing (NAAT)

Real-time RT-PCR

Real time reverse transcriptase PCR is the gold standard test for diagnosis of COVID-19.

- ❖ The average time taken is around 4-5 hours from receipt of sample to generation of the result
- ❖ The advantage of this platform lies in its accuracy of detection as well as the ability to run up to 90 samples in a single run. Therefore, if available, this platform should be used as a frontline test for the diagnosis of SARS-CoV-2
- ❖ **Gene targets:** Most of the commercial kits target two genes, performed in a single reaction—one for screening and other for confirmatory

Gene targets for screening are genus specific; i.e. specific for *Sarbecovirus* (*Betacoronavirus*):

- ❑ Spike protein (S)
- ❑ Envelope protein (E)
- ❑ Membrane protein (M)
- ❑ Nucleocapsid protein (N)

Gene targets for confirmation are species specific; i.e. specific for SARS-CoV-2

- ❑ RNA-dependent RNA polymerase (*RdRp*)
- ❑ Open reading frames (ORF1a/b)
- ❑ N2 nucleocapsid

- ❖ **Principle:** Most commercial kits available are based on qualitative real-time PCR
 - The target gene/s in the specimen is amplified in the thermocycler

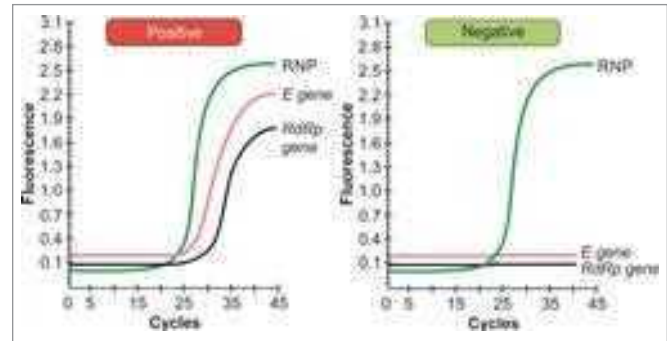


Fig. 67.5: Interpretation of real-time RT-PCR result for COVID-19 diagnosis.

- When the amplicon binds with the probe, a fluorescence is generated. The point at which the fluorescence starts is the cycle threshold (Ct) of the run
- A sample is considered positive when both screening, as well as confirmatory genes, are detected with a Ct value ≤ 40 cycles (Fig. 67.5).
- **Detectable:** NAAT becomes positive as early as day 1 of onset of symptom (usually after 5 days of infection) and starts to decline by 3rd week and subsequently becomes undetectable (Fig. 67.6).

Automated real-time RT-PCR

Several automated real-time PCR are commercially available such as—Truenat and CBNAAT (Cartridge based nucleic acid amplification test, e.g. GeneXpert). Both these systems are already in use for the diagnosis of tuberculosis.

- ❖ **Advantages** of these systems include:
 - These platforms have widespread availability even at district and primary health center level as these systems are already in use for the diagnosis of tuberculosis and other infectious diseases
 - They have a quick turnaround time (30-60 minutes)

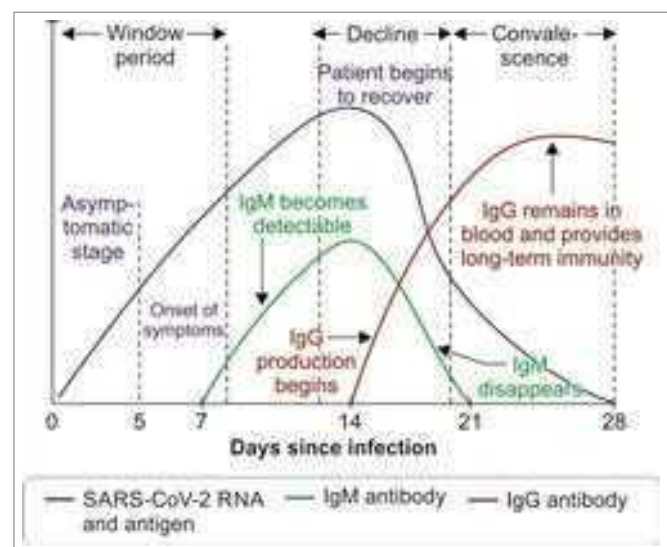


Fig. 67.6: Course of the diagnostic markers in COVID-19.

- Fully-automated, involves minimal handling; therefore poses minimum biosafety hazard. Safety is further augmented by the closed nature of these platforms.
- ❖ **Gene targets used are:**
 - **CBNAAT:** Two targets are used; *E* gene for screening and *N2* gene for confirmation
 - **Truenat:** Two targets are used; *E* gene for screening and *RdRp* gene for confirmation.
- ❖ However, **disadvantages** of these systems include: Only 1-4 samples can be tested in one run, therefore suitable only for laboratories with less sample load (24-48 samples/day)

Antigen Detection

A rapid chromatographic immunoassay is commercially available (SD Biosensor) for qualitative detection of specific antigens (nucleocapsid protein) to SARS-CoV-2.

- ❖ **Nasopharyngeal swab**, after collection should be immersed and squeezed in the viral extraction buffer, provided with the kit. This buffer inactivates the virus, releasing the antigen
- ❖ It is a **point-of-care test**, conducted at the bedside within one hour, as the antigen in the extracted buffer is stable only for an hour
- ❖ **Performance:** It is highly specific (99–100%), with moderate sensitivity (50–84%). Therefore, symptomatic but negative patients should be essentially referred for a real-time RT-PCR test.

There are various other antigen detection kits under validation, which may be marketed in near future.

Antibody Detection

IgG antibodies generally start appearing after two weeks of the onset of infection, once the individual has recovered and last for several months. Therefore, the IgG test should not be used for clinical diagnosis. ELISA, chemiluminescence and immunochromatographic test formats are available for the detection of IgG antibodies. They may be useful in the following situations:

- ❖ **Sero-surveillance purpose**, to estimate the proportion of population exposed to infection with SARS-CoV-2 including asymptomatic individuals; so that appropriate public health interventions can be planned and implemented for prevention and control of the disease
- ❖ **The survey in high-risk or vulnerable populations** such as health care workers, frontline workers, immunocompromised individuals, individuals in containment zones, police and security personnel, etc. to know who has been infected in the past and has now recovered.

Sequencing

Sequencing methods played a major role in the initial identification of SARS-CoV-2.

- ❖ It is not used routinely for diagnostic purpose
- ❖ However, next-generation sequencing and metagenomic next-generation sequencing will be needed for determining mutations in the genome of SARS-CoV-2.

Viral Culture

Although not recommended for diagnostic purpose, viral culture can be used for research purpose such as understanding the properties of the virus and development of vaccine.

Nonspecific Tests

- ❖ **Prognostic markers:** There are several prognostic markers which can be used in the setting of ARDS, include:
 - Elevated IL-6 level: Indicates cytokine storm
 - Elevated D-dimer: Indicates the presence of high level of fibrin degradation products, thus suggesting an underlying coagulopathy
 - Elevated serum ferritin: Indicates inflammation
 - Severe lymphopenia
 - Elevated C-reactive protein: Marker of acute inflammation.
- ❖ **CT scan** of lungs shows *ground glass appearance* (Chapter 59, Fig. 59.4) and/or consolidation.

TREATMENT

COVID-19

Currently, there is no definitive therapy available for COVID-19; however many clinical trials are ongoing (Table 67.3).

Symptomatic management

The mainstay of treatment is an early supportive therapy for symptomatic management

- In patients with severe respiratory distress
 - Supplemental oxygen therapy is given immediately
 - High-flow nasal cannula oxygenation (HFNO)
 - Non-invasive mechanical ventilation
 - Mechanical ventilation: In patients with moderate or severe ARDS, higher PEEP (positive end-expiratory pressure) instead of lower PEEP is suggested.
- Management of septic shock by—vasopressors, fluid replacement by crystalloids such as normal saline and Ringer's lactate.

Investigational therapy

Few drugs are now recommended for treatment by the Government of India based on limited available evidence. The recommendations may be changed or updated as on when more data is available.

Remdesivir

It interferes with the action of viral RNA-dependent RNA polymerase

- **Indication:** May be considered in patients with moderate disease (those on oxygen)
- **Dosage:** 200 mg IV on day 1 followed by 100 mg IV daily for 5 days
- **Contraindicated in:** Children, pregnancy, lactation, liver or renal impairment.

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TREATMENT

COVID-19

Convalescent plasma therapy

The plasma of patients recovered from COVID-19 contains neutralizing antibodies against SARS-CoV-2 virus. These antibodies are believed to eliminate the virus completely.

- **Indication:** May be considered in patients with the moderate disease who are not improving (oxygen requirement is progressively increasing) despite the use of steroids
- **Dose** is variable ranging from 4 to 13 mL/kg (usually 200 mL, single dose), given slowly over not less than 2 hours.

Tocilizumab

It is a monoclonal antibody against IL-6 receptor, the most important cytokine raised in cytokine storm

- **Indication:** May be considered in patients with moderate disease with progressively increasing oxygen requirements and in mechanically ventilated patients not improving despite the use of steroids
- **Dose:** 8 mg/kg (maximum 800 mg at one time) given slowly in 100 mL normal saline over 1 hour
- **Special considerations:**
 - It is given only when there is raised inflammatory markers (e.g. CRP, ferritin, IL-6)
 - Should be monitored for secondary bacterial infections and neutropenia
 - Active infections and tuberculosis should be ruled out before use.

Hydroxychloroquine (HCQ)

It may be effective in the early course of the disease and should be avoided in patients with severe disease.

Discharge policy

Upon clinical and/or microbiological recovery (negative result by real time RT-PCR), the patient can be discharged and transmission-based precautions can be discontinued.

Table 67.3: Treatment under research for COVID-19.

Drugs	Mechanism of action
Favipiravir	It is converted into an active form in cells which inhibits RNA polymerase activity
Remdesivir	It is converted into an active form (GS-441524), which inhibits viral RNA-dependent RNA polymerase
Lopinavir/Ritonavir	Protease inhibitors; block viral replication
Hydroxychloroquine	Used for prophylaxis against COVID-19
Anti-cytokines	Useful for ARDS, prevents cytokine storm
Tocilizumab or sarilumab	IL-6 receptor blocker
Tofacitinib	Jak3 inhibitor
Convalescent plasma therapy	
The plasma of patients recovered from COVID-19 contains neutralizing antibodies against SARS-CoV-2 virus. These antibodies are believed to eliminate the virus completely.	
Other drugs	
Camostat mesylate	Inhibits TMPRSS2
Hesperidin	Inhibit binding of spike protein to ACE-2

PROPHYLAXIS

Chemoprophylaxis

Hydroxychloroquine (HCQ) has been recommended by the Government of India for prophylaxis for SARS-CoV-2 infection. However, it is reiterated that the intake of HCQ should not instill a sense of false security and should practice infection control measures as recommended.

❖ **Indication:** HCQ prophylaxis is indicated in:

- Asymptomatic household contacts of laboratory confirmed cases
 - All asymptomatic healthcare workers
 - Asymptomatic frontline workers
 - Paramilitary/police personnel involved in COVID-19 related activities
- ❖ **Dosage:** 400 mg twice a day on day 1, followed by 400 mg once weekly for next 7 weeks (except for household contacts, given for 3 weeks)
- ❖ **Contraindication:** The following are excluded from HCQ prophylaxis—known case of retinopathy, hypersensitivity to HCQ, glucose-6-phosphate dehydrogenase (G6PD) deficiency, pre-existing cardiomyopathy and cardiac rhythm disorders, children <15 years age, in pregnancy and in lactation
- ❖ **Precaution:** ECG should be done before prescribing HCQ prophylaxis and during the course to look for prolongation of QT interval.

COVID Vaccine

Currently, there are several vaccines available against COVID-19; which are approved for human use after proven safe and effective in large (phase III) clinical trials. More so, intense research is on-going and more than 200 additional vaccine candidates are in development, of which more than 60 are in the stage of clinical development.

❖ **Vaccine principles:** The COVID-19 vaccines available are based on one of these following principles:

1. **Inactivated or weakened virus vaccines**, which use a form of the virus that has been inactivated or weakened so it doesn't cause disease, but still generates an immune response (e.g., Covaxin, CoroVac and BBIBP-CorV)
2. **Protein-based vaccines**, which use harmless fragments of proteins or protein shells that mimic the SARS-CoV-2 to safely generate an immune response (e.g., Epi Vac Corona)
3. **Viral vector vaccines** (e.g., Adenovirus), which use a safe virus that cannot cause disease but serves as a platform to produce coronavirus proteins to generate an immune response (e.g., Covishield, Oxford-Astrazeneca, Sputnik V and Johnson & Johnson COVID -19 vaccine)
4. **RNA and DNA vaccines**, a cutting-edge approach that uses genetically engineered RNA or DNA to

generate a protein that itself safely prompts an immune response (e.g., Pfizer-BioNTech, Moderna).

- ❖ **Adverse effect:** The COVID-19 vaccines are proven to be safe, except for mild adverse reactions such as injection site pain, fever, tiredness, myalgia, fatigue and headache. For some vaccines (e.g., Pfizer and Moderna vaccine), the adverse effects are more pronounced after the second dose
- ❖ **Efficacy:** Most vaccines in use are shown to be efficacious (70–90%) in phase III clinical trial. However, the duration of protection will be known in due course of time
- ❖ **Individuals infected with COVID-19 in past** also should get the vaccine (complete schedule). However, person with active COVID-19 infection may increase the risk of spreading the same to others at vaccination site. For this reason, infected individuals should defer vaccination for 14 days after symptoms resolution
- ❖ **Storage:** Most vaccines can be stored at 2–8°C, except Pfizer vaccine which needs to be stored at -80 to -60°C
- ❖ **Schedule:** Most vaccines are given as two doses at 2–4 weeks gap by IM route; the exception being Johnson & Johnson vaccine, which is given as a single dose.
- ❖ **Contraindications:** (i) Anaphylaxis or allergy to previous dose, (ii) pregnancy and lactation, (iii) age <18yr.

COVID-19 Vaccine in India

Two vaccines are licenced in India; enrolled for use in a phased manner: (i) **Phase I (January 16th 2021):** For healthcare workers; (ii) **Phase II (March 1st 2021):** High-risk general public; which include persons > 60 years age and persons between 45 and 59 years of age with comorbid conditions such as cancer, diabetes, hypertension, etc.

- ❖ **Covaxin:** Initiated by ICMR-Bharat Biotech, India. It is a whole-virion inactivated vaccine, which uses spike protein as a target. It is administered in two doses (4 weeks apart) by IM route
- ❖ **Covishield:** It is prepared by Serum Institute of India, in collaboration with University of Oxford and AstraZeneca pharmaceuticals, UK. It is based on non-replicating adenovirus vector (modified Chimpanzee adenovirus, ChAdOx1) expressing spike protein. It is administered in two doses (4 weeks apart) by IM route.

INFECTION PREVENTION AND CONTROL

Infection prevention and control (IPC) is the most effective method currently available for the prevention of COVID-19. The following are the key IPC measures need to be strictly followed.

IPC Measures at Healthcare Facility

IPC measures of droplet and contact precautions need to be followed by the healthcare workers while handling

COVID-19 cases, except for aerosol-generating procedures (AGPs) when airborne precautions need to be followed (refer Chapter 21).

Hand Hygiene

As contact mode appears to be an important mode of transmission, absolute hand hygiene is probably the most effective method for the prevention of COVID-19.

- ❖ Hand hygiene needs to be performed when opportunity arises (as per WHO's 'my five moments of hand hygiene,' Chapter 21)
- ❖ Hand hygiene must be performed by the correct technique, and for appropriate duration (20-40 sec for hand rub).

Personal Protective Equipment

The following are the current recommendations:

- ❖ **HCWs giving care to the COVID-19 suspects:** Should wear a medical (3-ply) mask, a pair of gloves, gown, and a face shield. Medical mask should be replaced by a N95 respirator if AGPs are carried out (Fig. 67.7)
- ❖ **HCWs working in non-COVID areas:** Should wear a medical (3-ply) mask. This referred to as '*targeted continuous medical mask use*'
 - Should wear masks during all routine activities throughout the entire shift
 - Masks are only changed if they become soiled, wet or damaged, or at the end of shift
 - Front part of the mask should never be touched
 - Mask should never be hanged around the neck.
- ❖ **Anyone entering into a healthcare facility:** Must wear a face mask (e.g. cloth/fabric mask), regardless of the activities he is involved in. This is referred to as '*universal masking*' in healthcare facilities.



Fig. 67.7: Personal protective equipment recommended for healthcare workers when giving care to COVID-19 patients.

Environmental Cleaning

SARS-CoV-2 may survive on surface and floor for a variable period, ranging from few hours to as long as 9 days. This may be a potential source of transmission by indirect contact. Therefore, the following measures need to be followed.

- ❖ **Floor and surfaces:** Should be cleaned with a detergent, followed by disinfected with sodium hypochlorite (0.5%)
- ❖ **Cleaning of equipment** or patient care items such as stethoscope, BP apparatus, etc. should be done by using alcohol (70%)
- ❖ **High touch surfaces** such as lift button, rail of the staircase, patient trolley, bed rails, bed frames, bedside tables, door handles, etc. should be frequently disinfected with alcohol
- ❖ **Terminal disinfection** in patient care room after discharge/transfer of patients.

Other IPC Measures

- ❖ **Respiratory hygiene and cough etiquette** such as wearing a medical mask by all individuals who are symptomatic, hand wash immediately after sneezing or coughing, etc. (Chapter 21)
- ❖ **Biomedical waste management** should be carried out as per the 2016 guideline (Chapter 24). However, additional precautions are taken such as use of double bag, use of dedicated trolley and collection bins, label as “COVID-19 waste”, and disinfecting outer bag with hypochlorite before handing over
- ❖ **Laundry:** All linens used for COVID-19 patients should be washed at 60-90°C with laundry detergent followed by soaking in 0.1% sodium hypochlorite for approximately 30 minutes and dried.

IPC Measures for General Public

Hand Wash

Frequent hand wash is necessary after contact with other individuals, high-touch area, public places, after receipt of any items, or after blowing nose, coughing, or sneezing. Touching of eyes, nose, and mouth with unwashed hands must be avoided.

Social Distancing

Ideally, people should stay at home as much as possible. If not possible, 1 meter (2 arms) distance should be strictly maintained from other people at all times, no matter how close is the person and how important is the work involved. As droplets can travel a maximum of 1-meter distance, therefore the social distance of 1 meter would prevent the droplet transmission.

Environmental Cleaning

Frequently touched surfaces should be disinfected daily. This includes tables, doorknobs, light switches, countertops, handles, desks, phones, keyboards, toilets, faucets, and sinks. If surfaces are dirty, then it should be cleaned with detergent or soap and water prior to disinfection.

Cloth Mask (Non-medical Masks)

Everyone should wear a cloth face mask when they have to go out in public, e.g. for the grocery store. It primarily aims at **source control**, i.e. preventing transmission from the wearers to others).

- ❖ Cloth masks are made from a variety of fabrics, such as polypropylene. It should comprise of at least two layers
 1. Internal layers are made up of water-absorbing (hydrophilic) fabrics to readily absorb droplets
 2. An external synthetic material (hydrophobic), which does not easily absorb liquid.
- ❖ They should not be used in hospital settings, as there is no filter used
- ❖ They can be washed and reused
- ❖ Should not be shared between individuals.

Measures taken by the Government

Quarantine

Quarantine refers to restriction on the movement of healthy people who are exposed to a confirmed case; aims at preventing the transmission if they develop disease subsequently.

- ❖ **Duration:** Quarantine period is usually kept as maximum incubation period (i.e. 14 days in case of COVID-19)
- ❖ **Quarantine centers:** There are two types of centers—
 - (i) **Facility quarantine:** Provided by the government. Various centers are converted to quarantine facilities such as schools, marriage halls mandaps, hotels, etc. High-risk contacts are usually kept in facility quarantine;
 - (ii) **Home quarantine:** Low-risk contacts are usually sent for home quarantine.

Indications for Quarantine

WHO has recommended the following exposures occurring from -2 to + 14 days of onset of symptoms in a COVID-19 patient should be considered as contacts and exposed persons should be sent for quarantine

- ❖ **Face-to-face contact** with a COVID-19 patient within 1 meter, for >15 minutes
- ❖ **PPE breach:** Providing direct care for COVID-19 patients without using PPEs or inappropriate use of PPE mounting to breach
- ❖ **Staying in the same close environment** as a COVID-19 patient (including sharing a workplace, classroom or household, cinema hall, or being at the same gathering) for any amount of time
- ❖ **Traveling in close proximity** with a COVID-19 patient in any kind of conveyance
- ❖ **Other situations**, as indicated by the local risk assessment

Lockdown

Lockdown refers to limiting the movement of the entire population as a preventive measure against the COVID-19 pandemic. It was adopted by several countries worldwide including India.

Group	Definition	Benefit of lockdown
A	COVID-19 positive cases	They are admitted in the COVID care facility
B	Pass-by contacts <ul style="list-style-type: none"> • Include anyone who is exposed to the case, but are beyond the memory re-call (e.g. all individuals of a movie theater) • This is the most vulnerable group; nobody including themselves knows who is category B • It is impossible to trace category B, except by performing passive surveillance of the whole community which is practically very difficult 	Lockdown aims at immobilizing category B <ul style="list-style-type: none"> • If anyone developed symptoms, will be referred to the hospital • Others, who do not develop symptoms are either <ul style="list-style-type: none"> ➢ Un-exposed individuals, or ➢ Asymptomatic contacts: They get recovered during lockdown period and therefore will no longer transmit the infection
C	Close contacts of COVID-19 positive cases (e.g. family members and office staff)	Traced easily by active surveillance as they are immobilized at home
D	Rest of the community , who are not exposed to a COVID-19 positive case	The most important objective of lockdown is immobilizing category D, so that they will no longer come in contact with others (especially category B)

- ❖ **India** adopted nationwide lockdown in four phases from 25th March to 31st May 2020. Subsequently, state-specific lockdown was followed in hot spot areas
- ❖ **Shut down:** All services including offices, shops, private and government organizations were shut down, except for emergency services such as food and health.
- ❖ **Objective of lockdown:** Based on exposure risk, the entire population can be divided into four groups. Lockdown has a definite objective for each of these groups (Table 67.4).

Cluster Containment Strategy

The government has adapted cluster containment strategy in areas where cases are reported either in singly or in clusters (local transmission).

- ❖ **Objective:** The objective is to contain the disease within a defined geographic area by early detection of cases, breaking the chain of transmission and thus preventing its spread to new areas causing community transmission

- ❖ **Components:** The components of this strategy include geographic quarantine, social distancing measures, enhanced active surveillance, testing all suspected cases, isolation of cases, quarantine of contacts and risk communication to create awareness among public on preventive public health measures
- ❖ **Containment zone** is determined by four factors—(i) the index case/cluster, (called as epicenter), (ii) the listing and mapping of contacts, (iii) geographical distribution of cases and contacts around the epicenter, and (iv) administrative boundaries within urban cities/towns/rural areas. A buffer zone of additional 5 km radius will be identified.

The knowledge about COVID-19 is evolving and intense research are ongoing on various areas of the disease. Therefore, the reader should update oneself with the latest information from the official websites of WHO and Government of India.

EXPECTED QUESTIONS

I. Write essay on:

1. A 65-year-old patient (without wearing any mask) with complaints of dry cough, sore throat and fever visited a hospital. The security guided him to go to the casualty. The resident doctor (without mask) took history, examined the patient. His throat swab was sent for COVID-19 testing which came positive. Subsequently the security and the resident doctor were also turned positive for COVID-19.
 - a. Identify the infection control breaches.
 - b. Discuss the laboratory diagnosis of this disease.
 - c. Discuss the infection control measures to prevent the transmission

II. Write short notes on:

1. Pathogenesis of COVID-19 disease.
2. Epidemiology of COVID-19.

III. Multiple Choice Questions (MCQs):

1. **While examining a stable patient with COVID-19, all the following PPE are required, except:**
 - a. N 95 mask
 - b. Gown
 - c. Goggles
 - d. Gloves
2. **Gene targets for confirmation of COVID-19 include:**
 - a. Spike protein (S)
 - b. Envelope protein (E)
 - c. Membrane protein (M)
 - d. RNA-dependent RNA polymerase (*RdRp*)
3. **Which of the following is not used for clinical diagnosis of COVID-19 disease:**
 - a. Real time OPCR
 - b. Truenat
 - c. Antigen detection
 - d. Antibody detection

Answers

1. a 2. d 3. d

Miscellaneous Viral Infections of Respiratory Tract

CHAPTER 68

CHAPTER PREVIEW

- Infectious Mononucleosis
- Adenovirus Infections
- Rhinovirus Infection (Common Cold)

This chapter will cover various viral infections of respiratory tract such as infectious mononucleosis, adenovirus infections and rhinovirus infection (common cold).

INFECTIOUS MONONUCLEOSIS

Epstein-Barr Virus (EBV) causes infectious mononucleosis and is also associated with several human tumors, including nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, and B cell lymphoma.

Morphology of EBV

EBV is a member of γ sub-family of Herpesviridae (Chapter 56). They possess dsDNA, are enveloped with an icosahedral symmetry. EBV expresses three classes of antigens.

1. **Latent phase antigens:** They are synthesized during the period of latency, e.g.
 - EBV nuclear antigen (EBNA)
 - Latent membrane protein (LMP).
2. **Early antigens:** They are non-structural proteins which help in viral replication
3. **Late antigens:** They are the structural proteins that form viral capsid and envelope.

Pathogenesis

EBV is transmitted by oropharyngeal contact through infected salivary secretions.

- ❖ **EBV receptors:** EBV binds to specific receptors present on B cell (CD21 or CR2) which are also receptors for C3b component of complement. Such receptors are also present on pharyngeal epithelial cells
- ❖ **Primary infection** occurs in the oropharynx. EBV replicates in epithelial cells or surface B lymphocytes of the pharynx and salivary glands
 - Infected B cells become *immortalized* by the virus and synthesize large number of variety of immunoglobulins (polyclonal), many of those are autoantibodies (e.g. heterophile antibody to sheep RBC antigen)
 - In response to this, the bystander CD8 T lymphocytes are stimulated and appear atypical, a feature which is characteristically seen in infectious mononucleosis.

- ❖ **Oncogenicity:** Persistent EBV infection can induce malignant transformation of infected B cells and epithelial cells by expressing latent EBV antigens such as LMP and EBNA (Chapter 80, for detail).

Clinical Manifestations

Infectious Mononucleosis

It is also called kissing disease (transmitted through salivary contact) or glandular disease. It usually affects young adults, not children. It is characterized by (Table 68.1):

- ❖ Headache, fever, malaise
- ❖ Pharyngitis
- ❖ Cervical lymphadenopathy
- ❖ Hepatosplenomegaly
- ❖ Rashes following ampicillin therapy
- ❖ Atypical lymphocytosis (CD8 T cells)
- ❖ Autoantibodies reactive to sheep RBC antigens (detected by Paul-Bunnell test).

Table 68.1: Comparison of infectious mononucleosis and mononucleosis-like syndrome.

Features	Infectious mononucleosis	Mononucleosis-like syndrome
Agent	Epstein-Barr virus (EBV)	CMV (20–50%) HHV-6, <i>Toxoplasma</i> , <i>Ehrlichia</i> , HIV
Atypical lymphocytosis	Seen	Seen
Clinical symptoms	Fever, myalgia, hepatosplenomegaly, exudative pharyngitis, cervical lymphadenopathy, rashes following ampicillin therapy	Similar presentation, except that exudative pharyngitis, cervical lymphadenopathy are absent
Heterophile antibodies	Elevated (detected by Paul-Bunnell test)	Negative
Specific antibodies	Antibodies to specific EBV antigens are elevated	Antibodies to CMV or other agents may be elevated

EBV-associated Malignancies

EBV is associated with several malignancies such as Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma and non-Hodgkin's lymphoma (discussed in detail in Chapter 80).

Other Conditions Associated with EBV

- ❖ **Lymphoproliferative disorder:** It is seen among immunodeficient patients, e.g. Duncan syndrome which is an X-linked recessive disease affecting young boys
- ❖ **Oral hairy leukoplakia:** Wart-like growth of epithelial cells of the tongue developed in some HIV-infected patients and transplant recipients
- ❖ Chronic fatigue syndrome.

Epidemiology

EBV is worldwide in distribution.

- ❖ **Age:** EBV infections are most common in early childhood, with a second peak during late adolescence. However, infectious mononucleosis is common among young adults of developed countries
- ❖ **Prevalence:** EBV is common in all parts of the world, with >90% of adults being seropositive and develop antibodies to EBV
- ❖ **Transmission:**
 - Intimate and prolonged oral contact is required for effective transmission. EBV is spread by direct contact with oral secretions, e.g. salivary contact during kissing
 - Other modes are blood transfusion and following bone marrow transplantation.
- ❖ **Source:** Asymptomatic seropositive individuals shed the virus in oropharyngeal secretions. Shedding is more in immunocompromised patients.

LABORATORY DIAGNOSIS Epstein-Barr virus infections

- ❑ **Antibody detection:**
 - Nonspecific heterophile antibody detection:
 - ◆ Paul-Bunnell test
 - ◆ Differential absorption test
 - ◆ Monospot test
 - EBV specific antibody detection—ELISA and indirect IF assay detect antibody to viral capsid antigen, EBNA and early antigen.
- ❑ **Molecular methods:**
 - Detects EBV DNA (by PCR)
 - Quantifies EBV DNA (by real-time PCR)—detecting genes BamH1W, EBNA1 and LMP
 - Detects EBER RNA (by RT-PCR).
- ❑ **EBV antigen:** By direct IF assay.

Laboratory Diagnosis

Antibody Detection

Heterophile Agglutination Test (Paul-Bunnell Test)

Paul-Bunnell test: It is a tube agglutination test that uses sheep RBCs to detect heterophile antibodies in patient's serum.

- ❖ **Procedure:** Serial dilutions of inactivated (56°C for 30 minutes) patient's serum are mixed with equal volumes of 1% sheep RBCs, and then the tubes are incubated at 37°C for four hours
- ❖ **Result:** Agglutination titer of >256 is considered as significant
- ❖ **False positive:** Heterophile antibodies are non-specific, may also be present following serum therapy or even in some normal individuals. Therefore, the result needs to be confirmed
- ❖ **Differential absorption test** and Monospot test are available to confirm the result of Paul-Bunnell test.

EBV-specific Antibody Detection

Various formats such as ELISA and indirect immunofluorescence techniques are available to detect specific EBV antibodies. These tests have become more popular and are almost replacing the traditional heterophile antibody tests.

- ❖ **Antibody to viral capsid antigen (VCA):**
 - IgM type: Indicates current infection
 - IgG type: Is a marker of past infection and indicates immunity.
- ❖ **Antibodies to early antigen (EA):** These also indicate current viral infection. They are elevated in patients with Burkitt's lymphoma or nasopharyngeal carcinoma
- ❖ **Antibodies to EBNA** (Epstein-Barr nuclear antigen) reveal past infection, but four fold rise of titer may suggest current infection.

Other Tests

- ❖ **Detection of EBV DNA** (by PCR), **EBER RNA** (EBV encoded small RNAs, by reverse transcriptase PCR), or **EBV antigens** (by direct-IF technique) have been useful for detecting the virus in various malignancies and in infectious mononucleosis
- ❖ **Real-time PCR** quantifying EBV DNA load in blood is extremely useful to monitor the treatment response in patients with lymphoproliferative disease. The various genes targeted are BamH1W, EBNA1 and LMP
- ❖ **Virus isolation:** It is laborious, time-consuming (6–8 weeks) and highly sophisticated, hence not routinely performed.

TREATMENT Epstein-Barr virus infections

- ❑ **Supportive measures** such as analgesics are used in the treatment of infectious mononucleosis
- ❑ **Acyclovir** is useful in the treatment of oral hairy leukoplakia, though relapse is common. It reduces EBV shedding from the oropharynx, but it has no effect on the immortalized B cells, hence, it is not effective for infectious mononucleosis and other malignancies
- ❑ **Antibody to CD20** (rituximab) has been effective in some cases.

Prevention

The isolation of patients with infectious mononucleosis is not needed as temporary contact does not transmit the

infection. No vaccine is currently available. A vaccine trial using EBV glycoprotein was found to be ineffective.

ADENOVIRUS INFECTIONS

Adenoviruses are non-enveloped DNA virus. It has icosahedral symmetry with fiber proteins projecting from each vertex, which gives a unique **space vehicle shaped** appearance (Fig. 68.1).

Clinical Manifestations

Adenoviruses infect and replicate in the epithelial cells of the respiratory tract, eye, gastrointestinal tract, urinary bladder and liver. Though one-third of the serotypes can cause human diseases, **types 1–7** are most common worldwide.

- ❖ **Respiratory diseases** are the most common manifestation of adenoviruses
 - Upper respiratory tract infection in children—mainly caused by serotypes 1,2 and 5. Among adolescents serotypes 3, 4 and 7 cause mild respiratory infections
 - **Pneumonia:** Adenoviruses particularly types 3, 7, and 21 are responsible for about 10–20% of pneumonia in childhood. Serotype 14 is associated with severe pneumonia in healthy young adults
 - Acute respiratory disease syndrome outbreaks among military recruit—are commonly associated with type 4, 7 and occasionally type 3.
- ❖ **Ocular infections** (Chapter 78, for detail):
 - **Pharyngoconjunctival fever:** It tends to occur in outbreaks, at children’s summer camps (also called swimming pool conjunctivitis), and is associated with types 3 and 7
 - **Epidemic keratoconjunctivitis or shipyard eye:** It occurs mainly in adults and is highly contagious, caused by types 8, 19 and 37.

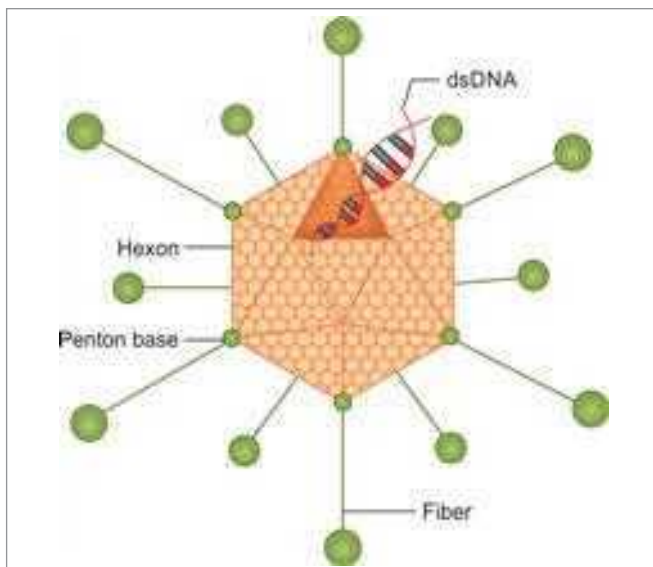


Fig. 68.1: Adenovirus (schematic diagram).

- ❖ **Infantile gastroenteritis:** Serotype 40 and 41 may account for 5–15% of cases of viral gastroenteritis in young children
- ❖ **Acute hemorrhagic cystitis** in children, especially in boys—caused by serotypes 11 and 21
- ❖ **Immunocompromised** patients are at higher risk of developing serious pneumonia
- ❖ **Transplant recipients** may develop pneumonia, hepatitis, nephritis, colitis, encephalitis and hemorrhagic cystitis. Types 34 and 35 are isolated commonly from transplant recipients.

Laboratory Diagnosis

Depending on the manifestations, various specimens such as throat swab, conjunctival swab, stool or urine may be collected

- ❖ **Virus isolation:** Primary human embryonic kidney cell line and A 549 cell line are the most susceptible cell lines
- Viral growth** can be detected by:
 - Characteristic cytopathic effect: Rounding and grape-like clustering of swollen cells
 - Antigen detection by direct-IF test.
- ❖ **Serotyping:** Type specific antigens (viral capsid proteins) can be identified by hemagglutination test (targeting HA antigens) and neutralization test (targeting capsid proteins)
- ❖ **Direct-IF test:** It can be employed to detect adenoviral antigens from clinical samples such as throat or conjunctival secretions by using fluorescent tagged anti-hexon antibody
- ❖ **Fastidious enteric serotypes** such as type 40 and 41 from stool: They can be detected by electron microscopy or by antigen detection by ELISA
- ❖ **Molecular methods:** PCR has been available targeting group-specific conserved hexon or fiber genes. Multiplex PCR followed by sequencing is done for detection of adenovirus types. PCR is rapid and more sensitive than conventional culture
 - Real-time PCR is used to monitor viral load, which is useful for immunocompromised and transplant recipients
 - The BioFire FilmArray respiratory and gastrointestinal panels are available for simultaneous detection of many microbial pathogens including Adenovirus.
- ❖ **Serum antibody detection:** It can be done by ELISA.

Treatment and Control

Symptomatic treatment is given; only in severe cases of pneumonia cidofovir is recommended. General preventive measures are:

- ❖ Effective hand washing
- ❖ Sodium hypochlorite to disinfect environmental surfaces
- ❖ Chlorination of swimming pools and waste water
- ❖ Strict asepsis during eye examinations.

Adenoviruses used for Gene Therapy

Replication defective adenoviruses can also be used as live-virus vectors for the delivery of vaccine antigens and for gene therapy; e.g. trials on adenovirus vectored *M. tuberculosis* (using 85A antigen), HIV and COVID-19 vaccines.

RHINOVIRUS INFECTION (COMMON COLD)

Rhinoviruses are the most common cause of common cold. They belong to Picornaviridae family, which also include enteroviruses (Chapter 73).

- ❖ They use host cell intercellular adhesion molecule-1 (ICAM-1) as receptor
- ❖ More than 100 antigenic types have been identified
- ❖ They are similar to enteroviruses in structure and properties except that:
 - Acid-labile (unstable below pH 6)
 - Transmitted by respiratory route.

- ❖ **Clinical features:** The incubation period is about 2–4 days
 - **Common cold syndrome:** Rhinoviral symptoms are similar to that of any other viruses causing common cold syndrome such as coronaviruses, adenoviruses, enteroviruses, parainfluenza viruses, and influenza viruses
 - The primary disease in adults presents as rhinosinusitis. Usual clinical symptoms include sneezing, nasal obstruction, nasal discharge, and sore throat, but no fever
 - Secondary bacterial infection may produce otitis media, sinusitis, bronchitis, or pneumonitis, especially in children.
- ❖ **Relapse:** The average adult gets 1–2 attacks each year
- ❖ **Laboratory diagnosis:** Rhinoviruses can be grown in human diploid cell lines such as WI-38 and MRC-5 cell lines. Most of the strains grow better at 33°C (nasopharynx temperature) but not at 37°C
- ❖ **Treatment** is supportive (i.e. symptomatic treatment).

EXPECTED QUESTIONS**I. Write short notes on:**

1. Infectious mononucleosis.
2. Adenovirus infections.
3. Rhinovirus infections

II. Multiple Choice Questions (MCQs):

1. Which of the following virus is the agent of infectious mononucleosis?
 - a. Epstein-Barr virus
 - b. Human herpesvirus-6
 - c. Cytomegalovirus
 - d. Varicella-zoster virus
2. Which of the following tumor is not caused by Epstein-Barr virus?
 - a. Post-transplant lymphomas
 - b. Hodgkin's disease
 - c. Burkitt's lymphoma
 - d. Kaposi's sarcoma
3. A 25-year-old female has developed fever, sore throat, and lymphadenopathy accompanied with

atypical lymphocytosis and an increase in sheep cell agglutinins. The diagnosis is most likely:

- a. Hepatitis
 - b. Infectious mononucleosis
 - c. Chickenpox
 - d. HSV infection
4. Which of the following adenovirus serotypes cause epidemic keratoconjunctivitis?
 - a. Serotypes 3 and 7
 - b. Serotypes 8, 19, and 37
 - c. Serotypes 40 and 41
 - d. Serotypes 11 and 21
 5. All of the following are true about rhinovirus, except:
 - a. More than 100 antigenic types have been identified
 - b. Acid-labile
 - c. Incubation period is 10–14 days
 - d. Transmitted by respiratory route

Answers

1. a 2. d 3. b 4. b 5. c

Parasitic and Fungal Infections of Respiratory Tract

CHAPTER 69

CHAPTER PREVIEW

- Parasitic Infections of Respiratory Tract
 - Paragonimiasis
 - Tropical Pulmonary Eosinophilia (TPE)
- Fungal Infections of Respiratory Tract
 - *Pneumocystis* Pneumonia
 - Zygomycosis
- Aspergillosis
- Penicilliosis

PARASITIC INFECTIONS OF RESPIRATORY TRACT

Respiratory tract infections (RTI) can be produced by number of parasitic agents.

- ❖ *Paragonimus westermani*: This human parasite is a primary pathogen of lungs
- ❖ Parasites that pass through lungs during their life cycle: e.g. intestinal nematodes such as *Ascaris*, hookworm, and *Strongyloides*
- ❖ Parasites causing hypersensitivity in lungs: Filarial nematodes causing tropical pulmonary eosinophilia (TPE)
- ❖ Parasites that infect elsewhere, rarely infect lungs: *E. histolytica*, *Toxoplasma*, *Balantidium coli*, *Cryptosporidium parvum* and *Echinococcus granulosus*
- ❖ Parasites of lower animals, that rarely infect man, e.g. *Mammomonogamus*.

PARAGONIMIASIS

Paragonimus westermani is a trematode, also known as **oriental lung fluke**. It causes endemic hemoptysis in man.

Epidemiology

About 10 species of *Paragonimus* infect humans, with a global prevalence of 22 million.

- ❖ **World:** *P. westermani* is the most important species infecting humans, found in the Far East, principally Korea, Japan, Taiwan, China, and Philippines
- ❖ **India:** Paragonimiasis is endemic in Northeast states of India. Many cases are reported from **Manipur** with a prevalence of 6.7%.

Life Cycle

Life cycle of *Paragonimus* is similar to other trematodes, except schistosomes (discussed in Chapter 46, Fig. 46.12)

- ❖ **Morphology:** Similar to other trematodes, they have adult worm (leaf-like), operculated eggs, and larvae (in five stages) (Figs 69.1A and B)



Figs 69.1A and B: *Paragonimus* species: **A.** Adult worm (carminestained); **B.** Egg (in sputum wet mount).

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Hosts:** Lung flukes have three hosts
 - **Definitive host:** Humans are definitive host
 - **Intermediate hosts:** Snails are the first intermediate host, whereas the second intermediate host are crabs or crayfishes.
- ❖ **Transmission:** Human gets infection by ingestion of second intermediate host carrying metacercaria larvae (infective form)
- ❖ The larvae penetrate the intestinal wall to reach peritoneal cavity and then reach the lungs, where they develop into adult worms
- ❖ Adult worms undergo fertilization to produce eggs, which are passed in sputum (commonly) or rarely eggs are swallowed and excreted in feces. Eggs are the diagnostic form.

Pathogenesis and Clinical Manifestations

Pathogenesis is due to multiplication of the adult worm in lungs (most common) or occasionally at other extrapulmonary sites.

Pulmonary Paragonimiasis (Endemic Hemoptysis)

The adult worm initially causes eosinophilic granulomatous inflammation in the lungs and subsequently forms cysts surrounding the worms. The cysts are commonly found in the right lung.

- ❖ Symptoms appear with moderate to heavy infection. The common presenting features are productive cough with brownish blood tinged rusty sputum with an offensive fishy odor
- ❖ Sometimes, frank hemoptysis occurs along with peripheral blood eosinophilia
- ❖ In chronic cases, bronchitis, bronchiectasis, pneumonia or lung abscess may be seen.

Extrapulmonary Paragonimiasis

The worms migrate from the ruptured cysts to various sites such as liver, spleen, abdominal wall and less commonly in brain. Extrapulmonary infections are usually associated with *P. mexicanus*, *P. heterotremus*, *P. skrjabini*, *P. hueitungenensis* and occasionally with *P. westermani*.

- ❖ **Cerebral paragonimiasis:** It is the most severe form of paragonimiasis, discussed in Chapter 75
- ❖ **Cutaneous paragonimiasis** such as migratory subcutaneous nodules and larva migrans, discussed in Chapter 57.

Laboratory Diagnosis

Sputum Microscopy

Early morning, deeply coughed sputum sample is collected for microscopy. The saline mount of sputum sample (particularly blood-tinged flecks) is examined for characteristic operculated eggs. If the egg burden is less, then:

- ❖ Multiple sputum examination (up to seven samples) should be done
- ❖ Sputum can be concentrated by formalin-ether sedimentation technique, but not by floatation method
- ❖ Stool microscopy may be done to detect eggs, especially in children as the collection of sputum is difficult in them.

Eggs of *Paragonimus*

The eggs are oval, operculated and golden-brown in color and measures 80–120 µm × 45–65 µm. They are unembryonated when laid (Fig. 69.1B).

Serological Tests

Serological tests are useful in the early part of the disease, where the microscopy has failed to detect eggs in sputum and stool and also for epidemiological purpose.

- ❖ **Antibody detection:** Various tests available are—
 - **DIGFA:** Serum antibodies to *P. westermani* can be detected by rapid test such as dot-immunogold filtration assay
 - **ELISA** using purified adult excretory-secretory antigen to detect parasite specific IgG or IgE has

shown a high sensitivity, especially with pleural fluid than serum

- **Western blot** test using adult worm homogenate is also highly sensitive and specific.
- ❖ **Antigen detection:** Dot ELISA format has been developed to detect species specific and stage specific antigens by using monoclonal antibodies. Detection of antigens indicates active infection.

Other Tests

- ❖ Peripheral blood eosinophilia
- ❖ **Radiological tests:** MRI and CT scan are preferred to locate the cysts in the CNS or other sites. Even chest X-ray may demonstrate the characteristic pulmonary lesions, including patchy densities, cavities, pleural effusion.

TREATMENT

Paragonimiasis

- ❑ Praziquantel (25 mg/kg/dose, three doses per day for 2 days) is the drug of choice for treatment of paragonimiasis
- ❑ Surgical management may be needed for pulmonary or cerebral lesions.

Prevention

Paragonimiasis can be prevented by: (i) sanitary disposal of sputum, (ii) control of snails, (iii) treatment of cases and (iv) health education.

TROPICAL PULMONARY EOSINOPHILIA (TPE)

It is a distinct syndrome that develops in some infected individuals with *Wuchereria bancrofti* and *Brugia malayi* in endemic places (Chapter 37). It is also called as occult filariasis or Weingarten's syndrome. It differs from classical filariasis in various ways (Table 69.1).

Pathogenesis

It represents a hypersensitivity reaction to microfilaria antigen. Microfilariae are rapidly cleared from the blood stream and filtered, lodged and destroyed in the lungs initiating an allergic response. Hence, microfilariae are not detected in the peripheral blood.

Clinical Features

Common features include nocturnal paroxysmal cough and wheezing weight loss, low-grade fever. Occasionally, microfilariae are entrapped in other organs like spleen, liver and lymph node leading to hepatosplenomegaly and lymphadenopathy. This is sometimes called as **Meyers Kouwenaar Syndrome**.

Epidemiology

The majority of cases have been reported from India, Pakistan, Sri Lanka, Brazil, Guyana, and Southeast Asia. Males are affected more than females (4:1), mainly in the third decade of life.

Table 69.1: Differences between classical and occult filariasis.

Characters	Classical filariasis	Occult filariasis
Probable etiology	Inflammatory changes to adult worm	Hypersensitivity reaction to microfilaria antigen
Diagnostic form	Microfilaria in blood and in fluid	Microfilaria absent in blood
Organs affected	Lymph nodes and lymphatic vessels	Lungs, liver and spleen
Pathology	Lymphangitis and lymphadenitis	Eosinophilic granuloma
Serology	Antibody detection is not diagnostic	IgE antibody detection is diagnostic

Laboratory Diagnosis

Occult filariasis is diagnosed by:

- ❖ Blood eosinophilia (absolute eosinophil count more than 3000/ μ L)
- ❖ **Chest X-ray:** Shows diffuse infiltration
- ❖ Elevated serum IgE levels
- ❖ Pulmonary function test shows obstructive changes in lungs.

TREATMENT

Occult filariasis

It responds well to diethylcarbamazine (DEC), 4–6 mg/kg for 14 days. Relapse may occur in 12–25% of cases.

MIGRATORY STAGE OF INTESTINAL NEMATODES

Intestinal nematodes such as *Ascaris*, hookworm and *Strongyloides* pass through the lungs during their life cycle (Refer Chapter 46).

In Ascariasis

Pulmonary symptoms are observed in the second week after ingestion of eggs. The eggs hatch out to liberate the larvae in the duodenum, which molt to develop L3 larvae; later penetrate the intestine, reach right side of heart via portal circulation and finally enter the lungs via pulmonary capillaries.

- ❖ Migrating larvae in lungs provoke an immune-mediated hypersensitivity response. Common symptoms include a non-productive cough, chest discomfort and fever
- ❖ **Loeffler's syndrome:** In severe cases, patients develop dyspnea and a transient patchy infiltrates seen on chest X-ray along with peripheral eosinophilia. This stage is called as eosinophilic pneumonia (Loeffler's syndrome)
- ❖ **Diagnosis:** Eosinophilia is prominent during the early lung stage, but disappears later. This should be differentiated from that of *Toxocara larva migrans*; where the pulmonary infection is long-term with persistent eosinophilia
 - Presence of Charcot-Leyden crystals in sputum and stool, a nonspecific finding

- Larvae can be found in sputum or gastric aspirates. However, eggs are characteristically absent in stool examination (as the life cycle would not have proceeded to that stage).

In Hookworm Infection

Migrating larva through the lungs occasionally cause mild transient pneumonitis, asthma and bronchitis; but the severity and frequency of lung manifestation is less compared to ascariasis.

In Strongyloidiasis

Pulmonary symptoms are uncommon compared to ascariasis and hookworm. It occurs only secondary to underlying chronic obstructive lung disease.

RARE PARASITIC PULMONARY INFECTIONS

- ❖ **Pulmonary amoebiasis:** Lungs is the second most common site of extraintestinal amoebiasis (next to liver) by *E. histolytica*
- ❖ **Pulmonary toxoplasmosis:** In patients with HIV, *Toxoplasma* occasionally cause pulmonary infections. It mainly causes encephalitis (Chapter 75) and congenital infection (Chapter 79)
- ❖ **Pulmonary balantidiasis:** It mainly causes intestinal disease (Chapter 45)
 - In immunocompromised and malnourished people trophozoites of *Balantidium coli* may perforate the large intestine and produce extraintestinal manifestations like pneumonia
 - Trophozoites may be demonstrated in broncho-alveolar lavage, but should be differentiated from ciliated epithelial cells; which are smaller in size (<30 μ m) with fewer cilia than that of *B. coli*.
- ❖ **Respiratory cryptosporidiosis** may occur occasionally in HIV-infected individuals (Chapter 45)
- ❖ **Pulmonary hydatidosis:** This is the second most common form of hydatid disease (next to liver), occurs in 20–30% of cases infected with *Echinococcus granulosus*. However, lung involvement in alveolar hydatid disease is rare (1%) (Chapter 49)
- ❖ **Parasites of lower animals** that occasionally infect man include:
 - *Mammomonogamus:* Infects larynx and trachea, producing chronic cough and hemoptysis
 - *Capillaria aerophila:* Infects trachea and bronchus, causing tracheobronchitis
 - *Ascaris suum:* Infects lungs and intestine.

FUNGAL INFECTIONS OF RESPIRATORY TRACT

Respiratory tract infections (RTI) can be produced by number of fungi.

- ❖ **Opportunistic fungal agents:** They are major fungal agents that cause respiratory infections
 - *Pneumocystis jirovecii* pneumonia
 - Zygomycoses
 - Aspergillosis
 - Penicilliosis.
- ❖ **Fungi causing systemic mycoses:** They involve multiple organs. They are saprophytic fungi present in the environment. Human infection occurs by inhalation of spores leading to pulmonary infection. From lungs, they disseminate to cause various systemic manifestations (discussed in detail in Chapter 38). There are four agents of systemic mycoses, all are dimorphic fungi
 - *Blastomyces dermatitidis*
 - *Histoplasma capsulatum*
 - *Paracoccidioides brasiliensis*
 - *Coccidioides immitis*.
- ❖ **Yeast:** *Cryptococcus neoformans* (Chapter 75).
Note: Isolation of *Candida* species in sputum culture is a common finding; but it represents colonization. It is almost never indicative of underlying pulmonary candidiasis and therefore does not warrant antifungal treatment.

PNEUMOCYSTIS PNEUMONIA

Pneumocystis pneumonia (PcP) has been increasingly reported after the discovery of HIV/AIDS.

Taxonomy

Recently, the taxonomy of *Pneumocystis* has been changed (2002). Once thought to be a protozoan, now it is classified under fungus based on nucleic acid sequence studies.

- ❖ Taxonomists renamed the human species of *Pneumocystis* as *Pneumocystis jirovecii*
- ❖ The previously used species name *P. carinii* has been assigned to describe the rat species of *Pneumocystis*.

Pathogenesis

Like protozoa, *Pneumocystis* exists in cyst and trophozoite forms. The cysts are found in the environment, whereas in human tissues both cysts and trophozoites (containing 4–8 sporozoites) are found.

Once inhaled, the cysts are carried to the lungs where they transform into the trophozoite stage. The trophozoites induce an inflammatory response, that leads to recruitment of plasma cells resulting in formation of **frothy exudate** filling the alveoli. Hence, this condition is also called **plasma cell pneumonia**.

Laboratory Diagnosis

Histopathological examination of lung tissue or fluids obtained by bronchoscopy, bronchoalveolar lavage (BAL), or open lung biopsy reveals **cysts**.

- ❖ Gomori's methenamine silver (GMS) staining is the method of choice to demonstrate the cysts of *P. jirovecii*. The cysts resemble black colored **crushed**

ping-pong balls, against the green background (Fig. 69.2A)

- ❖ *Pneumocystis* is not cultivable and there is no serological test available
- ❖ PCR assay has been developed for detection of *P. jirovecii* specific genes
- ❖ Detection of 1, 3 β -D-glucan in serum
- ❖ **Radiology:** Chest X-ray depicts classical finding of bilateral diffuse infiltrates. CT of the lung may reveal **ground-glass opacities** at the early stage (Chapter 59, Fig. 59.4). However various atypical manifestations (nodular densities, cavitory lesions) have also been reported (Fig. 69.2B).

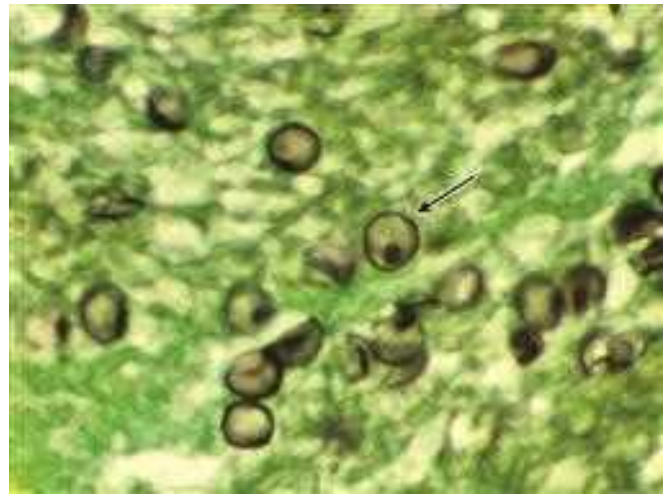


Fig. 69.2A: Cysts of *Pneumocystis jirovecii* in lung tissue of an AIDS patient (methenamine silver stain) (arrow showing).

Source: Public Health Image Library/Dr Edwin P Ewing, Jr. ID#: 960/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Fig. 69.2B: CT scan of lungs suggestive of *Pneumocystis* pneumonia (soft tissue density nodule with reverse halo sign in the right upper lobe).

Source: Dr Sunitha V. Department of Radiology, JIPMER, Puducherry (with permission).

TREATMENT

Pneumocystis pneumonia

Cotrimoxazole (trimethoprim/sulfamethoxazole) is the drug of choice for *Pneumocystis pneumonia*.

- It is given for 14 days in non-HIV patients and 21 days in patients with HIV.
- It is also the recommended drug for primary and secondary prophylaxis in patients with HIV (Chapter 33).

ZYGOMYCOSIS

Zygomycosis represents group of life-threatening infections caused by aseptate fungi belonging to the phylum Zygomycota. Agents of zygomycosis fall into two orders:

1. Order mucorales: Causes mucormycosis
 - *Rhizopus* (*R. arrhizus* and *R. microsporus*)
 - *Mucor racemosus*
 - *Rhizomucor pusillus*
 - *Lichtheimia corymbifera* (previously called as *Absidia corymbifera*)
 - *Apophysomyces elegans*.
2. Order entomophthorales: Causes entomophthoromycosis
 - *Basidiobolus ranarum*
 - *Conidiobolus coronatus*.

Mucormycosis

Pathogenesis

Spores of fungi causing mucormycosis are found ubiquitously in the environment. Transmission occurs via inhalation, inoculation or rarely ingestion of spores. Spores develop into mycelial form containing wide aseptate hyphae which are angioinvasive in nature resulting in spread of infection.

Predisposing factors: Agents of mucormycosis require iron as growth factor. Hence conditions with increased iron load are at higher risk of developing invasive mucormycosis.

- ❖ Diabetic ketoacidosis (DKA) is the most important risk factor. Acidosis causes release of iron from the sequestered proteins in serum
- ❖ End-stage renal disease
- ❖ Patients taking iron therapy or deferoxamine (iron chelator)
- ❖ Defects in phagocytic functions (e.g. neutropenia or steroid therapy).

Clinical Manifestations

Agents of mucormycosis are angioinvasive in nature. Mucormycosis has six types of clinical presentations.

1. **Rhinocerebral mucormycosis:** It occurs commonly in patients with diabetic ketoacidosis. It is the most common form; starts as eye and facial pain, may progress to cause orbital cellulitis, proptosis and vision loss (Figs 78.11A and B)

2. **Pulmonary mucormycosis** is the second most common form, occurs in patients with leukemia. Symptoms include dyspnea, cough, chest pain and variable fever
 - **Angioinvasion** can lead to necrosis, cavitation, and/or hemoptysis
 - **Chest-X ray:** May reveal lobar consolidation, isolated masses, nodular disease, cavities, or wedge-shaped infarcts
 - **Differential diagnosis:** Mucormycosis needs to be differentiated from pulmonary aspergillosis as quickly as possible, as treatment for both these infections differ. The presence of ≥ 10 pulmonary nodules, pleural effusion, or concomitant sinusitis favors the diagnosis towards mucormycosis.
3. Cutaneous mucormycosis (Chapter 58)
4. Gastrointestinal mucormycosis such as necrotizing enterocolitis; seen commonly in premature neonates
5. Disseminated mucormycosis: Brain is the most common site of dissemination, but can affect any organ
6. Miscellaneous forms: Any body site may be randomly affected such as bones, trachea and kidneys, etc.

Laboratory Diagnosis

- ❖ **Histopathological staining** such as H&E or methenamine silver stain of tissue biopsies shows broad aseptate hyaline hyphae with wide angle branching (Fig. 69.3)
- ❖ **Culture on SDA at 25°C:** Reveals characteristic white cottony woolly colonies with tube filling growth (hence called **lid lifters**) (Figs 69.4A and B). In some species, e.g. *Rhizopus* the colonies become brown black later, due to sporulation giving rise to **salt and pepper** appearance (Fig. 69.4A)
- ❖ **Microscopic appearance:** LPCB mount of the colonies reveals broad aseptate hyaline hyphae, from which

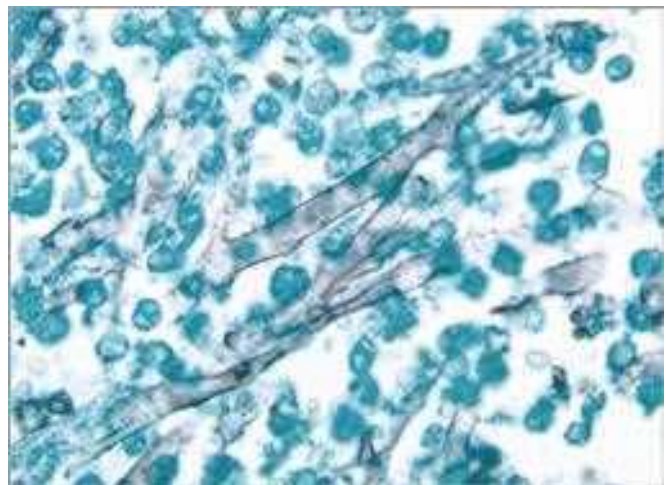
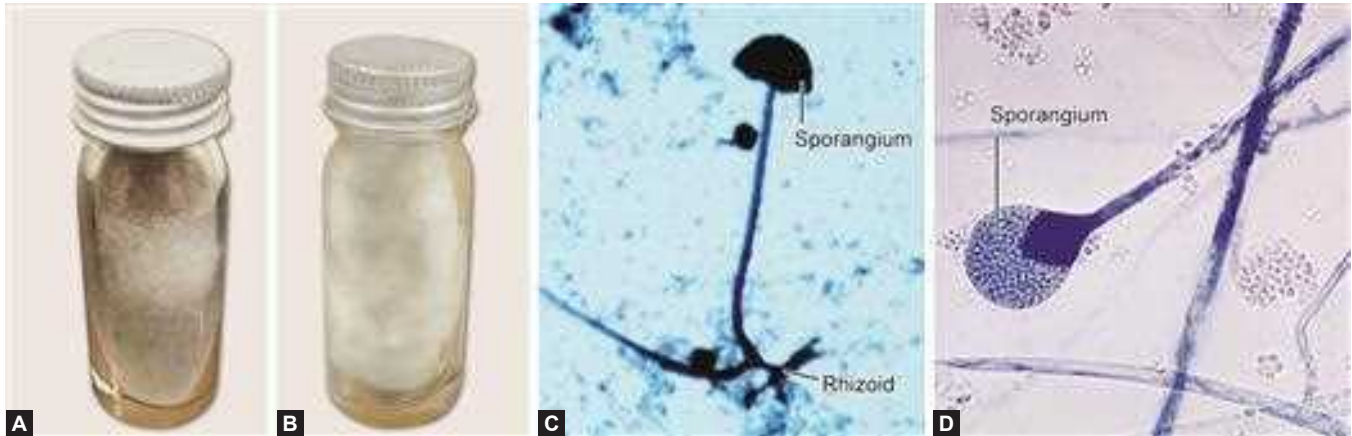


Fig. 69.3: Zygomycosis—histopathology of tissue section shows aseptate broad hyphae (Methenamine silver stain).

Source: Public Health Image Library/Dr Libero Ajello, ID#:4234/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Figs 69.4A to D: **A.** *Rhizopus* colony on SDA shows white cottony woolly colonies with black spores (salt and pepper appearance); **B.** *Mucor* on SDA—white cottony woolly colonies; **C.** LPCB mount of colonies of *Rhizopus* shows sporangium with rhizoid present; **D.** LPCB mount of colonies of *Mucor* shows sporangium (absence of rhizoid).

Source: A to C. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; D. Public Health Image Library/Dr Lucille K Georg, ID#:3960/ Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

sporangiophore arises and then ending at sporangium which contains numerous sporangiospores (Figs 69.4C, D and 69.5A to C)

- ❖ **Rhizoid:** Some species bear a unique root like growth arising from hyphae called rhizoid which provides initial clue for identification of the fungus. Species can be differentiated depending on the position of the rhizoids with respect to sporangiophore (Figs 69.5A to C)
 - *Rhizopus* bears nodal rhizoids
 - *Lichtheimia* bears internodal rhizoids
 - *Mucor*: rhizoids absent.

TREATMENT

Zygomycosis

Amphotericin B deoxycholate remains the drug of choice for all forms of mucormycosis. Posaconazole or isavuconazole can be given alternatively. For mild localized skin lesions in immunocompetent patients, which can be removed surgically.

Entomophthoromycosis

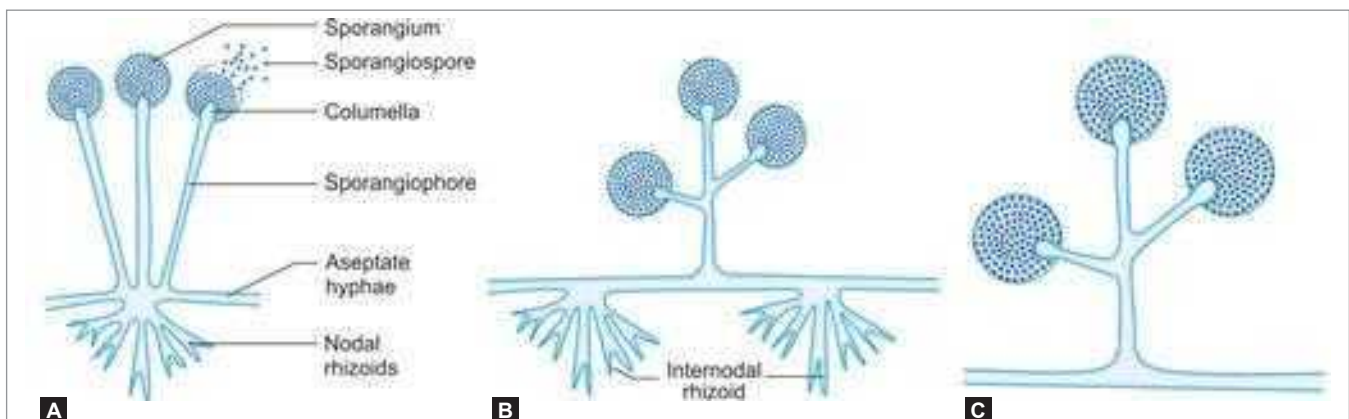
This includes the subcutaneous lesions produced by members of the order Entomophthorales, i.e. *Conidiobolus* and *Basidiobolus*; the latter is also associated with visceral involvement.

ASPERGILLOSIS

Aspergillosis refers to the invasive and allergic diseases caused by a hyaline mold named *Aspergillus*. There are nearly 35 pathogenic and allergenic species of *Aspergillus*, important ones being—*A. fumigatus*, *A. flavus* and *A. niger*.

Pathogenesis

Aspergillus species are widely distributed in nature, most commonly growing on decaying plants, producing chains of conidia. Transmission occurs by inhalation of airborne conidia.



Figs 69.5A to C: Microscopic schematic diagram: **A.** *Rhizopus*; **B.** *Lichtheimia*; **C.** *Mucor*.

Risk factors for invasive aspergillosis are:

- ❖ Glucocorticoid use (the most important risk factor)
- ❖ Profound neutropenia
- ❖ Neutrophil dysfunction
- ❖ Underlying pneumonia, chronic obstructive pulmonary disease, tuberculosis or sarcoidosis
- ❖ Anti-tumor necrosis factor therapy.

Clinical Manifestations

The incubation period varies from 2 to 90 days. Depending upon the site of involvement, *Aspergillus* produces various clinical manifestations such as:

- ❖ **Pulmonary aspergillosis:** It is the most common form of aspergillosis; includes various manifestations such as:
 - Allergic bronchopulmonary aspergillosis (ABPA)
 - Severe bronchial asthma
 - Extrinsic allergic alveolitis
 - Aspergilloma (fungal ball)
 - Acute angioinvasive pulmonary aspergillosis
 - Chronic cavitory pulmonary aspergillosis.
- ❖ **Other forms of aspergillosis include:**
 - Invasive sinusitis of various types may occur.
 - ◆ Invasive sinusitis (acute and chronic form)
 - ◆ Chronic granulomatous sinusitis
 - ◆ Maxillary fungal ball
 - ◆ Allergic fungal sinusitis.
 - Cardiac aspergillosis: Endocarditis (native or prosthetic) and pericarditis
 - Cerebral aspergillosis: Brain abscess, hemorrhagic infarction, and meningitis
 - Ocular aspergillosis: Keratitis and endophthalmitis
 - Ear infection: Otitis externa
 - Cutaneous aspergillosis: Described in detail in Chapter 58
 - Nail bed infection: Onychomycosis
 - Mycotoxicosis: Various *Aspergillus* species produce several fungal toxins; e.g. *A. flavus* produces aflatoxin, which causes liver carcinoma (see Chapter 40).

Clinical manifestations also depend on the species involved:

- ❖ *A. fumigatus* accounts for most of the cases of acute pulmonary and allergic aspergillosis
- ❖ *A. flavus* is more common in hospitals and causes more sinus, skin and ocular infections than *A. fumigatus*
- ❖ *A. niger* can cause invasive infection but more commonly colonizes the respiratory tract and causes otitis externa.

Laboratory Diagnosis

Specimens such as sputum and tissue biopsies may be collected.

Direct Examination

KOH (10%) mount or histopathological staining (Fig. 69.6) of specimens reveals characteristic narrow septate hyphae with acute angle branching.



Fig. 69.6: Hematoxylin-eosin stained (H and E) lung section shows septate narrow hyphae with acute angle branching—confirms invasive aspergillosis.

Source: Public Health Image Library/Armed Forces Institute of Pathology; Dr Hardin, ID#:15630/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Culture

Specimens are inoculated onto SDA and incubated at 25°C. Species identification is done based on macroscopic and microscopic (LPCB mount) appearance of the colonies (Table 69.2).

- ❖ Colonies consist of hyaline septate hyphae from which conidiophores arise, which end at vesicles. Vesicles are either tubular or globular in shape
- ❖ From the vesicle, finger-like projections of conidia producing cells arise called **phialides** or **sterigmata**. Phialides are arranged either in one or two rows, the first row is called **metulae**
- ❖ Conidia arise from the vesicles either on their entire surface or only on the upper half (Figs 69.7A to C).

Table 69.2: Identification features of *Aspergillus* species.

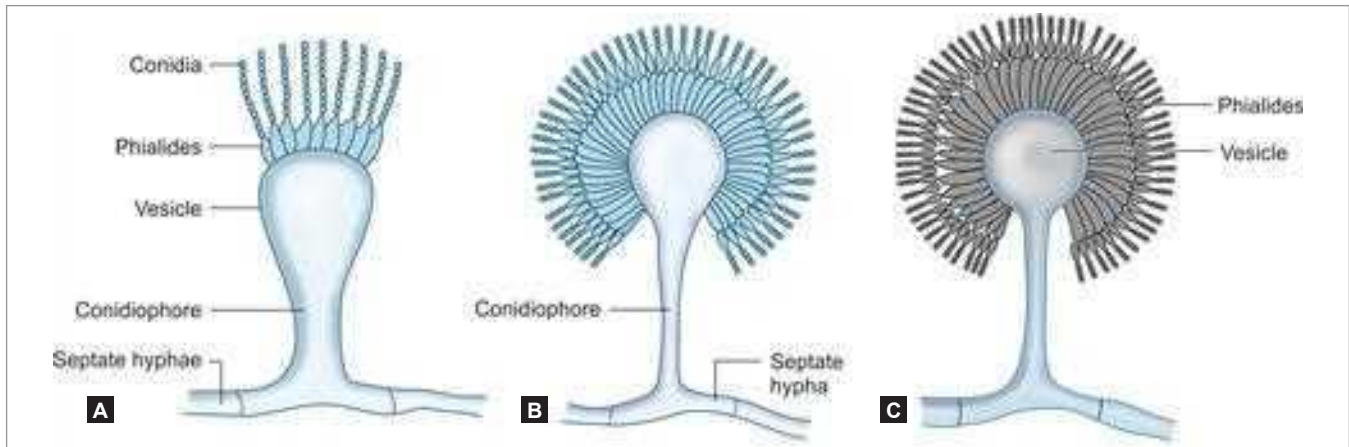
<i>Aspergillus</i> species	Macroscopic appearance of colony	Microscopic appearance of colony (LPCB mount)
<i>A. fumigatus</i> (Figs 69.7A, 69.8A, 69.9A)	Colonies—smoky green, velvety to powdery, reverse is white	<ul style="list-style-type: none"> • Vesicle is conical-shaped • Phialides are arranged in single row • Conidia arise from upper third of vesicle • Conidia are hyaline
<i>A. flavus</i> (Figs 69.7B, 69.8B, 69.9B)	Colonies—yellow green, velvety, reverse is white	<ul style="list-style-type: none"> • Vesicle is globular-shaped • Phialides in one or two rows • Conidia arise from upper two-third to entire vesicle • Conidia are hyaline
<i>A. niger</i> (Figs 69.7C, 69.8C, 69.9C)	Colonies—black, cottony type, reverse is white	<ul style="list-style-type: none"> • Vesicle is globular-shaped • Phialides in two rows • Conidia arise from entire vesicle • Conidia are black in color

Antigen Detection

- ❖ **β-d-Glucan antigen assay:** It is a marker of invasive fungal infections, raised in most invasive fungal infection including invasive aspergillosis
- ❖ **Galactomannan antigen:** This is an *Aspergillus* specific antigen, can be detected by ELISA in patient's sera or urine. It is useful for establishing early diagnosis.

Antibody Detection

- ❖ Detection of serum antibodies is very useful for chronic invasive aspergillosis and aspergilloma, where the culture is usually negative. Titer falls rapidly following clinical improvement
- ❖ In allergic syndromes such as ABPA and severe asthma, specific serum IgE levels are elevated.

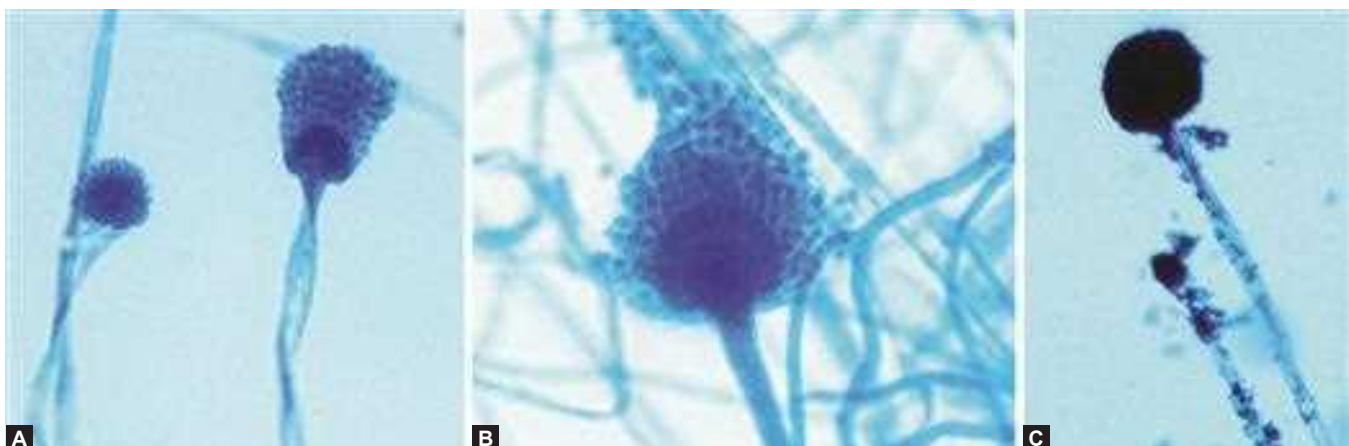


Figs 69.7A to C: Conidiation of various *Aspergillus* species: **A.** *A. fumigatus*; **B.** *A. flavus*; **C.** *A. niger*.



Figs 69.8A to C: *Aspergillus* (colonies on SDA): **A.** *Aspergillus fumigatus*; **B.** *Aspergillus flavus*; **C.** *Aspergillus niger*.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).



Figs 69.9A to C: *Aspergillus* microscopic picture (LPCB mount): **A.** *Aspergillus fumigatus*; **B.** *Aspergillus flavus*; **C.** *Aspergillus niger*.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Skin Test

Positive skin test to various antigen extracts of *Aspergillus* indicates hypersensitivity response and is usually positive for various allergic type of aspergillosis.

TREATMENT

Aspergillosis

Following are the first line treatment recommended in different forms of aspergillosis.

- ❑ For invasive aspergillosis—voriconazole is the drug of choice
- ❑ For ABPA—itraconazole is the drug of choice
- ❑ For single aspergilloma—surgery is indicated
- ❑ For chronic pulmonary aspergillosis—itraconazole or voriconazole is the drug of choice
- ❑ For prophylaxis, posaconazole is indicated.

PENICILLIOSIS

Penicilliosis denotes the group of infections caused by pathogenic *Penicillium* species.

Clinical Significance

Penicillium has more than 250 species, most are found as saprophytes in the environment. However, some species are associated with human diseases such as:

- ❖ *Penicillium marneffeii*: It is a dimorphic fungus, produces wart like skin lesions, described in Chapter 58
- ❖ Mycotoxicoses is caused by toxins released by certain species of *Penicillium* such as *P. cyclospium*, *P. verrucosum* and *P. puberulum* (Table 40.3, Chapter 40)

❖ Other *Penicillium* species are usually found in environment and are isolated as common laboratory contaminants Rarely they are associated with human diseases such as:

- Invasive penicilliosis: For example, endophthalmitis and endocarditis
- Superficial disease: Such as otomycosis, keratitis and onychomycosis
- Allergic disease: For example, asthma and allergic pneumonitis.

Laboratory Diagnosis

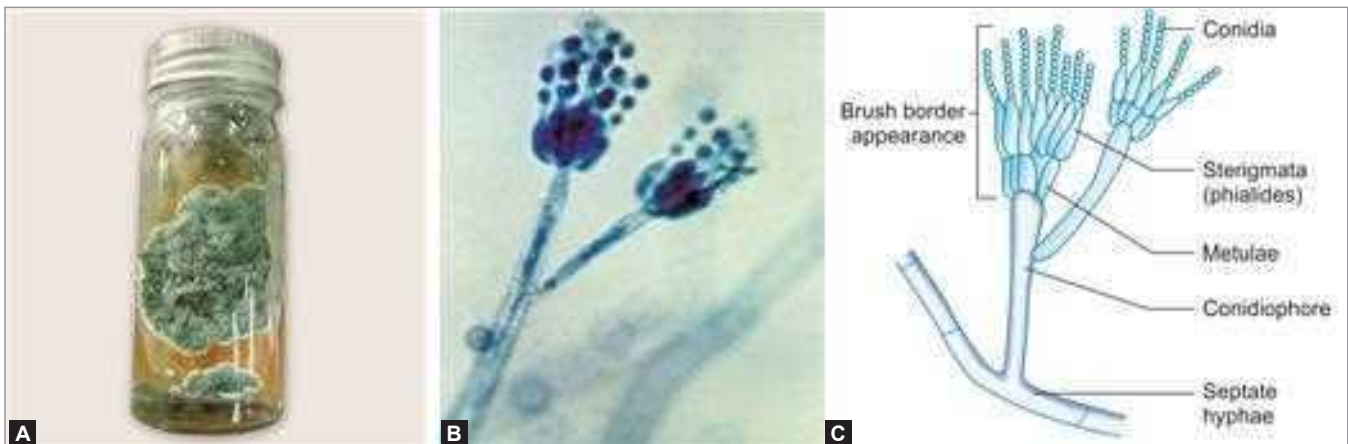
Except for *P. marneffeii* which is a dimorphic fungus, all other *Penicillium* species occur only as molds, can grow easily on SDA at 25°C.

- ❖ **Colonies** are rapidly growing, flat with velvety to powdery texture and greenish in color (Fig. 69.10A)
- ❖ **Microscopic appearance:** LPCB mount of the colonies reveals hyaline thin septate hyphae, and the vesicles are absent. The conidiophore and its branches give rise to elongated metulae, from which flask-shaped phialides originate which bear chain of conidia. Such an arrangement is called as **brush border appearance** (Figs 69.10B and C).

PULMONARY CRYPTOCOCCOSIS

Cryptococcus neoformans is a capsulated yeast that causes meningitis in HIV-infected individuals (Chapter 75).

- ❖ Infection is acquired by inhalational route
- ❖ In immunocompetent individuals, the lungs exhibit defense mechanisms which usually limit the infection
- ❖ However, in people with low immunity, pulmonary infection occurs first, followed by dissemination through blood to distant sites such as CNS.



Figs 69.10A to C: *Penicillium* species: **A.** Colonies on SDA; **B.** Microscopic picture (LPCB mount); **C.** Schematic microscopic picture.

Source: A. Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry; B. Public Health Image Library/Lucille Georg, ID#:8398/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

EXPECTED QUESTIONS

I. Write short notes on:

1. Paragonimiasis.
2. Mucormycosis.
3. *Pneumocystis pneumonia* (PcP).
4. Aspergillosis.

II. Multiple Choice Question (MCQ):

1. The drug of choice for *Pneumocystis pneumonia* is:
 - a. Amphotericin B
 - b. Flucytosine
 - c. Cotrimoxazole
 - d. Voriconazole

Answer

1. c

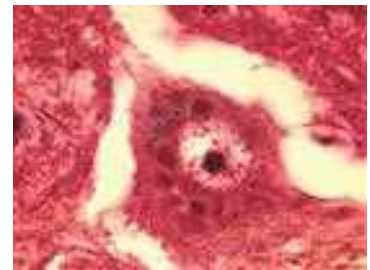
Central Nervous System Infections

SECTION

9

SECTION OUTLINE

70. Infective Syndromes of Central Nervous System
71. Bacterial Meningitis
 - Acute Bacterial (Pyogenic) Meningitis: *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Haemophilus influenzae* and *Listeria*
 - Chronic Bacterial Meningitis: Tubercular Meningitis, Spirochetal Meningitis, Lyme disease and Others
72. Tetanus
73. Viral Meningitis and Myelitis: Poliomyelitis, Coxsackie, Mumps, and Others
74. Viral Encephalitis and Encephalopathy: Rabies, HSV Encephalitis, Arboviral Encephalitis (Japanese Encephalitis and West Nile), Nipah and Hendra, Slow Virus and Prion Disease, and Others
75. Parasitic and Fungal Infections of Central Nervous System
 - Parasitic Infections: Neurocysticercosis, Free-living Amoeba Infections, Toxoplasmosis and Others
 - Fungal Infections: Cryptococcal Meningitis and Others



Rabies is a major public health problem



Fatal once symptoms appear



One death every 15 min worldwide



99% human cases result from **dog bites**



4 out of 10 deaths are in children



Rabies is 100% vaccine preventable

VACCINATE



TO SAVE LIVES

VACCINATE



TO STOP TRANSMISSIONS

No bites = No rabies



Zero by 30

28 September - World Rabies Day

Infective Syndromes of Central Nervous System

CHAPTER 70

CHAPTER PREVIEW

- Meningitis
- Encephalitis and Encephalopathy
- Space-occupying Lesions

Infections of the central nervous system (CNS) account for significant morbidity and mortality. Most of these infections present as a medical emergency; need prompt treatment.

The CNS consists of the brain and spinal cord; covered by three layers of meninges—dura mater (outermost), arachnoid and pia mater (the latter two are collectively called as leptomeninges). Between and around the meninges are spaces that include the epidural, subdural, and subarachnoid spaces (Fig. 70.1).

INFECTIVE SYNDROMES OF CNS

The various infective syndromes of CNS are meningitis, encephalitis and encephalopathy, space occupying lesions and others (Table 70.1).

- ❖ **Bacterial agents:** The bacterial agents of CNS mainly cause meningitis (Chapter 71), also cause other CNS infections such as brain abscess or shunt infections (discussed in this chapter) and neurotoxin mediated disease, tetanus (Chapter 72)

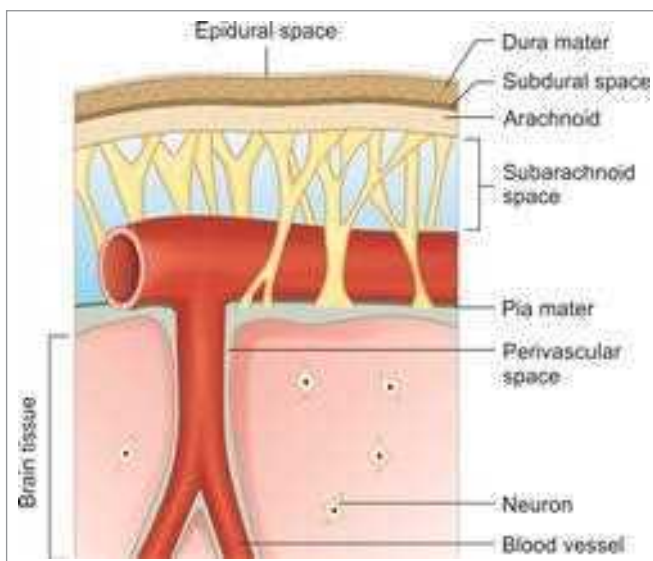


Fig. 70.1: Anatomy of central nervous system.

- ❖ **Viral agents:** They predominantly cause either meningitis (Chapter 73) or encephalitis (Chapter 74)
- ❖ **Parasitic agents:** Neuroparasites most often cause space-occupying lesions, encephalitis or rarely meningitis; discussed in Chapter 75
- ❖ **Fungal agents of CNS:** They cause mainly meningitis, discussed in Chapter 75.

Routes of Infection

The organisms may gain access to the CNS by several routes.

- ❖ **Hematogenous spread:** This is the most common route, where entry into the subarachnoid space is gained through the choroid plexus or through other blood vessels of the brain
- ❖ **Direct spread** from an infected site present close to meninges—otitis media, mastoiditis, sinusitis, etc.

Table 70.1: Infective syndromes of CNS.

Meningitis: It can be classified into—

- **Acute bacterial meningitis:** Caused by pneumococcus, meningococcus, *H. influenzae*, *Listeria*, *Streptococcus agalactiae*, gram-negative bacilli such as *E. coli*
- **Acute viral meningitis:** Mainly caused by enteroviruses, herpesviruses and others
- **Chronic meningitis:** Caused by bacterial agents (e.g. *M. tuberculosis*), viral, parasitic and fungal agents (e.g. *Cryptococcus*)

Encephalitis and encephalopathy

- **Encephalitis:** Viral agents like herpes, rabies, arboviruses (e.g. Japanese encephalitis), parasites such as *Toxoplasma*
- **Encephalopathy** of infectious etiology such as slow virus infections, cerebral malaria, etc.

Space-occupying lesions: Include

- **Focal CNS lesions:** such as brain abscess, subdural empyema, and epidural abscess
- **Cystic parasitic diseases:** such as neurocysticercosis, cystic echinococcosis, etc.

Other infective syndromes of CNS

- Suppurative intracranial thrombophlebitis
- CSF shunt infections
- Myelitis (inflammation of spinal cord): e.g. poliomyelitis
- Neurotoxin mediated: Tetanus and botulism

- ❖ **Anatomical defect in CNS:** It may occur as a result of surgery, trauma, congenital defects which can allow organisms for ready and easy access to CNS
- ❖ **Direct intraneural spread** along the nerve: This is the least common route, occurs in cases of rabies virus or herpes simplex virus (HSV) infection.

Meningitis

Meningitis is an inflammation of the leptomeninges (arachnoid and pia mater) surrounding the brain and spinal cord, with involvement of the subarachnoid space.

Types of Meningitis

Based on the onset, meningitis can be classified into:

- ❖ **Acute meningitis:** Presents as an acute fulminant illness that progresses rapidly in a few hours. It can be further grouped into acute bacterial (or pyogenic) and acute viral meningitis
- ❖ **Chronic meningitis:** Progressively worsens over weeks (>4 weeks). This includes bacterial, parasitic, fungal agents and viral agents (Table 70.2). Some of the agents of chronic meningitis have a **subacute course** that progressively worsens over several days.

The host inflammatory response in meningitis may vary—acute bacterial meningitis is characterized by elevated neutrophil count in CSF, whereas both acute viral meningitis and chronic meningitis are predominantly lymphocytic; although exceptions to this general rule do exist.

Acute Bacterial (or Pyogenic) Meningitis (Chapter 71)

Acute bacterial meningitis is characterized by elevated polymorphonuclear cells (i.e. neutrophils) in CSF (hence called as pyogenic meningitis); except for *Listeria*, where CSF is predominantly lymphocytic. According to the age, the agents involved may vary.

- ❖ Overall *Streptococcus pneumoniae* is the most common cause of pyogenic meningitis
- ❖ Others agents include meningococcus, Group B *Streptococcus*, *Haemophilus influenzae*, *Listeria monocytogenes* and occasionally gram-negative bacilli such as *Escherichia coli*.

Acute Viral Meningitis (Chapter 73)

Acute viral meningitis is caused by a number of viruses, among which enteroviruses account for the majority of cases (>85%). Others include herpesviruses, arboviruses (encephalitis group), HIV and rarely lymphocytic choriomeningitis (LCM) virus, mumps virus.

- ❖ The CSF is predominantly lymphocytic
- ❖ Although they usually develop meningitis in few days after the infection; many of these viruses progress slower and can also occasionally cause chronic meningitis.

Chronic Meningitis

Chronic meningitis is defined as persistence of meningitis exists for >4 weeks; associated with a persistent

inflammatory response in CSF (white blood cell count >5/ μ L)

- ❖ **Etiology:** Although infections (bacterial, viral, parasitic or fungal) account for a significant proportion of cases (Table 70.2), chronic meningitis may also be caused by non-infectious causes such as malignancy, autoimmune diseases, and chemical meningitis
- ❖ **Clinical forms:** Chronic meningitis occurs in two clinical forms
 - *Chronic persistent meningitis:* In most of the cases, the symptoms are chronic and persistent
 - *Chronic recurrent meningitis:* Characterized by discrete episodes of illness along with the absence of symptoms with normal CSF parameters between episodes. It is particularly seen in Mollaret's meningitis, caused by HSV type 2.
- ❖ **CSF findings:** In chronic meningitis, the CSF is predominantly lymphocytic; although elevated neutrophil in CSF is observed in some of these infections.

Note: Aseptic meningitis: The term 'aseptic meningitis' was traditionally used to describe those meningitis, where the infectious etiology is unidentified (aseptic meaning

Table 70.2: Agents of chronic meningitis.

Bacterial agents (Chapter 71)

Common bacterial agents

- Partially treated suppurative meningitis
- Parameningeal infections (e.g. otitis media)
- *Mycobacterium tuberculosis*
- *Borrelia burgdorferi* (Lyme disease)
- *Treponema pallidum* (secondary, tertiary syphilis)

Rare bacterial agents: *Nocardia*, *Actinomyces*, *Tropheryma whipplei*, *Leptospira*, *Brucella*

Viral agents

Some of the agents of acute viral meningitis may also present as chronic meningitis

Examples: Mumps, echoviruses, herpesviruses, HIV and lymphocytic choriomeningitis virus

Parasitic agents

- *Toxoplasma gondii*
- Free-living amoebae
- *Trypanosoma brucei* (African sleeping sickness)
- *Angiostrongylus cantonensis* (Eosinophilic meningitis)
- *Gnathostoma spinigerum*
- *Baylisascaris procyonis*
- Cysticercosis, schistosomiasis, echinococcal disease
- *Toxocara canis* and *Trichinella*

Fungal agents

- *Cryptococcus neoformans*
- *Candida albicans*
- *Blastomyces dermatitidis*
- *Histoplasma capsulatum*
- *Coccidioides immitis*
- *Aspergillus* species
- *Sporothrix schenckii*
- *Pseudallescheria boydii*
- *Cladophialophora bantiana*

Note: Some of these agents may present as a subacute form of meningitis, that progresses over several days to <4 weeks.

lack of infection). This used to include agents other than acute bacterial meningitis; such as the agents of acute viral meningitis and chronic meningitis. However in the modern era of sophisticated diagnostic facility (e.g. molecular methods), it is now possible to detect most of these agents. Therefore, the term ‘aseptic meningitis’ is no longer correct, although in clinical practice it may still be used widely by the clinicians.

Clinical Manifestations

Patients with meningitis present with high-grade fever, vomiting, headache, neck rigidity and certain unique signs which can be elicited such as Kernig’s and Brudzinski’s signs (Chapter 71).

