

4th
EDITION

Most Updated and Latest Edition 2022
Covering all Recent Updates & Qs up to June 2022 Exams

Most Comprehensive Fully-Colored Book on Microbiology with an Innovative and Simplified Way to Study
First Book having Integrated Approach with Various Clinical Subjects, like Pharmacology, Pathology, Surgery and Medicine

4th
EDITION

MICRONS
Microbiology Simplified

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Microbiology Simplified

As per the New Pattern Exams (NEXT) with many Clinical Case-based Questions

*References and updates from Ananthanarayan & Paniker's 10/e,
Jawetz 27/e, Arora 5/e, Harrison's 21/e, Mandell's 8/e*

Papers/Questions Covered

INI-CET 2022 - 20

Recent Qs (Jan) 2022 - 2011

AIIMS June 2020 - 2002

Expected NEXT/Clinical Pattern Qs

Sample Video Qs

CBME-Based Subjective Qs with
Chapter References

- Written and Compiled by Leading Faculty and Subject Expert of Microbiology
- Enriched with Recent/Latest Updates

2000+
MCQs of
Recent Exams

50+
CBQs

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IBQs

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4th Edition

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Preface

Dear readers,

We all know that COVID-19 has changed our perspective of life completely. An invisible microbe that emerged as a pandemic in 2020 and still causing a surge in the number of new cases globally has turned the human life topsy-turvy. Even with the development of vaccines and ongoing research on treatment modalities, we haven't overcome this pandemic till date.

The knowledge on different microbes and evolution of their species and variants is essential. Microbiology deals with the evolution process, structure, pathogenesis, diagnosis, and treatment of human infections caused by bacteria, virus, parasites, and fungi. As you have completed your MBBS, it will be easier now to correlate the infectious disease condition with the microbial pathogenesis. An integrated clinical learning will help you to remember the features of a genus and species.

When it comes to NEET or INI CET or any new competitive exams that you are planning to face in forthcoming years, your main aim should be to crack the MCQs by using your sharp memory and presence of mind. Every reader who goes through MICRONS will become smart enough to clear these exams because this book will make it easier for the students to have a quicker revision.

I am grateful to the readers for their constant support to *MICRONS-Microbiology Simplified* and happy to share that the new 4th edition is updated with recent questions and an *exclusive chapter on COVID-19*.

As I always say, *Confidence and Self-Motivation is the Key to Success*.

Always ask this to yourself "If not me, then who else?"

All the best for your exams!

Keep sharing your feedback on my mail Id or Facebook page.

With love!

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CBSPD

Dedicated to Education

Contents

<i>Preface</i>	<i>v</i>
<i>Acknowledgments</i>	<i>vii</i>
<i>CBME-Based Subjective Questions with Chapter References</i>	<i>xi</i>
<i>Recent Updates—Coronavirus Disease-19</i>	<i>xv</i>
<i>Latest Exam Questions 2022–2019</i>	<i>xix</i>
<i>Sample Video Questions</i>	<i>lxix</i>
<i>Image-Based Concept Zone</i>	<i>lxxiii</i>

UNIT 1 GENERAL MICROBIOLOGY

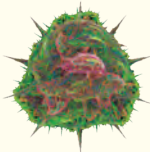
Chapter 1. Introduction, History and Microscopes	3–10
Chapter 2. Morphology and Physiology of Bacteria	11–25
Chapter 3. Sterilization and Disinfection	26–38
Chapter 4. Culture Media and Culture Methods	39–48
Chapter 5. Bacterial Genetics, Resistance and Susceptibility Testing	49–59

UNIT 2 BACTERIOLOGY

Chapter 6. Staphylococcus	63–72
Chapter 7. Streptococci	73–84
Chapter 8. Pneumococcus	85–89
Chapter 9. Neisseria	90–95
Chapter 10. Corynebacterium	96–102
Chapter 11. Bacillus	103–108
Chapter 12. Clostridium	109–118
Chapter 13. Enterobacteriaceae	119–134
Chapter 14. Vibrio	135–141
Chapter 15. Pseudomonas, Acinetobacter and Burkholderia	142–146
Chapter 16. Haemophilus, Francisella and Pasteurella	147–151
Chapter 17. Brucella and Bordetella	152–155
Chapter 18. Mycobacterium	156–166
Chapter 19. Spirochaetes	167–175
Chapter 20. Rickettsia and Chlamydia	176–186
Chapter 21. Helicobacter and Campylobacter	187–189
Chapter 22. Mycoplasma and Legionella	190–193
Chapter 23. Miscellaneous Bacteria	194–202

UNIT 3 VIROLOGY

Chapter 24. Introduction and General Properties of Viruses	205–213
Chapter 25. Bacteriophages	214–217
Chapter 26. Poxviruses	218–221
Chapter 27. Herpesviruses	222–230
Chapter 28. Adenovirus	231–232
Chapter 29. Picornaviruses	233–238
Chapter 30. Orthomyxoviruses	239–243
Chapter 31. Paramyxovirus	244–249
Chapter 32. Arthropod- and Rodent-Borne Viral Infections	250–256
Chapter 33. Rhabdovirus	257–261
Chapter 34. Hepatitis Virus	262–271
Chapter 35. Human Immunodeficiency Virus	272–279
Chapter 36. Miscellaneous Viruses	280–286

**UNIT 4 PARASITOLOGY**

Chapter 37.	Introduction to Parasitology	289–295
Chapter 38.	Flagellates-I	296–299
Chapter 39.	Hemoflagellates	300–304
Chapter 40.	Leishmania	305–308
Chapter 41.	Apicomplexa	309–317
Chapter 42.	Toxoplasma, Ciliate Protozoa	318–321
Chapter 43.	Coccidian Intestinal Parasites	322–325
Chapter 44.	Helminthology Cestodes	326–332
Chapter 45.	Trematodes	333–338
Chapter 46.	Nematodes	339–347
Chapter 47.	Filarial Nematode	348–353

UNIT 5 MYCOLOGY

Chapter 48.	Characteristics and Laboratory Diagnosis of Fungi	357–363
Chapter 49.	Superficial Mycoses	364–370
Chapter 50.	Endemic/Systemic Mycoses	371–375
Chapter 51.	Opportunistic Mycoses	376–383
Chapter 52.	Miscellaneous Fungi	384–386

UNIT 6 IMMUNOLOGY

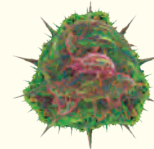
Chapter 53.	Immunity	389–392
Chapter 54.	Structure and Functions of Immune System	393–400
Chapter 55.	Antigens	401–403
Chapter 56.	Antibodies	404–410
Chapter 57.	Complement System	411–415
Chapter 58.	Antigen-Antibody Reactions	416–421
Chapter 59.	Immune Response	422–426
Chapter 60.	Hypersensitivity	427–431
Chapter 61.	Immunodeficiency Diseases	432–435
Chapter 62.	Autoimmunity	436–437
Chapter 63.	Transplantation and Tumor Immunology	438–440
Chapter 64.	Immunoematology	441–444

UNIT 7 APPLIED MICROBIOLOGY

Chapter 65.	Applied Microbiology	447–450
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UNIT 8 INFECTIOUS DISEASES