The details of meningitis along with its laboratory diagnosis and treatment have been discussed in Chapters 71 and 73.

Encephalitis and Encephalopathy

Encephalitis

Encephalitis is an acute inflammation of the brain parenchyma by invasion of infectious agents, most often the viruses.

- ❖ **Manifestations:** The common manifestations include altered consciousness, behavioral changes, seizures, focal neurological deficits and sometimes, extrapyramidal signs such as involuntary movements
- ❖ **Agents:** The common etiological agents of encephalitis include viruses, followed by parasites
 - **Viruses:** Include rabies, herpes, and arboviruses such as Japanese encephalitis and West Nile viruses
 - **Parasites:** Such as *Toxoplasma gondii* and *Naegleria fowleri*.
- ❖ The cellular infiltrate present in the CSF is typically **lymphocytic** rather than neutrophilic
- ❖ Very often, encephalitis is associated with concomitant meningitis; called as **meningoencephalitis** (e.g. primary amoebic meningoencephalitis, caused by a free-living amoeba called *Naegleria fowleri*).

Encephalopathy

Encephalopathy is a general term used for any diffuse disease of the brain that alters the brain function or structure.

- ❖ **Etiology:** Encephalopathy usually have non-infective etiology, rarely may also be caused by infections
 - **Infectious agent:** Most infections of brain usually cause encephalitis; while for some infections still the word ‘encephalopathy’ is widely used—slow virus disease, prions or cerebral malaria, etc.
 - **Non-infectious causes** such as metabolic encephalopathy, hepatic encephalopathy or uremic encephalopathy, brain tumor, etc.
- ❖ **Clinical features:** The hallmark of encephalopathy is an altered mental state. Other symptoms vary depending upon the etiology and may include progressive loss of

memory and personality changes, inability to concentrate, lethargy, and progressive loss of consciousness

- ❖ **Diagnosis:** CSF examination, imaging studies, electroencephalograms, are some of the useful tests to determine the etiology.

Acute encephalitis syndrome is discussed at the end of this chapter. The agents of encephalitis are discussed in Chapters 74 and 75.

Space Occupying Lesions of CNS

Space-occupying lesions of CNS include focal CNS lesions and cystic parasitic diseases.

Focal CNS Lesions

Focal CNS lesions include brain abscess, subdural empyema and epidural abscess.

1. Brain Abscess

Brain abscesses are an uncommon but serious life-threatening condition; characterized by the formation of localized collection of pus within a cavity in the brain, which is formed by the breakdown of tissue.

- ❖ **Source of infection:** Brain abscesses result from one of the following sources
 - *Contiguous infection* of the adjacent structures such as sinuses, middle ear or mastoids (45%–50%)
 - *Hematogenous spread* from a remote site, especially in patients with cyanotic congenital heart disease (25%)
 - Through *direct inoculation* as a result of trauma or surgery (10%)
 - *Cryptogenic:* In at least 15% of cases, the source of the infection is unknown.
- ❖ **Location:** The most frequent intracranial locations are frontal-temporal, followed by frontal-parietal, parietal, cerebellar, and occipital lobes. Brain abscesses may rupture into the subarachnoid space, producing severe meningitis with a high mortality rate
- ❖ **Etiology:** The organism implicated depends upon the site of primary infection, and the patient’s immune status
 - **In immunocompetent individuals**—the most important pathogens are:
 - ♦ Streptococci such as anaerobic, aerobic, and viridans group (e.g. *S. anginosus*): Most common cause (40%)
 - ♦ Anaerobes such as *Bacteroides fragilis*, *Fusobacterium* (30%)
 - ♦ Enterobacteriaceae such as *Proteus*, *E. coli*, *Klebsiella* (25%)
 - ♦ *Staphylococcus aureus* (10%): Usually in post-trauma or post-neurosurgery cases.
 - **In immunocompromised hosts** with underlying HIV infection, organ transplantation, cancer, or immunosuppressive therapy—most brain abscesses are caused by *Nocardia*, *Toxoplasma gondii*, *Aspergillus*, *Candida*, and *Cryptococcus*

- *Taenia solium* (neurocysticercosis): Common cause of brain abscess in Latin America
- *M. tuberculosis* (tuberculoma): Remains a major cause of focal CNS mass lesions in India and East Asia.
- ❖ **Clinical features:** The *triad* of fever, severe headache (unilateral, on the side of the abscess), and focal neurologic deficit constitute the major manifestations. Other features are seen rarely; include mental status changes (due to cerebral edema), seizures, nausea and vomiting, nuchal rigidity and papilledema
- ❖ **Diagnosis:** Clinical diagnosis is made by neuroimaging studies such as MRI and CT scans (Fig. 70.2A). Abscess aspirate (obtained via CT-guided or surgery) may be subjected to:
 - Culture: Aerobic, anaerobic, fungal and mycobacterial culture
 - Direct microscopy such as Gram stain, acid-fast stain, and special fungal stains
 - Histopathological examination of the brain tissue
 - Serology: Anti-anticysticercal antibodies for the diagnosis of neurocysticercosis
 - Molecular tests: PCR detecting 16S ribosomal genes
 - Blood cultures: About 10% of patients will also have positive blood cultures.

Note: CSF analysis contributes nothing to diagnosis or therapy, more so lumbar puncture (LP) increases the risk of herniation. Therefore, LP should not be performed in focal intracranial infections such as brain abscess, epidural or subdural empyema.

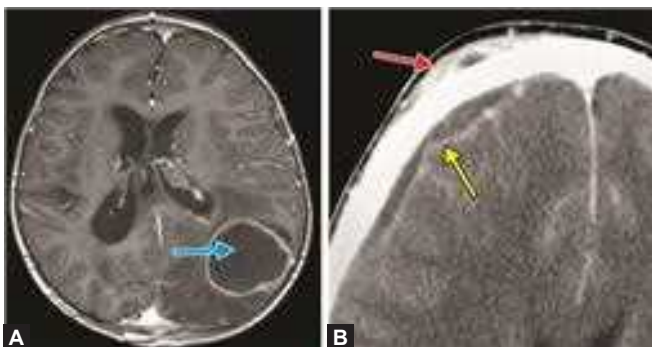
TREATMENT

Brain abscess

- Surgical excision or drainage of the abscess combined with prolonged antibiotics (for 6-8 weeks) remains the treatment of choice
- Ceftriaxone plus metronidazole is the preferred regimen, if bacterial etiology is suspected.

2. Subdural Empyema

Subdural empyema (or abscess) is an intracranial focal collection of purulent material located between the dura



Figs 70.2A and B: A. Brain abscess; B. Epidural abscess (red arrow) and subdural empyema (yellow arrow).

Source: A. Hellerhoff, B. James Heilman, Wikipedia (with permission).

mater and the arachnoid mater (Fig. 70.2B); majority (95%) of which are confined to frontal lobe. The source of infection, etiological agents and clinical presentation are similar to that described for brain abscess.

3. Epidural Abscess

It is a rare but potentially life-threatening disease that requires early detection and prompt management. It is defined as an inflammation that involves a collection of pus between the dura mater and the bones of the skull or spine (Fig. 70.2B). It is of two types.

- ❖ **Intracranial epidural abscess:** The causative agents may vary; upper respiratory flora (e.g. viridans streptococci) are the common cause for the cases that develop secondary to sinusitis, whereas nosocomial pathogens are of concern in cases that develop after craniotomy. The symptoms include fever, headache, malaise, lethargy, nausea, and vomiting
- ❖ **Spinal epidural abscess:** *S. aureus* (60%) accounts for the majority of cases; followed by enteric gram-negative bacilli and others. It presents with fever, spinal pain (back or neck pain) and neurological deficits.

Cystic Parasitic Diseases of CNS

There are various parasitic infections that can produce cystic disease in CNS; the most important of which is neurocysticercosis (Chapter 75).

Other Infective Syndromes of CNS

Suppurative Intracranial Thrombophlebitis

It refers to septic venous thrombosis of cortical veins and sinuses such as cavernous sinus, the lateral sinus, or the superior sagittal sinus.

- ❖ Cavernous sinus thrombophlebitis occurs secondary to infection of sinuses (sphenoid and ethmoid sinuses) and oral cavity
- ❖ Septic phlebitis of the lateral sinuses is associated with mastoiditis and otitis media
- ❖ Thrombophlebitis of the superior sagittal sinus is the rarest and is primarily associated with bacterial meningitis.

TREATMENT

Suppurative intracranial thrombosis

This condition is treated with antibiotics, hydration, and removal of infected tissue and thrombus in septic lateral or cavernous sinus thrombosis.

CSF Shunt Infections

Ventricular shunt is a neurosurgical procedure by which a channel is created to divert the excess CSF into other body cavities (e.g. ventriculoperitoneal shunt) where it can be reabsorbed. These shunts are useful in conditions such as hydrocephalus, where CSF is formed abnormally excess.

- ❖ The common organisms implicated in shunt infections are normal skin flora such as coagulase-negative

staphylococci, *S. aureus* and *Propionibacterium acnes*, which may be introduced at the time of surgery

- ❖ Gram-negative organisms (e.g. *Acinetobacter*, *Pseudomonas*, *Klebsiella*, *Escherichia coli*, and *Serratia*) and *Candida* species have also been recovered from shunt infections
- ❖ Positive cultures are most often associated with specimens such as shunt tip, shunt valves, and cerebrospinal fluid (CSF).

Infectious Myelitis

Myelitis is inflammation of the spinal cord which can result in disruption of the connection from the brain to the rest of the body, and vice-versa. It may have either infectious or autoimmune etiology.

- ❖ **Viral myelitis:** They are the most common cause of infectious myelitis; e.g. poliovirus (which causes poliomyelitis) and rarely by few other viruses (Chapter 73)
- ❖ **Non-viral causes** of infectious myelitis are rare and include:
 - Bacterial myelitis: *Mycoplasma pneumoniae*, tuberculosis, syphilis, and brucellosis
 - Fungal myelitis: Primary pathogens include the following: *Cryptococcus*, *Coccidioides*, *Blastomyces*, and *Histoplasma*
 - Parasitic myelitis, may be reported with *Schistosoma*, *Toxocara*, *Echinococcus*, *Taenia solium*, *Trichinella*, and *Plasmodium*.

Neurotoxin Mediated Infections

Tetanus and botulism are the classical examples of neurotoxin mediated diseases.

- ❖ **Tetanus:** It is caused by *Clostridium tetani*, which produces a neurotoxin (tetanus toxin) that causes skeletal muscle spasm and autonomic nervous system disturbance (Chapter 72)
- ❖ **Botulism:** It is caused by *Clostridium botulinum*, which produces a powerful neurotoxin called botulinum

toxin that causes flaccid paralysis of muscles. It mainly causes food poisoning; discussed in Chapter 40.

ACUTE ENCEPHALITIS SYNDROME (AES)

AES is a serious public health problem in India. The disease most commonly affects children and young adults. It occurs either sporadically or causes outbreaks; can lead to considerable morbidity and mortality.

- ❖ **Clinical presentations:** AES presents with acute-onset of fever and a change in mental status (mental confusion, disorientation, delirium, or coma) and/or new-onset of seizures
- ❖ **Etiological agents:** Viruses are the main causative agents, although bacteria, fungi, parasites and noninfectious agents have also been reported. The etiology in a large number of AES cases still remains unidentified.
 - Japanese encephalitis virus (JEV) is the major cause of AES in India (ranging from 5%-35%).
 - Other viral agents include herpes simplex virus, West Nile virus, Chandipura virus, mumps, measles, dengue, Parvovirus B4, enteroviruses, Epstein-Barr virus, Influenza A virus, Nipah virus and Zika virus
 - Bacterial agents include *Orientia* (scrub typhus), *S. pneumoniae*
 - **Litchi:** There has been cases of AES in Muzaffarpur, Bihar; associated with consumption of litchi. Cases mostly occur during April to June particularly in children who are undernourished with a history of visiting litchi orchards.
- ❖ The National Vector Borne Disease Control Programme (NVBDCP) in India has set up country wide surveillance for AES through sentinel sites with a focus on detecting JEV and other causes of AES. During 2018, 10,485 AES cases and 632 deaths (6% mortality) were reported, mainly from Assam, Bihar, Jharkhand, Karnataka, Manipur, Meghalaya, Tripura, Tamil Nadu, and Uttar Pradesh.

EXPECTED QUESTIONS

I. Write short notes on:

1. Brain abscess.
2. Encephalitis.
3. Chronic meningitis.
4. Acute encephalitis syndrome.

CHAPTER PREVIEW

- Acute Bacterial Meningitis
- Chronic Bacterial Meningitis

Meningitis is a life-threatening infection of the leptomeninges (arachnoid and pia mater) surrounding the brain and spinal cord, with involvement of the subarachnoid space. The disease can be caused by several pathogens including bacteria, viruses, fungi or parasites (enlisted in Chapter 70), but the highest global burden is seen with bacterial meningitis.

ACUTE BACTERIAL MENINGITIS

Acute bacterial meningitis (also called as pyogenic meningitis), is an acute purulent infection within the subarachnoid space. It is characterized by elevated polymorphonuclear cells in CSF.

The agents implicated in pyogenic meningitis may vary according to the age.

- ❖ **Overall:** *Streptococcus pneumoniae* is the most common cause of pyogenic meningitis (~50%). Other agents include meningococcus (~25%), *Streptococcus agalactiae* (~15%), *Listeria* (~10%) and *Haemophilus influenzae* (<10%)
- ❖ **Neonates:** The common agents of neonatal meningitis include *Streptococcus agalactiae*, gram-negative bacilli such as *Escherichia coli* and *Klebsiella*, and *Listeria monocytogenes*
- ❖ **Elderly (>60 years):** Common agents are *Streptococcus agalactiae* and *Listeria monocytogenes*.

PATHOGENESIS

The bacteria that cause acute meningitis are transmitted from person-to-person through droplets of respiratory secretions from cases or nasopharyngeal carriers. Close and prolonged contact—kissing, sneezing or coughing on someone, or living in close quarters with an infected person facilitate the spread of the disease.

Routes of Infection

Organisms may gain access to the meninges by several routes:

- ❖ **Hematogenous spread:** This is the most common route, where entry into the subarachnoid space is gained through the choroid plexus or through other blood vessels of the brain
- ❖ **Direct spread from an infected site** present close to meninges—otitis media, mastoiditis, sinusitis, etc.
- ❖ **Anatomical defect in central nervous system (CNS):** It may occur as a result of surgery, trauma, congenital defects, which can allow organisms for ready and easy access to CNS.

Predisposing Factors

Pathogenesis of meningitis depends upon various host and microbial factors.

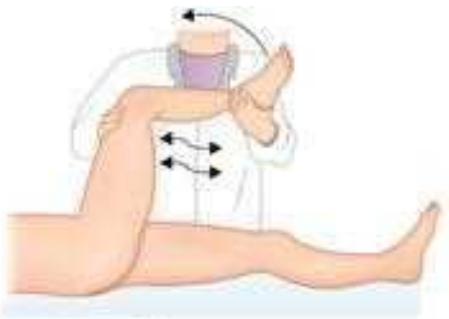
- ❖ **Age:** Neonates have the highest prevalence of meningitis; probably due to—(1) their immature immune system, (2) acquiring the colonized organisms from mother's birth canal (e.g. *Listeria* or *Streptococcus agalactiae*), (3) increased permeability of blood brain barrier
- ❖ **Vaccination:** Widespread vaccination is shown to reduce the incidence of meningitis due to the particular agent—e.g. decreased incidence of *H. influenzae* meningitis following implementation of Hib vaccination
- ❖ **Factors that promote infection at primary site:** Because respiratory tract is the primary portal of entry for many etiological agents of meningitis, the factors that predispose to respiratory infections can also increase the likelihood of development of meningitis—e.g. alcoholism, diabetes, immunosuppression, splenectomy, etc.
- ❖ **Presence of CSF shunts** can also directly facilitate the pathogen entry into meninges, e.g. staphylococci, *Pseudomonas*, *Acinetobacter*, etc.
- ❖ **Breach in the blood-brain barrier (BBB):** Organism can gain access through BBB by:
 - Loss of capillary integrity by disrupting the tight junctions of BBB
 - Transport within circulatory phagocytes

- Crossing the endothelial cells by transport within the endothelial cell vacuoles.

CLINICAL MANIFESTATIONS

The average incubation period is 4 days but can range between 2 and 10 days. Patients with meningitis develop various manifestations such as:

- ❖ **Important symptoms** include fever, vomiting, intense headache, altered consciousness and occasionally photophobia
- ❖ **Signs of meningism** (meningeal irritation) such as:
 - **Nuchal rigidity** (“stiff neck”) is the pathognomonic sign of meningeal irritation and is present when the neck resists passive flexion
 - **Kernig’s sign:** Severe stiffness of the hamstrings causes an inability to straighten the leg when the hip is flexed to 90° (Fig. 71.1A)
 - **Brudzinski’s sign:** When the neck is passively flexed, results in spontaneous flexion of the hips and knees (Fig. 71.1B).



A



B

Figs 71.1A and B: Signs seen in meningitis: **A.** Kernig’s sign; **B.** Brudzinski’s sign.

- ❖ **In infants:** Pyogenic meningitis in infants may have a slower onset, signs may be nonspecific, and neck stiffness may not be present. Babies usually present with fever, irritability and bulging fontanelle
- ❖ **Complications:** In due course, the disease may involve brain parenchyma leading to meningoencephalitis—that may result in decreased consciousness, seizures, raised intracranial pressure, and stroke
- ❖ **Organism specific finding**—e.g. purpuric rashes seen in meningococcal meningitis.

LABORATORY DIAGNOSIS

Specimen Collection and Transport

CSF is the most ideal specimen for pyogenic meningitis. Blood culture is another useful specimen for culture.

- ❖ **CSF collection:** CSF is obtained by lumbar puncture under strict aseptic conditions. It is divided into three sterile containers; one each for cell count, biochemical analysis and bacteriological examination
- ❖ **CSF transport:** CSF being the most precious specimen should be examined immediately
 - When the bacteriological examination (culture) is required, CSF should never be refrigerated as delicate pathogens such as *H. influenzae*, pneumococci or meningococci may die. Therefore if a delay is expected, it may be kept in an incubator at 37°C
 - However for molecular diagnosis, CSF can be kept inside the freezer.

Other useful specimens for isolation of etiological agents of pyogenic meningitis are:

- ❖ **Blood culture:** Blood should be collected in automated blood culture bottles such as BacT/ALERT
- ❖ **For suspected meningococcal meningitis:** Other useful specimens are nasopharyngeal swabs, pus or scrapings from rashes; which should be carried in transport media (such as Stuart’s medium). These specimens are inoculated onto selective media, such as Thayer Martin medium or New York City medium, to suppress the growth of normal flora.

Cytological and Biochemical Analysis

Biochemical analysis and cell count of CSF give a preliminary clue about the type of meningitis (Table 71.1)

Table 71.1: Cytological and biochemical parameters in CSF of normal individuals and in different types of meningitis.				
Characteristics	Normal individual	Pyogenic meningitis	Tuberculous meningitis	Viral meningitis
CSF pressure (mm of water)	Normal (50–150)	Highly elevated (>180)	Moderately elevated	Slightly elevated/normal
Total leukocyte count (per mm ³)	0–5	100–10,000	10–500	25–500
Predominant cell type	Lymphocytes	Neutrophils	Lymphocytes	Lymphocytes
Glucose (mg%)	40–70	<40 mg/dL (decreased to absent)	20–40 mg/dL (slightly decreased)	Normal
Total proteins (mg%)	15–45	>45 mg/dL (usually >250; markedly increased)	100–500 mg/dL (moderate to markedly increased)	20–80 mg/dL (normal or slightly elevated)

Table 71.2: Preliminary clue about the etiological agents of pyogenic meningitis based on CSF Gram stain.

Appearance in CSF Gram stain	Suggestive of
Gram-positive diplococci, flame or lanceolate-shaped with clear halo (capsulated) (Fig. 61.1)	<i>Streptococcus pneumoniae</i>
Gram-negative diplococci, capsulated, with adjacent sides flattened (lens or half-moon shaped) (Fig. 71.2)	<i>Neisseria meningitidis</i>
Pleomorphic gram-negative coccobacilli, capsulated (Fig. 61.4A)	<i>Haemophilus influenzae</i>
Gram-negative bacilli, arranged singly	<i>Escherichia coli</i> or others
Gram-positive cocci in short chain	<i>Streptococcus agalactiae</i>
Gram-positive short bacilli, often confused with diphtheroids	<i>Listeria monocytogenes</i>

In acute bacterial (pyogenic) meningitis:

- ❖ CSF usually contains >1000 leukocytes/ μL and predominantly neutrophils (90–95%). However in *Listeria* meningitis, there is increased lymphocyte count in CSF
- ❖ The total protein content is elevated and the glucose level is markedly diminished or even absent
- ❖ CSF pressure is highly elevated.

CSF Microscopy (Gram Staining)

Microscopic examination of gram-stained smear may give a preliminary clue about the etiological agent of pyogenic meningitis based on the morphology of the bacteria (Table 71.2).

- ❖ This helps in early initiation of appropriate empirical antimicrobial therapy
- ❖ **Heaped smear:** As the bacterial load in CSF may be very low, to increase the sensitivity, several drops of CSF should be placed at the same spot on the slide, each drop being allowed to air dry before the next is added
- ❖ **Centrifugation:** Alternatively, CSF can be centrifuged (by cytopspin) and the deposit is examined for Gram staining.

Direct Antigen Detection

From CSF: After centrifugation of CSF, the supernatant can be used for antigen detection. Latex agglutination test is performed using latex beads coated with anti-capsular antibodies.

- ❖ It is available for detection of capsular antigens of common agents of meningitis such as *S. pneumoniae*, *S. agalactiae*, *N. meningitidis*, *H. influenzae* or *E. coli*
- ❖ Detection of capsular antigens in CSF is more sensitive than CSF microscopy.

From urine: Antigen detection in urine is useful for pneumococcal meningitis. Immunochromatographic test (ICT) is available to detect the C-polysaccharide antigen of *S. pneumoniae* in urine.

Culture

Ideal media for bacteriological culture of CSF are enriched media like chocolate agar and blood agar, and differential media like MacConkey agar

- ❖ **Enriching:** As the bacterial load is very low, a part of the CSF can be inoculated into enriched media such as blood culture bottles at the bed side (preferred) or brain heart infusion (BHI) broth in the laboratory
- ❖ **Blood culture** can be collected in conventional blood culture bottles such as BHI broth/agar or preferably in automated blood cultures (e.g. BacT/ALERT)
- ❖ **Culture plates** (blood agar and chocolate agar) are incubated at 37°C, preferably in candle jar (provides 5% CO₂) for 48 hours

Table 71.3: Culture and identification properties of common bacterial agents of pyogenic meningitis.

<p><i>Streptococcus pneumoniae</i> (Chapter 61)</p> <ul style="list-style-type: none"> • Culture: It produces α-hemolytic colonies on blood agar, described as draughtsman-shaped or carrom coin appearance • Biochemical identification: It shows bile soluble, ferments inulin and sensitive to optochin
<p><i>Neisseria meningitidis</i> (discussed in this chapter)</p> <p>It produces non-hemolytic colonies on blood agar, which on smear shows gram-negative diplococci (Fig. 71.2)</p> <ul style="list-style-type: none"> • Biochemical identification: Meningococci are catalase and oxidase positive. They ferment glucose and maltose but not sucrose • Serogrouping: Slide agglutination serogrouping (SASG) test is done to identify the serogroups of meningococci isolates by using appropriate antisera
<p><i>Haemophilus influenzae</i> (Chapter 61)</p> <ul style="list-style-type: none"> • Culture: Blood agar with <i>S. aureus</i> streak line shows satellitism. • Biochemical identification: Disk test for X and V factor requirement shows growth surrounding combined XV disk
<p><i>Streptococcus agalactiae</i> (Chapter 52)</p> <ul style="list-style-type: none"> • Culture: It produces β-hemolytic colonies on blood agar, which on smear shows gram-positive cocci in short chain • Biochemical identification: It shows CAMP test positive and resistance to bacitracin • Serogrouping with group specific antisera shows Lancefield group B
<p>Gram-negative bacilli meningitis</p> <ul style="list-style-type: none"> • <i>Escherichia coli</i> and <i>Klebsiella</i> produce lactose-fermenting colonies on MacConkey agar; identified by ICUT tests • Non-fermenters: <i>Pseudomonas</i> is oxidase positive, whereas <i>Acinetobacter</i> is oxidase negative. They produce non-lactose fermenting colonies; identified by ICUT tests (Chapter 65)
<p><i>Listeria monocytogenes</i> (discussed in this chapter)</p> <ul style="list-style-type: none"> • Motility: It shows tumbling type of motility at 25°C but non-motile at 37°C (called differential motility, which is due to temperature dependent flagella expression) • Culture: It grows on blood agar (β-hemolytic colonies), and chocolate agar. <p><i>Note:</i> Selective media such as PALCAM agar (containing mixture of antibiotics) may be useful for isolation of <i>Listeria</i> from specimens such as food and environmental samples.</p>

Abbreviations: ICUT tests, indole, citrate, urease and triple sugar iron agar test; CAMP, Christie, Atkins, and Munch-Peterson test.

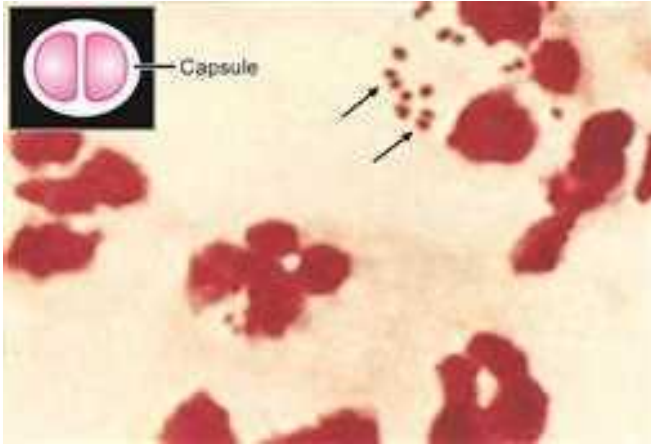


Fig. 71.2: Meningococci in CSF smear (gram-negative diplococci, lens-shaped) (arrows showing).

Source: Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Identification:** Colonies grown on solid media are processed for identification of the organism either by automated identification system such as MALDI-TOF or VITEK, or by conventional biochemical tests (Table 71.3)
- ❖ **Antimicrobial susceptibility test** should be done to initiate definitive antimicrobial therapy. It is carried out by disk diffusion test or preferably by automated MIC-based methods such as VITEK
- ❖ **Sensitivity:** CSF and blood cultures may take >48 hours for organism identification and are positive in 70-85% of patients with bacterial meningitis. However, sensitivity drops rapidly in case of prior antimicrobial therapy or delay in processing
- ❖ Therefore, **rapid diagnostic tests** such as antigen detection or molecular test should be considered to determine the bacterial etiology of pyogenic meningitis.

Serology

Antibodies to capsular antigens of meningococci can be detected in patient's serum by ELISA. This is useful to study seroprevalence and to know the response to vaccination; not for diagnosis.

Molecular Methods

Molecular tests are highly sensitive, detect even few bacteria in CSF with less turnaround time than culture and also help in serogroup identification.

- ❖ **Formats:** Multiplex PCR and multiplex real-time PCR can be used for simultaneous detection of common agents of pyogenic meningitis
- ❖ **BioFire FilmArray** is an automated nested multiplex PCR commercially available, which can simultaneously detect 14 common agents of meningitis (both pyogenic and viral) in CSF, with a turnaround time of 1 hour
- ❖ **Common genes** targeted include:
 - For meningococcus: *ctrA* (capsule transport gene) and *sodC* (Cu-Zn superoxide dismutase gene)

- For pneumococcus: *lytA* (autolysin gene), *ply* (pneumolysin) and *psaA* (pneumococcus surface antigen A)
- For *H. influenzae*: Conserved capsular gene *bexA*.

TREATMENT

Pyogenic meningitis

The mortality of pyogenic meningitis is very high (~20% for pneumococci) and among the survivors, up to 50% develop complications. Therefore, treatment should be initiated as early as possible.

The choice of antimicrobial agent is based on the type of organism suspected and good CSF penetration ability of the agent.

Empirical therapy comprises of:

- ❑ **Adult:** IV cefotaxime or ceftriaxone and vancomycin is the recommended regimen. If *Listeria* is suspected, IV ampicillin can be added to the regimen
- ❑ **For neonates:** IV ampicillin plus gentamicin is the recommended regimen
- ❑ **IV dexamethasone** is added to the regimen to reduce intracranial pressure.

Definitive therapy: After the culture report is available, the empirical therapy is modified based on the organism isolated and its antimicrobial susceptibility pattern.

AGENTS OF PYOGENIC MENINGITIS

Pneumococcal Meningitis

Streptococcus pneumoniae (or pneumococcus) is the leading cause of meningitis in adults (>20 years of age), accounting for 50% of all cases. It is also the most common agent of pneumonia, discussed in detail Chapter 61.

- ❖ They may present as commensals in human nasopharynx, from where the bacteria spread locally to cause otitis media or pneumonia and subsequently via the bloodstream to distant sites to cause invasive pneumococcal disease such as bacteremia and meningitis
- ❖ The principle virulence factors include capsular polysaccharide, C-carbohydrate antigen, pneumolysin and autolysin
- ❖ **Risk factors:** The predisposing factors that increase the risk of pneumococcal meningitis include underlying pneumococcal pneumonia (most important) or otitis media, alcoholism, diabetes, splenectomy, complement deficiency, hypogammaglobulinemia, and head trauma
- ❖ The mortality rate remains high (~20%), despite antibiotic therapy.

Laboratory diagnosis and treatment of pneumococcal infections have been discussed in detail in Chapter 61.

Meningococcal Meningitis

Meningococcal meningitis is of particular importance due to its potential to cause large outbreaks and epidemics.

- ❖ It is caused by *Neisseria meningitidis* (or meningococcus); which appears capsulated gram-negative diplococci with adjacent sides flattened (lens-shaped/half-moon shaped) (Fig. 71.2)

Table 71.4: Differences between *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

<i>N. meningitidis</i>	<i>N. gonorrhoeae</i>
Capsulated	Noncapsulated
Lens-shaped/half moon-shaped (diplococci with adjacent sides flattened) (Fig. 71.2)	Kidney-shaped (diplococci with adjacent sides concave) (Chapter 77, Fig. 77.7)
Ferments glucose and maltose	Ferments only glucose
Rarely have plasmids	Usually possess plasmids, coding for drug-resistant genes
Exist in both intra- and extracellular forms	Predominantly exist in intracellular form
Habitat—nasopharynx	Habitat—genital tract (urethra, cervix), rarely pharynx

- ❖ *Neisseria* has another pathogenic species, *N. gonorrhoeae* (causes gonorrhoea, Chapter 77), both differ from each other in various aspects (Table 71.4)
- ❖ Other species are commensals of the genital tract or oral cavity, such as *N. lactamica*, *N. flavescens*, *N. mucosa*, *N. sicca*, *N. subflava*, etc.; although they can be occasionally pathogenic to humans.

Virulence Factors

Capsular Polysaccharide

It is the principal virulence factor of meningococci; protects the bacteria from complement-mediated phagocytosis.

- ❖ Based on the antigenic nature of the capsule, meningococci can be typed into 13 serogroups [A–D, X–Z, 29E, W, H–J and L], among which only 6 serogroups—**A, B, C, X, Y, and W** (formerly W135)—account for the majority of cases of invasive disease
- ❖ Other capsular serogroups and noncapsulated meningococci (16% of isolates are noncapsulated) commonly colonize the nasopharynx of asymptomatic carriers and are rarely associated with invasive disease.

Other Virulence Factors

- ❖ **Outer membrane proteins:** They are the porin proteins present beneath the capsule, embedded in the outer membrane
- ❖ **LPS and endotoxin:** Like other gram-negative bacteria, meningococci possess LPS (lipopolysaccharide) and endotoxins in their cell wall. Endotoxin induces the release of various inflammatory mediators, which in turn damage the vascular endothelium. Endothelial injury is central to many clinical features of meningococemia, such as:
 - Increased vascular permeability leading to loss of fluid and shock
 - Intravascular thrombosis (due to activation of pro-coagulants) leading to disseminated intravascular coagulation (DIC)
 - Myocardial dysfunction.
- ❖ **Others** include IgA proteases, transferrin binding proteins and adhesins.

Epidemiology

Worldwide, nearly 5 lakh cases of meningococcal disease occur each year, and 5–16% of those die.

- ❖ **Patterns of disease:** Meningococcus causes several patterns of invasive disease ranging from sporadic infection, to endemic, hyperendemic and explosive epidemics
- ❖ **High prevalence area:** The sub-Saharan belt of Africa (from Ethiopia to Senegal) is the most prevalent area for meningococcal infections. Around 30,000 cases are still reported each year from this area
- ❖ **World:** The serogroups distribution vary among various regions of the world
 - **Group A:** It was the leading cause of epidemic meningitis worldwide. With the advent of vaccination, the occurrence of group A has been reduced considerably
 - **Group B and C** are currently the major serogroups causing invasive disease worldwide
 - ◆ Group B can cause hyperendemic disease (>10 cases per 100,000 population)
 - ◆ Group C continues to cause outbreaks in Nigeria (>4000 cases in 2018).
- ❖ **Group X, Y and W** are less commonly reported worldwide. Group W (formerly W135) can cause outbreaks in mass gatherings; has caused the global outbreak in 2000 in Hajj pilgrimage
- ❖ **India:** Sporadic cases have been occurring every year, mainly from North India with occasional outbreaks
 - Serogroup A has been reported from few places, though accurate data on serogroup distribution is lacking
 - In 2015, >12,000 cases were reported, maximum from Bihar (>8,000 cases).
- ❖ **Seasonality:** Meningococcal infections are common in winter and spring (cold and dry climate)
- ❖ **Age:** Meningitis is common in early childhood (3 months to 5 years) with a second peak occurring in adolescents (15–25 years of age)
- ❖ **Risk factors that promote colonization include:**
 - Overcrowding and semiclosed communities, such as schools, military and refugee camps
 - Travelers (e.g. Hajj pilgrims)
 - Smoking
 - Recent upper respiratory tract infection.
- ❖ **Risk factors that promote disease include:**
 - Deficiency of terminal complement components (C5–C9)
 - Eculizumab or ravulizumab therapy—a terminal complement inhibitor
 - Hypogammaglobulinemia and hyposplenism.
- ❖ **Carriers:** 5–10% of populations are asymptomatic nasopharyngeal carriers at any given time.

Pathogenesis

Humans are the only natural host for meningococci. Most common source of infection is nasopharyngeal carriers.

- ❖ **Mode of transmission:** It is by droplet inhalation and the portal of entry is through the nasopharynx
- ❖ **Spread of infection:** From nasopharynx, meningococci reach the meninges either by (1) hematogenous route causing septicemia (most common); or (2) by direct spread along olfactory nerve through cribriform plate; or (3) rarely through conjunctiva.

Clinical Manifestations

The majority of infected individuals become carriers. The remainders develop the following manifestations:

- ❖ **Rashes:** A non-blanching rash (petechial or purpuric) develops in more than 80% of the cases (Chapter 55)
- ❖ **Septicemia:** It is attributed to endotoxin induced endothelial injury leading to increased vascular permeability and intravascular thrombosis
- ❖ **Waterhouse–Friderichsen syndrome:** It is a severe form of fulminant meningococemia, characterized by large purpuric rashes (purpura fulminans), shock, disseminated intravascular coagulation (DIC), bilateral adrenal hemorrhage and multiorgan failure (Fig. 55.6).
- ❖ **Pyogenic meningitis:** It commonly affects young children (3–5 years of age). Presentation includes fever, vomiting, headache, neck stiffness—similar to any other bacterial meningitis, except for the presence of rashes
- ❖ **Chronic meningococemia:** It occurs rarely and is characterized by repeated episodes of petechial rashes, fever, arthritis, and splenomegaly
- ❖ **Postmeningococcal reactive disease:** Immune complexes (made up of capsular antigens and their antibodies) develop 4–10 days later, lead to manifestations like arthritis, rash, iritis, pericarditis, polyserositis, and fever.

Laboratory diagnosis is discussed in Table 71.3.

TREATMENT

Meningococcal meningitis

Meningococcal meningitis is associated with high fatality (up to 50% when untreated) and high frequency (>10%) of severe sequelae. Early antibiotic treatment may reduce fatality to <10%.

- ❑ **Drug of choice** is third-generation cephalosporins, such as ceftriaxone or cefotaxime, given for 7 days. Penicillin can also be given; however, reduced meningococcal sensitivity to penicillin has been reported from few countries
- ❑ **Symptomatic treatment**, such as aggressive fluid resuscitation (for shock) and measures to decrease intracranial pressure should be instituted.

Prevention

Chemoprophylaxis

Chemoprophylaxis is indicated to the close contacts of primary cases, regardless of their vaccination status. Close contacts refers to household contacts and others who are directly exposed to patient's oral secretions, in the 7 days before symptom onset.

- ❖ Ceftriaxone (single dose, IM) is the drug of choice
- ❖ Alternatively, rifampicin or ciprofloxacin can be given.

Vaccine Prophylaxis

Meningococcal polysaccharide vaccines are currently formulated as either bivalent (serogroups A and C) or quadrivalent (serogroups A, C, Y, and W135).

- ❖ **Schedule:** Administered as two doses, 2–3 months apart to children of 3–18 months of age or as a single dose to older children or adults
- ❖ **Efficacy:** It has a protective efficacy rate of >95%. The duration of protection lasts for 3–5 years
- ❖ **Indication:** It is recommended for high-risk people such as (i) contacts of patients during outbreaks, (ii) splenic dysfunction, (iii) terminal complement component deficiency, (iv) taking eculizumab therapy, (v) laboratory staff at risk, (vi) international travellers, including students going to study abroad
- ❖ **Capsular vaccine is not available for serogroup B as:**
 - Capsule of serogroup B (made up of sialic acid) is less immunogenic
 - It is also encephalitogenic due to expression of similar cross reactive antigens on neural cells.
- ❖ **Not given below 3 years:** Similar to pneumococcal vaccine, meningococcal capsular vaccine is also an example of T cell-independent antigen and is poorly immunogenic to children; hence not given to children of less than 2–3 years of age
- ❖ **Conjugated vaccine:** However, conjugated meningococcal capsular vaccine is available which can be given to young children. Addition of a protein carrier (adjuvant) increases the immunogenicity of the capsular vaccine.

Vaccine for Group B (MenB Vaccine)

Recently, recombinant vaccine for Group B meningococcus has been licensed.

- ❑ Vaccine contains four recombinant proteins: Adhesin A, heparin binding antigen, factor H binding protein and outer membrane vesicles (OMV)
- ❑ Schedule: Two doses, given IM route 1 month apart
- ❑ Indication: 16–25 years age.

Haemophilus influenzae Meningitis

Haemophilus influenzae was previously an important agent of pyogenic meningitis; the incidence has been dramatically reduced after the start of its effective vaccination.

- ❖ **Virulence factor:** Capsular polysaccharide is the most important virulence factor, acts by inhibiting phagocytosis
- ❖ **Hib:** Out of the six capsular serotypes, *H. influenzae* serotype b (Hib) is the most virulent types; responsible for pneumonia, which subsequently spread by hematogenous route to cause meningitis
- ❖ **CNS infections:** Hib causes various CNS infections such as:
 - Pyogenic meningitis: It mainly occurs in children less than 2 years of age

- Subdural effusion: It is a common complication following meningitis, characterized by seizures or hemiparesis
- Mortality rate is high if untreated. Survivors develop neurologic sequelae, such as partial hearing loss and delayed language development.

H. influenzae is discussed in detail in Chapter 61.

Group B Streptococcal (*S. agalactiae*) meningitis

Group B *Streptococcus* has been recognized as a major cause of neonatal sepsis and meningitis. Neonatal sepsis can be of two types—early-onset and late-onset type (Table 71.5).

- ❖ **Early-onset:** Occurs in the first week of life and due to transmission of organism from the maternal genital tract during or before birth
 - ❖ **Late-onset:** Occurs from 7–90 days of birth, transmitted by contact with a colonized mother or nursing personnel.
- In addition, group B *Streptococcus* has been reported with increasing frequency in individuals aged >50 years, particularly those with underlying diseases.

TREATMENT Group B Streptococcal meningitis

Meningitis should be treated with penicillin for a duration of 14 days, as shorter courses may lead to relapse.

Group B *Streptococcus* is discussed in detail in Chapter 52.

Gram-negative bacilli Meningitis

Gram-negative bacilli cause meningitis in individuals with chronic and debilitating diseases such as diabetes, cirrhosis, or alcoholism and in those with chronic UTI. Gram-negative meningitis can also occur secondary to neurosurgical procedures, particularly craniotomy, and head trauma with CSF rhinorrhea or otorrhea.

- ❖ *Escherichia coli* and *Klebsiella* are one of the common cause of pyogenic meningitis in neonates

Table 71.5: Early and late-onset group B *Streptococcus* disease in neonates.

Characteristics	Early-onset disease	Late-onset disease
Age of onset	0–6 days of birth	7–90 days of birth
Increased risk following obstetric complications	Prematurity and prolonged labor	Not associated
Mode of transmission to the baby	During or before birth from the colonized maternal genital tract	Contact with a colonized mother and nursing personnel
Common clinical manifestations	Pneumonia and/or respiratory distress syndrome (most common), bacteremia and meningitis	Meningitis (most common) and bacteremia
Common serotypes	Ia, III, V, II, Ib	Type III
Case fatality rate	4.7%	2.8%

- ❖ *Pseudomonas* can cause meningitis in postoperative or post-traumatic patients (head trauma with CSF rhinorrhea or otorrhea)
- ❖ *Acinetobacter* is known to be associated with post-neurosurgical meningitis
- ❖ *Elizabethkingia meningosepticum* can cause meningitis in neonates.

Listeriosis

Listeriosis is caused by *Listeria monocytogenes*, which is a food-borne pathogen that can cause serious infections, particularly in neonates, pregnant women and elderly people. It is also a ubiquitous saprophyte and known to cause epizootic disease in birds and animals.

Human Infection

- ❖ **Mode of transmission:** It is transmitted most commonly through contaminated food followed by vertical transmission (mother to fetus, during birth)
- ❖ **Common food sources** include contaminated coleslaw, milk, soft cheeses, and several types of “ready-to-eat” foods, including delicatessen meat and uncooked hotdogs
- ❖ **Age:** Listeriosis is common among extremes of age (neonate and old age)
- ❖ Use of **proton pump inhibitors** increases the risk by reducing the gastric acid mediated killing of *Listeria*
- ❖ **Other risk factors:** Pregnant women and immunocompromised individuals are at higher risk
- ❖ Due to its ability to survive refrigeration (4°C), it is **commonly found in stored foods** especially aged soft cheeses, packaged meats, milk and cold salads
- ❖ Listeriosis is most often sporadic, although outbreaks do occur.

Pathogenesis

- ❖ **Intracellular survival:** After entry into the intestinal epithelium, it survives inside the host cell, mainly due to lysis of phagosome by forming pores (mediated by listeriolysin O)
- ❖ Then it causes **host cell actin polymerization**, which helps the bacterium to reach near the cell membrane
- ❖ Finally, it migrates to the adjacent epithelial cells/macrophages by **direct cell-to-cell spread**, mediated by listeriopods.

Clinical Manifestations

Clinical manifestation depends on the age of the patient and other risk factors, such as immunosuppression. Most of the human infections are caused due to serotypes 1/2a, 1/2b, and 4.

- ❖ **Neonatal listeriosis:** Two clinical presentations are recognized—early-onset and late-onset neonatal disease (Table 71.6)
- ❖ **In pregnant women:** It affects both mother and the fetus. (1) Fetal complications, such as abortion, preterm

Table 71.6: Differences between early- and late-onset neonatal listeriosis.

Early-onset neonatal disease	Late-onset neonatal disease
Occurs <5 days of birth (Mean age 1.5 days)	Occurs >5 days of birth (Mean age 14.2 days)
Acquired from maternal genital flora	Acquired from environment
Associated with obstetrical complications like premature delivery and low birth weight	Not associated
Most common form is neonatal sepsis	Most common form is neonatal meningitis
Granulomatosis infantiseptica: Occurs rarely, characterized by miliary microabscesses and granulomas, mostly in skin, liver and spleen	Not seen
Mortality rate is up to 50%	Mortality rate is <10%
Does not cause nosocomial outbreaks	Nosocomial outbreaks are seen

delivery lead to early onset disease; (2) Maternal complications, such as flu-like symptoms, bacteremia and rarely meningitis

- ❖ **Adults:** It produces manifestations such as bacteremia and meningitis in elderly individuals (>60 years)
 - Common risk factor is immunosuppression (steroid therapy, HIV, diabetes, malignancy)
 - *Listeria* can cause meningitis in kidney transplanted patients 1-month after the transplantation
 - It also causes gastroenteritis following consumption of contaminated milk, meat and salads.

Laboratory diagnosis of listeriosis is discussed in Table 71.3.

TREATMENT

Listeriosis

- **Meningitis:** Ampicillin is the drug of choice, given for 2–3 weeks in combination with gentamicin for synergistic effect
- Cotrimoxazole is given for patients with penicillin allergy
- In febrile gastroenteritis, Amoxicillin is recommended in immunocompromised, elderly or pregnant patients
- **Antibiotics to be avoided:** Cephalosporins, chloramphenicol are not active in vivo; vancomycin, linezolid, macrolides and tetracyclines are not recommended.

Staphylococcal Meningitis

S. aureus and coagulase-negative staphylococci can cause meningitis following invasive neurosurgical procedures, particularly ventricular shunting (Chapter 51).

CHRONIC BACTERIAL MENINGITIS

Several bacterial meningitis present as chronic stage, characterized by persistence of signs and symptoms as well as the CSF abnormality for >4 weeks. The bacterial agents causing chronic meningitis include the following.

- ❖ Partially treated pyogenic meningitis
- ❖ Parameningeal infections (e.g. otitis media)

- ❖ *Mycobacterium tuberculosis*
- ❖ *Borrelia burgdorferi* (Lyme disease)
- ❖ *Treponema pallidum* (tertiary syphilis)
- ❖ Rare bacterial agents such as *Nocardia*, *Actinomyces*, *Tropheryma whipplei*, *Leptospira* and *Brucella*.

Clinical Manifestations

Neurologic manifestations of chronic meningitis are determined by the anatomic location of the inflammation and its consequences. Cardinal features include:

- ❖ Persistent headache and neck or back pain/stiffness (similar to pyogenic meningitis)
- ❖ Hydrocephalus: Accumulation of CSF within the ventricles causes increased pressure inside the skull
- ❖ Cranial neuropathies: Leads to facial weakness, diminished vision, papilledema, optic atrophy, hearing loss
- ❖ Myelopathy or radiculopathy: Leads to arm or leg weakness or numbness, urinary retention/incontinence
- ❖ Changes in the personality such as altered mental status—drowsiness, inattention, disorientation, and memory loss.

AGENTS OF CHRONIC MENINGITIS

Mycobacterium tuberculosis Infections of CNS

Tuberculosis of the CNS accounts for ~5% of extrapulmonary cases. It is seen most often in young children but also develops in adults, especially those infected with HIV. It presents in two clinical forms: tuberculous meningitis and tuberculoma.

Tuberculous Meningitis (TBM)

TBM results from the hematogenous spread of primary or post-primary pulmonary TB. Typically, the disease evolves over 1–2 weeks or longer, which differentiates it from bacterial meningitis.

Clinical Features

TBM often presents subtly as headache, slight mental changes, low-grade fever, malaise, night sweat, anorexia, and irritability.

- ❖ Subsequently, it may evolve acutely with severe headache, confusion, lethargy, altered sensorium, and neck rigidity
- ❖ Cranial nerves paresis (ocular nerves in particular) is a frequent finding. Stroke may occur due to involvement of cerebral arteritis
- ❖ Ultimately, it progresses towards coma, with hydrocephalus and intracranial hypertension.

Laboratory Diagnosis

- ❖ **CSF analysis:** Examination of CSF reveals—
 - High leukocyte count (up to 1,000/ μ L), mostly lymphocytic. However, neutrophils may be elevated in the early stage

- Protein content of 100–800 mg/dL
 - Low glucose concentration.
- However, it should be noted that any of these three parameters can be within the normal range.
- ❖ **Cobweb coagulum:** When CSF is kept in a tube for 12 hours, a coagulum forms in the form of a cobweb due to higher fibrin content in the fluid
 - ❖ **Acid-fast staining of CSF:** Direct smear of CSF sediment may reveal long slender beaded acid-fast bacilli
 - However, the sensitivity of acid fast stain is very low (10–40%) and may require repeated lumbar punctures to increase the yield
 - Acid-fast staining of cobweb may give better yield as TB bacilli may be trapped in the cobweb. However, fibrin strands in the cobweb may be mistaken as fungi.
 - ❖ **Culture of CSF** is diagnostic in up to 80% of cases and remains as the gold standard test. However, culture is time consuming, takes 4–8 weeks by Lowenstein-Jensen medium and 2–3 weeks by automated liquid culture, such as Mycobacteria Growth Indicator Tube (MGIT)
 - ❖ **GeneXpert assay:** It is an automated real-time PCR, has a sensitivity of up to 80% and is the preferred initial diagnostic option. In addition to detection of *M. tuberculosis*, it can also detect resistance to rifampicin
 - ❖ **Imaging studies** (CT and MRI) may show hydrocephalus and abnormal enhancement of basal cisterns. In more than half of cases, evidence of old pulmonary lesions or a miliary pattern is found on chest X-ray.

TREATMENT

Tuberculous meningitis

If unrecognized, TBM is invariably fatal.

- ❑ Treatment should be initiated immediately upon a positive GeneXpert MTB/RIF result. A negative result does not exclude a diagnosis of TB and requires further diagnostic workup
- ❑ It responds well to anti-tubercular therapy, if started early. However, neurologic sequelae may occur in 25% of treated cases, if initiation of treatment is delayed
- ❑ Adjunctive glucocorticoids may be used to reduce the CSF pressure, resulting in faster resolution.

Tuberculoma (or Tuberculous Granulomas)

It is an uncommon manifestation of tubercular infection of CNS; presents as space-occupying lesions (firm nodule with central caseous necrosis), and usually causes seizures and focal signs. CT or MRI reveals contrast-enhanced ring lesions, but biopsy is necessary to establish the diagnosis and to differentiate it from malignancies.

Neuroborreliosis (Lyme Disease)

Lyme disease (Chapter 32) is caused by *Borrelia burgdorferi*, transmitted by tick bite. Apart from cutaneous lesions and arthritis; Lyme disease may also present with various CNS infections.

- ❖ **Manifestations:** After several weeks or months, ~15% of untreated patients develop frank neurologic abnormalities, including meningitis, subtle encephalitic

signs, cranial neuritis (including bilateral facial palsy), and radiculoneuropathy

- ❖ **CSF findings** include elevated lymphocytes (~100 cells/ μ L), elevated protein levels and normal or slightly low glucose level
- ❖ **Meningopolyneuritis:** This is seen in cases from Europe and Asia, which presents as radicular pain with CSF pleocytosis, without meningeal or encephalitic signs. This is called as **Bannwarth's syndrome**
- ❖ These early neurologic abnormalities usually resolve completely within a month, but rarely **chronic neurologic disease** may occur later
- ❖ **Treatment:** IV ceftriaxone for 14–28 days is recommended for neuroborreliosis. Alternatively IV cefotaxime or IV penicillin G can be given.

Neurosyphilis (*Treponema pallidum*)

Neurosyphilis is a type of tertiary form of syphilis, which develops in about 10% of untreated patients.

- ❖ Syphilis is a sexually transmitted disease, discussed in detail in Chapter 77
- ❖ Invasion of CNS occurs early within first few weeks of infection, which is followed by years of asymptomatic period.

Clinical Manifestations

Neurological manifestations appear usually several decades after the initial infection. Common manifestations include:

- ❖ **Asymptomatic neurosyphilis:** This may be seen in up to 25–40% of cases; may present with CSF abnormalities without any clinical manifestations. Very rarely, acute meningitis may be seen in 1–2% of cases of secondary syphilis
- ❖ **Meningeal syphilis:** Presents as features of chronic meningitis
- ❖ **Meningovascular syphilis:** Presents with vasculitis of arteries leading to embolic stroke
- ❖ **General paresis of insane:** It is a late manifestation, presents with features of parenchymal damage such as (mnemonic '*paresis*)—defects in **personality**, **affect**, **reflexes** (hyperactive), **eye** (e.g. Argyll Robertson pupils), **sensorium** (illusions, delusions, hallucinations), **intellect** (recent memory loss), and **speech**
- ❖ **Tabes dorsalis:** It is also a late manifestation, presents with features of demyelination of the posterior columns such as ataxia, foot drop, paresthesia, bladder disturbances and impotence.

Laboratory Diagnosis

Neurosyphilis is diagnosed by the following tests.

- ❖ **CSF analysis:** CSF examination reveals increased lymphocytes (>5/ μ L), and increased protein level (>45 mg/dL)
- ❖ **CSF VDRL test:** Venereal disease research laboratory (VDRL) is a nontreponemal test, detects antibodies

against a nonspecific cardiolipin antigen derived from bovine heart

- It is highly specific and, when reactive, is considered diagnostic of neurosyphilis. However, it has low sensitivity
- Other nontreponemal test like RPR (rapid plasma reagin) test cannot be performed on CSF, therefore should not be used in substitution of VDRL for neurosyphilis.
- ❖ The **FTA-ABS test** (fluorescent treponemal antibody absorption) on CSF is reactive far more often than the CSF VDRL test in all stages of syphilis, but reactivity may reflect passive transfer of serum antibody into the CSF. A nonreactive FTA-ABS test on CSF however may be used to rule out asymptomatic neurosyphilis

- ❖ **PCR-based tests** have a high reliability; can amplify *T. pallidum* specific genes, such as gene coding for 47-kDa surface antigen (lipoprotein) and 39-kDa basic membrane.

TREATMENT

Neurosyphilis

Aqueous crystalline or procaine penicillin G is given for 10–14 days for neurosyphilis or in cases of abnormal CSF in any stage.

- Consider re-treatment if non-treponemal titres in CSF do not decrease by four-folds within 2 years of completion of treatment
- In patients with penicillin allergy, desensitization to penicillin has to be done, following which penicillin is administered.

EXPECTED QUESTIONS

I. Write essays on:

1. A 7-year-old girl was admitted to the hospital with complaints of high-grade fever, headache, vomiting, altered mental status, seizure and neck rigidity. CSF sample was collected by lumbar puncture in a sterile container and sent to the laboratory for biochemical analysis, direct microscopic test, culture and sensitivity testing.

Questions:

- a. What is the probable clinical diagnosis?
 - b. What are the etiological agents, pathogenesis and clinical manifestations of this disease?
 - c. Describe the laboratory diagnosis in detail?
 - d. What are the treatment modalities according to the etiological agents?
2. Alisha, A 4-year-old girl from Bhubaneswar was brought to the emergency room by her parents due to an acute onset of fever, neck rigidity and altered sensorium for the past 2 days. Physical examination showed that when her neck was passively flexed, her legs also flexed (positive Brudzinski's sign). Direct examination of the CSF showed gram-positive, lanceolate-shaped diplococci surrounded by a clear halo.
 - a. Identify the clinical diagnosis of this condition and the most likely etiologic agent?
 - b. How will you confirm the etiological diagnosis in the laboratory?

II. Write short notes on:

1. Listeriosis.
2. Tubercular meningitis.

III. Multiple Choice Questions (MCQs):

1. Neonatal meningitis acquired through infected birth canal is due to:

- a. *S. pyogenes*
- b. Viridans streptococci
- c. *S. agalactiae*
- d. *S. pneumoniae*

2. Carrom coin appearance of colonies is seen for:

- a. *S. pyogenes*
- b. Viridans streptococci
- c. *S. agalactiae*
- d. *S. pneumoniae*

3. Which is not a property of *S. pneumoniae*?

- a. Bile solubility
- b. Animal pathogenicity in mice
- c. Growth in presence of 40% bile
- d. Optochin sensitivity

4. Serotyping and serosubtyping of meningococci are based on:

- a. Outer membrane proteins
- b. Endotoxin
- c. Capsular polysaccharide
- d. Transferrin binding proteins

5. Which of the following is not a common cause of neonatal meningitis?

- a. *E. coli*
- b. *S. agalactiae*
- c. *Listeria*
- d. *S. pneumoniae*

6. Biochemical analysis of pyogenic meningitis reveals all of the following, except:

- a. CSF pressure: highly elevated
- b. Total leukocyte count: Highly elevated, neutrophilic
- c. Glucose: highly elevated
- d. Total proteins: markedly increased

Answers

1. c 2. d 3. c 4. c 5. d 6. c

CHAPTER PREVIEW

- Pathogenesis
- Clinical Manifestations
- Epidemiology
- Laboratory Diagnosis
- Treatment
- Prevention

TETANUS (CLOSTRIDIUM TETANI)

Clostridium tetani is an obligate anaerobic, gram-positive bacillus with terminal round spore (drum stick appearance).

- ❖ It is the causative agent of ‘tetanus’—an acute disease, manifested by skeletal muscle spasm and autonomic nervous system disturbance
- ❖ Tetanus has been known since ancient time; however, its causative organism was isolated later by Kitasato (1889)
- ❖ *C. tetani* is ubiquitous in nature, widely distributed in soil, hospital environment and in the intestine of man and animals.

Pathogenesis**Virulence Factors**

C. tetani produces two exotoxins—tetanolysin and tetanospasmin.

1. **Tetanolysin:** It is a hemolysin, has no role in the pathogenesis of tetanus
2. **Tetanospasmin (or tetanus toxin):** It is a neurotoxin responsible for the disease manifestations
 - It is antigenic and is specifically neutralized by its antitoxin
 - It gets toxoided spontaneously or by formaldehyde. The toxoid form is antigenic, but loses its virulence property, hence, it is used for vaccine preparation
 - Tetanus toxin is plasmid coded, its mechanism of action is given below.

Mechanism of Action of Tetanus Toxin

Tetanus toxin binds to receptors (polysialogangliosides) present on motor nerve terminals which results in toxin internalization.

- Following internalization, tetanus toxin gets transported in retrograde way to the gamma-aminobutyric acid (GABA) and glycine producing inhibitory neuron terminals
- The toxin prevents the presynaptic release of inhibitory neurotransmitters glycine and GABA, which leads to spastic muscle contraction.

Mode of Transmission

Tetanus bacilli enter through:

- ❖ Injury (superficial abrasions, punctured wounds, road traffic accidents)
- ❖ Surgery done without proper asepsis
- ❖ **Neonates:** Following abortion/delivery, due to unhygienic practices
- ❖ Otitis media (otogenic tetanus)

It is noninfectious: There is no person-to-person spread.

Clinical Manifestations

The incubation period is about 6–10 days. Shorter the incubation period, graver is the prognosis. Muscles of the face and jaw are often affected first (due to shorter distances for the toxin to reach the presynaptic terminals).

- ❖ **First symptom:** Increase in the masseter tone leading to **trismus** or **lockjaw**, followed by muscle pain and stiffness, back pain, and difficulty in swallowing
- ❖ **In neonates**, difficulty in feeding is the initial presentation
- ❖ **As the disease progresses**, painful muscle spasm develops which may be:
 - **Localized:** Involves the affected limb
 - **Generalized** painful muscle spasm → leads to descending spastic paralysis.
- ❖ Hands, feet are spared and mentation is unimpaired. Deep tendon reflexes are exaggerated
- ❖ **Autonomic disturbance** is maximal during the second week of severe tetanus—characterized by low or high blood pressure, tachycardia, intestinal stasis, sweating, increased tracheal secretions and acute renal failure.

Complications

Eventually, the following complications may be developed.

- ❖ **Risus sardonicus:** It is characterized by an abnormal, sustained spasm of the facial muscles that appears to produce grinning (Fig. 72.1A)



Figs 72.1A to C: **A.** Lockjaw and the facial spasms (risus sardonius); **B.** Patient with opisthotonos seen in tetanus; **C.** Gram stained smear of *Clostridium tetani* showing round terminal spore bearing gram-positive bacilli.

Source: A. Wikia/Hoidkempuhtust; B. Public Health Image Library, ID# 6373; C. ID# 12056/Dr Holdeman/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Opisthotonos position:** It is an abnormal posture of the body, occurs due to generalized spastic contraction of the extensor muscles (Fig. 72.1B)
- ❖ Respiratory muscles spasm: May cause airway obstruction.

Epidemiology

Tetanus is more common in developing countries including India, which is attributed various risk factors such as—(i) warm climate, (ii) rural area with fertile soil, and (iii) unhygienic surgeries or deliveries.

- ❖ In 2018 (WHO report), total of 15,103 cases of tetanus have been reported worldwide; out of which 7,000 (~46%) cases were from India
- ❖ However, the incidence in India has been reduced to a large extent (>45,000 cases in 1980), which is due to widespread immunization of infants and pregnant mothers.

Laboratory Diagnosis

Treatment should be started immediately based on clinical diagnosis. Laboratory diagnosis provides supportive evidence for confirmation.

Specimen

Excised tissue bits from the necrotic depths of wounds are more reliable than wound swabs.

Gram Staining

- ❖ Gram staining reveals gram-positive bacilli with terminal and round spores (drum stick appearance) (Fig. 72.1C)
- ❖ However, microscopy alone is unreliable as it cannot distinguish *C. tetani* from morphologically similar non-pathogenic clostridia like *C. tetanomorphum* and *C. sphenoides*.

Culture

Culture is more reliable than microscopy.

- ❖ **Robertson cooked meat broth:** *C. tetani*, being proteolytic turns the meat particles black and produces foul odor (Fig. 53.1C, Chapter 53)
- ❖ **Blood agar with polymyxin B:** These plates are incubated at 37°C for 24–48 hours under anaerobic condition. *C. tetani* produces characteristic **swarming growth**.

Toxicogenicity Test

As pathogenesis of tetanus is toxin mediated, the association of the isolated organism can only be established when its toxin production is demonstrated. Toxin assay can be performed by **in vivo mouse inoculation test** on specimens such as serum and urine.

TREATMENT

Tetanus

Passive immunization (tetanus immunoglobulin)

It is the treatment of choice for tetanus.

- ❑ **Two preparations are available:**
 1. HTIG (Human tetanus immunoglobulin), prepared in Serum Institute of India, Pune
 2. ATS (Antitetanus serum, equine derived)
- ❑ **Dosage:** 250 IU of HTIG or 1,500 IU of ATS is given as a single IM dose. Intrathecal route is more effective
- ❑ **Duration of protection:** Effect of HTIG and ATS last for 30 days and 7–10 days respectively
- ❑ HTIG is preferred over ATS as the latter is associated with side effects such as serum sickness and anaphylactoid reactions.

Combined Immunization

(Both active and passive immunization):

In nonvaccinated person, it is ideal to immunize with first dose of tetanus toxoid (TT) vaccine in one arm along with administration of ATS or HTIG in another arm, followed by a complete course of TT vaccine, as per the schedule described later.

Antibiotics:

Antibiotics play only a minor role as they cannot neutralize the toxins which are already released.

- ❑ However, they are useful:
 - In early infection, before expression of the toxin (<6 hours)
 - To prevent further release of toxin.

Contd...

Contd...

TREATMENT Tetanus

- Metronidazole is the drug of choice; given for 7–10 days. Penicillin or doxycycline can be given alternatively.

Other measures:

- Symptomatic treatment: (i) **Endotracheal intubation** and **early tracheostomy** may be useful to protect the airway; (ii) **anti-spasmodic** (e.g. diazepam) to eliminate the reflex spasms; (iii) **Beta-blockers** to control sympathetic hyperactivity
- Surgical debridement: Entry wound should be identified, cleaned and debrided of necrotic material, so as to remove the anaerobic foci of infection
- Patient should be isolated in a separate room as any noxious stimulus (e.g. light) can aggravate the spasm.

Note: Clinical tetanus does not confer immunity and therefore, vaccine series should be completed after recovery.

Prevention

Active Immunization (Vaccine)

It is the most effective method of prophylaxis.

- ❖ **Tetanus toxoid (TT)** is commonly used for active immunization. It is available either as:
 - Monovalent vaccine: Tetanus toxoid is (TT) is prepared by incubating toxin with formalin to become toxoid and then adsorbed on to alum
 - Combined vaccine: Refer Chapter 60 for detail—
 - ◆ **DPT vaccine** (consists of diphtheria toxoid, pertussis whole cell killed preparation and tetanus toxoid)
 - ◆ **Td vaccine** (tetanus toxoid and adult diphtheria toxoid)
 - ◆ **Pentavalent vaccine** (DPT, hepatitis B and Hib)
- ❖ **Primary immunization of children:** Tetanus toxoid is given under National Immunization Schedule of India. Total ‘seven doses’ are given—
 - Three doses of pentavalent vaccine at 6, 10 and 14 weeks of birth, followed by
 - Two booster doses of DPT at 16–24 weeks and 5 years followed by
 - Two additional doses of Td at 10 years and 16 years
- ❖ **Adult immunization:** If primary immunization is not administered in childhood, then adults can be immunized with Td (tetanus toxoid and adult diphtheria toxoid) (Chapter 60)
- ❖ **Site:** Tetanus vaccine is given by deep intramuscular route at anterolateral aspect of thigh (children) and in deltoid (adults)
- ❖ **Protective titer:** Persons are said to be protected if tetanus antitoxin titre is ≥ 0.01 IU/mL.

Prevention of Tetanus after Injury

All types of wounds need surgical toilet followed by immunization which depends on the wound type and immunization status of the individual (Table 72.1).

Table 72.1: Recommendation for prevention of tetanus after injury.

Complete course of vaccine	Not tetanus-prone wound	Tetanus-prone wound*
Taken within 5 years	Nothing required	Nothing required
Taken within >5 to <10 year	Td 1 dose	Td 1 dose
Taken >10 years back	Td 1 dose	Td 1 dose + HTIG
Unknown/ incomplete immunization	Td complete dose	Td complete dose + HTIG

***Tetanus-prone wound** includes- age of wound > 6 hours, penetrating wound (missiles, crush, burn, bite), depth of wound >1 cm, presence of devitalized tissue and contaminants (dirt, saliva, etc.)

Abbreviations: Td, tetanus toxoid and adult diphtheria toxoid; HTIG, human tetanus immunoglobulin.

Prevention of Neonatal Tetanus

Neonatal tetanus is defined by WHO as ‘an illness occurring in a child who loses ability to suck and cry between day 3 and 28 of life and becomes rigid and has spasms’. It is also known as “**8th day disease**” as the symptoms usually start after 1 week of birth (Fig. 72.2).

- **Most common reason:** Unhygienic practices during deliveries such as infected umbilical stumps due to application of cow dung, rarely by circumcision or by ear piercing
- **Seasonal:** Neonatal tetanus is seasonal—more common in July, August and September months
- **Neonatal tetanus can be prevented by:**
 - Discouraging home deliveries and promoting hospital or attended deliveries
 - Following aseptic **clean practices** are followed during deliveries—clean hand, clean surface, clean blade for cutting cord, clean cord tie, clean cord stump, clean towel and clean water
 - Td (2 doses) are given to all pregnant women during 2nd trimester at 1 month gap.
- **Neonatal tetanus elimination is based on:**
 - Neonatal tetanus rate: <1/1,000 live births in every districts of country
 - Td coverage to pregnant women >90%
 - Attended deliveries >75%.
- **Situation in India:** India has achieved the elimination status for neonatal tetanus; as declared by WHO in 2016. In 2018, around 129 cases of neonatal tetanus were reported in India; compared to 1,803 cases in the world.



Fig. 72.2: Neonatal tetanus (neonate displaying a bodily rigidity).

Source: Public Health Image Library, ID#6374/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

EXPECTED QUESTIONS

I. Write essay on:

1. 3–5 days following a bullet injury, a person developed trismus followed by muscle pain and stiffness, back pain, and difficulty in swallowing. Excised tissue bits from the necrotic depths of the wound revealed gram-positive bacilli with terminal and spherical spores.
 - a. What is the probable diagnosis of this clinical condition?
 - b. Describe in detail the pathogenesis and clinical manifestations, laboratory diagnosis of this condition.
 - c. Add a note on vaccination to prevent this condition.

II. Multiple Choice Questions (MCQs):

1. **The most effective way of preventing tetanus:**
 - a. Hyperbaric oxygen
 - b. Antibiotics
 - c. Tetanus toxoid
 - d. Surgical debridement and toilet
2. **Spore with drum stick appearance is produced by:**
 - a. *C. bifermentans*
 - b. *C. perfringens*
 - c. *C. tetani*
 - d. *C. tertium*
3. **A 32-year-old male has got clean wound without laceration. He had a booster dose of TT 6 years back. What is next line of management?**
 - a. Wound care with single dose of tetanus toxoid
 - b. Wound care with human tetanus Ig with tetanus toxoid single dose
 - c. Wound care with complete course of tetanus toxoid
 - d. Wound care with no immunization
4. **A patient is presented with trismus with opisthotonus position. The probable causative agent is:**
 - a. *Clostridium tetani*
 - b. *Clostridium perfringens*
 - c. *Clostridium difficile*
 - d. *Clostridium tetanomorphum*
5. **Mechanism of action of tetanospasmin:**
 - a. Inhibition of GABA release
 - b. Inhibition cAMP
 - c. Inactivation of ACh receptors
 - d. Inhibition of cGMP
6. **A 25-year-boy is presented with deep injury and abrasions on the left shoulder, thigh and leg with immunization status unknown. What is to be given now?**
 - a. DTaP only
 - b. DTaP + Ig
 - c. Td only
 - d. Td + Ig
7. **A person has received complete immunization against tetanus 12 years ago. Now he presents with a contaminated wound with lacerations from an injury sustained 8 hours ago. He should now be given:**
 - a. Full course of tetanus toxoid
 - b. Single dose of tetanus toxoid
 - c. Human Tet globulin
 - d. Human tetanus globulin and single dose of toxoid

Answers

1. c 2. c 3. a 4. a 5. a 6. d 7. d

CHAPTER PREVIEW

■ Viral Meningitis

■ Viral Myelitis

● Poliomyelitis

VIRAL MENINGITIS

Viral meningitis, inflammation of subarachnoid space due to viral etiology. It is the second most common type of meningitis, next to acute bacterial meningitis. However, it is often less severe than bacterial meningitis and has a better prognosis.

ETIOLOGY

Enteroviruses are the most common cause of viral meningitis, accounting for >85% of cases.

Other less common viral agents causing acute meningitis are:

- ❖ **Herpesviruses**, including herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus and others
- ❖ **Arboviruses**: The encephalitis group of arboviruses can also occasionally cause meningitis, especially in people having travel history
- ❖ **LCM virus**: Lymphocytic choriomeningitis virus affects people with history of contact with rodent droppings or urine
- ❖ **Other causes**: Mumps virus, measles virus, influenza virus and human immunodeficiency virus (HIV).

EPIDEMIOLOGY

- ❖ **People at risk**: Although people of any age can get viral meningitis, some have a higher risk of acquiring the disease, including:
 - Children (< 5 years old) and old age
 - Immunocompromised individuals, chemotherapy or transplant recipients.
- ❖ **Transmission**: Close contacts with infected patients is necessary for the transmission to set in
- ❖ **Seasonality**: Most cases of viral meningitis occur from nonwinter months (from late spring to fall), the time when enteroviruses and arbovirus spread most often.

CLINICAL MANIFESTATIONS

Common symptoms in children and adults include fever, headache (frontal or retro-orbital), stiff neck (milder than bacterial meningitis), photophobia, sleepiness or trouble in waking up from sleep, nausea, irritability, vomiting, lack of appetite (poor eating in babies), and lethargy.

Note: Seizures or focal neurologic signs, or profound alterations in consciousness such as coma, or marked confusion does not occur in viral meningitis and suggest the presence of encephalitis or other alternative diagnoses.

LABORATORY DIAGNOSIS

Laboratory diagnosis of viral meningitis includes the following investigations.

CSF Analysis (Cytological and Biochemical)

Examination of CSF reveals the following (Table 71.1.):

- ❖ Normal or slightly elevated protein level (20–80 mg/dL)
- ❖ Normal glucose level (rarely low glucose level may occur, as in cytomegalovirus meningitis)
- ❖ Normal or mildly elevated CSF pressure (100–350 mm H₂O)
- ❖ **Cell count** is typically 25–500/μL, although in some viral meningitis (e.g. LCM virus and mumps) the cell counts of several thousands/μL may be seen
- ❖ **Pleocytosis**: Lymphocytes are typically the predominant cell type, although neutrophils may predominate in the first 48 h of illness in some viral meningitis (e.g. West Nile virus)
- ❖ Organisms are not seen on Gram staining of CSF.

Molecular Methods

Molecular methods have become the gold standard method for diagnosing viral meningitis, appears to be more sensitive than viral cultures.

- ❖ Amplification of specific viral DNA or RNA from CSF by PCR based method provides definitive diagnosis

- ❖ PCR of throat washings or stool specimen may assist in the diagnosis of enterovirus infections
- ❖ **Formats:** Multiplex PCR and multiplex real-time PCR can be used for simultaneous detection of common agents of viral meningitis
- ❖ **BioFire FilmArray** is an automated nested multiplex PCR commercially available that can simultaneously detect 14 common agents of meningitis in CSF, which includes agents of pyogenic and viral meningitis. It is extremely sensitive and specific, with a turnaround time of 1 hour.

Viral Culture

The sensitivity of CSF cultures for the diagnosis of viral meningitis is generally poor.

- ❖ In addition to CSF, specific viruses may also be isolated from throat swabs, stool, blood, and urine
- ❖ However, isolation of enteroviruses from stool is not diagnostic as it may also result from residual fecal shedding from a previous infection.

Antibody Detection

Antibody detection is important for the diagnosis of less prevalent arboviruses such as West Nile virus, however it is of less useful for viruses that have a high seroprevalence in the general population, such as HSV and VZV.

Oligoclonal Gamma Globulin Bands

They may be detected in CSF in a number of viral infections; although can also be raised in other conditions such Lyme disease and multiple sclerosis.

TREATMENT

Viral meningitis

Treatment of almost all cases of viral meningitis is **primarily symptomatic**, which includes use of analgesics, antipyretics, antiemetics and fluid and electrolyte replacement. Antivirals may be useful for certain viral agents.

- ❑ Oral or intravenous acyclovir may be of benefit in patients with meningitis caused by HSV-1 or 2 and in cases of severe EBV or VZV infection
- ❑ Patients with HIV meningitis should receive highly active antiretroviral therapy.

AGENTS CAUSING VIRAL MENINGITIS

Non-polio Enterovirus Infections

Non-polio enteroviruses are the most common cause of viral meningitis, accounting for >85% of cases in which a specific etiology can be identified.

- ❖ Cases may either be sporadic or occur in clusters
- ❖ Although cases can occur throughout the year, affecting any age, but majority occur in the summer and rainfall, especially in children
- ❖ Examples include Coxsackieviruses, echoviruses, parechoviruses and Enterovirus 71.

Taxonomy (Picornaviridae)

Enteroviruses belong to picornaviruses (family Picornaviridae); which include two major groups of human pathogens: enteroviruses, and rhinoviruses. Morphology of picornaviruses has been discussed subsequently under polioviruses.

1. **Enteroviruses:** They are transmitted by feco-oral route, but do not cause any intestinal manifestations. They are associated with various systemic manifestations. They comprise of the following viruses which further divide into several (>115) human serotypes
 - Polioviruses—cause myelitis, discussed subsequently in this chapter
 - Coxsackieviruses, echoviruses, parechoviruses and Enterovirus 71— cause aseptic meningitis, discussed below.
 - Other enteroviruses include—
 - ♦ Enterovirus 68—causes pneumonia
 - ♦ Enterovirus 70—causes acute hemorrhagic conjunctivitis (Chapter 78). It uses the CD55 as host cell receptor, same as for poliovirus
 - ♦ Enterovirus 72—is reclassified as hepatitis A virus (discussed in Chapter 48).
2. **Rhinoviruses** comprise of >100 antigenic types. They are transmitted by respiratory route and cause common cold (Chapter 68).

Coxsackieviruses

Coxsackieviruses (named after the place of discovery; Coxsackie village in USA) can be divided into two groups, A and B, based on their pathogenic potentials for suckling mice.

Serotypes: Group A coxsackieviruses are typed into serotypes 1–24 (except 15, 18 and 23) and group B are typed into serotypes 1–6.

Clinical Manifestations

Coxsackieviruses can spread through an infected person's nasal and throat secretions, fluid from blisters or scabs. They produce a variety of clinical illnesses in humans associated with different serotypes. The incubation period ranges from 2 to 9 days.

- ❖ **Aseptic meningitis:** It is caused by all types of group B coxsackieviruses and by many group A coxsackieviruses (most commonly A7 and A9)
- ❖ **Herpangina:** It is a severe febrile vesicular pharyngitis that is caused by certain group A viruses (type 2–6, 8, 10)
- ❖ **Hand-foot-and-mouth disease:** It is characterized by oral and pharyngeal ulcerations and vesicular rashes of the palms and soles which heal without crusting. It is particularly associated with coxsackievirus A16 (Chapter 56)
- ❖ **Pleurodynia** (also known as **Bornholm disease** or epidemic myalgia): It is caused by coxsackie B viruses. It is characterized by fever and abrupt onset of stabbing chest pain

- ❖ **Cardiac:** Myocarditis and pericarditis are caused by coxsackievirus B types 1–5
 - ❖ **Respiratory:** Coxsackieviruses A and B have been associated with common colds. Pneumonia may be caused by coxsackieviruses B4 and 5
 - ❖ **Acute hemorrhagic conjunctivitis:** It is caused by coxsackievirus A24 and enterovirus 70
 - It is a self-limiting subconjunctival hemorrhage. Incubation period is about 1 day. Complete recovery occurs within 8–10 days
 - It had caused explosive epidemics among adults, during 1969–71 in Africa and Southeast Asia.
 - ❖ **Generalized disease of infants:** It is an extremely serious disease involving multiple organs, caused by group B coxsackieviruses
 - ❖ **Pancreatitis** leading to juvenile diabetes mellitus is caused by coxsackievirus B4.
- The differences between group A and B coxsackieviruses are given in Table 73.1.

Laboratory Diagnosis

- ❖ **Specimen collection** depends on the type of infection. Important specimens include throat swabs, stool and CSF
- ❖ **Isolation of the virus:** Coxsackieviruses can be recovered by:
 - Intracerebral inoculation into suckling mice (obsolete now):
 - ♦ Coxsackie-A produce flaccid paralysis
 - ♦ Coxsackie-B produce spastic paralysis.
 - **Inoculating into tissue culture:** Cytopathic effect can be observed within 5–14 days.
- ❖ **PCR** targeting specific genes (e.g. VP1) is highly useful as it is rapid, more sensitive and serotype-specific
- ❖ **Serology** is performed to detect neutralizing antibodies.

Echoviruses

Echoviruses (enteric cytopathogenic human orphan viruses) infect humans by feco-oral route. They were named

‘orphan’ viruses because at the time of their discovery, they were not attributed to any disease.

- ❖ Echoviruses are further typed into serotypes 1–33 (there are no types—8, 10, 22, 23 or 34), but not all cause human illness
- ❖ They are associated with aseptic meningitis, encephalitis, rashes, common cold, and ocular disease
- ❖ They can cause outbreaks in summer especially among children.

Parechoviruses

Parechoviruses have 16 serotypes:

1. Serotype 1 and 2 were previously classified as echoviruses 22 and 23 respectively
2. Their capsid consists of three viral proteins (in contrast to four proteins in most picornaviruses)
3. They have been rarely associated with aseptic meningitis, respiratory and neonatal diseases.

Enterovirus 71

Enterovirus 71 has caused large epidemic of meningitis in Southeast Asia. In addition, it can also cause encephalitis, hand-foot-and-mouth disease and herpangina (similar to coxsackieviruses) and paralysis resembling poliomyelitis.

Herpesvirus Meningitis

A number herpesviruses can cause meningitis.

- ❖ The common agents are herpes simplex viruses, varicella-zoster virus (VZV) and Epstein-Barr virus (EBV)
- ❖ Rare causes include cytomegalovirus and human herpesvirus 6.

HSV Meningitis

Herpes simplex viruses (HSV) are the second most common cause of viral meningitis, next to enteroviruses; accounting for 5% of cases. Adults are commonly affected than children.

- ❖ HSV-2 is a more frequent cause of meningitis than HSV-1; in contrast to HSV encephalitis, where HSV-1 accounts for >90% of cases
- ❖ **History of genital herpes** may be an important clue as HSV meningitis can occur in ~25–35% of women and ~10–15% of men at the time of an initial (primary) episode of genital herpes.

Mollaret meningitis

HSV typically produces a chronic recurrent lymphocytic meningitis, called as **Mollaret meningitis** (named after Pierre Mollaret, who described it in 1944). It is characterized by repeated episodes of meningitis, typically lasting two to five days; occurring weeks to years apart. It can also be caused by EBV.

Other neurological manifestations caused by HSV include:

Table 73.1: Differences between group-A and group-B Coxsackieviruses.

Group A coxsackieviruses	Group B coxsackieviruses
Suckling mouse intracerebral inoculation	
<ul style="list-style-type: none"> • Flaccid paralysis • Generalized myositis 	<ul style="list-style-type: none"> • Spastic paralysis in mice • Focal myositis and necrosis of brown fat
Manifestations	
<ul style="list-style-type: none"> • Aseptic meningitis (A7, A9) • Herpangina (vesicular pharyngitis) • Hand-foot-and-mouth disease (also caused by enterovirus 71) • Acute hemorrhagic conjunctivitis: Caused by coxsackievirus-A24 (and also by enterovirus 70) 	<ul style="list-style-type: none"> • More organ involvement seen • Aseptic meningitis (B1 to 6) • Pleurodynia (epidemic myalgia or Bornholm disease) • Myocarditis, pericarditis • Hepatitis • Pancreatitis leading to Juvenile diabetes mellitus: Coxsackie B4 • Pneumonia • Generalized disease of infants

- ❖ **HSV encephalitis:** HSV is the most common cause of acute sporadic viral encephalitis (Chapter 74)
- ❖ **Bell's palsy:** HSV-1 and 2 are one of the most common cause of facial nerve (VII cranial nerve) palsy
- ❖ **Autonomous system involvement** (sacral region) is common; characterized by numbness, tingling of the buttock areas, urinary retention, constipation, and (in males) impotence
- ❖ **Transverse myelitis** may occur rarely; manifested by a rapidly progressive symmetric paralysis of the lower extremities
- ❖ Guillain-Barré syndrome
- ❖ Peripheral nervous system involvement.

HSV mainly produces cutaneous and mucocutaneous lesions; discussed in detail in Chapter 56.

Other Herpesviruses

- ❖ **VZV meningitis** should be suspected in the presence of concurrent chickenpox or shingles; although cases of VZV meningitis and encephalitis can occur in patients without history of rash
- ❖ **EBV meningitis:** Patients may have history of associated infectious mononucleosis and may demonstrate presence of atypical lymphocytes in the CSF or peripheral blood.

Arboviral Meningitis

The encephalitic arboviruses (Chapter 74) can sometime cause meningitis, especially in individuals who have recently travelled to the areas where these viruses are endemic. They are transmitted by the bite of their arthropod vectors.

- ❖ **West Nile virus:** Endemic in Americas, Africa, West Asia; mainly transmitted by *Culex*
- ❖ **Saint Louis encephalitis virus:** Endemic in United States, transmitted by *Culex*
- ❖ **Tick-borne encephalitis** viruses such as Powassan virus or Colorado tick fever virus
- ❖ **California encephalitis virus:** Endemic in United States, transmitted by *Aedes*.

Non-encephalitic arboviruses which can cause meningitis are Zika virus (Brazil) and Oropouche virus.

HIV Meningitis

Meningitis in HIV infection (Chapter 33) may occur following primary infection in 5–10% of cases and less commonly at later stages of illness. Cranial nerve palsies—especially involving cranial nerves V, VII, or VIII, are more common in HIV meningitis than in other viral infections.

Mumps Meningitis

Mumps mainly cause parotitis (Chapter 66). Meningitis in mumps may occur secondary to parotitis, or rarely in about 10% of cases, patients may directly develop meningitis without an underlying history of parotitis (called as atypical mumps). Mumps meningitis is more common in the late winter or early spring, especially in unvaccinated children with a male preponderance.

LCM Virus Meningitis

Lymphocytic choriomeningitis (LCM) virus affects people with history of contact with rodent droppings or urine. Some patients have an associated rash, pulmonary infiltrates, alopecia, parotitis, orchitis, or myopericarditis.

VIRAL MYELITIS

Myelitis is inflammation of the spinal cord which can result in disruption of the connection from the brain to the rest of the body, and vice-versa. **Viral myelitis** is the most common infectious cause of myelitis; which may be of two types.

- ❖ **Gray matter myelitis:** It is usually caused by infections of the anterior horn of the spinal cord; results in acute flaccid paralysis
 - It is most often caused by poliovirus (the disease is known as poliomyelitis)
 - Also caused by other viruses such as other enteroviruses (enteroviruses 70 and 71, echoviruses, coxsackieviruses A and B) and the arboviruses (West Nile, Japanese encephalitis, tick-borne encephalitis).
- ❖ **Transverse myelitis** or leukomyelitis, or white matter myelitis: Often caused by the herpesviruses and influenza virus, rarely HIV and HTLV. It can result from direct viral invasion or via immune-mediated mechanisms.

Poliomyelitis being the most important type of viral myelitis is discussed below. The other viruses causing myelitis, principally produce infections of other systems and therefore discussed elsewhere.

POLIOMYELITIS

Polioviruses cause a highly infectious childhood disease called polio (or poliomyelitis) causing acute flaccid paralysis due to involvement of nervous system. Polio is in the verge of eradication globally.

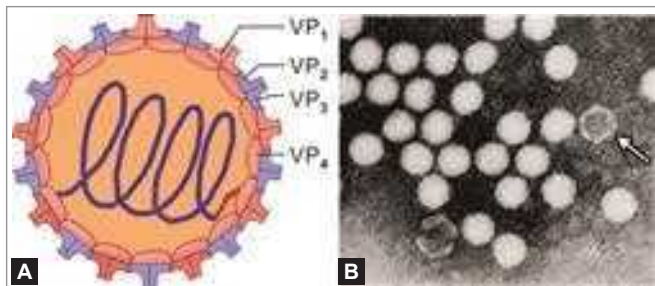
Morphology

Polioviruses are type of enteroviruses, belong to the family Picornaviridae. Poliovirus and other picornaviruses are simple in structure, appear very small (28–30 nm size) and nonenveloped.

- ❖ They are spherical shaped and have icosahedral symmetry (Figs 73.1A and B)
- ❖ Capsid is composed of 60 subunits, each consisting of four viral proteins (VP1-VP4), except parechoviruses (have three proteins)
- ❖ Possess single-stranded positive sense linear RNA.

Antigenic Types

Polioviruses can be classified into wild polioviruses; which cause natural disease and vaccine derived poliovirus (VDPV), which are the vaccine strains that have regained neurovirulence and are capable of producing disease in man. VDPV has been described subsequently in this chapter.



Figs 73.1A and B: Poliovirus: **A.** Schematic diagram; **B.** Transmission electron micrograph (arrow showing).

Source: B. Public Health Image Library, ID#235/Dr Joseph J Esposito, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Wild Poliovirus (WPV)

There are three wild poliovirus strains: Wild poliovirus type 1 (WPV1), wild poliovirus type 2 (WPV2) and wild poliovirus type 3 (WPV3).

- ❖ All three strains are identical, produce similar manifestations and severity of illness
- ❖ However, they are genetically and immunologically-distinct; differ from each other in VP1 region. The antibody response is type-specific and not cross-protective. Therefore, each strain need to be eradicated individually
- ❖ Currently all the natural cases are caused by WPV1. It has also been the common serotype to cause poliomyelitis till now
- ❖ Both WPV2 and WPV3 are globally eradicated, in the years 1999 and 2019 respectively.

Pathogenesis

Polioviruses are transmitted by feco-oral route (most common), followed by respiratory droplets via inhalation or rarely by conjunctival contact

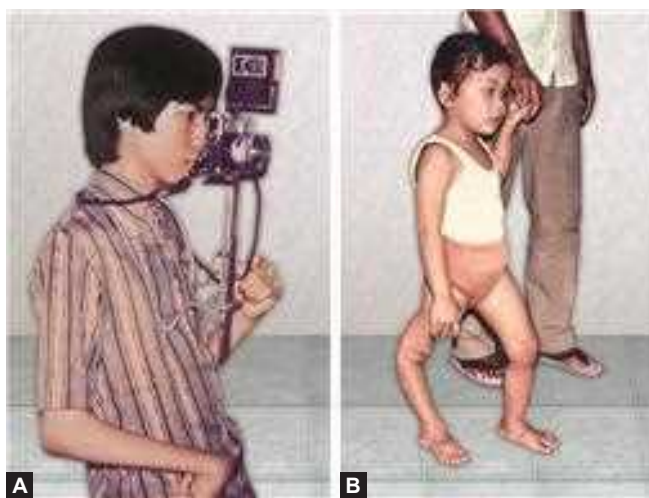
- ❖ **Multiply locally:** They multiply in intestinal epithelial cells, submucosal lymphoid tissues, tonsils and Peyer's patches
- ❖ **Receptor:** Viral entry into the host cells is mediated by binding to *CD155* receptors present on the host cell surface
- ❖ **Spread to CNS/spinal cord:**
 - **Hematogenous spread** (most common): Virus spreads to the regional lymph nodes and spills over to the bloodstream (primary viremia). After further multiplying in the reticuloendothelial system, the virus enters the bloodstream again, causing secondary viremia. Then it is carried to the spinal cord and brain
 - **Neural spread:** Virus may also spread directly through nerves. This occurs especially following tonsillectomy where the virus may spread via glossopharyngeal nerve present in the tonsillar fossa.
- ❖ **Site of action:** The final target site for poliovirus is the motor nerve ending, i.e. anterior horn cells of the spinal cord which leads to muscle weakness and flaccid paralysis

- ❖ **Neuron degeneration:** Virus-infected neurons undergo degeneration. Earliest change in neuron is the degeneration of **Nissl body** (aggregated ribosomes, normally found in the cytoplasm of neurons)
- ❖ Pathological changes are always more extensive than the distribution of paralysis.

Clinical Manifestations

The incubation period is usually 7–14 days. The manifestations may range from asymptomatic stage to the most severe paralytic stage.

- ❖ **Inapparent infection:** Following infection, the majority (91–96%) of cases are asymptomatic
- ❖ **Abortive infection:** About 5% of patients develop minor symptoms such as fever, malaise, sore throat, anorexia, myalgia, and headache
- ❖ **Nonparalytic poliomyelitis:** It is seen in 1% of patients, presented as aseptic meningitis
- ❖ **Paralytic poliomyelitis** is the least common form (<1%) among all the stages
 - It is characterized by descending asymmetric acute flaccid paralysis (AFP) (Figs 73.2A and B)
 - Proximal muscles are affected earlier than the distal muscles; paralysis starts at hip → proceeds towards extremities; which leads to the characteristic **tripod sign** (child sits with flexed hip, both arms are extended towards the back for support)
 - **Sites involved** can be spinal, bulbo-spinal and bulbar. Accordingly, the nature of paralysis varies (e.g. respiratory insufficiency or dysphagia are common in bulbar involvement)
 - **Biphasic course:** In children, the disease progression is typically biphasic; aseptic meningitis occurs first → recovery → return of fever with paralytic features 1–2 days later



Figs 73.2A and B: Deformities seen in poliomyelitis.