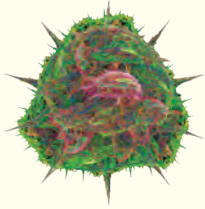
Chapter 66.	Introduction	453–456
Chapter 67.	Fever of Unknown Origin	457–458
Chapter 68.	Infections of Ear, Nose and Throat	459–466
Chapter 69.	Infections of Eye	467–470
Chapter 70.	Infection in Lower Respiratory Tract – Pneumonia	471–472
Chapter 71.	Gastrointestinal Infections	473–478
Chapter 72.	Cardiovascular Infection – Endocarditis	479–481
Chapter 73.	CNS Infections	482–484
Chapter 74.	Skin and Soft Tissue Infections	485–486
Chapter 75.	Infections of Bones and Joints	487–489
Chapter 76.	Sexually Transmitted Infections	490–491
Chapter 77.	Urinary Tract Infections	492–493
Chapter 78.	Infections Related to Obstetrics and Gynecology	494–496
Chapter 79.	Surgical Site and Related Infections	497–500
Chapter 80.	Infections in Special Hosts	501–503
Chapter 81.	Antimicrobial Chemotherapy – A Short Review	504–507
Chapter 82.	Multiple Choice Questions	508–510
	Self-Assessment	511–527



CBME-Based Subjective Questions with Chapter References*

Number	COMPETENCY The student should be able to	Chapters
MICROBIOLOGY		
Topic: General Microbiology and Immunity		
MI 1.1	Describe the different causative agents of Infectious diseases, the methods used in their detection, and discuss the role of microbes in health and diseases	2,4
MI 1.2	Perform and identify the different causative agents of Infectious diseases by Gram Stain, ZN stain and stool routine microscopy	2
MI 1.3	Describe the epidemiological basis of common infectious diseases	66–80
MI 1.4	Classify and describe the different methods of sterilization and disinfection. Discuss the application of the different methods in the laboratory, in clinical and surgical practice	3
MI 1.5	Choose the most appropriate method of sterilization and disinfection to be used in specific situations in the laboratory, in clinical and surgical practice	3
MI 1.6	Describe the mechanisms of drug resistance, and the methods of antimicrobial susceptibility testing and monitoring of antimicrobial therapy	5
MI 1.7	Describe the immunological mechanisms in health	53
MI 1.8	Describe the mechanisms of immunity and response of the host immune system to infections	53, 54
MI 1.9	Discuss the immunological basis of vaccines and describe the Universal Immunization schedule	65
MI 1.10	Describe the immunological mechanisms in immunological disorder (hypersensitivity, autoimmune disorders and immunodeficiency states) and discuss the laboratory methods used in detection.	61, 62
MI 1.11	Describe the immunological mechanisms of transplantation and tumor immunity	63
Topic: CVS and Blood		
MI 2.1	Describe the etiologic agents in rheumatic fever and their diagnosis	7
MI 2.2	Describe the classification, etio-pathogenesis, clinical features and discuss the diagnostic modalities of Infective endocarditis	72
MI 2.3	Identify the microbial agents causing Rheumatic Heart Disease & infective Endocarditis	2, 72
MI 2.4	List the common microbial agents causing anemia. Describe the morphology, mode of infection and discuss the pathogenesis, clinical course, diagnosis and prevention and treatment of the common microbial agents causing Anemia	–
MI 2.5	Describe the etio-pathogenesis and discuss the clinical evolution and the laboratory diagnosis of kala azar, malaria, filariasis and other common parasites prevalent in India	40, 41
MI 2.6	Identify the causative agent of malaria and filariasis	41, 47
MI 2.7	Describe the epidemiology, the etio- pathogenesis, evolution complications, opportunistic infections, diagnosis, prevention and the principles of management of HIV	35
Topic: Gastrointestinal and Hepatobiliary System		
MI 3 1	Enumerate the microbial agents causing diarrhea and dysentery. Describe the epidemiology, morphology, pathogenesis, clinical features and diagnostic modalities of these agents	71
MI 3.2	Identify the common etiologic agents of diarrhea and dysentery	71, 13, 14, 21
MI 3.3	Describe the enteric fever pathogens and discuss the evolution of the clinical course and the laboratory diagnosis of the diseases caused by them	13
MI 3.4	Identify the different modalities for diagnosis of enteric fever. Choose the appropriate test related to the duration of illness	13

* Important competency-based topics covered



RECENT UPDATES

Coronavirus Disease-19

BACKGROUND

Novel Coronavirus disease originated from Wuhan, China during December 2019. It was then renamed as Coronavirus disease-19 (COVID-19) and the virus was named as Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) on February 11, 2020.

EPIDEMIOLOGY OF COVID-19



CHARACTERISTICS OF SARS-COV-2

- Coronaviruses are enveloped positive-stranded RNA viruses.
- SARS-CoV-2 is an enveloped beta-coronavirus with genetic sequence similar to SARS-CoV-1 (80%).
- Closest RNA sequence similarity is to two bat coronaviruses, and it appears likely that bats are the primary source; whether COVID-19 virus is transmitted directly from bats or through some other mechanism (e.g., through an intermediate host) remains unknown.
- The viral envelope is coated by spike (S) glycoprotein, envelope (E) and membrane (M) proteins.
- S protein is the virulence factor that helps in host cell binding and entry into host cells.
- The virus binds the host cell through its target receptor. The S1 subunit of the S protein has the receptor binding domain which binds to the peptidase domain of angiotensin converting enzyme 2.
- The cellular protease TMPRSS2 also appears important for SARS-CoV-2 cell entry (Fig. 1).

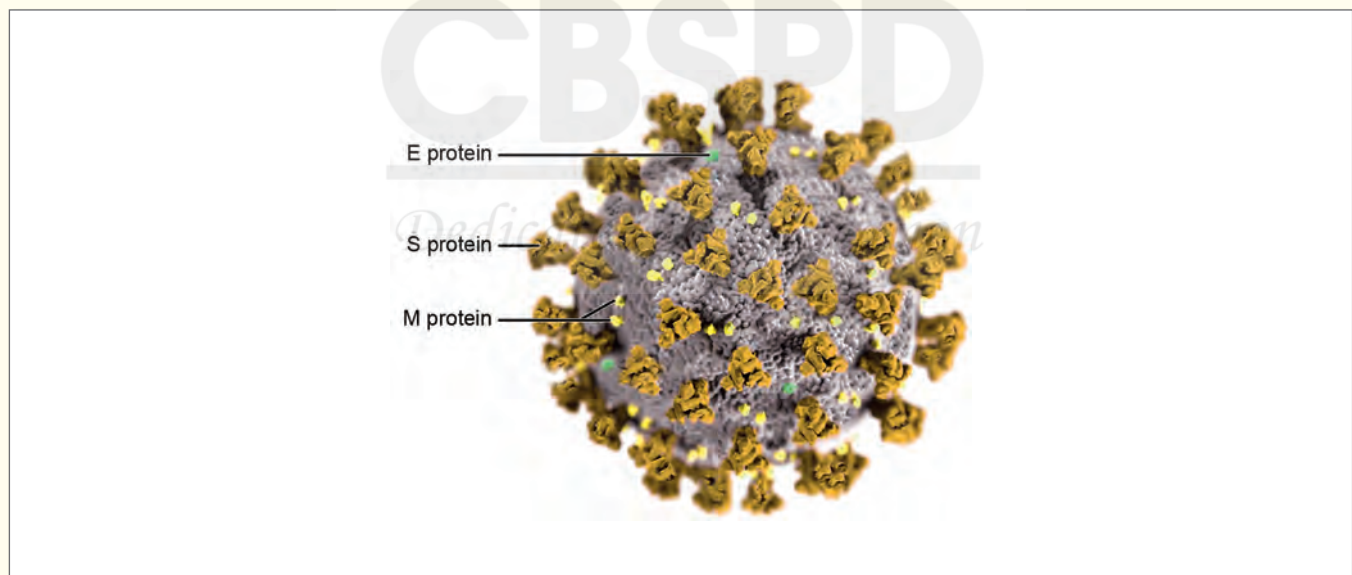
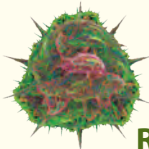