Source: Public Health Image Library, A. ID#: 5579; B. ID#: 5578, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- Cranial nerves are also involved occasionally
- However, there is no sensory loss.
- ❖ **Risk factors:** Paralytic disease is more common among:
 - Older children and adults
 - Pregnant women
 - Following heavy muscular exercise
 - Persons undergoing trauma at the time of CNS symptoms
 - Tonsillectomy: It predisposes to bulbar poliomyelitis
 - IM injections: They increase the risk of paralysis in the involved limb.

LABORATORY DIAGNOSIS

Poliomyelitis

- Virus isolation in primary monkey kidney cell line from throat swabs, rectal swabs or stool samples—viral growth is detected by CPE, viral antigen or viral gene in cell line
- Antibody detection—by neutralization test

Laboratory Diagnosis

Virus Isolation

- ❖ **Specimen:** Poliovirus may be recovered from the throat swabs (up to 3 weeks of illness) and from rectal swabs or stool samples (up to 12 weeks). However, long-term excretion has been observed in immunodeficient persons. Virus isolation from CSF or blood is very rare
- ❖ **Sewage testing:** Screening of sewage for detection of poliovirus (wild or vaccine virus) is routinely conducted under polio eradication program. This is to verify whether the transmission is on-going or interrupted
- ❖ **Transport:** Specimens should be kept frozen during transport to the laboratory
- ❖ **Cell line:** Primary monkey kidney cells are the most recommended cell lines. Virus growth can be identified by various methods
 - Cytopathogenic effects appear in 3–6 days; described as crenation and degeneration of the entire cell sheet
 - Antigen detection: Isolated virus can be identified and serotyped by neutralization with specific antiserum or by immunofluorescence test
 - Polymerase chain reaction (PCR) assay is available targeting VP1 region of poliovirus.

Antibody Detection

A rise in antibody titer in paired sera collected at 1–2 weeks interval is suggestive of poliomyelitis

- ❖ Neutralization test is the recommended method, which detects neutralizing antibodies
- ❖ Only first infection with poliovirus produces strictly type-specific responses
- ❖ Subsequent infections induce antibodies against group-specific antigen common to all the three serotypes.

Molecular Method

Real-time multiplex reverse-transcriptase PCR has been developed using primers from VP1 region, which can

detect and differentiate between various types of wild and vaccine polioviruses (VAPP and VDPV strains) directly from stool specimen.

Vaccine

Both inactivated and live attenuated polio vaccines are available; both have their unique useful properties as well as drawbacks (Table 73.2).

Injectable Polio Vaccine (IPV, Salk Vaccine)

- ❖ **Discovery:** Jonas Salk had prepared IPV in 1952
- ❖ **Preparation:** Virus is grown in **monkey kidney cell line** and inactivated by formalin. Each dose (0.5 mL) of vaccine contains total 80 units of antigen of all the three poliovirus serotypes (40 units of type 1, 8 units of type 2, and 32 units of type 3)
- ❖ **Dose:** IPV can be given either as (i) full dose (0.5 mL/dose), intramuscular (IM) route at thigh; or (ii) as fractional dose (f-IPV): 1/5th of full dose (0.1 mL), intradermal(ID) route at upper arm
- ❖ **National immunization schedule (India):**
 - In 2015, IPV was introduced in national immunization program as single full dose (IM route) at 14th week along with bivalent OPV
 - Since 2017, fractional dose IPV (f-IPV) is administered by ID route, scheduled at 6th and 14th weeks of age along with bivalent OPV
 - This change was made because (i) 2 f-IPV given by ID route at 6 and 14 weeks had shown to provide higher seroconversion rates than a single full dose (IM) given at 14 weeks, and (ii) cost saving (0.2 mL/2 doses of f-IPV vs 0.5 mL of full dose IPV).
- ❖ **Efficacy** of 80–90% is achieved after the full course of vaccination
- ❖ **Advantages:**
 - IPV is much safer than OPV, safer even in immunocompromised people
 - It does not cause vaccine-associated paralytic polio (VAPP)
 - It is more stable, does not require stringent storage conditions.
- ❖ **Disadvantages:**
 - It does not provide herd immunity: Being inactivated vaccine, it cannot spread by feco-oral route
 - It is not useful during epidemics; as there is no community protection. Instead, it can precipitate paralysis
 - It does not induce mucosal IgA production, hence, the local immunity is absent
 - It is relatively expensive than OPV.

Oral Polio Vaccine (OPV, Sabin Vaccine)

- ❖ **Discovery:** OPV was developed by Albert Sabin, Koprowski and Cox who prepared OPV independently almost around the same time (1955)

Polio vaccine	Injectable polio vaccine (Salk)	Oral polio vaccine (Sabin)
Formulations	IPV contains three serotypes 1, 2 and 3 It can be given in two doses : Full dose IPV and fractional-dose (f-IPV)	OPV is available as: <ul style="list-style-type: none"> • Trivalent OPV (serotypes 1, 2 and 3) • Bivalent OPV (serotype 1 and 3) • Monovalent OPV (any one serotype)
National immunization schedule (India, 2020)	Two fractional doses (intra-dermal route at upper arm, 0.1 mL/dose): Given at 6th and 14th weeks of age along with bivalent OPV	Total five doses (2 drops/dose, oral) of bivalent OPV (serotype 1 and 3): <ul style="list-style-type: none"> • Zero dose—given at birth • 1st, 2nd and 3rd dose —given at 6th, 10th, 14th week of age; booster given at 16–24 months
Safety	Relatively safer than OPV	Safe except in immunocompromised patients, pregnancy, old age
Efficacy	80–90% by full course of IPV Immune response is slower than OPV	90–100% efficacy is achieved even by 1 or 2 doses of OPV Efficacy decreases by: <ul style="list-style-type: none"> • Interference by other enteroviruses • Diarrheal diseases and breastfeeding
Economy	Relatively expensive	Economical
Duration of protection	Short, need booster doses periodically	Long-lasting
In epidemics	Can precipitate paralysis	Can be used safely
Herd immunity	Not provided	Provided due to feco-oral spread of vaccine virus
Local immunity	Weakly stimulated	Strongly stimulated (due to IgA antibody)
Can prevent	Only paralysis	Paralysis and intestinal re-infection
Storage condition	Relatively stable Does not require stringent condition	Should be stored at (–20°C) Stabilized in MgCl ₂ , pH<7
VAPP and VDPV	Zero chance	Relatively more chance

Abbreviations: OPV, oral polio vaccine; IPV, injectable polio vaccine; VAPP, vaccine-associated paralytic poliomyelitis; VDPV, vaccine-derived polioviruses.

- ❖ **Formulations:** OPV is available as (i) trivalent OPV (contains serotypes 1, 2 and 3); (ii) bivalent OPV (contains serotype 1 and 3), and (iii) monovalent OPV (contains any one serotype)
- ❖ **Preparation:** Each dose (0.5 mL) contains type 1 virus (3 lakh), type 2 virus (1 lakh, absent in bivalent OPV), type 3 virus (3 lakh) of TCID₅₀ (tissue culture infective dose-50)
- ❖ **National immunization schedule:** OPV is the vaccine recommended under national immunization schedule of India and most other countries of the world
 - Earlier trivalent OPV was used. As serotype 2 has been eradicated from the world since 1999, and there is a high-risk of causing vaccine virus induced paralysis, trivalent OPV is stopped and is replaced by bivalent OPV (1 and 3) since 2015
 - It is administered orally (two drops/dose). Total five doses are given
 - ◆ Zero dose: Given at birth; if missed, can be given within first 15 days
 - ◆ 1st, 2nd, 3rd doses: Given at 6, 10 and 14th weeks. (at 6th and 14th week, fractional IPV is given along with bivalent OPV)
 - ◆ Booster dose: Given at 16–24 months of birth.
- ❖ **Efficacy** is about 90–100%, which is achieved much faster (with one or two doses than IPV)
- ❖ **Advantages:** OPV has the several advantages over IPV
 - **Herd immunity:** OPV strains being live, can shed in the feces and spread in the community by feco-oral route, hence, it can induce herd immunity. It can provide both individual and community protection
 - OPV is the vaccine of choice during epidemics
 - **Local immunity:** OPV induces mucosal IgA production, hence provides local or mucosal immunity
 - Cheaper than IPV
 - Easy to administer (given by oral route).
- ❖ **Disadvantages:** OPV has the following drawbacks
 - **Safety:** OPV is otherwise safe, but it is risky to give in immunocompromised people, during pregnancy, and in old age
 - **Stability:** OPV is unstable vaccine, requires stringent conditions such as: (i) by storage at –20°C, (ii) stabilized in MgCl₂, and (iii) by maintaining pH <7
 - **Efficacy of OPV decreases by:**
 - ◆ **Interference** by other enteroviruses
 - ◆ **Diarrhea:** OPV gets washed away in diarrheal stool. Therefore, OPV given during diarrhea should not be counted. After recovery again a repeat dose should be given
 - ◆ **Breastfeeding:** OPV gets washed away in stool if given immediately before or after breastfeeding. Hence, breastfeeding should be avoided for 30 min before or after administration of OPV.

- OPV can cause vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived polioviruses (VDPV) (described below).

VAPP and VDPV

Vaccine-associated paralytic poliomyelitis (VAPP)

VAPP denotes all the cases of paralytic poliomyelitis that occur following OPV administration.

- VAPP strains are **OPV-like isolates**, which show limited genetic divergence from their parental OPV strains (<1%)
- VAPP can occur among OPV recipients as well as to their close contacts due to feco-oral spread
- However, VAPP strains are not capable of circulating in the community and do not cause secondary cases or outbreaks. This is largely because the spread of OPV-related virus is largely limited by high population immunity
- **VAPP rate:** VAPP occurs approximately at a rate of one case per 2.7 million doses of OPV
- **Most common serotype** associated with VAPP is Sabin type 3 (60%) followed by Sabin type 2 (40%).

Vaccine-derived polioviruses (VDPVs)

The live-attenuated vaccine strains present in oral polio vaccine may undergo genetic changes to become VDPV.

- VDPV is capable of producing poliomyelitis; clinically indistinguishable from wild polioviruses (due to regain of neurovirulence)
- **Three types** of VDPVs have been recognized; out of which circulating VDPVs (cVDPVs) is the most common types. Other two varieties are immunodeficiency-associated VDPVs (iVDPVs) and ambiguous VDPVs (aVDPVs)
- **cVDPV:** These strains can circulate in the community, spread person-to-person by feco-oral transmission and can cause outbreaks. They pose the same threat to the community as that of WPV
 - The genetic divergence (> 1%) is more than the strain that causes VAPP.
 - The occurrence of cVDPV cases are more common than VAPP as it can spread in community
 - Since 2000, cVDPV outbreaks have occurred in several countries including non-endemic countries which are already declared eradicated
 - Majority (90%) of cVDPV cases are due to type 2 followed by type-1
 - **In 2019:** 365 cases of cVDPV were reported; 325 of which were from non-endemic countries.

Epidemiology

- ❖ **Reservoir:** Man is the only known reservoir. Most cases are subclinical
- ❖ **Clinical-subclinical ratio:** For every clinical case, there may be 1,000 children and 75 adults of subclinical cases
- ❖ There are **no chronic carriers**. However, immunodeficient individuals may excrete the virus for longer periods
- ❖ **Source:** Infective materials such as stool and oropharyngeal secretions are the sources of infection
- ❖ **Age:** Younger children and infants are more susceptible to infection than adults. However in developed countries, there is shift of age; affecting older children

- ❖ **Period of communicability:** Patients are infectious, shedding the virus in the feces from 7–10 days before the onset of symptoms up to 2–3 weeks thereafter, sometimes as long as 3–4 months.

Polio Eradication

Poliomyelitis is now at the verge of eradication. This is attributed to the extensive immunization program being conducted globally.

Pulse Polio Immunization (PPI) was initiated globally to eradicate poliomyelitis. In India, it is in operation since 1995–96.

- ❖ Two rounds of PPI (6 weeks apart) are scheduled every year during the winter season (for e.g. 18th January and 11th March in 2018), where all children under the age of five years are vaccinated with bivalent-OPV irrespective of their OPV vaccination status
- ❖ PPI doses are considered as extra doses and they do not replace the OPV doses received under the routine national immunization schedule
- ❖ **AFP surveillance:** Acute flaccid paralysis surveillance has been conducted to identify all remaining infected areas and to monitor progress towards eradication.

Polio Situation in the World

Based on the status of polio transmission, the countries are classified into: (i) endemic country, (ii) outbreak country, (iii) key at-risk country, and (iv) polio free country (Table 73.3).

- ❖ **Endemic (PAN) countries:** Currently, wild polio is endemic only in three countries—Pakistan, Afghanistan and Nigeria (abbreviated as PAN countries). Nigeria reported the last wild case on August 2016
- ❖ **WPV cases:** In 2019, 174 wild cases were reported globally (Pakistan-145, Afghanistan-29). In addition, there were

Table 73.3: Classification of countries based on their polio transmission status as of August 2020*.

Endemic countries	Ongoing transmission of indigenous wild poliovirus Examples: PAN countries Pakistan, Afghanistan and Nigeria
Outbreak countries	Stopped indigenous wild poliovirus but are experiencing re-infection either by: <ul style="list-style-type: none"> • Importation of wild poliovirus or VDPV from another country, or • Emergence and circulation of VDPV There are 17 outbreak countries as of August 2020. To stop these outbreaks, it is necessary to implement outbreak response guidelines
Key at-risk countries	Low levels of immunity and surveillance leave countries at risk of polio returning There are 12 key at-risk countries, as of August 2020
Polio free countries	No transmission of indigenous wild poliovirus; No wild case has been reported for ≥3 years India and rest 178 countries fall in this category

*Source: Global Polio Eradication Initiative (GPEI).
Abbreviation: VDPV, vaccine-derived poliovirus.

365 cVDPV cases; out of which 325 were reported from non-endemic countries such as Angola and Congo

- ❖ **India** has been declared polio-free since March 2014, the last natural case was detected three years back (January 2011)
- ❖ **The Global Polio Eradication Initiative (GPEI)** had launched 'The Polio Endgame Strategy 2019–2023', which aims at eradication, integration, containment and certification.

Polio Endgame Strategy (2019–2023)

GPEI had initiated an end-game strategy for polio eradication. It has three goals; aiming at eradication of polio by 2023.

Goal-1: Eradication

Contd...

- ❑ Interrupt transmission of all wild poliovirus (WPV)
- ❑ Stop all circulating vaccine-derived poliovirus (cVDPV) outbreaks within 120 days of detection and eliminate the risk of emergence of future VDPVs

Goal-2: Integration

- ❑ Contribute to strengthening immunization and health systems to help achieve and sustain polio eradication
- ❑ Ensure sensitive poliovirus surveillance through integration with comprehensive vaccine preventable disease (VPD) and communicable disease surveillance systems
- ❑ Prepare for and respond to future outbreaks and emergencies

Goal-3: Certification and Containment

- ❑ Certify the countries for eradication of WPV
- ❑ Contain all polioviruses including from laboratories

Contd...

EXPECTED QUESTIONS

I. Write essay on:

1. A 9-week-old baby named Sweetie was brought to the emergency room with weakness in her right leg. On examination, her right leg appeared flaccid and no deep tendon reflex or Babinski reflex can be elicited, although sensation was intact. The tone, movement, sensation, and reflexes of her other limbs were normal. Her immunization records were up-to-date according to the National Immunization Schedule of India. CSF demonstrates elevated protein with normal glucose levels. Fecal sample was collected and then sent to the referral center where the poliovirus is identified as a vaccine strain (not the "wild-type" strain) of poliovirus type-1 was isolated.
 - a. What is the probable diagnosis of this clinical condition?
 - b. Add a note on the laboratory diagnosis.
 - c. Mention the types of vaccines available against this etiological agent.

II. Write short notes on:

1. Polio vaccine.
2. Polio eradication.
3. Coxsackievirus.

III. Multiple Choice Questions (MCQs):

1. Zero dose of OPV is given:

- a. At one month
- b. At birth
- c. When child is having diarrhea
- d. When child is having polio

2. Enterovirus 72 is:

- a. Hepatitis A virus
- b. Hepatitis E virus
- c. Hepatitis B virus
- d. Hepatitis C virus

3. Not true about salk vaccine:

- a. Expensive than OPV
- b. Not useful in epidemics
- c. Contraindicated in low immunity
- d. Booster doses are required

4. The most common viruses that can cause meningoencephalitis in children are:

- a. Arboviruses
- b. Herpesviruses
- c. JE virus
- d. Enteroviruses

5. As of 2020, Polio is endemic in all the following countries, except:

- a. India
- b. Pakistan
- c. Afghanistan
- d. Nigeria

6. Hand-foot-and-mouth disease is caused by:

- a. HSV
- b. Coxsackie virus
- c. Measles
- d. Chickenpox

7. Acute hemorrhagic conjunctivitis is caused by:

- a. Adenovirus
- b. Coxsackie virus A24
- c. Coxsackie virus A16
- d. Coxsackie virus B6

8. Most common serotype associated with VAPP:

- a. Type 1
- b. Type 2
- c. Type 3
- d. Any of the above

9. Which is not a vaccine-derived polioviruses (VDPVs)?

- a. mVDPV
- b. cVDPV
- c. iVDPV
- d. aVDPV

Answers

1. b 2. a 3. c 4. d 5. a 6. b 7. b 8. c 9. a

CHAPTER PREVIEW

- HSV Encephalitis
- Nipah and Hendra Viral Encephalitis
- Slow Virus and Prion Diseases
- Arbovirus Encephalitis
- Rabies
- Borna Disease

INTRODUCTION

Encephalitis

Encephalitis is an acute inflammation of the brain parenchyma, caused by invasion of infectious agents—most often viruses; rarely by other infectious agents such as parasites or non-infectious causes such as autoimmune encephalitis. This chapter will focus on **viral encephalitis**. The common etiological agents of viral encephalitis is enlisted in Table 74.1. The parasitic causes of encephalitis (e.g. *Toxoplasma*) is discussed in Chapter 75.

Encephalopathy

Encephalopathy is a general term used for any diffuse disease of brain that alters the brain function or structure. It usually refers to non-infective causes of encephalopathy (e.g. metabolic or hepatic encephalopathy). However, for certain chronic infections of brain, the word ‘encephalopathy’ still is used, such as slow virus and prion diseases.

Clinical Manifestations

In addition to the acute febrile illness, the patients with encephalitis commonly present with the following features depending upon the site of involved.

- ❖ Altered level of consciousness (confusion) or a depressed level of consciousness ranging from mild lethargy to coma
- ❖ Seizures: Focal or generalized seizures
- ❖ Neuropsychiatric manifestations such as hallucinations, agitation, personality change, behavioral disorders, and, at times, a frankly psychotic state
- ❖ Focal or diffuse neurologic signs: The common focal findings are:
 - Aphasia, ataxia, upper or lower motor neuron patterns of weakness
 - Involuntary movements (e.g. myoclonic jerks, tremor)
 - Cranial nerve deficits (e.g. ocular palsies, facial weakness)

Table 74.1: Agents of viral encephalitis and encephalopathy.

Acute viral encephalitis
Herpesviruses <ul style="list-style-type: none"> • Herpes simplex virus (HSV-1>HSV-2): The most common cause of sporadic encephalitis • Cytomegalovirus (in immunocompromised host) • Human herpesvirus 6 • Varicella-zoster virus • Epstein-Barr virus
Arboviruses: Important ones in India are: <ul style="list-style-type: none"> • Japanese encephalitis virus (the most common cause of epidemic encephalitis in India) • West Nile virus (the most common cause of epidemic encephalitis in USA)
Rabies virus: Causes encephalitis secondary to dog bite
Nipah and Hendra viruses
Rare causes: Enteroviruses and mumps virus
Encephalopathy
Slow virus diseases (chronic encephalitis) <ul style="list-style-type: none"> • Progressive multifocal leukoencephalopathy • Measles encephalitis • Rubella encephalitis
Prion disease
Borna virus disease

- ❖ Features of meningitis (if involved) such as neck rigidity
- ❖ Involvement of the hypothalamic-pituitary axis may result in temperature dysregulation, diabetes insipidus, etc.

Laboratory Diagnosis

CSF analysis: The characteristic CSF profile in encephalitis is indistinguishable from that of viral meningitis; typically consists of a lymphocytic pleocytosis, a mildly elevated protein level, and a normal glucose level.

The specific laboratory diagnosis of viral encephalitis has been discussed subsequently in this chapter under the respective etiological agents.

TREATMENT

Viral encephalitis

Symptomatic management is the mainstay of treatment. Antiviral drugs may be useful for some of the etiological agents.

- ❑ Acyclovir is the drug of choice for HSV encephalitis
- ❑ Ganciclovir, foscarnet or cidofovir either alone or in combination are often used in the treatment of CMV encephalitis

HSV ENCEPHALITIS

HSV is the most common cause (10–20%) of acute sporadic viral encephalitis, most frequently involving temporal lobe. HSV-1 is more common (95%) than HSV-2.

- ❖ Children generally get primary HSV infection, acquired exogenously and invades CNS via the olfactory bulb; whereas adults get recurrent infections due to reactivation of HSV in trigeminal nerve
- ❖ Clinical manifestation is same as for viral encephalitis described earlier
- ❖ **Laboratory diagnosis:** Detection of viral DNA in CSF remains the mainstay of diagnosis. Detail is discussed in Chapter 56
- ❖ **Treatment:** IV acyclovir (10 mg/kg q8h) is given for 10 days or until HSV DNA is no longer detected in CSF.

ARBOVIRAL ENCEPHALITIS

The arboviruses are arthropod transmitted viruses that can cause various clinical syndromes including hemorrhagic fever, arthritis and encephalitis. A number of arboviruses

can cause encephalitis; which are taxonomically diverse (belonging to five different families) and geographically restricted (Table 74.2).

The discussion in this chapter will be limited to Japanese encephalitis and West Nile viruses, which are prevalent in India. The hemorrhagic fever group of arboviruses that are common in India are discussed in Chapter 34.

Japanese Encephalitis

Japanese encephalitis virus is the leading cause of vaccine-preventable viral encephalitis in Asia, including India. It belongs to family Flaviviridae. It is an enveloped virus, containing ssRNA.

History

JE virus was so named because the disease was first seen in Japan (1871) as “Summer encephalitis epidemics” (however, it is now uncommon in Japan) and named ‘B’ to distinguish it from encephalitis A (encephalitis lethargica/von Economo disease), which was endemic in Japan during that time.

Epidemiology

- ❖ **Vector:** JE virus is transmitted by bite of *Culex* mosquito
 - *C. tritaeniorhynchus* is the major vector worldwide including India
 - *C. vishnui* is the next common vector found in India.
- ❖ **Transmission cycle:** JE virus infects several non-human hosts, e.g. animals and birds. Two transmission cycles are predominant

Table 74.2: Arboviruses causing encephalitis.

Virus	Distribution	Vector	Reservoir
Family: Togaviridae			
Eastern equine encephalitis virus	Eastern part of North America	<i>Aedes, Culex</i>	Birds
Western equine encephalitis virus	Western part of North America	<i>Culex tarsalis, Aedes</i>	Birds
Venezuelan equine encephalitis virus	South and Central America	<i>Aedes, Culex</i>	Horses
Family: Flaviviridae			
Japanese B encephalitis virus	South East Asia	<i>Culex tritaeniorhynchus</i>	Pigs, Birds
St Louis encephalitis virus	United States	<i>Culex</i>	Wild birds
West Nile encephalitis virus	East Africa (Uganda), Algeria, Romania	<i>Culex, Aedes, Anopheles</i>	Birds
Murray Valley encephalitis virus	America	<i>Culex annulirostris</i>	Birds
Rocio virus	São Paulo, Brazil	<i>Culex</i>	*
Russian spring-summer encephalitis virus	Central Europe, Russia	Tick	Rodents, other mammals, birds
Powassan virus	America	Tick	Rodents
Louping-ill virus	Europe	Tick	Sheep
Family: Bunyaviridae			
California encephalitis virus	USA	<i>Aedes triseriatus</i>	Rodents
Family: Reoviridae			
Colorado tick fever virus	America (mountains)	Tick	Rodents
Family: Rhabdoviridae			
Chandipura virus	India	Sandfly	*

* Not yet identified. For viruses like dengue, some studies have shown domestic dog can be infected with dengue virus.

- Pigs → *Culex* → Pigs
- Ardeid birds → *Culex* → Ardeid birds.

- ❖ **Animal hosts:** JE virus has several animal hosts
 - **Pigs** have been incriminated as the major vertebrate host for JE. JE virus multiplies exponentially in pigs without causing any manifestation. Pigs are considered as the **amplifier host** for JE
 - **Cattle and buffaloes** may also be infected with JE virus; although they are not the natural host. They may act as **mosquito attractants**
 - **Horses** are probably the only animals to be symptomatic and develop encephalitis following JE virus infection
 - **Humans** are considered as dead end; there is no man → mosquito → man cycle (unlike in dengue).
- ❖ **Bird hosts:** Ardeid (wading) birds such as herons, cattle egrets, and ducks can also be involved in the natural cycle of JE virus
- ❖ **Age:** About 85% of cases occur in children below 15 years (but infants are not affected) and about 10% occur in the elderly
- ❖ **Seasonal variation:** Infection is common in rainy season which coincides with maximum mosquito activity
 - Temperate areas (summer-autumn)
 - Tropical areas including India (June-October).

Geographical Distribution

Currently, JE is endemic in Southeast Asian region.

- ❖ It is increasingly reported from India, Nepal, Pakistan, Thailand, Vietnam and Malaysia
- ❖ Because of immunization, its incidence has been declining from Japan and Korea
- ❖ It is estimated that nearly 50,000 cases occur every year globally with 10,000 deaths
- ❖ **In India:** JE has been reported since 1955
 - JE is endemic in 15 states; Uttar Pradesh (Gorakhpur district) accounted for the largest burden in past
 - Between 2010–19, a total of 14,933 cases (average 1,493 cases/year) and 2,230 deaths (average 223 deaths/year) have been reported
 - In 2019, nearly 2,545 cases of JE were reported from India with 266 deaths. Maximum cases were reported from Assam followed by Manipur and UP.

Clinical Manifestations

JE is the most common cause of epidemic encephalitis.

- ❖ **Incubation period:** In persons who develop symptoms, the incubation period is 5–15 days
- ❖ **Subclinical infection is common:** JE typically shows iceberg phenomenon
 - Cases are much less compared to subclinical/inapparent infections with a ratio of 1:300 to 1:1000
 - Even during an epidemic, the number of cases are just 1–2 per village.

- ❖ **Clinical course** of the disease can be divided into three stages:

1. **Prodromal stage** is a febrile illness; the onset of which may be either abrupt (1–6 hours), acute (6–24 hours) or more commonly subacute (2–5 days)
2. **Acute encephalitis stage:** JE is the most common cause of acute encephalitis syndrome (AES) in India; characterized by an acute onset of fever, mental confusion, disorientation, delirium, seizures (among children), or coma
3. **Late stage and sequelae:** It is the convalescent stage in which the patient may be recovered fully or retain some neurological deficits permanently (up to 50%). Case fatality rate is about 20–40%.

Laboratory Diagnosis

- ❖ **IgM capture antibody (MAC) ELISA** supplied by NIV, Pune has been the recommended method for diagnosis of JE. It is a two-step sandwich ELISA, uses JERA (JE recombinant antigen) to detect JE-specific IgM antibody in serum. Refer Figure 12.7B (Chapter 12) for detail
- ❖ **Molecular methods:** Reverse-transcriptase (RT) PCR and real time RT-PCR have been developed to detect JE virus specific envelope (E) gene in blood.

TREATMENT

Japanese encephalitis

Treatment of JE is only by supportive measures; no specific antiviral drugs are available.

Vaccine Prophylaxis for Japanese Encephalitis

It is recommended to the children residing in rural area and only for individuals living in endemic areas. The following JE vaccines are licensed in India.

Live attenuated SA 14-14-2 vaccine:

- It is prepared from SA 14-14-2 strain of JE virus
- It is cell line-derived; primary hamster kidney cell lines are commonly used
- It is manufactured in China, but now licensed in India
- Under National Immunization Program, it is given to children (1–15 years) targeting 231 endemic districts of states such as—UP, Bihar, Assam, West Bengal and Karnataka
- Schedule: Two doses; 1st at 9 completed months-12 months of age and 2nd at 16–24 months
- Administered: 0.5 mL/dose, subcutaneously at left upper arm.

Inactivated JE vaccine

- It is inactivated, Vero cell culture-derived vaccine
- It is manufactured in many countries including India
- Schedule: Two doses; 4 weeks interval, for children >3 years and adults aged ≥18 years
- Administered: 0.5 mL/dose, intramuscular.

Catch up vaccination: All susceptible children up to 15 years should be administered during disease outbreak/ahead of anticipated outbreak in campaigns

Note: Mouse brain-derived inactivated vaccines (Nakayama strain and Beijing strain), were used previously, now discontinued.

Contd...

Contd...

Combined vaccine: A genetically engineered JE vaccine that combines the attenuated SA14-14-2 strain and yellow fever vaccine strain 17D (YF 17D) virus as a vector for genes encoding the protective antigenic determinants, has been tested in several clinical trials.

West Nile Encephalitis

West Nile virus (WNV) is a flavivirus related to JE virus. It is mainly transmitted by *Culex* mosquito. It is zoonotic, maintained in nature by transmission between birds and mosquitoes.

- ❖ **Clinical feature:** The incubation period is about 3-14 days. 80% of people remain asymptomatic. The rest may develop disease
 - **West Nile fever:** Common symptoms include fever, headache, tiredness, body aches, nausea, vomiting, sometimes skin rash (on the trunk of the body) and swollen lymph glands
 - **West Nile encephalitis** or meningitis may develop rarely; in 1 in 150 cases. It is a severe form of disease, with a mortality of 10%.
- ❖ **Epidemiology:** WNV was first isolated in the West Nile district of Uganda (1937)
 - **World:** It has caused an epidemic in Israel in 1951 and large outbreak in USA from 1999-2010
 - **India:** WNV is highly prevalent in India in various states and has caused several outbreaks in the past, such as in Kerala (2011) and Assam (2006).
- ❖ **Diagnosis:** The various laboratory tests available are:
 - IgM antibody capture ELISA detecting IgM antibodies in serum and CSF is available. Serum IgM antibody may persist for more than a year
 - IgG ELISA demonstrating seroconversion in two serial specimen collected at a one-week interval is also diagnostic
 - Viral RNA detection by RT-PCR.
- ❖ **Treatment** is only by supportive measures; no specific antiviral drugs are available.

NIPAH AND HENDRA VIRAL ENCEPHALITIS

They are zoonotic paramyxoviruses. Hendra virus was first isolated in 1994 in Hendra (Australia) and Nipah virus was discovered in 1999 in Malaysia.

- ❖ **Reservoir:** Fruit bats (flying foxes) are the natural host for both Nipah and Hendra viruses
- ❖ **Geographical distribution:**
 - Hendra virus infections are confined to horses in Australia, whereas Nipah viruses cause infection of pigs in Malaysia
 - Human infection of Nipah virus is emerging especially in Southeast Asia including Bangladesh (mainly), India, Thailand and Malaysia. 477 cases were reported so far with 52% mortality.

Nipah Virus in India

Two outbreaks of Nipah virus in humans were reported from West Bengal (Siliguri in 2001 and Nadia in 2007); together accounted for 71 cases with 50 deaths. In 2018, cluster of 18 cases of Nipah encephalitis (with 16 deaths, 89% mortality) have been reported from Kozhikode, Kerala.

❖ Transmission:

- Hendra virus is transmitted by exposure to infected body fluids and excretions of horses
- Transmission of Nipah virus to humans may occur after direct contact with infected fruit bats, pigs, or persons. Consumption of infected raw date palm sap is thought to be another mode of transmission. Human-to-human transmission has been reported among family members and care givers of infected patients.

- ❖ **Clinical manifestations:** The incubation period is 4 to 14 days. Both the viruses can produce **encephalitis** in humans; patients present with fever, headache, myalgia, and CNS symptoms such as dizziness, drowsiness, altered consciousness and seizure. Severe cases progress to coma within 48 hours and death

❖ Laboratory diagnosis:

- Real-time PCR from throat and nasal swabs, CSF, urine and blood should be performed in the early stages of disease
- Antibody detection by ELISA (IgM and IgG) can be used at a later stage
- Immunohistochemistry is performed on tissues collected during autopsy, which confirms the postmortem diagnosis
- They are also prone to cause laboratory acquired infections and are classified as biosafety level 4 pathogens.

- ❖ **Treatment:** No antiviral drug is available

- ❖ **Vaccine:** A subunit vaccine, using the Hendra G protein, produces cross-protective antibodies against Hendra and Nipah viruses. It has been recently used in Australia to protect horses against Hendra virus. It can be used in humans as well.

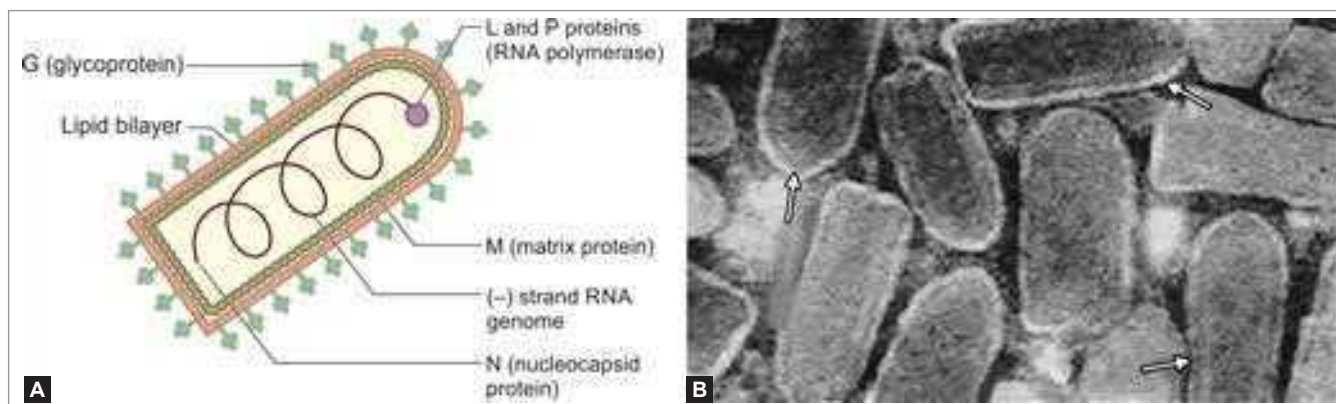
RABIES

Rabies virus causes a rapidly progressive, acute infectious disease of the central nervous system (CNS) in humans and animals, transmitted from another rabid animal. Although the human cases are few in number, rabies is still considered as a major public health problem because it is almost always fatal.

Morphology

Rabies virus belongs to Rhabdoviridae family, which has a unique morphology.

- ❖ **Bullet-shaped** (75 nm in width and 180 nm in length)



Figs 74.1A and B: Rabies virus: **A.** Schematic diagram; **B.** Electron micrograph (arrows showing).

Source: Public Health Image Library, B. ID#5611/Fred. A. Murphy/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Enveloped:** They have a lipid envelope in which 10 nm long peplomers or spikes (glycoprotein-G) are embedded. The envelope is lined internally by a layer of matrix protein (Figs 74.1A and B)
- ❖ **Nucleocapsid** has a helical symmetry and comprises a single-stranded, negative-sense RNA, nucleoprotein and polymerase proteins
- ❖ **Antigens:** Glycoprotein-G and nucleocapsid are the major antigens of rabies. They differ from each other in their role in pathogenicity, diagnosis, immunity and vaccination (Table 74.3).

Street vs Fixed Virus

Rabies virus undergoes certain changes when it is serially propagated in animals.

- ❖ **Street viruses:** These are freshly isolated strains in the laboratory. They mimic the wild viruses; show long and variable incubation periods and produce intracytoplasmic inclusion bodies
- ❖ **Fixed viruses:** When street viruses are propagated in rabbits by serial brain-to-brain passage; they lose certain properties and become fixed strains
 - They do not produce inclusion bodies
 - They do not multiply in extraneural tissues
 - They do not infect salivary gland
 - They multiply rapidly, and the incubation period is shortened to 4–6 days, hence these strains are best used for vaccination.

Pathogenesis

Transmission

Rabies virus is usually transmitted to humans by:

- ❖ **Bite:** Following a deep bite or scratch from an infected animal with rabies
 - Rabid dogs account for 99% of cases
 - Bats are now the major source in America
 - Other animal bites such as foxes, raccoons, skunks, jackals, mongooses and other wild carnivore host.

Table 74.3: Major antigens of rabies virus.

Glycoprotein G	Nucleoprotein
These are peplomers or spikes embedded in the envelope	These are capsid proteins associated with viral RNA
Species-specific	Group-specific and it cross reacts with that of rabies-related viruses
Role in pathogenesis: It binds to acetyl choline receptors in neural tissues, which is the first step of pathogenesis (attachment)	Does not have any role in pathogenesis
Diagnostic role: Antibody detection: It is used in hemagglutination inhibition test (obsolete now), to detect antibody in patient's serum	Antigen detection: Detected in patient's serum by direct-IF test
Role in immunity: It induces neutralizing antibodies which are protective in nature	Antibodies are not protective
Role in vaccination: Purified form of the antigen can be used in subunit vaccine	Not used for vaccination

- ❖ **Non-bite exposures:** These include—
 - Direct contact with saliva of infected animals with mucosa or fresh skin wounds
 - Inhalation of virus-containing aerosols (important for laboratory workers)
 - Cornea or other organ transplantation.
- The following types of contacts are not reported to transmit rabies.
- ❖ Human-to-human transmission through bites or saliva (e.g. kissing) or other non-bite exposures are theoretically possible but have never been confirmed
 - ❖ Via consumption of raw meat or milk of infected animals
 - ❖ Bites from rodents
 - ❖ Petting a rabid animal or contact with the blood, urine or feces of a rabid animal

- ❖ Contact with people/animals who have already received rabies vaccination.

Spread of the Virus (Fig. 74.2)

- ❖ **Multiply locally:** Virus starts replicating locally at the site of inoculation in muscle or in connective tissue
- ❖ **Viral entry to peripheral neurons:** Virus binds to nicotinic acetylcholine receptors present at neuromuscular junctions
- ❖ **Neuronal spread:** Rabies virus spreads centripetally along the peripheral motor nerves via retrograde fast axonal transport, at a rate up to 250 mm/day
- ❖ It reaches dorsal root ganglia of the spinal cord, and then ascends upward towards CNS
- ❖ **Central nervous system (CNS) infection:** It rapidly disseminates to various parts of CNS, most common sites are hippocampus and cerebellum
- ❖ **Centrifugal spread:** From CNS, the virus spreads along the sensory and autonomic nerves to various tissues such as salivary glands followed by pancreas, kidney, heart, retina, and cornea. However, viremia does not occur
- ❖ **Shed in saliva:** Rabies virus is shed in the saliva of rabid animals which acts as the source of infection to other animals. Viral shedding also occurs in human saliva, but human to human transmission is not documented yet
- ❖ **Pathological changes:** Presence of **Negri bodies** (intracytoplasmic eosinophilic inclusions) composed of rabies virus proteins and viral RNA, in the brain parenchyma is an important pathological finding of Rabies.

Clinical Manifestations

Incubation Period

Incubation period is prolonged and variable, average being 20–90 days (ranges from 1 week to 19 years).

It is directly related to the distance for the virus to travel from the site of inoculation to CNS. Hence the incubation period is usually shorter in:

- ❖ Children than in adults
- ❖ Bites on head, neck and upper limbs than legs
- ❖ Short people
- ❖ Severe lacerations
- ❖ Presence of genetic predisposition
- ❖ Low host immunity
- ❖ **Virus:** High dose of inoculum, ↑ virulence of the strain.

The clinical spectrum is divided into 3 phases as follows.

1. Prodromal Phase

It lasts for 2–10 days, characterized by non-specific symptoms such as fever, malaise, anorexia, nausea, vomiting, photophobia, sore throat, abnormal sensation (paresthesia, pain, or pruritus) around the wound site.

2. Acute Neurologic Phase

This may be either encephalitic type (80%) or paralytic type (20%).

- ❖ **Encephalitic or furious rabies:** It lasts for 2–7 days, and is characterized by:
 - **Hyperexcitability:** Anxiety, agitation, hyperactivity, bizarre behavior and hallucinations may be seen
 - **Lucid interval:** Period of hyperexcitability is typically followed by complete lucidity that becomes shorter as the disease progresses
 - **Autonomic (sympathetic) dysfunction** features may be seen such as ↑lacrimation, ↑salivation (leads to **foaming** at the mouth), ↑perspiration, gooseflesh, cardiac arrhythmia and priapism
 - **Hydrophobia** (fear of water) or **aerophobia** (fear of air)—The act of swallowing precipitates an involuntary, painful spasm of the respiratory, laryngeal, and pharyngeal muscles. These symptoms are probably due to dysfunction of infected brainstem neurons.
- ❖ **Paralytic or dumb rabies:** This occurs in 20% of cases, especially in people who are partially vaccinated or infected with bat rabies virus. It is characterized by flaccid paralysis, often begins in the bitten limb and progressing to quadriplegia with facial paralysis. However, hydrophobia and other features of encephalitic rabies are typically absent.

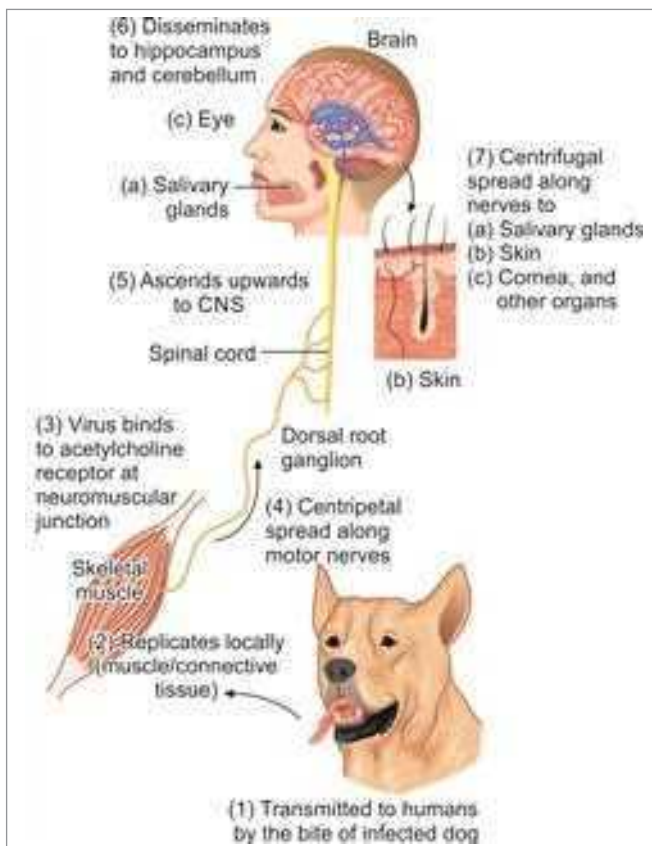


Fig. 74.2: Pathogenesis of rabies.

3. Coma and Death

Following acute neurological phase, patient develops coma that eventually leads to death within 14 days. Patients with paralytic rabies may survive longer up to 30 days. However, death is almost certain. Recovery and survival are extremely rare.

LABORATORY DIAGNOSIS

Rabies

- ❑ Antigen detection from hair follicles at nape of the neck and from corneal smear—by direct IF test
- ❑ Viral Isolation by:
 - Mouse inoculation
 - Cell lines inoculation—Mouse neuroblastoma and BHK cell lines.
- ❑ Antibody detection from serum and CSF—by MNT, RFFIT, FAVN, and IFA
- ❑ Viral RNA detection—by RT-PCR
- ❑ Negri body detection in histopathological staining of brain biopsies (hippocampus)—for postmortem diagnosis of rabies.

Laboratory Diagnosis

Rabies Antigen Detection

Direct immunofluorescence test (direct-IF); also called as direct fluorescent antibody (DFA) test can be performed to detect rabies nucleoprotein antigens in specimens by using specific monoclonal antibodies tagged with fluorescent dye.

Because of its high sensitivity and specificity, DFA test is considered as the “**gold standard**” method for rabies diagnosis.

- ❖ The best specimen is hair follicle of the nape of the neck (most sensitive)
- ❖ Corneal impression smear can also be used. It is usually positive in late stage with a sensitivity of 30%.

Viral Isolation

- ❖ **Mouse inoculation:** Intracerebral inoculation into suckling mice can cause encephalitis and death. The brain biopsies of the inoculated animal are examined for the presence of Negri bodies and rabies antigen
- ❖ **Cell lines:** Mouse neuroblastoma cell lines and baby hamster kidney (BHK) cell lines are the preferred cell lines for rabies virus isolation
 - They can yield virus (2–4 days) much faster than that of mice inoculation
 - Viral growth in the cell lines can be detected by direct-IF test using specific antiserum.

Antibody Detection

Detection of CSF antibodies is more significant than serum antibodies.

- ❖ Serum antibodies appear late and can also be present after vaccination
- ❖ CSF antibodies appear early and they are produced only in rabies-infected individuals but not in response to vaccination

- ❖ Several test formats are available, which detect antibodies by employing antigens such as glycoprotein or nucleoprotein

- Mouse neutralization test (MNT)
- Rapid fluorescent focus inhibition test (RFFIT)
- Fluorescent antibody virus neutralization test (FAVN)
- Indirect fluorescence assay (IFA).

Viral RNA Detection

Reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify genes of rabies virus (e.g. genes coding for nucleoprotein or large structural protein) from brain tissue, saliva and skin biopsy samples. It is the most sensitive and specific assay available at present for the diagnosis of rabies.

Negri Body Detection

It is useful to confirm postmortem diagnosis of rabies.

- ❖ It is an intracytoplasmic eosinophilic inclusion with characteristic basophilic inner granules, composed of rabies virus proteins and viral RNA
- ❖ **Location:** Negri bodies are commonly observed in Purkinje cells of the cerebellum and in pyramidal neurons of the hippocampus, and are less frequently seen in cortical and brainstem neurons
- ❖ **Stains:** Histological stains such as H and E (Fig. 74.3) and Sellers stains are commonly used to demonstrate Negri bodies
- ❖ **Immunohistochemistry:** Peroxidase labeled specific antibodies are used to detect the viral inclusions in formalin-fixed tissues. It is more sensitive and specific than histological staining methods
- ❖ Negri body detection is pathognomonic of rabies.

However, it may not be detected in 20% of cases. Therefore, the absence of Negri bodies does not rule out the diagnosis of rabies.



Fig. 74.3: Negri bodies in brain biopsy by H and E stain (arrows showing).

Source: Public Health Image Library, ID# 33771/Dr. Daniel P. Perl/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

TREATMENT

Rabies

There is no specific treatment for rabies. Symptomatic treatment may prolong life, but the outcome is almost always fatal.

- ❑ Isolation: Patient should be isolated in a quiet room, protected as far as possible from external stimuli such as bright light, noise, water or cold air which can precipitate spasms
- ❑ Sedatives and anti-anxiety drugs such as morphine can be used
- ❑ Hydration and urination should be properly maintained.

Prognosis

Mortality in rabies is almost 100%; however, it is preventable by administration of post-exposure therapy during the early incubation period. There are seven well-documented cases who survived from rabies—mostly because of taking rabies vaccine in the early incubation period.

Prevention of Human Rabies (WHO Guideline 2018)

Rabies is prevented by providing prophylactic measures such as post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP).

Post-exposure Prophylaxis (PEP) (For Individuals Not Received PEP/PrEP Previously)

PEP consists of three components—local wound care, rabies vaccine and rabies immunoglobulin (RIG). Inclusion of these components in PEP depends upon the risk of exposure. WHO has classified the exposures into three categories (Table 74.4).

- ❖ **For category I exposures:** Require only wound care. Vaccine or RIG are not required
- ❖ **For category II exposures:** Require local wound care and rabies vaccine. RIG is not required except for immunodeficient individuals who need RIG in addition
- ❖ **For category III exposures:** All three components of PEP are required such as local wound care, rabies vaccine and RIG.

Local Wound Care

It consists of the following measures.

- ❖ **Physical cleansing:** All bite wounds and scratches should be washed thoroughly with soap and water for 15 minutes
- ❖ **Chemical inactivation:** Antiseptics such as povidone iodine or alcohol can be used to inactivate the residual viruses
- ❖ **Suturing:** Suturing causes local tissue damage, which may help in spreading of the virus. Therefore, suturing should be avoided. Deeper wounds that definitely require suturing should be sutured loosely and only after RIG is infiltrated into the wound
- ❖ **Other general measures** include: (i) debridement of devitalized tissues, (ii) tetanus prophylaxis, (iii) antibiotic treatment to prevent secondary bacterial infection
- ❖ Do not touch the wound(s) with bare hand

Table 74.4: Risk categorization and recommended anti-rabies prophylaxis (WHO, 2018).

Category of risk	Type of exposure	Recommended prophylaxis (WHO**)
Category I (No risk)	<ul style="list-style-type: none"> • Touching, or feeding of animal • Licks on intact skin 	<ul style="list-style-type: none"> • No treatment needed if history is reliable
Category II (Minor risk)	<ul style="list-style-type: none"> • Minor scratches or abrasions without bleeding or nibbling of uncovered skin 	<ul style="list-style-type: none"> • Wound management • Rabies vaccine • Observe the dog for 10 days
Category III (Major risk)	<ul style="list-style-type: none"> • Single or multiple transdermal bites with oozing of blood • Licks on broken skin (fresh wounds) or mucous membrane • Direct contact with bats or wild animals 	<ul style="list-style-type: none"> • Wound management • Rabies immunoglobulin • Rabies vaccine • Observe the dog for 10 days*

*Vaccine may be discontinued if animal (dogs and cats) is healthy after 10 days of bite. Other animals are humanely killed and tissue is examined for detection of rabies antigen/Negri body in brain biopsies.

**In India post-exposure prophylaxis is indicated following exposure to any animal bite except rodents and bat bite.

Note:

- Though human-to-human transmission is rare; still PEP is offered as a precautionary measure following an exposure.
- Immunodeficient individuals must receive both Ig and vaccine for category II and III exposures and should be checked for serum antibody after 14 days of vaccination if facility available.
- There is no contraindications to PEP including in pregnancy, lactation, infancy, old age and any associated concurrent illness.

- ❖ Do not apply irritants like soil, oil, lime, herbs, chalk, betel leaves, etc.

Rabies Vaccine

A series of rabies vaccine injections should be administered promptly after the exposure.

- ❖ **Type of vaccines:** Cell line derived non-neural vaccines are recommended. Three vaccines are available
 1. Purified chick embryo cell (PCEC) vaccine: It is prepared from chicken fibroblast cell line
 2. Purified Vero cell (PVC) vaccine: It is prepared from Vero cell line
 3. Human diploid cell (HDC) vaccine: It is derived from WI-38 (human embryonic lung fibroblast cell line).

Note: The **neural vaccines** derived from brain of infected animals such as Semple vaccine (sheep brain), beta-propiolactone (BPL) vaccine and infant mouse brain vaccines are encephalitogenic and therefore no longer in use. Similarly, the **egg-derived non-neural vaccines** (e.g. purified duck embryo vaccine) and **recombinant non-neural vaccines** (containing surface glycoproteins) are also not in use for humans

- ❖ **Routes:** Rabies vaccines can be administered by two different routes; intradermal (ID) or intramuscular (IM)

- ❖ **Site:** The preferred site of administration is in the deltoid area of the arm for adults and the anterolateral area of the thigh for children (aged < 2 years); never administered in gluteal region
- ❖ **Dose:** One ID dose is 0.1 mL of vaccine and one IM dose is considered as an entire vial of vaccine, irrespective of the vial size
- ❖ **Schedule of PEP regimen:** ID regimens are cost-effective; dose-sparing and time-sparing and therefore are preferred over IM regimens
 - **ID PEP regimen (2-2-2):** 2-site ID vaccine is given on days 0, 3 and 7
 - **IM PEP regimens:** Total four doses are given. Two schedules are available
 - ◆ 1-site IM vaccine given on days 0, 3, 7 and the fourth dose between days 14 to 28 or
 - ◆ 2-site IM vaccine given on day 0 and 1-site IM on days 7 and 21.
- ❖ **Interchange:** Changes in vaccine product and/or the route (IM ↔ ID) during the same PEP course are acceptable if unavoidable, to ensure PEP course completion
- ❖ **If delayed:** If a vaccine dose is delayed for any reason, the PEP regimen should be resumed (not restarted).

Rabies Immunoglobulin (RIG)

RIG provides passive immunization, by neutralizing the virus.

- ❖ **Timing:** It should be administered only once, as soon as possible and not beyond day 7 after the first dose of vaccine. Vaccines should never be withheld, regardless of the availability of RIG
- ❖ **Preparations:** It is available in two forms; human RIG (hRIG) and equine RIG (eRIG). Both have shown similar clinical outcomes in preventing rabies
- ❖ **Dose:** The maximum dose is 20 IU (hRIG) or 40 IU (eRIG) per kg body weight. There is no minimum dose
- ❖ **Administration:** It should be infiltrated as much as possible into the wound; the leftover dose is not needed to be administered IM at a different site, but can be used for other patients, if retained aseptically
- ❖ If correctly administered, RIG neutralizes the virus at the wound site within a few hours.

PEP for Individuals Previously Vaccinated (PrEP or PEP)

For individuals who previously received rabies vaccine (either PEP or PrEP), RIG is not necessary regardless of exposure category. They need local wound care and an accelerated vaccine regimen; consisting of any of the following three schedules.

- ❖ 1-site ID vaccine given on days 0 and 3 or
- ❖ 1-site IM vaccine given on days 0 and 3 or
- ❖ 4-site ID vaccine given on day 0 only (left and right deltoids, thigh or suprascapular areas).

Note: If repeat exposure occurs within 3 months of receiving PEP, only local wound treatment is required; neither vaccine nor RIG are needed.

Pre-exposure Prophylaxis (PrEP)

PrEP is recommended in two conditions.

- ❖ **For individuals at higher occupational risk** such as laboratory staff handling the virus and infected material, clinicians attending to human rabies cases, veterinarians, animal handlers and travellers to endemic areas
 - ❖ **For sub-populations in remote endemic areas**, which have limited access to PEP and if annual dog bite incidence is > 5% or vampire bat exposures prevail.
- PrEP regimen** can be given to individuals of all ages.
- ❖ **Schedule:** Two schedules are available
 - 2-site ID vaccine given on days 0 and 7
 - 1-site IM vaccine given on days 0 and 7.
 - ❖ **Booster:** PrEP is likely to provide lifetime protection, no need to take PrEP booster periodically. A routine PrEP booster or serology for neutralizing antibody titres would be recommended only if a continued, high-risk of rabies exposure remains
 - ❖ **Following exposures**, an accelerated vaccine regimen is indicated. RIG is not necessary.

Epidemiology

Rabies is an enzootic and epizootic disease of both wild and domestic animals worldwide.

- ❖ **Worldwide**, rabies is endemic in >150 countries. About 55,000 deaths occur due to human rabies each year, maximum in rural areas of Asia and Africa. India accounts for 20,000 deaths/year. However, rabies may be grossly underreported in many countries including India
- ❖ **Source:** Infected dog is the source of infection in 99% of cases. Virus present in saliva from 3–4 days before the onset of symptoms till death of the dog
- ❖ **Age:** Though all age groups are affected, children aged 5–15 years are at greater risk.

Rabies in Dogs

As 90% of human rabies is caused by dog bites, control of rabies in dogs is the most important step to prevent human rabies.

- ❖ **Clinical features:** Incubation period varies from 3–8 weeks (ranges from 10 days to few years). Like in humans, two types of manifestations may be seen
 - **Furious Rabies (or Mad Dog syndrome):** It is the most common type; characterized by:
 - ◆ Changes in behavior: Dog loses fear of people, become very aggressive, bites without provocation
 - ◆ Running amok-wandering aimlessly
 - ◆ Change in tone of the dog's bark
 - ◆ Fever and loss of appetite
 - ◆ Excessive salivation and foaming at mouth
 - ◆ Paralytic stage towards the later stage

- ◆ Coma and death within a week.
- **Dumb or paralytic rabies:** Predominantly paralytic features are seen, but excitation symptoms are absent.
- ❖ **Control of rabies in dogs:** Most logical and cost-effective approach for control of urban rabies is elimination of stray dogs and mass immunization of at least 80% dogs in an area
- ❖ **Immunization of dogs:** It is the most important method of rabies control. All dogs should receive a primary immunization at 3–4 month of age, followed by boosters as per the type of vaccine used. Vaccines commonly used in dogs are:
 - BPL inactivated neural vaccine
 - Oral recombinant glycoprotein vaccine.

SLOW VIRUS AND PRION DISEASES

Slow virus diseases and prion diseases are a group of neurodegenerative conditions affecting both humans and animals, and characterized by:

- ❖ **Long incubation period**, ranging from months to years because of their long doubling time of 5.2 days or more
- ❖ Predilection for CNS
- ❖ Invariably fatal
- ❖ Strong **genetic** predisposition
- ❖ **The lack antigenicity**; hence, there is lack of immune response against viral proteins and lack of associated inflammation
- ❖ Does not produce cytopathologic effect in vitro.

Slow Virus Disease

Slow virus diseases are caused a number of conventional viruses (Table 74.5). They produce a chronic form of encephalitis/encephalopathy—described as chronic, progressive demyelinating disease of the CNS.

Table 74.5: Slow virus and prion diseases.	
Slow virus disease	Hosts
Progressive multifocal leukoencephalopathy (JC virus)	Human
Subacute sclerosing panencephalitis (Measles)	Human
Progressive rubella panencephalitis	Human
Visna virus encephalitis	Sheep
Maedi virus* progressive pneumonia	Sheep
Prion disease	Hosts
Kuru	Humans, monkeys
Creutzfeldt-Jakob disease	chimpanzees
Gerstmann-Sträussler-Scheinker disease	Humans
Fatal familial insomnia	Humans
Scrapie	Sheep, goats
Bovine spongiform encephalopathy	Cattle
Transmissible mink encephalopathy	Mink
Chronic wasting disease	Mule deer, elk

Abbreviation: JC, John Cunningham.

*Does not produce CNS disease, causes progressive pneumonia.

Progressive Multifocal Leukoencephalopathy (PML)

PML is caused by a polyoma virus called JC virus; named after the initials of the patient (John Cunningham) in whom it was described first.

- ❖ **Pathology:** PML is characterized by multifocal areas of demyelination distributed throughout the brain, along with deformation of both astrocytes and oligodendrocytes
- ❖ **Risk factors:** Most patients are immunodeficient with comorbidity such as AIDS (80%), hematologic malignancies or transplantation. Up to 5% of AIDS patients develop PML
- ❖ **Manifestations:** Patients often present with visual deficits, mental impairment, weakness and ataxia and sometimes seizures
- ❖ **Diagnosis:** PML is diagnosed by:
 - MRI scan detects multifocal asymmetric, lesions in the white matter of brain
 - PCR targeting JC virus DNA from CSF provides the definitive tool for diagnosis. It is highly specific, but variably sensitive (low in patients on antiretroviral therapy)
 - CSF is typically normal, with occasional lymphocytic pleocytosis.
- ❖ **Treatment:** No definite therapy for PML is available. Cidofovir may be effective. Antiretroviral therapy is given in case of HIV infection.

Measles Encephalitis

Measles (Chapter 56) produces various CNS complications, of which the most important is **subacute sclerosing panencephalitis (SSPE)**. Others include post-measles encephalomyelitis and measles inclusion body encephalitis.

Subacute Sclerosing Panencephalitis (SSPE)

It is a slowly progressive disease characterized by seizures and progressive deterioration of cognitive and motor functions; caused by a defective measles virus.

- ❖ **Occurrence** of SSPE is rare, 1 in 1–5 lakh measles cases
- ❖ **Age:** Children of <2 years of age acquiring the primary measles infection are usually prone to develop SSPE, than older children. SSPE usually develops after 7–13 years after primary measles infection
- ❖ **Manifestations:** Patients develop progressive intellectual deterioration, focal and/or generalized seizures, myoclonus, ataxia, and visual disturbances. It is fatal within 1–3 years of onset with mortality rate of 10–20%
- ❖ **Diagnosis:** The CSF analysis shows markedly elevated gamma globulin level (>20% of total CSF protein)
 - High titers of antibody to measles virus in CSF is diagnostic
 - Viral antigen can be identified immunocytochemically
 - Viral genome can be detected by in situ hybridization or PCR amplification
 - Measles virus can be cultured from brain tissue using special co-cultivation techniques.
- ❖ **Treatment:** No effective therapy is available.

Rubella Encephalitis

Progressive rubella panencephalitis is a SSPE like condition, but occurs extremely rare. It primarily affects males with congenital rubella syndrome; manifests after a latent period of 8–19 years.

Visna and Maedi

They cause slowly developing infections in sheep.

- ❖ Visna virus causes demyelination of CNS
- ❖ Maedi virus causes a slow progressive fatal hemorrhagic pneumonia of sheep.

Prion Disease

Prions are infectious protein particles that lack any nucleic acid. They are filterable like viruses; but are resistant to wide range of chemical and physical agents of sterilization. There are several prion diseases of humans and animals; *Scrapie* being the prototype.

Mechanism of Prion Diseases

It was described by Stanley B Prusiner (Nobel prize winner, 1997).

- ❖ Following infection, the infectious protein particles are carried to brain, and induce misfolding of normal cellular prion proteins (**PrP^C**) to form its disease-causing isoform (**PrP^{Sc}**) (Table 74.6)
- ❖ The **PrP^{Sc}** are aggregated as amyloid-like plaques in the brain and then internalized by neurons and get accumulated inside the **cytoplasmic vacuoles** giving the cell a spongiform appearance
- ❖ PrP^{Sc} are internalized by neurons and get accumulated inside the **cytoplasmic vacuoles** giving the cell a spongiform appearance
- ❖ PrP^{Sc} are transmissible (i.e. infectious), capable self-propagating either to other individuals or inheritable to offsprings.

Clinical Manifestations of Prion Diseases

Incubation period of prion diseases varies from months to years (longest being 30 years). But once the disease sets in, progression is fast.

Table 74.6: Differences between PrP ^C and PrP ^{Sc} .		
Features	PrP ^C	PrP ^{Sc}
Full form	Prion protein cellular	Prion protein scrapie
	Normal isoform of prion protein present in man/animals	Prion protein that causes prion disease in man/animals
Structure	Elongated polypeptide, rich in α-helix and has little β-structure	<ul style="list-style-type: none"> • Globular polypeptide • Contains less α-helix but more β-structure
Location	Anchored to the cell membrane	Cytoplasmic vesicles
Protease	Sensitive	Resistant
Turnover	Hours	Days

- ❖ Prodromal phase lasts for 3–5 months, followed by appearance of manifestations such as loss of muscle control, shivering, myoclonic jerks, tremors, loss of coordination and rapidly progressive dementia
- ❖ Death occurs within 1 year of onset of disease.

Prion Diseases of Animals

- ❖ **Scrapie:** It is the prototype of prion diseases that has been extensively studied. It is a prion disease of sheep, which occurs naturally in sheep (natural scrapie) or can be experimentally transmitted to various animals by injection of neural tissues of infected sheep
- ❖ **Mink encephalopathy:** It is a scrapie-like disease of mink transmitted by feeding the minks on scrapie infected sheep meat
- ❖ **Mad cow disease:** It is also called as bovine spongiform encephalopathy (BSE); has been enzootic in cattle in Great Britain since 1986
 - The epidemic peaked in 1993 infecting over 1 million cattle with infection spreading to European countries
 - BSE is transmitted due to the practice of feeding the cattle with meat and bone meal contaminated with scrapie or BSE prions.

Human Prion Diseases

The various human prion diseases are as follows:

- ❖ **Kuru:** It was seen only in the Eastern Highlands of New Guinea and was spread by customs surrounding ritual cannibalism of dead relatives infected with the disease. Since this practice has ceased, the disease has disappeared now
- ❖ **Creutzfeldt-Jakob disease (CJD):** It is the most common form of prion disease in humans. It typically presents with dementia and myoclonus, is relentlessly progressive, and generally causes death within a year of onset. Types of CJD include:
 - **Classical or sporadic (sCJD):** It is caused by the spontaneous misfolding of prion-protein in an individual. This accounts for 85% of cases of CJD
 - **Familial (fCJD):** It accounts for the majority of the other 15% of cases of CJD. fCJD and its variants Gerstmann Sträussler-Scheinker syndrome and fatal familial insomnia are **hereditary**
 - **Iatrogenic (iCJD):** It affects people of 50–75 years age; caused by contamination with tissue from an infected person, usually as the result of a medical or surgical procedures such as blood transfusion, use of human-derived pituitary growth hormones, gonadotropin hormone therapy, and corneal and meningeal transplants
 - **Variant (vCJD):** In contrast to the classical CJD, vCJD occurs below 30 years and is believed to be transmitted through the consumption of contaminated

beef with BSE prions. More than 190 cases of vCJD have occurred, mainly from Britain (where BSE is prevalent).

Laboratory Diagnosis

The following laboratory diagnosis methods are useful for prion diseases.

- ❖ **Measurement of PrP^{Sc}** by conformation dependent immunoassay is the definitive diagnostic tool for prion diseases
- ❖ **Neuropathological diagnosis** in brain biopsies: The pathologic hallmarks of prion diseases seen under light microscopy, are spongiform degeneration with lack of inflammatory response
- ❖ **Sequencing the PRNP gene** to identify the mutation: This is important in familial forms of prion diseases
- ❖ **Abnormal EEG** (electroencephalogram): In late stage of the disease, high-voltage, triphasic sharp discharges are observed.

TREATMENT

Prion diseases

There is no known effective therapy for preventing or treating prion diseases. Several trials using drugs such as quinacrine and anti-PrP antibodies have shown to eliminate PrP^{Sc} from the cultured cells, but they failed to do so in vivo.

Decontamination

Prions are extremely resistant to most of the common sterilization procedures. Recommended methods for sterilization of materials contaminated with prion proteins are:

- ❑ Autoclaving at 134°C for 1–1.5 hour
- ❑ Treatment with 1 N NaOH for 1 hour
- ❑ Treatment with 0.5% sodium hypochlorite for 2 hours.

Prions if bound to the stainless steel should be treated with an acidic detergent solution prior to autoclaving; rendering them susceptible to inactivation.

BORNA DISEASE

Borna disease virus (BDV) is a highly neurotropic virus which causes neuropsychiatric disorders in horses and sheep, manifested by behavioral abnormalities usually ending in death.

- ❖ It is seen in certain areas of Germany
- ❖ The disorder is immune-mediated; characterized by deposition of inflammatory cells in the brain
- ❖ It belongs to the family Bornaviridae. It is enveloped, contains negative sense ssRNA, which replicates in the nucleus
- ❖ Human infection has not been established yet, though serologic data suggest that BDV may be associated with neuropsychiatric disorders in humans.

EXPECTED QUESTIONS

I. Write essay on:

1. Mr Michel, a 25-year-old Australian visited his local doctor complaining of difficulty in swallowing liquids, loss of appetite and restlessness. He had a travel history to India one month back and did mention being bitten by a street dog in Puducherry.
 - a. What is the most probable etiological diagnosis?
 - b. Draw a labeled diagram of the morphology of the causative agent of this condition.
 - c. Discuss the laboratory diagnosis.
 - d. Name the vaccines available for human use.
2. Name the encephalitogenic arboviruses along with their vectors and discuss in detail about pathogenesis, clinical features and laboratory diagnosis of Japanese B encephalitis virus.

II. Write short notes on:

1. Vaccines for Japanese B encephalitis.
2. Prion diseases.
3. Post-exposure prophylaxis against rabies.

III. Multiple Choice Questions (MCQs):

1. **Anti-rabies vaccine is prepared from:**
 - a. Street virus
 - b. Fixed virus
 - c. Live virus
 - d. Wild virus
2. **For the treatment of case of class III dog bite, all of the following are correct, except:**
 - a. Give immunoglobulin for passive immunity
 - b. Give anti-rabies vaccine

- c. Immediately tightly stitch the wound
- d. Immediately wash wound with soap and water

3. All of the following rabies vaccines are commercially available for human use, except:

- a. Purified chick embryo cell vaccine (PCEC)
- b. Human diploid cell vaccine
- c. Vero continuous cell vaccine
- d. Recombinant glycoprotein

4. Rabies is identified by:

- a. Guarneri bodies
- b. Negri bodies
- c. Cowdry A bodies
- d. Paschen body

5. False statement regarding Japanese encephalitis:

- a. It is caused by flavivirus
- b. Transmitted by *Aedes* mosquito
- c. Endemic in India
- d. Man is dead-end host

6. Which of the following disease shows iceberg phenomena:

- a. Rabies
- b. Japanese encephalitis
- c. Measles
- d. HSV encephalitis

7. Which of following is correct about prions?

- a. Destroyed by autoclaving at 121°C
- b. Long incubation period
- c. Immunogenic
- d. Nucleic acid present

Answers

1. b 2. c 3. d 4. b 5. b 6. b 7. b

Parasitic and Fungal Infections of Central Nervous System

CHAPTER 75

CHAPTER PREVIEW

- Parasitic Infections of CNS
 - Free-living Amoebae Infections
 - Toxoplasma Encephalitis
- Neurocysticercosis
- Rare Parasitic Infections of CNS
- Fungal Infections of CNS
 - Cryptococcal Meningitis
 - Other Fungal Infections of CNS

PARASITIC INFECTIONS OF CNS

A number of parasites can infect central nervous system (CNS), which include agents from protozoans, cestodes, trematodes and nematodes. The major neuroparasitic infections* have been discussed first, followed by the parasitic agents that rarely infect CNS (Table 75.1).

FREE-LIVING AMOEBAE INFECTIONS

Free-living amoebae are small, freely living, widely distributed in soil and water and can cause opportunistic infections in humans. Among the many free-living amoebae that exist in nature, only four genera have an association with human disease.

1. *Naegleria fowleri* is a causative agent of primary amoebic meningoencephalitis (PAM)
2. *Acanthamoeba* species causes granulomatous amoebic encephalitis (GAE) and amoebic keratitis in contact lens wearers

3. *Balamuthia mandrillaris* causes GAE
4. *Sappinia* species: Causes encephalitis.

Naegleria fowleri Infection

Naegleria is a free-living amoeba, typically found in warm fresh water, such as ponds, lakes, rivers and swimming pools. It exists in nature as cyst and trophozoite forms (Figs 75.1A to C).

- ❖ **Trophozoite stage:** The trophozoites occur in two forms:
 - Amoeboid form: 20 µm size, found in humans
 - Flagellated form (10-18 µm): Amoeboid form when subjected to distilled water, transforms to flagellated form.
- ❖ **Cyst stage:** Cysts measure 7-15 µm in size and is surrounded by a thick, smooth double wall. It is found in the environment (Fig. 75.3B).

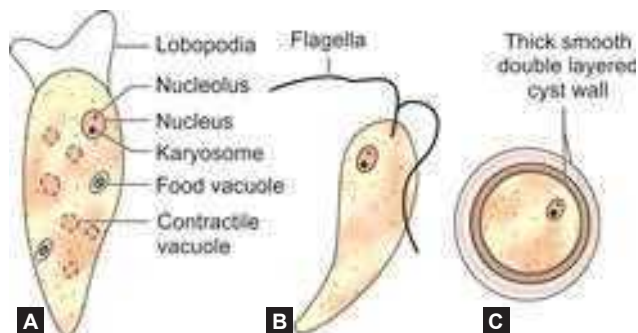
Life Cycle and Pathogenicity (Fig. 75.2)

- ❖ **Infective form:** Amoeboid form is the invasive form and also the usual infective form of the parasite
- ❖ **Mode of transmission:** Man acquires infection by nasal contamination during swimming in fresh hot water bodies like ponds, river, swimming pools or lakes. Rarely, if the flagellated or cyst form enters, soon it reverts back into amoeboid form
- ❖ **CNS invasion:** The amoeboid form invades the nasal mucosa, cribriform plate and travels along the olfactory

Table 75.1: Parasitic infections of central nervous system.

Protozoan infections of CNS
<ul style="list-style-type: none"> ● Free-living amoebae infections of CNS* ● <i>Toxoplasma</i> encephalitis* ● Cerebral malaria (<i>Plasmodium falciparum</i>)* ● African sleeping sickness (<i>Trypanosoma brucei</i>)* ● Chagas' disease (meningoencephalitis, <i>Trypanosoma cruzi</i>) ● Cerebral amoebiasis (<i>E. histolytica</i>)
Cestode infections of CNS
<ul style="list-style-type: none"> ● Neurocysticercosis (<i>Taenia solium</i>)* ● Others: <i>Taenia multiceps</i>, <i>Spirometra</i> and <i>Echinococcus</i>
Trematode infections of CNS
<ul style="list-style-type: none"> ● <i>Schistosoma mansoni</i> and <i>S. japonicum</i> infections ● Cerebral paragonimiasis
Nematode infections of CNS
<ul style="list-style-type: none"> ● Hyperstrongyloidiasis syndrome (<i>Strongyloides</i>) ● Eosinophilic meningitis (<i>Angiostrongylus cantonensis</i>) ● Others: <i>Loa loa</i> (meningoencephalitis), <i>Trichinella spiralis</i>, <i>Toxocara</i>, <i>Baylisascaris procyonis</i> and <i>Gnathostoma</i> infections

*Include the major parasitic infections of CNS.



Figs 75.1A to C: *Naegleria fowleri* (schematic diagram): **A.** Amoeboid trophozoite; **B.** Flagellated trophozoite; **C.** Cyst stage.

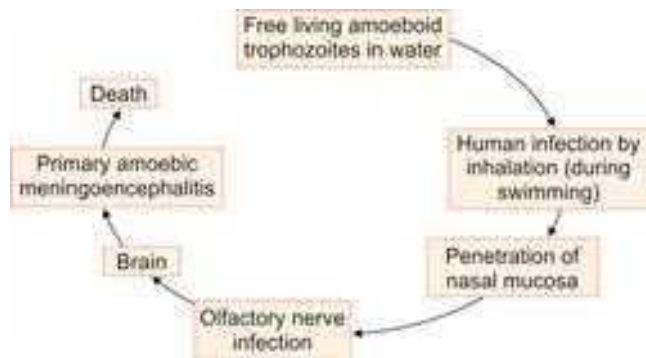


Fig. 75.2: Life cycle of *Naegleria fowleri*.

nerve to reach brain. The penetration initially results in significant necrosis of the nasal mucosa and olfactory bulbs

❖ **Tissue destruction** is mainly mediated by two mechanisms:

1. Direct ingestion of the brain tissue by producing food cups or **amoebostome** into which the cytopathic enzymes are liberated
2. Contact dependent cytolysis mediated by hemolytic proteins, cytolysins and phospholipase enzymes.

Primary Amoebic Meningoencephalitis

N. fowleri causes acute suppurative fulminant infection of CNS known as primary amoebic meningoencephalitis (PAM).

- ❖ It is so named because to distinguish it from the secondary invasions of CNS caused by *E. histolytica*
- ❖ PAM usually occurs in healthy children or young adults with recent history of swimming in fresh hot water
- ❖ Incubation period: 1–2 days to 2 weeks after exposure
- ❖ Clinical course is acute and fulminant
- ❖ The initial symptoms include changes in the taste and smell (due to olfactory nerve involvement) followed by headache, anorexia, nausea, vomiting, high fever, and signs of meningeal involvement like stiff neck and a positive Kernig's sign
- ❖ Secondary symptoms include confusion, hallucinations, lack of attention, ataxia, and seizures
- ❖ The mortality rate is nearly 98%. Death occurs within 7–14 days after exposure.

Epidemiology

- ❖ Till now more than 300 cases of PAM have been reported; mainly from USA (>100 cases) and also from other parts of the world
- ❖ In India, it is reported from Mangalore, Kolkata and Rajasthan (>20 cases reported so far).

Laboratory Diagnosis

CSF is the specimen of choice. CSF should be examined immediately. Refrigeration is not recommended as it will destroy the morphology of the parasite.

❖ **CSF analysis:** CSF is thick purulent, with pus cells >20,000/ μ L, elevated protein and reduced sugar level (mimic bacterial meningitis)

❖ **CSF microscopy:** Detection of characteristic trophozoites in CSF confirms the diagnosis. Cysts are not seen in CSF. The various microscopic methods are as follows

- **Wet mount:** Motile amoeboid trophozoites can be demonstrated in wet mount preparation of CSF
- **Phase contrast microscope** yields better result than light microscope
- Trophozoites can also be demonstrated by **direct fluorescent antibody** staining of centrifuged CSF using monoclonal antibody.

Amoeboid Trophozoite of *Naegleria*

It measures 20 μ m (7–35 μ m) in size (Figs 75.1A and 75.3A)

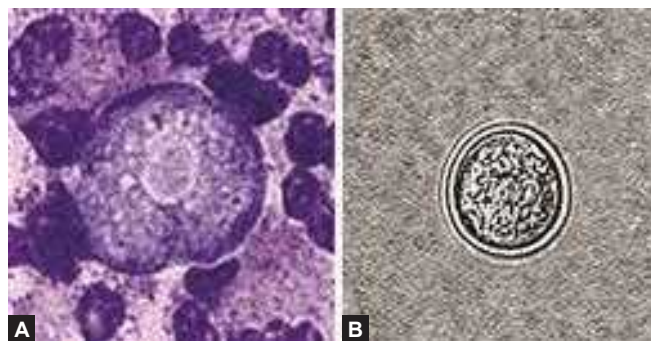
- ❑ Cytoplasm is granular with food vacuoles; nucleus shows large central karyosome and no peripheral chromatin
- ❑ It possesses lobular pseudopodia (called as lobopodia)
- ❑ If the parasite load is low, then CSF can be centrifuged at low speed (150 g for 5 minutes). Trophozoites won't get damaged, they only lose their pseudopodia
- ❑ Care should be taken to differentiate the trophozoites from leukocytes

❖ **Histopathology:** Brain biopsied tissue may be stained with hematoxylin and eosin and Giemsa stain to demonstrate trophozoites having sky blue cytoplasm with a pink nucleus (Fig. 75.3A)

❖ **Culture:** CSF sample can be cultivated on **non-nutrient agar** (Page's saline and 1.5% agar), lawn cultured with bacterial supplement like *E. coli*. *Naegleria* feeds on bacteria and crawls over the lawn culture of *E. coli* to produce trails (**Trail sign**)

❖ **Enflagellation test:** When the scrapping of the non-nutrient agar is transferred to sterile tubes containing distilled water, the amoeboid form undergoes transformation to a pear shaped flagellate form

❖ **Isoenzyme analysis:** Useful for specific identification of *N. fowleri* cultured from CSF and brain specimens



Figs 75.3A and B: *N. fowleri*: A. Trophozoite in CSF, stained with hematoxylin and eosin (H&E); B. Cyst grown in culture.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Molecular methods:** Multiplex real-time PCR is available targeting three regions of 18S rRNA of *Acanthamoeba* species, *Balamuthia*, and *N. fowleri* in CSF
- ❖ **Imaging methods:** CT scan and MRI show obliteration of cisterns, and diffuse enhancement around midbrain, subarachnoid space and over cerebrum.

TREATMENT Primary amoebic meningoencephalitis

No effective treatment is available for PAM

- ❑ Amphotericin B has considerable anti-*Naegleria* effect. Four cases were treated successfully with amphotericin B
- ❑ Other drugs like rifampicin, sulfisoxazole and antifungals like miconazole, fluconazole and miltefosine are also found to be effective.

Acanthamoeba Infections

Acanthamoeba species are free-living amoebae that infect CNS, skin and eye.

- ❖ They are ubiquitous and present worldwide. They have been isolated from soil, fresh and brackish waters
- ❖ It is so named because of the spine like pseudopodia present in trophozoite (called as acanthopodia)
- ❖ More than 24 species have been identified. Important ones that cause human infection include *Acanthamoeba astronyxis*, *A. castellanii*, *A. culbertsoni* and *A. polyphaga*
- ❖ Reservoir for bacteria: *Acanthamoeba* may harbor bacterial pathogens such as *Legionella* and may serve as a potential reservoir and act as **Trojan horse** of the microbial world.

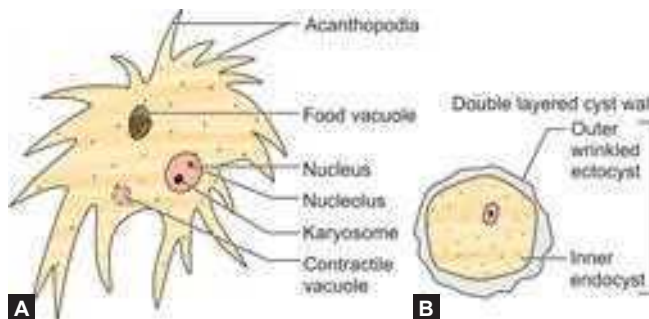
Morphology

Acanthamoeba species exist in nature as cyst and trophozoite forms (Fig. 75.4). There is no flagellated form.

Life Cycle and Pathogenesis (Fig. 75.5)

Man acquires infection by inhalation of aerosol contaminated with cyst or trophozoite, or rarely by direct spread through broken skin or infected eye.

- ❖ Primary sites of infection are sinuses and lungs. From lungs, trophozoites reach CNS by hematogenous route
- ❖ It causes two important clinical syndromes



Figs 75.4A and B: *Acanthamoeba* species (schematic diagram):
A. Trophozoite; B. Cyst.

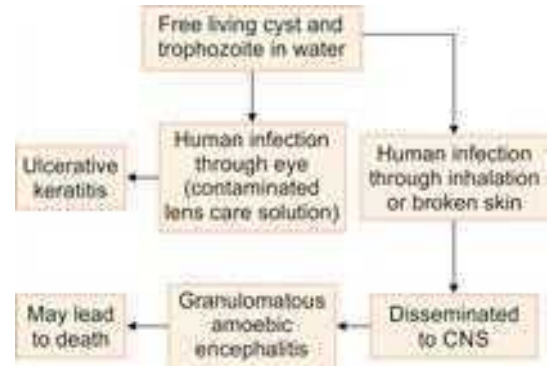


Fig. 75.5: Life cycle of *Acanthamoeba* species.

Abbreviation: CNS, central nervous system.

- GAE (granulomatous amoebic encephalitis) in immunocompromised patients like HIV positive patients, and
- Keratitis among contact lens users (discussed in Chapter 78).

Granulomatous Amoebic Encephalitis (GAE)

GAE has insidious onset, incubation period varies from several weeks to months.

- ❖ **Subacute to chronic course:** Lasts for months to years
- ❖ **Pathology:** It produces focal granulomatous lesions in brain
- ❖ **Lymphocytosis** of CSF can be seen. However, in patients with AIDS, no cells are seen in CSF
- ❖ **Symptoms:** Confusion, dizziness, nausea, headache, stiff neck and sometimes seizure and hemiplegia
- ❖ **Epidemiology:** More than 400 cases of GAE due to *Acanthamoeba* have been reported so far, half of those were from USA. From India, few cases were reported from Vellore, Chandigarh, Puducherry, Hyderabad and other places
- ❖ **In HIV patients:** In addition to GAE, *Acanthamoeba* produces nasal ulcers, cutaneous ulcers and musculo-skeletal abscesses.

Laboratory Diagnosis

- ❖ **CSF microscopy:** CSF is the specimen of choice for GAE. It should be examined immediately. Presence of characteristic trophozoites (or occasionally cyst) confirms the diagnosis. Wet mount examination and phase contrast microscopy are performed

Trophozoites and Cyst of *Acanthamoeba*

Trophozoite: Measures 30 μm (25–40 μm) size, characterized by presence of thorn or spine like pseudopodia called acanthopodia (Figs 75.4A and 75.6A)

- ❑ Nucleus is single with central karyosome and no peripheral chromatin
- ❑ Trophozoites should be carefully differentiated from pus cells by their motility observed in wet mount examination.

Cyst: Measures 10–25 μm , double walled, with an outer wrinkled cyst wall (Figs 75.4B and 75.6B)



Figs 75.6A and B: *Acanthamoeba* species: **A.** Trophozoite in CSF wet mount; **B.** Cyst in brain tissue (hematoxylin and eosin stain).

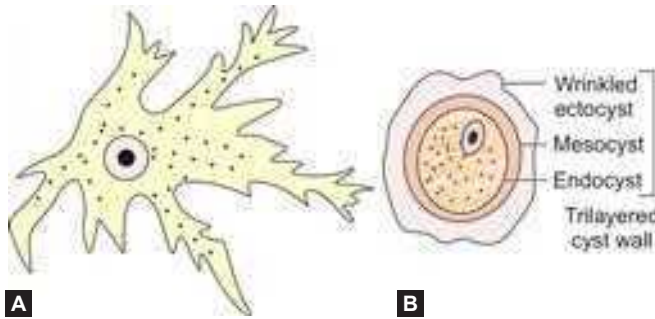
Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Imaging method:** CT scan or MRI reveals space-occupying or ring enhancing lesions in brain.
- TREATMENT** **Granulomatous amoebic encephalitis**
- ❑ Unfortunately, there are no therapies with proven efficacy against this disease. Only three cases have survived so far
 - ❑ The combination therapy recommended include pentamidine, an azole, sulfonamide (e.g. cotrimoxazole) and possibly flucytosine.
- Differences in the characteristics of *Naegleria* and *Acanthamoeba* are discussed in Table 75.2.
- Balamuthia mandrillaris Infection**
- Balamuthia mandrillaris* is a free-living, heterotrophic amoeba which causes GAE. It is named after its discoverer W. Balamuth.
- ❖ **Epidemiology:** It is distributed in the temperate regions of the world. Till now more than 200 cases have been reported, half of them being reported from USA and South America. So far, three cases have been reported from India (Delhi and Chandigarh)
 - ❖ **Life cycle:** It is similar to *Acanthamoeba*. It has trophozoite and cyst form (no flagellated form)
 - The trophozoite is approximately 12–60 μm , irregular with extensive branching, single nucleus, centrally located karyosome with no peripheral chromatin (Fig. 75.7A)
 - The cyst measures 13–30 μm , surrounded by a three layered cyst wall, and an abnormally large, vesicular nucleus (Fig. 75.7B).
 - ❖ **Clinical features:** It may enter the body through the respiratory tract or through open wounds. In CNS, it causes GAE. It can also cause skin lesion. Unlike *Acanthamoeba*, *Balamuthia* encephalitis has been found in immunocompetent individuals

Table 75.2: Differences in the characteristics of *Naegleria* and *Acanthamoeba*.

Character	<i>Naegleria fowleri</i>	<i>Acanthamoeba</i>
Disease	Primary amoebic meningoencephalitis	Granulomatous amoebic encephalitis Ulcerative keratitis, cutaneous lesions, sinusitis
Risk factor	Swimming in contaminated water	Immunodeficiency
Clinical course	Acute	Sub-acute to chronic
Pathology	Diffuse suppurative changes	Focal granulomatous inflammation
Trophozoites	<ul style="list-style-type: none"> • Two forms, amoeboid and flagellated form • Lobular and blunt pseudopodium (lobopodia) • 8–15 μm size 	<ul style="list-style-type: none"> • One form, no flagellated form • Thorn like pseudopodium (acanthopodia) • 25–40 μm size
Cyst	<ul style="list-style-type: none"> • Not present in tissue or CSF • Small (7–15 μm), thick and smooth double wall 	<ul style="list-style-type: none"> • Can be found in tissue • Larger (10–25 μm), double walled, with an outer wrinkled cyst wall
Spread	Direct neural spread	Hematogenous spread
CSF leukocytes	Neutrophils	Lymphocytes
Culture	<ul style="list-style-type: none"> • Require bacterial supplement • Don't grow with > 0.4% NaCl 	<ul style="list-style-type: none"> • May grow without bacterial supplement • Not affected by NaCl
CT scan	Unremarkable (such as basal arachnoiditis), no specific feature	Space occupying lesion is seen

Abbreviations: CSF, cerebrospinal fluid; NaCl, sodium chloride; CT, computed tomography.



Figs 75.7A and B: *Balamuthia mandrillaris*:
A. Trophozoite; B. Cyst.

- ❖ **Laboratory diagnosis:** Both trophozoites and cysts of *Balamuthia* are found in CSF and brain biopsy. *Balamuthia* can be differentiated from *Acanthamoeba* species by:
 - Cyst: Nucleus contains more than one nucleoli and cyst wall is tri-layered (Fig. 75.7B)
 - Indirect fluorescent antibody (IFA) test using specific antisera
 - Culture on cell lines but not on agar plate
 - PCR targeting mitochondrial small subunit rRNA gene.
- ❖ **Treatment:** Multidrug therapy has been recommended. The regimens include some combinations of pentamidine, azithromycin, fluconazole, amphotericin, and miltefosine.

Sappinia Infection

It is a newly recognized pathogenic free-living amoeba found in soil and water.

- ❖ Two species have been recognized—*S. diploidea* and *S. pedata*
- ❖ One confirmed case of *S. pedata* encephalitis has been documented so far while the pathogenicity of *S. diploidea* in man is unknown
- ❖ The characteristic feature—both trophozoite and cyst stages are bi-nucleated.

TOXOPLASMA ENCEPHALITIS

Toxoplasma gondii is an obligate intracellular parasite affecting a wide range of mammals and birds including

humans. Though human infection is common; very few progress to disease, mostly restricting to immunocompromised persons such as with HIV/AIDS (developing encephalitis) and congenital infection in fetus.

Morphology

It exists in three morphological forms—two asexual forms (tachyzoite and tissue cyst) and a sexual form (oocyst).

Tachyzoites

They are actively multiplying form (trophozoite), usually seen in acute infection in peripheral blood.

- ❖ They are **crenate shaped**, 4–8 μm in length (Fig. 75.8A)
- ❖ Some time, host cell becomes distended by the proliferating tachyzoites and appears as *pseudocyst* (Fig. 75.8B).

Tissue cyst

It is the resting stage of the parasite, usually seen in chronic infections.

- ❖ **Organs:** They are found in various organs; most common sites—muscles and brain
- ❖ **Shape:** They are round to oval—appear spherical in the brain and oval inside the muscle tissue
- ❖ **Size:** The cysts vary in size (2–100 μm)
- ❖ The tissue cysts comprise of several bradyzoites, surrounded by a cyst wall (Fig. 75.8C)
- ❖ **Bradyzoites** are crescent shaped and slowly multiplying trophozoites. They measure 7 μm in length and contain several strongly periodic acid-Schiff stain (PAS) positive granules.

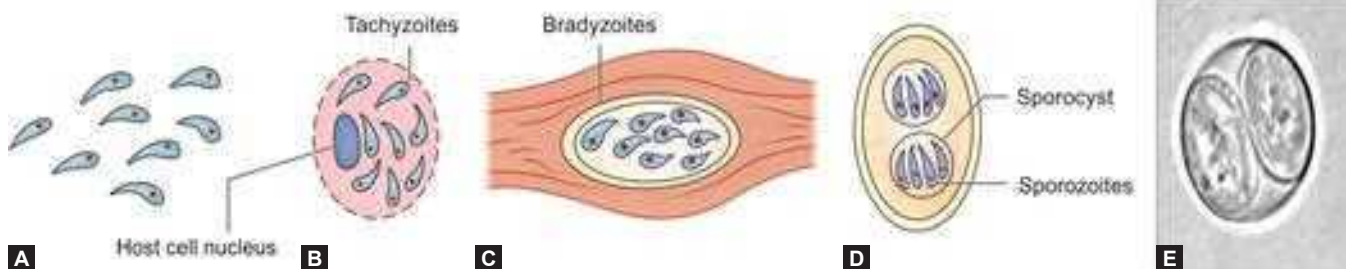
Oocyst

It is the sexual form of the parasite found in cats and other felines. It is oval shaped, measures 11–14 μm long; contains two sporocysts, each containing four elongated sporozoites (Figs 75.8D and E).

Life Cycle (Fig. 75.9)

Host: The life cycle involves two hosts:

1. **Definitive hosts** are cat and other felines; where the sexual cycle takes place



Figs 75.8A to E: *Toxoplasma gondii* (schematic diagram): A. Tachyzoites; B. Pseudocyst; C. Tissue cyst; D. Sporulated oocyst; E. Sporulated oocyst in cat's feces (saline mount).

Source: E. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

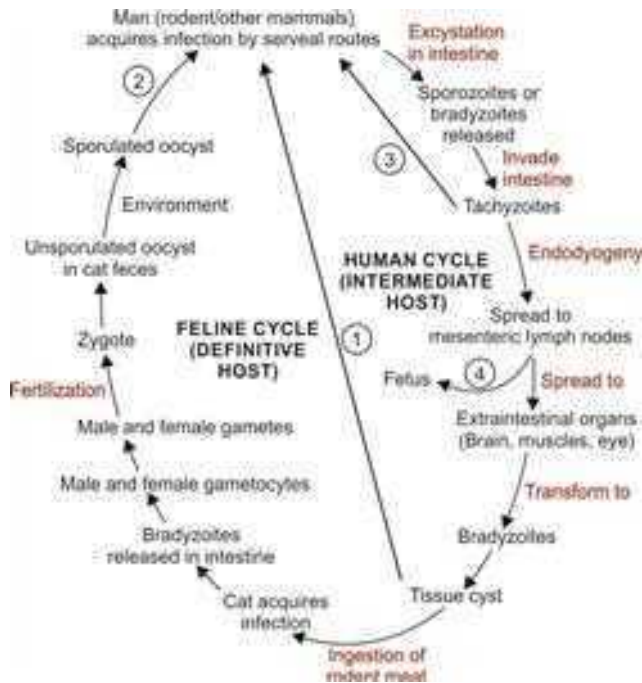


Fig. 75.9: Life cycle of *Toxoplasma gondii*.

Note: The routes of transmission are—(1) ingestion of contaminated undercooked meat (most common route), (2) ingestion of sporulated oocysts from contaminated soil, food, or water, (3) blood transfusion, organ transplantation, and (4) mother-to-fetus.

2. Intermediate hosts are man and other mammals (e.g. rodents); where the asexual cycle takes place.

Human (Asexual) Cycle

- ❖ **Transmission:** *T. gondii* is unique among the protozoa as all the three morphological forms can transmit the infection. Transmission to man occurs (in the decreasing order of frequency):
 - Ingestion of tissue cyst from undercooked meat (most common route)
 - Ingestion of sporulated oocysts from contaminated soil, food, or water
 - By blood transfusion, organ transplantation, or mother-to-fetus: Tachyzoites are the infective form.
- ❖ **Transform into tachyzoites:** In the intestine, sporozoites are released from sporulated oocyst and bradyzoites are released from the tissue cyst. They invade the intestinal epithelium and transform into tachyzoites
- ❖ **Transform into tissue cyst:** Tachyzoites multiply actively by a process called endodyogony and spread locally to the mesenteric lymph node. Subsequently, they also spread to distant extraintestinal organs like brain, muscles, eye, liver, etc. where they transform into bradyzoites which subsequently encysted to form tissue cysts.

Sexual Cycle (The Feline Cycle)

Cat and other felines (definitive host) acquire infection by ingestion of tissue cysts in the meat of rodents and other animals.

- ❖ Bradyzoites are released from the tissue cysts in the intestine, which undergo several cycles of schizogony (asexual cycle) followed by gametogony (sexual cycle) to form male and female gametes, which then fertilize to form zygotes
- ❖ Zygotes develop a cyst wall to transform to oocysts that are excreted in cat's feces
- ❖ Freshly passed oocysts are unsporulated and non-infective, which become sporulated within 1-5 days in humid environment. The mature sporulated oocyst is infectious to man for about 1 year.

Pathogenicity

Toxoplasmosis is one of the most common parasitic zoonotic infections affecting a wide range of mammals and birds.

Prevalence: Global prevalence is about 25–30%. High prevalence (>50%) is found in Latin America and tropical African countries. Southeast Asia including India has a low seroprevalence (10–30%).

Various risk factors for infection are:

- ❖ **Immune status:** Patients associated with HIV, malignancies and other immunocompromised conditions are at high-risk
- ❖ Patients undergoing blood transfusion, and organ transplantations are at higher risk
- ❖ **Age:** It commonly affects elderly and fetus
- ❖ **Food habits:** Ingestion of uncooked cat and other animal meat (seen in countries like France)—at higher risk
- ❖ **Genetic factor:** HLA DQ3 is associated with encephalitis in AIDS patient and hydrocephalus in fetus infected with *Toxoplasma*.

Clinical Manifestations

The clinical manifestations of toxoplasmosis can vary depending upon the patient population affected.

Immunocompetent Patients

In the immunocompetent host, the host immune response controls the infection. Therefore, acute toxoplasmosis in the immunocompetent host is usually asymptomatic and self-limited

- ❖ **Lymphadenopathy:** The most common manifestation is cervical lymphadenopathy. Other lymph nodes may also be affected like suboccipital, supraclavicular and inguinal nodes
- ❖ **Other symptoms** include headache, malaise, fatigue and fever.

Immunocompromised Patients

In immunocompromised hosts such as patients infected with HIV, heart transplant recipients, malignancies or in fetus, the clinical manifestations are more severe due to the lack of the immune system to control the infection. The tachyzoites are disseminated to a variety of organs,

particularly lymphatic tissue, skeletal muscle, myocardium, retina, placenta and CNS.

Toxoplasmosis in Patients with HIV

Toxoplasmosis is one of the common opportunistic parasitic infections in patients with AIDS (15–40%).

- ❖ Infection occurs either due to reactivation of latent infection (more common) or as a newly acquired infection from an exogenous source such as blood or transplanted organs
- ❖ It mainly targets CNS leading to *Toxoplasma* encephalitis (TE). Other manifestations include pulmonary infections and chorioretinitis.

Toxoplasma Encephalitis (TE)

It is the most common presentation of toxoplasmosis in HIV-infected individuals.

- Most common areas involved are the brainstem, basal ganglia, pituitary gland and corticomedullary junction
- TE develops when the CD4+ T cell count falls below 100/μL
- Pathogenesis is due to the direct invasion by the parasite leading to necrotizing encephalitis and also due to secondary pressure effects on the surrounding area of the CNS
- Patients may present with altered mental status, seizures, sensory abnormalities, cerebellar signs and focal neurologic findings including motor deficits, cranial nerve palsies and visual-field loss.

Congenital Toxoplasmosis

Transplacental transmission of *T. gondii* can cause various congenital malformations such as chorioretinitis, hydrocephalus, and intracranial calcifications; discussed in detail in Chapter 79.

Laboratory Diagnosis

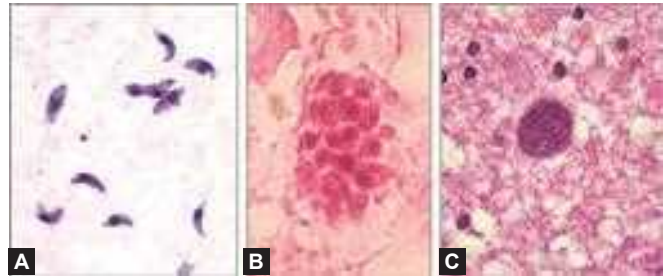
Direct Microscopic Examination

The specimens frequently examined are peripheral blood, body fluids, lymph node aspirate, bone marrow aspirate and biopsy material from spleen, liver and brain. These specimens are stained with Giemsa, H & E and PAS.

- ❖ Comma-shaped tachyzoites are detected in the smear made from blood, body fluids and tissue; their presence indicates acute infection (Figs 75.10A and B)
- ❖ Tissue cyst containing strongly PAS positive bradyzoites can be detected in various tissues like brain or muscle (Fig. 75.10C). This denotes the presence of infection but cannot differentiate acute and chronic infection.

Antibody Detection

Antibody detection remains the most widely used method for diagnosis of acute toxoplasmosis in immunocompetent individuals. Antibodies are produced at a very low level and irregularly in immunocompromised patients; hence, antibody detection methods are not reliable for diagnosis of *Toxoplasma* encephalitis.



Figs 75.10A to C: *Toxoplasma gondii*. **A.** Giemsa stain showing comma-shaped tachyzoites in the blood smear; **B.** Histopathology of brain shows pseudocyst containing numerous tachyzoites; **C.** Tissue cyst containing bradyzoites (section of brain).

Source: A and C. DPDx Image Library; B. Public Health Image Library, ID# 575/ Dr Edwin P Ewing; Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Various methods are available to detect isotype specific antibodies such as capture ELISA and immunosorbent agglutination assay (ISGA).

- ❖ **IgG antibody:** IgG cannot be used to diagnose congenital infection (as IgG crosses placenta). It also cannot differentiate between recent and past infection or recovery as IgG appears early in infection and persists for long
 - **Fourfold rise in IgG titer** is necessary for diagnosis of acute toxoplasmosis
 - **IgG avidity test:** Avidity of IgG with its antigen increases with time. Detection of low IgG avidity indicates recent infection (<12 weeks); whereas a strong avidity indicates past infection.
- ❖ **IgM antibody:** It appears early, within 1-2 weeks; therefore, indicates acute infection, but it is not a reliable indicator as it persists up to 6 months. The detection of IgM antibodies in baby's serum confirms congenital infection
- ❖ **IgA antibody:** IgA may be detected in sera of acutely infected adults. It is also useful for diagnosis of congenital toxoplasmosis
- ❖ **IgE antibody:** IgE is useful for *Toxoplasma* encephalitis in HIV infected patients, congenital and in acute infection. When IgG and IgM tests are performed simultaneously, interpretation is done as follows.
 - ❖ IgG (+) and IgM (+): Indicates acute infection, but needs further confirmation by IgG avidity testing
 - ❖ IgG (+) and IgM (-): Indicates infection with *T. gondii* >6 months before (remote infection)
 - ❖ IgG (-) and IgM (+): Indicates false-positive IgM.

Sabin-Feldman Dye Test

This is the gold standard antibody detection method, usually done in the reference laboratories. Other serological tests are evaluated taking this test as standard.

- ❖ It is a complement mediated neutralization test that requires live tachyzoites
- ❖ Because of its technical difficulty and inability to differentiate between recent and past infection; this test is seldom used nowadays in routine diagnostics.

Detection of *Toxoplasma* Antigens

Antigen detection is less commonly used as it lacks sensitivity. It may have a role in situations where antibodies are low—(i) immunocompromised, (ii) early acute stage, and (iii) monoclonal gammopathies.

Molecular Diagnosis

Polymerase chain reaction (PCR) can be employed to detect *Toxoplasma* specific DNA from various clinical samples like blood, CSF, bronchoalveolar lavage or amniotic fluid.

- ❖ PCR is highly sensitive, specific; can be used to diagnose TE or congenital infections in resource poor settings
- ❖ Molecular method is also useful for detection of *Toxoplasma* genotypes. It has three genotypes, I to III.

Other Methods

- ❖ **Animal inoculation:** *T. gondii* can be isolated from mice by intraperitoneal inoculation of the clinical samples
- ❖ **Tissue culture:** *T. gondii* can be isolated by inoculating into cell lines such as human foreskin fibroblast, continuous cell lines (HeLa and Vero)
- ❖ **Imaging methods:** CT or MRI scan of brain can be done to demonstrate multiple ring enhancing lesions in basal ganglia or corticomedullary junction to diagnose TE in HIV patients
- ❖ **CSF examination:** Evaluation of CSF of patients with TE reveals an elevation of intracranial pressure, lymphocytosis, and a slight increase in protein concentration, occasional increase in the gamma globulin level and a normal glucose level.

TREATMENT

Toxoplasmosis

Immunocompetent patients

Immunocompetent patients with only lymphadenopathy do not require specific therapy unless they have persistent, severe symptoms.

Immunocompromised patients

Toxoplasmosis is rapidly fatal in immunocompromised patients. If not treated, it may progress to encephalitis. So treatment is essential.

i. Primary prophylaxis:

AIDS patients with *Toxoplasma* infection, having CD4+ T lymphocyte count of less than 100/μL should receive prophylaxis against TE.

- ❑ Trimethoprim-sulfamethoxazole (cotrimoxazole) is the drug of choice
- ❑ Dapsone-pyrimethamine, atovaquone with or without pyrimethamine can be given alternatively
- ❑ Prophylaxis can be discontinued in patients who have responded to antiretroviral therapy (ART) and whose CD4+ T lymphocyte count has been more than 200/μL for 3 months.

ii. Secondary prophylaxis (Long-term maintenance therapy):

Required for HIV positive patients who are previously treated for toxoplasmosis.

- ❑ Should be started if the CD4+ T lymphocyte count decreases to less than 200/μL

Contd...

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TREATMENT

Toxoplasmosis

- ❑ However, it can be discontinued if the patient is asymptomatic, and have a CD4+ T lymphocyte count of more than 200/μL for at least 6 months.

Prevention

The various methods recommended to prevent toxoplasmosis include:

- ❖ Consumption of thoroughly cooked meat
- ❖ Proper hygiene maintenance and hand cleaning of people handling cats and other felines
- ❖ Regular prenatal and antenatal screening to detect *Toxoplasma* infection in women of child-bearing age
- ❖ Avoiding cat's feces (oocyst) contaminated materials (like a cat's litter box)
- ❖ Screening of blood banks or organ donors for antibody to *T. gondii*
- ❖ No vaccine trials are going on currently.

CEREBRAL MALARIA

Cerebral malaria occurs as a complication of *Plasmodium falciparum* infection (Chapter 35), not by any other *Plasmodium* species.

- ❖ **Pathogenesis:** *P. falciparum* exhibit unique property of being sequestered inside the brain capillaries by:
 - Expressing a protein called PfEMP (*P. falciparum* erythrocyte membrane protein-1), by virtue of which, the parasitized RBCs adhere to vascular endothelium and also adhere to nonparasitized RBCs
 - This results in plugging of brain capillaries by the rosettes of sequestered parasitized RBCs leading to vascular occlusion and cerebral anoxia
- ❖ **Clinical manifestations:** Cerebral malaria manifests as diffuse symmetric encephalopathy characterized by repeated seizures, reduced muscle tone and tendon reflexes
 - Seizures are more common in children (up to 50%) than in adults (10%)
 - Other defects are retinal hemorrhages, neurologic sequelae, and rarely deep coma
 - Signs of focal neurologic and meningeal irritations are absent
 - Associated with high mortality rate of 15–20%.
- ❖ **Laboratory diagnosis:** The various diagnostic methods include (Chapter 35):
 - Peripheral blood smear examination is the gold standard method to identify *P. falciparum*
 - ♦ Banana-shaped gametocytes
 - ♦ Ring forms- multiple ring forms, accolae forms and head-phone shaped ring forms
 - ♦ Schizonts are not seen.
 - Quantitative buffy coat examination
 - Rapid diagnostic tests: ICT detecting *P. falciparum* specific HRP-II antigen.

- ❖ **Treatment:** Artesunate, artemether, arteether and quinine are the drugs used for treatment of cerebral malaria (Chapter 35).

AFRICAN SLEEPING SICKNESS

Trypanosoma brucei (T.b.) gambiense and *T. b. rhodesiense* are the causative agents of Western and Eastern African sleeping sickness respectively (Chapter 36). It is transmitted by tsetse fly, inoculating the infective form—metacyclic trypomastigotes.

- ❖ **Pathogenesis:** It begins with hemolymphatic stage (i.e. dissemination of the parasite through the lymphatics and bloodstream), which proceeds to the next stage with invasion of CNS particularly pons, medulla and frontal; which leads to steady progressive chronic meningoencephalitis
- ❖ **Clinical manifestations:** Patients develop characteristic progressive daytime somnolence (hence called as “sleeping sickness”), with restlessness and insomnia at night which may be related to increased prostaglandin D2 level in the body
 - Behavioral and personality changes with apathy, confusion, fatigue and loss of coordination may be observed
 - Other features include listless gaze, loss of spontaneity, and abnormal speech with few extrapyramidal signs like choreiform movements, tremors and fasciculations
 - In the terminal stage, the patient becomes emaciated progressing to coma and death, usually from secondary infection.
- ❖ **Duration:** West African form is a chronic disease, lasting for months to years; whereas East African disease runs an acute course with duration <9 months (before that the death occurs)
- ❖ **Diagnosis:** The disease is diagnosed by detection of trypomastigote form of the parasite in peripheral blood, parasite specific antigen and antibody detection in blood and CSF
- ❖ **Treatment:** Eflornithine or melarsoprol are the drugs of choice for West and East African trypanosomiasis respectively.

NEUROCYSTICERCOSIS

Neurocysticercosis (NCC) is a neurologic infection caused by the larval stage of the tapeworm *Taenia solium*.

Life Cycle of *Taenia solium*

Life cycle of *T. solium* causing cysticercosis is different than its life cycle when it causes intestinal taeniasis (Chapter 46, Fig. 46.4).

- ❖ **Host:** Man acts as both **definitive** and **intermediate host**
- ❖ **Infective stage:** Eggs of *T. solium*
- ❖ **Mode of transmission:** Firstly man acquires the infection by—(1) ingestion of contaminated food or

water containing eggs of *T. solium*, and (2) autoinfection (described below)

Autoinfection

Eggs excreted in the feces reinfect the same individual. Autoinfection can be of two types:

- 1. External autoinfection:** Due to unhygienic personal habit, e.g. contaminated finger.
- 2. Internal autoinfection:** Due to reverse peristaltic movements by which the gravid segments throw the eggs back into the stomach (equivalent to swallowing of the eggs).

Note: A prior episode of intestinal taeniasis is a prerequisite for development autoinfection. Intestinal taeniasis is transmitted by ingestion of uncooked pork meat contaminated with larvae (*cysticercus cellulosae*), which develop into adult worms that sexually mature (self-fertilize) to produce eggs in the gut.

- ❖ **Human cycle:** The embryo or oncosphere is released from the eggs, penetrates the intestine and enters into the portal circulation or mesenteric lymphatics and reaches to various organs like subcutaneous tissue, muscle, eye and brain where it is transformed to the larval stage; *cysticercus cellulosae* in 7–9 weeks and deposited as cyst. Full development to mature cysts takes 2–3 months of time.

Cysticercus Cellulosae

A mature *cysticercus cellulosae* measures 5 mm long and 8–10 mm wide, spherical (or slightly oval), yellowish white, separated from the host tissue by a thin collagenous capsule.

- ❖ **It contains two chambers:** Outer one is a bladder like sac filled with 0.5 mL of vesicular fluid and the inner chamber contains the growing scolex (Fig. 75.11)
- ❖ **Racemose cysticerci:** In some cases, when the parasites are lodged in spacious area, they grow and transform



Fig. 75.11: *Cysticercus cellulosae* (surgically removed).

Source: Head, Department of Microbiology, Meenakshi Medical College, Chennai (with permission).

into larger lobulated cysticerci (>20 cm), containing 60 mL of vesicular fluid

- They do not contain scolices within the bladder, resemble metastatic tumor
- Mainly found in brain (fourth ventricle and subarachnoid space), and cervical spinal cord
- It is associated more frequently with HIV infected patient
- Patients present with increased intracranial pressure and frequently require surgery
- The prognosis of racemose cysticerci is poor.

Clinical Manifestations (Cysticercosis)

Clinical spectra of the disease depend upon the localization of the cyst. Though it is discovered from any site of the body, but the common sites are CNS, subcutaneous tissue, skeletal muscle and eyes.

Neurocysticercosis

Neurocysticercosis (NCC) is the most common form of cysticercosis; accounts for 60–90% cases of cysticercosis.

- ❖ NCC is considered as the most common parasitic CNS infection of man and the most common cause of adult onset epilepsy throughout the world
- ❖ **Age:** Adults of 30–50 years of age are affected commonly
- ❖ **Types:** Based on the site of involvement, NCC is of two types:
 1. **Parenchymal:** Involves brain parenchyma; considered as the most common site of NCC
 2. **Extraparenchymal** sites are meninges, ventricles, spinal cord and subarachnoid space.
- ❖ **Asymptomatic NCC:** Sometimes NCC remains in the brain without causing any apparent symptoms
- ❖ **Seizure** is the most common manifestation (70% of cases). NCC accounts for 50% cases of late-onset epilepsy
- ❖ **Hydrocephalus:** It is the most common extra parenchymal feature. Its presence carries a bad prognosis
- ❖ **Other clinical features include:**
 - Increased intracranial pressure and hypertension—presented as headache, vomiting and vertigo
 - Chronic meningitis
 - Focal neurological deficits
 - Psychological disorders and dementia
 - Cerebral arteritis (associated with subarachnoid cysticercosis)
 - Basal and ventricular involvement: Carries poor prognosis.
- ❖ **NCC exists in four morphological stages:** It starts as vesicular form, gradually develops into necrotic, followed by nodular and finally into calcified stage
- ❖ The clinical presentation is variable and depends on number, location and size of the cyst, the morphological stage of the cyst and the host immune response
- ❖ **NCC and HIV:** NCC co-infection is likely to be increasingly recognized in patients with HIV and should be included

in the differential diagnosis of CNS infections in these patients.

Other Forms of Cysticercosis

- ❖ **Subcutaneous cysticercosis:** It is frequently asymptomatic but may manifest as palpable nodules
- ❖ **Muscular cysticercosis:** Manifest as muscular pain, weakness or pseudohypertrophy
- ❖ **Ocular cysticercosis:** Can involve eyelids, conjunctiva and sclera. Common symptoms like proptosis, diplopia, loss of vision and slow growing nodule with focal inflammation.

Epidemiology

World

Cysticercosis is a major public health problem, especially in the developing world.

- ❖ *T. solium* infection is endemic in Mexico, Central America, South America, Africa, Southeast Asia, India, Philippines, and Southern Europe
- ❖ NCC is the most frequent preventable cause of epilepsy worldwide. According to WHO, 30% of all epilepsy cases in endemic countries and 3% epileptic cases globally may be due to NCC
- ❖ However, it is reported less from the Muslim countries (as pork eating is not allowed).

India

Cysticercosis is highly prevalent in the northern states such as Bihar, Odisha, Uttar Pradesh and Punjab.

- ❖ It is believed that NCC is largely underreported in India, accounting for 2–3% of epileptic cases
- ❖ The underreporting is because of lack of systematic population-based studies and unavailability of imaging techniques in rural areas.

LABORATORY DIAGNOSIS

Cysticercosis

- ❑ **Radiodiagnosis**—CT scan and MRI (useful for detecting number, location, size of the cysticerci and the stage of the disease)
- ❑ **Antibody detection** in serum or CSF—
 - ELISA (using crude extract of cysticerci)
 - Western blot (using 13 kDa LLGP Ag)
- ❑ **Antigen detection** in serum or CSF—ELISA
- ❑ Lymphocyte transformation test
- ❑ **Histopathology** of muscles, eyes, subcutaneous tissues or brain biopsies—can detect cysticerci
- ❑ **FNAC** of the cyst and then staining with Giemsa
- ❑ **Fundoscopy** of eye—detects larvae
- ❑ Modified Del Brutto diagnostic criteria.

Laboratory Diagnosis

Radiodiagnosis (Imaging Methods)

CT scan and MRI are the two important imaging methods used to detect cysticerci in brain.

- ❖ Because of the vesicular structure, live cysticerci appear hypodense (low signal intensity) area and the scolex is present eccentric inside the vesicle and gives a hyperdense (high signal intensity) area
- ❖ Imaging methods are useful to identify:
 - The number of cysts (single or multiple cysticerci)
 - Location of the cyst (parenchymal or extra-parenchymal)
 - Size of the cyst (small—cysticercus cellulosae and big cyst—cysticercus racemosus)
 - The stage of the disease (vesicular, necrotic, nodular and calcified)
 - Extent of the lesion
 - Active or dormant lesion: Associated inflammation and edema gives a ring-like enhancement surrounding the cysts, indicates acute infection.
- ❖ CT scan is useful to detect calcified cysts (appears as hyperdense dots) (Fig. 75.12)
- ❖ MRI has a higher contrast resolution, which makes the lesion clearer. It is superior than CT scan to detect the:
 - Extraparenchymal cysts in ventricle and cisterns
 - Inflammatory changes
 - Vesicular and necrotic lesions
 - Noncystic lesions.

Immunodiagnosis

It has the advantage of lower cost than CT and MRI and confirms the etiology.

1. Antibody Detection

ELISA: ELISA detects antibodies in serum and CSF by using crude extract of cysticerci or vesicular fluid.

- ❖ It is highly sensitive (75–90%) in serum. CSF ELISA also gives better results
- ❖ Moreover, recent ELISA method using purified glycoprotein antigens has shown better sensitivity but

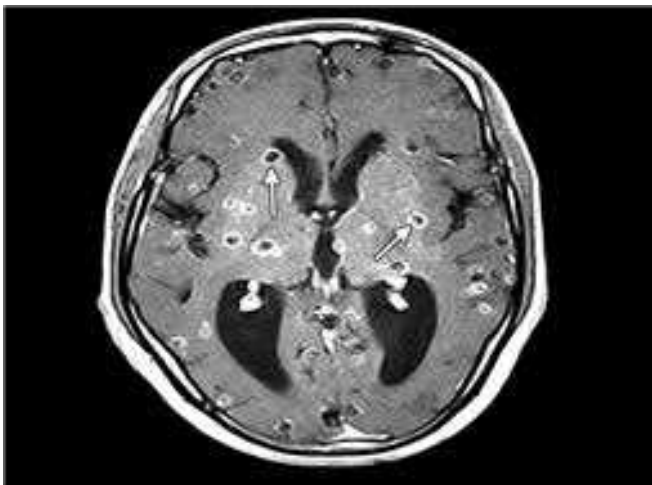


Fig. 75.12: CT scan of brain showing multiple ring enhancing lesions with eccentric scolex (neurocysticercosis).

Source: Dr A Subathra. Department of Radiodiagnosis, JIPMER, Puducherry (with permission).

its specificity is low as it gives false positive results in cross reacting helminthic infections such as echinococcosis

- ❖ **QuickELISA:** It is a quantitative ELISA, available commercially. It detects antibodies in serum against T24H recombinant antigen. Results are comparable to western blot, has a sensitivity of 96% and specificity 99%.

Western blot: Western blot assay [also called as enzyme immune transfer blot (EITB)] uses highly specific 50–13 kDa lentil lectin-purified seven glycoprotein (LLGP) antigenic fractions; hence its specificity approaches 100%.

- ❖ Presence of **one to seven Gp bands** confirms the diagnosis
- ❖ It can be performed on serum, blood, dried blood spot or CSF
- ❖ The sensitivity and specificity are 98% and 100% respectively in persons with multiple viable cyst
- ❖ The sensitivity is directly proportional to the number of live cysticerci.

However, antibody detection methods have disadvantages—(1) cannot differentiate active and past infection, (2) antibodies persist even after recovery of the patient.

2. Antigen Detection

Antigen detection in CSF or serum by ELISA has been developed using monoclonal *T. solium* antibodies. Antigen disappears following treatment hence, can be used for monitoring.

Histopathology

Cysticerci can be detected in muscles, eyes, subcutaneous tissues (or brain during postmortem) by biopsy following surgical removal or fine needle aspiration of the cyst followed by microscopic demonstration of the parasite (Fig. 75.13).

Fine-needle Aspiration Cytology (FNAC)

Fine-needle aspiration of the cyst can be done followed by staining with Giemsa, or Ryan's modification of trichrome stain.

- ❖ Microscopically, it can differentiate between viable, necrotic and calcified cysticerci through their morphological pattern
- ❖ Cholesterol crystals are frequently seen which may be attributed to the high lipid content of the lesions. Charcot-Leyden crystals are characteristically absent.

Fundoscopy

Ocular cysticercosis can usually be diagnosed by fundoscopy for the visual identification of the movements and morphology of the larval worm.

Revised DelBrutto's Diagnostic Criteria

DelBrutto's criteria has been widely used for the diagnosis of NCC in endemic countries since 2001. It has been revised



Fig. 75.13: *Cysticercus cellulosae* in biopsy from the brain (hematoxylin and eosin stain)—An entire cysticercus seen within the bladder walls, [Parenchymatous portion of the cysticercus can be better observed. The extensive folding of the spiral canal and one sucker of the scolex (Arrow)].

Source: Head, Department of Pathology, Meenakshi Medical College, Chennai (with permission).

in 2016. It is based on clinical, imaging, immunological and epidemiological data.

TREATMENT

Cysticercosis

Antiparasitic agents:

- ❑ For brain parenchymal lesions:
 - Albendazole (15 mg/kg per day for 8–28 days) or
 - Praziquantel (50–100 mg/kg daily in three divided doses for 15–30 days)
- ❑ Longer courses are often needed in patients with multiple subarachnoid cysticerci

Symptomatic treatment of:

- ❑ Seizures by antiepileptic drugs
- ❑ High-dose glucocorticoids should be used to reduce the inflammatory reactions caused by dead cysticerci
- ❑ Hydrocephalus: Attempts should be made to reduce intracranial pressure. In the case of obstructive hydrocephalus, cysticerci can be removed by endoscopic surgery or ventriculo-peritoneal shunting.

Surgery:

- ❑ Open craniotomy to remove cysticerci is rarely required nowadays
- ❑ Surgery is indicated for ocular, spinal and ventricular lesions because antiparasitic drugs can provoke irreversible inflammatory damage.

Prevention

The prevention of cysticercosis involves:

- ❖ **Good personal hygiene** to prevent autoinfection with eggs
- ❖ **Effective fecal disposal** to prevent contamination of food and water with eggs
- ❖ Treatment and prevention of human intestinal taeniasis
- ❖ **Vaccines to prevent porcine cysticercosis:** Various antigens like *T. solium* oncosphere antigen, *T. crassiceps*

and *T. ovis* recombinant antigens are attempted for vaccination of pigs. They are under development.

RARE PARASITIC INFECTIONS OF CNS

The following are the rare parasitic agents infecting CNS. They are either—(1) common human pathogens producing infections elsewhere and occasionally infect CNS (e.g. *E. histolytica*, *Strongyloides*) or (2) primary pathogens of lower animals and human infection is rare (e.g. *Angiostrongylus*).

Rare Protozoan Infections of CNS

***Entamoeba histolytica*:** Hematogenous spread of trophozoites can occur from liver affecting brain causing brain abscess and secondary amoebic encephalitis (Chapter 45).

Rare Cestode Infections of CNS

❖ ***Taenia multiceps*:** It is a cestode, infecting dog and other animals

- Human infection is rare, producing space occupying lesion in CNS similar to NCC due to deposition of larval stage called as coenurus in CNS. Therefore, the disease is called as coenurosis
- Nearly 175 cases have been reported in humans so far; mainly from developing countries where the dog population is not controlled such as African countries.

❖ ***Spirometra*:** It is another cestode of dogs and cats

- Human infection is extremely rare, results in deposition of its larva (called sparganum) in various tissues such as subcutaneous tissues, muscles, eyes and visceral organs like brain (frontal and parietal lobes) and lymphatics. The disease is called as sparganosis
- Presents with various neurological symptoms including weakness, headache, seizure, and abnormal skin sensations, such as numbness or tingling.

❖ ***Echinococcus*:** Hydatid cyst may get deposited in brain very rarely (3% of cases) and spinal cords. (discussed in detail in Chapter 49).

Rare Trematode Infections of CNS

❖ ***Neuroschistosomiasis*:** It is rare complications seen with *Schistosoma* infection (Chapter 46). Myelopathy of the lumbosacral region is the most common neurological manifestation of *S. mansoni* or *S. haematobium* infection, whereas acute encephalitis is the most common feature of *S. japonicum*

Cerebral Schistosomiasis

Acute encephalitis is the common CNS presentation of *S. japonicum* infection.

- ❑ It occurs in 2–4% of cases due to migration of eggs
- ❑ Parietal lobe is the most common site
- ❑ Symptoms include Jacksonian convulsions and grand mal seizures

Contd...

Contd...

- The higher incidence of CNS involvement of *S. japonicum* compared to other schistosomes is attributed to their small size eggs; which are released in higher numbers from the worm, and can be carried easily to CNS.

❖ **Cerebral paragonimiasis:** It is the most severe form of paragonimiasis (Chapter 69). Encapsulated cysts in the brain parenchyma present as space-occupying lesions. Symptoms include fever, headache, vomiting, motor weakness or epilepsy.

Rare Nematode Infections of CNS

Hyperstrongyloidiasis Syndrome

In *Strongyloides* infection, repeated autoinfection cycles occur occasionally which result in generation of large number of filariform larvae, which penetrate the GIT and migrate to various organs including CNS causing disseminated strongyloidiasis.

- ❖ CNS invasion causes brain abscess and meningitis. Larvae can be seen in the CSF occasionally
- ❖ CSF examination shows pleocytosis, elevated protein, normal glucose and negative for bacterial culture
- ❖ The mortality rate in untreated patients approaches 100% and even with treatment it may exceed 25%
- ❖ **Risk factors:** Glucocorticoid therapy is the main risk factor. Other risk factors include immunosuppressive conditions such coinfection with human T cell lymphotropic virus type-1 (HTLV-1).

Strongyloidiasis is discussed in detail in Chapter 46.

Angiostrongylus cantonensis Infection

Also called as the rat lung worm, produces a form of visceral larva migrans (Chapter 49) affecting CNS called, **eosinophilic meningitis**. This infection occurs principally in Southeast Asia.

- ❖ Human acquires infection by ingestion of L₃ larvae in undercooked intermediate host or in contaminated water. The larvae penetrate the intestine and through the blood circulation, migrate to the CNS and develop into adult worms
- ❖ As man is an abnormal host, the life cycle gets arrested, worms die producing a local eosinophilic inflammation and granuloma formation around the dying worms
- ❖ Patients usually present with headache, neck stiffness, nausea and vomiting, and paresthesia
- ❖ **Diagnosis:** Examination of CSF can reveal—increased CSF pressure, protein and WBC count, eosinophilic pleocytosis of more than 20%, and normal glucose level
 - Larvae or young adult worms can often be recovered in the CSF
 - Peripheral blood eosinophilia may be mild.
- ❖ **Treatment:** Management consists of supportive measures, including analgesics, sedatives and glucocorticoids.

Other rare nematode infections of CNS are:

- ❖ *Loa loa:* It is a filarial nematode causing cutaneous and ocular infections (Chapter 57). It rarely can cause meningoencephalitis
 - It occurs in DEC treated patients with higher microfilaremia; antigens released from the dead larvae may induce inflammatory reaction
 - It can be prevented by administration of anti-inflammatory drugs along with DEC
 - DEC should be stopped if any neurological symptoms appear.
- ❖ *Trichinella spiralis:* It is a somatic nematode that usually produce cystic lesions in muscle (Chapter 57). Occasionally the larvae may migrate to CNS producing encephalitis
- ❖ *Toxocara:* It is a parasite of lower animals, causes visceral larva migrans, mainly affects liver, occasionally involve brain, resulting in seizures (Chapter 49)
- ❖ *Baylisascaris procyonis:* It is also parasite of lower animals, and also a rare cause of neural larva migrans, producing fatal eosinophilic meningoencephalitis
- ❖ *Gnathostoma spinigerum:* It is also a parasite of lower animals that rarely infects man. It usually produces cutaneous swellings. Rarely, the larvae may spread to CNS producing eosinophilic meningoencephalitis.

FUNGAL INFECTIONS OF CNS

Various fungal agents can cause CNS infections (mainly chronic meningitis), out of which *Cryptococcus neoformans* is the most important. Other fungi infecting CNS predominantly cause infections of other systems and therefore discussed elsewhere (Table 75.3).

CRYPTOCOCCAL MENINGITIS

Cryptococcal meningitis is caused by a capsulated yeast called *Cryptococcus neoformans*, which is capable of producing potentially fatal meningitis in HIV infected people.

Species and Serotypes

Cryptococcus has two species, *C. neoformans* and *C. gattii* and four serotypes A, B, C and D.

Table 75.3: Fungal agents causing CNS infections.

Primary CNS pathogen: *Cryptococcus neoformans*

Fungi which primarily cause infections of other body sites, rarely cause CNS infections

- **Agents of systemic mycoses:** *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Coccidioides immitis*
- **Agents of subcutaneous mycoses:** *Sporothrix schenckii*, *Pseudallescheria boydii* and *Cladophialophora bantiana*
- **Opportunistic fungal agents:** *Candida albicans*, *Aspergillus* species and *Mucor* (rhinocerebral mucromycosis)
- **Others:** Microsporidia

- ❖ *C. neoformans* occurs in two varieties—*C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*; which correlate with serotypes A and D, respectively
- ❖ *C. gattii* is antigenically diverse and corresponds to the serotypes B and C
- ❖ Most laboratories do not routinely distinguish between the types, and report all isolates simply as *C. neoformans*.

Pathogenesis

Infection is acquired by inhalation of aerosolized forms of *Cryptococcus*. Both yeast cells as well as basidiospores (the sexual stage of *Cryptococcus*) are infectious.

- ❖ In immunocompetent individuals, the lungs exhibit defense mechanisms which usually limit the infection
- ❖ However, in people with low immunity, pulmonary infection occurs followed by dissemination through blood
- ❖ **CNS spread:** The unique feature of *Cryptococcus* is its ability to cross blood-brain barrier which occurs by the yeast cells either they migrate directly across the endothelium or carried inside the macrophages as “Trojan horse”
- ❖ **Virulence factors** of *Cryptococcus* that favor invasion and spread of infection include:
 - Polysaccharide capsule—It is the principal virulence factor of the fungus. It is antiphagocytic and also inhibits the host’s local immune responses
 - Ability to make melanin by producing an enzyme called phenyl oxidase
 - Production of other enzymes such as phospholipase and urease.
- ❖ **Risk factors:** Individuals at high-risk for cryptococcosis include:
 - Patients with advanced HIV infection with CD4 T cell counts less than 200/ μ L: They are at high-risk of acquiring *C. neoformans* infection. However, *C. gattii* is not associated with HIV infection. It usually causes infection in immunocompetent individuals
 - Patients with hematologic malignancies
 - Transplant recipients
 - Patients on immunosuppressive or steroid therapy.

Clinical Manifestations

Various clinical manifestations of cryptococcosis include:

- ❖ Pulmonary cryptococcosis: It is the first and the most common presentation
- ❖ Cryptococcal meningitis: It presents as chronic meningitis, with headache, fever, sensory and memory loss, cranial nerve paresis and loss of vision (due to optic nerve involvement)
- ❖ Skin lesions: They are commonly seen with *C. neoformans* var. *neoformans* (serotype D)
- ❖ Osteolytic bone lesions.

Epidemiology

Worldwide, cryptococcosis accounts for nearly 1 million cases, with more than 600,000 deaths annually.

- ❖ **Geographical distribution:** *C. neoformans* var. *grubii* (serotype A) strains are found worldwide; however, *C. neoformans* var. *neoformans* (serotype D) strains are restricted to Europe and *C. gattii* is confined to tropics; outbreaks occurred in Vancouver in 1999
- ❖ **Habitat:** *C. neoformans* is frequently found in soils contaminated with avian excreta and pigeon droppings. In contrast, *C. gattii* inhabits in eucalyptus tree.

Laboratory Diagnosis

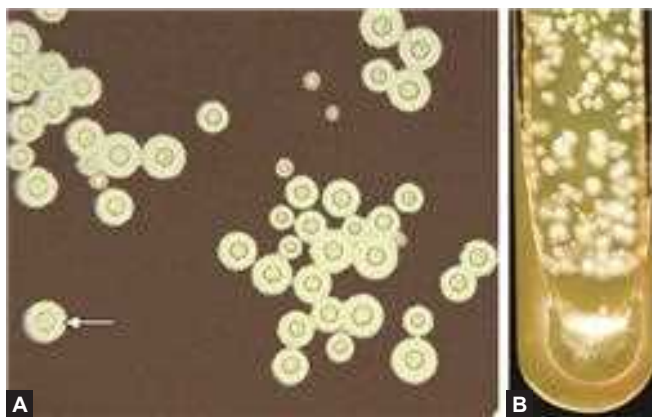
Specimens such as CSF, blood or skin scrapings can be collected.

Direct Detection Methods

- ❖ **Negative staining:** Modified India ink stain (added with 2% mercurochrome) and nigrosin stain are used to demonstrate the capsule, which appears as refractile delineated clear space surrounding the round budding yeast cells against black background
 - Capsules may be twice as thick as the diameter of yeast cells (Fig. 75.14A)
 - India ink stain is less sensitive (60–70%).
- ❖ **Gram staining** may show gram-positive round budding yeast cells
- ❖ **Other stains include:**
 - Mucicarmin stain: It stains the carminophilic cell wall of *C. neoformans*
 - Masson-Fontana stain: It demonstrates the production of melanin
 - Alcian blue stain to demonstrate the capsule.
- ❖ **Antigen detection:** The capsular antigens can be detected from CSF or serum by latex agglutination test. It is a rapid and sensitive (95% sensitivity) and specific method.

Culture

CSF is inoculated onto SDA without antibiotics, blood agar or chocolate agar and incubated at 37°C. Blood is



Figs 75.14A and B: *Cryptococcus neoformans*: **A.** India ink staining shows clear refractile capsules surrounding round budding yeast cells (arrow showing); **B.** Growth on SDA at 37°C—shows creamy white mucoid colonies.

Source: Public Health Image Library/A. Dr Leonor Haley, ID#:3771 B. Dr William Kaplan, ID#:3199/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

inoculated in biphasic blood culture bottles. Colonies appear as mucoid creamy white and yeast like (Fig. 75.14B).

Confirmation of *Cryptococcus* species is made by:

- ❖ Niger seed agar and bird seed agar: They are used to demonstrate melanin production (brown colored colonies)
- ❖ Growth at 37°C
- ❖ Urease test is positive
- ❖ Assimilation of inositol and nitrate
- ❖ Mouse pathogenicity test
- ❖ Automated identification system such as MALDI-TOF.

TREATMENT

Cryptococcosis

Treatment depends upon the type of cryptococcosis.

- ❑ Cryptococcosis without CNS involvement: Fluconazole is the drug of choice
- ❑ HIV-infected patients with CNS involvement: The recommended regimen is induction phase for two weeks (amphotericin B ± flucytosine) followed by oral fluconazole therapy till CD4 T cell count raises >200 / μ L for 6 months.

OTHER FUNGAL INFECTIONS OF CNS

Other fungi that infect CNS include:

- ❖ **Rhinocerebral mucormycosis:** It is a rare infection of the sinuses, eyes, orbits, oral cavity, and brain; caused by opportunistic saprophytic fungi of Zygomycetes family such as *Mucor* and *Rhizopus* (Chapter 69)
 - It occurs commonly in patients with diabetic ketoacidosis
 - It starts as eye and facial pain, may progress to cause orbital cellulitis, proptosis and vision loss (Chapter 78). The infection can rapidly result in death.
- ❖ ***Cladophialophora bantiana*:** It is a dematiaceous or phaeoid fungus. It is neurotropic, produces brain abscess; frontal lobe being the most common site affected
- ❖ **Microsporidia:** Their taxonomy recently changed from parasite to fungus. *Encephalitozoon cuniculi* and *Trachipleistophora* can cause brain abscess. They usually cause intestinal and ocular infections; discussed in Chapters 45 and 78.

EXPECTED QUESTIONS

I. Write essay on:

- a. A 61-year-old person reactive for HIV, presented with altered mental status, seizures, sensory abnormalities. The bone marrow aspirate collected was sent for Giemsa stain which revealed crescent shaped tachyzoites ($6 \times 2 \mu\text{m}$ in size).
 1. Identify the etiological agent and diagnose the clinical condition.
 2. Write briefly about the life cycle of the etiological agent.
 3. What are the various diagnostic modalities?
 4. How will you treat this condition?
- b. A 21-year-old vegetarian female presented with recurrent episodes of seizure, headache vomiting and vertigo. MRI scan of brain showed cystic lesion in brain parenchyma, following which surgery was performed. The cysts were surgically removed which appeared yellowish white in color, measuring 0.5–1.5 cm size, slightly oval in shape, containing a bladder like sac with a white spot.
 1. What is the etiological diagnosis?
 2. Write briefly about the life cycle of the etiological agent.
 3. What are the various diagnostic modalities?
 4. How will you treat this condition?

II. Write short notes on:

1. Cryptococcal meningitis.
2. Cerebral malaria.

III. Multiple Choice Questions (MCQs):

1. **Oocysts of *Toxoplasma gondii* are excreted in the feces of:**
 - a. Cat
 - b. Sheep
 - c. Cattle
 - d. Humans
2. **Most common manifestation of *Toxoplasma gondii* in immunocompetent adult:**
 - a. Lymphadenopathy
 - b. Chorioretinitis
 - c. Myocarditis
 - d. Encephalitis
3. **Most common manifestation of *Toxoplasma gondii* in immunocompromised adult:**
 - a. Lymphadenopathy
 - b. Chorioretinitis
 - c. Myocarditis
 - d. Encephalitis
4. **Humans acquire *Cysticercus cellulosae* infection by all, except:**
 - a. Ingestion of contaminated vegetables
 - b. Autoinfection
 - c. Reverse peristalsis
 - d. Ingestion of contaminated pig's meat

Answers

1. a 2. a 3. d 4. d

As long as a single child remains infected with poliovirus, children in all countries are at risk of contracting the disease.



Material is developed by CDC

Urogenital Tract Infections

SECTION

10

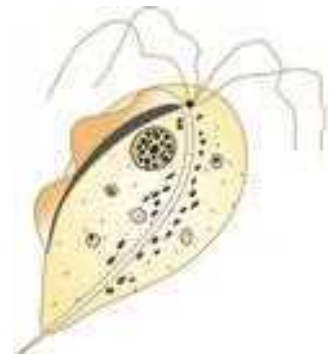
SECTION OUTLINE

76. Infective Syndromes of Urinary Tract

- Bacterial Infections: Enterobacteriaceae, *Enterococcus* and Others
- Viral (BK Virus), Parasitic (*Schistosoma haematobium*) and Fungal Infections

77. Infective Syndromes of Genital Tract and Sexually-transmitted Infections

- Ulcerative Genital Disease: Syphilis, Lymphogranuloma Venerum, Granuloma Inguinale, Soft Chancre and Genital Herpes
- Gonorrhoea and Non-gonococcal Urethritis (*Chlamydia trachomatis* and Others)
- Vulvovaginitis (Trichomoniasis, Bacterial Vaginosis, Vaginal Candidiasis)
- Other Genital Tract Infections of Females and Males



Prevention of Sexually Transmitted Infections is in your hands

S

- Safe sex: Use Condoms

- Avoid Multiple Partners

- Talk to Your Partner

- Test Appropriately

- Treat Both Partners

- Abstinence Till

Recovery

- Get Vaccinated

(e.g. HBV, HPV)



T

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Infective Syndromes of Urinary Tract

CHAPTER PREVIEW

- Urinary Tract Infection (UTI)
- Etiological Agents of UTI
- Bacterial Infections
- Viral Infections
- Parasitic Infections
- Fungal Infections

URINARY TRACT INFECTION

Urinary tract infection (UTI) is defined as a disease caused by microbial invasion of the urinary tract that extends from the renal cortex of the kidney to the urethral meatus. UTI is the leading cause of morbidity and healthcare expenditures in persons of all ages.

Classification

- ❖ UTIs may be broadly classified into two types—lower UTI and upper UTI (Table 76.1) depending upon the anatomical sites involved
- ❖ Depending upon the source of infection, UTI can be of two types: healthcare-associated (e.g. CAUTI) and community-acquired.

Catheter-associated UTI (CAUTI)

CAUTI is the most common type of healthcare-associated infection. Presence of indwelling urinary catheter is the single most important risk factor to develop UTI in hospitalized patients. CAUTI has been discussed in detail in Chapter 22.

Normal Commensals of Urinary Tract

Urinary tract in a healthy man is sterile, except its distal portion of urethra, which is colonized with resident microbial flora such as lactobacilli, diphtheroids, coagulase-negative staphylococci, anaerobes and also with potential pathogens, including Enterobacteriaceae

and *Candida* species. Invasion of some of these pathogens (e.g. *E. coli*) into bladder can result in UTI.

Epidemiology

Urinary tract infections (UTIs) are among the most common bacterial infections that need medical care; accounting for the second most common infection after respiratory tract infections in the community. Whereas in hospitals, they are the most common HAIs (hospital acquired infections) accounting for 35% of total HAIs.

Predisposing Factors

- ❖ **Prevalence:** About 10% of humans develop UTI in some part of their life
- ❖ **Gender:** UTI is predominantly a disease of females
 - An estimated 50% of women report to have at least one episode of UTI at some point in their lives
 - The higher prevalence in females is due to the anatomical structure of female urogenital system—(1) short urethra, and (2) close proximity of urethral meatus to anus; so that there is more chance of introduction of endogenous bacteria into the urinary tract.
- ❖ **Age:** Incidence of UTI increases with age
 - During first year of life, the prevalence is around 2% in both females and males
 - After that, the incidence of UTI decreases in males until old age, where they again show an increased prevalence because of the prostate enlargement which interferes with the emptying of the bladder
 - Whereas in females, the incidence keeps increasing after first year of life
 - ◆ During 5–17 years, the incidence of bacteriuria is about 1–3%
 - ◆ Thereafter in adult life, the incidence is around 10–20%
 - ◆ Reinfection is common in females (20–40 years of age), as many as 50% would suffer from reinfection within one year.

Table 76.1: Comparison between lower and upper UTIs.

	Lower UTI	Upper UTI
Sites involved	Urethra, and bladder	Kidney and ureter
Symptoms	Local manifestations: dysuria, urgency, frequency	Local and systemic manifestations (fever, vomiting, abdominal pain)
Route of spread	Ascending route	Both ascending (common) and descending route
Occurrence	More common	Less common

- ❖ **Pregnancy:** Anatomical and hormonal changes in pregnancy favor the development of UTIs. Most females develop asymptomatic bacteriuria during pregnancy. In some cases, it can lead to serious infections in both mother and fetus
- ❖ **Structural and functional abnormality** of urinary tract may obstruct the urine flow, that can lead to urinary stasis; which predisposes to infection
 - **Structural obstruction:** For example, urethral stricture, renal and ureteric stones, prostate enlargement, tumors, renal transplants, etc.
 - **Functional obstruction:** For example, neurogenic bladder due to spinal cord injury or multiple sclerosis.
- ❖ **Bacterial virulence** such as expression of pili helps in bacterial adhesion to uroepithelium
- ❖ **Vesicoureteral reflux:** If the normal valve-like mechanism at the vesicoureteric junction is weakened, it allows urine to flow from the bladder up into the ureters and sometimes into the renal pelvis
- ❖ **Genetic factors:** Genetically determined receptors present on uroepithelial cells may help in bacterial attachment. UTI is the leading cause of gram-negative sepsis (urosepsis) especially in hospitalized patients and the urinary catheters are the origin of nearly 50% of nosocomial UTIs.

Etiology

Escherichia coli (uropathogenic *E. coli*) is by far the most common cause of all forms of UTIs (i.e. community-acquired and healthcare-associated UTI and upper and lower UTI); accounting for 70% of total cases.

- ❖ The endogenous flora such as gram-negative bacilli (e.g. *E. coli*, *Klebsiella*, *Proteus*, etc.) and enterococci are the important agents
- ❖ In healthcare-associated UTIs, the agents are often multidrug resistant. In addition to the members of Enterobacteriaceae, other organisms such as staphylococci, *Pseudomonas*, *Acinetobacter* are also increasingly reported.

Bacterial pathogens are the major cause of UTI; their pathogenesis, clinical features, laboratory diagnosis and treatment have been discussed below. In general, viruses, parasites and fungi infrequently infect the urinary tract; discussed subsequently in this chapter (Table 76.2).

Pathogenesis

Bacteria invade the urinary tract mainly by two routes—ascending and descending routes (Fig. 76.1).

Ascending Route

It is the most common route; the enteric endogenous bacteria (*E. coli*, other gram-negative bacilli and enterococci) enter the urinary tract which is facilitated by sexual intercourse, or instrumentation (e.g. catheterization), etc.

Table 76.2: Common microorganisms causing UTIs.

Bacterial agents	Other agents
Gram-negative bacilli: Enterobacteriaceae <ul style="list-style-type: none"> • <i>Escherichia coli</i>: Most common (70%) • <i>Klebsiella pneumoniae</i> • <i>Enterobacter</i> species • <i>Proteus</i> species • <i>Serratia</i> species Non-fermenters <ul style="list-style-type: none"> • <i>Pseudomonas aeruginosa</i> • <i>Acinetobacter</i> species 	Fungus: <i>Candida albicans</i>
Gram-positive bacilli: <i>Mycobacterium tuberculosis</i>	Parasites: <ul style="list-style-type: none"> • <i>Schistosoma haematobium</i> • <i>Trichomonas vaginalis</i> • <i>Diocotophyme renale</i>
Gram-positive cocci: <ul style="list-style-type: none"> • <i>Enterococcus</i> species • <i>Staphylococcus saprophyticus</i>* • <i>Staphylococcus aureus</i> • <i>Staphylococcus epidermidis</i> • <i>Streptococcus agalactiae</i> 	Viruses: <ul style="list-style-type: none"> • BK virus • Adenovirus types– 11 and 21

Abbreviation: UTI, Urinary tract infection.

*Common in sexually active females.

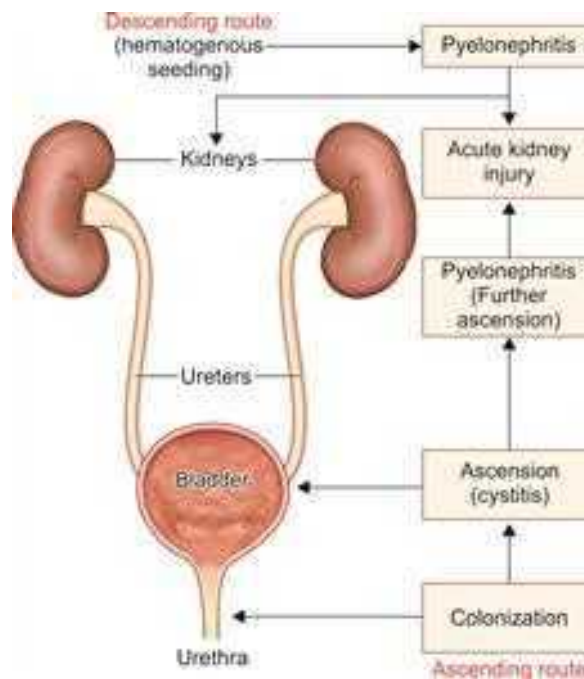


Fig. 76.1: Pathogenesis of urinary tract infection.

- ❖ **Colonization:** Adhesion to urethral epithelium is the first and the most important step in pathogenesis. A number of virulence factors (e.g. P fimbriae, mannose resistant fimbriae in *E. coli*) help in adhesion
- ❖ **Ascension:** Following colonization, pathogen ascends through urethra upwards towards bladder to cause cystitis. Bacterial toxins may facilitate ascension by inhibiting peristalsis (urinary stasis)

- ❖ **Further ascension** through the ureter may occasionally occur if there is vesicoureteral reflux leading to pyelonephritis (infection of renal parenchyma causing an acute inflammatory response)
- ❖ **Acute tubular injury:** If the inflammatory cascade continues, tubular obstruction and damage occurs which may lead to interstitial nephritis.

Descending Route

This refers to invasion of renal parenchyma through hematogenous seedling of the pathogen, which occurs as a consequence of bacteremia. This accounts for 5% of total UTIs. Although most infections affecting kidney are acquired by ascending route, certain organisms are particularly invasive and their association with pyelonephritis often indicates a descending route of origin; for example—*Staphylococcus aureus*, *Salmonella*, *Mycobacterium tuberculosis*, and *Leptospira*.

Host Defense Mechanisms

Host defense mechanisms play an important role in prevention of UTI. They can be grouped into—(1) factors related to urine, (2) activation of host's mucosal immunity by the uropathogens (Table 76.3).

Clinical Manifestations

UTIs may be presented in various forms:

- ❖ **Lower UTI:** Asymptomatic bacteriuria, cystitis, urethritis, acute urethral syndrome
- ❖ **Upper UTI:** Pyelonephritis, ureteritis, perinephric abscess, renal abscess, renal tuberculosis
- ❖ **Immunological sequela:** Post-streptococcal glomerulonephritis (PSGN).

Lower UTI

1. Asymptomatic Bacteriuria

It refers to isolation of specified quantitative count of bacteria in an appropriately collected urine specimen, obtained from a person without symptoms of UTI. It is more common in females and its incidence increases with age (1% among school girls to more than 20% in old age).

Table 76.3: Host defense mechanisms against UTIs.

Urinary factors	Mucosal immunity
Acidic urine: inhibits pathogens	Uroepithelial secretion of cytokines (induced by bacterial LPS)
High urine osmolality: inhibits pathogens	Mucosal IgA—prevent attachment of pathogen to uroepithelium
Urinary inhibition of bacterial adherence	Tamm-Horsfall protein (uromodulin)—a glycoprotein secreted by epithelial cells of kidney, serves as anti-adherence factor by binding to type-I fimbriae of <i>E. coli</i>
Mechanical flushing by urine flow	In men: (1) Zinc in prostatic secretion is bactericidal, (2) long urethra

Clinical Significance

- ❖ **Asymptomatic UTI** is clinically significant in a certain group of people such as pregnant women (as chances of complication in mother and fetus are more), people undergoing prostatic surgery or any urologic procedure where bleeding is anticipated. Therefore, in this group, routine screening and treatment for asymptomatic UTI is highly recommended
- ❖ In contrast, **asymptomatic UTI** is not clinically significant in non-pregnant, pre-menopausal women, old age, catheterized patient, or patients with spinal injury. In such cases, neither screening nor treatment of asymptomatic UTI is needed.

2. Cystitis (Infection of Bladder)

It is characterized by localized symptoms such as:

- ❖ Dysuria (pain while micturition), frequency, urgency, and suprapubic tenderness (over the bladder area)
- ❖ Urine becomes cloudy, with bad odor, and in some cases grossly bloody (hematuria)
- ❖ There is no associated systemic manifestation.

3. Acute Urethral Syndrome

This is another form of lower UTI seen in young sexually active females, characterized by:

- ❖ Presence of classical symptoms of lower UTI as described for cystitis
- ❖ Bacterial count is often low (10^2 to 10^5 CFU/mL)
- ❖ Pyuria is present
- ❖ **Agents:** Mostly due to the usual agents of UTI, a few cases may be caused by gonococcus, *Chlamydia*, herpes simplex virus, etc.

Upper UTI (Pyelonephritis)

Pyelonephritis refers to inflammation of kidney parenchyma, calyces and the renal pelvis, i.e. the part of ureter present inside the kidney (Table 76.1).

- ❖ Associated with systemic manifestations such as—fever, flank pain, vomiting
- ❖ Lower tract symptoms such as frequency, urgency and dysuria may also be present.

Other conditions are discussed subsequently.

Laboratory Diagnosis

Specimen Collection

Urine should be collected in a wide mouth screw capped sterile container by various methods.

- ❖ **Clean voided midstream urine:** It is the most common specimen for UTI; collected after properly cleaning the urethral meatus or glans
- ❖ **Suprapubic aspiration** of urine from the bladder: It is the most ideal specimen. It is recommended for patients in coma or infants
- ❖ **In catheterized patients,** urine should be collected from the catheter tube (after clamping distally and disinfecting); but not from the uro bag.

Transport

Urine sample should be processed immediately. If delay is expected for more than 1–2 hours, then it can be stored in refrigerator or stored by adding boric acid for maximum 24 hours.

Direct Examination

The screening tests done are as follows:

- ❖ **Wet mount examination:** It is done to demonstrate the pus cells in urine. Pyuria of more than 8 pus cells/mm³ is taken as significant
- ❖ **Leukocyte esterase test:** It is a rapid and cheaper method that detects leukocyte esterases secreted by pus cells present in urine
- ❖ **Nitrate reduction test (Griess test):** Nitrate reducing bacteria like *E. coli* gives a positive result
- ❖ **Gram staining** of urine is not a reliable indicator as— (1) the bacterial count in urine is usually low, (2) pus cells rapidly deteriorate in urine and may not be seen well. Gram staining may be limited to pyelonephritis and invasive UTI cases and a count of ≥ 1 bacteria/oil immersion field is taken as significant.

Culture

- ❖ **Culture media:** Urine sample should be inoculated onto CLED agar (cysteine lactose electrolyte deficient agar) or combination of MacConkey agar and blood agar. CLED agar is preferred in laboratories with higher sample load
- ❖ **Kass concept of significant bacteriuria:** This is based on the fact that, though the normal urine is sterile it may get contaminated during voiding, with normal urethral flora. However, the bacterial count in contaminated urine would be lower than that caused by an infection

Significant bacteriuria

- ❑ A count of $\geq 10^5$ colony forming units (CFU)/mL of urine is considered as significant—indicates infection (referred as 'significant bacteriuria' developed by Kass)
 - ❑ **Count between 10^4 to 10^5 CFU/mL** indicates doubtful significance; should be clinically correlated
 - ❑ **Low count of $< 10^4$ CFU/mL** is due to presence of commensal bacteria (due to contamination during voiding) and is of no significance. However, low counts can be significant in the following conditions:
 - Patient on antibiotic or on diuretic treatment
 - Infection with some gram-positive organisms such as *S. aureus*
 - Pyelonephritis and acute urethral syndrome
 - Sample taken by suprapubic aspiration
 - In catheterized patients: If the patient is symptomatic, then a count of $\geq 10^3$ CFU/mL is considered significant
- ❖ **Quantitative culture:** This is done to count the number of colonies. Each colony on plate corresponds to one bacterium in urine sample. Quantitation is done by:
 - Semi-quantitative method such as standardized loop technique
 - Quantitative method such as pour plate method.
 - ❖ **Colony appearance:** It depends upon the organism grown (Table 76.4). For example, lactose fermenters such as *E. coli* and *Klebsiella* produce pink colonies on MacConkey agar and yellow colonies on CLED agar; whereas non-lactose fermenters such as *Proteus*, *Pseudomonas* and *Acinetobacter* produce pale colonies
 - ❖ **Identification:** The colonies grown are identified either by automated identification systems such as MALDI-TOF or VITEK, or by conventional biochemical tests as summarized in Table 76.4

Table 76.4: Culture identification features of common organisms causing UTI.

	Culture	Culture smear and motility testing	Biochemical reactions
<i>Escherichia coli</i> (Chapter 41, Fig. 41.1)	MAC or CLED: flat lactose fermenting colonies BA: gray moist colonies	Gram-negative bacilli Motile	Catalase positive, oxidase negative ICUT tests: I ⁺ C ⁻ U ⁻ TSI (acidic slant/acidic butt, gas ⁺ , H ₂ S ⁻)
<i>Klebsiella pneumoniae</i> (Chapter 61, Fig. 61.6B)	MAC or CLED: mucoid lactose fermenting colonies BA: gray mucoid colonies	Gram-negative bacilli Non-motile	Catalase positive, oxidase negative ICUT tests: I ⁻ C ⁺ U ⁺ TSI (acidic slant/acidic butt, gas ⁺ , H ₂ S ⁻)
<i>Proteus</i> species (Fig. 76.2)	MAC or CLED: lactose non-fermenting colonies BA: swarming type of growth	Gram-negative pleomorphic bacilli Motile	Catalase positive, oxidase negative ICUT tests: I ^{-/+} C ^{+/+} U ⁺ TSI (alkaline slant/acidic butt, gas ^{+/+} , H ₂ S ⁺ , PPA test positive)
<i>Enterococcus</i> (Fig. 76.3)	MAC: magenta pink colonies BA: translucent non-hemolytic colonies	Gram-positive cocci in pair, spectacle shaped Non-motile	Catalase negative Bile aesculin test positive
<i>Staphylococcus aureus</i> (Chapter 51, Fig. 51.3B)	BA: golden yellow hemolytic colonies	Gram-positive cocci in clusters Non-motile	Catalase positive, coagulase positive
<i>Staphylococcus saprophyticus</i>	BA: white non-hemolytic colonies	Gram-positive cocci in clusters Non-motile	Catalase positive, coagulase negative, Resistant to novobiocin

Abbreviations: I, indole test; C, citrate test; U, urease test; TSI, triple sugar iron agar test; +, positive; -, negative; MAC, MacConkey agar; BA, blood agar; CLED, cysteine lactose electrolyte-deficient agar; PPA, phenylalanine deaminase.

- ❖ **AST:** Antimicrobial susceptibility test is essential to guide the appropriate treatment. It is performed conventionally by disk diffusion test (on Mueller-Hinton agar) or by automated MIC based methods such as VITEK.

Antibody Coated Bacteria Test

This test is done to differentiate upper and lower UTI.

- ❖ In **upper UTI**, as the route of spread is hematogenous, bacteria coated with specific antibodies are found in urine. Such bacteria coated with specific antibody are detected by immunofluorescence method using fluorescent labeled antihuman globulin
- ❖ In **lower UTI**, bacteria found in urine are never coated with specific antibodies.

Culture identification features of common organisms causing UTI is described in Table 76.4.

TREATMENT

Urinary tract infections

Treatment should be based on antimicrobial susceptibility testing report. Quinolones (e.g. norfloxacin), nitrofurantoin, cephalosporins, and aminoglycosides are among the preferred drugs.

Higher antibiotics such as carbapenem (e.g. meropenem), β -lactam/ β -lactamase inhibitor combinations (e.g. piperacillin-tazobactam) or fosfomycin are used for treatment of healthcare-associated UTIs caused by multidrug resistant gram-negative bacilli.

ETIOLOGICAL AGENTS OF UTI

BACTERIAL UTI

Enterobacteriaceae Causing UTI

Several members of the family Enterobacteriaceae can cause UTI; among which uropathogenic *E. coli* is most important.

1. Uropathogenic *E. coli*

Uropathogenic *E. coli* (UPEC) is the single most common pathogen of UTI, accounting for 70–75% of all cases. UPEC serotypes O1, O2, O4, O6, O7 and O75 are responsible for most UTIs. The virulence factors of UPEC include:

- ❖ Cytotoxins (CNF 1–cytotoxic necrotizing factor 1 and SAT: Secreted autotransporter toxin)
- ❖ Hemolysins
- ❖ Fimbriae (e.g. P fimbriae)–specific for strains causing lower UTI
- ❖ Capsular K antigen–specific for strains causing upper UTI.

Escherichia coli can also cause diarrhea, pyogenic infections including skin and soft tissue infections, meningitis, etc. (Chapter 41, for detail).

2. *Klebsiella pneumoniae* UTI

Klebsiella pneumoniae is usually found as commensal in human intestines; causes infections similar to *E. coli*

such as urinary tract infections, meningitis (neonates), septicemia and pyogenic infections such as abscesses and wound infections. Detail is discussed in Chapter 61.

3. *Enterobacter* UTI

Enterobacter species have become increasingly important nosocomial pathogens. They are opportunistic pathogens, implicated in infected wounds, UTI and pneumonia (discussed in Chapter 61).

4. *Citrobacter* Infections

Citrobacter species are mostly environmental contaminants isolated from water, soil, food and feces of man and animals. *C. freundii* and *C. koseri* are the important species causing human infections.

- ❖ They occasionally cause urinary tract, gallbladder and middle ear infections and neonatal meningitis
- ❖ **Identification** is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests
- ❖ **Treatment:** Most *Citrobacter* isolates are MDR; and the guideline for treatment is same as that for *E. coli*.

5. *Proteaeae* Infections

Tribe Proteaeae comprises of three genera: *Proteus*, *Morganella* and *Providencia*. They exhibit a unique tribe character of being positive for phenylalanine deaminase (PPA) test. They are part of commensals in human intestine. However, they can cause nosocomial outbreaks of UTI, wound infections, etc.

Proteus

Proteus species show pleomorphism, i.e. they vary in size. It is named after the Greek God 'Proteus' who was able to assume any shape.

Naming of H and O antigens: In general, the terms H and O antigens are used to denote the flagellar and somatic antigens of any organism respectively. However, the naming of these antigens were linked historically to the properties of *Proteus*.

- ❖ The **H antigen is named** from the ability of flagellated strains of *Proteus* to grow on agar as a thin film resembling the film of breath on glass (from German word 'Hauch', meaning 'film of breath')
- ❖ **Naming of O antigen:** The thin film is not observed when strains carrying only the somatic antigen (non-flagellated strains) grow on media (from German word 'Ohne Hauch' meaning 'without film of breath').

Pathogenesis

Proteus mirabilis and *P. vulgaris* are the most commonly encountered species.

- ❖ **Saprophytes:** Most of the species are widely distributed in nature and are isolated from decomposing animal matter, sewage and soil

- ❖ **Commensals:** They are also frequently present on the moist areas of the skin, intestine of humans and animals
- ❖ **Infections produced:** They are opportunistic pathogens, commonly associated with urinary, wound and soft tissue infections and septicemia
 - *Proteus* species are often involved in nosocomial outbreaks
 - **Struvite stones in bladder:** *Proteus* produces urease enzyme, which breaks down urea to form ammonia that damages the renal epithelium and makes the urine alkaline. Alkaline urine predisposes to the deposition of phosphate, which leads to the formation of renal calculi
 - Other *Proteus* species such as *P. penneri* and *P. myxofaciens* are rarely encountered in clinical specimens.

Proteus as the basis of Weil–Felix Reaction

Somatic antigen of certain non-motile *Proteus* strains (called **X strains**) cross react with the alkali-stable antigen of some *Rickettsia* species.

- Thus, *Proteus* antigens can be used to detect heterophile antibodies in sera of patients suffering from rickettsial infections
- Three non-motile *Proteus* strains: OX2, OX19 (from *P. vulgaris*) and OXK (from *P. mirabilis*) are used in this agglutination test.

Laboratory Diagnosis

- ❖ **Pleomorphism:** *Proteus* species are gram-negative coccobacilli occasionally appear bacillary and in filamentous forms
- ❖ **Odor:** They produce characteristic putrid fishy or seminal odor in cultures
- ❖ **Swarming:** *Proteus* has an ability to swarm (or spread) on solid media such as blood agar (Fig. 76.2). It is a uniform film of growth, may extend on the whole plate (continuous swarming) or present as concentric circles of growth surrounding the point of inoculum (discontinuous swarming)
- ❖ **Identification:** Identification of *Proteus* from colonies is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests as described below
 - It is catalase positive and oxidase negative
 - **ICUT tests:** Indole test (positive for *P. vulgaris* and negative for *P. mirabilis*), citrate test (variably positive), urease test (positive) and TSI (triple sugar iron agar) test shows alkaline/acid, gas variably present, H₂S present.

Morganella

Morganella has only one species, *M. morganii*.

- ❖ It is commonly found in human and animal feces
- ❖ It is rarely associated with urinary tract infection, pneumonia and wound infection. Most of the infections are nosocomial.



Fig. 76.2: *Proteus* on blood agar, showing swarming growth (arrow showing).

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

Providencia

Providencia species are associated with nosocomial infections of the urinary tract, wounds and burns. It consists of five species; *P. rettgeri*, *P. stuartii*, *P. alcalifaciens*, *P. rustigianii* and *P. heimbachae*.

TREATMENT

Tribe Proteeae infections

Members of the tribe Proteeae are often multidrug resistant. They are also resistant to many disinfectants.

- They show intrinsic resistance to ampicillin, first and second generation cephalosporins, nitrofurantoin, tetracyclines, tigecycline and polymyxins (colistin and polymyxin B)
- They produce various β -lactamases such as extended spectrum β -lactamases (ESBL) and AmpC β -lactamases. As a result, they are resistant to most of the β -lactam drugs
- Drug of choice depends on the antimicrobial susceptibility testing. In general, aminoglycosides, fourth generation cephalosporins (cefepime), and carbapenems are effective in treatment
- In general, *P. mirabilis* is more susceptible to antibiotics than *P. vulgaris*.

Non-fermenters Causing UTI

Non-fermenters such as *Pseudomonas*, *Acinetobacter* are important cause of healthcare-associated UTI. They also cause skin and soft tissue infections and pneumonia (Chapter 65).

Enterococcal Infections

Enterococci are the most common gram-positive cocci to cause UTI. They were initially grouped under group D

Streptococcus, but later, have been reclassified as a separate genus *Enterococcus*. Based on the molecular structure, they are now placed under a new family; Enterococcaceae.

Virulence Factors

Enterococci are part of normal flora of human intestine, biliary tract and to lesser extent vagina and male urethra. At the same time, they are also becoming increasingly important agents of human disease especially in hospitals, which is attributed to their resistance to several antibiotics and also due to exhibiting a number of virulence factors such as:

- ❖ **Aggregation substances or pheromones:** They help in clumping of adjacent cells to facilitate plasmid exchange (transfers drug resistance)
- ❖ **Extracellular surface protein (ESP):** It helps in adhesion to bladder mucosa
- ❖ **Common group D lipoteichoic acid antigen:** It induces cytokine release such as tumor necrosis factor α (TNF- α).

Clinical Manifestations

E. faecalis and *E. faecium* are the two species that are clinically important. *E. faecalis* is the most common species isolated from the clinical specimens, whereas *E. faecium* is more drug resistant than *E. faecalis*. Enterococci are one of the major healthcare-associated pathogens, produce various infections such as:

- ❖ **Urinary tract infections:** Healthcare-associated UTI (cystitis) is the most common infection caused by enterococci; usually associated with indwelling urinary catheterization, or instrumentation. Rarely, it may progress into pyelonephritis and perinephric abscesses
- ❖ **Chronic prostatitis:** Enterococci can cause chronic prostatitis, when urinary tract is manipulated surgically or endoscopically. As antibiotics penetrate poorly into prostatic tissue, this can be a source of recurrent enterococcal bacteremia
- ❖ **Bacteremia** and left-sided endocarditis involving mitral and aortic valves (common in intravenous drug abusers)
- ❖ **Intra-abdominal**, pelvic and soft tissue infections, including surgical site infections following intra-abdominal surgeries
- ❖ **Neonatal infections:** Such as sepsis (mostly late-onset), bacteremia, meningitis, and pneumonia.

Laboratory Diagnosis

Specimen collection depends upon the site of infection. Urine, blood (collected in blood culture bottles), exudate, peritoneal fluid, etc. are the useful specimens for culture. Enterococci show the following characteristics that help in their identification:

- ❖ They are gram-positive oval cocci (Fig. 76.3A) arranged in pairs; at an angle to each other (**spectacle-shaped** appearance)



Figs 76.3A to C: *Enterococcus*: **A.** Gram-positive oval cocci in pairs; **B.** translucent non-hemolytic colonies on blood agar; **C.** Bile esculin hydrolysis test (left—negative, right—positive result, black color due to esculin hydrolysis).

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puduchery (with permission).

- ❖ **Blood agar:** It produces non-hemolytic (Fig. 76.3B), translucent colonies (rarely produces α or β -hemolysis)
- ❖ **MacConkey agar:** It produces minute magenta pink colonies
- ❖ Bile esculin hydrolysis test is positive (Fig. 76.3C)
- ❖ They can grow in presence of extreme conditions such as—6.5% NaCl, 40% bile, pH 9.6, 45°C and 10°C
- ❖ *E. faecalis* and *E. faecium* can be differentiated by arabinose fermentation test
- ❖ Accurate species identification can be made by automated methods such as VITEK and MALDI-TOF.

TREATMENT

Enterococcal infections

The treatment options vary, depending upon the type of infections and presence of drug resistance.

- ❑ **For less serious infections**
 - UTI: Oral therapy with ampicillin, nitrofurantoin or fosfomycin are the drug of choice
 - Intra-abdominal and soft tissue infections: Ampicillin, vancomycin or linezolid can be given.
- ❑ **For invasive infections** such as endocarditis, bacteremia, and meningitis: **Combination therapy** with a cell wall-active agent (e.g. penicillin or ampicillin) and an aminoglycoside (e.g. gentamicin) is recommended
 - This synergistic effect is due to cell wall alterations produced by cell wall-active agents, which facilitate increased penetration of aminoglycoside into the bacterial cell
 - This combination therapy fails if the isolate is found resistant to either penicillin or high level aminoglycoside in vitro. In such case, alternative drugs such as vancomycin, linezolid or daptomycin can be considered.
- ❑ **If resistant to vancomycin**, then treatment options available are linezolid, streptogramins (only active against *E. faecium*) and daptomycin
- ❑ **Intrinsic resistance:** Enterococci are intrinsically resistant to aminoglycosides (monotherapy), clindamycin, cephalosporins, cotrimoxazole, vancomycin (for *E. gallinarum* and *E. casseliflavus*) and streptogramins (for *E. faecalis*); therefore, should not be included in the treatment regimen.

Vancomycin Resistant Enterococci (VRE)

Vancomycin resistance in enterococci has been increasingly reported now a days.

- The **prevalence** of VRE varies with time and place.
 - **World:** A report in 2016 revealed that among hospitalized patients the VRE frequency is high in America (35%) and low in Europe (4%) and moderate (10–15%) in Asian countries.
 - **In India,** the VRE rate reported was 9.7% (overall); 2.5% for *E. faecalis* and 17.4% for *E. faecium* (ICMR, 2019)
- **Mechanism:** VRE is mediated by *van* gene, which alters the target site for vancomycin present in the cell wall; i.e. D-alanyl-D-alanine side chain of peptidoglycan layer (which is the usual target site for vancomycin), is altered to D-alanyl-D-serine or D-alanyl-D-lactate. This altered side chains have less affinity for binding to vancomycin
- **Van gene** has several genotypes; out of which *van A* and *van B* are common types; expressed by *E. faecalis* and more commonly by *E. faecium*.

VRE Carriers

VRE often colonizes the intestine and poses a risk of transmitting to other patients.

- **Screening for VRE:** It is recommended for high risk patients such as from ICUs and transplantation units
- **Detection:** Rectal swab is collected and subjected to (i) Sodium azide agar with vancomycin or (ii) PCR for detection of *van* gene
- **Management:** Ensure infection control measures such as hand hygiene and isolation precautions (refer Chapter 21). Treatment (i.e. decolonization) is not recommended for VRE carriers.

Other Gram-positive cocci Causing UTI

- ❖ **Staphylococcus aureus:** *S. aureus* is an important cause of UTI. It predominantly causes skin and soft tissue infections (Chapter 51)
- ❖ **Staphylococcus saprophyticus:** It causes UTI in sexually active young women. This is due to expression of a 160 kDa hemagglutinin/adhesin protein that can adhere to uroepithelial cells. It can be differentiated from other staphylococci in being resistant to novobiocin disk (5 µg)
- ❖ **Streptococcus agalactiae:** Group B *Streptococcus* (GBS) can cause UTI—cystitis and asymptomatic bacteriuria. It usually colonizes in female genital tract; can cause more often postpartum infection, neonatal sepsis, skin and soft tissue infections (Chapter 52).

Other Bacterial Infections of Urinary Tract**Renal Tuberculosis**

Genitourinary TB, which accounts for 10–15% of all extrapulmonary tuberculosis, may involve any portion of the genitourinary tract. Up to 75% of patients have chest X-ray suggesting previous or concomitant pulmonary tuberculosis (Chapter 63).

- ❖ **Symptoms:** Urinary frequency, dysuria, nocturia, hematuria, and flank or abdominal pain are common presentations

- ❖ **Urinalysis** gives abnormal results in 90% of cases, revealing pyuria and hematuria
- ❖ **Sterile pyuria:** Presence of pus cells in urine but negative routine bacterial urine culture raises the suspicion of TB
- ❖ **Urine culture for TB:** Three consecutive early morning urine specimens yield a definitive diagnosis in nearly 90% of cases. Following centrifugation, the deposit is sent for culture in MGIT (Mycobacteria Growth Indicator Tube) or LJ (Lowenstein-Jensen) medium
- ❖ **Radiology:** Abdominal CT or MRI scan may show deformities, obstructions, calcifications and ureteral strictures. Severe ureteral strictures may lead to hydronephrosis and renal damage
- ❖ **Treatment:** Genitourinary TB responds well to antitubercular therapy (Chapter 63).

Post-streptococcal Glomerulonephritis (PSGN)

PSGN is a non-suppurative sequela of group A streptococcal infection (Chapter 52). The antibodies developed against streptococcal antigens cross react with glomerular basement membrane resulting in glomerulonephritis.

- ❖ **Serotypes involved:** PSGN typically occurs following either streptococcal pyoderma (usually by M serotypes—47, 49, 55, 57, 60) or rarely following pharyngitis (caused by M serotypes 1-4, 12, 25)
- ❖ PSGN due to impetigo develops 2–6 weeks after skin infection; whereas it develops 1–3 weeks after streptococcal pharyngitis
- ❖ **Pathology:** PSGN results from the lodging of antigen antibody complexes on the glomerular basement membrane (appear as “humps”), followed by complement activation. Streptococcal pyogenic exotoxin-B (SPE-B) may be the main nephritogenic antigen involved
- ❖ **Clinical presentations:** Urine retention and renal insufficiency occurs that leads to edema, hypertension, hematuria, pyuria, proteinuria and oliguria
- ❖ **Diagnosis:** Patients usually have elevated streptococcal anti-DNase B antibodies (70%), compared to rise of ASO (30%) and anti-hyaluronidase (40%). Anti-DNase-B antibodies titer more than 300–350 units/mL is diagnostic of PSGN and pyoderma
- ❖ **Prognosis:** PSGN usually occurs in children (2–14 years) and has a good prognosis.

Apart from PSGN, another non-suppurative sequela is seen following streptococcal sore throat called **acute rheumatic fever** (described in Chapter 28); differences between which have been depicted in Table 76.5.

Perinephric and Renal Abscesses

Perinephric and renal abscesses develop secondary to a urinary tract infection rather than direct hematogenous spread.

- ❖ Infection ascends from the bladder (cystitis) to the kidney (pyelonephritis), then ultimately proceed to involve renal parenchyma (medulla to context) to produce abscess.

Table 76.5: Differences between acute rheumatic fever and post-streptococcal glomerulonephritis.

Features	Acute rheumatic fever (ARF)	Post-streptococcal glomerulonephritis (PSGN)
Prior history of infection with	Pharyngitis strains	Mainly pyoderma, or rarely pharyngitis strains
Serotypes responsible	Most of the strains of group A <i>Streptococcus</i>	Only nephritogenic strains
Immune response	Marked	Moderate
Complement level	Unaltered	Low (due to deposition in glomeruli)
Genetic susceptibility	Present	Absent
Repeated attack	Common	Uncommon
Penicillin prophylaxis	Indicated	Not indicated
Course	Progressive	Spontaneous resolution
Prognosis	Variable	Good
Hypersensitivity reaction	Type II	Type III

Areas of abscess within the parenchyma may rupture into the perinephric space

- ❖ Pre-existing renal stones obstructing urinary flow is the major risk factor associated with most of the cases
- ❖ The common etiologies include uropathogenic organisms such as *E. coli*, *Proteus* species and *Klebsiella* species
- ❖ Treatment involves drainage of pus and antibiotic therapy directed at the organism(s) recovered.

VIRAL INFECTIONS OF URINARY SYSTEM

Viral infections of urinary system is very rare. Only few viruses can infect urinary tract.

BK Virus Infection

BK virus causes nephropathy in kidney transplant recipients. It can also cause hemorrhagic cystitis in hematopoietic stem cell transplant recipients. Infection in other individuals may occur, but almost always asymptomatic.

- ❖ **Naming:** BK virus is named after the initials of the patient in whom it was described first. It is a polyomavirus, possesses dsDNA
- ❖ **Diagnosis:** The diagnostic modalities include:
 - **Renal biopsy:** Histologic examination of renal biopsy specimens showing characteristic histopathologic changes (inflammatory infiltrates, fibrosis and tubular injury) plus positive immunohistochemistry for BK virus provide definite diagnosis
 - **PCR:** Identification of BK viremia (BK virus DNA in blood) by PCR plus features of nephropathy is also suggestive of the diagnosis
 - **Asymptomatic BK viruria** (detection of BK virus DNA in urine) is a less specific finding; may not be clinically significant.

- ❖ **Treatment:** There is no specific treatment available. Cidofovir has been used for treatment of refractory cases.

Adenovirus Cystitis

Adenovirus serotypes 11 and 21 can cause acute hemorrhagic cystitis in children, especially in boys.

Certain viruses such as Cytomegalovirus and herpes simplex virus (HSV) are excreted in urine, however they usually do not cause urinary tract infections. Urinary retention may be seen in HSV infection secondary to autonomic nervous system dysfunction.

PARASITIC INFECTIONS OF URINARY SYSTEM

Urinary Schistosomiasis (*S. haematobium*)

Schistosoma haematobium is the causative agent of urinary schistosomiasis or Bilharziasis, named after its discoverer T. Bilharz in 1851.

- ❖ It is a blood trematode (or fluke), resides in venous plexus of urinary bladder and ureter
- ❖ The other two blood flukes *Schistosoma mansoni* and *S. japonicum* reside in venous plexus of intestine and mesentery, produce intestinal disease (Chapter 46).

Epidemiology

Approximately, 200–300 million individuals are infected with schistosomes globally across 74 countries. Large majority (85%) live in sub-Saharan Africa, where >2 lakh deaths per year occur due to schistosomiasis.

- ❖ *S. haematobium* infection is endemic in 53 countries in the Middle East, the African continent (across Nile river valley) and the Indian Ocean islands (Madagascar, Zanzibar and Pemba)
- ❖ **India:** Schistosomiasis is extremely rare in India. A confirmed endemic focus of urinary schistosomiasis was demonstrated in Gimvi village of Ratnagiri district, Maharashtra; transmitted by snail of genus *Ferrissia*.

Life Cycle

The life cycle of *S. haematobium* is similar to that of other schistosomes as discussed in Chapter 46 (Fig. 46.13). They exist in three morphological forms: adult worms, eggs and larvae.

- ❖ **Host:** There are two hosts; man is the definitive host and freshwater snails are the intermediate host
- ❖ **Mode of transmission:** Man acquires infection by penetration of skin by the infective form (cercaria larvae) present in the contaminated water
- ❖ **Development in man:** The cercariae penetrate the skin, travel via dermal veins to reach systemic circulation and then enter the portal system where they develop into adult worms
- ❖ Adult worms reach vesical and ureteric venous plexuses, where they undergo fertilization to produce eggs that are excreted in urine

- ❖ **Pre-patent period:** Human cycle takes around 3 months of time to produce eggs in urine.

Pathogenesis and Clinical Features

Acute Schistosomiasis

The invasion of cercariae in the skin causes dermatitis at penetration site followed by allergic pruritic papular lesion.

Chronic Schistosomiasis

- 1. Urogenital disease:** Light infection may be asymptomatic. Symptoms develop usually after 3–6 months
 - The adult worms are rarely pathogenic. The main pathogenic mechanism in schistosomiasis is due to the deposition of eggs in various tissues
 - The eggs have terminal spines which cause damage to the bladder mucosa that leads to dysuria and hematuria (seen up to 80% of children infected)
 - The soluble antigens released from the eggs provoke delayed type of hypersensitivity reaction that leads to the formation of egg granuloma, which later undergoes fibrotic changes
 - In heavy infection, male genital organs are frequently affected. Deposition of the eggs in scrotal lymphatics may cause elephantiasis in scrotum and penis
 - *S. haematobium* infection may be an important risk factor for potential activation and transmission of HIV.
- 2. Obstructive uropathies:** Fibrosis may cause obstruction of the lower end of the ureters that result in hydronephrosis and hydronephrosis, which may be seen in 25–50% of infected children
- 3. Bladder carcinoma:** The metaplastic changes in urinary mucosa may lead to carcinoma of bladder
 - **Predisposing factors** include—(1) Intake of diet containing nitroso-compounds, commonly found in Egyptian food (cheese, fava beans, raw salted fish); (2) Secondary bacterial infections, causing cystitis; (3) Genetic factors such as activation of *H-ras*, inactivation of p53 and retinoblastoma genes
 - **Type:** Squamous cell carcinoma is the most common type. It is seen with high to moderate worm burden; whereas transitional cell carcinoma may occur in areas with lighter worm load.
- 4. Involvement of other sites:** Eggs may be carried by venous blood to various parts of the body like spinal cord, liver, lungs or intestine and produce similar granulomas.

Laboratory Diagnosis

Urine Microscopy

Diagnosis of *S. haematobium* infection is made by detection of non-operculated terminal spined eggs in the urine or rarely in feces (Fig. 76.4A).

The terminal hematuria portion of urine is collected between 12 noon to 3 pm, concentrated by centrifugation or by membrane filtration and observed under microscope for the presence of elliptical shaped egg of size 112–170 × 40–70 μm, with a sharp terminal spine. It should be



Figs 76.4A and B: A. *Schistosoma haematobium* egg with terminal spine (red arrow); B. Egg of *Dioctophyme renale*.

Source: A. D# 4843, Public Health Image Library; B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

differentiated from *S. mansoni* egg, which has a lateral spine (Fig. 46.14B).

Histopathology

S. haematobium eggs can be demonstrated in bladder mucosal biopsy or wet cervical biopsy specimens (in females). The number of eggs present in crushed tissue correlates significantly with the size of the genital lesions.

Antibody Detection

The tests for the detection of antibody are useful for sero-epidemiology. Two assays are available to detect serum antibodies against *S. haematobium* adult worm microsomal antigen (HAMA).

- ❖ **HAMA-FAST-ELISA** (Falcon assay screening test ELISA)
- ❖ **HAMA-EITB** (Enzyme-linked immunotransfer blot)
- ❖ IgE and IgG4 are elevated in schistosomiasis like any other helminthic infections.

Antigen Detection

Detection of circulating antigen indicates recent infection and can be used for monitoring the treatment response. They are also useful when urine microscopy fails to detect eggs (chronic and ectopic cases).

- ❖ Circulating cathodic antigen (CCA) and circulating anodic antigen (CAA) can be detected in serum and urine by ELISA or dip stick assays
- ❖ CCA levels are much higher in urine than CAA.

TREATMENT

Urinary schistosomiasis

Praziquantel is the drug of choice; given 20 mg/kg/dose, two doses in single day.

Metrifonate can be give alternatively. It inhibits acetylcholine receptors on tegument surface of adult male worm. It is administered in multiple oral doses over weeks; hence not preferred in control programs.

Prevention

Preventive measures include:

- ❖ Proper disposal of human excreta and urine
- ❖ Eradication of snails by using molluscicides such as metal salts (iron or aluminum sulfate), metaldehyde, methiocarb and acetylcholine esterase inhibitors
- ❖ Treatment of infected persons.

Diectophyme renale Infection

Diectophyme renale is commonly known as “giant kidney worm” is a nematode of lower animals, human infection is extremely rare (only 23 cases, including 3 cases from India).

- ❖ **Life cycle:** Human gets infection by ingestion of fish infected with larva of *D. renale*. Larva penetrates the intestine and reach the kidney (right kidney affected commonly) and transform into adult worms. Adult worms are larger in size and can block the kidney and ureter. Adult worms lay eggs, that are passed in urine
- ❖ **Clinical features:** It includes hematuria and renal colic. Extensive destruction of kidney parenchyma may occur
- ❖ **Laboratory diagnosis:** Condition is diagnosed by demonstration of characteristic eggs in urine. Eggs are oval-shaped, measure 60–80 µm size, contain an embryo surrounded by characteristic thick sculptured or pitted egg shell (Fig. 76.4B)
- ❖ **Prevention:** Proper cooking of fish prior to consumption.

Trichomonas vaginalis Urethritis

T. vaginalis is a sexually-transmitted parasite that primarily cause urethritis. The trophozoites may be detected in urine sediment. It is discussed in detail in Chapter 77.

FUNGAL INFECTIONS OF URINARY SYSTEM

Candiduria

Isolation of of *Candida* species in urine is a common finding, which may result from contamination during collection, bladder colonization, or upper UTI (due to hematogenous or ascending infection from bladder).