Figure 1: SARS-CoV-2 virus structure
(Courtesy: CDC PHIL library)



REPLICATION CYCLE (FIG. 2)

The replication of SARS-CoV-2 virus in host cell is as follows:

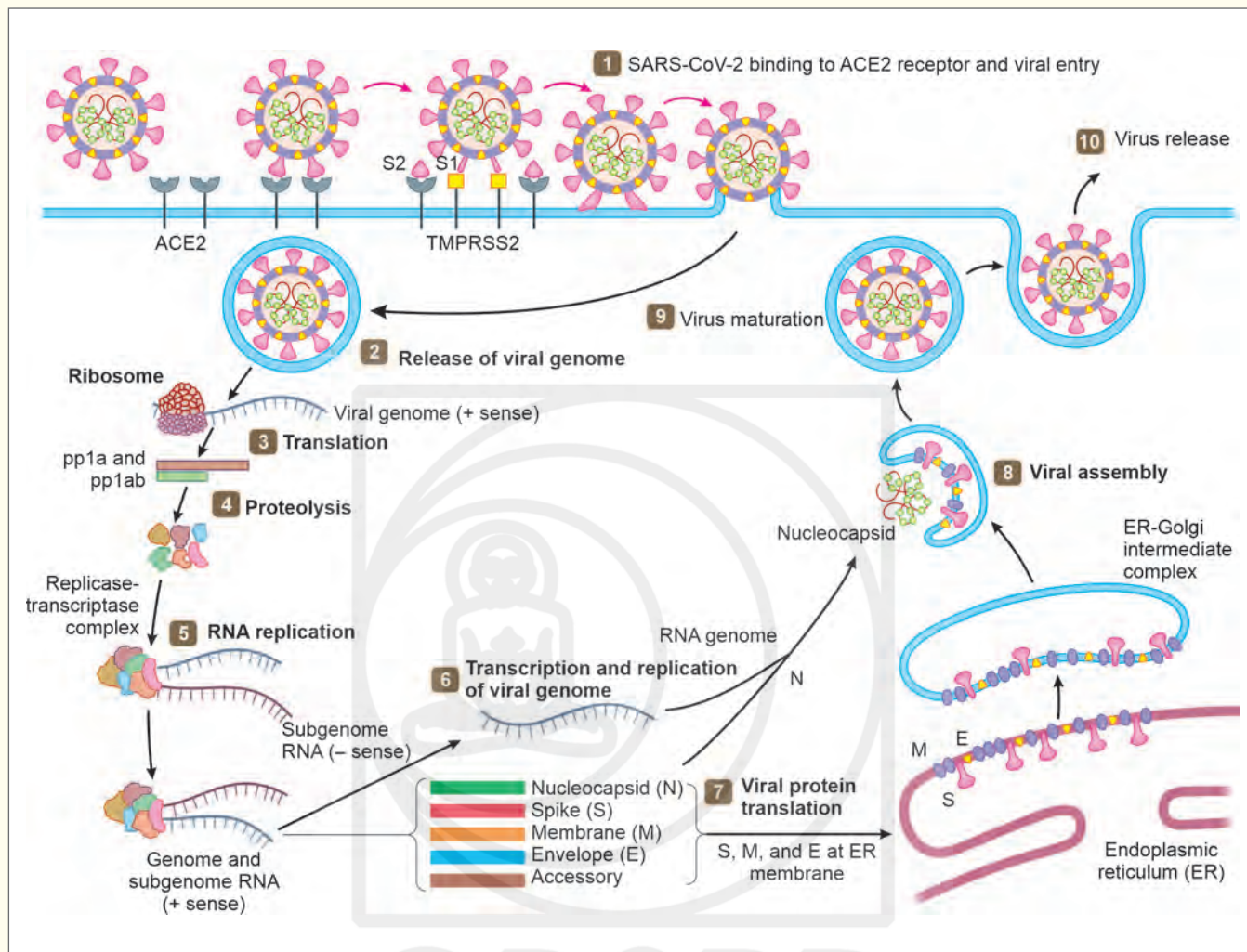


Figure 2: Replication cycle

Ref: Cevik M, Kuppalli K, Kindrachuk J, Peiris M. *Virology, transmission, and pathogenesis of SARS-CoV-2* BMJ 2020; 371 :m3862 doi:10.1136/bmj.m3862

MUTATION AND VARIANTS IN SARS-COV-2

Mutation:

- Substitution at a particular position in the protein (S:D614G) which means aspartic acid to glycine substitution at the 614 position of the spike (S) protein.
- There are several mutations that occur in RNA virus.

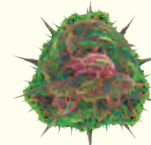
Variants:

- In SARS-CoV-2, there has been variants reported across the world. Two terminologies are used:
 1. Variants of concern—which means it is:
 - Highly transmissible and more infectious
 - More lethal
 - Can escape immunity due to previous infections
 - Can escape immunity due to vaccination
 - Can reduce the efficacy of drugs and monoclonal antibodies
 - Can reduce the sensitivity of PCR assays