Treatment of candiduria in asymptomatic patients is not recommended, but can be considered in the following situations:

- ❖ Symptomatic cystitis or pyelonephritis and patients at high-risk for disseminated disease
- ❖ Neutropenic or immunosuppressed patients
- ❖ Patients undergoing urologic manipulation
- ❖ If upper-pole or bladder-wall invasion or obstruction is associated
- ❖ Critically-ill patients (have higher risk for invasive candidiasis)
- ❖ Low birth weight infants.

Fluconazole (for 14 days) is the drug of choice, as it reaches high levels in urine. In case of fluconazole resistance, oral flucytosine and/or parenteral amphotericin B can be considered.

EXPECTED QUESTIONS

I. Write essay on:

- a. A 32-year-old female was admitted with dysuria (burning micturition) and increased frequency of micturition for the past 2 days. Culture of the urine specimens revealed lactose fermenting colonies on MacConkey agar.
 1. What is your clinical diagnosis and probable etiological agents?
 2. What are the risk factors associated, pathogenesis and clinical manifestations of this disease?
 3. Describe the laboratory diagnosis in detail.
 4. How will you treat this clinical condition?

II. Write short notes on:

1. A 28-year-old female was admitted with high grade fever, vomiting, flank pain with increased frequency of micturition for the past 3 days. What is your clinical diagnosis, etiological agents and laboratory diagnosis?
2. A 46-year-old man from Africa came to the OPD with abdominal pain, hematuria and dysuria. Urine culture was found as sterile. Urine wet mount examination

revealed oval non-operculated elongated eggs with terminal spine. What is the etiological diagnosis? Write briefly about the life cycle and various diagnostic modalities of this condition?

3. Significant bacteriuria
4. Difference between upper and lower UTI
5. Asymptomatic bacteriuria

III. Multiple Choice Questions (MCQs):

1. Which culture medium is preferred for processing of urine specimens?
 - a. TCBS agar
 - b. CLED agar
 - c. Chocolate agar
 - d. XLD agar
2. Which of the following is the most common etiological agent of UTI?
 - a. *Escherichia coli*
 - b. *Klebsiella*
 - c. *Proteus*
 - d. *Enterobacter*

Answers

1. b 2. a

CHAPTER PREVIEW

- Genital Tract Infections Common in Both Sexes
- Genito-ulcerative Disease
- Urethritis
- Genital Tract Infections in Females
- Genital Tract Infections in Males

SEXUALLY TRANSMITTED INFECTIONS

The sexually transmitted infections (STIs) are a group of communicable diseases which are transmitted by sexual contact. Causative agents of STIs may be classified into two groups (Table 77.1):

1. **Agents causing local manifestations**—called genital tract infections
 - Lesions common to both sexes: Such as genital ulcers, urethritis, and anorectal lesions
 - Female genital tract infections: Such as vulvovaginitis, cervicitis and others
 - Male genital tract infections: Such as prostatitis, epididymitis, and orchitis.
2. **Agents causing systemic manifestations** without producing local manifestations (e.g. HIV, hepatitis B and C)—these infections are discussed under the systems which they primarily affect.

GENITO-ULCERATIVE DISEASE

Genito-ulcerative disease comprises of five important STIs—syphilis, chancroid, genital herpes, lymphogranuloma venereum and donovanosis.

- ❖ It is important to clinically differentiate them from each other so that appropriate treatment can be initiated
- ❖ Differentiation is based on the characteristic of genital ulcer such as pain, induration, and associated lymphadenopathy (Table 77.2).

SYPHILIS (TREPONEMA PALLIDUM)

Treponema pallidum is the causative agent of an ancient sexually transmitted infection (STI) 'syphilis'. The name *pallidum* refers to its pale-staining property. It was discovered by Schaudinn and Hoffmann in 1905.

Genus Description

Spirochetes are thin, flexible, elongated spirally coiled helical bacilli. They include *Treponema*, *Borrelia* and

Table 77.1: Causative agents of sexually transmitted infections.

Agents causing local manifestations (genital tract infections)
In both sexes:
Genito-ulcerative disease: <ul style="list-style-type: none"> ● Syphilis: Caused by <i>Treponema pallidum</i> ● Chancroid: Caused by <i>Haemophilus ducreyi</i> ● Genital herpes: Caused by herpes simplex viruses ● Lymphogranuloma venereum: Caused by <i>Chlamydia trachomatis</i> ● Donovanosis: Caused by <i>Klebsiella granulomatis</i>
Urethritis: <ul style="list-style-type: none"> ● Gonococcal urethritis: Caused by <i>Neisseria gonorrhoeae</i> ● Non-gonococcal urethritis (NGU): Caused by <ul style="list-style-type: none"> ➢ <i>Chlamydia trachomatis</i> (D-K) ➢ Genital mycoplasmas: <i>Ureaplasma urealyticum</i>, <i>Mycoplasma genitalium</i>, <i>M. hominis</i> ➢ Herpes simplex virus ➢ <i>Candida albicans</i> ➢ <i>Trichomonas vaginalis</i>
Other genital tract infections common to both sexes <ul style="list-style-type: none"> ● Genital tuberculosis: Caused by <i>M. tuberculosis</i> ● Anorectal lesions: <ul style="list-style-type: none"> ➢ Proctitis: Caused by HSV, gonococcus, <i>C. trachomatis</i> ➢ Anogenital warts: Caused by human papilloma virus
In females only:
<ul style="list-style-type: none"> ● Vulvovaginitis: Bacterial vaginosis, trichomoniasis and candidiasis ● Mucopurulent cervicitis caused by gonococcus, <i>C. trachomatis</i> ● Pelvic inflammatory disease: Presents as— <ul style="list-style-type: none"> ➢ Endometritis, salpingitis, oophoritis, tubo-ovarian abscess ➢ Extension to peritoneum can lead to peritonitis, pelvic abscess and perihepatitis ● Infections after gynecologic surgery ● Infections in pregnancy/postpartum
In males only:
Prostatitis, epididymitis, and orchitis
Agents causing systemic manifestations, no local lesions
HIV, Hepatitis B virus (HBV), Hepatitis C virus (HCV)

Leptospira; the latter two have been discussed in Chapter 32.

Treponemes are slender spirochetes with fine spirals having pointed ends (*trepos*, meaning 'turn' and *nema*,

Features	Syphilis	Genital Herpes	Chancroid	LGV	Donovanosis
Incubation period	9–90 days	2–7 days	1–14 days	3 days–6 weeks	1–4 weeks (up to 6 months)
Genital ulcer	Painless, single, indurated	Painful, multiple, bilateral, tiny vesicular ulcers	Painful, soft, usually multiple, purulent, bleeds easily	Painless, firm single lesion	Painless, single/multiple, beefy-red ulcer, bleeds readily
Lymphadenopathy	Painless, non-indurated (firm), bilateral	Painful, firm, often bilateral with initial episode	Painful, soft, marked swelling leads to bubo formation, unilateral	Painful and soft, unilateral	Absent (pseudobubo may be present due to subcutaneous swelling)
Treatment	Penicillin (single dose)	Acyclovir (7–14 days)	Azithromycin (single dose)	Doxycycline (21 days)	Azithromycin (7 days)

meaning ‘thread’). Most of them are commensals in mouth and genitalia. Only a few species are pathogenic to men, which can be divided into two groups.

- Sexually-transmitted:** *Treponema pallidum*—it causes syphilis, discussed below
- Nonvenereal treponematoses:** *T. pertenue*, *T. endemicum* and *T. carateum*
 - They are almost identical with *T. pallidum* in their morphology, antigenic structure and in genetic composition
 - However, they differ from *T. pallidum*, being transmitted by non-sexual mode (by direct contact) and produce non-genital cutaneous manifestations (discussed in Chapter 55).

Pathogenesis of Syphilis

Syphilis is one of the ancient sexually transmitted infection known since fifteenth century. Name was derived from a famous poem in the year 1530 which described a legend of a shepherd boy named Syphilus, who had suffered from the disease.

- ❖ **Mode of transmission:** Venereal syphilis is acquired by sexual contact. However, it can also be transmitted by non-venereal modes such as direct contact, blood transfusion or transplacental transmission
- ❖ **Spread:** *T. pallidum* rapidly penetrates through the minute abrasions on the skin or mucosa and, within a few hours, enters the lymphatics and blood to produce systemic infection and metastatic foci long before the appearance of a primary lesion. Blood is infectious even during the incubation period or in the early stage of syphilis
- ❖ **Incubation period** can range from 10 to 90 days and is inversely proportional to the number of organisms inoculated. The median incubation period in humans is about 21 days which corresponds to an average inoculum of 500–1000 infectious organisms.

Clinical Manifestations of Syphilis

Approximately, 30% of persons who have sexual exposure with an infected partner develop syphilis. This disease has been called as “**The Great Pretender**”, as its clinical

manifestations can mimic many other diseases. Clinically, patients suffering from syphilis pass through four stages if left untreated: primary, secondary, latent and tertiary (or late) stages. Apart from this, if transmitted vertically, the newborn babies develop a congenital form of syphilis.

Primary Syphilis

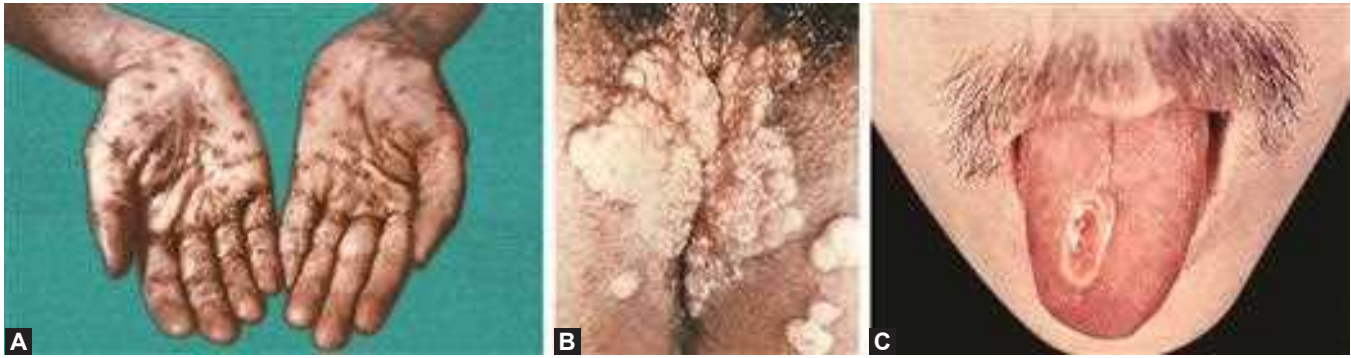
Primary syphilis is characterized by:

- ❖ **Primary (or hard) chancre:** It is characterized by single painless hard indurated ulcer; covered by thick exudate rich in spirochetes. The most common sites are penis (in males), cervix or labia (in females), and anal canal, rectum or mouth (in homosexuals) (Fig. 77.1)
- ❖ **Regional (usually inguinal) lymphadenopathy** appears within 1 week of onset of skin lesions. Lymph nodes are painless firm, non-suppurative, and often bilateral
- ❖ The chancre generally heals within 4–6 weeks (range 2–12 weeks), but lymphadenopathy may persist for months
- ❖ If acquired by non-venereal mode, then the primary syphilis is presented as follows:
 - If transmitted by direct contact→the primary chancre is extragenital, usually on the fingers
 - If transmitted by blood transfusion→the primary chancre does not occur.



Fig. 77.1: Primary syphilis (hard chancre).

Source: Public Health Image Library, ID# 6803, Dr/M. Rein/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Figs 77.2A to C: Clinical manifestations of secondary syphilis: **A.** Skin rashes; **B.** Condylomata lata; **C.** Mucosal patch.

Source: Public Health Image Library/A. ID# 6808; B. ID# 4098; C. ID# 4816/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Secondary Syphilis

Secondary syphilis usually develops 6–12 weeks after the healing of primary lesion. Skin and mucous membranes are commonly affected and characterized by:

- ❖ **Skin rashes** (palms and soles Fig. 77.2A)
- ❖ **Condylomata lata** (mucocutaneous papules which coalesce to form large pink to grey lesions in warm moist intertriginous areas such as perianal region, vulva, and scrotum) (Fig. 77.2B)
- ❖ **Mucous patches** (superficial mucosal erosions; Figure 77.2C)
- ❖ Generalized lymphadenopathy is seen. Chancre may also persist in up to 1/3rd cases.

Latent Syphilis

Secondary lesions usually subside within 2–6 weeks, and the infection proceeds to latent syphilis; which is characterized by absence of clinical manifestations of syphilis with positive serological tests for syphilis and normal CSF findings.

- ❖ Latent syphilis may be early latent syphilis (occurs within first year after infection) and late latent syphilis (occurs after the first year of infection)
- ❖ Patients are still infectious transmitting the infection either by bloodstream or in utero
- ❖ Latent syphilis may have one of the following fates:
 - Persistent lifelong infection (common)
 - Development of late syphilis (rare)
 - Spontaneous cure.

Late or Tertiary Syphilis

Several decades after the initial infection, about one-third of untreated patients develop tertiary syphilis, of which 15% develop gummatous lesions, about 10% develop cardiovascular lesions and remaining 10% develop neurosyphilis. The latter two stages are sometimes classified as *quaternary syphilis*.

- ❖ **Gumma (late benign syphilis):** Gummas are locally destructive granulomatous lesions. They can occur in any organ but most commonly seen in bone and skin

- ❖ **Neurosyphilis:** Common manifestations include—chronic meningitis, vasculitis, general paresis of insane and tabes dorsalis (Chapter 71 for detail)
- ❖ **Cardiovascular syphilis:** Characterized by aneurysm of ascending aorta and aortic regurgitation.

Congenital Syphilis

Mother-to-fetus transmission can lead to development of various congenital manifestations, discussed in Chapter 79.

Epidemiology

In 1940s, syphilis was considered as the most common type of genital ulcer. With increased education on safe sex practices, and widespread use of broad-spectrum antibiotics to treat STI-related syndromes; the incidence of syphilis has declining over past few decades.

- ❖ **Incidence:** Syphilis still remains a significant health problem globally; the number of new infections is estimated to be 11 million per year globally
- ❖ **Most affected regions:** The regions that are most affected include sub-Saharan Africa, South America, China, and Southeast Asia
- ❖ **In pregnancy:** Worldwide, 1.4 million cases of syphilis occur among pregnant women, with 5 lakh adverse pregnancy outcomes annually.

LABORATORY DIAGNOSIS

Syphilis

Microscopy

- ❑ Dark ground microscopy
- ❑ Direct IF staining for *T. pallidum* (DFA-TP)
- ❑ Silver impregnation method
 - Levaditi stain (for tissue section)
 - Fontana stain (smear)

Culture: Not cultivable, maintained in rabbit testes

Serology (antibody detection)

- ❑ **Non-treponemal or STS (standard tests for syphilis):** Reagin antibodies are detected by using cardiolipin antigen
 - VDRL (Venereal disease research laboratory) test
 - RPR (Rapid plasma reagin)

Contd...

Contd...

L LABORATORY DIAGNOSIS

Syphilis

- TRUST (toluidine red unheated serum test)
- USR (Unheated serum reagin test).
- ❑ **Specific/Treponemal test:** Specific antibodies are detected by using *T. pallidum* antigens
 - TPI (*Treponema pallidum* immobilization test)
 - FTA-ABS (Fluorescent treponemal antibody absorption test)
 - TPA (*T. pallidum* agglutination test)
 - TPHA (*T. pallidum* hemagglutination test)
 - TPPA (*T. pallidum* particle agglutination test).

Polymerase chain reaction (PCR)**Laboratory Diagnosis of Syphilis**

Laboratory diagnosis of syphilis consists of demonstration of treponemes, detection of antibodies and PCR.

Direct Microscopy (Demonstration of Treponemes)

Treponemes can be demonstrated from the superficial lesions of primary, secondary and congenital syphilis.

Surface of the chancre is cleaned with saline, gentle pressure is applied at the base of the lesion, and a drop of exudate is collected on a slide.

Dark Ground Microscopy (DGM)

Treponemes cannot be visualized by light microscope but can be seen by examining the wet film of specimen under dark ground (DGM) or phase contrast microscope.

- ❖ **Under DGM:** *T. pallidum* appears as slender, flexible, spirally coiled bacilli with tapering ends, measuring 6–20 µm in length and contains 6–20 spirals (Fig. 77.3A)
- ❖ **Motility:** *T. pallidum* shows typical: (i) slow to rapid *flexion-extension type* of movement with (ii) rotation around its longitudinal axis (**corkscrew motility**), (iii) rotation may be accompanied by a **soft bending** at right angle to the midpoint
- ❖ The **sensitivity** of DGM approaches 80% with a detection limit of 10⁴ bacilli/mL

- ❖ **Multiple specimens** should be examined on three consecutive days before declaring DGM to be negative
- ❖ **Saprophytic spirochetes:** It is difficult to differentiate *T. pallidum* from other saprophytic spirochetes of the genital area, such as *T. refringens* (shows very active serpentine-like movement), and *T. phagedenis* (shows jerky movement). Differentiation is based on size, spiral character and motility.

Direct Fluorescent Antibody Staining for *T. pallidum* (DFA-TP)

Smear made from the exudate or tissue sections is stained with fluorescent-labelled monoclonal antibody targeted against *T. pallidum* surface antigens.

- ❖ *T. pallidum* appears as distinct, sharply outlined, apple green fluorescent colored bacilli (Fig. 77.3B)
- ❖ Sensitivity of DFA-TP test approaches 100% when smear made from fresh lesions are examined.

Silver Impregnation Staining

Treponema do not take up ordinary stains as they are extremely thin and delicate (Fig. 77.3C).

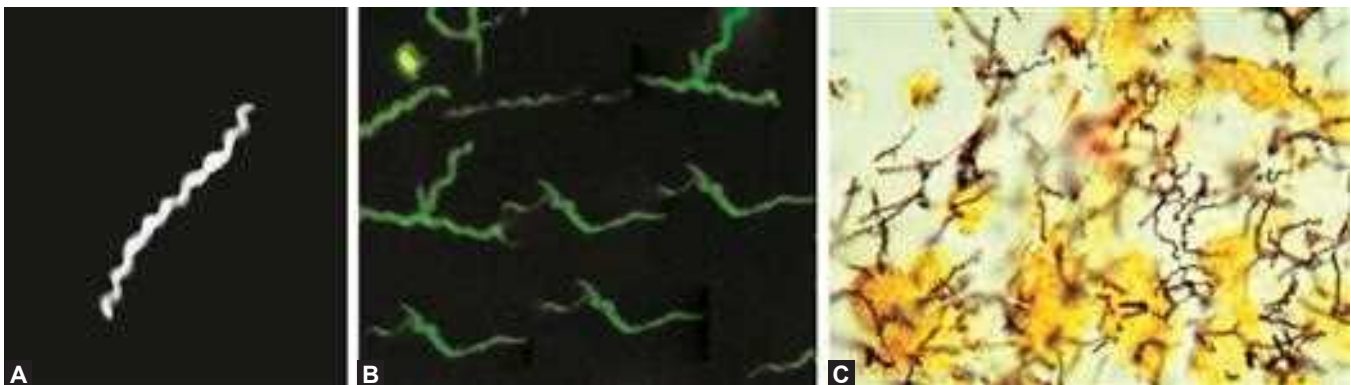
- ❖ However, silver impregnation methods can be used to increase their thickness
- ❖ Treponemes reduce silver nitrate to metallic silver that is deposited on the surface, making them thicker
- ❖ Levaditi stain is used for staining tissue section and Fontana stain is used for staining smears made from exudates.

Cultivation

Pathogenic treponemes including *T. pallidum* cannot be grown in artificial culture media but are maintained by subcultures in susceptible animals such as rabbit testes (e.g. Nichols strain).

Serology (Antibody Detection)

As microscopy is difficult and culture methods are not available, antibody detection methods are of paramount importance in the diagnosis of syphilis.



Figs 77.3A to C: Direct microscopy of *T. pallidum*: **A.** Dark ground microscope; **B.** Direct fluorescent antibody staining for *T. pallidum* (DFA-TP); **C.** Silver impregnation method.

Source: Public Health Image Library, A. ID# 2043; B. ID# 14967/Dr Russell; C. ID# 836, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Depending upon the type of antigen used, three types of tests are available to detect antibodies in patient's sera:

- ❖ **Non-treponemal tests:** Detect non-specific reagin antibody by using cardiolipin antigen derived from bovine heart
- ❖ **Treponemal tests:** Detect species-specific antibody by using *T. pallidum* specific antigen; which is polysaccharide in nature.

Non-treponemal or Lipoidal Tests or STS (Standard Tests for Syphilis)

Non-treponemal tests (or lipoidal tests) detect a characteristic non-specific antibody (called reagin antibody) in the sera of syphilitic patients by using cardiolipin antigen extracted from beef heart.

- ❖ Cardiolipin antigen is chemically a diphosphatidyl glycerol. Similar lipid haptens have been detected on the surface of *T. pallidum*
- ❖ Such reagin antibodies are IgG or rarely IgM type and are distinct from the IgE class of reagin antibodies seen in type I hypersensitivity reactions.

Examples of non-treponemal tests include various slide flocculation tests such as:

- ❖ Venereal Disease Research Laboratory (VDRL)
- ❖ Rapid Plasma Reagin (RPR)
- ❖ Unheated Serum Reagin (USR)
- ❖ Tolidine Red Unheated Serum Test (TRUST).

VDRL and RPR are the widely used tests and therefore are described below.

Venereal Disease Research Laboratory (VDRL)

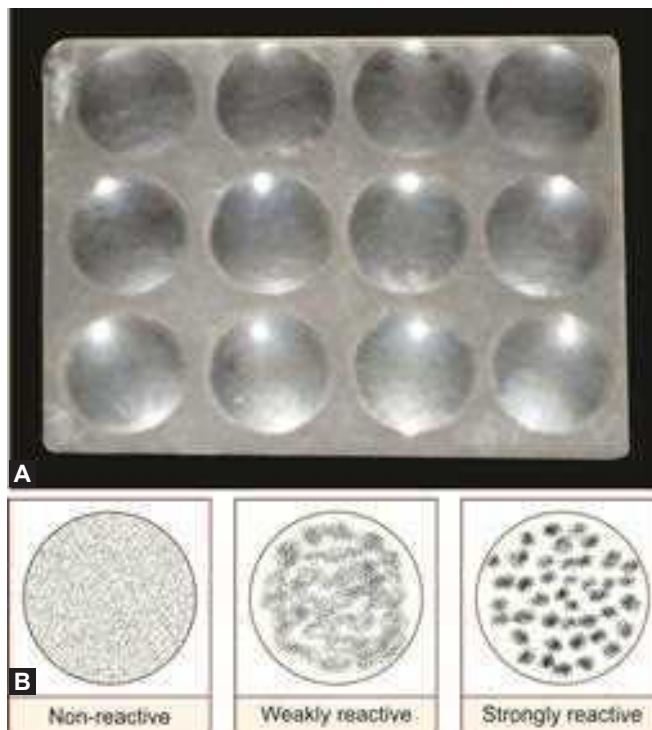
This test was named after Venereal Disease Research Laboratory (VDRL), New York, where the test was developed. It works on the principle of precipitation (slide flocculation) test.

- ❖ **Procedure:** 50 μ L of patient's serum (heat inactivated) is mixed with a drop of VDRL antigen on a concave slide, which is then mixed by rotating the slide for 4 minutes (Figs 77.4A and B)
- ❖ **Result:** Positive test (i.e. reactive) is indicated by formation of medium to large clumps of antigen antibody complexes; visualized by focusing the slide under microscope (10x)
- ❖ **CSF antibodies:** VDRL test can also be performed on CSF specimen to detect antibodies
- ❖ **Uses:** VDRL test is cheaper and preferred as a screening test for laboratory with higher sample load and for batch testing (e.g. antenatal screening) and also to monitor treatment response.

Rapid Plasma Reagin (RPR)

RPR is another slide flocculation test using disposable plastic cards having clearly defined circles. It is similar to VDRL test with some differences such as:

- ❖ RPR antigen has a prolonged shelf-life, therefore it is preferred to test individual sample (less sample load);



Figs 77.4A and B: A. VDRL slide; B. VDRL test results.

Source: Department of Microbiology, JIPMER, Puducherry (with permission).

where as VDRL is preferred when samples are tested in batches (large sample load)

- ❖ Results can be read in naked eyes, without the need of a microscope, as the clumps formed are bigger in size
- ❖ It can only be used for detecting antibodies in blood; not in CSF
- ❖ It is more expensive than VDRL.

Advantages of Non-treponemal Tests

- ❖ Non-treponemal tests are recommended to monitor the response to treatment
- ❖ Neurosyphilis: VDRL can also be used to detect CSF antibodies
- ❖ Reagin antibody becomes detectable 7-10 days after the appearance of primary chancre (or 3-5 weeks after acquiring the infection)
- ❖ **Utility:** The sensitivity of nontreponemal tests varies from 78 to 85% in primary stage, 100% in secondary stage, 71-73% in late stage and the specificity is around 98-99%.

Disadvantages of Non-treponemal Tests

Biological false-positive (BFP) reactions: Non-treponemal tests may give a false-positive result in the absence of syphilis and is also not due to technical faults. The incidence of BFP is generally 1-2%.

- ❖ This is because reagin antibodies may also be found in patients with unrelated diseases such as lepromatous leprosy, relapsing fever, malaria, viral hepatitis, HIV, pregnancy and IV drug abusers

- ❖ In these conditions, the reagin antibodies are induced against lipid haptens released from the damaged host tissues which may mimic cardiolipin antigens.

Other disadvantages include:

- ❖ **Prozone phenomena:** If antibody titer in patient's sera is high, it may lead to false negative result hence it is essential to test sera in dilutions
- ❖ **Sensitivity** of non-treponemal tests is low in late stage of syphilis. VDRL-CSF is more reliable for neurosyphilis than VDRL test of serum.

Treponemal or Specific Tests

Treponemal tests aim at detecting *T. pallidum* specific antibodies in patient's sera by using either live or killed *T. pallidum* or their antigenic extract. The various examples include:

- ❖ **TPI (*T. pallidum* immobilization test):** Uses live actively motile *T. pallidum* (Nichols strain); which become immobilized after they combine with specific antibodies
- ❖ **FTA-ABS (Fluorescent treponemal antibody absorption test):** Patient's serum is layered on a slide which is previously coated with killed *T. pallidum*. Fluorescent labeled anti-human immunoglobulin is added and then slide is examined under fluorescent microscope
 - It is highly sensitive and specific in all the stages of syphilis
 - It is the first serological test to be positive following infection
 - **IgM-FTA-ABS test** is a modification that detects only IgM antibodies and therefore is useful for congenital syphilis
 - It can also be used to detect CSF antibodies.
- ❖ **Tests that use extract of *T. pallidum***
 - TPHA (*T. pallidum* hemagglutination test)
 - TPPA (*T. pallidum* particle agglutination test)
 - Western blot and enzyme immunoassay.

The sensitivity of treponemal tests varies from 84 to 90 % in primary stage, 100% in secondary stage, 94–96% in late stage and the specificity is around 97–99%.

Molecular Methods

PCR-based techniques are available to amplify *T. pallidum* specific genes, such as gene coding for 47-kDa surface antigen (lipoprotein) and 39-kDa basic membrane protein. PCR is of paramount importance in the diagnosis of congenital and neurosyphilis.

A multiplex PCR has been developed for simultaneous detection of common agents of genital ulcers such as *Treponema pallidum*, *Haemophilus ducreyi*, and herpes simplex virus.

Testing Algorithm

CDC recommends to use a **testing algorithm** comprising of non-treponemal test (as screening test), followed by

treponemal test (for confirmation) for serodiagnosis of syphilis. However, in area with high-prevalence for syphilis, a strategy of **reverse algorithm** may be found cost-effective where a treponemal test is performed first, followed by non-treponemal test.

Testing for syphilis in pregnancy: Every pregnant woman should undergo a non-treponemal screening test at her first antenatal visit and, if there is high-risk of exposure, again retested at the third trimester and at delivery.

Syphilis and HIV

Both syphilis and HIV affect each other's pathogenesis.

- ❑ Genital syphilis facilitates the transmission of HIV through the abraded mucosa (2 to 5 fold increased risk)
- ❑ Patient with HIV, if develops syphilis later→there is rapid progression to late stages of syphilis and neurological involvement even after treatment of primary or secondary syphilis.

Problems in the diagnosis of syphilis in HIV infected people are:

- ❑ Confusing clinical signs and symptoms
 - Clinical overlap with different stages of syphilis may be present
 - CNS invasion and ocular manifestations (posterior uveitis) are common presentation.
- ❑ Lack of serologic response in a patient with a clinically confirmed case of active syphilis
- ❑ Unusually high titers in non-treponemal tests perhaps as a result of B cell activation
- ❑ Failure of non-treponemal test titers to decline even after treatment with standard regimens
- ❑ Disappearance of treponemal test reactivity over time.

TREATMENT

Syphilis

- ❑ **Penicillin** is the drug of choice for all the stages of syphilis:
 - Primary, secondary, or early latent syphilis: single dose of Penicillin G is given
 - Late latent CVS or benign tertiary stage: penicillin G is given single dose weekly for 3 weeks
 - Neurosyphilis or abnormal CSF in any stage or associated HIV—aqueous crystalline or procaine penicillin G is given for 10–14 days. Consider re-treatment if non-treponemal titres in CSF, do not decrease four fold within 2 years of completion of treatment.
- ❑ **Alternative drug is used** in patients with penicillin allergy:
 - For primary, secondary, latent, CVS or benign tertiary syphilis—tetracycline is recommended
 - For neurosyphilis or in pregnancy or associated HIV—desensitization to penicillin has to be done, following which penicillin is administered.

Note: **Jarisch-Herxheimer** is a condition that results due to a reaction to lipoproteins released by the death of *Treponema pallidum*, during the antibiotic treatment to syphilis.

Evaluation after Treatment

Non-treponemal tests, such as VDRL and RPR are preferred over treponemal tests for monitoring response to treatment. Antibody titers of treponemal tests remain

elevated even after clinical improvement. VDRL has to be done at 3 months' intervals for at least 1 year.

- ❖ **For primary and secondary syphilis:** Following clinical improvement, there should be at least fourfold decline in the titer by the third or fourth month and non-reactive by 12 months
- ❖ **For latent or late syphilis, or patients with multiple episodes of syphilis:** It may show a gradual decline in titer, low titers may persist for years.

Prevention

Prevention of syphilis includes:

- ❖ Treatment of cases and contacts (sexual partners)
- ❖ Education about safe sex practices
- ❖ Prophylactic use of barrier contraceptive methods.

CHANCROID (HAEMOPHILUS DUCREYI)

Haemophilus ducreyi is an etiologic agent of **chancroid (or soft chancre)**, a sexually transmitted infection (STI) characterized by:

- ❖ Painful genital ulceration (Fig. 77.5) that bleeds easily; no inflammation of the surrounding skin
- ❖ Enlarged, tender inguinal lymph nodes (bubo).

Incubation period can range from 4–7 days. There is no immunity following the infection; however, hypersensitivity may develop.

Epidemiology

Chancroid is a common cause of genital ulcers in developing countries.

- ❖ Transmission is predominantly heterosexual
- ❖ Male to female ratio is about 3:1 to 25:1
- ❖ Chancroid and HIV: Chancroid increases both the efficiency of transmission and the degree of susceptibility to HIV infection.



Fig. 77.5: Chancroid (painful ulcer).

Source: Public Health Image Library, ID# 15567/ Dr Pirozzi/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Laboratory Diagnosis

- ❖ **Specimens:** Exudate or swab from the edge of the ulcer and lymph node aspirate are the useful specimens
- ❖ **Direct microscopy:** *H. ducreyi* is a pleomorphic gram-negative coccobacillus; occurs in groups or in parallel chains
 - They frequently take bipolar staining
 - The arrangement has been described as **school of fish** or **rail road track** appearance.
- ❖ **Culture:** *H. ducreyi* requires factor X (hemin), but not factor V for its growth. Primary isolation is difficult. It can be grown on—
 - Rabbit blood agar or chocolate agar enriched with 1% isovitalex and made selective by adding vancomycin
 - It may also be grown on chorioallantoic membrane of the chick embryo.
- ❖ **Optimum conditions** required for isolation are 10% CO₂, high humidity and incubation at 35°C for 2–8 days
- ❖ **Colony morphology:** Colonies are small, gray, translucent, 1–2 mm in size in 2–3 days
- ❖ **Biochemical reactions:** *H. ducreyi* is biochemically inert. Growth surrounding X disk can be used for presumptive diagnosis
- ❖ **Slide agglutination test:** *H. ducreyi* is antigenically homogeneous and cultures can be confirmed by agglutination with the antiserum
- ❖ A **multiplex PCR** assay has been developed for simultaneous detection of common agents of STIs including *H. ducreyi* (targeting 16S rRNA).

TREATMENT

Chancroid

- ❑ Drug of choice: Azithromycin (1g oral; single dose)
- ❑ Alternative drugs: Ceftriaxone, ciprofloxacin or erythromycin
- ❑ Treatment of all the sexual partners is essential

HERPES GENITALIS

Genital herpes is caused by herpes simplex viruses (HSV-1 and 2). They produce widespread disease including cutaneous, mucocutaneous and systemic diseases (discussed in detail in Chapter 56).

- ❖ **Genital ulcers:** Characterized by multiple, painful, bilateral (widely spaced), tiny vesicular ulcers
- ❖ **Inguinal lymphadenopathy:** Enlarged, tender, firm, often bilateral
- ❖ **Recurrent episodes** are milder and recover faster than primary genital herpes. Recurrence is more common with HSV-2 than with HSV-1; the median number of recurrences is 4 and <1, respectively
- ❖ **Associated symptoms** include fever, headache, malaise, myalgia, itching, dysuria, vaginal and urethral discharge
- ❖ **Other genital infections** seen in herpes include: Urethritis, vulvovaginitis, cervicitis, endometritis and salpingitis, rectal (HSV proctitis) and perianal infections following rectal intercourse

- ❖ **Laboratory diagnosis:** Diagnostic modalities for genital herpes include the following:
 - **Staining of scrapings** from the base of the lesions with Giemsa's (Tzanck preparation), or Papanicolaou's stain can detect giant cells or intranuclear inclusions of HSV infection
 - **Viral antigen** (by direct IF) or **viral DNA** (by PCR) can be detected in scrapings from lesions
 - **Multiplex platforms** of PCR and real-time PCR have been developed for simultaneous detection of pathogens causing STIs including HSV in genital secretions/lesions
 - **Isolation** of the virus in scrapings from lesions.

TREATMENT

Genital herpes

The effective drugs in genital herpes are acyclovir, valacyclovir, or famciclovir.

- **First episode:** Oral acyclovir is given for 7-14 days. IV acyclovir is given for severe disease or associated neurologic complications
- **Recurrent genital herpes:** Short-course (1 to 3 day) regimens are preferred.

LYMPHOGRANULOMA VENEREUM (LGV)

Lymphogranuloma venereum (LGV) is an invasive systemic sexually transmitted infection, caused by *Chlamydia trachomatis* serovars L1, L2, and L3. The clinical course passes in three stages.

- ❖ **First stage:** Painless ulcer develops on the penis or vulva after an incubation period of 3 days to 6 weeks
- ❖ **Second stage:** Inguinal lymph nodes in the groin become enlarged, tender and soft (called **bubo**)
 - Fistulae: Bubo may breakdown and discharge may spread externally as chronic fistulae
 - Groove sign (unilateral) may be present, as a result of enlargement of nodes below the inguinal ligament
 - Systemic symptoms may develop such as fever, headache and myalgia.
- ❖ **Third stage:** Occurs in untreated cases, especially in women and homosexual men
 - Rectal stricture or rectovaginal and rectal fistulae may occur
 - Esthiomene—the vulva, scrotum or penis may undergo edematous granulomatous hypertrophy
 - Elephantiasis of the vulva or scrotum may occur due to impaired lymphatic drainage and is endemic in tropics, notably Africa and India.
- ❖ **Diagnosis** of LGV is usually based on serology; biopsy is contraindicated as there is a risk of sinus tract formation
 - **NAAT:** Nucleic acid amplification test (NAAT) for *C. trachomatis* will be positive
 - **Antibody detection** by ELISA or microimmunofluorescence (MIF)
 - Direct detection of **inclusion bodies** by direct IF or for culture confirmation

- **Frei test:** It is a skin test, used in the past to demonstrate type IV hypersensitivity reaction in LGV; now obsolete.

- ❖ **Treatment:** Longer treatment course is necessary; doxycycline for 21 days is drug of choice. Azithromycin (weekly once for 3 weeks) can be given alternatively. The detail of *Chlamydia trachomatis* has been discussed later in this chapter, under non-gonococcal urethritis.

DONOVANOSIS

Donovanosis, also called *granuloma inguinale* is a sexually transmitted disease caused by *Klebsiella granulomatis*; previously called as *Calymmatobacterium granulomatis*.

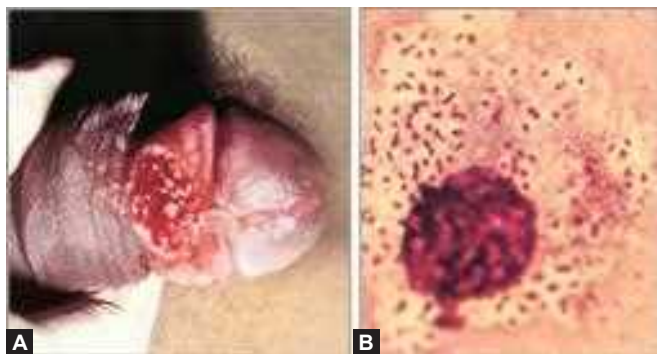
- ❖ Disease was first described in Kolkata by McLeod in 1882, and the characteristic pathological finding “Donovan bodies” in the genital lesion was recognized by Charles Donovan Chennai in 1905
- ❖ Donovanosis is prevalent in India, Brazil, Papua New Guinea and parts of South Africa
- ❖ Risk factors include poor hygiene, lower socioeconomic status and multiple sex partners
- ❖ Globally, the incidence of donovanosis has greatly decreased.

Clinical Features

- ❖ Incubation period is about 1–4 weeks (may be up to 6 months). It runs a chronic course
- ❖ Genital lesion starts as a painless papule which subsequently becomes a **beefy red ulcer** that bleeds readily when touched (Fig. 77.6A)
- ❖ Most common sites: Genitals are affected in 90% of patients; affecting prepuce, frenulum and glans in men and the labia minora in women
- ❖ Lymph node involvement is rare however, *pseudobuboes* (subcutaneous granulomas) may be seen in the inguinal region in 10% of cases due to subcutaneous abscess.

Laboratory Diagnosis

- ❖ **Clinical diagnosis** is made by the appearance of characteristic lesion
- ❖ **Specimen collection:** A swab should be rolled firmly over the genital ulcer previously cleaned with a dry swab to remove debris. Alternatively, a piece of granulation tissue crushed and spread between two slides can be used
- ❖ **Direct microscopy:** Smears can be examined after a rapid Giemsa or Wright's stain
 - Donovan bodies can be seen as large cyst like macrophages filled with deeply stained capsulated bacilli having a *safety-pin* (bipolar) appearance (Fig. 77.6B). These cysts eventually rupture releasing the bacilli
 - They are non-motile, capsulated and gram-negative bacilli.
- ❖ **Culture:** They can be grown on egg yolk medium and on HEp-2 cell lines



Figs 77.6A and B: Donovanosis: **A.** Beefy red ulcer; **B.** Donovan bodies: Cyst-like macrophages filled with deeply stained capsulated bacilli having a safety-pin appearance (Giemsa stain).

Source: Public Health Image Library: A. /ID#:5363/ Dr. Tabua; B. ID#: 18899, Susan Lindsley/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Molecular method:** PCR has been developed to differentiate *Klebsiella granulomatis* from other *Klebsiella* species by detecting unique base changes in the *phoE* gene.

TREATMENT

Donovanosis

- ❑ Azithromycin 1 g orally once per week or 500 mg daily for at least 3 weeks, until all lesions have completely healed
- ❑ Alternatively, doxycycline or co-trimoxazole is given for 14 days
- ❑ Both the sexual partners should be treated and all those who are diagnosed with granuloma inguinale should also be tested for HIV.

URETHRITIS

GONOCOCCAL URETHRITIS

Neisseria gonorrhoeae is noncapsulated, gram-negative kidney-shaped diplococcus. It causes 'gonorrhoea', a sexually transmitted infection (STI) which commonly manifests as cervicitis, urethritis and conjunctivitis.

Virulence Factors

- ❖ **Pili or fimbriae:** Pili are the principal virulence factors of gonococci that help in adhesion to host cells and prevent bacteria from phagocytosis
- ❖ **Outer membrane proteins:**
 - **Porin (protein I):** This accounts for more than 50% of total outer membrane proteins
 - ♦ They form transmembrane channels (pores) which help in exchange of molecules across gonococcal surface
 - ♦ There are two major serotypes: PorB.1A and PorB.1B serotypes. PorB.1A strains are often associated with both local and disseminated gonococcal infections (DGI), while PorB.1B strains usually cause local genital infections only

- **Opacity-associated protein (Protein II):** It helps in adhesion to neutrophils and other gonococci.

- ❖ **Others** include transferrin-binding and lactoferrin-binding proteins, IgA1 protease and lipo-oligosaccharide (LOS) with endotoxin.

Clinical Manifestations

Gonorrhoea is a venereal disease reported since ancient times; produces various infections in males, females and also in newborns.

- ❖ **In males:** Acute urethritis is the most common manifestation

- It is characterized by purulent urethral discharge (the word 'gonorrhoea' is derived from flow of seed resembling semen, coined by Galen in 130 AD)

- The incubation period is 2–7 days

- Untreated cases may go for complications, such as epididymitis, prostatitis, and balanitis

- Infection may spread to periurethral tissues causing abscess with sinus formation (**water-can perineum**).

- ❖ **In females:** Gonococcal infection is less severe in females, with more asymptomatic carriage than males

- **Mucopurulent cervicitis:** It is the most common presentation, characterized by scanty vaginal discharge

- **Vulvovaginitis:** It is not seen in adult females as the adult vagina is resistant to gonococcal infection (due to its low pH and thick stratified squamous epithelium). However, gonococcal vaginitis can occur in prepubertal girls and postmenopausal women where the vagina is lined by thinned out mucosa with higher pH

- **Spread:** Infection may spread to Bartholin's gland, endometrium and fallopian tube. Salpingitis and pelvic inflammatory disease may lead to sterility

- **Fitz-Hugh-Curtis syndrome:** It is a rare complication, characterized by peritonitis and associated perihepatic inflammation.

- ❖ **In both the sexes:** The following manifestations may occur in both the sexes:

- Anorectal gonorrhoea (spread by anal sex): Rectal isolates are usually multidrug-resistant

- Pharyngeal gonorrhoea, spread by orogenital sex (Chapter 60)

- Ocular gonorrhoea.

- ❖ **In pregnant women:** Gonococcal infection causes prolonged rupture of the membranes, premature delivery, chorioamnionitis, and sepsis in infant

- ❖ **In neonates (Ophthalmia neonatorum):** It is characterized by purulent eye discharge, occurs within 2–5 days of birth. Transmission occurs during birth from colonized maternal genital flora (Chapter 78)

- ❖ **Disseminated gonococcal infection (DGI):** It occurs rarely following gonococcal bacteremia; characterized by polyarthritides, and rarely dermatitis and endocarditis

- ❖ **In HIV-infected persons:** Nonulcerative gonorrhea enhances the transmission of HIV by three-to-five folds, possibly because of increased viral shedding.

Epidemiology

The incidence of gonorrhea has come down in developed countries; however, it still remains a public health problem in developing countries, and may play a role in enhancing transmission of HIV. Because of the associated social stigma, it is often under-reported.

- ❖ **Host:** Gonorrhea is an exclusively human disease, there are no animal reservoirs
- ❖ **Source:** The source of infection are asymptomatic female carriers or less often a patient
- ❖ **Transmission:** It is almost exclusively transmitted—(1) by sexual contact (venereal); transmission from males to females is more efficient than in the opposite direction, (2) from mother to baby during birth.

Laboratory Diagnosis

Specimen Collection

Urethral swab in men and cervical swab in women are the preferred specimens. Vaginal swab is not satisfactory.

- ❖ In males, the urethral meatus is cleaned with gauze soaked in saline. The purulent discharge is expressed out by pressing at the base of the penis and collected directly on to slides or swabs
- ❖ **Dacron or rayon swabs** are preferred, as cotton and alginate swabs are inhibitory to gonococci
- ❖ **In chronic urethritis:** As discharge is minimal, prostatic massage is done to collect the secretion; alternatively, the morning drop of secretion may be collected.

Transport Media

Specimens should be transported immediately. If not possible, then it should be collected in charcoal-coated swabs kept in **Stuart's** transport medium or, alternatively, charcoal containing medium (**Amies** medium) can be used. Currently, various commercial transport devices, such as JEMBEC or Gono-Pak system are available.

Microscopy

Gram staining of urethral exudates reveals gram-negative intracellular kidney-shaped diplococci (Fig. 77.7). Gram staining is highly specific and sensitive in symptomatic men. However in females, it is only 50% sensitive because, the presence of commensal *Neisseria* species may confound with interpretation. Hence, culture is recommended for diagnosis of gonorrhea in women.

Culture

Endocervical culture has a sensitivity of 80–90%. As cervical swabs contain normal flora, hence, selective media are preferred, such as **Thayer Martin medium** (chocolate agar added with antibiotics).



Fig. 77.7: Gonococcus (gram-negative diplococci, kidney-shaped).

Source: Public Health Image Library, ID# /2108, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Blood culture (using automated blood culture systems) and synovial fluid cultures should be done in suspected cases of DGI.

Identification

Species identification is important to differentiate gonococci from other commensal *Neisseria* species.

- ❖ Gonococci are catalase and oxidase positive
- ❖ They ferment only glucose, but not maltose and sucrose
- ❖ Automated systems such as MALDI-TOF can be used.

Molecular Method

Nucleic acid amplification tests (NAATs) such as PCR are available for detection of *N. gonorrhoeae* from the clinical specimens targeting 16s or 23s rRNA gene.

TREATMENT

Gonococcal urethritis

- ❑ **Drug of choice:** Third generation cephalosporins currently are the mainstay of therapy for uncomplicated gonococcal infection. Both the sexual partners should be treated
 - Ceftriaxone (250 mg given IM, single dose)
 - Cefixime (400 mg given orally, single dose).
- ❑ If coexisting chlamydial infection is present, then azithromycin or doxycycline can be added to the regimen.

Note: Gonococci were initially susceptible to most antibiotics, such as sulfonamides, penicillins, quinolones, but because of their continual usage, resistance has emerged over the time, for example, penicillinase producing strains of *N. gonorrhoeae*.

Prophylaxis

There is no vaccination available for gonococci. The general prophylactic measures include:

- ❖ Early detection of cases
- ❖ Treatment of both partners
- ❖ Tracing of contacts
- ❖ Health education about safe sex practices such as use of condoms.

Table 77.3: Differences between gonococcal and non-gonococcal urethritis.

Features	Gonococcal urethritis	Non-gonococcal urethritis
Onset	48 hours	Longer (>1 week)
Urethral discharge	Purulent (flow of seed-resembling semen)	Mucous to mucopurulent
Complication	DGI (polyarthritides and endocarditis) Water-can perineum	Reiter's syndrome: Characterized by conjunctivitis, urethritis, arthritis and mucosal lesions
Diagnosis	<ul style="list-style-type: none"> Gram stain Culture on Thayer Martin media 	<ul style="list-style-type: none"> For <i>Chlamydia</i>—culture on McCoy and HeLa cell lines For <i>Trichomonas</i>—detection of trophozoite For <i>Candida</i>—detection of budding yeast cells in discharge For PCR—can be done for HSV or <i>Chlamydia</i>
Treatment	Ceftriaxone	<ul style="list-style-type: none"> For <i>Chlamydia</i>—Doxycycline For <i>Trichomonas</i>—Metronidazole For <i>Candida</i>—Clotrimazole (as vaginal cream or tablet)

Abbreviations: DGI, disseminated gonococcal infection; HSV, herpes simplex virus; PCR, polymerase chain reaction.

NON-GONOCOCCAL URETHRITIS (NGU)

Chronic urethritis where gonococci cannot be demonstrated has been labeled as non-gonococcal urethritis. NGU is more common than gonococcal urethritis. Several agents are implicated in NGU such as:

- ❖ **Bacteria:** These agents are discussed below
 - *Chlamydia trachomatis*: Most common agent of NGU (has been discussed below)
 - Urogenital *Mycoplasma*: *Ureaplasma urealyticum* and *Mycoplasma hominis*.
- ❖ **Viruses:** Herpes simplex virus—genital herpes mainly presents as genital ulcer (described earlier in this chapter), but can also cause urethritis
- ❖ **Fungi:** *Candida albicans*—in addition to urethritis, it also causes vulvovaginitis, described later in this chapter
- ❖ **Parasites:** *Trichomonas vaginalis*—in addition to urethritis, it also causes vulvovaginitis, described later in this chapter.

Differences between gonococcal and non-gonococcal urethritis are given in Table 77.3.

CHLAMYDIA TRACHOMATIS INFECTIONS

Genus Description

Chlamydiae are obligate intracellular bacteria that cause a spectrum of diseases in man such as trachoma, lymphogranuloma venereum (LGV), conjunctivitis, pneumonia and psittacosis and can also cause widespread diseases in birds and mammals.

Classification

Based on genetic characteristics, family Chlamydiaceae has undergone recent taxonomic changes. Previously, *Chlamydia* was the only genus under the family. But now, it comprises of two genera:

1. *Chlamydia*: It has one pathogenic species, *C. trachomatis*
2. *Chlamydophila*: It has two pathogenic species—*C. psittaci* and *C. pneumoniae*. They cause interstitial (atypical) pneumonia (discussed in Chapter 62).

Chlamydiae are Bacteria, Not Viruses

Chlamydiae were once thought to be viruses because of possessing a few viral properties, such as:

- ❖ They are obligate intracellular
- ❖ They cannot be grown in artificial media
- ❖ Filterable—small enough to pass through bacterial filters
- ❖ Produce intracytoplasmic inclusions.

However, chlamydiae are now confirmed to be bacteria, because they have many other properties similar to that of bacteria, as follows:

- ❖ Possess both DNA and RNA
- ❖ Their cell wall is similar to that of gram-negative bacteria (although they lack peptidoglycan layer)
- ❖ Multiply by binary fission
- ❖ Susceptible to a wide range of antibacterial agents.

Life Cycle

Chlamydiae exist in two distinct morphological forms—elementary body (EB) and reticulate body (RB) (Fig. 77.8)

- ❖ EBs are the **extracellular** and **infective form**; attach to the specific receptors on the host cells (e.g. squamous epithelial cells)
- ❖ **EB→RB**: Following entry, they transform into reticulate bodies; which are the **intracellular form**, survive inside the cells by preventing phagosome-lysosome fusion
- ❖ RBs are **replicative form**; divide by binary fission. They are also the **metabolically active form** and can synthesize their own nucleic acid, lipids and proteins except ATP; hence, they are called as **energy parasites** (as they depend on host ATP for survival)
- ❖ RBs present inside the vacuole may enlarge to form **inclusion bodies**, that can be readily detected by histological stains
- ❖ RBs transform back to EBs, which are subsequently released from the host cells by 48 hours and then infect other host cells
- ❖ Sometimes, the development is arrested at the reticulate body stage, leading to persistent infection; which plays an important role in pathogenesis of ocular and chronic genital infections.

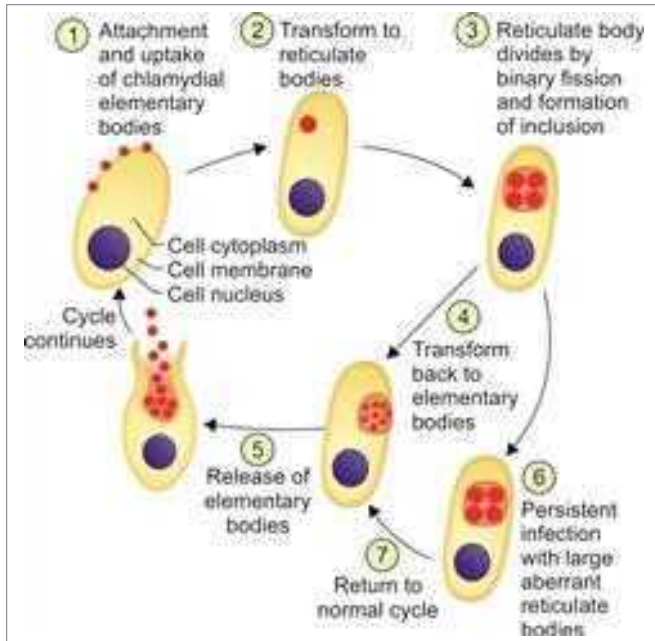


Fig. 77.8: Life cycle of *Chlamydia*.

Antigenic Structure

Chlamydiae possess the following antigens:

- ❖ **Genus/group specific antigen:** Chlamydial lipopolysaccharide (LPS) is genus specific. It plays an important role in the pathogenesis, acts by induction of TNF- α and other proinflammatory cytokines, which leads to scarring and fibrosis
- ❖ **Species specific protein antigens:** They are present at the envelope surface
- ❖ **Serovar-specific antigens:** They are the major outer membrane proteins (MOMP), encoded by *ompA* gene
- ❖ **Other antigens:** Such as outer membrane complex proteins and heat shock proteins, which play important role in pathogenesis.

Chlamydia trachomatis Infections

Chlamydia trachomatis is primarily a human pathogen, causing ocular, urogenital and neonatal infections.

Typing of *Chlamydia trachomatis*

Biovars

Historically, based on the disease produced, *C. trachomatis* was subdivided into two strains or biovars (Table 77.4).

1. TRIC (Trachoma-inclusion conjunctivitis)
2. Lymphogranuloma venereum (LGV) biovar.

Serotypes and Disease Produced

Based on antigenic structure of MOMP (and its gene *ompA*) of *C. trachomatis*, 18 serovars have been identified affecting humans.

- ❖ Serovars A, B, Ba and C are associated primarily with ocular disease called trachoma—a form of chronic keratoconjunctivitis (Chapter 78)
- ❖ Serovars D–K are associated with—(1) **genital tract infections** (described below), (2) **infant pneumonia** (interstitial pneumonia, Chapter 62) and (3) ocular disease, called **inclusion conjunctivitis**, which is of two types (Chapter 78)
 - Swimming pool conjunctivitis in adults
 - Ophthalmia neonatorum in new born.
- ❖ Serovars L1–L3 causes a sexually transmitted infection, lymphogranuloma venereum (LGV). It is an ulcerative genital disease, described earlier this chapter.

Genital Infections (*C. trachomatis* Serovars D–K)

The genital infections produced by *C. trachomatis* serovars D–K are as follows.

- ❖ **Nongonococcal urethritis (NGU):** *C. trachomatis* is the most common cause of nongonococcal urethritis (NGU), responsible for 30–50% of cases of NGU. It differs from gonococcal urethritis (GU) by:

Table 77.4: Features of *Chlamydia* infections.

Species	Character	Biovar	Serotype(s)	Disease
<i>C. trachomatis</i>	<ul style="list-style-type: none"> • Forms compact inclusions mixed with glycogen matrix • Sensitive to sulfonamide • Natural human pathogen • Leaves the host cell with a scar 	TRIC	A, B, Ba, C	Trachoma (Chapter 78)
			D–K (D, Da, E, F, G, H, I, Ia, J, Ja, K)	<ul style="list-style-type: none"> • Genital chlamydiasis • Inclusion conjunctivitis (Chapter 78) • Infant pneumonia (Chapter 62)
		LGV	L ₁ , L ₂ , L ₃	Lymphogranuloma venereum
<i>C. psittaci</i> (Chapter 62)	<ul style="list-style-type: none"> • Forms diffuse vacuolated inclusions without glycogen matrix • Resistant to sulfonamide • Natural pathogen of birds • Leaves the host cell by lysis 	Nil	Many serotypes	<ul style="list-style-type: none"> • Psittacosis (Atypical interstitial pneumonia) • Transmission is by inhalation route—pet birds (parrots) and poultry (turkeys and ducks) • No man-to-man transmission
<i>C. pneumoniae</i> TWAR agent (Chapter 62)	<ul style="list-style-type: none"> • Exclusive human pathogen • Forms inclusions without glycogen matrix • Resistant to sulfonamide 	Nil	Only 1 serotype	<ul style="list-style-type: none"> • Community-acquired atypical pneumonia • Associated with: atherosclerosis and asthma

Abbreviations: TRIC, trachoma inclusion conjunctivitis; LGV, lymphogranuloma venereum; TWAR agent, Taiwan acute respiratory agent.

- Onset of symptoms (incubation period is 7–10 days, compared to 2–5 days for GU)
- Symptoms: Mucopurulent discharge is followed by dysuria and urethral irritation (GU has purulent discharge).
- ❖ **Postgonococcal urethritis (PGU):** *C. trachomatis* is the most common cause of PGU.
 - Urethritis develops in men 2–3 weeks after recovery from GU
 - This occurs when patients with GU are treated with penicillin or cephalosporin alone without adding any antichlamydial drugs (such as azithromycin).
- ❖ **Epididymitis and proctitis:** *C. trachomatis* is the most common cause of epididymitis in males
- ❖ **Reactive arthritis (Reiter's syndrome):** It consists of conjunctivitis, urethritis (or, in females-cervicitis), arthritis, and characteristic mucocutaneous lesions
 - It occurs in 1–2% of cases of NGU, develops after 1–4 weeks after genital infection
 - Men are more frequently affected than women (10:1)
 - It is the most common cause of peripheral inflammatory arthritis in young men
 - Knee, ankle, small joints of feet and sacroiliac joints are commonly affected
 - Most of the patients possess HLA-B27 haplotype
 - **Mechanism:** It is an immune-mediated inflammatory response to an infection at a distant site. *C. trachomatis* may act as a trigger organism to initiate an aberrant hyperreactive immune response that can produce inflammation of the targeted joints in genetically predisposed individuals
 - Resolution usually occurs without specific treatment, but relapse is common.
- ❖ **In females:** It produces various manifestations.
 - Mucopurulent cervicitis is the most common manifestation
 - It may progress to endometritis, salpingitis (fallopian tube), PID (pelvic inflammatory disease) and finally pelvic peritonitis
 - Perihepatitis (Fitz-Hugh-Curtis syndrome).

LABORATORY DIAGNOSIS**Chlamydial infections**

- **Specimen:** Depends on the type of lesions
- **Microscopy:** Detects chlamydial inclusion bodies
 - Gram staining, Lugol's iodine and other stains such as Castaneda, Machiavello or Gimenez stains
 - Direct IF: Used for direct detection of inclusion bodies.
- **Antigen detection (LPS antigens):** By enzyme immunoassays
- **Culture:** It was the gold standard method in the past
 - Egg (yolk sac), mice inoculation and cell line culture
 - Cell lines of choice-
 - ◆ McCoy, HeLa (for *C. trachomatis*)
 - ◆ HEp2 (for *C. pneumoniae*).

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LABORATORY DIAGNOSIS**Chlamydial infections**

- **Nucleic acid amplification tests (NAAT),** e.g. PCR
 - The most sensitive and specific method
 - Currently the diagnostic assay of choice.
- **Serology (antibody detection):**
 - CFT or ELISA using group specific LPS antigen
 - Micro-IF test detects antibody against species and serovar specific MOMP antigen.

Laboratory Diagnosis of Chlamydial Infections

Laboratory diagnosis of various chlamydial infections is discussed here.

Specimen Collection

It depends up on the types infection associated.

- ❖ Scrapings or swabs from infected sites: As chlamydiae are intracellular, the sample must contain cells. Hence, firm scraping or swabbing of the site is required. Recommended specimens are:
 - Urethral swab for NGU
 - Endocervical swab for cervicitis
 - Conjunctival swabs for ocular infections-upper conjunctiva for trachoma and lower conjunctiva for ophthalmia neonatorum.
- ❖ First catch urine samples in the morning contain greatest amount of urethral secretions, hence it is the preferred specimen for urethritis or cervicitis
- ❖ Nasopharyngeal aspirate and respiratory secretions for suspected chlamydiae pneumonia
- ❖ Bubo aspirate for LGV.

Microscopy

- ❖ **Gram staining:** Though, chlamydiae are gram-negative they are poorly stained
- ❖ **Presumptive diagnosis:** Routine Gram staining often reveals sterile pyuria (i.e. elevated neutrophils without any organisms, including gonococci). In such a case any other diagnostic test should be performed for confirmation
- ❖ **Other stains:** Such as Castaneda, Machiavello or Gimenez stains are better methods to detect chlamydiae from the samples. The inclusion bodies can be detected in the cytoplasm
- ❖ **Lugol's iodine:** The inclusion bodies of *C. trachomatis* can be stained with Lugol's iodine because of the presence of glycogen matrix
- ❖ **Inclusion bodies:** They are given various names such as:
 - Halberstaedter-Prowazek (H-P) body in trachoma
 - Miyagawa corpuscle in LGV
 - LCL body (Levinthal-Cole-Lillie) body in psittacosis.

Direct Immunofluorescence Test (DIF)

DIF is used as for direct detection of inclusion bodies in clinical material, particularly from the genital tract and eye or can also be used for culture confirmation.

Enzyme Immunoassays (Antigen Detection)

EIA detects chlamydial group specific antigens (LPS) from the samples by using specific monoclonal antibodies.

Culture

Chlamydiae cannot be cultivated in artificial media. They can grow only in embryonated egg (yolk sac), animal (mice) and cell line.

- ❖ Both egg and mice inoculation methods are no longer in use
- ❖ **Cell line culture** was the traditional method of diagnosis in the past, was considered as the gold standard method
 - Though highly specific, it is less sensitive (90% compared with NAATs), time consuming, technically demanding and labor intensive
 - **Choice of the cell line** depends on the species:
 - ♦ *C. trachomatis*: McCoy, HeLa are the recommended cell lines (Figs 77.9 and 77.10)
 - ♦ *C. pneumoniae* can be isolated from HEp2 or human fibroblast cell line
 - ♦ *C. psittaci* although grow well in cell culture, isolation should not be attempted in the routine laboratory because of the risk of laboratory acquired infection.
- ❖ **Promote contact**: Pre-treatment of cell lines with diethylaminoethanol (DEAE) dextran or centrifugation after inoculation of specimen should be done to promote contact between chlamydiae and the cells, thus increasing the chance of isolation
- ❖ **Incubation and growth detection**: Cell lines are incubated in 10% CO₂ for 48–72 hours, and growth is detected by the presence of inclusions under microscopy, after staining (Fig. 77.10).

Nucleic Acid Amplification Tests (NAAT)

NAAT have revolutionized the diagnosis of chlamydial infections.

- ❖ **Advantages**: NAAT is highly sensitive and specific, takes less time, and detects even few copies of DNA from the sample. It can also differentiate the species and serovars
- ❖ NAATs are currently the diagnostic assays of choice for chlamydial infection as recommended by the CDC, replacing the so called gold standard culture methods
- ❖ **Genes targeted** are *C. trachomatis* specific genes such as opacity protein gene or 16S or 23S rRNA
- ❖ Various methods available are:
 - Polymerase chain reaction (PCR)
 - Real time PCR
 - FilmArray respiratory panel.

Serology (Antibody Detection)

Serological tests are useful for LGV, infant pneumonia and psittacosis (systemic infections).

- ❖ **Complement fixation test (CFT)** using LPS antigen was used in the past; now obsolete
- ❖ **ELISA** based formats are also available using recombinant LPS antigen
- ❖ **Microimmunofluorescence (MIF) test**: It uses the species and serovar specific MOMP (major outer membrane protein) antigen (Fig. 77.11)
 - Serovar and species-specific antigens are spotted onto slides and incubated with serial dilutions of patient's serum
 - After incubation and washing, antigen-antibody complex is detected by fluorescein tagged antihuman globulin.

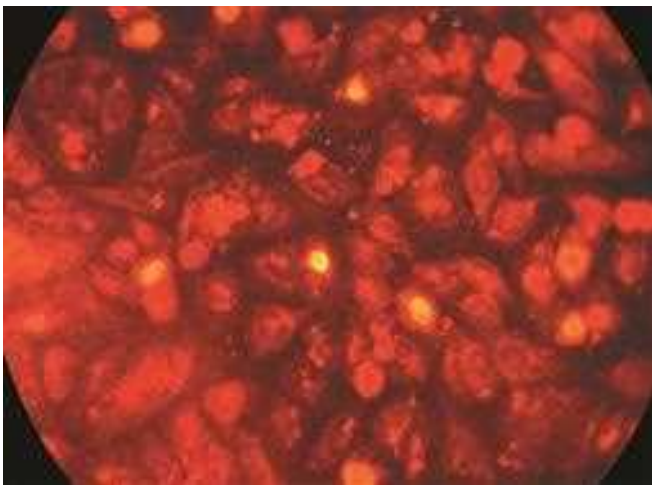


Fig. 77.9: HeLa cells infected with *Chlamydia trachomatis*.

Source: Public Health Image Library, ID#/3847/Joe Miller/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).



Fig. 77.10: *Chlamydia trachomatis* inclusion bodies (brown) in a McCoy cell culture.

Source: Public Health Image Library/ ID#: 3802, Dr E Arum; Dr N Jacobs, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

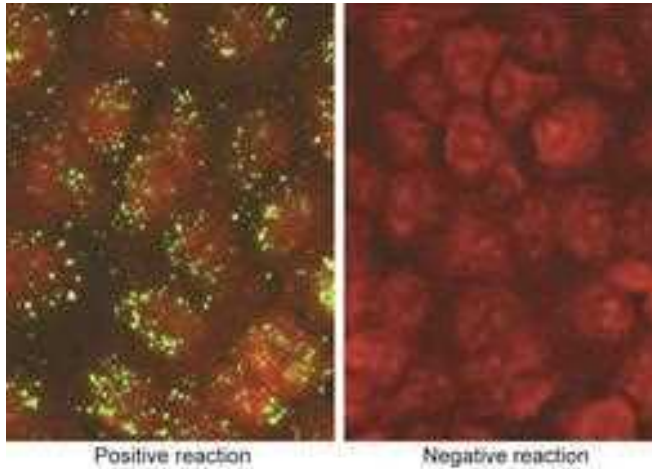


Fig. 77.11: Anti-*Chlamydia* microimmunofluorescence test (MIF).
Source: EUROIMMUN AG Pvt Ltd (with permission).

TREATMENT

C. trachomatis infections

- ❑ **For uncomplicated genital infection or trachoma or adult conjunctivitis:**
 - Azithromycin is the drug of choice given as single dose of 1 gram tablet, per oral
 - Alternatively doxycycline, tetracycline, erythromycin or ofloxacin can be given for at least a duration of 7 days
 - Both the sex partners should be treated
 - Ceftriaxone should be added to the regimen as co-infection with gonococcus may be present in most of the cases.
- ❑ **For complicated genital infection:** Doxycycline (100 mg twice daily), or erythromycin (500 mg four times daily) are the drugs of choice, given for:
 - 2 weeks for pelvic inflammatory disease and epididymitis
 - 3 weeks for LGV.

Prevention

Control measures for prevention of chlamydial genital infections include:

- ❖ Periodic screening of high-risk groups, such as young women having multiple sex partners
- ❖ Treatment of both the sex partners
- ❖ Use of barrier methods of contraception such as condoms
- ❖ Abstain from sex till 7 days after starting the treatment.

UROGENITAL MYCOPLASMA INFECTIONS

Mycoplasma (*M. hominis*, *M. genitalium*) and *Ureaplasma* (*U. urealyticum* and *U. parvum*) are associated with urogenital tract disease.

- ❖ They frequently colonize female lower urogenital tract such as vagina, periurethral area and cervix
- ❖ Transmission: They are transmitted mostly by sexual contact or mother to fetus during birth.

Clinical Manifestations

The manifestations of urogenital mycoplasmas are as follows:

- ❖ Non-gonococcal urethritis and epididymitis (mainly due to *Ureaplasma* and *M. genitalium*)
- ❖ Pyelonephritis (*M. hominis*), and urinary calculi (*Ureaplasma*)
- ❖ Pelvic inflammatory disease (mainly due to *M. hominis*)
- ❖ Postpartum and postabortal infection
- ❖ Non-urogenital infections (rare, due to *M. hominis*) such as: Brain abscess, wound infections or neonatal meningitis.

Laboratory Diagnosis

Culture and PCR are the appropriate methods for diagnosis of urogenital mycoplasmas. *Ureaplasma* forms very tiny colonies of 15–50 µm size, hence it was previously named as T-form *Mycoplasma*.

TREATMENT

Urogenital *Mycoplasma* infections

- ❑ Macrolides (azithromycin) are the drug of choice for *Ureaplasma* and *M. genitalium* infections
- ❑ Doxycycline is the drug of choice for *M. hominis*
- ❑ However, resistance has been reported to both the drugs.

OTHER GENITAL TRACT INFECTIONS COMMON TO BOTH THE SEXES

Apart from ulcerative genital disease and urethritis, the other genital tract infections common to both sexes include genital tuberculosis, and anorectal lesions.

GENITAL TUBERCULOSIS

Genital TB is diagnosed more commonly in female than in male patients.

- ❖ **In female patients**, it affects the fallopian tubes and the endometrium and can cause infertility, pelvic pain, menstrual abnormalities and adnexal swelling. Endometrial biopsy shows tuberculous granulomas, which can be sent for culture
- ❖ **In male patients**, genital TB preferentially affects the epididymis, producing a slightly tender mass that may drain externally through a fistulous tract. Other manifestations include orchitis and prostatitis.

ANORECTAL LESIONS

Anorectal lesions are frequently seen in—(1) women and men who practice of anal-genital intercourse; (2) HIV-infected and other immunocompromised patients. Common anorectal lesions include proctitis causing rectal ulcers, anal abscess and anogenital warts

- ❖ **Proctitis** (inflammation of the rectum): It can be caused by *N. gonorrhoeae* or *C. trachomatis* or HSV
 - The common manifestations include itching, mucopurulent anal discharge, anal pain, bleeding, and tenesmus (feeling of incomplete emptying of bowel)



Figs 77.12A and B: Condyloma acuminatum: **A.** Penis; **B.** Vagina.

Source: Public Health Image Library, A. ID# 3724; B. ID# 4097/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- Sigmoidoscopy reveals ulcerative lesions of the distal part of the rectal mucosa.
- ❖ **Anogenital warts:** Also called as condyloma acuminata is caused by human papilloma virus (HPV); the most common serotypes implicated being types 6 and 11. They have low malignant potential
 - **Site:** They may be found in the genital area such as the penile shaft, scrotum, or labia majora of the vagina or in the anal area (Figs 77.12A and B)
 - **Appearance:** They are generally pink in color and project out from the surface of the skin. Size may vary; usually appear small, but can merge into large masses. HPV is an oncogenic virus; causes carcinoma of cervix and other sites (Chapter 80).
- ❖ **In HIV-infected patients,** anorectal lesions tend to last longer, more severe, and are more difficult to treat compared with infections in the immunocompetent individuals
- ❖ **Subclinical infection in HSV:** Subclinical perianal shedding of herpes simplex virus (HSV) may occur in individuals without the history of rectal intercourse. This phenomenon is due to the establishment of latency in the sacral ganglia from prior genital tract infection, with subsequent subclinical reactivation in rectal epithelial cells
- ❖ **Unusual organisms:** Rarely, anorectal lesions are produced by enteric pathogens such as *Campylobacter*, *Shigella*, and *Entamoeba histolytica*.

FEMALE GENITAL TRACT DISEASE

Common infections of female genital tract include vulvovaginitis, mucopurulent cervicitis, pelvic inflammatory disease, infections after gynecologic surgery and infections associated with pregnancy.

VULVOVAGINITIS

Vulvovaginitis refers to inflammation of the vaginal mucosa (called vaginitis) and the external genitalia vulva (called vulvitis). It is the most common genital tract infection in females.

- ❖ Women present with vaginal symptoms such as abnormal discharge with/without offensive odor or itching
- ❖ The three most common causes of vaginitis in premenopausal women are trichomoniasis, bacterial vaginosis and vaginal candidiasis; can be differentiated from each other as given in Table 77.5.

Trichomoniasis

It is the most common parasitic sexually transmitted infection (STI), caused by a flagellated parasite *Trichomonas vaginalis*. It has only trophozoite stage; there is no cyst stage. Trophozoite has two forms:

- ❖ **Flagellated trophozoite:** It is the infective as well as the diagnostic form
- ❖ **Amoeboid trophozoite:** It is the actively replicating form, found in the tissue feeding stage of the life cycle.

Life Cycle

Asymptomatic females are the reservoir of infection. Humans acquire infection by sexual route. Flagellated trophozoites after entry, transform into amoeboid forms which multiply in the genital tract and cause infection. They again transform back to flagellated trophozoites that are discharged in vaginal/urethral secretions.

Clinical Feature

About 25–50% of individuals are asymptomatic, harboring the trophozoites and can transmit the infection; whereas others develop into disease after an incubation period of 4–28 days.

- ❖ **Acute infection (vulvovaginitis):** Adhesin proteins help in attachment to the vaginal epithelium. Females are commonly affected and present as vulvovaginitis, characterized by thin profuse foul smelling purulent vaginal discharge.
 - Discharge may be frothy (10% of cases) and yellowish green color mixed with pus cells
 - Strawberry appearance of vaginal mucosa (**Colpitis macularis**) is observed in 2% of patients. It is characterized by small punctate hemorrhagic spots on vaginal and cervical mucosa
 - Other features include dysuria and lower abdominal pain
 - In males, the common features are nongonococcal urethritis and rarely epididymitis, prostatitis and penile ulcerations.
- ❖ **Chronic infection:** In chronic stage, the disease is mild with pruritus and pain during coitus. Vaginal discharge is scanty, mixed with mucus

Table 77.5: Differential diagnosis of vulvovaginitis.			
Feature	Vulvovaginal Candidiasis	Trichomonal Vaginitis	Bacterial Vaginosis
Etiology	<i>Candida albicans</i>	<i>Trichomonas vaginalis</i>	<i>Gardnerella vaginalis</i> , various anaerobic bacteria
Typical symptoms	Vulvar itching and/or irritation	Profuse purulent discharge; vulvar itching	Malodorous, slightly increased discharge
Discharge	Scanty, white, thick and cheesy	Profuse, white or yellow	Moderate, thin, white to gray
pH of vaginal fluid	Usually ≤ 4.5	Usually ≥ 5	Usually >4.5
Fishy odor with 10% KOH	None	May be present	Present
Vaginal inflammation (erythema)	May be present	Colpitis macularis (strawberry appearance)	None
Microscopy of vaginal discharge	\uparrow Leukocytes, epithelial cells; budding yeast cell with pseudohyphae	\uparrow Leukocytes; trophozoites seen in 80–90% of symptomatic patients	Clue cells, few leukocytes, no/few lactobacilli (Nugent's score ≥ 7)
Other laboratory findings	Isolation of <i>Candida</i> spp.	Antigen detection or PCR	Culture, broad-range PCR
Treatment of the patient	Azole cream, tablet	Metronidazole or tinidazole	Metronidazole (tablet) and clindamycin cream
Treatment of sexual partner	None; topical treatment needed in case of <i>Candida</i> dermatitis of penis	Usually treatment needed	None

- ❖ **Complications:** Rarely, it is associated with complications like pyosalpinx, endometritis, infertility, low birth weight and cervical erosions. It increases the risk of transmission of HIV and HSV-2 infections.

Laboratory Diagnosis

Direct Microscopy

Vaginal, urethral discharge, urine sediment and prostatic secretions can be examined.

- ❖ **Wet (saline) mount** of fresh samples (within 10–20 minutes of collection) should be done to demonstrate the jerky motile trophozoites and pus cells (Fig. 77.13). Its sensitivity is variable (40–80%) and specificity is up to 100%
- ❖ **Other staining methods** include permanent stains (e.g. Giemsa and Papanicolaou stain), acridine orange

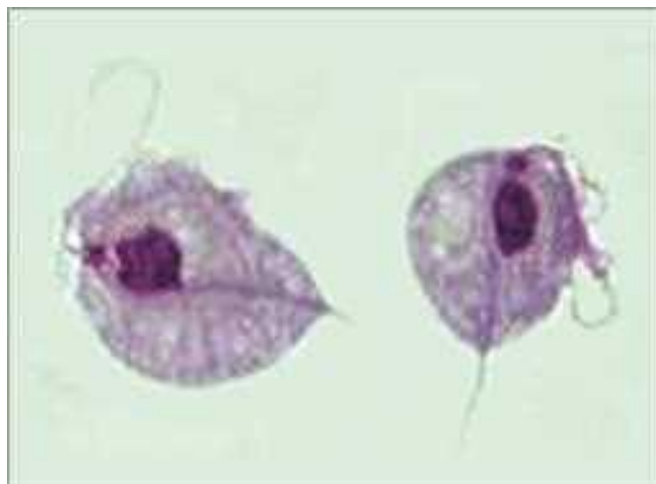


Fig. 77.13: *Trichomonas vaginalis* trophozoite (Giemsa stain).

Source: DPDx Image Library, Centers for Disease Control and prevention (CDC), Atlanta (with permission).

fluorescent stain and direct fluorescent antibody test (DFA). DFA test is more sensitive (70–90%) than wet-mount examination (Figs 77.13 and 77.14).

Trophozoite of *Trichomonas vaginalis*

It is pear shaped, measures 7–23 μm (Figs 77.13 and 77.14)

- ❑ It shows characteristic jerky or twitching motility in saline mount preparation
- ❑ It bears five flagella—four anterior flagella and one lateral flagellum called as recurrent flagellum which traverses the parasite as an **undulating membrane**, that in turn is supported on to the surface of the parasite by a rod like structure called as **costa**
- ❑ It has a single nucleus containing central karyosome with evenly distributed nuclear chromatin and the cytoplasm contains a number of siderophore granule along the axostyle.

Culture

Culture is the gold standard method for diagnosis. It is highly sensitive 75–96% and specific (100%).

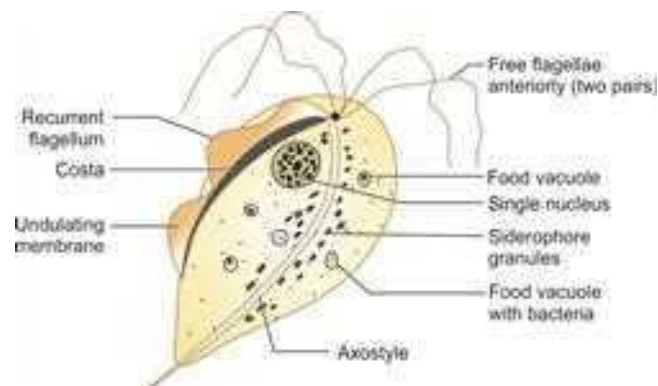


Fig. 77.14: Trophozoite (flagellated) of *Trichomonas vaginalis* (schematic diagram).

- ❖ Specimen should be processed immediately into media such as Lash's cysteine hydrolysate serum media. Special container like "InPouch TV" can be used for sample collection and culture
- ❖ Cultures should be incubated for 3–7 days, followed by mounting of the culture fluid to demonstrate the trophozoites.

Antigen Detection in Vaginal Secretion

Antigen detection methods are more sensitive than microscopy, easy to perform and indicates recent infection. Both rapid ICT and ELISA are available using monoclonal antibodies.

Antibody Detection

ELISA is available using whole cell antigen preparation and aqueous antigenic extract to detect antitrichomonal antibodies in serum and vaginal secretion of the patients. However, antibodies persist for longer time, hence cannot differentiate between current infection and past infection.

Molecular Methods

Molecular methods are highly sensitive, have replaced the culture techniques; target *T. vaginalis* specific genes such as beta-tubulin gene.

Other Supportive Tests

- ❖ **Raised vaginal pH (>4.5):** It is not specific as the vaginal pH is also raised in bacterial vaginosis. However, in vaginal candidiasis, the pH is not raised
- ❖ **Positive whiff test:** Fishy odor is accentuated when a drop of 10% KOH is added to vaginal discharge due to production of amine
 - It is positive in more than 75% of cases
 - It is also positive in bacterial vaginosis.
- ❖ **Increased pus cells** on wet mount examination is seen in >75% of cases.

TREATMENT

Trichomoniasis

Metronidazole or tinidazole are the drug of choice.

- ❑ Standard therapy: 2 g, single dose is usually effective
- ❑ Both the sexual partners must be treated simultaneously to prevent reinfection, especially asymptomatic males
- ❑ Resistance to metronidazole is rare, but reported (2.5–10%). Consider repeating the treatment course, if the standard therapy fails (with 5 days therapy).

Prevention

Trichomoniasis can be prevented by:

- ❖ Treatment of both the sexual partners
- ❖ Safe sex practices like use of condoms
- ❖ Avoidance of sex with infected person.

Bacterial Vaginosis

Bacterial vaginosis affects women of reproductive age. This condition is associated with an alteration of the normal vaginal flora, which is as follows:

- ❖ **Increase in the concentrations of:**
 - *Gardnerella vaginalis*: It is normally isolated from the female genital tract in low numbers; but in bacterial vaginosis, it outnumbers other organisms
 - *Mobiluncus* (motile, curved, gram-variable or gram-negative, anaerobic rods)
 - Several other anaerobes (*Prevotella* and some *Peptostreptococcus*)
 - *Mycoplasma hominis*.
- ❖ **Decrease in the concentrations** of lactobacilli (lactobacilli maintain the acidic pH of the vagina, thereby inhibiting the growth of pathogenic organisms).

Risk Factors

Bacterial vaginosis can occur in presence of the following risk factors:

- ❖ Coexisting other infections such as HIV, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*
- ❖ Recent unprotected vaginal intercourse
- ❖ Vaginal douching
- ❖ Premature rupture of membranes and preterm labor.

Clinical Diagnosis

Bacterial vaginosis is so named because there is no associated inflammation. It is clinically diagnosed by *Amsel's criteria*.

Amsel's Criteria

Bacterial vaginosis is diagnosed if any 3 of the following 4 findings are present:

1. Slight to moderately increased thin (low viscous), white homogeneous vaginal discharge uniformly coated on vaginal wall
2. pH of vaginal discharge more than 4.5
3. Accentuation of distinct fishy odor (attributable to volatile amines such as trimethylamine) immediately after vaginal secretions are mixed with 10% solution of KOH (*Whiff test*)
4. **Clue cells:** They are vaginal epithelial cells coated with coccobacilli, which have a granular appearance and indistinct borders observed on a wet mount (Fig. 77.15).

Laboratory Diagnosis

- ❖ **Nugent's score:** It is a scoring system followed for the diagnosis of bacterial vaginosis; done by counting the number of *G. vaginalis*, *Mobiluncus* and lactobacilli present in the Gram stained smear of vaginal discharge. A score of more than or equal to 7 is diagnostic
- ❖ **Culture:** *G. vaginalis* requires enriched media such as chocolate agar, BHI broth with serum, etc.
 - It is gram-negative (appears gram-variable in smears), nonmotile, small pleomorphic rod, which shows metachromatic granules
 - It produces minute hemolytic colonies on blood agar, incubated aerobically under 5% CO₂ for 24–48 hours.



Fig. 77.15: Wet mount of vaginal secretion depicting clue cell.

Source: Public Health Image Library/ID#: 14574/ M. Rein /Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Identification** from colonies is made either by conventional biochemical tests or by automated identification systems such as MALDI-TOF or VITEK
- ❖ **Broad-range PCR** amplification of 16S rRNA in vaginal fluid can be performed, with subsequent identification of specific bacterial species by various methods.

TREATMENT

Bacterial vaginosis

Drug of choice is oral metronidazole, given twice daily for 7 days.

Vaginal Candidiasis

Candida albicans is the most common species to cause vaginal candidiasis (80% to 90% of cases), followed by *C. glabrata* and *C. tropicalis*.

- ❖ **Classical presentation:** Most patients present with perivaginal pruritus (itching), erythema and vaginal discharge—typically thick and “cheesy” in appearance with pH <4.5
- ❖ **Other symptoms** include vulvovaginal soreness, dyspareunia and dysuria
- ❖ **Risk factors** include pregnancy, hormone replacement therapy, steroid, diabetes or immunocompromised state
- ❖ **Laboratory diagnosis** include culture of vaginal secretions on Sabouraud dextrose agar (pasty or dry white colonies), followed by identification by conventional (e.g. germ tube test) or automated methods (VITEK or MALDI-TOF)
- ❖ **Treatment:** Primary treatment includes oral fluconazole or itraconazole (for 1 day). Topical cream of clotrimazole may be given in milder cases.
Candidiasis can present in various forms including systemic candidiasis. Detail is discussed in Chapter 38.

OTHER GENITAL TRACT INFECTIONS IN FEMALES

Mucopurulent Cervicitis

Mucopurulent cervicitis (MPC) refers to inflammation of the columnar epithelium of the endocervix.

- ❖ **Agents:** MPC is commonly caused by agents of urethritis such as *C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma genitalium*
- ❖ **Clinical diagnosis:** The three cardinal signs of MPC are—(1) yellow mucopurulent discharge from cervix, (2) endocervical bleeding upon gentle swabbing, and (3) edematous cervical ectopy; the latter two findings are more common in chlamydial infection. HSV cervicitis produces ulcerative lesions of ectocervix
- ❖ **Diagnosis:** Yellow cervical mucus on a white swab removed from the endocervix suggestive of the presence of pus cells
 - **Gram stain:** The presence of ≥ 20 pus cells per oil immersion field within strands of cervical mucus indicates endocervicitis
 - Intracellular gram-negative diplococci—may indicate gonorrhea, but is sensitive only 50% cases
 - **PCR** specific for *N. gonorrhoeae* or *C. trachomatis* is more useful.
- ❖ **Treatment:** Comprises of ceftriaxone (single dose IM) followed by doxycycline (for 10 days).

Pelvic Inflammatory Disease (PID)

PID refers to the infection that ascends from the cervix or vagina to involve the endometrium and/or fallopian tubes and sometimes can extend beyond the reproductive tract to involve peritoneum. PID can be either primary or secondary.

1. **Primary PID**, occurs spontaneously and usually sexually transmitted or
2. **Secondary PID**, occurs following invasive intrauterine procedures; e.g. dilatation and curettage or insertion of an intrauterine device.

Etiology

PID is most often caused by the agents causing cervicitis such as *N. gonorrhoeae* and *C. trachomatis*. Rare causes of PID include:

- ❖ Genital mycoplasmas such as *M. genitalium*
- ❖ Anaerobic (peptostreptococci) and facultative organisms (*Prevotella* species)
- ❖ *E. coli*, *Haemophilus influenzae*, and group B streptococci
- ❖ Secondary to hematogenous dissemination (e.g. tuberculosis or staphylococcal bacteremia).

Clinical Manifestations

Manifestations depend upon the site of infection.

- ❖ **Endometritis:** Can present as midline abdominal pain and abnormal vaginal bleeding

- ❖ **Salpingitis** (inflammation of the fallopian tube): Presents as bilateral lower abdominal and pelvic pain (dull or aching type of pain). On examination, reveals adnexal swelling and tenderness, and cervical motion tenderness. Other findings include fever, elevated ESR and WBC count
- ❖ **Oophoritis** (inflammation of ovary) and tubo-ovarian abscess
- ❖ **Extension to peritoneum** can cause peritonitis, perihepatitis, perisplenitis, or pelvic abscess

Fitz-Hugh–Curtis syndrome

It is a perihepatitis, seen secondary to gonococcal or chlamydial infection. It presents as right upper quadrant, edema and erythema of the liver capsule, progresses into formation of exudate with fibrinous adhesions between the liver and peritoneum

- ❖ **Late sequelae** include infertility (if tubes get occluded), ectopic pregnancy (if tubal scarring occurs without occlusion), chronic pelvic pain, and recurrent salpingitis
- ❖ **Prevention:** PID can largely be prevented by annual screening of sexually active women for genital chlamydial infection.

TREATMENT

Pelvic inflammatory disease

Women with suspected PID can be treated as either outpatients or inpatients. Hospitalization is necessary if associated with appendicitis, pregnancy, pelvic abscess or very sick patient.

- ❑ **Outpatient regimen:** Ceftriaxone (IM once) plus doxycycline (for 14 days) plus metronidazole (for 14 days)
- ❑ **Parenteral regimen:** In hospitalized patient, parenteral regimen is given for 48h or till clinical improvement, then switched over to outpatient regimen. There are two parenteral regimens
 - Cefotetan or cefoxitin plus doxycycline
 - Clindamycin plus gentamicin.

Surgery: It is indicated only in the presence of life-threatening infection (e.g. ruptured tubo-ovarian abscess) or for drainage of pelvic abscess.

Bartholinitis

Bartholin's gland abscess can result from infection of Bartholin gland and blockade of its duct.

- ❖ It is a mucus-producing gland present on each side of the vaginal orifice; opens through a duct on to the inner surface of the labia minora
- ❖ Anaerobic and polymicrobial infections originating from normal genital flora are more common cause although it can also be caused by *N. gonorrhoeae* and *C. trachomatis*.

Infections after Gynecologic Surgery

Postoperative infections such as pelvic cellulitis or abscesses are common following gynecologic surgery (e.g. vaginal hysterectomy). The common pathogens implicated include the normal vaginal flora organisms such as anaerobes (peptostreptococci), genital mycoplasmas, aerobic gram-positive cocci and gram-negative bacilli.

Infections in Pregnancy/Postpartum

Infections can also occur in women during pregnancy (prenatal), during birth (natal) or following the birth (postpartum) of a child.

- ❖ **Prenatal infections** may be acquired from:
 - **Hematogenous route** and then cross placenta to infect fetus or
 - **Ascending genital tract route** from the vagina through ruptured membranes resulting in **chorioamnionitis**. Maternal vaginal flora organisms are frequently isolated from chorioamnionitis such as anaerobic bacteria, genital mycoplasmas and group B streptococci.
- ❖ **Natal (during birth) infections:** Infections transmitted through the infected birth canal during delivery include—
 - **Bacteria:** Group B streptococci, *E. coli*, *Listeria monocytogenes*, *N. gonorrhoeae*, *C. trachomatis*
 - **Viruses:** CMV, HSV, enteroviruses, hepatitis B virus, HIV.
- ❖ **Postpartum infections:** Puerperal sepsis is common in mother during postpartum period. All the organisms listed under natal infection can also cause postpartum infection. In addition, various organisms from the nursery environment (e.g. multidrug resistant gram-negative bacilli) can cause postpartum infection. These infections during birth or postpartum period can be transmitted to the newborn to cause postnatal infections.

Certain organisms are teratogenic (e.g. rubella); infections during pregnancy can be transmitted to the fetus which can lead to various congenital manifestations (discussed in Chapter 79).

Group B Streptococcal Infection in Pregnancy

Streptococcus agalactiae is a commensal in maternal genital tract.

- ❖ Infection in pregnancy can lead to peripartum fever, endometritis and puerperal sepsis
- ❖ Transmission of organism to the neonate during birth can lead to neonatal sepsis and meningitis (Chapter 71)
- ❖ **Prevention:** Screening for anogenital colonization of GBS by rectal/vaginal swab culture is recommended at 35–37 weeks of pregnancy. Culture and identification is discussed in Chapter 52
- ❖ **Chemoprophylaxis** with penicillin is given to carrier mothers during delivery to reduce the risk of infection to the newborn.

GENITAL TRACT INFECTIONS IN MALES

Prostatitis

Prostatitis (inflammation of prostate gland) is caused by both infectious (bacterial agents) and noninfectious means. Bacterial prostatitis may present in acute or in chronic form.