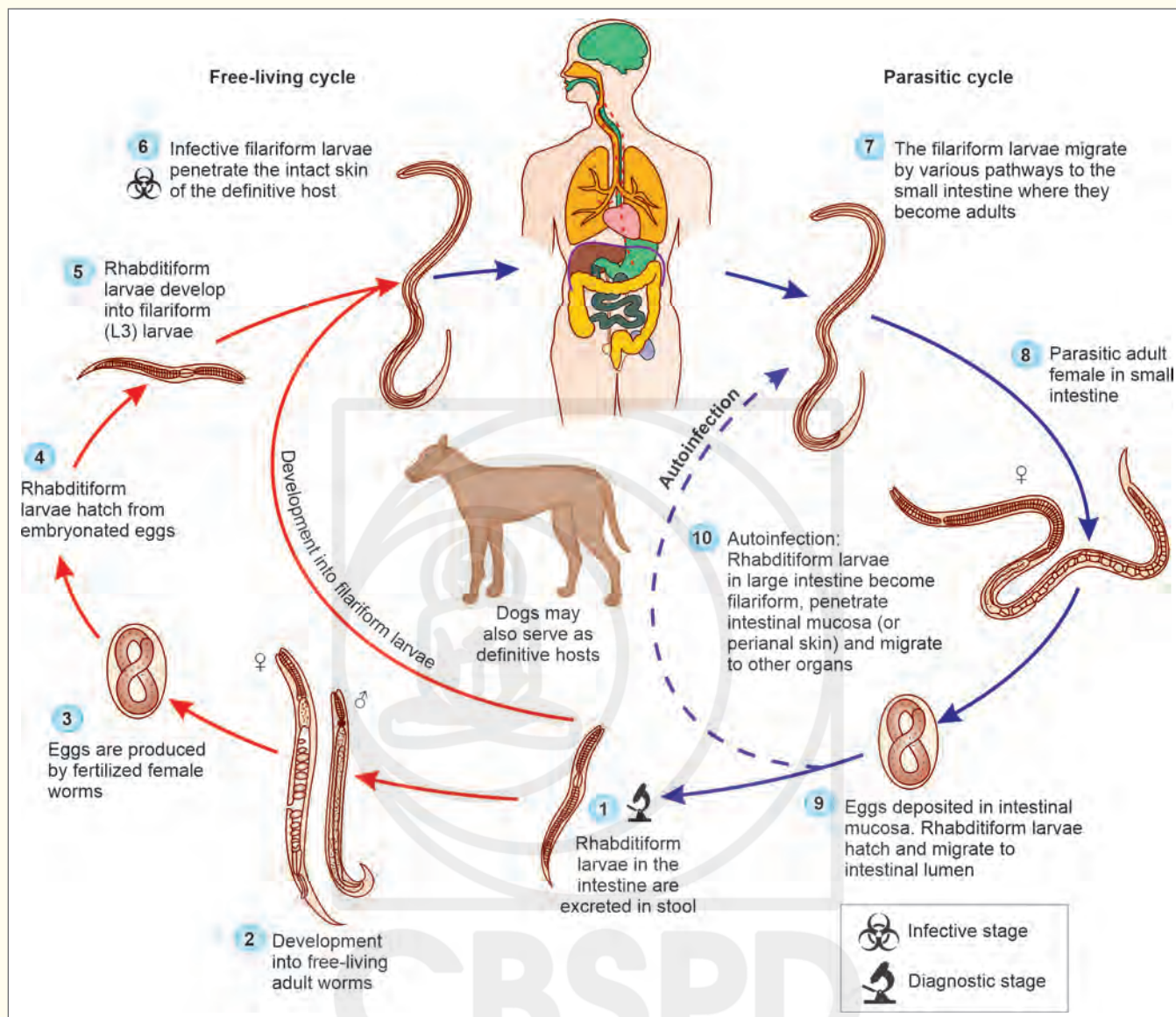
2. Variants of interest—which means it is under investigation

Mutants/variants of concern	Properties
D614G variant	<ul style="list-style-type: none"> • Identified from parent Wuhan virus (19B) in February 2020 • D614G variant occurs due to single mutation in gene coding for spike protein • Changes from aspartic acid to glycine hence, less bulky allowed it to bind better to ACE2 receptor • More transmissible than original Wuhan virus • Not more lethal

Contd...



8. Identify the organism that has the following life cycle.



- a. Enterobius vermicularis b. Ancylostoma duodenale c. Strongyloides stercoralis d. Dracunculus medinensis

9. Which of the following is correct regarding the organism that excretes the egg given in the image below?



- a. The drug of choice is albendazole
 b. It is a leaf-shaped parasite
 c. Water plants is the source of infection
 d. Most of the patients are asymptomatic

10. Which of the following virus undergoes genetic reassortment as influenza virus?

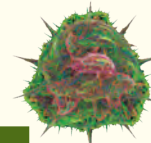
- a. Ebola virus
 b. Rhabdo virus
 c. Adeno virus
 d. Rota-virus

11. Which of the following is wrongly matched between primary immune response (PIR) and secondary immune response (SIR)?

- a. Antibody involved: PIR - IgM; SIR- IgG
 b. Lag period: PIR 4-7days; SIR 1-2 days
 c. Antibody affinity: PIR - High; SIR - Low
 d. Longevity: PIR - Short lived; SIR - Prolonged

12. If an antigen binds to MHC class I, it activates the following:

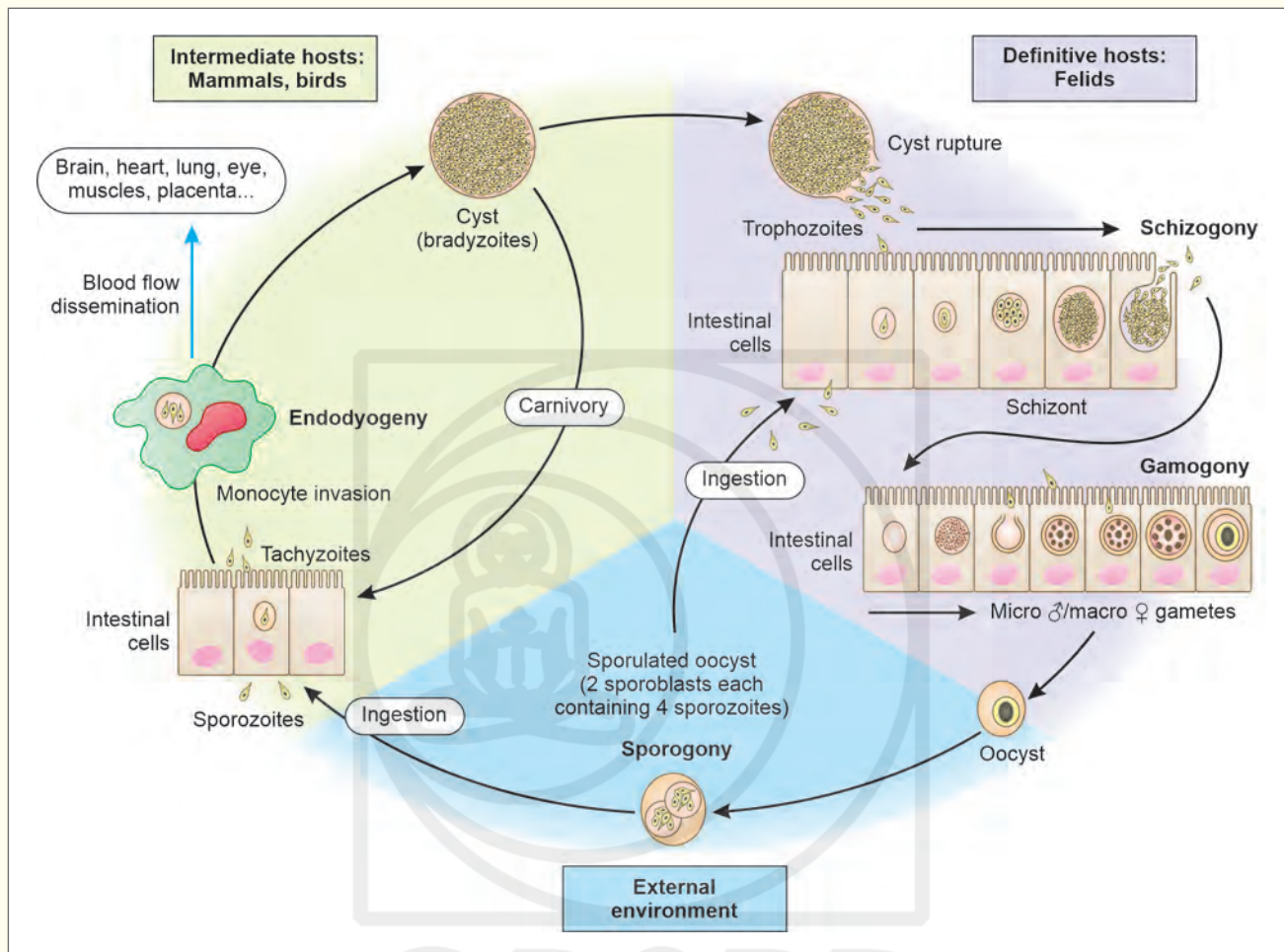
- a. B cell
 b. Cytotoxic T cell
 c. Helper T cell
 d. NK cell