Acute Bacterial Prostatitis

It is usually caused by *N. gonorrhoeae* and *C. trachomatis* in males of age <35 years. However, in males of >35 years, the common agents are Enterobacteriaceae and *Enterococcus*.

- ❖ **Manifestations:** It typically presents with fever, chills, malaise, myalgia, dysuria, pelvic/perineal pain and cloudy urine
- ❖ **Complications** may develop such as bacteremia, epididymitis, prostatic abscess, extension to joints, or rarely proceeds to chronic prostatitis
- ❖ **Treatment:** Ceftriaxone (IM, single dose), followed by doxycycline (for 10 days).

Chronic Prostatitis

It is usually caused by agents of lower UTI such as Enterobacteriaceae (80%) and *Enterococcus* (15%), occasionally by *Pseudomonas*.

- ❖ **Manifestations:** Presents with low grade fever, urinary frequency, dysuria, urgency and perineal discomfort. It is an important cause of persistent bacteriuria that leads to recurrent UTI
- ❖ **Treatment:** Ciprofloxacin or levofloxacin is given for 4 weeks.

Epididymitis

Acute epididymitis presents with pain, swelling, and inflammation of the epididymis that lasts <6 weeks.

- ❖ **Young men:** It is caused most frequently by *C. trachomatis* and less commonly by *N. gonorrhoeae*; usually associated with urethritis
- ❖ **In older men,** it may be seen following urinary tract instrumentation and is usually caused by urinary pathogens
- ❖ **In homosexual males:** Epididymitis following insertive rectal intercourse is often caused by Enterobacteriaceae. These men usually have bacteriuria, but no urethritis.

TREATMENT

Epididymitis

- ❑ Ceftriaxone (single dose IM) followed by doxycycline (for 10 days) is the regimen given for epididymitis caused by *N. gonorrhoeae* or *C. trachomatis*.
- ❑ Oral levofloxacin is given when infection with Enterobacteriaceae is suspected.

Orchitis

Orchitis (inflammation of the testicles) is uncommon and generally acquired by the blood-borne dissemination of viruses. **Mumps** is the etiological agent in most cases. Patients exhibit testicular pain and swelling following infection. Infection ranges from mild to severe. Mumps orchitis is usually unilateral and therefore infertility following mumps orchitis is very rare.

EXPECTED QUESTIONS

I. Write essay on:

1. A 27-year-old woman had developed mucopurulent discharge, followed by development of dysuria and urethral irritation. She had a history of multiple sexual partners. Microscopy of the urethral swab revealed sterile pyuria and presence of compact inclusion bodies which are later stained by Lugol's iodine.
 - a. What is the most probable etiological diagnosis?
 - b. What are the other manifestations produced by the causative agent?
 - c. How is this infection diagnosed in the laboratory?

II. Write short notes on:

1. A 23-year-old male having a history of sexual exposure with a commercial sex worker is presented to a STD clinic with painless hard indurated genital ulcer and painless hard lymph node. What is the clinical diagnosis? Discuss the laboratory diagnosis.
2. A 25-year-old heterosexual male with history of dysuria and noted some 'pus like' drainage in his underwear and at the tip of his penis. Gram staining of yellow urethral discharge revealed pus cells with gram-negative diplococci. What is the probable clinical diagnosis? Discuss briefly the laboratory diagnosis.
3. Lymphogranuloma venereum.
4. Nongonococcal urethritis.

III. Multiple Choice Questions (MCQs):

1. Lugol's iodine is used to stain the inclusion body of:

- a. *Chlamydia trachomatis*
- b. *Chlamydomydia psittaci*
- c. *Chlamydomydia pneumoniae*
- d. All of the above

2. Gonococcal infection in females, all are true, except:

- a. Less severe than male
- b. Mucopurulent cervicitis is the most common presentation in females
- c. Vulvovaginitis is seen frequently
- d. Fitz-Hugh-Curtis syndrome seen

3. The most commonly used method for isolation of *Chlamydia*:

- a. Culture on artificial media
- b. Culture on Vero cell line
- c. Inoculation into guinea pig
- d. Culture on McCoy cell line

4. The most sensitive and specific test for *Chlamydia* diagnosis:

- a. Direct immunofluorescence test (DIF)
- b. Culture on McCoy cell line
- c. Nucleic acid amplification tests (NAAT)
- d. Microimmunofluorescence (MIF) test

5. Wrong about Bacterial vaginosis is:

- a. Discharge has offensive smell
- b. pH >4.5
- c. Causative agent is *Chlamydia trachomatis*
- d. Clue cell is diagnostic

Answers

1. a 2. c 3. d 4. c 5. c

Miscellaneous Infective Syndromes

SECTION

11

SECTION OUTLINE

78. Ocular and Ear Infections

79. Congenital Infections

Cytomegalovirus Infections, Congenital Varicella, Neonatal Herpes, Congenital Rubella, Congenital Toxoplasmosis, Congenital Syphilis, Zika Virus Infections and Others

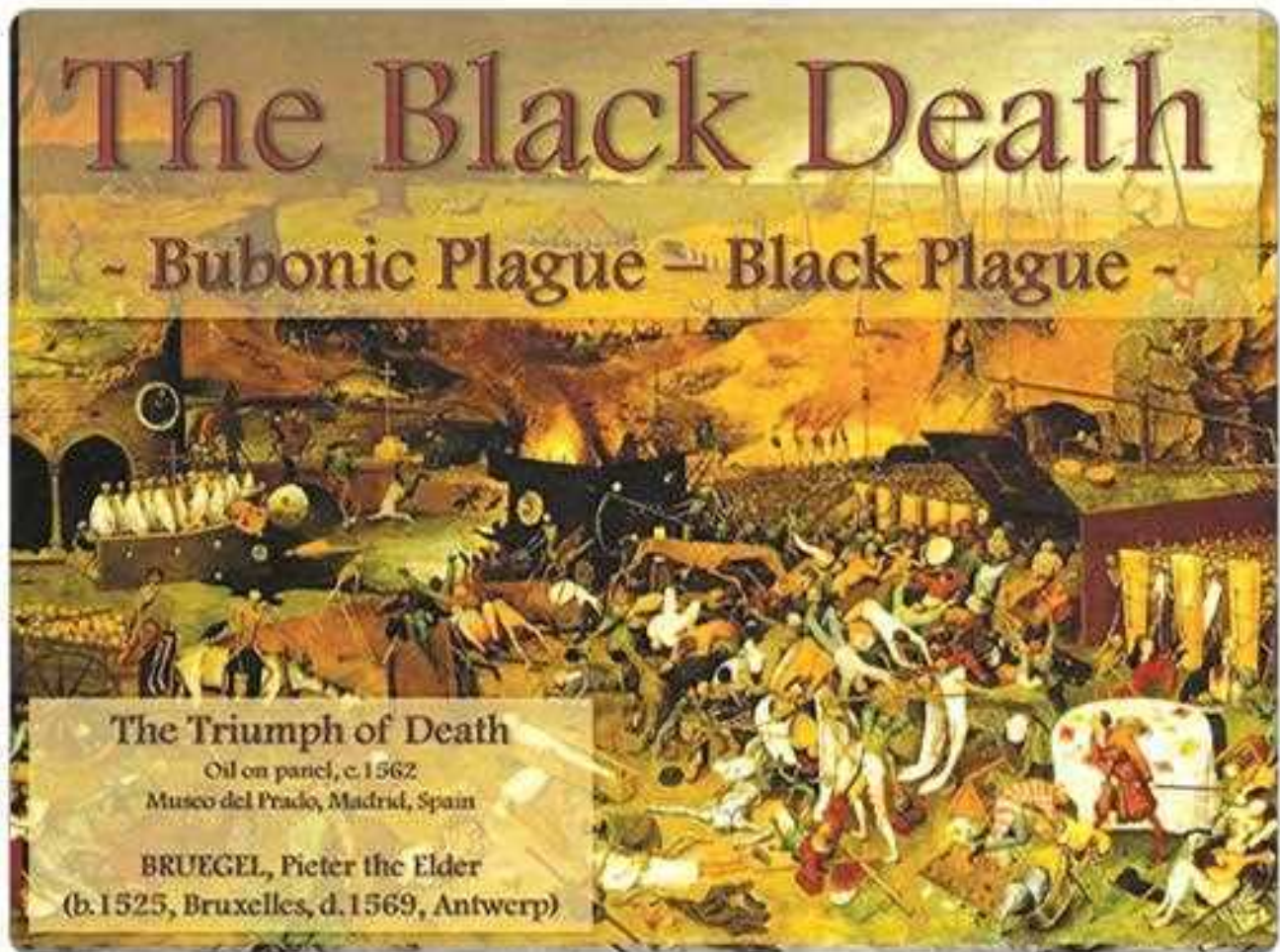
80. Organisms with Oncogenic Potential

Human Papillomavirus, Kaposi Sarcoma, HTLV and HIV, Epstein-Barr Virus, Hepatitis B and C, and Others

81. Zoonotic Infections: Plague, Tularaemia and Bite Wound Infections



The Largest Pandemic of the World



- Occurred in 14th century
- Mainly in Europe, Asia and North Africa
- Killed over 50 million lives
- Caused by *Yersinia pestis* biotype 'Antiqua'

CHAPTER PREVIEW

- Ocular Infections
 - Ocular Bacterial Infections
 - Ocular Viral Infections
- Ocular Parasitic Infections
- Ocular Fungal Infections
- Infections of Ear
- Otitis Externa
- Otitis Media
- Chronic Suppurative Otitis Media

OCULAR INFECTIONS

Ocular infective syndromes include infection of various parts of eyes such as conjunctiva, cornea, uvea, retina, eyelid, lacrimal gland and orbit (Fig. 78.1).

- ❖ **Infective conjunctivitis:** Infection of the conjunctiva is the most common ocular infection. It should be differentiated from non-infectious etiologies such as allergic conjunctivitis
- ❖ **Infective keratitis:** Infection of the cornea leads to formation of corneal ulcers
- ❖ **Uveitis:** It is a rare ocular infection, refers to inflammation of the uveal tissue which includes:
 - Iris and ciliary body anteriorly (leading to iridocyclitis)
 - Choroid posteriorly (choroiditis)
 - Most often choroid is co-infected with retina—a condition called chorioretinitis.
- ❖ **Endophthalmitis:** It refers to a purulent inflammation of the intraocular fluids (vitreous and aqueous)
- ❖ **Retinitis:** Infection of the retina is most often presented with choroiditis

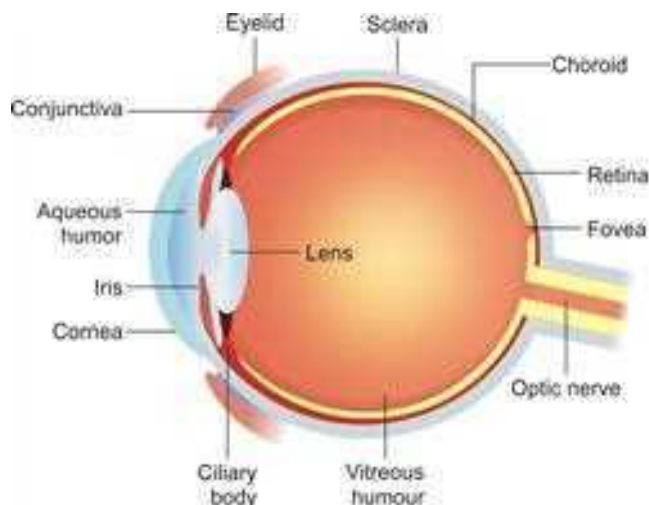


Fig. 78.1: Different anatomical parts of eye.

- ❖ **Infection of eyelid:** Infection of eyelid margin is called as *blepharitis*, caused by bacteria. Other eyelid infections include hordeolum (external and internal hordeolum)
- ❖ **Infections of lacrimal gland:** Includes *dacryocystitis* (infection of the lacrimal sac) and *dacryoadenitis* (infection of lacrimal glands)
- ❖ **Infections of orbit:** Includes *orbital cellulitis*; caused by either bacteria or fungi.

Ocular infections are caused by a wide range of organisms including bacteria, virus, parasites and fungi.

OCULAR BACTERIAL INFECTIONS

Bacterial infections of the eyes include conjunctivitis, keratitis, uveitis, endophthalmitis, retinitis, infections of eyelid, lacrimal gland and orbit.

Bacterial Conjunctivitis

Bacterial conjunctivitis, one of the commonest ocular infections can present in an acute form (most common) or rarely in a hyperacute or chronic form (Fig. 78.2A).

- ❖ The disease is highly infectious, can occur as both *sporadic* and *epidemic*; the latter is frequent during monsoon season
- ❖ **Predisposing factors** which help in establishing the infection include poor hygienic conditions, hot dry climate, poor sanitation and dirty habits.



Figs 78.2A and B: A. Acute bacterial conjunctivitis; B. Angular conjunctivitis.

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.63(A), p.66(B).

1. Acute Bacterial Conjunctivitis (ABC)

Acute bacterial conjunctivitis (ABC) is caused by a wide range of bacteria.

- ❖ *Staphylococcus aureus*: It is the most common cause of bacterial conjunctivitis
- ❖ *Streptococcus pneumoniae*: Conjunctivitis produced by pneumococcus is usually associated with petechial subconjunctival hemorrhages
- ❖ Other gram-positive cocci such as *Streptococcus pyogenes* and *Staphylococcus epidermidis*
- ❖ *Haemophilus aegyptius*: It can cause epidemics of mucopurulent conjunctivitis

Haemophilus aegyptius Infections

It is also called Koch-Weeks bacillus; closely resembles *H. influenzae* biotype III. However, it differs from the latter in having more predilection for conjunctiva and not occurring as a pharyngeal carrier. *Haemophilus aegyptius* causes:

- ❑ **Red-eye** (Egyptian ophthalmia): It classically causes epidemics of mucopurulent conjunctivitis, especially in semitropical countries
- ❑ **Brazilian purpuric fever**: A fulminant condition, characterized by fever, purpura, hypotension and shock

It requires both factors X and V, similar to *H. influenzae*, but can be differentiated from the latter by certain biochemical tests.

- ❖ *Moraxella lacunata* (*Moraxella*-Axenfeld bacillus): It is the most common cause of **angular conjunctivitis**; characterized by chronic mild grade inflammation of the lateral angle of the conjunctiva and nearby lid margins (Fig. 78.2B)
- ❖ *Pseudomonas aeruginosa*: It is a virulent organism, which readily invades the cornea
- ❖ *Neisseria gonorrhoeae*: It typically produces an acute purulent conjunctivitis—called **hyperacute bacterial conjunctivitis** (described later in this chapter)
- ❖ *Neisseria meningitidis*: It may produce mucopurulent conjunctivitis
- ❖ *Corynebacterium diphtheriae*: It rarely causes conjunctivitis, called acute pseudomembranous conjunctivitis.

Clinical Manifestations

Acute bacterial conjunctivitis is usually bilateral, although one eye may become affected 1–2 days before the other (Fig. 78.2A).

Symptoms: Common symptoms include:

- ❖ Mucopurulent discharge from the eyes
- ❖ Discomfort, foreign body sensation
- ❖ Redness of sudden onset (due to engorgement of vessels)
- ❖ Mild photophobia, i.e., difficulty to tolerate light
- ❖ Sticking together of lid margins with discharge—occurs during sleep
- ❖ Slight blurring of vision may occur due to presence of mucous flakes in front of cornea.

Signs: On examination of eyes, the following findings are noticed—conjunctival congestion, matted eye lashes, flakes of mucus discharge in eyes, swollen conjunctiva and eyelid, and petechial hemorrhages (in pneumococcus).

Laboratory Diagnosis

Swabs premoistened with sterile saline are used to collect conjunctival samples from both the eyes and sent to laboratory within 2 hr. The specimen is subjected to gram-staining, followed by culture on blood agar, chocolate agar and MacConkey agar.

TREATMENT

Acute bacterial conjunctivitis

Topical broad spectrum antibiotics are the treatment of choice; given as eye drops 3–4 hourly in a day and ointment is used at night.

- ❑ Topical gentamicin or tobramycin or quinolones such as ciprofloxacin, moxifloxacin are the preferred agents
- ❑ Irrigation of conjunctival sac with sterile warm saline once or twice a day will help by removing the deleterious material
- ❑ No bandage should be applied to the eyes
- ❑ No steroids should be applied.

Prevention

Preventive measures to reduce risk of transmission to the close contacts include—(1) frequent hand washing, (2) avoid eye rubbing, (3) avoid sharing towel, handkerchief and pillow with others, (4) disinfection of ophthalmic instruments such as tonometer should be frequently performed as they may serve as source of infection.

2. Chronic Bacterial Conjunctivitis

It is characterized by mild catarrhal inflammation of the conjunctiva, which may last for months.

- ❖ **Predisposing factors:** It occurs only in presence of predisposing factors such as chronic exposure to dust, smoke, alcohol and chemical irritants, eye strain (due to refractive errors), or any other local cause of irritation
- ❖ **Agents:** *S. aureus* is the commonest cause. Gram-negative bacilli such as *Proteus mirabilis*, *Klebsiella* and *E. coli* are other rare causes.

3. Hyperacute Bacterial Conjunctivitis

It is caused by *Neisseria gonorrhoeae*, which principally causes urethritis (Chapter 77). Gonococcal infection of eye occurs in two forms: acute purulent conjunctivitis in adults and ophthalmia neonatorum in newborn.

- ❖ **Acute purulent conjunctivitis:** Transmitted by oculo-genital contact, either by direct or indirect contact through fingers
 - **Presentation** is more severe than ABC, characterized by—(1) purulent secretion in eyes, (2) capable of invading intact corneal epithelium; can produce corneal ulcers or even perforation (Fig. 78.3A)
 - **Treatment:** Systemic antibiotic such as IV ceftriaxone (single dose) is given. Oral doxycycline for one week



Figs 78.3A and B: Hyperacute conjunctivitis: **A.** Acute purulent conjunctivitis; **B.** Gonococcal ophthalmic neonatorum.

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.65(A), p.78(B).

or azithromycin (single dose) is added to the regimen to cover co-existing chlamydial infection. In addition, topical eye drops (e.g. ciprofloxacin) should be given more frequently than for ABC.

- ❖ **Ophthalmia neonatorum (ON):** The infection occurs due to transmission of gonococci to the newborn from infected mother's birth canal
 - Gonococcal ON is characterized by an acute purulent discharge, and crusted lesions within 12-48 h of birth (Fig. 78.3B)
 - Treatment comprises of topical bacitracin 4 times a day plus IV ceftriaxone single dose, given within 7 days. Topical penicillin therapy is not reliable and should be avoided because of emergence of resistance.

Note: Gonococcal ON must be differentiated from chlamydial ON (described subsequently) as both require different treatment regimen.

4. Chlamydial Conjunctivitis

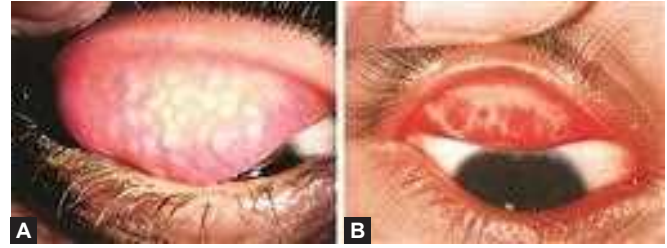
Chlamydia trachomatis principally causes genital tract infections, discussed in Chapter 77. It comprises of several serotypes, which can cause a wide range of ocular infections.

- ❖ *C. trachomatis* serotypes A, B, Ba and C: Cause a follicular conjunctivitis, called trachoma
- ❖ *C. trachomatis* serotypes D to K: Cause inclusion conjunctivitis affecting both adults and newborn.

Trachoma

Trachoma is a chronic keratoconjunctivitis, caused by *C. trachomatis* serovars A, B, Ba and C.

- ❖ **Mode of transmission:** Trachoma is transmitted through direct contact (fingers and fomites) with discharges from the eyes of the infected patients or indirect contact through contaminated clothes or flies
- ❖ **Age:** Infection is acquired by 2-3 years of age and active disease is common in preschool children, aged 3-5 years
- ❖ **Acute infection:** It presents as follicular conjunctivitis (inflammation of conjunctival lymphoid follicles) and papillary hyperplasia (Fig. 78.4A)
- ❖ **Late stage:** Recurrent infection leads to conjunctival scarring or cicatrization (Fig. 78.4B), concretions (whitish



Figs 78.4A and B: Trachoma: **A.** Follicular conjunctivitis; **B.** Conjunctival scarring.

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.69(A), p.70(B).

deposits), trichiasis (eyelashes falling on eyeball) and corneal ulcers progressing to opacity and blindness

- ❖ **Epidemiology:** Trachoma is the leading infectious cause of blindness (mainly in developing nations) and is responsible for the blindness or visual impairment of about 1.9 million people worldwide
- ❖ **Worldwide,** the hyperendemic areas of trachoma include sub-Saharan Africa, Middle East, and Southeast Asia including India
- ❖ **Prevention:** WHO has initiated an action plan called **SAFE strategy**, aiming towards global elimination of trachoma, and prevention of blindness. It has four components
 - **Surgery:** Needed in advanced stage to prevent corneal blindness
 - **Antibiotic (azithromycin single dose):** Mass administration is given; the target population chosen depends upon the prevalence of trachoma in children in the area
 - ◆ Given to all community: in areas with prevalence of trachoma >10%
 - ◆ Given only to family members and close contacts: if prevalence is 5-10%.
 - **Facial hygiene** such as frequent face wash and avoidance of use of common towel, handkerchief
 - **Environmental sanitation** to reduce transmission.

TREATMENT

Trachoma

Treatment of trachoma comprises of topical therapy with/without systemic therapy.

- ❑ **Topical therapy** such as tetracycline or erythromycin eye ointment, given 4 times a day for 6 weeks
- ❑ **Systemic therapy:** Azithromycin is the drug of choice, given for 4 days. Alternatives include tetracycline or doxycycline. Systemic antibiotic is indicated if the ocular infection is severe or when there is associated genital infection.

Inclusion Conjunctivitis

C. trachomatis serovars D-K cause two important ocular infections.

1. **Ophthalmia neonatorum** (or inclusion blennorrhoea): It occurs in the newborn
 - *C. trachomatis* is a more common cause of ophthalmia neonatorum than gonococcus

- Incubation period is longer (6–21 days) and discharge is mucopurulent compared to gonococcal infection
- **Treatment:** Comprises of topical ointment (erythromycin or tetracycline) plus oral erythromycin given for 21 days.

Prevention of Ophthalmia Neonatorum

The incidence of ophthalmia neonatorum (both gonococcal and chlamydial) has been reduced dramatically after institution of appropriate preventive measures during delivery.

- ❑ **Antenatal measures** such as treatment of genital infections when suspected
- ❑ **Measures during birth** include: Conducting deliveries under aseptic conditions and thorough cleaning of the newborn baby's closed lids
- ❑ **Measures after birth:** Use of topical ointments (erythromycin or tetracycline) into the eyes of the babies immediately after birth

2. **Adult inclusion conjunctivitis:** It is an acute follicular conjunctivitis that may occur in adults following swimming—called *swimming pool conjunctivitis*. General laboratory diagnosis of chlamydial infections including ocular infections is discussed in Chapter 77.

5. Granulomatous Bacterial Conjunctivitis

Granulomatous inflammations of the conjunctiva usually tend to remain localized to one eye and are associated with regional lymphadenitis. Common diseases where granulomatous conjunctivitis may be seen include tuberculosis, syphilis, leprosy and tularemia.

Bacterial Keratitis

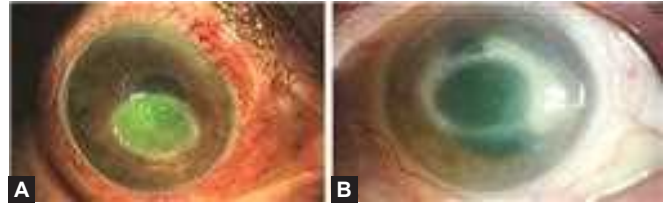
Bacterial keratitis is the most common form keratitis (corneal ulcer), although it may also be caused by viruses, parasites and fungi.

- ❖ **Etiology:** Common bacteria associated with corneal ulceration include *S. aureus*, *Pseudomonas*, *Streptococcus pneumoniae*, Enterobacteriaceae and *Neisseria*
- ❖ **Transmission:** Infection spreads to cornea either—(1) endogenously from underlying infection of adjacent structures—conjunctiva, eyelid or lacrimal gland; or (2) exogenously from water or airborne infection
- ❖ **Invasion:** The bacteria may invade corneal epithelium, leading to formation of purulent corneal ulcer. Invasion usually occurs through abraded cornea, except for *Neisseria* and *C. diphtheriae*, which can invade the intact corneal epithelium.

Clinical Features

Broadly bacterial corneal ulcers may manifest as:

- ❖ **Purulent corneal ulcer:** Present with eye pain, foreign body sensation, watering, photophobia, blurred vision, redness of eyes. Corneal ulcers are observed on examination as yellowish-white lesions, oval or irregular in shape (Fig. 78.5A)



Figs 78.5A and B: Corneal ulcer: **A.** Oval-shaped; **B.** Ring.

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.105.

- *Pseudomonas*: Produce an irregular sharp ulcer with thick greenish mucopurulent discharge, spreads rapidly and may cause perforation
- Enterobacteriaceae (*E. coli*) usually produce a shallow ulcer with greyish white suppuration and ring-shaped corneal infiltrate (Fig. 78.5B).
- ❖ **Hypopyon corneal ulcer:** Hypopyon (presence of pus in anterior chamber) is a typical feature of pneumococcal keratitis; although it may also be associated in other bacterial corneal ulcers.

Laboratory Diagnosis of Keratitis

Laboratory diagnosis of keratitis is necessary to identify causative organism (bacteria, viruses, fungi or parasites); thereby guiding appropriate treatment.

- ❖ **Corneal scraping:** Material from corneal ulcers may be obtained by scraping the base and margins of the ulcer (under local anesthesia) with the help of a spatula or bent tip of a hypodermic needle
- ❖ **Bedside inoculation:** The specimen should be processed immediately (within 15 min) for culture; therefore, bedside inoculation is recommended. Blood agar, chocolate agar and MacConkey agar are preferred for bacterial keratitis. Sabouraud dextrose agar is used if fungal keratitis is suspected
- ❖ **Direct microscopy:** A part of the specimen is also sent to the laboratory for direct microscopy
 - Gram staining and Giemsa staining for direct identification of infecting organisms
 - 10% KOH wet preparation for identification of fungal hyphae
 - Calcofluor white stain preparation is viewed under fluorescence microscope for fungal filaments, the walls of which appear bright apple green
 - Saline mount examination is performed for detection of any parasite, e.g. *Acanthamoeba* cyst.
- ❖ **Molecular tests:** Useful for direct detection of the nucleic acid of the causative agents (e.g. agents of viral keratitis).

TREATMENT

Bacterial keratitis

Topical antibiotics

Combination therapy is given with both gram-negative and gram-positive coverage.

- ❑ Cefazolin, plus tobramycin eye drops or
- ❑ Vancomycin and quinolones (ciprofloxacin, moxifloxacin) eye drops

Contd...

Contd...

TREATMENT**Bacterial keratitis**

Frequency of instillation: The chosen two drugs should be instilled alternately—every 15 minutes for 2 hours, then hourly for first 48 hours, 2 hourly till healing occurs.

Systemic antibiotics

Usually not required, except for fulminant cases (e.g. corneal perforation or sclera involvement). Here, cephalosporin and an aminoglycoside or oral ciprofloxacin is the primary regimen of choice.

Nonspecific treatment

- ❑ **Cycloplegic drug:** 1% atropine eye drops should be used to reduce pain from ciliary spasm
- ❑ **Analgesics** and anti-inflammatory drugs such as paracetamol can be given to reduce pain and edema
- ❑ **Others:** Hot fomentation, dark goggles (to reduce photophobia), rest and fresh air.

Bacterial Uveitis

Bacterial uveitis is relatively less common, which include the following:

- ❖ **Granulomatous uveitis:** For example, tubercular, leprotic, syphilitic, and brucellosis
 - **Tubercular uveitis:** It can present as both anterior uveitis (iritidocyclitis) or posterior uveitis causing choroiditis (characterized by multiple miliary tubercles on choroid)
 - **Leprotic uveitis:** Leprosy is caused by *Mycobacterium leprae*. Leprosy in eyes predominantly involves anterior uvea causing iridocyclitis. It is seen more commonly in lepromatous than in the tuberculoid form of disease
 - **Syphilitic uveitis:** It is a sexually transmitted disease caused by *Treponema pallidum* (Chapter 77). Ocular involvement is rare and may occur as acute plastic iritis, gummatous anterior uveitis and diffuse choroiditis.
- ❖ **Pyogenic uveitis:** Can be caused by streptococci, staphylococci, pneumococci and gonococcus
- ❖ **Rickettsial uveitis** may occur in scrub typhus and epidemic typhus.

Uveitis is more commonly caused by viruses and rarely by parasites such as *Toxoplasma* and fungi (discussed later in this chapter).

Endophthalmitis

Endophthalmitis is defined as an inflammation of the inner structures of the eyeball, i.e. uveal tissue and retina associated with pouring of exudates in the vitreous cavity, anterior chamber and posterior chamber. Panophthalmitis is an intense purulent inflammation of the whole eyeball.

Causative Organisms

Bacterial endophthalmitis: The most frequent pathogens are gram-positive cocci, i.e. *Staphylococcus epidermidis* (most common, 60%) and *S. aureus* (5-10%). Other causative

bacteria include streptococci, *Enterococcus*, *Pseudomonas*, pneumococci and *Corynebacterium*. *Propionibacterium acnes* and *Actinomyces* are gram-positive organisms capable of producing slow grade endophthalmitis.

Fungal endophthalmitis is relatively rare. It is caused by *Aspergillus*, *Fusarium*, *Candida*, etc.

Clinical Feature

Endophthalmitis most commonly occurs as a catastrophic complication of **intraocular surgery**, or secondary to intraocular **trauma**.

- ❖ The source of the infection in most of the cases is—patient's own bacterial flora present in the eyelids, conjunctiva, and lacrimal sac. Less common source of infection includes contaminated solutions and instruments, environmental flora and skin flora of the operating team
- ❖ **Onset of illness** may be—**acute** (occurs between 1–7 days of operation, most common type) or **delayed** (occurs a week to a month after surgery; fungi are the most common cause, followed by *Propionibacterium acnes*)
- ❖ **Symptoms:** Characterized by severe ocular pain, redness, lacrimation, photophobia and loss of vision
- ❖ **Signs:** Common signs include swollen lids, conjunctival congestion, corneal edema, hypopyon in anterior chamber and vitreous exudate.

TREATMENT**Endophthalmitis**

An early diagnosis and vigorous therapy is the hallmark of the treatment of endophthalmitis.

Antibiotic therapy

- ❑ **Intravitreal antibiotics** should be made as early as possible
Regimen: Vancomycin plus ceftazidime or amikacin is the empiric regimen of choice; provide both gram-positive and gram-negative coverage
- ❑ **Topical concentrated antibiotics** should be started immediately and used frequently (every 30 minute to 1 hourly)
Regimen: Vancomycin or ceftazolin plus amikacin or tobramycin
- ❑ **Systemic antibiotics** have limited role in endophthalmitis. The options available are:
 - IV ciprofloxacin
 - IV vancomycin plus ceftazidime
 - IV ceftazolin plus amikacin.

Steroid therapy

It aims at reducing inflammation. Intravitreal injection of dexamethasone is recommended, along with topical and systemic steroid therapy.

Evisceration

It refers to surgical removal of the contents of the eyeball leaving behind the sclera. It is indicated in panophthalmitis.

Infections of Eyelids**Bacterial Blepharitis**

Bacterial blepharitis is a subacute or chronic inflammation of the lid margins, most often caused by *Staphylococcus aureus*. It is an extremely common disease of eye. Clinical manifestations include yellow crusts seen at eyes lashes, red,

thickened lid margins, chronic irritation, mild lacrimation and mild photophobia.

TREATMENT

Blepharitis

Treatment of blepharitis consists of lid hygiene and antibiotics.

- ❑ **Lid hygiene** is essential and should include: warm compresses for 5–10 minutes to soften the crust, followed by crust removal and lid margin cleaning. Rubbing of the eyes or the lids should be avoided
- ❑ **Topical antibiotics:** Antibiotic ointment should be applied at the lid margin, immediately after removal of the crusts
- ❑ Topical steroid and ocular lubricants are useful.

Hordeolum

It is an acute focal infection of eyelid (lash follicles), usually caused by *Staphylococcus aureus*.

- ❖ **Risk factor:** It usually occurs among children and young adults with habitual rubbing of the eyes
- ❖ **Two types:** It is an acute suppurative inflammation of lash follicles and its associated glands
 1. External hordeolum or stye— inflammation of glands of Zeis (Fig. 78.6A)
 2. Internal hordeolum— inflammation of Meibomian gland (Fig. 78.6B).
- ❖ **Symptoms** include acute pain associated with swelling of lid, mild watering and photophobia
- ❖ **Treatment:** Includes hot compresses 2–3 times a day, evacuation of the pus or surgical incision, antibiotic eye drops (3–4 times a day) and systemic analgesics to reduce pain and edema.

Molluscum Contagiosum of Eyelid

It is a poxvirus, that can produce multiple, pearly white wart-like umbilicated lesions over eyelid (Fig. 78.7A) and other body surfaces (Chapter 56).

- ❖ **Complications** such as chronic follicular conjunctivitis and superficial keratitis may occur
- ❖ **Treatment:** The skin lesions are incised and the interior is cauterized with application of tincture of iodine.

Infections of Lacrimal Apparatus

It includes infections of lacrimal sac (dacryocystitis) and lacrimal gland (dacryoadenitis).



Figs 78.6A and B: Hordeolum: **A.** External hordeolum (stye) (upper eyelid); **B.** Internal hordeolum (lower eyelid).

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.383(A), p.385(B).

Dacryocystitis

Dacryocystitis in adults may occur in an acute or a chronic form.

Acute Dacryocystitis

Acute dacryocystitis is an acute suppurative inflammation of the lacrimal sac, characterized by the presence of a painful swelling in the region of lacrimal sac (Fig. 78.7B).

- ❖ **Etiology:** The common causative organisms involved are *Streptococcus pyogenes*, pneumococcus and *Staphylococcus* species
- ❖ **Clinical features:** Can be divided into 3 stages:
 1. Lacrimal cellulitis: Characterized by painful swelling of lacrimal sac
 2. Lacrimal abscess: Sac is filled with pus
 3. Fistula formation: Discharges the pus externally.
- ❖ **Treatment:** It consists of systemic and topical antibiotics and drainage of the pus with a small incision. Systemic anti-inflammatory analgesics are given to reduce pain and inflammation.

Chronic Dacryocystitis

Chronic dacryocystitis is more common than the acute dacryocystitis.

- ❖ The etiology is multifactorial, causing stasis and mild infection of long duration. Disease is more common in females (80%)
- ❖ The organisms implicated are same for acute dacryocystitis. Rarely, it can also be caused by chronic granulomatous infections like tuberculosis, syphilis, leprosy and occasionally rhinosporidiosis.

Dacryoadenitis

Dacryoadenitis refers to inflammation of the lacrimal gland.

- ❖ **Etiology:** It usually develops secondary to some local or systemic infection
 - **Local infections** such as trauma induced, erysipelas of the face, conjunctivitis (especially gonococcal and staphylococcal) and orbital cellulitis
 - Systemic infections such as mumps, influenza, infectious mononucleosis and measles.
- ❖ **Clinical features:** It is characterized by red painful swelling in the upper lid over the gland area.



Figs 78.7A and B: **A.** Molluscum contagiosum of the lids; **B.** Acute dacryocystitis with lacrimal abscess.

Courtesy: Dr AK Khurana. Comprehensive Ophthalmology, 7th Edn, New Delhi, Jaypee Brothers Medical Publishers, 2019. p.385(A), p.413(B).

Complications can develop such as painful proptosis of eyeball and fistula formation in upper lid

- ❖ **Treatment:** It consists of a course of appropriate systemic antibiotic, anti-inflammatory drugs along with hot fomentation. When pus is formed, incision and drainage should be carried out.

Infections of Orbit (Orbital Cellulitis)

Orbital cellulitis refers to an acute infection of the soft tissues of the orbit behind the orbital septum. It may progress to cause orbital abscess. *Periorbital* (or *preseptal*) *cellulitis* refers to the infection of the subcutaneous tissues present anterior to the orbital septum.

- ❖ **Etiology:** Causative organisms are usually *S. aureus*, *S. pyogenes*, *S. pneumoniae* and occasionally *Haemophilus influenzae*. Orbital cellulitis due to fungi (mucormycosis) is discussed later
- ❖ **Clinical features:** Swelling of lids and severe eye pain (which is increased by movements of the eyeball or pressure) are the main symptoms. Loss of vision may occur in later stage.

TREATMENT

Orbital cellulitis

Orbital cellulitis is an emergency; the patient should be hospitalized for aggressive management.

- ❑ Intensive antibiotic therapy should be initiated to overcome the infection
- ❑ Surgical intervention is indicated in case of unresponsiveness to antibiotics, decreasing vision and presence of an orbital abscess.

OCULAR VIRAL INFECTIONS

Viruses can cause wide range of ocular infections such as keratoconjunctivitis, uveitis (chorioretinitis) and retinitis.

Viral Keratoconjunctivitis

Most of the viruses affecting conjunctival epithelium also infect cornea and vice-versa, to produce keratoconjunctivitis, as a common presentation. However, they vary in their extent of involvement. In some viral infections, conjunctival involvement is more prominent (e.g. adenovirus); while in others, cornea is more involved (e.g. herpes simplex virus) (Table 78.1).

1. Herpes Keratoconjunctivitis

The disease is commonly caused by herpes simplex virus (HSV)-1 and spreads by kissing or other close personal contacts. HSV-2 is associated with genital infections, may also involve the eyes in adults, through oculogenital contact.

- ❖ **Conjunctivitis:** It presents as follicular conjunctivitis, associated with other herpes lesions such as vesicular lesions on face and eyelids (Fig. 56.2B). Preauricular lymphadenopathy is a usual co-manifestation
- ❖ **Keratitis:** Corneal involvement can lead to formation of corneal ulcers, which may be of various patterns

Table 78.1: Viral infections of eye.

Viral infections of conjunctiva and cornea

Acute follicular conjunctivitis

- Herpes simplex keratoconjunctivitis
- Varicella-zoster keratoconjunctivitis
- Adenovirus conjunctivitis
- Molluscum contagiosum conjunctivitis
- Myxovirus conjunctivitis: Following influenza, parainfluenza, measles and Newcastle disease infections
- Corona virus conjunctivitis including COVID-19

Acute hemorrhagic conjunctivitis

- Enterovirus 70 and Coxsackie virus A 24

Viruses causing posterior eye infections such as iridocyclitis, chorioretinitis and acute retinal necrosis

- Herpes simplex virus
- Varicella-zoster
- Cytomegalovirus

- **Epithelial keratitis:** Such as epithelial punctate keratitis, dendritic ulcer (irregular, zigzag linear branching shaped) and geographical ulcer
- **Stromal keratitis:** Involvement of corneal stroma.

- ❖ **Uveitis:** Presents as non-granulomatous iridocyclitis.

TREATMENT

Herpes keratoconjunctivitis

Herpes conjunctivitis

It is usually self-limiting. Topical antiviral drugs can be given to prevent recurrences.

Herpes keratitis

- ❑ **Topical antiviral drugs** are the first choice presently—acyclovir ointment, ganciclovir gel or trifluridine drops; given for 5 days
- ❑ **Systemic antiviral drugs** such as acyclovir, for a period of 10 to 21 days are increasingly being considered for recurrent and even acute cases
- ❑ **Mechanical debridement** of the involved area of cornea along with a rim of surrounding healthy epithelium, helps by removing the virus-laden cells. It may be reserved for resistant cases

2. Zoster Ophthalmicus

Zoster ophthalmicus is an acute infection of gasserian ganglion of the fifth cranial nerve by the varicella-zoster virus (VZV). It constitutes approximately 10% of all cases of zoster. It occurs more commonly in immunocompromised individuals.

The disease is characterized by sudden onset of—(1) severe neuralgic pain along the course of the affected nerve, (2) unilateral crops of painful skin rashes surrounding the eyes, (3) ocular manifestations, which are as follows:

- ❖ **Mucopurulent conjunctivitis**
- ❖ **Zoster keratitis:** Occurs in 40% of all patients. It may occur in several forms such as epithelial keratitis, nummular keratitis and disciform keratitis
- ❖ **Other ocular lesions** such as scleritis, iridocyclitis, acute retinal necrosis, secondary glaucoma and PORN

❖ **Progressive outer retinal necrosis (PORN):** It is a very rare devastating necrotizing retinitis caused by a VZV infection in usually HIV infected patients with CD4 T cell counts $<50/\mu\text{L}$. It presents as rapid painless loss of vision of both the eyes.

3. Adenoviral Conjunctivitis

Adenoviruses are the commonest causes of viral conjunctivitis. These are non-enveloped, DNA viruses; also cause respiratory infections (Chapter 68). There are four types of adenoviral conjunctivitis.

1. **Nonspecific acute follicular conjunctivitis:** Most common form of acute follicular conjunctivitis; caused by adenovirus serotypes 1 to 11 and 19
2. **Pharyngoconjunctival fever:** It tends to occur as outbreaks in children's summer camps (causing *swimming pool conjunctivitis*), and is associated with adenovirus types 3 and 7. It is co-presented with pharyngitis, fever, rarely keratitis (30% of cases) and pre-auricular lymphadenopathy
3. **Epidemic keratoconjunctivitis or shipyard eye:** It occurs mainly in adults and is highly contagious, caused by types 8, 19 and 37. It is mostly associated with keratitis (80% of cases) and pre-auricular lymphadenopathy
4. Chronic relapsing adenoviral conjunctivitis.

4. Acute Hemorrhagic Conjunctivitis

The disease is caused by picornaviruses—Enterovirus 70 and also by Coxsackie virus A 24 (Chapter 73).

- ❖ It is an extremely contagious disease; transmitted by direct hand-to-eye contact. It has caused epidemics in far East, Africa and England
- ❖ It is an acute inflammation of conjunctiva characterized by conjunctival hemorrhages, hyperemia and mild follicular hyperplasia.

5. Newcastle Disease (Conjunctivitis)

It is a rare type of acute follicular conjunctivitis caused by an avian parainfluenza virus—called Newcastle disease virus (or Ranikhet virus in India).

- ❖ Produces pneumoencephalitis in young chickens and mild flu like illness in older birds
- ❖ Human infection is rare and occupational (occurring in poultry workers); characterized by mild self-limiting conjunctivitis.

Cytomegalovirus Retinitis

Cytomegalovirus (CMV) retinitis usually occurs in immunocompromised patients, e.g. those suffering from AIDS, on chemotherapy, or following renal transplantation.

- ❖ It is the most frequent ocular opportunistic infection in patients with AIDS; occurs when CD4 T cell count is <50 per μL
- ❖ **Clinical features:** Initially asymptomatic, gradually develops decreased vision and/or floaters in one or both eyes. On examination, the following findings are noticed

- Hemorrhagic retinitis
- Granular retinitis
- Complications include retinal detachment retinal atrophy and optic nerve disease.

TREATMENT

CMV Retinitis

- ❑ Antiretroviral therapy is recommended to reduce retroviral load and to increase CD4 T cell count in patients with AIDS.
- ❑ Specific anti-CMV drugs can be given such as ganciclovir, foscarnet and cidofovir.

OCULAR PARASITIC INFECTIONS

The following parasitic infections are important causes of ophthalmic disease worldwide.

- ❖ *Acanthamoeba* keratitis
- ❖ Ocular infections in Chagas' disease
- ❖ Ocular toxoplasmosis (*Toxoplasma gondii*)
- ❖ Ocular malaria and leishmaniasis
- ❖ Ocular cysticercosis
- ❖ Filarial nematode infections of eye: *Onchocerca*, *Loa loa*, *Mansonella perstans*
- ❖ Trichinellosis (stage of larval migration)
- ❖ Nematodes of lower animals: *Toxocara* (ocular larva migrans), *Dirofilaria conjunctivae* and *Thelazia* species.

Acanthamoeba Keratitis

Ocular infection by *Acanthamoeba* is associated with the use of contact lenses. *Acanthamoeba* spp. are small, free-living amoebae which exist in both fresh and marine environments and are resistant to chlorination. They also cause a CNS infection called granulomatous amoebic encephalitis (Chapter 75).

- ❖ **Transmission:** *Acanthamoeba* spreads to cornea either by (i) trauma (onset is rapid) or (ii) contact lens use, especially present in the lens cleaning solution (onset is slow) or (iii) contaminated water (onset is slow)
- ❖ **Adhesion:** Mannose binding protein on *Acanthamoeba* adheres to glycoprotein receptors on corneal epithelium. Corneal injury is a predisposing factor and may occur secondary to use of contact lenses or corneal surgery
- ❖ **Manifestations:** Various ocular manifestations are ring infiltrate around the cornea, with possible corneal penetration leading to corneal ulcers, iritis, scleritis, hypopyon (pus in anterior chamber), severe pain, and loss of vision
- ❖ **Incidence:** From developed countries, about 1 to 33 cases of *Acanthamoeba* keratitis occurs per million of contact lens wearers
- ❖ **In India,** *Acanthamoeba* accounts for 2% of microbiology-proven cases of keratitis; most of which are associated with contact lens use (80–85%). However, recent data had shown an increasing trend of *Acanthamoeba* keratitis in noncontact lens wearers too.

Laboratory Diagnosis

Corneal scrapings are the specimen of choice.

- ❖ **Wet mount examination** of corneal scraping reveals:
 - Trophozoite with acanthopodia
 - Cyst with double layered cyst wall; with an outer wrinkled cyst wall.
- ❖ **Permanent staining** such as hematoxylin and eosin stain, PAS stain are used to visualize characteristic morphology of the trophozoite such as prominent nucleolus, contractile vacuole and cytoplasmic vacuole
- ❖ **IFAT** (indirect fluorescent antibody technique) with specific antisera can be used for speciation of *Acanthamoeba*. *A. polyphaga* and *A. castellanii* are commonly recovered from corneal scapping
- ❖ **Molecular methods** such as PCR for speciation of *Acanthamoeba* species.

TREATMENT

Acanthamoeba keratitis

Topical antiseptic agents such as 0.1% propamidine, polyhexamethylene biguanide or chlorhexidine (0.02%) are used.

- In early cases confined to epithelium, debridement is sufficient
- In severe cases of vision impairment may need penetrating keratoplasty.

Prevention

Acanthamoeba keratitis can be prevented by: (i) regular cleaning of contact lens case by commercial cleaning solution (avoid homemade saline), (ii) contact lens cases should be allowed to air dry, (iii) changing the case once in 3 months.

Acanthamoeba life cycle, pathogenesis and laboratory diagnosis in detail has been discussed in Chapter 75, along with granulomatous amoebic encephalitis.

Ocular Toxoplasmosis

Ocular toxoplasmosis may result from—congenital transmission or ingestion of undercooked meat contaminated with tissue cysts or ingestion of water contaminated with oocysts of *Toxoplasma gondii*.

- ❖ Congenital infection in newborns and infection in patients with HIV, *T. gondii* may cause necrotizing chorioretinitis (Chapter 79)
- ❖ **Ocular lesions:** It mainly presents as **necrotizing chorioretinitis**, characterized by whitish, fluffy lesion surrounded by retinal edema (Fig. 78.8). Optic nerve involvement may lead to optic neuritis or papillitis
- ❖ **Ocular symptoms:** May include strabismus, nystagmus, and blindness in congenital infection, whereas the acquired disease is associated with scotoma, photophobia, and loss of central vision due to macular involvement
- ❖ **Ocular involvement:** Without history of congenital infection, ocular involvement is mostly unilateral in presentation; whereas ocular involvement in congenital toxoplasmosis is mostly bilateral



Fig. 78.8: Chorioretinitis in toxoplasmosis

Source: DPDx Image Library; Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **Diagnosis:** The diagnosis of chorioretinitis is based on the combination of slit lamp examination and serologic confirmation (IgG antibodies)
 - In congenital toxoplasmosis, detection of IgM antibodies confirms the diagnosis
 - Antibody production in ocular fluid can also be used for the diagnosis
 - Antigen detection or PCR can be done for the diagnosis.
- ❖ **Treatment:** Triple drug therapy comprising of pyrimethamine + sulfadiazine or clindamycin + prednisone for one month is recommended.

Ocular Infections in Chagas' Disease

Chagas' disease is caused by *Trypanosoma cruzi*, transmitted by reduviid bugs (Chapter 36). Ocular manifestations are seen usually at its early stage. It is characterized by:

- ❖ **Chagoma:** An erythematous subcutaneous nodule is formed at the site of deposition of bug's feces. It is painful, commonly occurs on face and may take 2–3 months to resolve
- ❖ **Romana's sign:** When the parasites enter through conjunctiva, there occurs a unilateral painless edema of the eyelid and conjunctivitis (Fig. 78.9)
 - Though pathognomonic, it is observed only in 48% of cases
 - The edema is painless and is frequently followed by constitutional symptoms such as fever, malaise, and anorexia
 - It is self-limiting, without any long-term sequelae.

Ocular Leishmaniasis

In visceral leishmaniasis or kala-azar (Chapter 36), the ocular manifestations are relatively uncommon and include chorioretinitis, central retinal vein thrombosis, iritis, papillitis, and keratitis.

Cutaneous leishmaniasis may present with ocular features if the sandfly bite site is near the eye; presents



Fig. 78.9: Romana's sign in the eyelid.

Source: Centers for Disease Control and Prevention (CDC), Atlanta and WHO/TDR (with permission).

as ptosis with inflamed eyelid. If the initial bite occurs on the conjunctival mucosa, it may result in mucocutaneous leishmaniasis, which may lead to severe ulceration and possible loss of the eye.

Ocular Malaria

In cerebral malaria (caused by *Plasmodium falciparum*, Chapter 35), ocular disease is due to vascular obstruction and hemolysis releasing the toxic pigments.

- ❖ The most-common ocular finding in malaria is retinal hemorrhage and is a poor prognostic indicator
- ❖ Other eye-related findings include optic neuritis, glaucoma, uveitis, oculomotor paralysis, and cortical blindness.

Ocular Cysticercosis

It is caused by *T. solium*, transmitted by ingestion of food/water contaminated with eggs or by autoinfection. Eggs develop into larvae (cysticercus cellulosae) that get deposited in CNS, eye and muscle forming cysts (Chapter 75).

- ❖ In eye, it can involve eyelids, conjunctiva and sclera
- ❖ Common symptoms like proptosis, diplopia, loss of vision and slow growing nodule with focal inflammation
- ❖ Ocular cysticercosis can usually be diagnosed by fundoscopy for the visual identification of the movements and morphology of the larvae.

Ocular Onchocerciasis

It is caused by *Onchocerca volvulus*, seen in Africa (Chapter 57). It is transmitted by bites of infected blackflies (*Simulium* spp.). It is associated with various ocular manifestations.

- ❖ **Bilateral blindness (river blindness):** It is the most serious complication of onchocerciasis. Lesions may develop in all parts of the eye
- ❖ **Conjunctivitis with photophobia:** It is the most common early finding
- ❖ **Punctate keratitis:** It is a self-resolving, acute inflammatory reactions to surrounding dying microfilariae

seen in younger patients and presented as “snowflake opacities”

- ❖ **Sclerosing keratitis** occurs in 1–5% of infected persons and is the leading cause of blindness due to onchocerciasis in Africa
- ❖ **Other manifestations:** Anterior uveitis and iridocyclitis (Africa), retinal pigmentation, secondary glaucoma (seen in Latin America).

Diagnosis: Sitting with head placed between the knees for 10 minutes may help the microfilariae to concentrate in the anterior chamber of eyes behind the cornea; which may be visualized by slit lamp examination. Microfilariae measure 254 μm long, have pointed tail tip without any nuclei. They are unsheathed, nonperiodic.

Ocular manifestations may be seen with other filarial nematode such as *Mansonella perstans*, producing acute periorbital inflammation; known as **bung-eye** or **bulge-eye**.

Ocular Trichinellosis

Trichinella spiralis may infect eyes during its stage of larval migration. The larvae form cysts in the extraocular muscles (Chapter 57).

- ❖ Periorbital and facial edema is common
- ❖ Hemorrhages are seen in the subconjunctiva, retina and nail beds (“splinter” hemorrhages).

Ocular Larva Migrans (OLM)

Larva migrans results from accidental infection by larvae of lower animal nematodes. Humans being the accidental host, the life cycle gets arrested and the parasitic larvae may wander aimlessly in the body, infecting various organs; most common being liver (visceral larva migrans, Chapter 49), rarely eyes producing OLM.

- ❖ The most common cause of OLM is *Toxocara larva*; usually older children (around eight years) are affected
- ❖ **Presentation:** Unilateral painless chorioretinal granuloma in the posterior pole is the most common presentation. But in some cases, diffuse panuveitis, retinal detachment and unilateral visual loss may occur.

Other nematodes of lower animals such as *Dirofilaria conjunctivae* and *Thelazia* species may also infect eyes.

OCULAR FUNGAL INFECTIONS

Various fungal infections of eye include:

- ❖ **Mycotic keratitis :** Caused by *Aspergillus* (most common), *Candida* and *Fusarium*
- ❖ **Orbital mucormycosis:** Caused by Zygomycetes such as *Mucor* and *Rhizopus*
- ❖ **Fungal endophthalmitis** is relatively rare. It is caused by *Aspergillus*, *Fusarium*, *Candida*, etc.
- ❖ **Fungal uveitis:** It is rare and may accompany systemic aspergillosis, candidiasis and blastomycosis. It also includes a condition, that is believed to occur following

histoplasmosis, called as presumed ocular histoplasmosis syndrome (POHS)

- ❖ **Microsporidia** infections of eye.

Mycotic Corneal Ulcer

The incidence of suppurative corneal ulcers caused by fungi has increased in the recent years due to injudicious use of antibiotics and steroids.

- ❖ **Antibiotics and steroid use:** Antibiotics suppress the commensal bacteria in eyes and steroid suppresses immune system—favoring fungi which are usually saprophytes to cause ocular infections
- ❖ **Agents:** Fungi commonly responsible for mycotic corneal ulcers are *Aspergillus* (most common, Chapter 69), *Candida* and *Fusarium* (discussed below). Less common agents include *Penicillium*, *Rhizopus*, and *Mucor*
- ❖ **Modes of infection:** Fungi enter the eyes through *ocular injury* by vegetative material such as leaf, branch of a tree; common among field workers during harvesting season. *Secondary fungal ulcers* are common in patients who are immunosuppressed
- ❖ **Clinical features:** Symptoms are similar to the bacterial corneal ulcer, but in general mycotic ulcers are less marked and the overall course is slow in progress compared to bacterial keratitis.

TREATMENT

Mycotic keratitis

Topical antifungal eye drops such as natamycin, amphotericin B and azoles (e.g. fluconazole, or voriconazole) are used. They should be instilled initially hourly, then taper slowly over 6 to 8 weeks.

Systemic antifungal drugs (e.g. fluconazole or ketoconazole or voriconazole) may be required for severe cases of deeper fungal keratitis; given for 2–3 weeks.

Nonspecific treatment: Same as for bacterial keratitis.

Fusariosis

Fusarium species are soil and plant saprophytes found worldwide. They rarely cause human infections. Important species infecting humans are—*F. solani* (most common), followed by *F. oxysporum* and *F. verticillioides*.

- ❖ In immunocompetent individuals, they cause:
 - Keratitis in contact lens wearers
 - Onychomycosis.
- ❖ In immunocompromised patients—they are angioinvasive; cause pulmonary and sinus infection
- ❖ In patients with neutropenia and hematologic malignancies, disseminated fusariosis occurs with frequent skin lesions.

Laboratory Diagnosis

Fusarium is a filamentous fungus, grows rapidly on SDA at 25°C and produces woolly to cottony, flat, spreading white to pink colonies. LPCB mount of the colony reveals hyaline septate hyphae bearing round microconidia, sickle-shaped large macroconidia and chlamydo spores (Fig. 78.10).

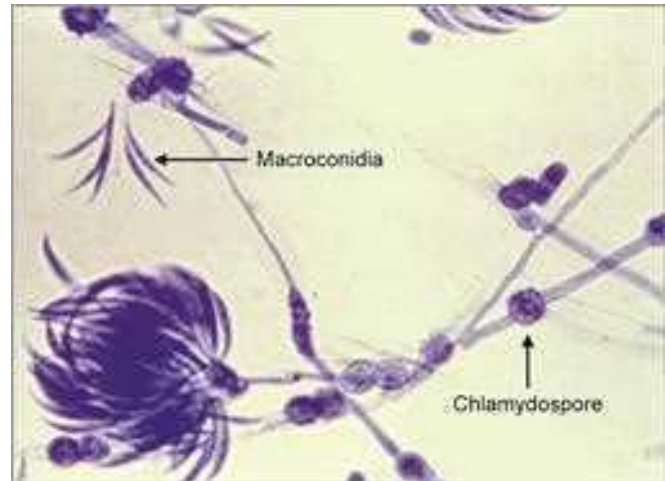


Fig. 78.10: *Fusarium* species (LPCB mount).

Source: Public Health Image Library, ID#: 17970/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

TREATMENT

Fusariosis

Fusarium species are resistant to many antifungal agents. Liposomal amphotericin B, voriconazole or posaconazole are recommended.

Orbital Mucormycosis

Orbital mucormycosis is a severe fungal infection of the orbit, caused by Zygomycetes such as *Mucor* and *Rhizopus*.

- ❖ **Pathogenesis:** Infection usually begins in the sinuses and erodes into the orbital cavity. They have a tendency to invade vessels and cause ischemic necrosis
- ❖ **Risk factors:** Include patients with diabetic ketoacidosis or immunocompromised conditions and on steroid therapy
- ❖ **Clinical features:** Most of the patients are seriously ill and present with severe pain, proptosis, and necrotic areas with black eschar formed on the palate, and nasal septum (Figs 78.11A and B)
- ❖ **Complications:** If not treated energetically, patient may develop meningitis, brain abscess and die within days to weeks
- ❖ **Laboratory diagnosis:** The biopsy tissue of the involved area is sent for microbiological confirmation (Chapter 69)
 - Direct microscopy by 10% KOH mount: Reveals nonseptate broad branching hyphae
 - Culture on Sabouraud dextrose agar reveals white cottony woolly colonies.

TREATMENT

Orbital mucormycosis

Treatment is often difficult and inadequate, leads to frequent recurrences. Treatment modalities include:

- Correction of underlying disease (e.g. diabetic ketoacidosis)
- Surgical excision of the involved tissue
- Intravenous amphotericin B or other appropriate antifungal drug



Figs 78.11A and B: Mucormycosis manifestations: **A.** Orbital cellulitis; **B.** Proptosis.

Source: Public Health Image Library/A. Dr Thomas F Sellers, ID#: 2831; B. Lucille K Georg, ID#: 14554 /Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Presumed Ocular Histoplasmosis Syndrome (POHS)

Histoplasma capsulatum is presumed to be associated with uveitis, though it has not been isolated from the affected eyes (Chapter 38).

- ❖ It is characterized by: (1) histospots (atrophic spots scattered in the periphery of retina), and (2) macular lesions in retina
- ❖ >90% of patients with POHS show positive histoplasmin skin test
- ❖ POHS is more common in the areas where histoplasmosis is endemic; e.g. Mississippi-Ohio-Missouri river valley.

Ocular Candidiasis

It is an opportunistic infection caused by *Candida albicans* (Chapter 38). It occurs in immunocompromised individuals such as patients with HIV/AIDS and malignancies. Ocular candidiasis is a very rare condition; may occur as:

- ❖ Anterior uveitis (associated with hypopyon)
- ❖ Multifocal chorioretinitis, or
- ❖ Endophthalmitis with severe retinal necrosis and abscesses—vitreous exudates present as ‘puff ball’ or ‘cotton ball’ colonies, which when joined by exudative strands form ‘string of pearls.’

Microsporidial Infections of Eye

Several *Microsporidium* species such as *Nosema*, *Encephalitozoon*, *Vittaforma corneae* can infect eyes.

- ❖ The microsporidia are either directly inoculated into eye structures or by systemic dissemination; the latter occurs in patients with AIDS
- ❖ Ocular findings are limited to conjunctiva and cornea; which include conjunctival hyperemia, punctate epithelial keratitis, necrotizing keratitis, and corneal ulcers
- ❖ **Diagnosis** is made by detection of Microsporidia spores in corneal scrapings or biopsy specimens staining or when examined by electron microscopy (Chapter 45)

- ❖ **Treatment:** Albendazole has shown some promise in the treatment of corneal disease. Topical agents can be applied for the corneal lesions like topical itraconazole, metronidazole and topical propamidine.

Microsporidia species also cause infections of other systems such as gastrointestinal (Chapter 45) and musculoskeletal systems (Chapter 58).

OCULAR FEATURES IN INTRACRANIAL INFECTIONS

Apart from local infections of eyes, the ocular involvement is frequent in certain systemic infections. Ocular manifestations (e.g. vision loss) are one of the common presentation in various intracranial infections such as meningitis, encephalitis and brain abscess. This is as a result of optic nerve involvement (papillitis) and paralysis of other cranial nerves.

INFECTIONS OF EAR

Ear infections include infections of external ear (otitis externa) or middle ear (otitis media).

OTITIS EXTERNA

Otitis externa refers to the infection of the external ear. The external ear consists of auricle or pinna, external auditory canal and tympanic membrane which separates external ear from middle ear. The otitis externa may have a bacterial, viral or fungal etiology.

- ❖ **Bacterial:** Localized otitis externa (furuncle), diffuse otitis externa and malignant otitis externa
- ❖ **Viral:** Herpes zoster oticus, otitis externa hemorrhagica
- ❖ **Fungal:** Otomycosis.

Localized Otitis Externa (Furuncle)

Furuncle is an infection of hair follicle, caused by *S. aureus*. It is confined only to the cartilaginous part of the external ear as hair are seen only in this part.

Clinical Features

Patient presents with severe ear pain and tenderness; aggravated by movement of the pinna. Jaw movements, as in chewing also cause pain in the ear. Furuncles appear usually single, but sometimes in multiple. Periauricular lymph nodes may also be enlarged and tender.

TREATMENT

Localized otitis externa

- ❑ **In early cases:** Treatment consists of systemic antibiotics, analgesics and local heat. Glycerine ear pack may provide splintage and reduce pain and edema
- ❑ **If abscess has formed,** incision and drainage should be done
- ❑ In case of *recurrent furunculosis*, diabetes should be excluded. Patients may be a carrier of *S. aureus* in the nose and skin, which may be a source of infection and therefore should be treated if present.

Diffuse Otitis Externa

It is diffuse inflammation of the skin of pinna which may spread to involve the entire external ear and adjacent tympanic membrane.

- ❖ **Etiology:** More often the infection is mixed, common organisms implicated being *Staphylococcus aureus*, *Pseudomonas*, and *Escherichia coli*
- ❖ **Risk factors:** Disease is commonly seen in hot and humid climate and in swimmers
- ❖ **Pathogenesis:** Most cases are associated with trauma to the ear skin, which can result from scratching the ear canal with hair pins or matchsticks; following which there occurs invasion by pathogenic organisms.

Clinical Features

It presents in two phases; acute or chronic with varying degrees of severity.

- ❖ **Acute phase** manifests as hot burning sensation in the ear, followed by ear pain and oozing of thick and purulent ear discharge; inflamed and swollen ears. In severe cases, conductive hearing loss occurs along with enlarged and tender regional lymph nodes
- ❖ **Chronic phase:** Presents with irritation in the ear and itching, scanty discharge which may dry up to form crusts. External ear skin becomes thick and swollen, shows scaling and fissuring.

TREATMENT

Diffuse otitis externa

In acute phase, treatment comprises of:

- ❑ **Ear toilet** is the mainstay of treatment; aims to remove exudate and debris. It is done by dry mopping, suction clearance or irrigating the ear canal with warm, sterile normal saline
- ❑ **Medicated gauze piece** soaked in antibiotic-steroid preparation is placed in the ear canal, which is changed daily for 2–3 days and subsequently, is substituted by ear drops
- ❑ **Antibiotics:** Broad-spectrum systemic antibiotics are used when there is cellulitis and acute tender lymphadenitis
- ❑ **Analgesics:** For relief of pain and inflammation.

Chronic phase is treated with:

- ❑ A gauze piece soaked in 10% glycerine is placed into the canal, which helps to reduce swelling. This is followed by ear toileting as discussed above
- ❑ Topical antibiotic steroid cream to reduce itching
- ❑ If the meatal skin is thickened, causing obstruction (chronic stenotic otitis externa), it is surgically excised.

Malignant (Necrotizing) Otitis Externa

It is an inflammatory condition caused by *Pseudomonas* infection; usually in the elderly diabetics, or in those on immunosuppressive drugs.

- ❖ **Clinical features:** It resembles diffuse otitis externa but there is excruciating pain and appearance of granulations in the ear canal. Infection may spread to the skull base causing multiple cranial nerve palsies, commonest being facial nerve paralysis

- ❖ **Laboratory diagnosis:** When clinically suspected, ear discharge may be collected and sent for culture and susceptibility testing
 - Any crust if present should be wiped away with sterile saline
 - The swab moistened with sterile saline is firmly rotated in the external auditory canal. Specimen should be sent to the laboratory within two hours of collection
 - Specimen is subjected to Gram staining and culture on blood agar, chocolate agar and MacConkey agar.
- ❖ **Radiology:** CT scan may show bony destruction. Gallium-67 scan is more useful in diagnosis and for follow-up of the patient.

TREATMENT

Malignant otitis externa

Treatment comprises of:

- ❑ **Ear canal toileting:** For removing discharge and granulations
- ❑ **Systemic antibiotic** for *Pseudomonas*, given for 6–8 weeks; such as gentamicin plus ceftazidime. Quinolones (ciprofloxacin) can also be given orally
- ❑ Control of underlying diabetes is crucial.

Zoster Oticus

Varicella-zoster virus infection is characterized by formation of vesicles on the tympanic membrane and meatal skin. The seventh and eighth cranial nerves may be involved.

Otitis Externa Hemorrhagica

It is characterized by formation of hemorrhagic bullae on the tympanic membrane and deep meatus; probably seen in viral infections. It causes severe pain in the ear and blood-stained discharge due to rupture of bullae.

Otomycosis

Otomycosis or fungal infection of the ear canal is usually caused by *Aspergillus niger*, *A. fumigatus* or *Candida albicans*.

- ❖ **Risk factors:** Otomycosis is seen in hot and humid climate and usually occurs in patients using topical antibiotics in ear
- ❖ **Clinical features:** It presents with intense itching, discomfort or pain in the ear, watery discharge with a musty odor and ear blockage
- ❖ **Ear examination** with an otoscope: The fungal mass may appear white, brown or black when removed by a wet piece of filter paper
 - *A. niger* appears as black headed filamentous growth
 - *A. fumigatus* as pale green growth
 - *Candida* appears as white or creamy deposit.
- ❖ **Diagnosis:** The discharge after collection can be sent for microscopy and culture
- ❖ **Treatment** consists of thorough ear toilet to remove all discharge and debris. It can be done by syringing, suction

or mopping. Topical antifungal agents such as nystatin or clotrimazole drops should be applied for a week.

OTITIS MEDIA

Middle ear infections can be broadly classified into two types—(1) acute suppurative otitis media (ASOM), and (2) chronic suppurative otitis media (CSOM).

Acute Suppurative Otitis Media

It is an acute inflammation of middle ear by pyogenic organisms, leading to formation of pus. It is more commonly seen in infants and children of lower socioeconomic group and with underlying viral infection of upper respiratory tract.

Etiology

The common organisms implicated in ASOM are *Streptococcus pneumoniae* (most common agent, 30%), followed by *Haemophilus influenzae* (20%) and *Moraxella catarrhalis* (12%). Other organisms include *S. pyogenes*, *S. aureus* and rarely *Pseudomonas*.

Clinical Features

Acute otitis media runs through three stages.

- Stage of tubal block:** ASOM begins with eustachian tube blockade, manifests as retracted tympanic membrane, mild earache, decreasing hearing and minimal discharge
- Suppuration stage:** This is marked by formation of pus in the middle ear, bulging tympanic membrane, severe excruciating ear pain, marked deafness and signs of systemic involvement such as fever and vomiting

Acute Necrotizing Otitis Media

It is a variety of ASOM, caused by β -hemolytic *Streptococcus* that occurs among children suffering from measles or influenza. There is rapid destruction of whole of tympanic membrane and profuse otorrhoea.

- Resolution stage:** The bulged tympanic membrane sometimes ruptures with release of pus and subsidence of symptoms.

Laboratory Diagnosis

Pus evacuated from middle ear after the rupture of tympanic membrane is sent for culture and antibiotic susceptibility testing.

- ❖ First, the ear canal is cleaned with mild soap solution. Then **myringotomy** (puncture of the ear drum) is performed and the pus material behind the drum is aspirated with syringe. If ear drum intact; a moistened swab is used to collect material from ruptured ear drum
- ❖ Specimen should be transported within two hours to the laboratory. It is subjected to Gram staining and culture on to blood agar, chocolate agar and MacConkey agar

- ❖ Anaerobic culture can be performed for the aspirated specimen, but not for the swab specimens.

TREATMENT

Acute suppurative otitis media

Treatment of ASOM comprises of the following:

- Antibiotic:** It is indicated in all cases with fever and severe earache.
 - Ampicillin or amoxicillin are the first choice
 - Amoxicillin-clavulanate is given if β -lactamase-producing *H. influenzae* or *M. catarrhalis* are isolated
 - Duration of therapy is minimum for 10 days, till tympanic membrane regains normal appearance and hearing returns to normal.
- Myringotomy:** If the drum is bulging and there is acute pain, it is incised to evacuate pus
- Symptomatic treatment** is given such as decongestant nasal drops (e.g. ephedrine) to relieve eustachian tube edema; analgesics and antipyretics and ear toileting to remove any discharge.

Chronic Suppurative Otitis Media (CSOM)

Chronic suppurative otitis media (CSOM) is a long-standing infection of a part or whole of the middle ear cleft.

- ❖ **Etiology:** CSOM may be caused by both aerobic and anaerobic bacteria
 - Common aerobic organisms associated with CSOM are *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli* and *Staphylococcus aureus*
 - Anaerobes include *Bacteroides fragilis* and anaerobic streptococci.
- ❖ **Clinical features:** CSOM is characterized by ear discharge and permanent perforation
 - The edge of the perforation is covered by squamous epithelium so that it does not heal spontaneously
 - A permanent perforation can be connected to an epithelium-lined fistulous track.
- ❖ **Two clinical types:** Clinically, CSOM is divided into two types
 - Tubotympanic type:** Also called the *safe* or *benign* type; characterized by profuse, mucoid, odourless discharge and central perforation
 - Atticoantral:** Also called *unsafe* or *dangerous* type; characterized by scanty purulent foul smelling discharge with attic or marginal perforation. Risk of complications is high in this variety (e.g. eroding of ear bones, cholesteatoma, etc).

TREATMENT

Chronic suppurative otitis media

Treatment depends upon the type of CSOM.

- ❑ Tubotympanic type is mainly managed medically by ear toileting, antibiotics (topical and systemic); similar to as followed for ASOM
- ❑ In contrast, the atticoantral type of CSOM is managed surgically, aiming to remove the disease and render the ear safe.

EXPECTED QUESTIONS

I. Write short notes on:

- Acute bacterial conjunctivitis (ABC).
- Ophthalmia neonatorum.
- Ocular viral infections.
- Ocular fungal infections.
- Acanthamoeba keratitis.
- Ocular toxoplasmosis.
- Acute suppurative otitis media.

CHAPTER PREVIEW

- Congenital Toxoplasmosis
- Congenital Rubella Syndrome (CRS)
- Congenital Cytomegalovirus Infection
- Neonatal Herpes
- Other Teratogenic Agents

INTRODUCTION

Vertical transmission refers to the spread of infections from mother-to-baby, which may occur either by transplacental route (congenital infection), during delivery, or after delivery.

Congenital Infections

Congenital infections refers to infections that cross the placenta to infect the fetus, and thereby results in developmental defects in the fetus (congenital malformation). The agents of congenital infections are abbreviated by an acronym ‘TORCH’ Infections.

TORCH Infections

‘TORCH’ is an acronym used for some common congenital infections. These are:

- ❑ Toxoplasmosis
- ❑ Other infections:
 - Congenital syphilis: *Treponema pallidum*
 - Varicella-zoster virus infection
 - Human parvovirus infection
 - Zika virus disease
 - Congenital trypanosomiasis: *Trypanosoma cruzi*
 - Malaria: *Plasmodium falciparum*
- ❑ Rubella
- ❑ Cytomegalovirus (CMV)
- ❑ Herpes simplex virus

There are various other organisms which can pass through placenta to infect fetus, however does not produce any fetal malformation. Examples include Hepatitis B, Coxsackie viruses, and Epstein-Barr virus, etc.

Perinatal Infections (During Delivery)

Perinatal infections occur while the baby passes through an infected birth canal. These infections are usually caused by the agents of sexually transmitted infections (STIs, Chapter 77). These also include the infections transmitted through contamination with fecal matter during the delivery. Common examples of agents causing perinatal infections include:

- ❖ **Bacteria:** Group B streptococci, *E. coli*, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*
- ❖ **Viruses:** Herpes simplex virus, cytomegalovirus, enteroviruses, hepatitis B virus, HIV.

Postnatal Infections (After Delivery)

These infections spread from mother to baby following delivery, usually during breastfeeding. All the organisms listed under perinatal infection can also cause postnatal infection. In addition, various organisms from the nursery environment such as multidrug resistant gram-negative bacilli can also cause postnatal infections.

The discussion in this chapter will be confined only to those infections which produce congenital malformation in fetus.

CONGENITAL TOXOPLASMOSIS

Toxoplasma is the most common parasite to be teratogenic. The incidence of congenital toxoplasmosis is approximately 1 per 1000 live births. It also causes encephalitis in HIV-infected individuals, the detail is discussed in Chapter 75.

Transmission

Mother acquiring *Toxoplasma* infection in pregnancy is usually asymptomatic. However, she can transmit the infection to the fetus.

- ❖ Transplacental transmission of *T. gondii* from mother-to-fetus can occur at any time during the pregnancy. Tachyzoites are the infective form
- ❖ **Gestational age:** It is the main factor that influences the fetal outcome. As the gestation proceeds, the chance of transmission of infection increases but the severity of the infection declines
 - If the mother becomes infected during the first trimester, the incidence of transplacental infection is lowest (15%), but the disease in the neonate is most severe

- If maternal infection occurs during the third trimester, the incidence of transplacental infection is maximum (65%), but the infant is usually asymptomatic at birth
- If the mother is infected before pregnancy, then the fetus is mostly uninfected, except when the mother is immunocompromised.

Clinical Manifestations

Initially though asymptomatic, but the persistence of infection in the newborn can result in severe disease.

- ❖ The **classical triad** comprises of chorioretinitis, hydrocephalus, and intracranial calcifications
- ❖ **Other manifestations** include stillbirth, psychomotor disturbance and microcephaly
- ❖ **Ocular involvement:** Eyes are involved later in life (2nd-3rd decade) when the cysts ruptures (Chapter 78)
 - Most frequently, it causes bilateral chorioretinitis leading to profound visual impairment. Other ocular manifestations include blurred vision, scotoma, photophobia, strabismus and glaucoma
 - In contrast, if ocular involvement occurs without history of congenital infection, it is mostly unilateral.
- ❖ Congenital toxoplasmosis is an important cause of repeated abortion and infertility. Hence, routine antenatal screening for *Toxoplasma* antibodies is advised in many developed countries.

Laboratory Diagnosis

Antenatal Diagnosis

If acute infection is documented in a pregnant women, then the following diagnostic algorithm should be followed.

- ❖ **Ultrasonography** of fetus should be done at 20–24 weeks of gestation and repeated every 2–4 weeks for detecting the lesions of congenital infection
- ❖ **PCR and/or isolation:** Amniotic fluid sample is collected, centrifuged and the pellet is subjected to PCR and/or isolation in mouse or tissue culture
 - If either or both found positive, then antenatal diagnosis is confirmed
 - If both negative: Warrants evaluation of the neonate to rule out any remote possibility of infection.

Postnatal Diagnosis

The postnatal diagnosis is made by the following laboratory tests.

- ❖ **Isolation of the parasite** at the time of delivery must be attempted from amniotic fluid, placenta and cord leukocyte
- ❖ **IgM and IgG:** Newborn and maternal sera are subjected to detect IgG (Sabin-Feldman dye test, IFA or ELISA) and IgM (ELISA or IFA)
 - IgG titer of $\geq 1,000$ in neonate: Indicates possible diagnosis which should be confirmed by IgM testing

- IgM titer of neonate $\geq 1:4$ after 2 weeks of age indicates probable diagnosis and guides the clinicians to initiate the treatment to the neonate.

❖ **Other tests** for congenital toxoplasmosis include:

- **IgA detection** (neonatal and maternal blood): IgA appears to be more sensitive than IgM for the diagnosis of congenital toxoplasmosis. IgA antibodies usually disappear within 10 days of birth, hence persistence of IgA beyond 10 days confirms the postnatal infection
- **IgE detection** (neonatal and maternal blood)
- **PCR** in neonatal and maternal blood detecting specific genes of *T. gondii* also confirms the diagnosis
- **Fundus examination** should be performed to rule out chorioretinitis.

TREATMENT

Congenital toxoplasmosis

Neonates with congenital toxoplasmosis are treated with daily oral pyrimethamine (1 mg/kg) and sulfadiazine (100 mg/kg) with folic acid for 1 year.

CONGENITAL RUBELLA SYNDROME (CRS)

Rubella is a highly teratogenic virus. The risk of transmission of infection to the fetus and the severity of congenital infection—both are maximum if the mother acquires the infection during first trimester of pregnancy. Risk after 5th month of pregnancy is almost negligible; 90% risk at 11 weeks vs 20% risk at 20 weeks of gestation.

Rubella is a RNA virus, belongs to Togaviridae family. In children, it mainly causes exanthematous lesions; discussed in detail Chapter 56.

Clinical Manifestations

Clinical manifestations of CRS can be grouped into permanent congenital defects and transient reversible congenital changes.

Permanent Congenital Defects

The classical triad consists of:

- ❖ **Ear defect:** Sensory neural deafness (most common defect of CRS)
- ❖ **Ocular defects:** The most common ocular defect is salt-and-pepper retinopathy, followed by cataract (See Figures 56.10 B)
- ❖ **Cardiac defect:** Patent ductus arteriosus (PDA) is the most common cardiac defect followed by pulmonary artery stenosis and ventricular septal defect.

Other features include—CNS defects may occasionally be seen such as microcephaly, mental retardation, motor delay and autism.

Transient Congenital Changes

The transient changes such as hepatosplenomegaly, bone lesions and intrauterine growth retardation (IUGR) may

be seen. Occasionally, thrombocytopenia with petechiae (**Blueberry muffin syndrome**) may also be seen.

Outcome in the fetus may be miscarriage, fetal death, or premature birth with congenital defects.

Laboratory Diagnosis

The various laboratory diagnosis parameters include:

- ❖ **IgM antibodies:** They do not cross placenta; their presence in a neonate is diagnostic of congenital rubella infection
- ❖ **IgG antibodies:** They cannot differentiate between maternal transfer and congenital infection. However, IgG antibodies persisting in baby's serum beyond the expected time of disappearance of maternal IgG (9 months of age) can also be used as a criterion to diagnose congenital rubella infection
- ❖ **Isolation of virus** can be done especially from the throat swab, to lesser extent from urine (excreted up to 1 year) and CSF. It is more likely to be positive in the first six months after the birth
- ❖ **Reverse transcriptase PCR** can be performed to detect the viral RNA.

Epidemiology

Congenital rubella remains an important public health problem globally. WHO Southeast Asia Region has implemented a program for elimination of rubella/congenital rubella syndrome (CRS) along with measles by introducing a 'Strategic Plan for achieving and maintaining **Measles and Rubella/CRS** elimination in 2020–2024.'

Prevention

Congenital rubella can be prevented by universal immunization of all woman of reproductive age (first priority group) and all children of 1–14 years age with rubella vaccine. It is live-attenuated vaccine (RA 27/3), discussed in detail in Chapter 56. Infection control measures such as airborne precautions (Chapter 21) should be taken to prevent transmission from rubella patients to pregnant woman.

CYTOMEGALOVIRUS INFECTIONS

Cytomegalovirus (CMV) is the largest virus in Herpesviridae family (Chapter 56). It is so named because it causes massive enlargement of infected host cells.

Properties of CMV are similar to any other herpesviruses described in Chapter 56, with some minor differences.

- ❖ CMV belongs to β -subfamily
- ❖ Its dsDNA is the largest among herpesviruses, which consists of 240 kbp nucleotides
- ❖ **Host specificity:** Cytomegaloviruses are strictly species-specific. Human CMV does not infect animals. Similarly, a number of animal CMVs exist, which do not infect humans
- ❖ **Cell-type specificity:** CMV infects kidney and salivary glands; where it undergoes latency

- ❖ **Cell-to-cell spread:** CMV is almost always closely associated with the cells and spread primarily cell-to-cell, so that very little virus may be cell-free.

Clinical Manifestations

CMV causes an array of clinical syndromes such as congenital and perinatal infections, CMV mononucleosis in adults and severe infection in immunocompromised and transplant recipients.

Congenital CMV Infection

CMV is probably the most common intrauterine infection associated with congenital defects.

- ❖ **Cytomegalic inclusion disease:** It develops in about 5% of the infected fetus. The remaining are although asymptomatic at birth, 5–20% of them may develop significant psychomotor, hearing, ocular, or dental defects over the next few years
- ❖ **Congenital defects include:**
 - Most common defects are petechiae, hepatosplenomegaly, and jaundice (60–80% of cases)
 - Less common defects include: Microcephaly, cerebral calcifications, intrauterine growth retardation, and prematurity (30–50% of cases)
 - Sensorineural hearing loss may occur in up to 10–15% of asymptomatic infants
 - Occasional defects are inguinal hernias and chorioretinitis.
- ❖ **Risk is maximum** if the infection occurs in early pregnancy and if the mother is primarily infected during pregnancy (one-third of the primarily infected mothers transmit the virus to the fetus in contrast to 1% of reactivated mothers)
- ❖ Mortality rate is very high (20%).

Perinatal CMV Infection

- ❖ Transmission to the newborn occurs either during:
 - Delivery—through infected birth canal or
 - Postnatal—through infected breast milk/secretions from mother.
- ❖ Most of the infected infants remain asymptomatic, but shed virus in urine from 8–12 weeks of age, up to several years
- ❖ Few infants, especially premature babies develop interstitial pneumonitis.

Immunocompetent Adults

In healthy adults, CMV produces an infection following blood transfusion called **mononucleosis like syndrome**.

- ❖ This condition is clinically similar to infectious mononucleosis caused by EBV, characterized by the presence of atypical lymphocytes
- ❖ However unlike EBV mononucleosis, the heterophile antibodies (detected by Paul Bunnell test) are typically absent

- ❖ Mononucleosis like syndrome can be seen in various infections, CMV being the most common cause (20-50% of cases). See Chapter 68, Table 68.1.

Immunocompromised Host

CMV produces markedly severe infection in immunosuppressed individuals; most of which are due to reactivation of their own latent viruses. They present with fatigue, fever, malaise, anorexia, night sweats, and arthralgias or myalgia, known as **CMV syndrome**; which may progress to end-organ disease without treatment.

- ❖ **In AIDS patients** with CD4 T cell count <50/μL: CMV may cause chorioretinitis (leading to blindness, Chapter 78), gastroenteritis, progressive dementia, cranial nerve defects, and other disseminated CMV infections
- ❖ **Organ transplant recipients:** CMV is probably the most common viral infection that occurs in transplant recipients. Infection occurs usually between 1 and 4 months following transplantation and presents in various forms such as:
 - Bilateral interstitial pneumonia, a life-threatening complication seen in 15–20% of bone marrow and solid organ transplant recipients
 - Febrile leukopenia is seen among solid organ transplant recipients
 - Obliterative bronchiolitis in lung transplants
 - Graft atherosclerosis in heart transplants
 - Rejection of renal allografts.

Epidemiology

- ❖ **Transmission:** Close person-to-person contact is required for transmission (unlike HSV). Various modes of transmission include:
 - Oral and respiratory spread (most common mode)
 - Transplacental route (from mother to fetus)
 - Blood transfusion
 - Organ transplantation
 - Sexual contact (in young adults).
- ❖ **Reservoir:** Humans are the only known host for CMV
- ❖ **Source:** Virus may be shed in urine, saliva, semen, breast milk, and cervical secretions, and is carried in circulating white blood cells
- ❖ **Endemic:** CMV is endemic worldwide, present throughout the year without any seasonal variation
- ❖ **Risk factors** such as low socioeconomic status and poor personal hygiene facilitate the infection
- ❖ **Prevalence** is high in underdeveloped nations with 90% of people being seropositive in contrast to 40–70% seropositivity in developed nations.

LABORATORY DIAGNOSIS Cytomegalovirus infections

- ❑ **Cytopathology**—detects inclusion bodies with owl's eye appearance
- ❑ **Virus isolation:**
 - Human fibroblasts cell line culture—detects CPE after 2–3 weeks

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LABORATORY DIAGNOSIS Cytomegalovirus infections

- Shell vial culture—detects viral antigens in infected cell lines by IF (1–2 days).
- ❑ **Antibody detection**—by ELISA using pp150, pp65, pp52, glycoprotein antigens
- ❑ **Antigen detection** such as pp65 antigen
- ❑ **Molecular methods**—PCR and real-time PCR targeting UL54, UL55 and UL83 genes.

Laboratory Diagnosis

Detection of Inclusion Bodies

In urine, CMV produces characteristic perinuclear cytoplasmic inclusions in addition to the usual intranuclear inclusions seen in other herpesviruses (Owl's eye appearance) (Fig. 79.1).

Virus Isolation

CMV can be isolated from throat washings and urine.

- ❖ **Human fibroblasts** are the most ideal cell lines, specific for CMV
- ❖ **Cytopathic effect:** After 2–3 weeks of incubation, the following CPE may be observed in the infected cell line:
 - Typical CMV inclusions (as described above)
 - Multinucleated giant cells are seen
 - Enlargement of infected host cells.
- ❖ **Shell vial technique** can be followed for early growth detection (1–2 days).

Antibody Detection

ELISA has been available for detecting serum antibodies against various antigens such as matrix phosphoproteins pp150 and pp65, glycoproteins gB and gH, and the major DNA binding protein (pp52).



Fig. 79.1: Histopathology of kidney shows cytomegalic host cell containing characteristic Owl's eye inclusions (arrows showing).

Source: Public Health Image Library, ID# /1155 Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ **CMV IgM antibodies** appear in 1–2 weeks after infection and indicates recent or on-going infection. It drops to below detectable levels within several months after infection. IgM detection in pregnancy indicates congenital infection, thus guiding initiation of appropriate treatment
- ❖ **CMV IgG antibodies** are produced few days after IgM and persist life-long. Its presence indicates either a recent or past infection; which can be differentiated by performing IgG avidity test.

Antigen Detection

CMV-specific **pp65 antigen** (a major matrix phosphoprotein) can be detected in peripheral-blood leukocytes by indirect immunofluorescence test using specific monoclonal antibody. This test has been in use since long; but is less preferred as it is time-consuming and technically demanding.

Molecular Methods

Molecular methods such as PCR can be used to detect specific CMV DNA in blood or body fluids such as CSF.

- ❖ Various genes targeted are glycoprotein B (UL55), DNA polymerase (UL54) and pp65 (UL83)
- ❖ **Quantitative nucleic acid testing (QNAT)** by real-time PCR is highly sensitive and specific, have replaced gold standard culture technique in most laboratories. It can quantitate the viral DNA load, hence it is the method of choice to monitor the treatment response.

TREATMENT

Cytomegalovirus infections

CMV does not respond to acyclovir. The following drugs are used for the treatment of CMV infections.

- ❑ **Ganciclovir:** It is the drug of choice for cytomegalic inclusion disease or neonatal CMV infection or for severe CMV infections such as retinitis or transplant infections
 - It is given by intravenous route
 - Mechanism of action: It is converted by viral phosphotransferase encoded by CMV UL97 gene to form ganciclovir triphosphate that inhibits CMV DNA polymerase.
- ❑ **Valganciclovir:** It is a pro-drug of ganciclovir that can be given orally
- ❑ **Foscarnet:** It inhibits CMV DNA polymerase, without undergoing phosphorylation. Hence, it is effective against most ganciclovir-resistant isolates
- ❑ **Cidofovir:** It is also given alternative to ganciclovir, but is nephrotoxic
- ❑ **CMV immunoglobulin:** It is given along with ganciclovir for the treatment of CMV infections in bone marrow transplant recipients.

Antiviral resistance can occur rarely due to mutations in target genes. For example, UL97 mutation confers resistance to ganciclovir, while UL54 mutation leads to resistance to ganciclovir, cidofovir, and foscarnet.

Prophylaxis

- ❖ Both ganciclovir and valganciclovir have been used successfully for prophylaxis and pre-emptive therapy in transplant recipients

- ❖ CMV immunoglobulin has shown to be effective in preventing congenital infection, when given to mother during pregnancy.

NEONATAL HERPES

Neonatal herpes (caused by herpes simplex virus) is rare, occurring in an estimated 10 out of every 100,000 births globally, but is a serious condition that can lead to neurologic disability or death.

- ❖ **Transmission:** Newborns acquire HSV infection most commonly during birth from the maternal genital tract. However, transmission can also occur in utero or after birth
- ❖ **Risk** of developing neonatal herpes is maximum (10 times more) if the mother recently acquires the virus (primary infection) than those who present with recurrent infection
- ❖ **HSV-2** is more common to cause neonatal herpes (50–70% of total cases) than HSV-1
- ❖ **Clinical features:** It presents as two forms: neonatal herpes or congenital herpes
- ❖ **Neonatal herpes:** Babies are almost always symptomatic and present in one of the three forms:
 1. **Local lesions** involving skin, eye and mouth (SEM) are the most frequent manifestations; typically presents at 9–11 days of life
 2. **Disseminated:** Presents with septic appearance, with deranged liver function tests; typically presents at 9–11 days of life
 3. **CNS infections:** Among HSV infected people neonates are at higher risk of CNS infections (encephalitis).
- ❖ **Congenital herpes:** In utero infection presents with vesicles or scarring, eye disease microcephaly or hydrocephalus
- ❖ **Mortality:** Neonatal herpes is associated with high mortality (65% without treatment)
- ❖ **Diagnosis:** It is done by PCR of vesicle lesions, blood or CSF. For asymptomatic infants born to women with active herpes obtain swabs from conjunctiva, nasopharynx, mouth or anus
- ❖ **Treatment:** Neonates with presumed herpes infection should be treated with antiviral drug such as acyclovir for 6–12 months
- ❖ **Prevention:** Treatment of genital herpes in mother before delivery and prevention of acquiring a new genital herpes infection in late pregnancy are the key measures to prevent neonatal herpes.

OTHER TERATOGENIC AGENTS

Varicella-zoster Virus (VZV) Infection

Chickenpox in pregnancy can affect both mother and the fetus.

- ❖ Mothers are at high-risk of developing varicella pneumonia
- ❖ Fetus can develop two types of infection; depending upon the gestational period at which it acquires infection.

Fetal or Congenital Varicella Syndrome (Infection in Early Pregnancy)

Transmission: Varicella-zoster virus (VZV) is highly teratogenic. Risk of transmission is maximum when mother:

- ❖ Acquires primary infection during pregnancy
- ❖ Acquires infection at early pregnancy (risk of transmission is 25%, whereas risk of developing into disease is 0.4% in <12 weeks and 2% in 13–20 weeks of gestation).