INI-CET QUESTIONS – JULY 2021

49. The following figure shows life cycle of:

- a. Cryptosporidium b. Toxoplasmosis c. Plasmodium d. Cystoisospora



50. Which of the following is true regarding trematodes?

- a. Fasciolopsis buski is seen in intrahepatic biliary radicles
 b. Schistosoma are monoecious
 c. Triclabendazole is the treatment of choice for all trematode infections
 d. Fasciola hepatica is acquired through contaminated water plants

51. An old age man undergone tooth extraction few days back. He now presents with mass in the submandibular region. There is no pain or fever. Upon drainage, foul smelling discharge with yellow granules were seen. On microscopic examination, Gram-positive filamentous rods were seen. What is the most likely organism causing this clinical condition?

- a. Chromoblastomycosis b. Mucormycosis
 c. Actinomyces israelii d. Histoplasmosis

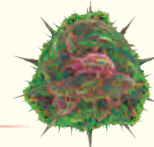
52. A 35-year-old man presents to the emergency department with rice watery stools. In the last 12 hours, there was 7-8 episodes. Which of the following is disrupted in the intestine?

- a. Hemidesmosomes b. Gap junctions
 c. Zona occludens d. Zona adherens

53. A man who went into the forest for trekking and few days later, he presents with the following skin lesion (eschar). All of the following could be the etiologies; except:



- a. Anthrax
 b. KFD
 c. Scrub typhus
 d. Spider bite



ANSWERS WITH EXPLANATIONS

INI-CET QUESTIONS – 2022

1. Ans. (c) *Aspergillus fumigatus*

Ref: Ananthanarayan and Paniker's T.B. of Microbiology – 10th edition – page 619

The cell wall given in the image has Beta-glucan. Beta glucan is seen in *Aspergillus*, *Candida*, and *Pneumocystis*. Another clue is Chitin, which is unique for cell wall of fungi.

2. Ans. (d) Dengue

Ref: <https://www.cdc.gov/bloodsafety/basics.html>

The screening tests before blood transfusion are:

- HIV
- Hepatitis B
- Hepatitis C
- Syphilis

Dengue is a mosquito borne illness and hence, does not need screening by blood.

3. Ans. (c) Chromoblastomycosis

Ref: Ananthanarayan and Paniker's T.B. of Microbiology – 10th edition – page 605

The given image shows copper penny or mulberry bodies which is characteristic of Chromoblastomycosis. Chromoblastomycosis is caused by Dematiaceous fungi. The infection occurs usually after trivial trauma.

4. Ans. (c) Malignant melanoma

Ref: Harrison's T.B. of Internal Medicine – 19th edition – page 1215

Among the given options, Non-Hodgkin's lymphoma is caused by Epstein-Barr virus. Kaposi sarcoma is caused by HHV8. Invasive cervical cancer is caused by HPV 16,18. All of these are opportunistic infections except malignant melanoma which is not associated with AIDS.

5. Ans. (a) *Ureaplasma urealyticum*

Ref: Jawetz T.B. of Medical Microbiology – 27th edition – page 31

Molliculate are group of bacteria that do not have cell wall and called L forms.

Species: *Mycoplasma pneumoniae*, *Ureaplasma urealyticum* and other species in the family.

6. Ans. a, b, d

Ref: Ananthanarayan and Paniker's T.B. of Microbiology – 10th edition – page 13

Organisms that are acid fast in nature are:

- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Nocardia*
- Oocysts of coccidian parasites
- Bacterial spores
- Eggs of *Taenia saginata*
- *Rhodococcus*
- *Legionella micdadei*

7. Ans. (d) Tuberculosis prophylaxis mandatory, if CD4 count is <1000

Ref: <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5108a1.htm>

CD4 count	Organisms
0–100	Disseminated MAC infection Histoplasmosis CMV retinitis CNS lymphoma
100–250	<i>Pneumocystis</i> Toxoplasmosis Cryptococcosis Coccidioidomycosis Cryptosporidiosis
250–500	Bacterial infections Tuberculosis Herpes simplex Herpes zoster Kaposi sarcoma

8. Ans. (c) *Strongyloides stercoralis*

Ref: T.B. of Medical Parasitology – S.C. Parija – 4th edition – page 271

In the given flowchart, the identification points are:

- Morphological forms: Filariform larvae and Rhabditiform larvae.
- Another clue is autoinfection.
- Route of entry is through skin penetration.

9. Ans. (d) Most of the patients are asymptomatic

Ref: T.B. of Medical Parasitology – S.C. Parija – 4th edition – page 213

- The given image is *Hymenolepis nana* egg.
- *H. nana* is a cestode that is acquired by ingestion of eggs. The definitive hosts are humans, rats and mice. Intermediate host is nil.
- Drug of choice is praziquantel.
- The parasite is an intestinal cestode and called dwarf tapeworm.

10. Ans. (d) Rotavirus

Ref: Ananthanarayan and Paniker T.B. of Microbiology – 10th edition – page 434

Genetic reassortment can occur in segmented RNA viruses. BORA:

- Bunyaviridae
- Orthomyxoviridae
- Reoviridae
- Arenaviridae

Rotavirus comes under viruses with segmented RNA.

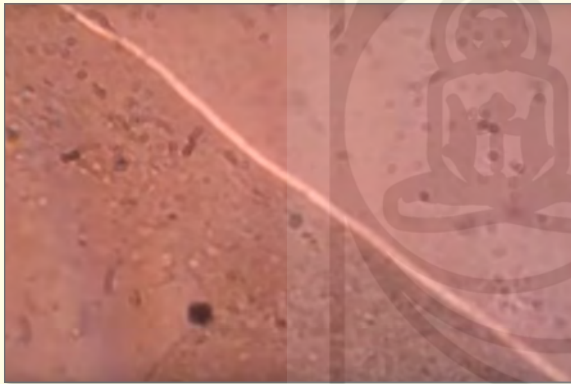
11. Ans. (c) Antibody affinity PIR: High; SIR – Low

Ref: Ananthanarayan and Paniker T.B. of Microbiology – 10th edition – page 148



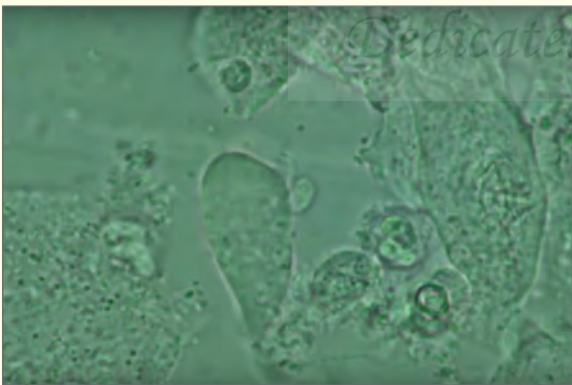
Sample Video Questions

1. In a war conflict zone, 150 patients got acute watery diarrhea. On stool examination, the following motility pattern is seen. Which culture media is ideal for identifying the organism?



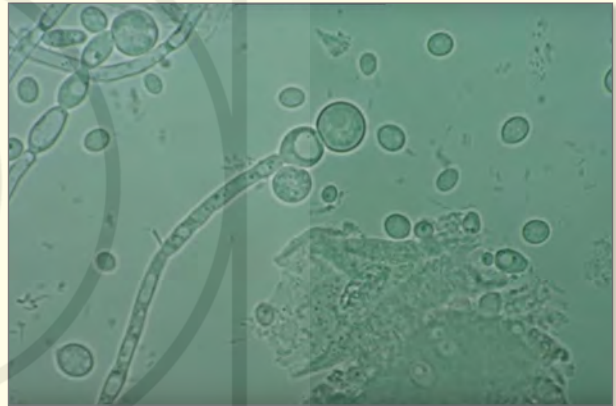
- a. DCA agar
- b. TCBS agar
- c. MacConkey agar
- d. Blood agar

2. A 28-year-old newly married female presented with complaints of intense itching per vagina and greenish discharge. On wet mount of vaginal discharge, the following organism is seen. What morphological stage is this?



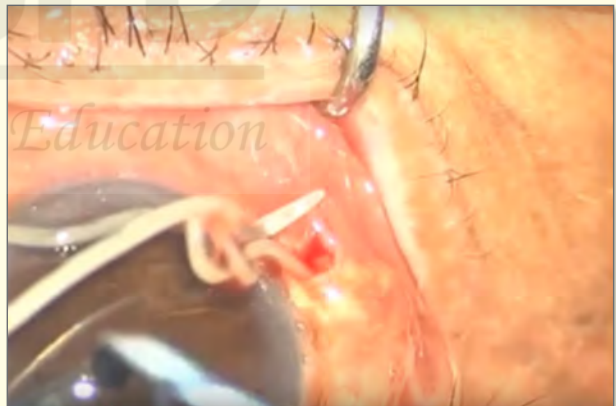
- a. Cyst
- b. Trophozoites
- c. Tachyzoites
- d. Oocysts

3. Identify the following organism visualized by wet mount of vaginal discharge:

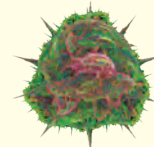


- a. *Candida albicans*
- b. *Cryptococcus neoformans*
- c. *Trichomonas vaginalis*
- d. *Gardnerella vaginalis*

4. Vector for this African eye worm is:



- a. *Simulium*
- b. *Chrysops*
- c. *Anopheles*
- d. *Xenopsylla*



9. What type of culture streaking method is shown here:



- a. Stab culture
- b. Streak culture
- c. Lawn culture
- d. Liquid culture

10. Identify the organism:



- a. *Strongyloides*
- b. *Hookworm*
- c. *Borrelia*
- d. *Wuchereria*

• Sample Video Questions

For video, scan this QR Code



ANSWERS FOR VIDEO QUESTIONS

1. Ans. (b) TCBS agar

- Video shows typical darting motility of *Vibrio cholerae*
- *Vibrio cholerae* has polar flagella and exhibits fast motility
- Clinical features shown as acute watery diarrhea causing cholera outbreak in a camp
- *Vibrio cholerae* is a Gram-negative bacilli which grows in selective medium called Thiosulfate-citrate-bile salt - sucrose agar (TCBS). Colonies of *V. cholerae* are yellowish in color because of sucrose fermentation.

2. Ans. (b) Trophozoites

- A sexually active female with greenish discharge indicates Trichomoniasis
- Video showing twitching motility of *Trichomonas vaginalis*
- *Trichomonas vaginalis* has only trophozoite stage; it does not have cystic stage.

3. Ans. (a) *Candida albicans*

- Vaginal discharge shows yeast cells with pseudohyphae
- True yeast: Cryptococcus
- Yeast with pseudohyphae: *Candida albicans*
- Hyphae are seen in other fungi like dermatophytes, *Aspergillus*, *Rhizopus*, *Mucor*

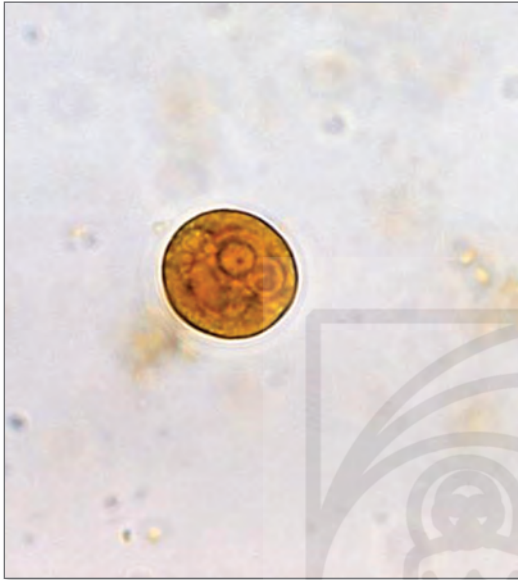
4. Ans. (b) *Chrysops*

- Loiasis, called African eye worm by most people, is caused by the parasitic worm *Loa loa*.
- It is transmitted to humans by the repeated bites of deerflies of the genus *Chrysops*.
- These flies are seen in West and Central Africa.
- Infection with the parasite can also cause repeated episodes of itchy swellings of the body known as Calabar swellings.
- In some patients, visible movement of worm is seen inside the eye; but it won't cause much effects to the eye.