Manifestations: Congenital varicella syndrome is characterized by:

- ❖ Cicatricial skin lesions and limb hypoplasia (most characteristic anomalies)
- ❖ CNS defects: Microcephaly, mental retardation and seizures
- ❖ Ocular defects: Chorioretinitis, microphthalmia, and cataract
- ❖ Renal system defects: Hydroureter and hydronephrosis
- ❖ Autonomic system defect: Neurogenic bladder
- ❖ Low birth weight.

Neonatal Varicella (Infection Near Delivery)

- ❖ If mother develops chickenpox more than 5 days before delivery—then baby is mostly asymptomatic due to protective maternal antibody which prevents the transmission of the virus during delivery
- ❖ If mother develops chickenpox 5 days before to 2 days after the delivery—maternal antibodies would not have produced in such a short time. Therefore, there occurs dissemination of virus in the baby to cause **neonatal varicella**; a severe form of chickenpox with mortality rate exceeding 30%.

VZV causes exanthematous lesions in children (called chickenpox) and shingles or zoster (in adults); discussed in detail in Chapter 56.

Parvovirus Infection

Parvovirus can cause **non-immune hydrops fetalis** in fetus, which results in fatal anemia and fetal death. Transplacental transmission occurs in 30% of cases and maximum risk is in the second trimester. In children, it causes exanthematous lesions (called fifth disease), discussed in Chapter 56.

Zika Virus Disease

Zika virus (ZIKV) has recently gained attention due to recent large outbreaks that occurred in 2015–16 worldwide. It is an arbovirus; possesses ssRNA virus, belongs to family Flaviviridae. Monkeys are the reservoirs. It is named after its place of discovery (1947), Zika Forest in Uganda.

Transmission

ZIKV is primarily transmitted by *Aedes aegypti* mosquito. Other modes include—mother-to-child transmission (common in first trimester) and rarely through sexual contact, blood transfusion and organ transplantation.

Epidemiology

Till date, a total of 87 countries have had evidence of ZIKV infection. Outbreaks have been recorded in Africa, the Americas, Asia and the Pacific; the largest outbreak occurred in 2015–16 in Brazil.

- ❖ **Brazil outbreak (2015–2016):** It began in Brazil in April 2015 and then subsequently spread to other countries in America, the Caribbean, Europe and Australia
 - **Worldwide**, >5 lakh cases have been reported with 18 deaths; out of which Brazil alone witnessed >2 lakh cases
 - In February 2016, the WHO declared the Zika virus outbreak a public health emergency of international concern.
- ❖ **Situation in India:** In 2018, about 290 cases were reported in India, which include an outbreak in Rajasthan (153 cases), Madhya Pradesh (130 cases) and one case from Gujarat.

Clinical Manifestations

Incubation period ranges from few days to 1 week. Majority (>80%) of infections are asymptomatic.

- ❖ **Zika Fever:** Symptomatic people develop minor illness such as fever with rash and conjunctivitis
- ❖ **Congenital Zika syndrome (CZS):** It is characterized by microcephaly and other neurological manifestations
- ❖ **Neurological complications** may rarely be seen such as Guillain-Barré syndrome, neuropathy and myelitis.

Laboratory Diagnosis

The following laboratory tests are performed for confirmation of ZIKV disease.

- ❖ **Nucleic acid testing (NAT):** RT-PCR has been the investigation of choice. It can detect ZIKV RNA in blood and urine up to 7 days of onset of symptoms

Multiplex real time RT-PCR has been commercially available in India targeting the non-structural 5 (NS5) region of ZIKV, non-structural protein 4 (nsP4) from chikungunya virus (CHIKV) and 3' untranslated region (3'UTR) of dengue viruses (DENV 1–4).

- ❖ **IgM antibody detection:** It appears in blood after 1 week of symptoms and remains positive up to several months. It cross reacts with dengue antibodies
 - Enzyme immunoassays (EIAs) and immunofluorescence assays (IFA) are available, which use viral lysate, cell culture supernatant or recombinant proteins
 - They are investigation of choice, if the symptoms last ≥ 7 days.
- ❖ **Plaque-reduction neutralization test** is a more specific serological (antibody detection) test; but it is cumbersome, not widely used.

TREATMENT

Zika virus disease

No effective treatment and vaccine is available so far. Only symptomatic treatment is available such as fluid replacement and analgesic such as acetaminophen.

Prevention

ZIKV Vaccine

Though no vaccine has been licensed yet; several vaccine trials are going on for ZIKV such as ZIKV DNA vaccine (VRC 705) and killed ZIKV vaccine.

General Preventive Measures

The following general preventive measures are recommended.

- ❖ Mosquito control measures
- ❖ Infected patients should prevent mosquito bites for the first week of illness
- ❖ **Sex restriction:** CDC recommends for condom use or abstinence for at least 3 months for males and 2 months for females after travel to area with Zika outbreak or developing symptoms or ZIKV diagnosis
- ❖ **Pregnant women** should NOT travel to areas with Zika virus outbreaks.

Congenital Syphilis (*Treponema pallidum*)

Though transmission of *T. pallidum* across the placenta may occur at any stage of pregnancy, but fetal damage occurs only after fourth month of gestation. Untreated cases of early maternal syphilis are at higher risk. Antenatal screening and treatment of positive cases during pregnancy may prevent congenital syphilis.

Manifestations

Manifestations of congenital syphilis include:

- ❖ **Earliest manifestations** occur within 2 years of age. Affected children are infectious and they suffer from rhinitis (or snuffles), mucocutaneous lesions, bone changes, hepatosplenomegaly and lymphadenopathy
- ❖ **Late congenital syphilis** occurs after 2 years and is non-infectious. It is characterized by interstitial keratitis, eighth-nerve deafness, bilateral knee effusions (Clutton's joints). **Residual stigmata** may remain for long time such as:
 - Hutchinson's teeth (notched central incisors)
 - Mulberry molars (molars with poorly developed cusps)
 - Saddle nose, and saber shins.

Diagnosis of Congenital Syphilis

Definitive Diagnosis

Demonstration of *T. pallidum* by dark ground microscopy (DGM) of umbilical cord, placenta, nasal discharge, or skin lesion material provides the definitive diagnosis.

Presumptive Diagnosis

Infant born to a mother who had syphilis at the time of delivery regardless of findings in the infant *and*

- ❖ Reactive treponemal test in infant *plus*
- ❖ One of the following additional criteria in infant:
 - Clinical signs/symptoms of congenital syphilis
 - Abnormal CSF findings without other cause
 - Reactive VDRL-CSF test
 - Reactive IgM antibody test specific for syphilis (IgM FTA ABS or IgM ELISA).

Note: As IgM does not cross the placenta, its presence in neonatal serum is suggestive of the diagnosis of congenital syphilis.

Syphilis in adults presents as genital ulcers, discussed in detail in Chapter 77. It may develop into complications decades later, such as neurosyphilis (Chapter 71).

Congenital Trypanosomiasis

Rarely, *T. cruzi* can be transmitted transplacentally both in acute and chronic stages of the disease. It manifests as low birth weight, stillbirth, rarely myocarditis and neurological alterations. The diagnosis is made by the following methods.

- ❖ **Antibody detection:** Detection of IgM and IgA is useful in arriving diagnosis of congenital infection
- ❖ **Antigen detection:** *T. cruzi* specific antigens from serum and urine of the infected patients are detected by ELISA which are very useful for diagnosing acute infection and congenital transmission
- ❖ **Molecular methods:** PCR is available that detects *T. cruzi* specific kinetoplast or nuclear DNA in blood of the neonate.

T. cruzi causes Chagas' disease in adults, discussed in detail in Chapter 36.

Malaria in Pregnancy

Plasmodium falciparum can be transmitted by transplacental route; merozoites (or trophozoites) are the infective form.

- ❖ Malaria during pregnancy increases the risk of fetal distress and can result in premature labor, low birth weight and still birth. In areas with high malaria transmission, pregnant women are particularly vulnerable to severe anemia, hypoglycemia and acute pulmonary edema
- ❖ HRP2 antigen is a reliable marker to diagnose malaria in pregnancy
- ❖ In pregnant women, cord blood and placental impression smears are used to make thin and thick smears.

Malaria parasites are discussed in detail in Chapter 35.

EXPECTED QUESTIONS

I. Write short notes on:

1. Congenital toxoplasmosis.
2. Congenital rubella syndrome.
3. Congenital cytomegalovirus infection.
4. Congenital varicella syndrome.
5. Neonatal herpes.
6. Zika virus disease.

CHAPTER PREVIEW

- Oncogenic Viruses
 - Overview of Viral Oncogenesis
- Oncogenic RNA Viruses
- Oncogenic DNA Viruses
- Nonviral Oncogenic Organisms

Among the organisms with oncogenic potential, viruses account for the majority. Bacteria, parasites and fungi, which contribute to a minor proportion of malignancies of infectious origin are described subsequently in this chapter.

ONCOGENIC VIRUSES

Viruses account for 15% of all human malignancies. There are several oncogenic viruses found worldwide, which include the agents of two major vaccine preventable malignancies—Human papillomavirus causing carcinoma cervix and Hepatitis B virus causing liver cancer (Table 80.1).

OVERVIEW OF VIRAL ONCOGENESIS

Viral oncogenesis is a complex and multistep process requiring prolonged time (years to decades) and occur only in a small percentage of the infected individuals. There are multiple oncogenic events that take place in order to transform the host cells into cancer cells. Viruses contribute to only a portion of those oncogenic events. In addition, there are other factors necessary for oncogenicity to set in, such as host immunity and host genetic susceptibility, etc.

Before understanding the detailed mechanism of viral oncogenesis (which is discussed under individual viruses subsequently in this chapter), a knowledge about oncogenes and normal host genes regulating cellular growth is essential.

Oncogenes

Oncogenes encode certain proteins (oncoproteins) that trigger the transformation of normal cells into cancer cells.

- ❖ **V-onc (viral oncogenes):** Oncogenes present in the viral genome are called as **viral oncogenes (V-onc)**. They are essential for the replication of the virus. Viral oncogenes are expressed only by certain retroviruses (called as **acutely transforming retroviruses**)

Table 80.1: Human oncogenic viruses and associated malignancies.

Virus Family	Human Cancer
DNA oncogenic viruses	
Papillomaviridae/ Polyomaviridae	
Human papillomaviruses	<ul style="list-style-type: none"> ● Cervical carcinoma ● Other genital tract carcinomas: Anal, vulval/vaginal, penile ● Esophageal carcinoma ● Laryngeal carcinoma ● Oropharyngeal carcinoma
Merkel cell virus	<ul style="list-style-type: none"> ● Merkel cell carcinoma of skin
Herpesviridae	
Epstein-Barr virus	<ul style="list-style-type: none"> ● Burkitt's lymphoma ● Hodgkin's disease ● Nasopharyngeal carcinoma ● B cell lymphoma
Human herpesvirus-8	<ul style="list-style-type: none"> ● Kaposi's sarcoma ● Castleman's disease ● Primary effusion lymphoma
Hepadnaviridae	
Hepatitis B virus	Hepatocellular carcinoma
RNA oncogenic viruses	
Retroviridae	
HTLV-I	Adult T cell leukemia/lymphoma
HIV	AIDS-related malignancies
Flaviviridae	
Hepatitis C virus	Hepatocellular carcinoma

Note: The association of herpes simplex virus-2 with cervical cancer and cytomegalovirus with prostate cancer have not been proved yet. Molluscum contagiosum virus is not an oncogenic virus as the lesion produced (molluscum contagiosum) is a benign condition.

- ❖ **C-onc (cellular oncogenes):** They are the cellular counter part of viral oncogenes present in the cancer cells
- ❖ **Proto-oncogenes:** They are the cellular counter part of viral oncogenes present in the normal host cells.

Genes Regulating Host Cell Growth

There are four categories of genes present in the host cell, which regulate the cellular growth and proliferation. Defect in any of these regulatory genes would lead to transformation of the normal host cells into abnormal tumor cells.

1. **Proto-oncogenes:** They promote the host cell growth and proliferation that are essential for life. However, over activation of proto-oncogenes may lead to transformation of the host cells
 2. **Anti-oncogenes or tumor suppressor genes:** They continuously check cellular growth and proliferation, and suppress any abnormal proliferation of cells. Inactivation of tumor suppressor genes permits the abnormal event to occur resulting in cell transformation
 3. **Apoptosis-regulatory genes:** They control the programmed cell death by either upregulating or downregulating apoptosis depending on the requirement. Hence, they may act as proto-oncogenes or tumor suppressor genes. Mutations in apoptosis-regulatory genes are another mechanism by which the cellular transformation is accelerated
 4. **DNA repair genes:** They are the normal host genes that repair any mutations occurring during the cell growth. Failure of DNA repair genes lead to inability to repair the damaged DNA and may lead to persistent mutation.
- Host cells permissive for one virus may be non-permissive for another
 - Though oncogenicity can occur both in permissive and non-permissive cells, but the risk is more when a non-permissive cell is infected by a tumor virus as the virus tries different ways to maintain its survival in a non-permissive cell and by doing so it may undergo some changes, which makes the cell immortal
 - This holds true especially for DNA tumor viruses. In a permissive cell, the DNA tumor viruses are released by host cell lysis. Hence, the DNA tumor viruses are not oncogenic to a permissive cell, unless the viral replicative cycle that normally results in death of the host cell is blocked in some way; and grow indefinitely
 - In contrast, RNA tumor viruses do not cause cell lysis, hence, they can be oncogenic to both permissive and non-permissive cells.
- ❖ **Retention of viral nucleic acid inside the host cells** is essential to maintain a stable genetic change that occurs in a tumor cell

Events that Must Occur Before Oncogenesis

- ❖ **Establishing persistent infection:** Prolonged interaction between the tumor virus and the host cell is essential for oncogenesis to develop and this is possible only when the oncogenic virus establishes a long-term persistent infection in host cells
 - ❖ **Evades host immune response:** Host immune response plays an important role in viral clearance. The oncogenic virus follows various evasion mechanisms to bypass the host immune response, which are as follows:
 - By restricting the expression of viral genes which go unnoticed by the immune cells [e.g. Epstein-Barr virus (EBV) in B cells]
 - Infecting the sites that are relatively inaccessible to immune responses [e.g. human papillomavirus (HPV) infecting epidermis]
 - Undergoing mutation of certain genes that allows the virus to escape from the host cellular and humoral responses (e.g. HIV)
 - Infection and suppression of essential immune cells (e.g. CD4 T cell by HIV).
 - ❖ **Immunosuppression** of the host allows the cancer cells to proliferate and escape the host immune response. Immunosuppressed organ transplant recipients and HIV-infected individuals are at increased risk of EBV and HPV associated malignancies
 - ❖ **Host cell susceptibility:** Host cells may be permissive or non-permissive for replication of a given virus
 - Permissive cells support viral growth and replication of a progeny virus; non-permissive cells do not
 - Non-permissive cells refer to the host cells that either do not have surface receptors for viral attachment or do not support the viral replication or the release of virus progeny
- The DNA copies of DNA tumor viruses are integrated within the host cell chromosome
 - RNA of retroviruses gets reverse transcribed into DNA
 - Hepatitis C virus is an exception, its RNA is neither reverse transcribed, nor integrated into the host chromosome; but are maintained in the tumor cells.

ONCOGENIC RNA VIRUSES

Oncogenic Retroviruses

Retroviruses are classical example of oncogenic virus. They possess a unique reverse transcriptase enzyme, that reverse transcribes the viral RNA to DNA, which is subsequently inserted into host chromosome (Chapter 33). Oncogenic retroviruses are of two types.

- ❖ **Acutely-transforming viruses:** They possess **viral oncogenes** (*V. onc*) in their RNA which after being integrated with the host chromosome, are directly capable of inducing oncogenesis
 - They are highly oncogenic and cause malignancy within weeks to months
 - Examples include animal retroviruses such as Rous sarcoma virus. They do not infect man.
- ❖ **Slow transforming viruses:** Retroviruses infecting humans such as HIV and HTLV-1 are classical examples
 - They possess additional regulatory gene (e.g. *tax* gene for HTLV-1 and *tat* gene for HIV) which after inserting into host chromosome, activates the host cell machineries that subsequently induces oncogenesis
 - They are of low oncogenic potential, require a long latent period to develop malignancy.

Human T Cell Lymphotropic Virus (HTLV)

HTLV belongs to the family Retroviridae, under the genus *Deltaretrovirus*. Two important members are HTLV-I and HTLV-II.

Human T Cell Lymphotropic Virus-I (HTLV-I)

HTLV-I is a potential human oncogenic virus; associated with several malignancies—(1) Adult T cell leukemia/lymphoma, (2) cutaneous T cell lymphoma, and (3) tropical spastic paraparesis.

- ❖ **Transmission:** Occurs through various routes— (1) from mother to child especially via breast milk (most common), (2) sexual (men to women), (3) infected blood
- ❖ **Target cells:** Like HIV, HTLV-I also infects CD4 T cells. **GLUT1** (glucose transporter protein-1) on CD4 T cells acts as receptor for virus attachment
- ❖ **Mechanisms of oncogenesis:** HTLV-I expresses a unique gene called *Tax* gene which has oncogenic potential

Tax gene

- ❑ *Tax* gene is capable of **activating several cellular genes** involved in T cells proliferation such as gene for interleukin-2, myeloid growth factor, etc.
- ❑ **Promotes cell growth cycle:** *Tax* protein inactivates the cell cycle inhibitor p16/INK4a and activates cyclin D (a cell cycle enhancer), thus promoting the host cell growth cycle
- ❑ *Tax* gene activates **nuclear factor $\kappa\beta$** (NF- $\kappa\beta$), a transcription factor that regulates certain host anti-apoptotic genes
- ❑ *Tax* gene also interferes with **DNA-repair pathways** which leads to sustained DNA mutation.

- ❖ **Distribution:** HTLV-I is endemic in certain parts of Japan (10% prevalence) and the Caribbean, but also found sporadically elsewhere.

Human T Cell Lymphotropic Virus-II (HTLV-II)

HTLV-II is endemic in certain native American tribes and in Africa. Transmission and replication of HTLV-II is similar to that of HTLV-1. However, its pathogenic potential is uncertain.

AIDS Associated Malignancies

HIV/AIDS is associated several malignancies, which can be grouped into:

1. **AIDS-defining cancers:** These cancers have been enlisted in WHO clinical staging of HIV/AIDS (Chapter 33) which include:
 - Non-Hodgkin's lymphomas (NHLs): It remains the most common cancer in patients with HIV worldwide. Common NHLs include:
 - ♦ Burkitt's lymphoma (associated with EBV)
 - ♦ Diffuse large B cell lymphoma— often involving the CNS
 - ♦ Primary effusion lymphoma (associated with HHV-8)
 - Kaposi's sarcoma—in association with HHV-8
 - Invasive cervical carcinoma—in association with HPV.
2. **Non-AIDS-defining cancers:** Such as classical Hodgkin's lymphoma, anal cancers and lung cancers.

Mechanisms of Oncogenesis

There are several pathogenetic mechanisms that contribute to AIDS associated malignancies.

- ❖ Altered cytokine expression
- ❖ B cell stimulation
- ❖ Associated infections such as HPV, EBV, HHV-8 Genetic alteration
- ❖ *Tat* protein, an early nonstructural protein of HIV, necessary for virus replication, has also been linked to the pathogenesis of malignancies.

The introduction of antiretroviral therapy had led to a dramatic decline in the incidence of several AIDS-defining malignancies, particularly the incidence of Kaposi sarcoma.

Hepatitis C Virus Associated Malignancy

Hepatitis C virus (HCV, Chapter 48) is the only oncogenic virus that does not get integrated with host chromosome but its RNA remains in the host cell. It is strongly linked to the pathogenesis of liver cancer. The oncogenic mechanisms of HCV are less well defined than those of HBV.

- ❖ Similar to HBV, chronic liver cell injury and compensatory regeneration seems to be the main mechanism
- ❖ In addition, components of the HCV genome, such as the HCV core protein, may activate a number of growth promoting signal transduction pathways.

ONCOGENIC DNA VIRUSES

Oncogenic DNA viruses include EBV, HHV-8, HPV and HBV.

EBV Associated Malignancies

Epstein-Barr virus (EBV) mainly causes infectious mononucleosis (Chapter 68). In addition, it is associated with several malignancies:

- ❖ **Burkitt's lymphoma** (tumor of the jaw seen in children and young adults): EBV is associated with 90% of African and 20% of non-African cases of Burkitt's lymphoma
 - Most of the cases have pre-existing mutation [t(8;14)]
 - Falciparum malaria may impair host CMI and stimulates EBV-infected B cells.
- ❖ **Nasopharyngeal carcinoma:** It is seen among *Chinese* people who have history of intake of *salted fish* (nitrosamine) and herbal snuff (phorbol ester)
- ❖ **Hodgkin's lymphoma** (especially the mixed-cellularity type): EBV DNA is found in Reed-Sternberg cells, in at least 50% of cases of Hodgkin's lymphoma
- ❖ **NHL (Non-Hodgkin's lymphoma):** All CNS non-Hodgkin's lymphomas and 50% of systemic non-Hodgkin's lymphomas are EBV DNA or antibody positive.

Mechanism of Oncogenesis of EBV

Epstein-Barr virus infects B lymphocytes and possibly pharyngeal epithelial cells by attaching to the complement receptor (CR2) or CD21.

- ❖ EBV does not actively replicate inside the B cells thus does not cause lysis of B cells, but such latently infected B cells with EBV become immortalized and acquire the ability to grow indefinitely in cell lines
- ❖ Persistent EBV infection can induce malignant transformation of infected B cells and epithelial cells by expressing latent EBV antigens such as latent membrane protein (LMP) and EBNA (EBV nuclear antigen)
- ❖ Latent membrane protein-1 (**LMP-1**) is the most important viral oncogene
 - It is coated on the surface of the infected cells and behaves as active CD40 receptor, a key recipient of helper T cell signals that stimulate B cell growth
 - LMP-1 also activates the NF- κ B and JAK/STAT signalling pathways and promotes B cell survival and proliferation
 - LMP-1 prevents apoptosis by activating anti-apoptotic factor BCL2
 - It induces the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) which may contribute to the oncogenesis of nasopharyngeal carcinoma.
- ❖ **Viral EBNA-2** activates host cell **cyclin-D**, and the proto-oncogene *src*, thus promotes cell proliferation
- ❖ **VIL-10 (viral interleukin 10)**: It is a viral cytokine, which modulates the transformation of B cells.

Role of Host Immune Response and *c-MYC*

- ❖ Effective host immune response is crucial for preventing cell transformation. Oncogenicity is kept under control by anti-LMP-1 antibodies
- ❖ Thus, oncogenicity is markedly enhanced in immunosuppressed individuals who are not able to produce anti-LMP-1 antibodies
- ❖ More so, B cells in immunocompetent individuals can still undergo malignant transformation in presence of another pre-existing mutation (8;14) that in turn activates the growth promoting MYC oncogene.

Human Herpesvirus 8 Associated Malignancies

Human Herpesvirus 8 (HHV-8) was first discovered in 1994 in patients with Kaposi's sarcoma, hence also called Kaposi's sarcoma-associated herpesvirus (KSHV).

- ❖ **Epidemiology:**
 - **In high prevalence area:** HHV-8 is endemic in Africa, where it is transmitted by oral secretion
 - **In low prevalence areas** such as North America, Asia, northern Europe, it affects adults and it is transmitted by sexual route (homosexual men).
- ❖ **Disease association:** In immunocompromised individuals (e.g. HIV-infected people), HHV-8 is associated with:
 - **Kaposi's sarcoma** (Fig. 80.1): A soft tissue sarcoma of vascular origin; characterized by red to purple color growth under the skin, mouth, oral mucosa, lymph nodes, or in other organs



Fig. 80.1: Kaposi's sarcoma of the hard palate secondary to AIDS infection (arrow showing).

Source: Public Health Image Library, ID# 6070/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- **Primary effusion lymphoma** (body cavity-based lymphomas).

Mechanism of Oncogenesis of HHV-8

HHV8 usually infects the endothelial cells and/or hematopoietic progenitor cells.

- ❖ The transformation of malignant cells is directly related to the expression of early lytic genes of HHV-8 such as viral G protein-coupled receptor K1, **viral interleukin-6 (vIL-6)** and K15
- ❖ These genes induce the host cells to secrete the angiogenic, inflammatory and proliferative factors such as, vascular endothelial growth factor (VEGF), platelet derived growth factor- β , angiopoietin 2, IL-6 and IL-8 that amount to continuous growth and transformation of cells.

Human Papillomavirus (HPV) Infections

Human papillomavirus (HPV) has selective tropism for epithelium of skin and mucous membranes and produces an array of infections ranging from benign warts, to malignant neoplasia of cervix.

Morphology

Papillomaviruses are non-enveloped, measure 50–55 nm in size, have icosahedral capsids and contain a circular dsDNA.

Viral Genome

Viral genome consists of an early (E) region, a late (L) region, and a noncoding regulatory region.

- ❖ **Early region genes (E1-E7):** They code for early non-structural proteins. The E1 and E2 proteins modulate viral DNA replication. Products of early genes E6 and E7 have oncogenic potential, by following ways:

- *E6 protein* facilitates the degradation of the p53 tumor-suppressor protein
- *E7 protein* binds to the retinoblastoma gene product and related proteins.
- ❖ **Late region genes (L1 and L2):** They code for structural proteins such as capsid
- ❖ **Types:** More than 100 types of HPV are recognized based on DNA sequences of L1 region.

Clinical Manifestations

Human papillomaviruses typically infect skin (squamous epithelium) and mucous membranes and produce various benign and malignant lesions.

- ❖ **Benign warts:** They are small, hard, rough growth on the skin. They are of different types (Chapter 56), such as common skin warts, plantar warts, anogenital warts, etc.
- ❖ **Epidermodysplasia verruciformis:** It is a rare autosomal recessive benign condition, may progress to squamous cell malignancy (seen with serotypes 5, 8, 9, 12, 17, 20, 36, 47), particularly in sun-exposed areas
- ❖ **Cervical lesions:** Can produce both benign and malignant cervical lesions, depending upon the serotypes involved
 - CIN (Cervical Intraepithelial Neoplasia) is a benign condition, associated with low-risk serotypes 6 and 11
 - Carcinoma cervix (squamous cell) is associated with high-risk serotypes such as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. HPV serotype 16 accounts for up to 50% of cervical cancer (serotypes 16 and 18 together cause 70% of cervical squamous cell carcinomas)
 - High-risk serotypes can also cause squamous cell carcinoma in other genital regions such as penis, anus, vagina, and vulva.
- ❖ **Head and neck lesions:** Can produce both benign and malignant lesions
 - Benign lesions such as recurrent laryngeal papillomas in children (serotypes 6 and 11)
 - Malignant lesions such as laryngeal and esophageal carcinomas (serotypes 16 and 18).
- ❖ **Pityriasis versicolor** like lesions can be seen in immunosuppressed patients, particularly those who have undergone organ transplantation.

Mechanism of Oncogenesis (Fig. 80.2)

Human papillomavirus genome consists of an early (E) region, a late (L) region. The early region consists of seven genes (E1–E7), which code for early non-structural proteins. Products of early genes E6 and E7 have oncogenic potential.

- ❖ E6 enhances p53 degradation, thus inhibiting the activation of apoptosis promoting gene *bax*. It leads to inhibition of apoptosis and also inhibition of the p53 induced activation of tumor suppressor gene p21
- ❖ E7 inhibits the tumor suppressor gene RB (retinoblastoma gene) either by:

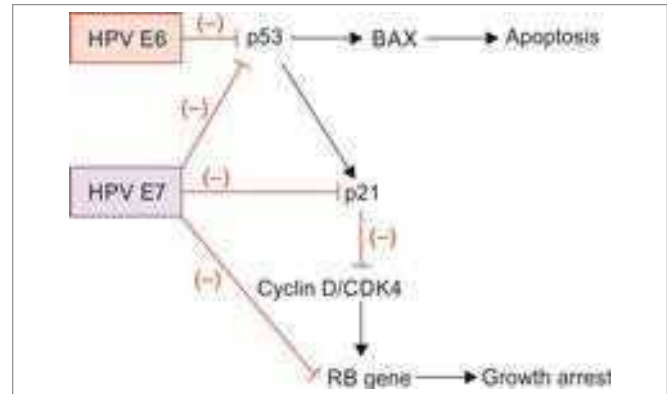


Fig. 80.2: Mechanism of oncogenesis by human papillomavirus

- Inhibiting p53
- Inhibiting p21
- Directly inhibiting RB gene.

However, HPV infection alone is not sufficient for carcinogenesis. Co-transfection with *v-fos* DNA results in full malignant transformation. There are several other factors that also have been implicated in the pathogenesis of HPV-induced malignancies such as:

- ❖ Presence of other genetic co-factors like mutated *RAS* gene
- ❖ Cigarette smoking
- ❖ Coexisting microbial infections
- ❖ Dietary deficiencies
- ❖ Hormonal changes.

Laboratory Diagnosis

- ❖ **Molecular methods:** PCR or the hybrid capture assay can be used to detect HPV DNA and to identify specific virus types by targeting genes coding for E6 and E7 regions
- ❖ Most lesions are visible to the naked eye. Solutions of **5% acetic acid** can be applied to improve visibility
- ❖ **Cytologic evidence** of HPV infection is detected by:
 - Papanicolaou smears prepared from cervical or anal scrapings
 - Histopathological staining of biopsies.
- ❖ **Antibody** detection is not much useful.

TREATMENT

HPV malignancies

- ❑ **Removal of the lesions:** Frequently used procedures for removal of lesions include cryosurgery, electrodesiccation, surgical excision and laser therapy
- ❑ **Topical preparations** of podophyllum, interferon or imiquimod (interferon inducer) can be used for genital warts
- ❑ Recurrence is common.

Prevention (HPV Vaccine)

Recently developed HPV vaccines have shown dramatic reduction in the rates of all HPV infections including cervical cancers. It is recommended to all adolescent boys and girls at ages 11–12 years.

- ❖ Subunit vaccine consists of virus-like particles composed of HPV L1 proteins which are produced in yeast by DNA recombinant technology
- ❖ Both nine valent and bivalent vaccines are licensed
 - **Nine valent vaccine** (Gardasil 9, Merck): Includes seven common cancer-causing serotypes (16, 18, 31, 33, 45, 52 and 58) and two noncancer causing serotypes (6 and 11)
 - ♦ It is given IM; at 11–12 years, as 2 doses (at least 6–12 months gap). If first dose is given after 15 years of age, then 3 doses are recommended (0, 2 and 6 months)
 - ♦ HPV vaccination is also recommended for everyone through age 26 years (if not vaccinated already)
 - ♦ Immune response is better if given earlier (<14 yrs)
 - ♦ Protection appears to be long lasting.
 - **Quadrivalent vaccine:** It was the earlier version, which included type 6, 11, 16 and 18. It is still in use
 - **Bivalent vaccine** (Cervarix, GSK) includes only the high-risk serotypes 16 and 18. It is given as single dose, IM.

Barrier methods of contraception can block sexual transmission, thus prevent anogenital HPV infections.

Hepatitis B Virus Associated Malignancy

Hepatitis B virus (HBV), in adjunction with hepatitis C is responsible for 70–85% of hepatocellular carcinomas worldwide. This can be largely prevented by HBV vaccination (Chapter 48).

Mechanism of Oncogenesis

Although not fully elucidated, there are several mechanisms proposed for the oncogenesis of HBV. The HBV genome does not contain any oncogenes, however; it gets integrated with the host genome randomly in the target cells.

- ❖ **Immunologically mediated chronic inflammation** appears to be the most dominant mechanism in the pathogenesis of viral-induced hepatocellular carcinoma
 - In chronic viral infection, hepatocellular injury occurs which is compensated by proliferation of hepatocytes. During the regenerative process, a plethora of growth factors, cytokines, chemokines, and other bioactive substances are produced by the activated immune cells which promote cell survival, tissue remodeling and angiogenesis
 - The activated immune cells also produce reactive oxygen species, that are genotoxic and mutagenic
 - One key molecular step seems to be activation of the NF- κ B pathway in hepatocytes which in turn blocks apoptosis, allowing the dividing hepatocytes to incur genotoxic stress and to accumulate mutations.
- ❖ **Hepatitis B X gene (HBx)**, a regulatory gene in HBV genome, can activate the transcription of cellular and viral genes

- ❖ **Deletion of tumor suppressor genes:** Integration of viral DNA with the host genome can cause secondary rearrangements of chromosomes which may lead to deletion of tumor suppressor genes.

NONVIRAL ONCOGENIC ORGANISMS

Bacteria, parasites and fungi contribute to a minor proportion of malignancies of infectious origin (Table 80.2).

HELICOBACTER PYLORI

Gastric colonization with *H. pylori* is associated with pathogenesis of peptic ulcer disease (Chapter 43). It also serves as the most important risk factor for several malignancies such as gastric adenocarcinoma and gastric MALT (mucosa-associated lymphoid tissue) lymphoma.

- ❖ In contrast, lifelong *H. pylori* colonization may offer some protection against certain cancers such as esophageal adenocarcinoma
- ❖ **Mechanisms:** *H. pylori* colonization induces genetic polymorphisms in man leading to enhanced activation of the innate immune response—polymorphisms in cytokine genes (IL-1) or genes encoding bacterial recognition proteins such as Toll-like receptors (TLRs)
- ❖ **Risk factors:** Environmental cofactors are important in pathogenesis of gastric cancer; such as smoking and diets high in salt and preserved foods. In contrast, diets high in antioxidants and vitamin C are protective
- ❖ **Treatment for *H. pylori*** is useful for patients with low-grade gastric MALT lymphoma. In contrast, it has no benefit in the treatment of gastric adenocarcinoma. However, prevention of *H. pylori* colonization could definitely contribute in preventing gastric malignancy.

Table 80.2: Malignancies associated with bacteria, parasites and fungi.

Bacteria	Malignancies
<i>Helicobacter pylori</i>	Carcinoma of stomach Marginal zone B cell lymphoma of stomach
<i>Bacteroides fragilis</i>	Colon cancer
Parasites	
<i>Schistosoma haematobium</i>	Carcinoma of bladder (squamous cell carcinoma)
<i>Schistosoma japonicum</i>	Colorectal carcinoma Carcinoma of liver
<i>Clonorchis sinensis</i>	Carcinoma of bile duct (Cholangiocarcinoma)
<i>Opisthorchis viverrini</i>	Cholangiocarcinoma Carcinoma of liver
Fungi	
<i>Aspergillus flavus</i> producing aflatoxin	Carcinoma of liver

SCHISTOSOMA HAEMATOBIMUM

S. haematobium (Chapter 76) induces metaplastic changes in urinary mucosa may lead to carcinoma of bladder.

- ❖ **Predisposing factors** include:
 - Diet containing nitroso-compounds intake, commonly found in Egyptian food (cheese, fava beans, raw salted fish)
 - Secondary bacterial infections, causing cystitis
 - Genetic factors: Such as activation of *H-ras*, inactivation of p53 and retinoblastoma genes
- ❖ **Type:** Squamous cell carcinoma is the most common type. It is seen with high to moderate worm burden; whereas transitional cell carcinoma may occur in areas with lighter worm load.

CLONORCHIS AND OPISTHORCHIS

Clonorchis sinensis and *Opisthorchis viverrini* cause chronic irritation of the bile duct for long periods, which can lead to bile duct carcinoma (cholangiocarcinoma). In addition, *Opisthorchis* is also associated with carcinoma of liver (Chapter 49).

- ❖ Risk factors for the bile duct carcinoma include elderly people (60–80 years old) and pre-existing primary sclerosing cholangitis
- ❖ Inhibition of tumor suppressor genes (p53) and release of cytokines such as IL-6 and TNF are the underlying mechanism of oncogenesis postulated.

EXPECTED QUESTIONS

I. Write short notes on:

1. Mechanism of viral oncogenesis.
2. Epstein-Barr virus associated malignancies.
3. Human papillomavirus associated malignancies.
4. Enumerate the organisms with oncogenic potential.

II. Multiple Choice Questions (MCQs):

1. All of the following are oncogenic RNA viruses **except**:
 - a. Hepatitis B virus
 - b. Hepatitis C virus
 - c. HIV
 - d. Varicella-zoster virus

2. Epstein-Barr virus is associated with the following malignancies **except**:

- a. Nasopharyngeal carcinoma
- b. Burkitt's lymphoma
- c. Carcinoma of cervix
- d. Non Hodgkin lymphoma

3. Highest risk of carcinoma cervix (squamous cell) is associated with which of the following HPV serotypes?

- a. Serotypes 6 and 11
- b. Serotypes 16 and 18
- c. Serotypes 2 and 4
- d. Serotypes 27 and 57

Answers

1. d 2. c 3. b

CHAPTER PREVIEW

- Common Zoonotic Infections
 - Plague (*Yersinia pestis*)
 - Tularaemia (*Francisella tularensis*)
 - Bite Wound Infections

INTRODUCTION

A zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans. Animals thus play an essential role in maintaining zoonotic infections in nature. Zoonoses may be associated with bacterial, viral, or parasitic, or due to fungal agents (Table 81.1).

Classification

Zoonotic diseases can be classified in terms of their reservoir hosts as:

- ❖ **Anthropozoonoses:** Infections transmitted from animals to man
- ❖ **Zooanthroponoses:** Infections that are transmitted from man to animals

- ❖ **Amphixenoses:** Infections that are maintained in both man and animals that may be transmitted in either direction.

COMMON ZOOONOTIC INFECTIONS

Few zoonotic infections are discussed here—plague (*Yersinia pestis*), tularaemia, and bite wound infections such as pasteurellosis, *Capnocytophaga* infection and rat bite fever. The reminders of the zoonotic infections have been discussed under the respective systems which they principally infect.

PLAGUE (YERSINIA PESTIS)

The tribe Yersiniaceae comprises of genus *Yersinia* which contains three well-established human pathogens.

Table 81.1: Important zoonotic infections affecting human beings and their usual sources.

Bacteria	Animals	Viruses	Animals	Fungi	Animals	Parasites	Animals
Anthrax	Herbivores	Rabies	Dogs	Zoophilic dermatophytoses		<i>Toxoplasma</i>	Cats
Plague	Rat	Yellow fever	Monkeys	<i>Trichophyton equinum</i>	Horse	<i>Leishmania</i>	Dogs
Brucellosis	Sheep, goat, camel	Japanese B encephalitis	Pigs	<i>Trichophyton simii</i>	Dogs, poultry	<i>Taenia</i>	Pigs, cattle
<i>Pasteurella</i> infection	Dog, cat	Kyasanur forest disease	Monkeys	<i>Microsporum canis</i>	Dogs	<i>Echinococcus</i>	Dog
<i>Capnocytophaga</i> infection	Dog	Chikungunya	Monkeys	<i>Microsporum equinum</i>	Horse	<i>Cryptosporidium</i>	Cattle
Rat bite fever	Rodents	Monkey pox	Monkeys	<i>Sporothrix</i>	Cats	<i>Fasciolopsis buski</i>	Pigs, cattle
Leptospirosis	Rodents	Prion diseases	Cattle	<i>Malassezia</i>	Dogs, cats	<i>Trichinella</i>	Pigs
Non-typhoidal salmonellosis	Poultry	Hemorrhagic fevers	Rodents, cattle, wild animals	<i>Cryptococcus</i>	Wide variety of animals, birds	Hookworms and Roundworms	Dogs and cats
Bovine tuberculosis	Cow	Influenza	Pigs, birds	<i>Penicillium marneffeii</i>	Bamboo rats	<i>Dirofilaria</i> and zoonotic <i>Brugia</i> species	Dogs, cats, raccoons, etc.
Endemic typhus	Rodents			<i>Lacazia loboi</i>	Dolphins		
Tularaemia	Rabbits			<i>Conidiobolus</i>	Horses		
				<i>Histoplasma</i>	Cattle, sheep		
				<i>Coccidioides</i>	Dogs		
				<i>Paracoccidioides</i>	Dogs		
				<i>Blastomyces</i>	Dogs		
				<i>Pneumocystis jirovecii</i>	Rodents		

- ❖ *Yersinia pestis*: It was isolated for the first time by Alexandre Yersin in 1894 in Hong Kong. It is the agent of **plague**; a fulminant systemic zoonosis, transmitted from rodents by arthropod vector (the rat flea)
- ❖ *Y. pseudotuberculosis* and *Y. enterocolitica*—both cause **yersiniosis**, a self-limiting gastrointestinal illness that may occasionally have serious complications in special circumstances (discussed in Chapter 41).

Epidemiology of Plague

Plague is the one of the greatest killer known to mankind.

Plague Pandemics

There were three pandemics reported in the history, each was associated with a different biotype of *Y. pestis*, differentiated by glycerol fermentation and nitrate reduction.

- ❖ **First pandemic (in AD 541)**: It occurred in the period of Roman Emperor Justinian; caused by biotype 'Medievalis'. It is estimated to have claimed nearly 100 million victims
- ❖ **Second pandemic** (in 14th century) was called **black death**, which had killed up to one-third of the European population (over 50 million deaths). It was caused by biotype 'Antiqua'
- ❖ **Third pandemic (1894)**: It started in Hong Kong. It mainly affected India and China causing more than 10 million deaths by 1918. It was caused by biotype 'Orientalis'.

Timeline of Plague in India

- ❖ **1896 to 1918**: Hong Kong pandemic entered India and millions of people were killed
- ❖ **1918 to 1967**: Plague gradually declined, occasional cases continued to be reported from endemic foci
- ❖ **1967 to 1994**: No plague cases were reported
- ❖ **1994 (Surat epidemic)**: It started as bubonic plague from Beed-Latur belt in Maharashtra. But, it soon became pneumonic plague and spread to Surat and adjoining regions of Gujarat. More than 6,000 suspected plague cases with 60 deaths were reported over a period of two months (August-September 1994)
- ❖ **In 2002 (Shimla outbreak)**: A small outbreak occurred at Rohru, near Shimla. Four deaths were reported
- ❖ **In 2004 (Uttarkashi outbreak)**: Localized outbreak of bubonic plague (8 cases and 3 deaths) was reported from Dangud village of Uttarkashi district, Uttaranchal
- ❖ **Four potential endemic foci** are there in India at present which include—(1) region near Kolar, Karnataka, (2) Beed-Latur belt in Maharashtra, (3) Rohru in Himachal Pradesh, and (4) Dangud village, Uttaranchal.

Current Situation in World

- ❖ Since 1990s, most human cases have occurred in **Africa**. Currently, three most endemic countries are the Democratic Republic of the Congo, Madagascar, and Peru

- ❖ From 2010 to 2015 there were 3,248 cases reported worldwide, including 584 deaths.

Epidemiological Factors

- ❖ **Reservoir**: Wild rodents, such as gerbils (*Tatera indica*), field mice and the bandicoot found in forests are the main reservoirs of *Y. pestis* in India than the domestic rats *Rattus rattus* as once thought
- ❖ **Source of infection** are infected wild rodents, rat fleas and cases of pneumonic plague
- ❖ **Vector**: Rat flea is the commonest vector of *Y. pestis*, which acquires infection by feeding on infected wild rodents
 - Several species of rat flea may act as vectors such as *Xenopsylla cheopis* (the most efficient vector, found in North India) and *Xenopsylla astia* (less efficient, found in South India) and *Xenopsylla brasiliensis*
 - Human flea (*Pulex irritans*) may rarely serve as vector.
- ❖ **Mode of transmission**: Human plague is frequently contracted from:
 - Bite of an infected rat flea (most common)
 - Direct contact with tissues of infected animal (rodents)
 - Droplet inhalation (man to man) from cases of pneumonic plague
 - Bite of an infected human flea (*Pulex irritans*).
- ❖ **Blocked flea**: In a blood meal, the fleas suck about 0.5 mL blood containing 5,000 bacilli from infected rodents
 - In the gut of the flea, the bacilli multiply enormously and may block the proventriculus. Such blocked flea eventually dies as it cannot obtain a blood meal
 - However, while making efforts to suck, it regurgitates the blood mixed bacteria into the bite, thus transmitting the infection
 - Infection may also be transmitted by contamination of the bite wound with the feces of infected fleas
 - A partially blocked flea is more dangerous than a completely blocked flea as it survives longer inside the burrows, may be up to 4 years in certain species
 - **Extrinsic incubation period** is the interval between the flea acquiring the infection through blood meal and becoming a blocked flea; which is usually about two weeks for *Xenopsylla cheopis*.
- ❖ **Cheopis index** (Average number of *X. cheopis* per rat) is the most significant flea index. Plague outbreak is likely to occur in places having cheopis index of more than 1
- ❖ **Seasonality**: Plague is seasonal in North India (September to May). However, in South India, it occurs throughout the year which maybe attributed to the climatic conditions of South favoring the rodents to breed.

Virulence Factors of *Y. pestis*

- ❑ **Fraction 1 (F1) antigen**: It is capsular protein antigen, encoded by a plasmid (pFra). It is the principle virulence factor; acts by inhibiting phagocytosis by macrophage. It is highly antigenic and is used as immunodiagnostic marker of infection

Contd...

Contd...

- ❑ **Other virulence factors** include—Phospholipase D (murine toxin), surface proteases, pH 6 antigen, lipopolysaccharide (endotoxin), pigments (hemin-containing), type III secretion system, adhesins (help in attachment), and siderophore (helps in acquisition of iron)

Human Plague: Clinical Types

Human plague occurs in three clinical forms—(1) bubonic (most common form), (2) pneumonic, and (3) septicemic. The case-fatality ratio is nearly 30–60% for the bubonic type, and is always fatal for the pneumonic type (30–100%), when left untreated.

Bubonic Plague

It is the most common type, transmitted by the bite of an infected rat flea.

- ❖ Bacilli pass through the local lymphatics to reach the regional lymph nodes, where they multiply
- ❖ Incubation period is about 2–7 days
- ❖ The onset is sudden and is characterized by fever, malaise, headache and painful lymphadenitis
- ❖ **Buboes:** Regional lymph nodes appear as tense, tender swellings called buboes; the most common site being inguinal (Fig. 81.1A), but can also be crural, axillary, cervical, or submaxillary, depending on the site of the bite. Children are most likely to present with cervical or axillary buboes
- ❖ Bubonic plague cannot spread from person to person as the bacilli are locked up in buboes
- ❖ Without treatment, dissemination occurs leading to pneumonia (secondary) and meningitis.

Pneumonic Plague

Primary pneumonic plague results from inhalation of bacilli in droplets expelled from another person or an animal with plague pneumonia.

- ❖ Incubation period is short, about 1–3 days



Figs 81.1A and B: Plague manifestations: **A.** Swollen inguinal lymph node (bubo); **B.** Gangrene of the toes turned the dead digits black (black death).

Source: Public Health Image Library, A. ID#2044, B. ID#16550, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- ❖ The onset is sudden and is characterized by fever, headache and respiratory symptoms (productive cough or hemoptysis, dyspnea, and chest pain)
- ❖ Though pneumonic plague is rare (<1%), it is highly infectious and highly fatal
- ❖ Agent of bioterrorism— aerosolized *Y. pestis* is a possible source of bioterrorism attack, especially in non-endemic regions.

Septicemic Plague

- ❖ Primary septicemic plague is rare except for accidental laboratory infections
- ❖ Secondary septicemic plague is more common. It develops from spread of bubonic or pneumonic plague
- ❖ Incubation period is about 2–7 days
- ❖ Massive involvement of blood vessels results in hemorrhages in the skin and mucosa which may lead to gangrene of the affected site; hence disease was named in the past as **black death** (Fig. 81.1B).

Agent of bioterrorism: Because of the highly infectious nature, *Y. pestis* is currently classified as category A agent of bioterrorism.

Laboratory Diagnosis

Specimen Collection

Depending upon the type of plague, the specimens collected are:

- ❖ Bubonic plague—pus or fluid aspirated from buboes
- ❖ Pneumonic plague—sputum and blood
- ❖ Septicemic plague—blood and splenic aspirate (post-mortem).

Transport medium (e.g. Cary–Blair medium) can be used if delay in transportation is expected.

Safety precautions such as biosafety level III must be used to handle clinical specimens to avoid the risk of laboratory-acquired infection.

Direct Microscopy

- ❖ **Gram staining:** Reveals presence of pus cells and gram-negative oval coccobacilli with rounded ends surrounded by capsule
- ❖ **Wayson stain** or methylene blue staining demonstrates the bacilli with typical **bipolar** or **safety pin** appearance. Two ends are darkly stained with clear central area (Fig. 81.2).

Culture

Y. pestis is aerobic and facultatively anaerobic. Various culture media used are:

- ❖ Blood agar: Colonies are non-hemolytic and **dark brown** pigmented due to the absorption of the hemin pigment
- ❖ MacConkey agar: Lactose non-fermenting colorless colonies are formed.

Culture Smear and Motility Testing

- ❖ Gram staining of culture smear reveals **pleomorphism**—coccid, coccobacillary, bacillary, filamentous and



Fig. 81.2: Wayson staining demonstrates bipolar appearance of *Yersinia pestis* (arrow showing).

Source: Centers for Disease Control and Prevention (CDC) Atlanta, USA and Jawetz, Melnick, & Adelberg's Medical Microbiology; McGraw-Hill Education (with permission).

giant forms. Involution forms are seen in older cultures

- ❖ *Y. pestis* is nonmotile both at 25°C and 37°C; in contrast to other *Yersinia* species which are motile at 25°C and nonmotile at 37°C.

Identification

Identification of *Yersinia pestis* from colonies is made either by automated identification systems such as MALDI-TOF; or by conventional biochemical tests as described below.

- ❖ It is catalase positive and oxidase negative
- ❖ **ICUT tests:** Indole test (negative), citrate test (negative), urease test (negative) and TSI (triple sugar iron agar) test shows alkaline/acid, gas absent, H₂S absent
- ❖ MALDI-TOF can be used for rapid accurate identification of *Y. pestis* and also to differentiate its three biotypes.

F1 Antigen Detection

It may be detected from bubo aspirate or sputum by direct immunofluorescence test, ELISA or immunochromatographic test (ICT) by using monoclonal antibodies.

Antibodies to F1 Antigen Detection

Antibodies may be detected by ELISA, or passive agglutination.

- ❖ Antibodies have a limited diagnostic value as they appear late. Only a retrospective diagnosis can be made if fourfold rise of titer is noted
- ❖ However, antibodies are useful epidemiological markers, as they remain positive for several years.

Molecular Methods

PCR is available targeting gene coding F1 antigen, *pesticidin* gene, and the *plasminogen activator* gene.

T TREATMENT

Plague

Early start of antibiotics is crucial for reducing mortality.

- ❑ Streptomycin has been the choice of treatment for plague in the past, given for 10 days
- ❑ Gentamicin is superior to streptomycin and currently recommended for treatment
- ❑ Levofloxacin has recently been approved for treatment and for post-exposure prophylaxis
- ❑ Alternative drugs, such as doxycycline and chloramphenicol are also effective
- ❑ β -lactams and macrolides are generally not recommended as the response is poor.

Prevention of Plague

Prevention and control of plague involves:

- ❖ **Control of cases** by early diagnosis, isolation and treatment of cases
- ❖ **Isolation precaution:** Contact precautions need to be followed for bubonic plague and droplet precautions for pneumonic plague (Chapter 21)
- ❖ **Control of fleas** by use of effective insecticides, such as DDT or BHC (β -hexachloro-cyclohexane)
- ❖ Control of rodents
- ❖ **Chemoprophylaxis** should be given to all contacts of pneumonic plague. Doxycycline or levofloxacin is the drug of choice, given for 7 days
- ❖ **Vaccine:** WHO recommends using vaccine only for prevention of an anticipated outbreak and not for general use
 - **Formalin killed vaccine** (Sokhey's modification of original Haffkine vaccine): It is prepared in Haffkine Institute, Mumbai
 - ◆ It is given subcutaneously, two doses 4 weeks apart and a booster given after 6 months. It is contraindicated in infants <6 months
 - ◆ Protection is short-lasting (<6 months)
 - ◆ It is not protective against pneumonic plague and has considerable side effects.
 - **Live attenuated vaccine** based on strain EV76 is still used in countries of the former Soviet Union but has significant side effects.

Plague in Rodents

Plague is primarily a disease of rodents in which man becomes accidentally involved. As an animal disease, plague is found in all continents, except Oceania. The plague bacillus is naturally parasite of rodents and the disease spreads among rodents by rat fleas. When a diseased rat dies (rat fall), the fleas leave the carcass and, in the absence of another rat, may bite human beings, causing bubonic plague. Plague in rodents is similar to that in man. The disease is mild or inapparent in resistant species.

TULARAEMIA (FRANCISELLA TULARENSIS)

Francisella tularensis is the causative agent of 'tularemia' primarily a plague-like disease of rodents and other small

animals. Human infection is zoonotic and usually results from:

- ❖ Interaction with biting or blood-sucking insects (especially ticks and tabanid flies)
- ❖ Ingestion of contaminated water or food
- ❖ Inhalation of infective aerosols.

Prevalence

F. tularensis has four subspecies: *tularensis*, *holarctica*, *novicida*, and *mediasiatica*.

- ❖ The first three subspecies are found in North America, whereas subspecies *mediasiatica* is found in central Asia
- ❖ Subspecies *tularensis* is the most common and the most virulent among all.

Clinical Manifestations

Tularemia is characterized by various clinical syndromes:

- ❖ **Ulceroglandular tularemia:** It is the most common form, accounting for 75–85% of total cases, characterized by ulcerative lesion at the site of inoculation, with regional lymphadenopathy
- ❖ **Other forms** include—Pulmonary, oropharyngeal, oculoglandular form and typhoid-like illness
- ❖ **Complications** such as: Suppurated lymph nodes, acute kidney injury, hepatitis, rhabdomyolysis, empyema, pericarditis, meningitis, osteomyelitis and endocarditis
- ❖ **Agent of bioterrorism:** Because of the highly infectious nature, *F. tularensis* is currently classified as category A agent of bioterrorism.

Laboratory Diagnosis

- ❖ **Culture:** Isolation is very difficult as *F. tularensis* is highly fastidious; needs special media, such as BCG agar (blood cysteine glucose agar)
 - **Specimen:** Ulcer scrapings, and lymph node biopsy are the preferred specimens
 - Safety precautions such as biosafety level III must be used to handle clinical specimens to avoid the risk of laboratory-acquired infection
 - **Species identification** from colonies is made either by conventional biochemical tests or by automated identification systems such as VITEK.
- ❖ **Antibody detection** is the mainstay of diagnosis as isolation is difficult. Agglutination tests (latex and tube agglutination) and ELISA formats are available
- ❖ **PCR assay** has been used to detect *F. tularensis* specific genes encoding the outer-membrane proteins. It can also differentiate subspecies.

TREATMENT

Tularemia

Gentamicin is considered as the drug of choice; given for 7–10 days. Doxycycline or ciprofloxacin can be given as alternatives.

BITE WOUND INFECTIONS

Bites and scratches from animals and humans allow the inoculation of microorganisms that are commonly found

in the animal's oral cavity, nose or nail. They cause a range of infections such as:

- ❖ Lodging of the organisms on the wound surface can cause bite-wound infections
- ❖ Can breach skin barrier and penetrate into the deeper tissue such as bone and joint, causing osteomyelitis and septic arthritis
- ❖ Invasion of lymphatics and blood can cause various systemic infections such as bacteremia, meningitis, brain abscess, and endocarditis. This is particularly common in individuals who have impaired hepatic function, undergone splenectomy, or who are immunosuppressed
- ❖ Rarely, infection of the cutaneous nerves can carry the organism to CNS (e.g. rabies causing encephalitis).

Dog Bites

Dog bites are responsible for 80% of all animal-bite wounds, of which about 15–20% become infected. Most of the dog bites are provoked by the victims/others and are inflicted by the victim's pet or by a dog known to the victim

- ❖ **Age/gender:** Victims are more often children than adults, and males than females
- ❖ **Site:** Bites most often involve an upper extremity, except for children <4 years who also get bites more frequently in head and neck region
- ❖ **Microbiology:** The organisms causing dog-bite wound infections are usually mixed
 - Common aerobes include β -hemolytic streptococci, *Pasteurella* species, *Staphylococcus*, *Eikenella corrodens*, and *Capnocytophaga canimorsus*
 - Many wounds also include anaerobic bacteria such as *Actinomyces*, *Fusobacterium*, *Prevotella*, and *Porphyromonas* species
 - Organisms causing systemic diseases: Rabies and tetanus.

Cat Bites

Cat bites and scratches though less common, are more likely to result in infection (>50% of cases) than dog bites.

- ❖ Compared to dog bite, they are also at a higher risk of causing **penetrating injury** leading septic arthritis and osteomyelitis (especially in the hand); owing to their narrow, sharp canine teeth
- ❖ Victims of cat bite are more often women than men
- ❖ **Microbiology:** The organism implicated in cat-bite wound infections is usually mixed, from cat's oropharynx; similar to that of dog bite
 - *Pasteurella multocida*
 - *Bartonella henselae*: Causes cat-scratch disease (Chapter 31)
 - Tularemia (*Francisella tularensis*)
 - *S. aureus*
 - Anaerobes
 - Organisms causing systemic disease such as rabies (rare) and tetanus.



Fig. 81.3: Clenched-fist injuries.

Human Bites

Human bites may take place during fights, domestic abuse, sexual activity or healthcare workers caring for patients.

- ❖ Human-bite wounds become infected less frequently (10–15% of the time) than the bites inflicted by animals
- ❖ **Types of human bites:** Human bites are categorized into two types
 - *Occlusional injuries*, which are inflicted by actual biting
 - *Clenched-fist injuries*: Occurs during fight, when the fist of one individual strikes the teeth of another. This is more common than occlusional injuries, result in serious deeper infections of hands, including the bones, joints, and tendons (Fig. 81.3).
- ❖ **Microbiology:** These infections reflect the diverse oral microbial flora of humans
 - Common aerobic isolates include viridans streptococci, *S. aureus*, *Eikenella corrodens* (common in clenched-fist injury), and *Haemophilus influenzae*
 - Anaerobic species, including *Fusobacterium nucleatum* and *Prevotella*, *Porphyromonas*, and *Peptostreptococcus* species
 - The oral flora of hospitalized patients often includes Enterobacteriaceae, non-fermenters in addition to the usual organisms.

Other Animal Bites

Other animal bites which are less common are bites from snakes, rats, monkeys and seals, etc.

- ❖ **Rat bite infections:** They are usually caused by *Streptobacillus moniliformis* (rat-bite fever), *Leptospira*, *Pasteurella multocida*
- ❖ **Snakebites** may become infected with *Pseudomonas*, *Proteus*, *Bacteroides fragilis*, *Clostridium*
- ❖ **Bites from Old World monkeys** (*Macaca*) may result in the transmission of B virus (*Herpesvirus simiae*), which is a cause of serious infection of the human central nervous system
- ❖ **Bites of seals**, walruses, and polar bears may cause a chronic suppurative infection known as **seal finger**,

which is probably caused by *Mycoplasma phocacerebrale*.

Laboratory Diagnosis for Bite Wound Infections

Bite wound infections usually involve relatively small lesions and minimal exudate. Surrounding of the bite wound skin should be thoroughly disinfected before the specimen is obtained.

- ❖ The best material for culture is **purulent exudate aspirated** from the depth of the wound or samples obtained during surgery involving incision and drainage or debridement (removal of all dead and necrotic tissue)
- ❖ The most common specimen is a **wound swab**, which is not suitable for anaerobic culture, unless immediately dipped into an anaerobic transport media
- ❖ Gram-stained smears should be prepared and examined
- ❖ For aerobic cultures, a minimum of blood, MacConkey and chocolate agar should be inoculated
- ❖ Anaerobic culture is necessary if abscesses, devitalized tissue, or foul-smelling exudate is present.

Agents causing Bite-wound Infections

Pasteurellosis

Pasteurella species are primarily harbored as normal flora in the oral cavity of cats and dogs; sometimes cause fatal diseases including hemorrhagic septicemia in animals. *Pasteurella multocida* is the most common species infecting man.

- ❖ **Clinical features:** In humans, *P. multocida* is the most common cause of wound infections after dog or cat bites
 - The affected area of bite becomes red, swollen and painful with regional lymphadenopathy and low grade fever
 - In more serious cases, bacteremia can result, causing an osteomyelitis or endocarditis or meningitis.
- ❖ **Laboratory diagnosis:** *P. multocida* is a gram-negative coccobacillus that readily grows in culture media. Identification is made biochemically or through automated methods such as MALDI-TOF or VITEK
- ❖ **Treatment:** Penicillin G or amoxicillin-clavulanate is considered as the drug of choice.

Capnocytophaga Infection

Several species such as *C. ochracea*, *C. gingivalis* and *C. sputigena* have been a part of human mouth flora.

- ❖ They occasionally cause periodontal diseases, and sepsis/meningitis in immunocompromised hosts
- ❖ Certain species such as *C. canimorsus* and *C. cynodegmi* are commensals in mouth of dogs and *C. canimorsus* can cause fulminant septicemia following dog bite (in asplenic patient)
- ❖ **Associated risk factors:** Include patients with anatomic or functional asplenia, heavy alcohol intake, or liver cirrhosis

- ❖ **Laboratory diagnosis:** They appear as fusiform or filamentous gram-negative coccobacilli
 - They are capnophilic (require CO₂), grow in enriched media (e.g. blood agar), and produce orange-pigmented colonies. They grow slowly, take up to 14 days
 - Identification from the colonies is made biochemically or through automated methods such as MALDI-TOF or VITEK.
 - ❖ **Treatment:** As they produce β-lactamases, β lactam/β-lactam inhibitor combination such as ampicillin-sulbactam is used as the drug of choice.
- Rat-bite Fever**
- Rat-bite fever (RBF) is characterized by septic fever, petechial rashes, and painful polyarthritis with frequent relapses. It is caused by either of the two pathogens: (1) *Streptobacillus moniliformis*, and (2) *Spirillum minus*.
- ❖ **Transmission:**
 - RBF is primarily transmitted by contact with rodents carrying these bacteria
 - Transmission also occurs through consumption of food or water contaminated with the urine and droppings of rodents carrying the bacteria. This is known as **Haverhill fever** or **epidemic arthritic erythema**.
 - ❖ *Streptobacillus moniliformis*: Causes RBF in North America
 - Gram-negative, highly pleomorphic nonmotile bacilli, which is frequently arranged in chains and tangled filaments with bulbous swellings. It has a tendency to form L-form
 - It can be isolated from blood, synovial fluid and other infected tissues.
 - ❖ *Spirillum minus*: Causes RBF in Asia (known as Sodoku)
 - They are rigid, spirally coiled motile bacilli
 - It doesn't grow in artificial media.
 - ❖ **Treatment:** Penicillin is the treatment of choice.

EXPECTED QUESTIONS

I. Write short notes on:

1. Clinical types of human plague.
2. Rat-bite fever.
3. Tularemia.
4. Bite wound infections.

II. Multiple Choice Questions (MCQs):

1. Rat bite fever is caused by:

- a. *Borrelia recurrentis*
- b. *Streptobacillus moniliformis*
- c. *Yersinia pestis*
- d. *Leptospira*

2. All the subspecies of *F. tularensis* are found in North America, except:

- a. *F. tularensis*
- b. *F. holarctica*
- c. *F. novicida*
- d. *F. mediasiatica*

3. Plague is transmitted by:

- a. Rat flea
- b. Soft tick
- c. Hard tick
- d. Louse

4. Bipolar staining is characteristic of:

- a. *Yersinia pestis*
- b. *Shigella*
- c. *Klebsiella*
- d. *Proteus*

5. Drug of choice for rat bite fever:

- a. Amikacin
- b. Cephalosporin
- c. Penicillin G
- d. Tetracycline

6. *Pasteurella multocida* mainly is transmitted by:

- a. Animal bite
- b. Insect bite
- c. Droplets
- d. Sexual contact

Answers

1. b 2. d 3. a 4. a 5. c 6. a

BACK-TO-COLLEGE TIPS

Protect Yourself from COVID-19

Watch your distance

Stay at least 6 feet apart from others, when possible



Wash your hands

or use hand sanitizer with at least 60% alcohol



Wear a mask

in public spaces and common areas



The more closely you interact with others and the longer that interaction, the higher the risk of COVID-19 spread



BEFORE YOU GO OUT,
TAKE THE FOLLOWING :

- Mask
- Tissues
- Hand sanitizer
- Disinfection wipes (if possible)

DORM

Avoid sharing items with roommates or others. If you do, clean and disinfect before sharing or using.

SHARED BATHROOM

Avoid placing toothbrushes directly on counter surfaces. Use totes for personal items to limit contact with other surfaces in the bathroom.

CLASSROOM

Enroll in online classes if they fit your educational needs. Wipe down your desk with a disinfectant wipe if possible. Skip seats or rows to create physical distance between other students. Avoid placing your personal items (e.g., cell phone) on your desk.

DINING HALL & MEALS

Avoid sharing food, drink, utensils or other items with people. Pick up grab-and-go options for meals, if offered. Avoid buffets and self-serve stations.

LAUNDRY ROOM

Clean and disinfect surfaces that others have touched (e.g., buttons on the washing machine). Wash masks in warmest appropriate water setting for the fabric.

Material is developed by CDC

Annexures

ANNEXURE OUTLINE

1. Opportunistic Infections
2. Post-transplantation Infections
3. Emerging and Re-emerging Infections
4. Bioterrorism—Biological Warfare
5. Laboratory-acquired Infections
6. National Health Programmes for Communicable Diseases
7. Vector-borne Infections and Ectoparasite Infestations
8. Transfusion-transmitted Infections
9. AETCOM in Microbiology
10. Pandemic Management

ROLE OF INDIAN MEDICAL GRADUATE (IMG)

A. Clinician who understands and provides preventive, promotive, curative, palliative and holistic care with compassion.

B. Leader and member of the health care team and system with capabilities to collect, analyze, synthesize and communicate health data appropriately.

C. Communicator with patients, families, colleagues and community.

D. Life-long learner committed to continuous improvement of skills and knowledge.

E. Professional, who is committed to excellence, is ethical, responsive and accountable to patients, community and profession.



*Adapted from Medical Council of India's
New Competency-based Curriculum for Medical Graduates*

Opportunistic Infections

Opportunistic infections are the infections which occur more often or are more severe in people with weakened immune systems than in people with healthy immune systems.

Risk factors for developing opportunistic infections include:

- ❖ **AIDS:** The most important clinical condition which is prone for acquiring opportunistic infections is HIV/AIDS. The list of opportunistic infections that frequently occur in patients with AIDS have been enlisted in Chapter 33, Table 33.3
- ❖ **Organ transplantation:** This is another important clinical condition, characterized by profound immunosuppression and therefore is prone for various opportunistic infections. This is discussed in Annexure 2
- ❖ **Diabetes mellitus:** The diabetes patients are at increased risk of acquiring infections, owing to various factors: (1) defective cell-mediated immunity, (2) hyperglycemia, (3) ketosis, and (4) poor vascularity. The common infections in diabetic patients are:
 - Mucocutaneous, vaginal candidiasis
 - Rhinocerebral mucormycosis (common in diabetic ketoacidosis)
 - Emphysematous infections of gallbladder
- Emphysematous pyelonephritis and emphysematous cystitis (common uropathogens like *E. coli* and *Candida*)
- Malignant otitis externa (*Pseudomonas*)
- Pneumonia (gram-negative organisms, *S. aureus*, *Mycobacterium tuberculosis*, etc.)
- Post-operative wound infections.
- ❖ **Steroid therapy:** Patients on prolonged systemic corticosteroid therapy are prone to develop various opportunistic infections including aspergillosis
- ❖ **Malignancies:** Patients with cancers are also at increased risk for various opportunistic infections (Table A1.1)
- ❖ **Immunodeficiency disorders:** T cell, B cell, and combined immunodeficiency disorders and granulocytopenia (Table A1.2) are at increased risk of infections (Chapter 18, Table 18.2)
- ❖ **Long-term hospitalization** and patients on a prolonged course of broad-spectrum antibiotics: They are prone to develop antibiotic-associated diarrhea, also called pseudomembranous colitis (caused by *Clostridioides difficile*)
- ❖ **Measles:** Patients with measles are at increased risk to develop secondary bacterial infections, diarrhea, etc.
- ❖ **Influenza:** Patients with influenza are more prone to develop secondary bacterial infections with *S. aureus*, *S. pneumoniae*, and *H. influenzae*.

Table A1.1: Opportunistic infections associated with malignancy.

Cancer	Underlying Immune Abnormality	Organism(s) Causing Infection
Multiple myeloma	Hypergammaglobulinemia	<i>S. pneumoniae</i> , <i>H. influenzae</i> , <i>N. meningitidis</i>
Chronic lymphocytic leukemia	Hypogammaglobulinemia	<i>S. pneumoniae</i> , <i>H. influenzae</i> , <i>N. meningitidis</i>
Acute myeloid or lymphocytic leukemia	Granulocytopenia, skin and mucous membrane lesions	Extracellular gram-positive and gram-negative bacteria, fungi
Hodgkin's lymphoma, leukemia	Abnormal T cell function	Intracellular pathogens: <i>M. tuberculosis</i> , <i>Listeria</i> , <i>Salmonella</i> , <i>Cryptococcus</i> and herpesviruses
Non-Hodgkin's lymphoma and acute lymphocytic leukemia	Glucocorticoid chemotherapy, T and B cell dysfunction	<i>Pneumocystis jirovecii</i>
Colon and rectal tumors	Local abnormalities	<i>Streptococcus bovis</i> biotype 1 (bacteremia)
Hairy cell leukemia	Abnormal T cell function	Intracellular pathogens (<i>M. tuberculosis</i> , <i>Cryptococcus</i> , etc.)
Renal, ovarian, biliary tree, metastatic diseases of many cancers	Occlusion of orifices: ureters, bile duct, colon	Rapid, overwhelming bacteremia; urinary tract infection

Table A1.2: Opportunistic infections in granulocytopenic patients.

Gram-positive cocci	<i>S. epidermidis</i> , <i>S. aureus</i> , viridans streptococci, <i>Enterococcus</i> , <i>S. pneumoniae</i>
Gram-negative bacilli	<i>Escherichia coli</i> , <i>Serratia</i> , <i>Klebsiella</i> , <i>Acinetobacter</i> , <i>Pseudomonas</i> , <i>Stenotrophomonas</i> , etc.
Gram-positive bacilli	Diphtheroids
Fungi	<i>Candida</i> , <i>Mucor/Rhizopus</i> , <i>Aspergillus</i>

Post-transplantation Infections

Transplant recipients (either hematopoietic or solid organ transplantation) are at higher risk for development of infectious complications during the course.

- ❖ Infection risk is higher in early post-transplant phase, compared to late phase (years following transplantation)
- ❖ **Risk factors:** The increased risk of infection in transplant recipients is attributed to several risk factors:
 - An allogeneic transplant has a higher risk than syngeneic graft
 - Immunosuppression before or after transplant
 - Bone marrow transplant has the highest risk than solid organ transplant
 - Infection risk is higher if the donor is unrelated compared to a fully matching sibling donor.
- ❖ **Sources of infections:** Include donor-derived (mostly unknown exposures), recipient-derived (activation of latent infection, colonization), endemic community-derived infections and healthcare-associated pathogens
- ❖ The list of various opportunistic infections in different phases of transplantation has been described in Table A2.1; of which the major infections of concern are CMV (Chapter 79), molds and respiratory viruses
- ❖ A robust and comprehensive infection control program comprising of enhanced screening, isolation precaution policy, and planned interventions tailored to curtail transmission must be in place to tackle the challenge of increased risk of infections seen in transplant patients.

Table A2.1: Common sources of infections after transplantation.

Infection Site	Period after Transplantation		
	Early (<1 month)	Middle (1-4 months)	Late (>6 months)
Common sources of infections after hematopoietic stem cell transplantation			
Disseminated	Aerobic gram-negative and gram-positive bacteria	<i>Candida</i> , <i>Aspergillus</i> , Epstein-Barr virus (EBV)	Encapsulated bacteria (pneumococcus, <i>H. influenzae</i> , <i>Neisseria meningitidis</i>)
Skin and mucosa	Herpes simplex virus	Human herpesvirus 6 (HHV-6)	VZV, human papillomavirus (warts)
Lungs	Aerobic gram-negative and gram-positive bacteria, <i>Candida</i> , <i>Aspergillus</i> , other molds, HSV	CMV, seasonal respiratory viruses, <i>Pneumocystis</i> , <i>Toxoplasma</i>	<i>Pneumocystis</i> , <i>Nocardia</i> , pneumococcus
Gastrointestinal tract	<i>Clostridium difficile</i>	CMV, adenovirus	EBV, CMV
Kidney	-	BK virus, adenovirus	-
Brain	-	HHV-6, <i>Toxoplasma</i>	<i>Toxoplasma</i> , JC virus (rare)
Bone marrow	-	CMV, HHV-6	CMV, HHV-6
Common sources of infections after solid organ transplantation (SOT)			
Donor organ	Bacterial and fungal infections of the graft, anastomotic site, and surgical wound	CMV infection	EBV infection (may present in allograft organ)
Systemic	Bacteremia and candidemia (often due to central line colonization)	Cytomegalovirus (CMV) infection	CMV infections (in patients given early post-transplant prophylaxis); EBV proliferative syndromes (in donor organs)
Lungs	Bacterial aspiration pneumonia with prevalent nosocomial organisms associated with intubation and sedation	<i>Pneumocystis</i> infection; CMV pneumonia, <i>Aspergillus</i> infection	<i>Pneumocystis</i> infection; granulomatous lung diseases (nocardial and reactivated fungal and mycobacterial diseases).
Kidney	Bacterial and fungal (<i>Candida</i>) infections (cystitis, pyelonephritis) associated with urinary catheters	BK virus nephropathy; JC virus infection	Bacterial infections (late UTI, not associated with bacteremia); BK virus infection
Liver, biliary tract	Cholangitis	CMV hepatitis	CMV hepatitis
Heart	-	<i>Toxoplasma gondii</i> Endocarditis (<i>Aspergillus</i> and GNB)	<i>Toxoplasma gondii</i>
Gastrointestinal tract	Peritonitis, especially after liver transplantation	Colitis secondary to <i>Clostridioides difficile</i>	Colitis secondary to <i>Clostridioides difficile</i> infection
CNS	-	<i>Listeria meningitis</i> , <i>Toxoplasma gondii</i> infection, CMV	<i>Listeria meningitis</i> , cryptococcal meningitis, nocardial abscess, JC virus associated PML

Source: Adapted and modified from Harrison's Principles of Internal Medicine, 20th edition, 2018.

Emerging and Re-emerging Infections

DEFINITIONS

Emerging Infections

They are the infectious diseases, whose incidence in humans has increased in the past two decades or threatens to increase in the near future (Tables A3.1

Year	Organism
1975	<i>Parvovirus B-19</i>
1976	<i>Cryptosporidium parvum</i>
1977	Ebola virus
1977	<i>Legionella pneumophila</i>
1977	Hantavirus
1977	<i>Campylobacter jejuni</i>
1980	Human T-lymphotropic virus I (HTLV-I)
1981	Toxin producing strains of <i>Staphylococcus aureus</i>
1982	<i>Escherichia coli</i> O157:H7
1982	Human T-lymphotropic virus II (HTLV-II)
1982	<i>Borrelia burgdorferi</i>
1983	Human immunodeficiency virus (HIV)
1983	<i>Helicobacter pylori</i>
1985	<i>Enterocytozoon bieneusi</i>
1986	<i>Cyclospora cayetanensis</i>
1988	Hepatitis E virus
1989	<i>Ehrlichia chaffeensis</i>
1989	Hepatitis C virus
1991	Guanarito virus
1991	<i>Encephalitozoon hellem</i>
1991	New species of <i>Babesia</i>
1992	<i>Vibrio cholerae</i> O139
1992	<i>Bartonella henselae</i>
1993	Sin Nombre virus
1993	<i>Encephalitozoon cuniculi</i>
1994	Sabia virus
1995	Human herpes virus 8 (HHV-8)
1999	Nipah virus
2002	SARS coronavirus (Severe acute respiratory syndrome coronavirus)
2003	Influenza A (H5N1)
2004	<i>Plasmodium knowlesi</i>
2009	Influenza A (H1N1), <i>Candida auris</i>
2012	Novel coronavirus or MERS-CoV (Middle East respiratory syndrome coronavirus)
2013	Severe fever with thrombocytopenia syndrome (SFTS) virus
2019-20	COVID-19 pandemic (Coronavirus disease 2019) due to SARS coronavirus-2

and A3.2). These diseases, with respect to no national boundaries, include:

- ❖ New infections resulting from changes or evolution of existing organisms
- ❖ Known infections spreading to new geographic areas or populations
- ❖ Previously unrecognized infections appearing in areas undergoing ecologic transformation.

Re-emerging Infections

They are old infections; which were clinically silent or reduced in incidence, have again re-emerged in the community, either as a result of—(1) Antimicrobial resistance in known agents or (2) Breakdown in public health measures. Chikungunya virus re-emergence in 2005 is the classical example (Table A3.2).

Drug Resistance and Re-emergence

The re-emerging infections that have increased in frequency in the last decade as a result of development of antimicrobial resistance include:

- ❖ MDR-TB (Multidrug-resistant tuberculosis)
- ❖ XDR-TB (Extensively drug-resistant tuberculosis)
- ❖ MRSA (Methicillin resistant *Staphylococcus aureus*)
- ❖ VRE (Vancomycin resistant enterococci)
- ❖ VRSA (Vancomycin resistant *Staphylococcus aureus*)
- ❖ Beta-lactamase producers such as Extended spectrum beta-lactamase producers and carbapenemase producers
- ❖ Colistin resistant gram-negative bacilli.

Year	Organism	Place
1992	<i>V. cholerae</i> O139	Chennai
1994	Plague	Surat
2000	Diphtheria	Delhi
2001	Nipah virus	Siliguri
2002	Plague	Shimla
2004	Plague	Uttarakhand
2003	Chandipura	Andhra Pradesh
2005	Chikungunya	Hyderabad
2007	Chandipura	Maharashtra
2009	Influenza A (H1N1), <i>Candida auris</i>	Almost all states
2011	Crimean-Congo hemorrhagic fever	Gujarat
2018	Nipah virus	Calicut (Kerala)
2019-20	COVID-19 Pandemic	All over India

DEFINITION

Bioterrorism is a form of terrorism (unlawful use of weapon against mankind) where there is intentional and deliberate release of biological agents (bacteria, viruses, fungi or their toxins) to cause mass illness or death of people, animals, or plants.

BIOLOGIC AGENTS USED AS BIOWEAPONS (TABLE A4.1)

The biologic agents used as bioweapons should have the following key features:

- ❖ Should produce high morbidity and mortality in the community
- ❖ Potential for person-to-person spread
- ❖ Should be of low infective dose
- ❖ Should be highly infectious by aerosol
- ❖ Lack of rapid diagnostic facilities
- ❖ Effective vaccine should not be available globally
- ❖ Potential to cause anxiety
- ❖ Availability of pathogen and feasibility of production
- ❖ Environmental stability—should have the potential to be “weaponized”.

DEVELOPMENT OF BIOWEAPON

Altering the genetic makeup of organisms has become easier and less expensive due to accessible advanced technologies for genetically modifying organisms. There are possibilities of misusing such technologies for the creation of pathogenic organisms or modification of existing microorganisms to make them more virulent. Various possible ways to create bioweapons include:

- ❖ Reconstruction of known pathogenic viruses using information on their genetic sequences
- ❖ Alteration of existing bacteria to make them more dangerous (e.g. introducing drug resistance genes)
- ❖ Reconstruction of microorganism that releases harmful biochemicals within the human body and alterations to

the human host (modification of human microbiome, decreasing immunity).

HISTORY OF BIOTERRORISM ATTACKS

The use of biological agents as weapons is not a new concept. They have been used since ancient time.

- ❖ The first bioweapon used was the fungus *Claviceps purpurea* (rye ergot) by the Assyrians, in the sixth century BC
- ❖ The plague bacilli were used in 14th century
- ❖ During World War I—Anthrax was used by Germany to infect the mules and horses of enemies
- ❖ During World War II—Japanese forces used anthrax and plague bacilli against prisoners
- ❖ **2001 USA World Trade Center attacks**—Anthrax spores were mailed to US media and government offices during a terrorist attack. There were 22 cases with five deaths.

PREVENTION AND PREPAREDNESS

After the 2001 anthrax attack, the US government has established an emergency preparedness and response network to address possible bioterrorism in the future. The network includes National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) as the main bodies along with several government agencies. It targets the following objectives:

- ❖ Understanding the basic biology of potential bioterrorism agents
- ❖ Understanding the interaction between the human immune system and these microorganisms
- ❖ Developing and improving drugs and vaccines that are effective against bioterrorism agents
- ❖ Developing tools to quickly and accurately diagnose diseases caused by these agents
- ❖ Establishing resources and biosafety laboratories to facilitate biodefense research.

Globally various agencies are working hard to curb such problems in future. India too is moving towards establishment of such a network.

Table A4.1: Classification of bioweapons.	
Category A: These agents are the highest priority pathogens which pose the greatest risk to national security	
<ul style="list-style-type: none"> • These agents can be easily disseminated or transmitted from person to person • Result in high mortality and have the potential for major public health impact • Might cause public panic and social disruption • Require special action for public health preparedness 	<ul style="list-style-type: none"> • Anthrax (<i>Bacillus anthracis</i>) • Botulism (<i>Clostridium botulinum</i> toxin) • Plague (<i>Yersinia pestis</i>) • Tularemia (<i>Francisella tularensis</i>) • Smallpox (<i>Variola major</i>) • Hemorrhagic viruses <ul style="list-style-type: none"> ➢ Arenaviruses: Old World virus (Lassa virus), New World viruses (Machupo, Junin, Guanarito, and Sabia) ➢ Bunyaviridae: Crimean-Congo virus ➢ Filoviridae: Ebola, Marburg virus
Category B: These agents are the second highest priority pathogens	
<ul style="list-style-type: none"> • Moderately easy to disseminate • Result in moderate morbidity rates and low mortality rates • Require specifically enhanced diagnostic capacity 	<ul style="list-style-type: none"> • Melioidosis (<i>Burkholderia pseudomallei</i>) • Glanders (<i>Burkholderia mallei</i>) • Brucellosis (<i>Brucella</i> species) • Psittacosis (<i>Chlamydomphila psittaci</i>) • Q fever (<i>Coxiella burnetii</i>) • Typhus fever (<i>Rickettsia prowazekii</i>) • Toxin: Ricin, <i>S. auerus</i> enterotoxin B, Epsilon toxin of <i>Clostridium perfringens</i> • Viral encephalitis [alphaviruses (e.g. Venezuelan, eastern, and western equine encephalitis)] • Food threats: <i>Salmonella</i>, <i>Shigella</i>, <i>E. coli</i> O157 • Water threats: <i>Vibrio cholerae</i>, <i>Cryptosporidium</i>
Category C: These agents are the third highest priority pathogens. They are the emerging pathogens, to which the general population lacks immunity	
<ul style="list-style-type: none"> • These agents could be engineered for mass dissemination in future because of availability, ease of production, and ease of dissemination • They have a potential for high morbidity and mortality rates 	<ul style="list-style-type: none"> • Nipah virus • Hantavirus • SARS and MERS coronaviruses • Pandemic influenza virus • Multidrug-resistant tuberculosis (MDR-TB) • Yellow fever virus

Source: Adapted from Centers for Disease Control and Prevention (CDC).

Laboratory-acquired Infections

Laboratory acquired infections (LAIs) are defined as all infections acquired through laboratory or laboratory-related activities, regardless whether they are symptomatic or asymptomatic in nature.

LAIs result from occupational exposure to infectious agents. The most common route of exposure and accidental inoculation are the following:

- ❖ Inhalation (see aerosols)
- ❖ Percutaneous inoculation (needle and syringe, cuts or abrasions from contaminated items and animal bites)
- ❖ Contact between mucous membranes and contaminated materials (hands or surfaces)
- ❖ Ingestion (aspiration through a pipette, smoking or eating).

The risk-based classification of potential organisms responsible for LAIs are summarized in Table A5.1.

Table A5.1: Risk based classification of agents causing laboratory-acquired infections.					
Group	Definition	Bacteria	Virus	Fungi	Parasite
Group-1	Biological agents that are unlikely to cause human disease	Non-pathogenic organisms	–	–	–
Group-2	Biological agents that can cause human disease and may be hazard to workers; but are unlikely to spread to community; effective treatment or prophylaxis is usually available	<ul style="list-style-type: none"> • <i>Bacillus</i> species (except <i>B. anthracis</i>) • <i>Clostridium</i> species • <i>Corynebacterium diphtheriae</i> • Enterobacteriaceae • <i>Staphylococcus</i> • <i>Streptococcus</i> • <i>Mycobacterium</i> (except <i>M. tuberculosis</i>) 	<ul style="list-style-type: none"> • Adenovirus • Calicivirus • Coronavirus (not SARS-CoV) • Herpesvirus • Influenza virus 	<ul style="list-style-type: none"> • <i>Cryptococcus</i> • <i>Candida</i> • Dermatophytes • <i>Aspergillus</i> 	All clinically important parasites
Group-3	Biological agents that can cause severe human disease and are a serious hazard to workers They may spread to the community; but effective treatment or prophylaxis is usually available	<ul style="list-style-type: none"> • <i>B. anthracis</i> • <i>Brucella</i> species • <i>Coxiella burnetii</i> • <i>Francisella tularensis</i> • <i>M. tuberculosis</i> 	<ul style="list-style-type: none"> • Prion • LCM virus (Lymphocytic choriomeningitis) • Hantavirus • SARS-CoV • Encephalitis virus such as: <ul style="list-style-type: none"> > St Louis > Japanese > West Nile > Western equine 	–	–
Group-4	Same as group 3 except that effective treatment or prophylaxis is usually not available	–	<ul style="list-style-type: none"> • Lassa virus • Ebola virus • Marburg virus • Herpes simiae virus 	–	–

National Health Programmes for Communicable Diseases

ANNEXURE 6

Several national health programmes are made available by the Government of India to address the prevalent health problems nationwide, which include both communicable and non-communicable diseases. These programs are evolved based on the changing pattern of diseases. They target various components such as health promotion,

prevention, treatment of diseases and rehabilitation. Some of these programs cover a particular infectious disease (tuberculosis or leprosy) while others address various aspects of a broader risk groups (pregnant women or children). Following is the list of national health programmes and policies related to infectious diseases.

Table A6.1: Major national health programmes in India.	
Diseases	National health programmes in India
Tuberculosis (Chapter 63)	<p>Revised National Tuberculosis Control Programme (RNTCP), established in 1992</p> <ul style="list-style-type: none"> It is renamed as National Tuberculosis Elimination Programme (NTEP) in 2020 National Strategic Plan, India (2020–2025): In parallel to WHO, Government of India has initiated National Strategic Plan (2020–2025), aiming at elimination of TB epidemic with a vision of TB-Free India with zero deaths, disease and poverty due to tuberculosis by 2025.
HIV/AIDS (Chapter 33)	<p>National AIDS Control Programme (NACP)</p> <ul style="list-style-type: none"> It was established in 1992, under National AIDS Control Organization (NACO) It aims at providing comprehensive care, support and treatment to all persons living with HIV/AIDS <p>National Strategic Plan for HIV/AIDS and STI (2017–2024): NACO has launched the national strategic plan 'Paving Way for an AIDS Free India'; going in line with United Nations Programme on HIV/AIDS (UNAIDS) for ending the AIDS epidemic by 2030.</p>
Vector borne diseases (Chapters 34 to 37 and 74)	<p>National Vector Borne Disease Control Programme (NVBDCP)</p> <p>It aims at control of six important vector borne diseases in India such as malaria (Chapter 35), kala-azar (Chapter 36), filaria (Chapter 37), dengue, chikungunya (Chapter 34), and Japanese encephalitis (Chapter 74) Earlier these programs were operated individually (e.g. National Anti-malaria Programme); but later on, they were merged in 2003.</p>
Viral hepatitis	<p>National Viral Hepatitis Control Programme (NVHCP)</p> <ul style="list-style-type: none"> Launched by Government of India on the World Hepatitis Day, 28th July 2018 It is an integrated initiative for the prevention and control of viral hepatitis in India Aims: It has the following three aims. <ol style="list-style-type: none"> Combat hepatitis and achieve country wide elimination of hepatitis C by 2030; Achieve significant reduction in the infected population, morbidity and mortality associated with hepatitis B and C, such as cirrhosis and hepatocellular carcinoma (liver cancer) Reduce the risk, morbidity and mortality due to hepatitis A and E.
Polio	National Polio Programme
Leprosy (Chapter 54)	National Leprosy Eradication Programme (NLEP)
Guinea-worm	Guinea Worm Eradication Programme
Rabies	National Rabies Control Programme
Yaws	Yaws Eradication Program
Epidemic-prone diseases	National Health Mission—Integrated Disease Surveillance Project (IDSP) Disease surveillance system for epidemic-prone diseases to monitor disease trends
Trachoma	National Programme for Control of Blindness—Trachoma
Emerging disease and bioterrorism	National emergency preparedness plan: disaster management Bioterrorism, SARS, Swine flu, Bird flu, Ebola, Zika, etc.
AMR	National Programme on Containment of Antimicrobial Resistance (AMR)

Vector-borne Infections and Ectoparasite Infestations

ANNEXURE 7

VECTOR-BORNE INFECTIONS

Vectors are living organisms that can transmit infectious pathogens between humans, or from animals to humans (Table A7.1). Vector-borne diseases are human illnesses caused by parasites, viruses and bacteria that are transmitted by vectors (Table A7.2).

Types of Transmission

Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 7,00,000 deaths annually. Vector-borne diseases occur primarily in tropical and subtropical regions. Vectors transmit infections by one of the following methods.

Mechanical Transmission

The disease agent does not replicate or develop in/on the vector; it is simply transported by the vector (e.g. housefly) from one animal or environment to man.

Biological Transmission

The microorganism is taken up by the vector, usually through a blood meal from an infected animal, following which it replicates and/or develops, and subsequently is regurgitated onto or injected into a susceptible animal. There are three types of biological transmission.

- ❖ **Propagative:** Pathogen only multiplies inside the vector (e.g. *Yersinia pestis* in rat fleas)
- ❖ **Cyclodevelopmental:** Pathogen only develops into the next stage inside the vector without multiplying (e.g. *Wuchereria bancrofti* in mosquitoes)
- ❖ **Cyclopropagative:** Pathogen multiplies and also advances to the next developmental stage (e.g. *Plasmodium species* in mosquito)
- ❖ **Salivaria** (anterior station): Pathogen multiplies and reaches the salivary glands of the vector. Infection is transmitted by releasing the pathogen by regurgitating/biting (e.g. *Trypanosoma brucei* in Tsetse fly)
- ❖ **Stercoraria** (posterior station): Pathogen multiplies and reaches the rectum of the vector and the infective forms

are released through feces (e.g. *Trypanosoma cruzi* in Reduviid bug)

- ❖ **Transovarian transmission:** Parent vector passes the pathogen to the offsprings and the latter can spread the infection to susceptible hosts (e.g. *Rickettsia rickettsii* in ticks).

ECTOPARASITE INFESTATIONS

Ectoparasites such as itch mite can cause cutaneous infestations.

Scabies (Itch mite or *Sarcoptes scabiei*)

Scabies is caused by itch mite or *Sarcoptes scabiei*. Transmission to man is through transfer of impregnated female mites through—(1) skin-to-skin contact with an infested person, or (2) rarely spread indirectly by sharing their items such as clothing, towels, or bedding. Scabies can spread easily under crowded conditions where close body and skin contact is common.