Image-Based Concept Zone

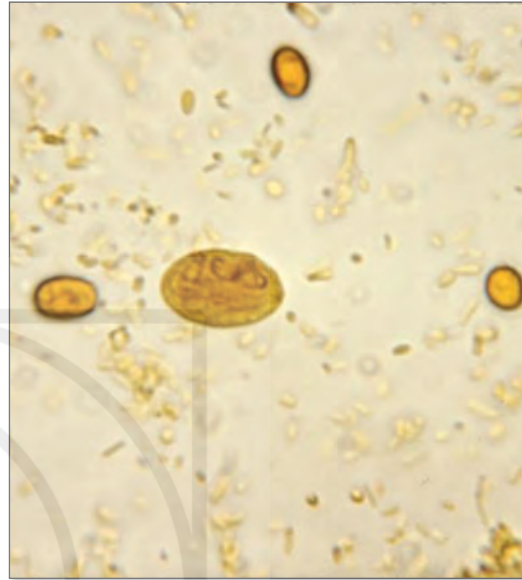
Parasitology

1. Cyst of *Entamoeba histolytica*



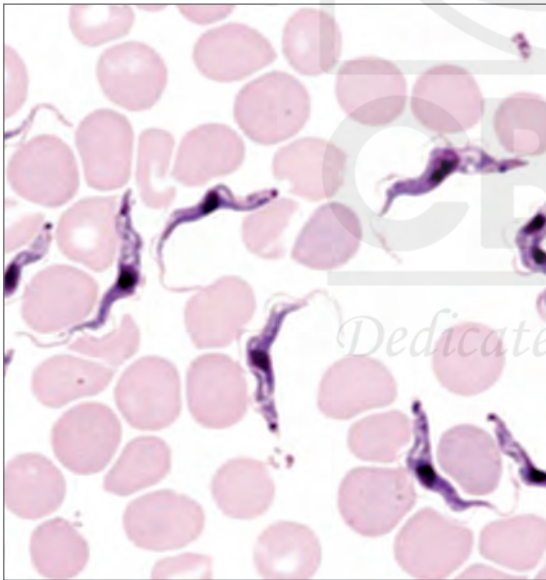
- *E. histolytica* is an intestinal protozoan
 - Mature cyst has four typical nuclei (Quadrinucleate)
 - One nucleus is clearly visible here
- DD:** Cyst of *Entamoeba coli*-it has 8 nuclei

2. Cysts of *Giardia lamblia*



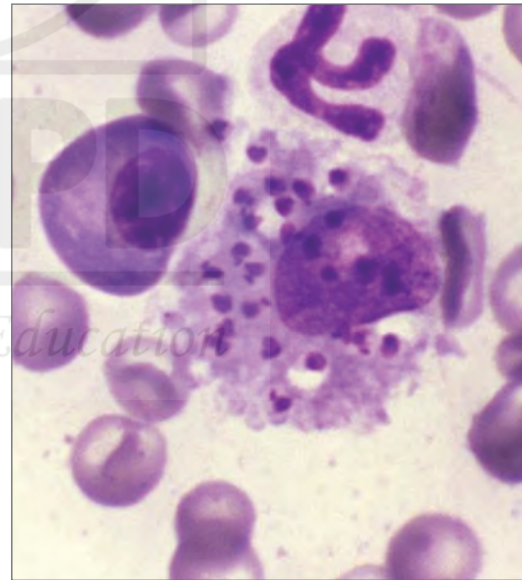
- Ellipsoidal in shape
- Mature cyst has four nuclei
- Identification is by correlation with clinical features like malabsorption, steatorrhea and by seeing cysts in feces.

3. *Trypanosoma brucei*



- Peripheral blood film shows *Trypanosoma brucei gambiense*
- **It occurs in three forms:** Long slender with flagellum, intermediate form and short stumpy form without flagellum.
- Metacyclic trypomastigote is the infective form for man
- Short stumpy form is the infective form for tsetse fly

4. Amastigotes of *Leishmania donovani*



- Image shows smear from bone marrow
- Many amastigotes are seen invading the REC and macrophages nearby
- Amastigotes seen near macrophages suggest *L. donovani*

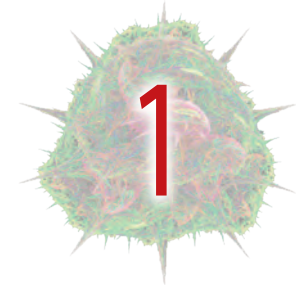


Dedicated to Education

GENERAL MICROBIOLOGY

1

Introduction, History and Microscopes



INTRODUCTION

General Microbiology

Medical microbiology is the study of microbial organisms that infect humans. Based on structures, replication and pathogenesis microbes are divided into Bacteria, Virus, Fungi and Parasites.

Types of Microorganisms

Bacteria

- Bacteria are simple and unicellular organisms
- They come under **prokaryotes**
- All the bacteria are covered by cell walls which are composed of **proteoglycans**
- They reproduce by a process called **binary fission** by which — bacteria divide into two equal cells
- Most of the bacteria derive food by photosynthesis (on its own) and some obtain nutrition by inorganic substances
- Many bacteria have the capacity to mobilize with the help of flagella.

Archaea

- **Prokaryotic cells**
- Do not have peptidoglycan
- They are classified into three main groups namely: Methanogens, Extreme halophiles and Extreme thermophiles.

Fungi

- Belong to **Eukaryotes**
- Cell walls are made up of **chitin**
- Most of the typical fungi grow as mold.

Protozoa

- They are unicellular, **eukaryotic** microbes
- They can move with the help of pseudopodia, flagellum or cilia
- They can reproduce sexually or asexually.

Algae

- Algae are photosynthetic **eukaryotes** which have characteristic presence of cellulose.

Viruses

- They are smaller than other microbes and acellular organisms
- Virus particle has a core that is made up either of DNA or RNA

- This core is surrounded by a protein coat
- Viruses can reproduce only with the help of cellular environment of other organisms (intracellular).

Prokaryotes	Bacteria and Archaea
Eukaryotes	Fungi, Protozoa and Algae

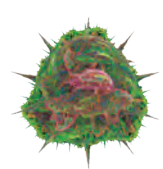
SCIENTISTS AND THEIR DISCOVERIES

Louis Pasteur

- He is known as the **father of microbiology**.
- After Leeuwenhoek discovered the invisible world of microbes, the dispute about spontaneous generation theory started.
- Pasteur conducted an experiment and **disproved the theory of spontaneous generation**.
- He then **proposed Germ theory of disease** by stating that invisible microbes in the air causes infection.
- He introduced many techniques in sterilization namely:
 - Steam sterilizer
 - Hot air oven
 - Autoclaving
 - Pasteurization of milk
- His contributions in vaccine designing are:
 - Anthrax
 - Cholera in fowls
 - Rabies
- He introduced liquid media (nutrient broth) for the growth of microbes.



Figure 1: Father of microbiology – Louis Pasteur



Robert Koch

- He is known as **father of medical microbiology**
- His contributions in identification of microbial organisms are as follows:
 - Solid media (Agar) for the growth of organisms
 - Methods to isolate organisms from pure culture
 - Hanging drop method to test the motility of an organism
 - **Staining techniques**
- He discovered the following organisms:
 - Anthrax bacilli
 - TB bacilli
 - Cholera bacilli

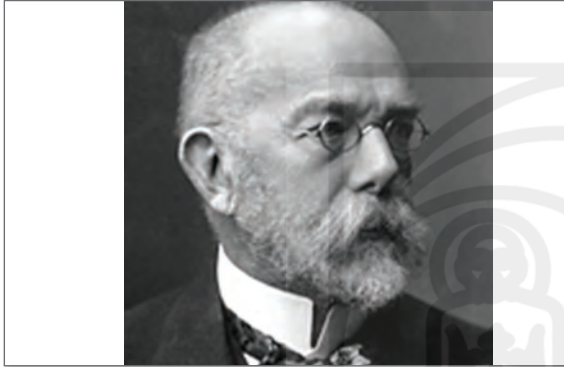


Figure 2: Father of medical microbiology – Robert Koch

Remember

- | | |
|-----------------------------------|-----------------|
| • Vaccination of Anthrax bacilli | – Louis Pasteur |
| • Organism per se Anthrax bacilli | – Robert Koch |
| • Liquid media | – Louis Pasteur |
| • Solid media | – Robert Koch |

Koch Postulates

- Bacterium should be constantly associated with the lesions of the disease
- It should be possible to isolate the bacterium in pure culture from the lesions
- Inoculation of the pure culture into suitable lab animals should produce lesions of the disease
- It should be possible to reisolate the bacterium in pure culture from the lesions produced in the lab animals
- Specific antibodies to the bacterium should be demonstrable in the serum of the patients with the disease.