Clinical Manifestations

Initial infestation is asymptomatic for two months although the person can still transmit scabies during this time. In case of reinfestation, symptoms appear much earlier in 1–4 days.

Primary infestation: The mites burrow into the upper layer of the skin but never below the stratum corneum.

- ❖ Mites burrowing under the skin cause a **rash**, which is most frequently found on the hands, particularly the finger **web spaces**; wrist folds, elbow or knee; the penis; the breast; and/or the shoulder blades (Fig. A7.1A)
- ❖ **Severe itching** is the most common presentation, especially at night and over body surface, including areas where mites are undetectable.

Crusted (Norwegian) scabies: This is a severe form of scabies, seen among persons who are immunocompromised, elderly, or institutionalized.



Figs A7.1A and B: A. Scabies (rashes on hands); B. *Sarcoptes scabiei* (itch mite).

Source: A. CDC Fact sheet; B. DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Table A7.1: Classification of arthropods (Phylum Arthropoda).

Class	Common names
Insecta	Mosquitoes, black flies, sand flies, deer flies, house flies, tsetse flies, fleas, cockroaches, lice, bugs, wasps, etc.
Arachnida	Hard ticks, soft ticks, itch mites, chiggers, etc.
Myriapoda	Centipedes, millipedes, etc.
Pentastomida	Tongue worms, etc.
Crustacea	<i>Cyclops</i> , crabs, crayfish, etc.

Table A7.2: Arthropods acting as vectors in transmission of medically important human diseases.			
Arthropods	Diseases transmitted		
	Parasitic	Viral	Bacterial
Mosquito	<ul style="list-style-type: none"> • Malaria (<i>Anopheles</i>) • Bancroftian filariasis (<i>Culex</i>, <i>Aedes</i> and <i>Anopheles</i>) • Malayan filariasis (<i>Mansonia</i>, <i>Anopheles</i>) 	<ul style="list-style-type: none"> • Yellow fever (<i>Aedes</i>) • Dengue fever (<i>Aedes</i>) • Chikungunya (<i>Aedes</i>) • Japanese B encephalitis (<i>Culex</i>) • Rift-Valley fever (<i>Aedes</i>) • O’Nyong-Nyong (<i>Anopheles</i>) • VEE, WEE and EEE (<i>Culex</i>, <i>Aedes</i>) • Zika fever (<i>Aedes</i>) • West Nile fever (<i>Culex</i>) 	–
Sandfly	<ul style="list-style-type: none"> • Kala-azar • Oriental sore 	<ul style="list-style-type: none"> • Sandfly fever (Papatasi fever) • Vesicular stomatitis virus • Chandipura virus 	Oroya fever (Carrion’s disease)
Tsetse fly	Sleeping sickness	–	–
Housefly (mechanical vector)	<ul style="list-style-type: none"> • Amoebiasis • Intestinal helminthiasis 	<ul style="list-style-type: none"> • Poliomyelitis • Enterically transmitted hepatitis (hepatitis A and E) 	<ul style="list-style-type: none"> • Typhoid fever • Paratyphoid fever • Cholera • Trachoma • Yaws
Blackfly (<i>Simulium</i> species)	Onchocerciasis	–	–
Deer fly	Loiasis	–	–
Rat flea (<i>Xenopsylla cheopis</i>)	<ul style="list-style-type: none"> • <i>Hymenolepis diminuta</i> • <i>Hymenolepis nana</i> 	–	<ul style="list-style-type: none"> • Bubonic plague • Endemic typhus
Cockroach (mechanical vector)	<ul style="list-style-type: none"> • Amoebiasis • Helminthiasis 	<ul style="list-style-type: none"> • Hepatitis • Poliomyelitis 	Enteric pathogens
Reduviid bug	Chagas’ disease	–	–
Louse	Ectoparasitic infection	–	<ul style="list-style-type: none"> • Epidemic relapsing fever • Epidemic typhus • Trench fever
Hard tick	Babesiosis	<ul style="list-style-type: none"> • Kyasanur Forest disease virus • Colorado tick fever virus • Russian spring-summer encephalitis virus • Powassan virus • Louping-ill virus • Omsk hemorrhagic fever virus • Crimean Congo hemorrhagic fever virus • Ganjam virus • Severe fever with thrombocytopenia syndrome virus 	<ul style="list-style-type: none"> • Tularemia • Lyme disease • Rocky Mountain spotted fever • Indian tick typhus • Ehrlichiosis
Soft tick	–	–	Endemic relapsing fever
Trombiculid mite	–	–	<ul style="list-style-type: none"> • Scrub typhus • Rickettsial pox
Itch mite	Scabies	–	–
<i>Cyclops</i>	<ul style="list-style-type: none"> • Dracunculiasis • Diphyllbothriasis • Gnathostomiasis 	–	–
Crabs and crayfish	Paragonimiasis	–	–

Abbreviations: VEE, venezuelan equine encephalitis; WEE, western equine encephalitis; EEE, eastern equine encephalitis.

- ❖ It is characterized by vesicles and formation of thick crusts over the skin, accompanied by abundant mites but only slight itching
- ❖ Secondary bacterial infections are common.

Laboratory Diagnosis

Scabies is clinically suspected based upon the appearance and distribution of the rash and the presence of burrows. It is confirmed by isolating the mites or ova in a skin scraping

at the burrows, especially on the finger web space and wrist folds.

Skin scrapping: Scrapings are best performed at the end of the burrows in non-excoriated and non-inflamed areas using a sterile scalpel blade containing a drop of mineral oil. The mineral oil enhances the adherence of the mites to the blade and can then be transferred to a glass slide.

Identification: *S. scabiei* is very small in size, just visible to naked eyes. Adult female mites measure 0.30–0.45 mm long; males are smaller at 0.20–0.24 mm long (Fig. A7.1B).

- ❖ Body is rounded above and flattened below
- ❖ The body surface is covered with short bristles
- ❖ It has two pairs of legs in front, and two pairs behind
- ❖ The front legs have suckers at the end and the hind legs have long bristles.

TREATMENT

Scabies

Medications

Scabies is treated with any of the following: (i) permethrin cream 5%, (ii) crotamiton lotion 10%, (iii) sulfur ointment 5%–10%, (iv) lindane lotion 1%, (v) oral ivermectin two doses (200 µg/kg/dose), one week apart and (vi) benzyl benzoate 25%, this is mainly for crusted scabies

Decontamination

- ❑ Bedding, clothing, and towels used by infested persons and also the people who are in close contact, should be decontaminated by washing them in hot water and drying in a hot dryer or dry-clean
- ❑ Items that cannot be washed should be stored in a sealed plastic bag for at least 72 hours
- ❑ Thoroughly clean and vacuum rooms.

Transfusion-transmitted Infections

DEFINITION

Transfusion-transmitted infections (TTIs) are infections resulting from the introduction of a pathogen into a person through transfusion of blood or blood products (packed RBCs, platelets, fresh frozen plasma, etc.)

With implementation of several stringent precautionary measures the risk of TTIs has reduced considerably. The threat of infectious agents entering the blood supply is not static and may evolve as new pathogens emerge or as old ones change their epidemiological pattern.

SOURCES OF TTIs

- ❖ Organisms present in donor's blood
- ❖ Contaminated blood/blood products – failure to maintain aseptic collection, storage or transfusion procedures.

ORGANISMS CAUSING TTIs (TABLE A8.1)

Theoretically, any organism circulating in the blood of the donor can pass on to the recipient. However, since only apparently healthy individuals are accepted as donors, chances of transmission by septicemic patients are very remote. Asymptomatic individuals with the organism in their blood are a more important threat.

- ❖ **Viruses:** The viruses most frequent transmitted through blood transfusion include hepatitis B (most common), HIV and hepatitis C
- ❖ **Bacterial contamination** is more frequent in platelet concentrates (PLT) than in red blood components. This is because bacteria can survive and propagate under the storage conditions typically used for platelets (20–24°C), but less so for RBC (1–6°C)
 - More commonly, contamination occurs during blood collection (improper disinfection of venipuncture site), or during handling of blood products (leaky seals)
 - Predominant bacteria isolated are usually commensals of the skin or intestine
 - As per Indian guidelines, routine sterility testing of blood should be done on 1% of the blood units collected or 4 per month whichever is higher.

STRATEGIES TO REDUCE TTIs

The various strategy to reduce TTIs include:

- ❖ **Donor eligibility:** Careful selection of donors should be done based on adequate history, physical examination, encouraging known donors (e.g. family members)

- ❖ **Processing, handling and storage:** Adequate donor skin disinfection and diverting initial 30 mL of blood can reduce bacterial contamination effectively
- ❖ **Screening for TTIs:** Screening for other TTIs varies depending upon the local policy guidelines based on local prevalence data. In India, screening is done for HIV, HBV, HCV, syphilis and malaria
- ❖ **Screening tests:** They should satisfy the following properties
 - Need to be highly sensitive, detect early in the phase of infection and rapid
 - There is a gradual shift from tests based on antigens or antibodies to highly sensitive tests based on nucleic acid amplification (NAAT) which helps in detecting infections in window period (the period of early infectivity when an immunologic test is non-reactive).
- ❖ **Storage:** Optimum storage condition (e.g. temperature) should be maintained
- ❖ **Pathogen inactivation:** Effective pathogen inactivation procedures without compromising the integrity of blood/blood-products should be followed
- ❖ **Quality control:** Quality audits and assessments should be carried out to ensure highest safety standards.

Table A8.1: Transfusion-transmitted infections.

Viruses	Tests (detection)	Frequency
Hepatitis B virus	HBsAg, NAAT	1 in 2.2 lakh
Hepatitis C virus	Antibody, NAAT	1 in 18 lakh
HIV	Antibody, NAAT	1 in 23 lakh
Other viruses (rare): Cytomegalovirus, human T cell lymphotropic viruses, Parvovirus B19, Zikavirus, West Nile virus		
Bacteria		
Bacterial contamination	Automated blood culture	1 in 1 lakh
<i>Treponema pallidum</i> (syphilis)	Anti-treponemal antibody: TPHA/ELISA If high prevalence: VDRL, RPR	
Others (rare): <i>Leptospira interrogans</i> , <i>Borrelia burgdorferi</i> , <i>Anaplasma phagocytophilum</i> , <i>Rickettsia rickettsiae</i>		
Parasites		
<i>Plasmodium</i>	Antigen (ICT)	< 1 in 3 million
Others: <i>Trypanosoma cruzi</i> , <i>Babesia</i> , <i>Toxoplasma gondii</i> , <i>Leishmania donovani</i>		
Prion disease: Variant Creutzfeldt-Jakob disease		

Abbreviations: NAAT, nucleic acid amplification test; TPHA, *Treponema pallidum* hemagglutination assay; ELISA, enzyme-linked immunosorbent assay; VDRL, Venereal Disease Research Laboratory test; RPR, rapid plasma regain; ICT, immunochromatography test.

INDIAN MEDICAL GRADUATE (IMG)

The healthcare needs of a community keep changing and therefore, the medical education system continuously evolves in order to adapt to the changing needs. To make the existing traditional medical education system more relevant and efficient in addressing current expectations from an Indian Medical Graduate (IMG), the Medical Council of India (MCI) has introduced Competency-Based Medical Education (CBME) from the year 2019—the first major revision to the medical curriculum since 1997 after 20 long years.

The overall **goal** of the undergraduate medical education program as envisaged by the MCI, in the revised Graduate Medical Education Regulations—2019 is, to create an “*Indian Medical Graduate (IMG)*” possessing requisite knowledge, skills, attitudes, values and responsiveness, so that she or he may function appropriately and effectively as a physician of first contact of the community while being globally relevant.

Roles of an IMG

In order to fulfil the above-mentioned goal, the IMG must be able to function appropriately, ethically and effectively in her/his five defined roles, which are as follows.

1. **Clinician** who understands and provides preventive, promotive, curative, palliative and holistic care with compassion
2. **Leader and member of the healthcare team and system** with capabilities to collect, analyze, synthesize and communicate health data appropriately
3. **Communicator** with patients, families, colleagues and community
4. **Lifelong learner** committed to continuous improvement of skills and knowledge
5. **Professional**, who is committed to excellence, is ethical, responsive and accountable to patients, community and profession.

An IMG is expected to gain competency to do justice to these roles. **Professional competence** is—‘the habitual and judicious use of communication, knowledge, technical skills, clinical reasoning, emotions, values, and reflection in daily practice for the benefit of the individual and community being served.’

AETCOM

AETCOM refers to the **soft skills** ‘*Attitude, Ethics & Communication*,’ that an IMG needs to learn along with the knowledge and clinical skills to provide holistic healthcare.

There should also be an assessment system in place to ensure that the learning about AETCOM has actually taken place. The essential components of AETCOM are as follows.

- ❖ **Attitude:** It is a settled way of thinking or feeling about something
- ❖ **Ethics:** Means moral principles that govern a person’s behavior or the conducting of an activity. Ethics in MBBS curriculum basically means bioethics or more specifically medical ethics, which has the 4 pillars—
 1. **Patient autonomy:** Allowing a capable person to exercise the right to make own decisions and only facilitating in such decision-making, e.g. informed consent
 2. **Beneficence:** Patients’ right to improvement or restoration of their health
 3. **Non-maleficence:** An obligation not to inflict harm on others
 4. **Social justice:** Fair and non-discriminatory distribution of the limited resources and, prioritising the need and distribution proportionate to the need.
- ❖ **Communication:** It is imparting or exchanging of information by speaking, writing, or using some other medium.

Need for AETCOM Competencies

Patients and members of the healthcare team value AETCOM skills as much as the clinical skills.

- ❖ Proficiency in AETCOM competencies is very much essential in carrying out routine healthcare activities including doctor-patient interactions, practicing informed decision making, breaking bad news (new diagnosis of chronic disease, malignancy, death, etc.), communication and documentation
- ❖ Lack of effective communication has direct bearing on medical errors, mistakes in diagnosis, inaccurate treatment, compromised patient safety, and patient noncompliance leading to stressful legal and sociocultural issues
- ❖ AETCOM competencies are essential to address issues such as growing distrust in healthcare, violence on doctors, litigations on doctors, etc.

AETCOM Learning in Current vs Previous Curriculum

AETCOM in earlier MBBS curriculum: Before CBME was introduced, medical education focussed mainly on gaining knowledge and clinical skills. AETCOM competencies were neither formally taught nor assessed. Some learning used

to happen by observing seniors (role modelling) or with self-experience.

AETCOM in current curriculum: AETCOM modules in current MBBS curriculum aim at acquisition of minimum essential skills such as communicating effectively and sympathetically with patients and their relatives by all the IMGs uniformly.

- ❖ **Longitudinally spread:** AETCOM competencies relevant to each subject are defined in the CBME and will be taught longitudinally spread over all the phases of MBBS
- ❖ **Increasing complexity:** The topics/competencies are interlinked and are of increasing complexity as one moves from the first year to the final professional year of MBBS.

Teaching-Learning Methods for AETCOM Competencies

Similar to gaining subject knowledge and learning clinical skills, AETCOM competencies can also be taught and learnt, though the approach needs appropriate modifications. Teaching-Learning sessions are planned in such a way that the students are provided with opportunities to learn the basic essential background knowledge, opportunities to learn by experiencing (mostly simulated) and reflect on the experiences. Innovative teaching-learning (TL) methods can be used for AETCOM competencies which will be more engaging and effective.

Problem-based Learning (PBL)

Problem-based learning (PBL) is suggested by the MCI as the main TL method for AETCOM. PBL helps students explore the various facets of “real life issues” that will confront them in their careers, develop problem solving skills. **Case discussions** promote collaborative learning and team work, reflection and self-directed learning.

- ❖ In this, first a case scenario is introduced to the students and they are guided to explore ethical, legal and sociocultural aspects involved in that case and prepare learning objectives
- ❖ Then the students are divided into groups and assignments are given for self-directed learning (SDL) by using various teacher-suggested or self-explored resources. Student learning may also be helped by an anchoring learning activity to increase relevant background knowledge (e.g. lecture, panel discussion) or experience (e.g. visit to hospital/laboratory, observation of working pattern of different types of healthcare workers, discussion with patients or their family members, etc.)
- ❖ After some days of learning time through SDL, the same case or another related case is discussed. The students provide suggestions and alternatives on the approach for doctors to follow and conclusions are drawn

- ❖ Finally the students write narrative about their learning experience which is expected to help themselves to develop the following essential skills:
 - To elicit, observe and record data
 - To reflect on the data at a higher level of thinking and derive opinions and conclusions
 - To communicate the observations and conclusions in a written and verbal form and expand on and defend the conclusions with colleagues and teachers, and
 - To form new experiences and conclusions based on this discussion.

Assessment of AETCOM Competencies

In the CBME, AETCOM competencies will be assessed formatively as well as summatively.

Formative Assessment

Formative assessment is done during and along with day-to-day TL sessions and its purpose is to provide feedback to the students and help them improve. Formative assessment is done based on the student participation in small group discussions, performance in assignments or internal assessment tests, etc.

Summative Assessment

Summative assessment is qualifying examination which decides pass or fail status.

- ❖ There will be questions on AETCOM competencies in the theory and practical examinations conducted at the end of each professional year
- ❖ Student needs to maintain a logbook as a record of his performance and acquisition of essential competencies
- ❖ Objective structured clinical/practical examination (OSCE/OSPE) or Kalamazoo skill rating scale may be used to make the assessment less subjective and to include all relevant aspects in the assessment.

Communications Skill Rating Scale

It is adapted from Kalamazoo consensus. The parameters assessed are: (i) builds relationship, (ii) opens the discussion, (iii) gathers information, (iv) understands the patient's perspective, (v) shares information, (vi) manages flow, (vii) overall rating. Rating: 1-3 Poor, 4-6 Satisfactory, 6-10 Superior.

Suggested AETCOM Topics in Microbiology

MCI has specified 8 AETCOM modules and 37 TL hours in the second professional year for all the subjects concerned. Following 2 competencies are specified in the Microbiology subject.

1. Demonstration of **confidentiality pertaining** to patient identity on laboratory results
2. Demonstration of **respect for patient samples** sent to the laboratory for performance of laboratory tests in the detection of microbial agents causing infectious diseases.

COMPETENCY-1**DEMONSTRATE CONFIDENTIALITY PERTAINING TO PATIENT IDENTITY ON LABORATORY RESULTS**

Competency: Demonstrate confidentiality pertaining to patient identity on laboratory results

Domain: Attitude

Level of learning: Knows how

Sample Case Scenarios**Case scenario 1 (Disclosing HIV result)**

A lady aged 20 years admitted for fever and breathlessness jumps from the 4th floor of the hospital and dies. On enquiry, it was revealed that she was recently diagnosed to be HIV reactive (one week ago) and "Retro positive" labels were put on her case file and bed. Her family members had come to know about her HIV status when they had enquired with a junior resident about the "Retro positive" label. They became stressed out and shouted at the patient. Except for her mother, all other family members had stopped visiting her then onwards.

Case scenario 2 (Not disclosing HIV result)

A 33-year man is admitted to the emergency ward with multiple limb fractures. With ongoing medical treatment, his vitals are stabilized, he is conscious and oriented. Emergency surgery is planned. His relative comes to the laboratory to collect the investigation reports, he is given all the reports except HIV results. He is suspicious and is specifically asking if the laboratory is not issuing only HIV report because it is reactive. Technician gets a call from the ward asking for the HIV report immediately over the phone, as the patient is being shifted to the operating room.

Case scenario 3 (Patient refuses to provide samples)

A nurse caring for an admitted patient gets a needle stick injury with a syringe used for the patient. The patient's HIV and HBV infection status unknown. To decide the need for post-exposure prophylaxis the nurse wants to get patient tested. The patient asks the nurse not to worry, refuses saying he has no such infection, hence no need to test and has financial constrain for performing the tests. The nurse is anxious. The nurse decides to use the patient's blood sample collected for some other tests for testing for HIV and HBsAg and bear the cost by herself.

Case scenario 4 (Social stigma in COVID-19)

A patient with influenza like illness (ILI) has come to the screening OPD. He had exposure to a confirmed COVID-19 case five days back. He wants to get tested and treated, as he is anxious. However, he does not want to be quarantined or discriminated, and has a fear of social stigma and losing the job. He is also the only caretaker of old parents at home.

Case scenario 5 (Occupational exposure)

A medical intern comes to the infection control division with a history of needle stick injury 1 hour back. The source patient's blood sample is collected and tested for HIV, hepatitis B and C. The intern is waiting in the reception of the infection control division to know about the test result of source sample. The test result shows it is reactive for HIV but negative for hepatitis B and C. The infection control officer discloses the source result to the intern immediately and provides appropriate counselling and post-exposure prophylaxis. He also forwards the source result to ICTC. The ICTC calls the source patient on next day and informs about the test result after providing counselling.

Essential Background Knowledge

1. Principles of medical ethics
2. Medicolegal aspects of confidentiality
3. Confidentiality and privileged communication related to laboratory results
4. Modes of transmission and diagnostic approach for HIV or COVID-19, etc.
5. Sociocultural issues related to sensitive infections like HIV or COVID-19 or needle stick injury
6. Post-exposure prophylaxis for needle stick injury when the source turns out to be positive for HIV or hepatitis B.

Specific Learning Objectives

1. Discuss the rights and responsibilities of patients (or healthcare worker in case scenario 5)
2. Discuss the rights and responsibilities of the laboratory with respect to the confidentiality of laboratory results
3. Analyze the ethical issues involved in confidentiality pertaining to patient identity
4. Describe the medicolegal consequences of a breach in confidentiality
5. Demonstration of sympathy when breaking through of result to healthcare workers, providing counselling and maintaining confidentiality pertaining to occupational hazards such as needle stick injury (in case scenario 5).

Teaching-Learning Method

1. **Introductory session:** Introduction of paper case in small group discussion, identification of various aspects involved, framing learning objectives and deciding assignments along with learning resources
2. **SDL:** Self-directed learning by the students

3. **Anchoring learning sessions:** This involves one or more of the following depending upon the case scenario:
 - Interaction with laboratory technician and counsellor of Integrated Counselling and Testing Center (ICTC)
 - Interaction with Microbiology laboratory technician involved in HIV/COVID-19 testing and report dispatch
 - Interaction with infection control officer involved in management of needle stick injury.
4. **Concluding session:** Small group discussion of various possible approaches for the case, their pros and cons, and justification for the best approach selected by each student. However, it may be possible that there may not be single best approach.
5. **Writing narratives** by the students about their learning experiences.

Resources: Standard medical microbiology and forensic medicine textbooks, NACO guidelines.

Assessment

Formative: Participation in the group discussion, assignments, reflection writing, MCQs to assess relevant background knowledge, OSPE, etc.

Summative (Theory): Short notes and short answer questions.

Summative (Practical): Includes OSPE with a simulated patient–HIV pre-test/post-test counselling, counselling following needle stick injury, informing positive COVID-19 report to the patient and informing needle stick injury test result to the healthcare worker.

Key Learning Points

1. **Confidentiality:** It is part of the professional secrecy, where a patient's personal/health information (history, clinical diagnosis, lab results, etc.) is not shared with others, including close relatives and employers, without the patient's consent. The person with HIV has the right to privacy, and the right to exercise informed consent in all decisions about disclosure of his/her HIV status except in circumstances when disclosure to another person is required by law or ethical or health considerations
2. **Informed consent:** The patient gives written permission to carry out HIV testing after understanding the potential implications, risks and advantages of the possible test results
3. **Counselling** is confidential communication limited to the patient and a counsellor with the purpose of understanding the risk of HIV infection, to help cope with the stress and to make personal decisions related to HIV/AIDS
4. **Privileged communication:** It is the unbiased, bonafide communication by a doctor (bypassing confidentiality) to an individual or an authority who has corresponding legal, social and moral obligations. The doctor first tries to convince the patient to disclose the information himself to the person(s) at risk and to avoid risky behavior and, informs the potentially affected/authority directly when in doubt. Such disclosures are made to the people at risk such as the spouse, healthcare workers involved in patient care, etc.
5. **The method followed in ICTC for HIV testing:** Only the counsellor interacts with the patient with privacy. Pre-test counselling is done and written informed consent is taken before sample collection and testing. Report will not have the patient's name written, instead will have identification marks (moles, scars, etc.), age and gender mentioned for patient identification. Irrespective of the result, the counsellor does the post-test counselling and hands over the report directly to the patient. Confidentiality regarding patient identity and HIV infection status is maintained throughout
6. **Method followed for other STDs (Syphilis, Gonorrhoea):** Confidentiality is maintained regarding patient identity and laboratory results. Patient is counselled to avoid risky behavior, get partner also tested and both advised to get treated simultaneously if required
7. **Method followed for COVID-19:** Patient is identified with an alphanumeric code instead of the name. Details are released in the media by the Government authorities with the code and not disclosing the patient identity. Labels are put on the patient house to identify and alert other people.

COMPETENCY-2

DEMONSTRATION OF RESPECT FOR PATIENT SAMPLES

Competency: Demonstration of respect for patient samples sent to the laboratory for performance of laboratory tests in the detection of microbial agents causing infectious diseases

Domain: Attitude

Level of learning: Shows how

Sample Case Scenarios

Case scenario 1 (Rejection due to improper transport)

Sequestrum from a chronic osteomyelitis case was debrided and sent for culture and sensitivity. The sample was rejected by the laboratory mentioning that it was received in formalin, hence unsuitable for culture. There is no more sample available for culture now.

Case scenario 2 (Specimen did not reach laboratory)

A critically ill 5-year-old child's CSF report is awaited for 3 days. On enquiry laboratory says it did not receive the sample. On further probing it was found that the nursing staff had kept the small bottle with the sample in his pocket and mistakenly taken it outside the

hospital and had dropped it somewhere, and did not submit it to the laboratory for testing. Now, the baby needs to undergo lumbar puncture again, results may not be the same as antibiotics are given and need to wait for some more days for the culture report.

Case scenario 3 (Misguided report due to inadequate information in requisition form)

Urologist calls up the laboratory to discuss about "Insignificant bacteriuria" culture report of a pyelonephritis patient. He says it was a percutaneous nephrostomy sample and asks for the organism and antimicrobial sensitivity. Microbiologist says it was written as urine sample on the request form, some gram-negative bacillus had grown and the count was less than 10,000 CFU/mL, so it was thought to be a periurethral commensal and the isolate was discarded, and hence further testing cannot be done.

Case scenario 4 (Specimen kept at wrong place)

Junior resident gets angry and yells at the patient on noticing a stool sample kept on the bedside table. The patient's attendant tries to explain that the container is covered in a plastic cover and all these days the junior resident herself used to keep collected blood and swab samples in that very same place, and he was not informed that stool sample was not to be kept on the side table.

Case scenario 5 (Rejection due to improper collection)

A suspected pulmonary tuberculosis patient, who would travel 30 km from his village to the private hospital with the attached laboratory in the city, had submitted spot sputum sample the previous day and an early morning sample today for acid-fast staining. Reports of both the samples mentioned "many epithelial cells suggestive of excessive salivary contamination. Repeat with the proper sample". Blood culture was also collected from the patient by the clinical team, the result of which came as contaminated blood culture specimen with patient's skin flora.

The doctor found it very difficult to convince the patient to submit proper samples again and pay for them too.

Case scenario 6 (Sample collected for culture and sensitivity in unsterile container)

Paired blood specimen (5 mL each) was sent to the laboratory in two vacutainers for blood culture. The laboratory rejected the specimen. The patient screams that he cannot allow to draw another set of blood specimen for investigation.

Case scenario 7 (Rejection due to lack of patient informations)

Microbiology laboratory rejects a bunch of specimens because one or the other relevant informations were missing in those specimens- patient's name, age or gender, ward, hospital number, sample type, clinical diagnosis, or treatment history. The clinical team screams at the laboratory that they could have called the ward or the patient's attendant and verified the details instead of rejecting.

Case scenario 8 (Rejection due to mismatch of name)

Microbiology laboratory rejects a blood culture specimen collected in BacT/ALERT bottle because the patient's name written on the bottle did not match with that of the requisition form. The clinical team asks the patient to pay again for a repeat blood culture investigation. The patient complains that he cannot afford the price for another test and neither he can give consent to draw another specimen.

Case scenario 9 (Prioritising a sample requiring immediate processing and reporting over the others)

In the midnight, the Microbiology laboratory receives three specimens (urine, sputum, CSF) from a patient for culture. The technician was already processing a huge load of investigations, therefore he informed the clinical team that these specimens can only be processed on the next day.

Essential Background Knowledge

1. Appropriate sample for the test planned: Sample type, amount, collection procedure, preservative if any, container type used – and its transportation and storage
2. Appropriate labelling for correct sample identification
3. Accompanying clinical information for correlation
4. Possible medicolegal issues following incomplete/incorrect sample identification
5. Sociocultural issues following incomplete/incorrect sample identification, relevant clinical information for correlation, improper storage or transportation
6. Ethical issues following incomplete/incorrect sample identification, relevant clinical information for correlation, improper storage or transportation.

Specific Learning Objectives

1. Choose an appropriate container for sample collection
2. Demonstrate an appropriate procedure for temporary storage and transportation of clinical sample
3. Discuss the information that shall be written in the request form and the sample container, completely and legibly
4. Discuss the judicious application of sample rejection criteria in the best interest of patient care
5. Discuss the importance of prioritising the specimen as relevant to the clinical situation
6. Discuss medical, ethical and socio-economical considerations of errors in sample collection and submission process.

Teaching-Learning Methods

1. **Introduction of scenarios** with the help of paper case/role plays/videos
2. **Small group discussion:** Identification of clinical, medicolegal, sociocultural and ethical issues involved
3. **Writing learning objectives**

4. **Writing narratives** by the students about their learning experiences
5. **Anchoring lecture** and demonstration of appropriate procedure of sample collection, transportation and reception at the laboratory. Discussion with nursing staff, phlebotomist, laboratory technicians to gather first-hand information
6. **Closing session** with small group discussion
7. **Writing narratives** by the students about their learning experiences.

Resources: Sample collection (refer in this book), Textbook of Forensic Medicine, Communicate-care-Cure by Dr Alexander Thomas.

Assessment

Formative: Participation in the group discussion, Assignments, reflection writing, MCQs to assess relevant background knowledge and OSPE.

Summative (Theory): Short notes and short answer questions.

Summative (Practical): OSPE can be conducted covering the following aspects

- Sample collection with care and empathy, instructing patients on appropriate sample collection (e.g. urine, sputum, blood culture, etc.)
- Labelling sample containers and filling request form for the clinical scenario provided.

Key Learning Points

- Specimen rejection criteria:** Microbiology samples that do not meet the required sample and test request requirements need to be rejected, so as to prevent inaccurate data and to ensure the safety of patients and laboratory personnel. Reasons for sample rejection may include the following:
 - Improperly labelled or unlabelled sample
 - Incomplete specimen-related or clinical information on the sample and/or on the requisition
 - Sub-optimal sample, i.e. leaking urine and/or stool containers, insufficient quantity, inappropriate sample for test request
 - Duplicate microbiology samples received on the same day, i.e. multiple stool, sputum samples
 - Sample delayed in transit more than the accepted limit.
- Specimen collection:** The method of collection and transport of various specimens has been discussed in detail in Chapter 3.3
- Prioritising the specimen for processing:** Certain precious specimens such as CSF and sterile body fluids, ocular specimens, tissue specimens, suprapubic aspirate and bone specimen should be processed immediately as soon as received, not more than 15 min delay. Similarly, blood culture bottles should be immediately incubated upon receipt. For detail, refer Chapter 3.3.

In the light of COVID-19 pandemic and to meet the unexpected health crisis in future, an acute necessity is being felt to train the Indian Medical Graduate (IMG) of the country. Therefore, Medical Council of India (MCI) has introduced a pandemic management module in MBBS curriculum. IMG should be able to recognize, diagnose,

investigate, treat and prevent newly emerging diseases that may result into outbreak, epidemic or pandemic.

This module comprises of several broad areas, to be covered under various subjects in a phase-based manner from phase I to phase III (part 2). The broad areas to be covered under Microbiology are depicted in Table A10.1.

Broad areas	Subject(s)
History of outbreaks, epidemics and pandemics (This is discussed in Chapter 8)	Pre-clinical Microbiology
Infection control practices—hand washing, decontamination, use of PPE, air borne precautions, contact precautions, infection control committee (This is discussed in Chapter 21)	Microbiology
Emerging and re-emerging infections, early identification and control of new infections (This is discussed in Annexure 3)	Community medicine Microbiology
Sample collection, microbial diagnosis, serologic tests and their performance parameters (This is discussed in Chapter 3.3)	Microbiology
<ul style="list-style-type: none"> • Vaccination strategies including vaccine development and implementation • Therapeutic strategies including new drug development • Outbreak management including quarantine, isolation, contact tracing • Interdisciplinary collaboration, principles of public health, administration, health economics, international health • Operational research, field work, surveillance • Electives epidemiology and research 	Community medicine
<ul style="list-style-type: none"> • Care of patients during pandemics • Emergency procedures • Death related management • Communications and media management • Intensive care management during pandemics • Palliative care during pandemics 	Clinical departments

Source: Adapted from Pandemic Management Module, Medical Council of India (MCI), August 2020.

Page numbers followed by *f* refer to figure and *t* refer to table.

A

Abiotrophia 290
 Abortive infection 88, 710
Absidia corymbifera 14, 680
Acanthamoeba 427, 729, 730
Acinetobacter baumannii 646
 Acquired immunity 133, 134*t*, 136, 138
 Acquired immunodeficiency syndrome 100, 212, 219, 325, 817
 Actinomycetoma 544, 579, 579*t*, 580
 Actinomycosis 543, 544
 Active immunity 134, 136, 137, 138*t*
 Active immunoprophylaxis 221
 Acute encephalitis syndrome 691
 Acute graft rejection 214, 215*f*
 Adansonian classification 13
 Adaptive immunity 136
 Adenovirus 97, 128, 237, 639, 674*f*, 675
 Adoptive immunity 139
Aeromonas infection 410, 418
 Aflatoxin 398
 African eye worm 567
 African hemorrhagic fever 343
 African sleeping sickness 368, 369, 727, 735
 Agglutination reaction 154
Aggregatibacter 291
 Airborne precautions 238
 Albert's stain 25, 29, 601
 Algid malaria 350
 Alloantigens 140
 Alpha chain disease 419
 Alveolar echinococcosis 496
 American trypanosomes 366
 Ames test 54
 Amoebiasis intestinal 427
 Amoebic liver abscess 474, 491, 491*f*, 492
 Amoebic meningoencephalitis, primary 110, 727-729
 Amoeboma 429
 Amsel's criteria 773
 Anaerobic bacteria 527
 Anaphylactic reactions 494
 Anaphylaxis 194
Anaplasma phagocytophilum 314
 Anchovy sauce pus 491
Ancylostoma 463
 braziliensis 463
 duodenale 113, 463, 572
 Andaman hemorrhagic fever 320
 Anemia 301*t*, 349, 619

Angiostrongylus cantonensis 739
Anisakis 499
 Anogenital warts 557
 Anoxomat anaerobic system 36
 Anthracoid bacilli 542
 Anthrax 539, 540, 542
 Antibiogram 49, 276
 Antinuclear antibody 206, 207*f*
 Antiretroviral therapy 100, 270, 334, 445
 Anti-streptolysin O antibodies 525
 Anti-tubercular drugs 631, 633*t*, 634
 Antiviral drugs 94*t*, 655
Apophysomyces elegans 680
 Arboviral infections 336, 338, 716
Arcanobacterium hemolyticum 605
 Arenavirus infections 345
 Arthus reaction 199
 Ascariasis 461, 463
 Ascaris 113, 461
 Ashdown's medium 645, 645*f*
 Aspergillosis 584, 681, 682, 684
 Asteroid body 581
 Ataxia telangiectasia 211
 Auramine staining 627
 Australia antigen 479
 Autofluorescent oocysts 440*f*
 Autograft 213
 Autoimmunity 203, 208
 Autoinfection 103, 459, 467
 Automated blood culture techniques 32
 Autotrophs 24
 Avian flu 651
 Avian parainfluenza viruses 656
 Axoneme 360
 Azithromycin 546

B

Babesiosis 346
 Bachman intradermal test 571
 Bacillary angiomatosis 315
 Bacillary dysentery 430*t*
 Bacillary peliosis 315
Bacillus
 anthracis 539
 atrophaeus 255, 262, 542
 calmette guerin 75, 635
 cereus 394, 395, 395*t*, 542
 stearothermophilus 256, 542
 thuringiensis 358, 542
 Bacterial endocarditis, subacute 288*f*, 290
 Bacterial endotoxin 71*t*, 164, 412
 Bacterial exotoxins 70*t*, 71*t*
 Bacterial genetics 51
 Bacterial growth 22, 23, 23*f*
 Bacterial peritonitis 474
 Bacterial pharyngitis 598, 605
 Bacterial pneumonia 593, 606
 Bacterial spore 21
 Bacteriological index 538
 Bacteriophages 97, 516
 Bacteriuria 748
 catheter-associated 241
 significant 748
Bacteroides 14, 246, 295, 532
 bile esculin agar 32
 fragilis 532
 Baermann funnel technique 466, 468
Balamuthia mandrillaris 727, 731*f*
Balantidium coli 441
 Bancroftian filariasis 371, 373, 375, 376
 Bannwarth's syndrome 700
 Bare lymphocyte syndrome 180, 210
 Barrett's esophagus 421
Bartonella 310
 bacilliformis 5, 548
 henselae 811, 819
Basidiobolus ranarum 680
 Battey bacillus 5
Baylisascaris procyonis 499, 727, 739
 Beaver fever 433
 Bedaquiline 632, 633
 Beefy red ulcer 764*f*
 Beige syndrome 219
 Bell's palsy 709
 Bence Jones proteins 149
 Bifidobacterium 124, 532
 Bile
 esculin hydrolysis test 38, 751*f*
 solubility test 38, 608
 Biofilm 253, 518
 formation 69, 243, 643
 BioFire FilmArray 47, 391, 432, 436, 441, 597, 613, 620, 695, 707
 respiratory panel 653, 656, 658
 Biomedical waste 230, 263
 Biopsy urease test 421
 Bioterrorism 820
 Bird-Fancier's disease 199
 Bite wound infections 506, 811, 812
 BK virus infection 753
 Black piedra 575
 Black water fever 350
 Blastocystosis 442

- Blastomycosis 381, 382
 Blepharitis 784
 Bloodstream infection 295
 Blotting techniques 60
 Blueberry muffin syndrome 795
 Borderline tuberculoid leprosy 535
Bordetella pertussis 638
 Borna virus disease 715, 726
 Bornholm disease 707
 Borreliosis 322
 Botryomycosis 514, 579
 Botulinum food poisoning 397
 Bovine spongiform encephalopathy 725
 Brazilian purpuric fever 780
 Break bone fever 339
Brevundimonas diminuta 257
 Brill-Zinsser disease 311
 Bronchiectasis 677
 Bronchiolitis 592, 655
 Bronchitis 592, 677
 Brood capsule 494
 Brown-Brenn modification 28, 543, 545
 Brownie nose appearance 342
 Brucellosis 316-318
 Brudzinski's sign 689, 693, 693f
 Brugian filariasis 375
 Brush border appearance 684
 Bruton's diseases 208, 209
 Bruton's tyrosine kinase 209
 Bubonic plague 809
 Buffered charcoal, yeast extract agar 622
 Bull neck appearance 601, 601f
 Bunyaviridae 336
Burkholderia 14
 cepacia 646
 mallei infections 645
 pseudomallei 645
 Burkitt's lymphoma 350, 672, 802
 Burn wound infections 506, 507, 548
 Buruli ulcer 547, 637
- C**
- C reactive protein 155
 Calabar swellings 567, 568
 Calcofluor white stain 116, 730
 California encephalitis virus 337, 709
Calymmatobacterium granulomatis 763
 Campylobacteriosis 419, 420
 Candidiasis 377, 378, 379, 574, 583, 583f
Capillaria
 aerophila 678
 hepatica 499
 philippinensis 469
Capnocytophaga canimorsus 811
 Capnophilic bacteria 36
 Carbuncle 514
 Card agglutination test 369
Cardiobacterium hominis 289, 291
 Cardiovascular system infections 3, 285, 287
- Carolus linnaeus 13
 Carrion's disease 315
 Cart wheel appearance 430
 Cary-Blair medium 414
 Cascade reaction 163
 Casoni test 494, 496
 Castaneda's biphasic media 317
 Catalase test 38, 38f, 515, 524
 Catheter associated urinary tract infection, risk factors for 242t
 Cathodic antigen, circulating 754
 Cat-scratch disease 315, 548
 Cavalier and Smith's classification 12
 C-carbohydrate antigen 521, 606
 Cellular immunodeficiency 208, 210
 Cellulitis 522
 anaerobic 506, 529
 Central lymphoid organs 169
 Central sterile supply department 240, 251, 252f
 Central venous catheter 242
 Centroblasts 190
 Centrocytes 190
 Cepacia syndrome 646
 Cephalosporins 644
 Cercaria-Huller reaction 455
 Cercarial dermatitis 455, 573
 Cerebral
 amoebiasis 727
 arteritis 736
 aspergillosis 682
 malaria 350, 727, 734
 paragonimiasis 677, 727, 739
 schistosomiasis 456, 738
 Cestodes 111, 111t
 Cetrimide agar 643
 Cetuximab 219
 Chagas' disease 182, 366, 367, 441, 443, 727
 Chagoma 366, 572
 Chancroid 610, 762f
 Charcoal culture method 466, 468
 Charcot-Leyden crystals 105f, 432
 Chediak-Higashi syndrome 211, 219
 Chemiluminescence-linked immunoassay 160
 Chemokines 180, 181
 Chemoorganoheterotrophs 24
 Chemotrophs 24
 Cheopis index 808
 Chickenpox 129, 238, 554, 555, 558, 558t
 rashes of 554f
 Chiclero ulcer 567
 Chiggerosis 313
 Chik sign 342
 Chikungunya 341, 342, 819
Chilomastix mesnili 437
Chlamydia 79, 156, 187, 596, 766
Chlamydomphila 766
 pneumoniae 79, 590, 592, 594, 618-620, 639
 psittaci 79, 289, 595, 620
 infection 128
 Chlorhexidine gluconate 260
 Chlorine 259
 Chocolate agar 30f, 608, 612, 780
 Cholangitis 474
 Cholecystitis 474
 Cholera 126, 410, 412
 Chorioallantoic membrane 558
 Christie-Atkins-Munch-Petersen test 38, 73, 525
 Chromista 12
 Chromoblastomycosis 119, 581
 Chronic meningitis, agents of 688t, 699
 Chyluria 373
 Citrate utilization test 38, 39
 Citrobacter 39, 400
 Cladophialophora 119
 bantiana 582, 739, 741
Cladosporium carrionii 581
 Clenched-fist injuries 812f
 Clinical pulmonary infection score 245
 Clonorchiasis 498
 Clonorchis 453, 806
 Clostridial myonecrosis 529
 Clostridial wound infections 529
Clostridioides difficile 14, 62, 78, 123, 392, 419, 422
Clostridium 28, 393
 botulinum 70, 78, 397, 691
 perfringens 5, 62, 70, 78, 279, 392, 394, 530f
 tetani 5, 62, 69, 70, 78, 127, 223, 702, 703f
 Coagglutination test 155
 Coagulase test 515
 Cobweb coagulum 700
 Coccidioides 119, 382f, 691
 immitis 116, 379, 595, 679, 739
 Coccidioidomycosis 382
 Coiling phagocytosis 621
 Cold agglutination test 141, 155, 620
 Coliform bacilli 400
 Colitis 389
 hemorrhagic 404
 Common cold syndrome 590, 655, 675
 Communicable diseases 125, 128, 823
 Complement protein 136
 deficiencies 167, 168
Condyloma acuminatum 557, 771f
Condylomata lata 758f
 Congenital rubella 795
 Congenital syphilis 758, 799
Conidiobolus 681
 coronatus 680
 Conjugation, bacterial 58f
 Conjunctivitis 785, 786
 Contact dermatitis 201, 202
 Continuous ambulatory peritoneal dialysis 475
 Convalescent plasma therapy 668

- Conventional biochemical tests 695
 Coombs antiglobulin test 155
 Coombs test 155
 Coprine poisoning 399
 Coproantigen 431, 436
 detection 571
 Coracidium 451
 Core polysaccharide 17
 Coronavirus 99, 588, 660
Corynebacterium 783
 diphtheria 5, 18, 29, 31, 49, 57, 62, 70,
 74, 154, 223, 547, 588, 598, 599,
 599f, 617, 780
 Covaxin trial 669
 COVID-19 128, 129, 588, 630, 660, 662,
 665, 668
 Cowpox 559
Coxiella burnetii 79, 289, 314, 595, 596,
 618, 622
 Coxsackievirus 99, 707, 708t
 C-polysaccharide antigen 694
 C-reactive protein 136, 273, 293, 606
 Creutzfeldt-Jakob disease 725, 827
 Crimean-Congo hemorrhagic fever 819
 Crohn's disease 389, 637
 Crushed ping-pong balls 679
 Cryoglobulinemia 149
 Cryptococcal meningitis 739, 740
 Cryptosporidium 29, 111, 187, 388, 427,
 432, 436-438, 439t
Ctenocephalides felis 312
Culex quinquefasciatus 371
 Cutaneous filariasis 567
 Cutaneous leishmaniasis 565-567
Cutibacterium acne 14, 532
Cyclospora 9, 29, 111, 388, 396, 427, 432,
 437, 439t
 Cystic echinococcosis 493
 Cysticercosis 107, 112, 161, 573, 736, 738
Cysticercus cellulosae 735, 735f, 738f,
 788
 Cystitis 747
 acute hemorrhagic 674
 Cystoisospora 29, 111, 427, 437, 439t
 Cytokine 135, 138, 176, 180, 182, 191t
 Cytomegalic inclusion disease 795
 Cytomegalovirus 94, 96, 129, 143, 211,
 296, 504, 556, 753, 793, 795
 Cytopathic viruses 93
 Cytotoxic T cells 172, 174, 184, 214, 217
- D**
- Dacryoadenitis 779, 784
 Dacryocystitis 779, 784, 784f
 Dakar vaccine 490
 Darling's disease 379
 Darting motility 414
 Daudi cells 179
 Decubitus ulcer 507
 Deep soft tissue infections 522
- Delayed-type hypersensitivity reaction,
 site of 200
 Delbrutto's diagnostic criteria, revised
 737
 Dendritic cells 134, 138, 177, 177f, 204
 Dengue 130, 336, 338, 339, 341
 Deoxycholate citrate agar 31, 34, 76, 305f,
 405, 408
 Dependoviruses 85
 Dermatolymphangitis 372
 Dermatomyositis 209
 Dermatophytes 575, 577
 Device-associated infections, prevention
 of 248, 248t
 Diabetic foot ulcer 507
 Diarrhea 274, 387, 391, 403
Dientamoeba fragilis 110, 437
 DiGeorge syndrome 170, 210
 Dimorphic fungi 115, 379
 Dioctophyme renale infection 755
 Diphtheria 128, 139, 236, 598-601, 603
 Diptheroids 604, 604f
Diphyllobothrium latum 111, 301, 450
Dipodascus geotrichum 14
Dipylidium caninum 446, 451f
 Direct acting antiviral agents 486, 487
 Direct agglutination test 154, 364, 364f
 Disk diffusion method 41
 Dispersed oil particulate test 283
 Diverticulitis 393
 Doderlein's bacilli 532
 Donovan bodies 764f
 Donovanosis 763, 764f
 DOTS plus program 634
 DOTS strategy 632
 Dracunculiasis 569, 570, 570f
Dracunculus medinensis 113, 457, 509
 Drosophila 133, 364
 Drug susceptibility testing 631
 Drum stick appearance 702
 Dry heat sterilizer 255f, 256
 Dumb rabies 720
 Dysentery 129, 387, 391t
 Dyspnea 606
 Dysuria 752
- E**
- Ear, infections of 643, 682, 779, 790
 Eastern blot 161
 Eastern equine encephalitis 825
 Eaton's agent 618
 Ebola virus 336, 343, 344f, 819
Echinococcus 112, 691, 727, 738
 Echinostoma ilocanum 457
 Echoviruses 707, 708
 Ecthyma gangrenosum 548
 Ectoparasite infestations 824
 Ectothrix 577f
Edwardsiella infections 406
 Effusion lymphoma, primary 802, 803
- Ehrlich phenomenon 5
Ehrlichia 5, 79, 314, 819
 Eichwald-Silmser effect 214
 Eijkman test 281
Eikenella corrodens 289, 291, 811
 Ekiri syndrome 405
 Elek's gel precipitation test 154, 602, 602f
 Elephantiasis 373, 373f
 Elispot test 158
Elizabethkingia
 anophelis 647
 meningosepticum 642, 698
 Empyema 596, 607
 Encephalitozoon 443, 790
 Encephalopathy 689, 715
 Endocarditis acute 287, 287t
 Endocardium 287
Endolimax nana 432
 Endotoxin 17, 69, 696
 Enflagellation test 728
Entamoeba coli 432, 433f
Entamoeba histolytica 102, 388, 389, 391,
 427, 428f, 436, 473, 491, 738, 771
 Enteric cytopathogenic human orphan
 viruses 708
 Enteritis necroticans 529
 Enterobacter 39, 65, 76, 279, 400
 Enterobacteriales 400
 Enterobacteriaceae 14, 44t, 75, 400, 547,
 614, 749, 782
 Enterobiasis 459, 460
 Enterobius 113, 457, 458
 Enterococcal infections 750, 751
Enterococcus 73, 241, 526, 751f
Enterocytozoon 443
 bieneusi 819
Enteromonas hominis 437
 Entero-test 435, 444, 468
 Enterovirus 588, 706-708
 Entomophthorales 680
 Enzyme
 immune transfer blot 737
 immunoassay 161, 597, 769
 linked fluorescence assay 159
 linked immunosorbant assay 156, 157,
 158f, 314, 318, 331, 495, 827
 Epidermodyplasia verruciformis 804
Epidermophyton floccosum 577f, 578f
 Epididymitis 776
 Epididymo-orchitis 657
 Epifluorescence microscope 9, 440f
 Epiglottitis 589, 611
 Epithelioid cells 201
 Epsilometer test 43, 44f
 Epstein-Barr virus 5, 94, 96, 141, 143, 167,
 205, 296, 504, 556, 588, 672, 708,
 801
 Erosio interdigitalis blastomycetica 583
 Erysipelas 522
 Erysipeloid 547
 Erysipelothrix rhusiopathiae 75

Erythema
 infectiosum 556
 marginatum 549
 migrans 323, 548, 548f
 multiforme 552, 619
 nodosum 408
 leprosum 536
 Erythroblastosis fetalis 197
 Erythrocytic schizogony 346, 347
 Erythrocytic schizont 347
 Erythrocytic vaccine 358
Escherichia coli 12, 14, 20, 22, 24, 31, 39,
 65, 68, 69, 75, 81f, 121, 122, 124,
 125, 182, 230, 279, 295, 388, 474,
 614, 691, 692, 698, 746, 749, 792,
 819
 Esculin hydrolysis 751f
 Eskape pathogens 230
 E-test 43, 44f
 Ethylene oxide sterilizer 255, 255f
 Eukaryotes 3
 Eumycetoma 579, 579t, 580, 580f
 Ewing's classification 400, 400t
 Exanthems 550
Exophiala jeanselmei 582
 Exotoxins 70

F

Falciparum malaria 349, 350
 Farmer's lung 199, 543
Fasciola gigantica 498
Fasciola hepatica 113, 453, 473, 491, 498f
Fasciolopsis buski 452f, 457f, 497
 Faucial diphtheria 600
 Fecal microbiota transplant 423
 Fibroblasts 136
 Figure of 8 appearance 381f
 Filariasis 126, 677
 Filmarray biothreat panel 318
 Filovirus 101, 336
 Fimbriae 15, 30, 69
 Fish tank granuloma 547, 636
 Fish tapeworm 450
 Fitz-Hugh-Curtis syndrome 764
 Flagella 15, 19
 Flash sterilization 255
 Flask shaped ulcers 429
 Flaviviridae 336, 337
 Floppy child syndrome 398
 Flu syndrome 650
 Fluctuation test 54
 Fluid mosaic model 17
 Fluorescence microscope 7, 9, 9f, 354
 Fluorescence resonance energy transfer
 48
 Fluorescent treponemal antibody
 absorption 701
 Fluoro-enzyme immunoassay 197
Fonsecaea pedrosoi 581
 Foot, mycetoma of 579f

Forchheimer spots 562
 Formaldehyde 258
 Fournier's gangrene 506
Francisella tularensis 69, 508, 581
 Free-living amoebae infections 727
 Freeze-etching technique 11
 Freund's complete adjuvant 142
 Freund's incomplete adjuvant 142
 Friderichsen syndrome 547
 Frustrated phagocytosis 199
 Fungi 7, 12, 127, 187, 475, 805t
 Fusariosis 789
 Fusobacterium 689, 811

G

Gaffky-Eberth bacillus 5
 Gambusia affinis 358
 Gardner and Venkatraman classification
 410, 410f
Gardnerella vaginalis 14, 29, 78, 532, 600,
 773
 Gas gangrene 527, 529-531
 Gaspak anaerobic system 37, 37f, 37, 527
 Gastritis 393
Gastrodiscoides hominis 457
 Gastroenteritis 389, 407, 424t
 Gastroesophageal reflux disease 421
 Gelatin stab agar 541
 Gell and Coombs classification 193
 Gene therapy 60, 675
 GeneXpert 630, 631
 Genital tract infections 3, 407, 770, 775
 Genito-ulcerative disease 756, 757t
Geobacillus stearothermophilus 24, 254
Geotrichum candidum 14
 Germ tube test 378
 German measles 562
 Ghon focus 626
 Giardia 127, 391, 432
 Global Polio Eradication Initiative 714
 Glomerulonephritis, acute 477
 Glutaraldehyde 253, 257
Gnathostoma infections 727
 Gomori's methenamine silver stain 116,
 596, 679
 Gomori's stained smear 543f
 Gonococcal infection 764, 766
 Gonococcal ophthalmic neonatorum
 781f
 Gonococcal pharyngitis 605
 Goodpasture syndrome 198, 205, 206
 Graft-versus-host reaction 217
 Gram stain 22, 25, 27
 Granulicatella 290
 Granulomatous disease, chronic 211
 Granzymes 187
 Graves' disease 198, 205, 408
 Gray matter myelitis 709
 Green nail syndrome 548
 Griess test 748

Ground itch 464, 572
 Ground-glass opacities 679
 Grubii strains 740
 Guanarito virus 819
 Guinea worm disease 127, 457, 569

H

HACEK endocarditis 291, 292
Haemaphysalis spinigera 343
Haemophilus 21, 28, 38, 244
aegyptius 5, 780
aphrophilus 14
ducreyi 68, 137, 549, 761, 762
influenzae 5, 19, 22, 31, 49, 69, 77, 142,
 171, 209, 223, 236, 594, 596, 606,
 612f, 688, 692, 694, 697, 812
 Halophilic vibrio 410
 Halzoun syndrome 497
 Hand hygiene 230, 231, 231f, 236, 237
 Hand rub 231
 Hand wash 670
 Hand-foot-and-mouth disease 563, 564f,
 707
 Hanging mask syndrome 233
 Hantavirus 345, 819
 Hapten 140
 Haptoglobin 136
 Hard chancre 757f
 Hard ticks 343, 358
 Hard tubercles 625
 Hashimoto's thyroiditis 206
 Hassall's corpuscles 170
 Hatching test 455
 Hathipaon Mukh Bharat 375
 Haverhill fever 813
 Hay fever 195
 Healthcare-associated
 infection 125, 229, 241, 249
 Heat
 labile toxin 70, 389
 stable toxin 70, 389
 tolerance test 38
 Heavy chain disease 149
Helicobacter pylori 26, 39, 78, 161, 393,
 805, 819
 Helminthic infections 111, 148
 Helper T cells 172, 174, 184, 186
 Hemagglutination assay, reverse passive
 155
 Hematuria 752
 Hemodigestion 415, 415f
 Hemoflagellates 110, 110f, 360
 Hemolytic streptococcal gangrene 522
 Hemoptysis, endemic 677
 Hemorrhagic fever 343, 345
 Hemorrhagic uremic syndrome 404
 Hendra viral encephalitis 718
 Hepadnaviruses 84
 Hepatic flukes 452
 Hepatitis A 126, 477, 478

- B 223, 271*t*, 478, 482, 483
 C 484, 486
 D 487, 487*t*, 488
 E 126, 488
 G 489
 Hepatobiliary system infections 3, 471, 473, 491
 Herd immunity 139
 Herpangina 707
 Herpes
 conjunctivitis 785
 genitalis 762
 gladiatorum 552
 keratitis 785
 keratoconjunctivitis 785
 simplex virus 94, 96, 129, 504, 550*f*, 551, 591, 688, 706, 753, 766, 771
 Heteroantigens 141
 Heterophile agglutination test 155, 673
 Heterophyes heterophyes 457
 Heterotrophs 24
 Hide Porter's disease 540
 Hidradenitis suppurativa 514
 Hikojima serotype 415
 Hinge region 145
 Hiss's serum sugar media 602
 Histidine rich protein-2 355
 Histoplasma 119, 187, 691
 Hodgkin's disease 672
 Hodgkin's lymphoma 673, 802
 Home quarantine 670
 Homocytotropism 148
 Honeycomb-like crusts 522
 Hookworm 113, 128, 391, 457, 465*f*, 468*f*
 Hordeolum 784, 784*f*
 Horror autotoxicus 203
Hortaea werneckii 575
 Hospital infection control 3, 227
 Hospital information system 273
 Hot air oven 255, 256
 Humoral immune response 536, 625
 Hybridoma technique 149, 150*f*
 Hydatid disease 107, 161, 494, 496
 Hydrocele 373, 373*f*
 Hydrocephalus 699, 736
 Hydrogen peroxide 156, 258
 Hydrophobia 720
Hymenolepis diminuta 446
Hymenolepis nana 103, 112, 450*f*
 Hyperacute graft rejections 214, 214*f*, 217
 Hypergammaglobulinemia 142, 143, 362, 365
 Hyperinfection syndrome 467
 Hypersensitivity 193, 201*t*
 Hyperstrongyloidiasis syndrome 727, 739
 Hypnozoites 347
 Hypochlorite 259, 266
 Hypopyon corneal ulcer 782
 Hyposplenism 696
 Hypotonic hyporesponsive syndrome 641
- I**
 Idiopathic thrombocytopenic purpura 205
 Immune reconstitution inflammatory syndrome 334
 Immune response 184, 185*f*, 367, 536
 Immune system 203, 205
 Immunochromatographic test 90, 161
 Immunodeficiency diseases 208, 221
 Immuno-electrophoresis 154
 Immunofluorescence assay 160, 160*f*
 Immunogenicity 140
 Immunoglobulin 94, 141, 144, 147, 224
 Immunological memory 138, 215
 Immunoperoxidase staining 160
 Immunoprophylaxis 221
 Impetigo 514, 522
 Indian tick typhus 312
 Indole test 38, 39, 39*f*, 415, 644, 646, 748
 Infectious mononucleosis 155, 672, 672*t*
 Infectious myelitis 691
 Infective conjunctivitis 779
 Infective endocarditis 289
 Infective keratitis 779
 Influenza 98, 128, 129, 148, 588, 639
 Innate immunity 133, 134*t*, 135*t*, 138
 Interferons 60, 93, 135, 177, 183, 186
 Interleukin 60, 70, 135, 172, 177, 178
 Interstitial pneumonia 594, 594*f*, 606, 618
 Intestinal amoebiasis 427, 430, 432
 Intestinal anthrax 542
 Intestinal botulism 398
 Intestinal cestode 446
 Intestinal coccidian infections 441
 Intestinal flagellate infections 433
 Intestinal flukes 446, 452
 Intestinal helminthic infections 446
 Intestinal nematodes 113, 446, 457, 469
 Intestinal protozoan infections 427, 441
 Intestinal sarcocystosis 442
 Intestinal taeniasis 112, 447, 448*f*, 449
 Intestinal trematode 446, 457
 Intracellular bacteria 69*t*
 Intradermal skin tests 108
 Inulin fermentation 38, 608
Iodamoeba butschlii 432
 Iodophor 259
 Irritable bowel syndrome 389, 419
 Isospora belli 14
 Isotypes 148
 Itch mite 824, 824*f*
- J**
 Janeway lesions 288, 289
 Japanese encephalitis 709, 716, 717
 Jeryl-Lynn strain 657
 Jewish housewives' disease 452
 Job's syndrome 212
- Johne's bacillus 5, 637
 Jones criteria 293
- K**
 Kala-azar 362, 365
 Kanagawa phenomenon 417
 Kaposi's sarcoma 219, 802, 803, 803*f*
 Katayama fever 455
 Kato-Katz technique 465
 Kauffmann-White scheme 302
 Kawamoto technique 354
 Kawasaki's disease 408
 Kawasaki's syndrome 143
 Kelsey-Maurer test 261
 Keratoconjunctivitis sicca 206
 Keratoconjunctivitis, epidemic 674, 786
 Kernig's sign 689, 693, 693*f*
 Kinetoplast 360
 Kingella kingae 289, 291
 Kinyoun's cold acid-fast staining 627, 628
 Kirby-Bauer disk diffusion 41, 42*f*
 Kleb-Loeffler bacillus 5
Klebsiella 40, 65, 75, 121, 124, 241, 244, 246, 279, 392, 400, 689, 691, 746
 Klebs-Loeffler bacillus 600
 Koch's postulates 4
 Koch's week bacillus 5
 Kopeloff and Beerman's modification 28
 Koplik's spots 560, 560*f*
 Kyasanur forest disease 100, 342
- L**
 Lactophenol cotton blue 117
 Lactose fermenters 400
 Langerhans cells 171, 202
 Larva 451
 currens 572
 migrans 499, 573
 Laryngitis 589
 Laryngotracheobronchitis 589, 655
 Latex agglutination test 155, 207, 645
 Lattice hypothesis 153
 Lazy leukocyte syndrome 212
 Lectin pathway 163, 166
Legionella pneumophila 230, 619, 819
 Legionnaires' disease 621, 622
 Leishman-Donovan bodies 364
 Leishmania 106, 108, 187, 360, 363, 365
Leishmania donovani 301, 361, 473
 Leishmanin test 365
 Leishmanoma 362
 Lemierre's syndrome 292, 533
 Lentivirus 325
 Leopard skin 568
 Lepra reactions 536, 536*t*
 Lepromatous leprosy 139, 535, 535*f*, 535*t*
 Lepromin test 538
 Leprosy 534, 538
 Leptospirosis 158, 319-322

- Leptotrichia buccalis* 533
Leuconostoc species 22
 Levofloxacin 633
Lichtheimia 119
Liponyssoides sanguineus 312
 Lipopolysaccharide 17, 70, 133, 142, 319
 Lipoteichoic acid 69, 521
 Listeria 14, 68, 75, 187, 396
 Live attenuated vaccines 221, 222, 478
Loa Loa infection 370, 373, 567, 727
 Lobar pneumonia 594, 594*f*, 607
 Local immunity 138
 Lockdown 670, 671*t*
 Loeffler's pneumonia 595
 Loeffler's serum slope 34, 602, 602*f*
 Loeffler's syndrome 461, 678
 Loiasis 568
 Lowenstein-Jensen medium 31, 31*f*, 544, 545, 629, 629*f*
 Lower respiratory tract infections 587, 588, 592
 Lower urinary tract infection 745*t*, 747
 Luciferase immunoprecipitation system 374
 Ludwig's angina 589
 Lumpy jaw 543
 Lupus band test 207
 Lupus vulgaris 547, 625
 Lyme disease 161, 323, 324, 548, 699
 Lymphadenitis 372, 508
 Lymphadenopathy 562, 568
 Lymphangitis 372, 508
 Lymphatic filarial nematodes 370
 Lymphatic filariasis 107, 370, 370*t*, 372
 Lymphedema 373
 Lymphocytic choriomeningitis virus 688, 706, 709
 Lymphogranuloma venereum 763, 766, 767
 Lymphoid cells 169, 171
 Lymphoid follicles 170, 190*f*
 Lymphoid organs 169
 Lymphokine 180
 Lymphoproliferative disorder 673
 Lysogenic conversion 56
- M**
- M'Faydean capsule stain 19
 MacConkey agar 32*f*, 400, 402, 402*f*, 408
 MacConkey purple broth 280, 280*f*
 Macroconidia 577
 Macrophage 70, 133, 134, 138, 171, 176
 Mad cow disease 725
 Mad dog syndrome 723
 Madura foot 579
Madurella grisea 14
Madurella mycetomatis 118
 Majocchi granuloma 575
 Malabsorption 434
 Malaria 130, 346, 348*t*, 350, 352, 356
 Malassezia furfur 143, 574, 574*f*, 575
 Malta fever 316
 Mammomonogamus 678
Mansonella 568
 Mansonia 376
 Mantoux test 627
 Marburg virus disease 343, 345
 Marrack's lattice hypothesis 153
 Marrara syndrome 497
 Mast cells 134, 178
 Mature cell 175
 Maxillary fungal ball 682
 Mayaro virus 337
 Mazzotti skin test 568
 McCoy cell culture 769*f*
 McFadyean's reaction 541, 541*f*
 McIntosh and Fildes's anaerobic jar 36*f*, 527
 Measles 129, 225, 559-561, 817
 Medlar bodies 581
 Medusa head appearance 541
 Meleney's ulcer 506
 Melioidosis 548, 644, 645
 Membrane attack complex 164, 164*f*, 192
 Memory cells 137, 137*t*, 172, 191
 Meningitis 236, 609, 688, 693, 698, 715
 Meningococcal meningitis 693, 695, 697
 Meningococcal rashes 546
 Meningococcus 47, 128, 155, 695
 Meningovascular syphilis 700
 Mercury drops appearance 639*f*
 Mesosomes 18
 Metachromatic granules 600
Metagonimus yokogawai 457
 Metal binding proteins 136
 Metallo-beta-lactamases 403
 Methenamine silver stain 583*f*, 679*f*, 680*f*
 Methicillin-resistant *Staphylococcus aureus* 25, 66, 121, 230, 236, 244, 512
 Meyers Kouwenaar syndrome 499, 677
 Microaerophilic bacteria 23
 Microfilaria 370, 371, 373, 374
 Microimmunofluorescence test 769
 Microscan walkaway system 40
 Microsporidia 427, 739, 741
 Microsporidial myositis 582
Microsporium canis 577*f*, 578, 578*f*
 Middle-east respiratory syndrome 661
 Miliary tuberculosis 626
 Molar tooth appearance 545
 Molecular mimicry 204, 205, 293, 421
 Molecular test 273, 313, 563, 597
 Mollaret meningitis 708
 Molluscum bodies 559, 559*f*
 Molluscum contagiosum 559, 559*f*
 Monkeypox virus 238, 559
 Monoclonal antibody 150
 Monocytes 134, 136, 176
 Mononucleosis like syndrome 672*t*, 795
 Monospot test 673
 Monozygotic twins 213
 Montenegro test 365, 566
Moraxella catarrhalis 617
Moraxella lacunata 780
 Moraxella-Axenfeld bacillus 780
Morganella 76, 241, 400, 749, 750
 Mouse foot pad cultivation 538
 Mouse inoculation 703, 721
 Mouse neutralization test 721
 Mucopurulent cervicitis 774
 Mucopurulent conjunctivitis 785
Mucor racemosus 680
 Mucormycosis 680
 Mucosal candidiasis 378, 583
 Mucosal immunity 148
 Mueller-Hinton blood agar 41
 Multibacillary leprosy 534
 Multidrug-resistant organisms 272
 tuberculosis 632-634, 819
 Mumps 128, 129, 592, 656, 709
 Muriform cells 581
 Muscular cysticercosis 736
 Muscular sarcocystosis 572
 Musculoskeletal infections 72, 501, 508, 514
 Mushroom poisoning 399
 Mutants, isolation of 54
 Mutational drug resistance 66*t*
 Myasthenia gravis 198, 205
 Mycetism 120, 398, 399*t*
 Mycetoma 118, 578, 579*f*, 580
Mycobacterium tuberculosis 14, 22, 28, 29, 33, 47, 65, 66, 69, 75, 129, 238, 595
Mycoplasma 14, 15, 79, 156, 596, 597
 Mycotic aneurysm 292
 Mycotic corneal ulcer 789
 Mycotic keratitis 788, 789
 Mycotic poisoning 396
 Mycotoxicoses 120, 398, 398*t*
 Myelitis, bacterial 691
 Myeloperoxidase deficiency 211
 Myocarditis 287, 292, 601, 619
 Myositis 508, 583
 Myxovirus 98, 648
- N**
- N95 respirator 233*f*
 N-acetyl glucosamine 16
 N-acetyl muramic acid 16
Naegleria 427, 728, 730
 Nagler's reaction 156, 531
 Naive lymphocytes 172
 Nasal cavity, infections of 590
 National AIDS Control Organization 100, 270, 330-333
 National Filariasis Day 375
 National Framework for Malaria Elimination 351

- National Immunization Schedule 223, 604, 711, 712
- Natural killer cell 134, 138, 172, 176, 187
- Nausea, acute 392
- Necator americanus* 113, 463, 572
- Necrotizing chorioretinitis 787
- Necrotizing enteritis 392
- Necrotizing enterocolitis 124, 392, 529
- Necrotizing fasciitis 506, 522, 523*f*, 584
- Needle stick injury 268
- Negri body 720, 721*f*
- Neil-Mooser reaction 313
- Neisseria* 38, 148, 696
- gonorrhoeae* 5, 14, 74, 128, 129, 167, 588, 598, 605, 696*t*, 764, 780, 793
- meningitidis* 5, 14, 69, 74, 171, 182, 237, 546, 695, 696*t*, 780
- Neobalantidium coli 14
- Neonatal listeriosis 699*t*
- Neonatal tetanus 704, 704*f*
- Neorickettsia sennetsu* 314
- Neotropical echinococcosis 497
- Nephropathia epidemica 345
- Neuroborreliosis 700
- Neurocysticercosis 727, 735, 736, 737*f*
- Neuroschistosomiasis 455, 738
- Neurosyphilis 700, 701, 758
- Neutrophil 134, 178, 187, 491
- Newcastle disease 656, 786
- Nezelof syndrome 211
- Nikolsky's sign 512
- Nikshay 635
- Nipah virus 99, 715, 718, 819
- Nitrate reduction test 748
- Nitroblue tetrazolium reduction test 211
- Nitrocellulose membrane 161, 162
- Nocardia* 14, 75, 542, 544*t*, 545*f*, 616
- Nomen system 316
- Nonbacterial thrombotic endocarditis 288
- Non-cholera vibrios 410
- Nonhalophilic vibrios 410
- Non-sporing anaerobes 14, 78, 531*t*
- Nontuberculous mycobacteria 14, 75, 547, 623, 636, 636*t*, 637*t*
- Non-typhoidal salmonella 76, 302, 309, 406, 617
- Non-venereal treponematoses 545, 546
- Normal flora 124*t*, 587
- Norovirus 392, 396
- Norwalk virus 223, 392, 425, 426
- Nurse cells 571
- Nutrient agar 30*f*, 515, 541, 643
- Nutritionally variant streptococci 290
- O**
- Occlusional injuries 812
- Occult filariasis 499, 678*t*
- Ochratoxin 398
- Ocular cysticercosis 736, 788
- Ocular histoplasmosis syndrome 790
- Ocular toxoplasmosis 786, 787
- Oesophagostomum* species 469
- Onchocerca 373, 457, 567, 568
- Onchocerciasis 568
- Onchocercoma 568
- Oncofetal antigens 218
- Oncogenic retroviruses 801
- Onychomycosis 583*f*, 789
- Ophthalmia neonatorum 781
- Opisthorchis* 453, 806
- Opsonization 166, 191, 197
- Optochin sensitivity 608
- Oral cavity infections of 588, 591
- Oral cholera vaccines 416
- Oral polio vaccine 86, 139, 222, 711, 712
- Oral thrush 583*f*, 591
- Orbital cellulitis 785, 790*f*
- Orbital mucormycosis 788, 789
- Orchitis 776
- Organ transplantation 817
- Oriental lung fluke 676
- Orientia tsutsugamushi* 79, 313, 618
- Original haffkine vaccine 810
- Oropouche virus 337, 709
- Oroya fever 315
- Orthomyxoviridae 98, 648
- Orthophthalaldehyde 258
- Orthotopic grafts 213
- Osler's nodes 288
- Osteomyelitis 72, 508, 509, 583
- Otitis externa 790
- Otitis media 609, 655, 792
- Otomycosis 791
- Ovoviviparous 457
- Owl's eye inclusions 796*f*
- Oxidase test 38, 39*f*
- P**
- Pancarditis 293
- Pancreas, infection of 475
- Pantoea 400
- Panton valentine toxin 512
- Papain digestion 145
- Papillomaviridae 800
- Paracoccidioides brasiliensis 116, 379, 382, 595, 679
- Paracoccidioidomycosis 382, 383, 383*f*
- Paraffin bait technique 545
- Paragonimiasis 676, 677
- Paragonimus* 677
- Paralytic poliomyelitis 710
- Paralytic rabies 720
- Paramyxoviridae infections 98, 648, 655
- Parapharyngeal infections 589
- Parapneumonic effusion 607
- Parapneumonic pleural effusion 596
- Paratope 140, 145
- Paratyphoid fever 406
- Paravaccinia 559
- Parvoviruses 707, 708
- Parvovirus 96, 129
- Paschen bodies 558
- Passive agglutination test 155, 155*f*
- Passive immunity 134, 136, 137, 151
- Pasteurella multocida* 508, 811, 812
- Paucibacillary leprosy 534
- Paul-Bunnell test 141, 155, 672, 673
- Peau D'orange skin 522, 522*f*
- Pediculus humanus 322
- Pelvic inflammatory disease 770, 774
- Pemphigus vulgaris 197
- Penicilliosis 120, 582, 684
- Pentatrichomonas hominis* 437
- Pepsin digestion 145
- Peptic ulcer disease 421
- Peptidoglycan 16, 133, 511
- Peptone water 30*f*, 515, 643
- Peracetic acid 258
- Perinephric abscess 752
- Periodic acid-Schiff 116, 431
- Periodontal infections 591
- Peritonitis 474
- Peritonsillar abscess 589
- Pernicious malaria 350
- Personal protective equipment 230, 231, 233*f*, 669
- Pertussis 128, 593, 638, 640
- Petroff's method, modified 627
- Pfeiffer's bacillus 5, 610
- Phaeohyphomycosis 119, 581
- Phagocytes 134
- Pharyngitis 522, 588, 598, 599, 672
- Pharyngoconjunctival fever 674, 786
- Phenol 259
- Phenylalanine deaminase test 748, 749
- Phialophora verrucosa* 581
- Phlebotomus argentipes* 361
- Phoenix system 43
- Picornaviruses 99
- Piedra 118, 575
- Pili 20, 69, 638, 764
- Pilot wheel appearance 383
- Pinta 546
- Pistia stratiotes* 376
- Pityriasis versicolor 574, 804
- Plague 126, 407, 807, 810, 819
- Plantar warts 557, 557*f*
- Plasma cell 175, 191
- pneumonia 679
- Plasma pyrolysis 267
- Plasma sterilization 255, 255*f*
- Plasmid 53
- Plasmodium* 10, 106, 108, 137, 187, 296, 346, 347, 351, 691
- Pleistophora* 443
- Pleomorphic bacteria 21
- Plesiomonas* 408
- Pleurodynia 707
- Pneumatocele 614
- Pneumococcal meningitis 695

- Pneumococcal pneumonia 606
 Pneumocystis 187, 211, 679
 Pneumocystosis 120
 Pneumonia 236, 593-595, 609, 616, 621
 Pneumonic plague 128, 237, 616, 809
 Polio 139, 148, 713*t*
 Poliomyelitis 709, 710*f*, 711
 Polyacrylamide gel electrophoresis 161
 Polyarteritis nodosa 477
 Polymerase chain reaction 5, 29, 45, 46, 46*f*,
 90, 108, 118, 630, 711, 734, 759, 766
 Polyomaviruses 97
 Pontiac fever 621
 Postgonococcal urethritis 768
 Post-herpetic neuralgia 555
 Post-kala-azar dermal leishmaniasis 360
 Poststreptococcal glomerulonephritis
 206, 523, 525, 598, 752, 753*t*
 Postzone phenomenon 153
 Potassium tellurite agar 31, 34, 602, 602*f*
 Pott's disease 625
 Povidone iodine 259
 Poxvirus 97, 557
 Preisz-Nocard bacillus 5, 604
Prevotella melaninogenica 527
 Prion disease 715, 724-726, 827
 Procalcitonin 276
 Proctitis 393
 Prodigiosin 615
 Progenitor T cells 170
Propionibacterium acnes 14, 503, 691
 Proptosis 790*f*
 Prostatitis 751, 775
 Prosthetic valve endocarditis 289
 Proteae 76, 749
 Proteus 400, 746, 749, 753, 792
 Proto-oncogenes 421, 800, 801
 Protozoa 102, 102*t*
Providencia 76, 241, 400, 749, 750
 Provirus 327
 Prozone 153
 Pruritic papules 546
Pseudallescheria boydii 739
 Pseudoappendicitis 408
 Pseudocowpox 559
 Pseudohyphae 115, 377, 378*t*
 Pseudomembrane 423, 592
 coat 600
 extension of 601
 Pseudomembranous colitis 422
 Pseudomembranous enterocolitis 392
 Pseudomonadaceae 38
Pseudomonas 14, 20, 21, 37-40, 49, 54,
 65, 70, 121, 130, 167, 211, 236, 244,
 246, 392, 691, 698, 746, 750, 782,
 791, 817
 aeruginosa 19, 66, 77, 230, 244, 279,
 280, 393, 548, 642-644, 644*f*, 780,
 792
 infections 642-644
 stutzeri 645
 Pseudopodia 427
 Psittacosis 128, 620
 Psoriasis 143, 204
 Psychrophiles 23
 Puerperal sepsis 523
 Pulex irritans 808
 Pulmonary amoebiasis 678
 Pulmonary anthrax 540, 540*t*, 616
 Pulmonary aspergillosis 238, 682
 Pulmonary balantidiasis 678
 Pulmonary cryptococcosis 684, 740
 Pulmonary edema 350
 Pulmonary function test 678
 Pulmonary histoplasmosis 380
 Pulmonary hydatidosis 678
 Pulmonary infection 407
 score 245*t*
 Pulmonary mucormycosis 680
 Pulmonary nocardiosis 544
 Pulmonary paragonimiasis 677
 Pulmonary toxoplasmosis 678
 Pulmonary tuberculosis 75, 595, 625, 626,
 626*f*, 626*t*, 631
 Pulmonary tularemia 622
 Pulp, white 171
 Pulse polio immunization 713
 Punctate keratitis 788
 Purified chick embryo cell 722
 Purine nucleoside phosphorylase
 deficiency 210
 Purpura fulminans 547
 Purulent corneal ulcer 782
 Pus
 cells 435
 blue green 548
 Pyelonephritis 747
 Pyoderma 522
 Pyogenic infections, recurrent 210
 Pyogenic liver abscess 473
 Pyogenic meningitis 692, 695, 697, 699
 agents of 694*t*, 695
 Pyogenic uveitis 783
 Pyomyositis 508, 514
 Pyridoxine 22
 Pyrogenic activity 181
 Pyrosequencing 456
- ## Q
- Q fever 314, 315, 597
 QBC capillary tube 354*f*
 Quantitative buffy coat 10, 107, 356*t*
 Quarantine centers 670
 Quartan malarial nephropathy 350
 Quaternary ammonium compound 260
 Quellung reaction 608
- ## R
- Rabbit pathogenicity tests 629
 Rabies 718, 719*t*, 721, 722
 Racemose cysticerci 735
 Radioallergosorbent test 197
 Radioimmunosorbent test 197
 Ramsay-Hunt syndrome 555
 Ranikhet virus 656
 Rapid diagnostic test 355, 355*f*
 Rapid fluorescent focus inhibition test 721
 Rapid plasma regain 45, 827
 Rash 304, 554, 555, 558, 562, 672, 697
 Rat bite fever 813
 Reactive arthritis 407, 619
 Reactive polyarthritis 509
 Red leg disease 418
 Reiter's syndrome 407
 Relapsing fever 322, 323
 Renal tuberculosis 752
 Reoviridae 336, 337
 Respiratory cryptosporidiosis 678
 Respiratory diphtheria 600
 Respiratory hygiene 237, 239, 670
 Respiratory syncytial virus 89, 99, 592
 infection 657, 658
 Respiratory viruses 426, 618
Retortamonas intestinalis 437
 Retroviral syndrome, acute 327, 588
 Reye's syndrome 555, 650
 Rhabditiform larva 468*f*
 Rhabdoviridae 336, 337
 Rheumatic fever 198, 293*t*, 294, 753*t*
 Rheumatic heart disease 293
 Rheumatoid arthritis 155, 204, 206, 207
 Rhinitis 590
 Rhinocerebral mucormycosis 680, 739
Rhinocladia aquaspersa 581
 Rhinoscleroma 590
 Rhinosinusitis, allergic 590
 Rhinosporidiosis 119, 582
Rhinosporidium seeberi 582*f*
 Rhinovirus 128, 237, 639, 707
Rhipicephalus sanguineus 312
 Rh-isoimmunization 225
 Rhizoid 681
 Rhizomucor 119
 Rhizomucor pusillus 680
 Rhizopus 115, 119, 120, 680, 741
Rhodococcus equi 617
 Ribosomes 18
 Rice water stool 412
Rickettsia 156, 187, 310, 372
 Rickettsiaceae 310, 311*t*
 Rickettsial pox 312, 548
 Rickettsiosis 313
 Ring worm infections 576*f*
 Risus sardonicus 702, 703*f*
 River blindness 568, 788
 Robertson's cooked meat
 broth 32, 527, 528*f*, 703
 medium 32*f*
 Roboviruses 345
 Rocky Mountain spotted fever 312, 504,
 548, 548*f*

Rodent-borne virus 101, 345
 Romana's sign 367, 787, 788
 Rose Gardner's disease 580
 Rose spot 304, 548
 Rose-Waaler test 207
 Ross river virus 337
 Rotavirus 390, 425
 Rubella 128, 129, 225, 237, 562, 563
 Runt disease 218
 Runyon's classification 636*t*

S

Sabia virus 819
 Sabin-Feldman dye test 156, 733
 Sabouraud's dextrose agar 117, 545
 Saccharomyces 124, 115
Saccharopolyspora species 199
 Saddle back fever 339
 Safe water, provision of 416
 Saint Louis encephalitis virus 709
 Saline mount 431*f*, 435*f*
 Salivary gland infections 592
Salmonella 279, 400, 414, 747
 Salmonellosis 406
 Salt tolerance test 417
 Sandfly 362
 Sandfly fever virus 337, 338
 Sapoviruses 392, 426
Sappinia infection 731
Sarcocystis hominis 442
 Sarcoidosis 212, 682
Sarcoptes scabiei 125, 824, 824*f*
 Satelliting streptococci 291
 Satellitism 612, 612*f*
 Scabies 824, 824*f*, 826
 Scalded skin syndrome 143
 Scarlet fever 598
 Schaeffer-Fulton stain 22
 Schick test 156, 604
Schistosoma 296, 452*f*, 691
 Schistosomula 454
 Scleroderma 206, 207
 Sclerosing keratitis 788
 Sclerotic bodies 581, 582*f*
 Scombroid food poisoning 399
 Scotochromogens 636
 Scrofuloderma 547, 625
 Scrub typhus 158, 311, 313, 314, 618
 Seasonal influenza 237, 652*t*
 Sebaceous gland, infections of 505
 Seborrheic dermatitis 574
 Sepsis 72, 297, 297*t*, 299
Serratia 76, 400, 691
 Severe acute respiratory syndrome 660
 Sewer-Swab technique 307
 Sexually transmitted infection 110, 329, 756, 762, 764
 Shanghai fever 643
 Shepherd's crook 569
 Shiga toxin 403, 405

Shigella 31, 39, 40, 68, 76, 127, 279, 389
 Shipyard eye 674, 786
 Simian immunodeficiency virus 325
 Simon's focus 626
 Sin nombre virus 819
 Sindbis virus 337
 Sjögren's syndrome 206, 207
 Skeletal tuberculosis 625
 Skirrow's media 420*f*
 Slapped cheek appearance 556, 556*f*
 Sleeping sickness 368
 Slide coagulase test 516
 Slide flocculation test 154
 Slim disease 329
 Slit skin smear 537
 Slow virus disease 715, 724, 724*t*
 Smallpox 126, 238, 557, 558, 558*t*
 Social distancing 670
 Socks syndrome 557
 Soil-transmitted helminth infections 466
 Somatic hypermutations 149, 190
 Sparganosis 573
 Spaulding's classification 253, 253*t*
 Spherules 382, 582
 Spidery molar teeth 544
 Spill management, steps of 235
 Spirillum 15, 20, 813
 Spirochetes 15, 78, 137, 531
 Spirometra 727, 738
 Splendore-Hoeppli phenomenon 581
Sporothrix schenckii 116, 508, 547, 580
 Spotted fever group 311
 Staining techniques 26
 Staphylococcal infections 511
 Steam sterilizer 253, 254, 254*f*
 Steatorrhea 434
Stenotrophomonas 14
 Sterile pyuria 752
 Sterilization control 254-257
 Streptobacillus 21, 78, 509, 812, 813
 Streptococcal pharyngitis 128, 237, 598
 Streptococcal skin infections 522*f*
Streptococcus agalactiae 73, 68, 524*f*, 525*t*, 617, 692, 752
Streptomyces 543
 String test 415, 415*f*, 435
 Stroke culture 35
 Stromal keratitis 785
 Strongyloides 113, 391, 457, 676, 727
 Subcutaneous mycoses 118, 574, 578
 Subdural empyema 690, 690*f*
 Subglottic secretions 244
 Sugar fermentation test 602
 Sulfur granules 543, 544
 Sun-ray appearance 544
 Superantigens 142, 143*f*, 143*t*
 Suppurative thrombophlebitis 292
 Surgical hand scrub 231
 Surgical mask 232, 233*f*
 Surgical site infection 230, 240, 241, 246
 Swarming growth 703, 750*f*

Swimmer's ear 643
 Swimmer's itch 455, 573
 Swimming pool
 chlorination of 674
 conjunctivitis 674
 granuloma 547, 636
 Swollen belly syndrome 468
 Symmers pipestem fibrosis 498
 Synergistic gangrene, progressive 506
 Synergohymenotropic toxins 512
 Synthesize vitamin 122
 Syphilis 62, 156, 756-758, 758*f*, 761
 Systemic lupus erythematosus 204, 206
 Systemic mycoses 119, 377, 379
 Systemic therapy 781

T

T cell 176*t*
 Tabes dorsalis 700
 Taenia 112
Talaromyces marneffeii 14, 582
 Tapir nose 567
 Teichoic acid 17, 512
Ternidens deminutus 469
 Tertiary syphilis 699
 Tetanolysin 702
 Tetanospasmin 702
 Tetanus 691, 702, 703, 704
 Thioglycollate broth 32*f*, 544
 Thumb print appearance 639, 640
 Thymus 169
 Tinea versicolor 118, 574, 574*f*, 575
 Tingible body macrophages 190
 Togaviridae 336, 337
 TORCH infection 129, 793
 Toroviruses 426
 Torres bodies 489
 Toxic shock syndrome 143, 182, 513
 Toxocara 691, 727, 739
 Toxoid 600
 Toxoplasma 108, 111, 156, 330, 676
 Tracheal cytotoxin 638
 Trachoma 781, 781*f*
 Transcytosis 191
 Transfusion malaria 350
 Transfusion transmitted infections 827
 Transposons 59
 Traveler's diarrhea 387, 389*t*, 403, 404
 Trematode 111*t*
 Trematodospaeria grisea 14
 Trench fever 315
Treponema 15, 319, 756, 759
 Trichinella 108, 457, 691
 Trichomonas 108
 Trichophyton 577*f*
Trichosporon beigeli 574*f*
Trichuris 113, 128, 130, 391, 457, 458
 Tridot test 331
 Triple sugar iron 40*t*
 Tripod sign 710

Trismus 702
Tropheryma whipplei 75, 289, 393, 699
 Tropical pulmonary eosinophilia 372, 676
 Tropical splenomegaly syndrome 350
Trypanosoma 108, 187, 296, 360
 Trypomastigote 366, 367*f*, 368
 Tsetse fly 368
 Tube coagulase test 516, 516*f*
 Tuberculin skin test 201, 627, 630
 Tuberculous leprosy 535, 535*f*, 535*t*
 Tuberculoma 700
 Tuberculosis 126, 595, 623, 632, 682
 Tuberculous granulomas 700
 Tuberculous lymphadenitis 625
 Tuberculous meningitis 699, 700
 Tuberculous pericarditis 625
 Tuberculous pleuritis 596
 Tuberculous skin lesions 547, 625
 Typhoid fever 126, 154, 317, 406
 Typhoidal salmonella 76, 302, 303, 406
 Typhus
 endemic 312, 548
 epidemic 311, 548
 Tzanck cell 553*f*

U

Urea breath test 421
 Urea hydrolysis test 38, 39
 Ureaplasma 618, 770
 Urethritis 764, 766*t*
 Urinary schistosomiasis 753, 754
 Urinary tract infection 3, 72, 230, 240,
 249*t*, 401, 514, 518, 745, 746, 747*t*,
 751
 Uropathogenic *Escherichia coli* 401, 749
 Uveitis 779

V

Vaccinia 557, 559
 Vacuolating cytotoxin 420
 Vaginal candidiasis 774, 817
 Vaginal secretion, wet mount of 774*f*
 Vaginosis, bacterial 124, 532, 773
 Varicella-zoster virus 96, 504, 554, 798
 Variolae vaccinae 4, 221
 Venereal disease research laboratory
 760
 Venezuelan equine encephalitis 825
 Ventilator-associated pneumonia 244
Vibrio alginolyticus infections 417
Vibrio cholerae 414
Vibrio parahaemolyticus 417
Vibrio vulnificus infections 417, 548
 Vietnam time-bomb disease 645
 Vincent's angina 322, 533, 591, 592
 Viral interference 86
 Virchow's lepra cells 537
 Viridans streptococci 72, 73, 290, 526
 Visceral larva migrans 499
 Visceral leishmaniasis 360, 361, 361*t*
 Vulvovaginitis 771

W

Wakana disease 464
 Waldenstrom's macroglobulinemia 149
 Waterhouse-Friderichsen syndrome
 697
 Watery diarrhea 412
 Wayson stain 809, 810*f*
 Weil's disease 79, 320, 321
 Weil-Felix test 45, 312, 314
 West Nile encephalitis 718

Western blot 161, 331, 495, 737
 Western equine encephalitis 825
 Whipple's disease 393
 Whipworm 458
 Whitmore's bacillus 5
 Whooping cough 593, 638
 Widal test 154, 305, 306, 306*f*, 307
 Wilson Blair's brilliant 305
 Winter vomiting disease 392, 426
 Winterbottom's sign 368
 Wiskott-Aldrich syndrome 211
 Wood's lamp examination 575, 576
 Wool Sorter's disease 540, 616
Wuchereria bancrofti 106, 370, 371, 374

X

Xenodiagnosis 367
 Xenograft 213
Xenopsylla 312, 808, 825*t*
 X-linked agammaglobulinemia 208
 X-linked recessive disease 211
 Xylose lysine deoxycholate 34, 76, 305*f*

Y

Yaws 545, 546
 Yellow fever 338, 489, 490
Yersinia pestis 807
 Yersiniosis 76, 407, 408

Z

Ziehl-Neelsen staining, modified 22
 Zika 798
 Zoonoses 817
 Zygomycoses 119, 679, 680, 680*f*, 681