Molecular Koch Postulates

- Additional postulates added to original Koch's postulate by Stanley Falkow.
- The phenotype or property under investigation should be significantly **associated with pathogenic strains** of a species and not with non pathogenic strains.
- Specific **inactivation of the gene** or genes associated with the suspected virulence trait should lead to a measurable **decrease in pathogenicity or virulence**
- Reversion or **replacement of the mutated gene** with the wild-type gene should lead to **restoration of pathogenicity** or virulence.

Exception to Koch's Postulates

- *Mycobacterium leprae* – cannot be cultured *in vitro*
- *Treponema pallidum* – cannot be cultured *in vitro*
- *Neisseria gonorrhoea* – no animal model for experimental inoculation
- **Partially satisfied Koch's postulates by *Escherichia coli*** – it showed bacterial pathogenicity in an *in vitro* model (Tissue cultures) rather than in an animal model.
- Rickettsia spp and Chlamydia spp – It can be grown only in cell culture media and very difficult to grow in agar plates.

Remember

Koch's Phenomenon

- It is seen in **tuberculosis**.
- Koch observed that guinea pig that has already got infected with TB bacillus will produce a hypersensitivity reaction when it is injected with TB bacilli or protein – this is called Koch's phenomenon.

Organism Not satisfying the Koch's postulates	Organism Partially satisfying the Koch's postulates
<ul style="list-style-type: none"> • <i>Mycobacterium leprae</i> • <i>Treponema pallidum</i> • <i>Neisseria gonorrhoeae</i> 	<ul style="list-style-type: none"> • <i>Escherichia coli</i>

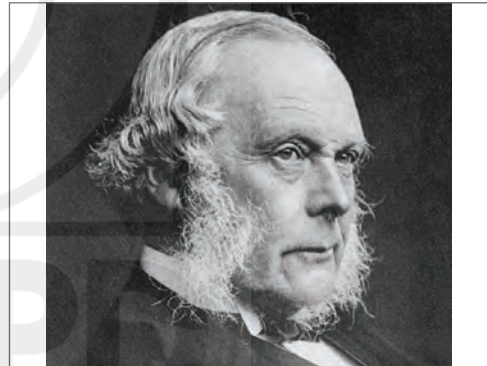


Figure 3: Father of aseptic surgery – Joseph Lister

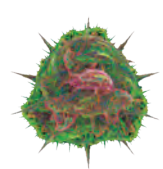
Remember

- | | |
|----------------------------------|-----------------|
| • Father of Microbiology | – Louis Pasteur |
| • Father of Medical Microbiology | – Robert Koch |
| • Father of Aseptic Surgery | – Joseph Lister |
| • Father of Chemotherapy | – Paul Ehrlich |

Table 1: Scientists and their contributions

Scientists	Contributions
Louis Pasteur (Father of Microbiology)	<ul style="list-style-type: none"> • Vaccine for rabies • Vaccine for anthrax • Technique of sterilization • Disproved theory of spontaneous generation • Proposed germ theory of disease • Coined the term vaccine

contd...



MULTIPLE CHOICE QUESTIONS

History

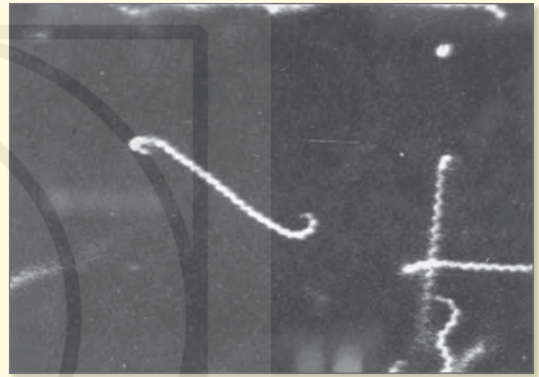
1. **Germ theory of disease was proposed by:**
(Recent Pattern Nov 15)
 - a. Louis Pasteur
 - b. James Lind
 - c. Aristotle
 - d. Pattenkoffer
2. **Technique of sterilization was introduced by:**
(Recent Pattern July 15)
 - a. Robert Koch
 - b. Edward Jenner
 - c. Louis Pasteur
 - d. Lister
3. **Mycobacterium tuberculosis was discovered by:**
(Recent Pattern Dec 15)
 - a. Louis Pasteur
 - b. Robert Koch
 - c. Lister
 - d. Jenner
4. **Microscope was invented by.**
(Recent Pattern Aug 13)
 - a. Ronald Ross
 - b. Robert Koch
 - c. Antonie van Leeuwenhoek
 - d. Louis Pasteur
5. **Who discovered electron microscope?**
 - a. Robert Koch
 - b. Paul Ehrlich
 - c. Elie Metchnikoff
 - d. Ernst Ruska

Microscopes

6. **Arrangement of lens from eye to source of light, in light microscope:**
(Recent Pattern Dec 13)
 - a. Ocular lens: objective lens: condenser lens
 - b. Subjective lens: ocular lens: condenser lens
 - c. Condenser lens: objective lens: ocular lens
 - d. Subjective lens: condenser lens: ocular lens
7. **Light microscopy resolution:**
(PGI May 12)
 - a. 200 nm
 - b. 20 nm
 - c. 0.2 nm
 - d. 300 nm
8. **Dark ground microscopy is used to see:**
(Recent Pattern Dec 14)
 - a. Refractile organism
 - b. Flagella
 - c. Capsule
 - d. Fimbriae
9. **Dye used for direct immunofluorescence:**
(Recent Pattern Dec 15)
 - a. India ink
 - b. Nigrosine
 - c. Rhodamine
 - d. Basic fuschin
10. **Shadow casting is used in:**
(Recent Pattern Dec 16)
 - a. Light microscopy
 - b. Electron microscopy
 - c. Optical microscopy
 - d. Fluorescence microscopy
11. **Bifringence polarization microscopy is used for:**
(Recent Pattern Dec 15)
 - a. Flagella
 - b. Intracellular structures
 - c. Capsule
 - d. Spores

12. **Side chain theory for antibody production is proposed by**
(Recent Pattern 2017)
 - a. Robert Koch
 - b. Paul Ehrlich
 - c. Elie Metchnikoff
 - d. Louis Pasteur
13. **Medium of travel in electron microscope**
(Recent Pattern 2017)
 - a. Air
 - b. Water
 - c. High vacuum
 - d. Oil

14. **Which method of diagnosis is shown here?**
(AIIMS May 2018)

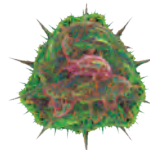


- a. Dark ground microscopy
- b. Phase contrast microscopy
- c. Fluorescent microscopy
- d. Electron microscopy

15. **Identify the condenser in the following microscope image:**
(AIIMS May 2018)



- a. Above the stage
- b. Below the stage
- c. Near the eyepiece
- d. Above the objective lenses



ANSWERS AND EXPLANATIONS

1. Ans. (a) Louis Pasteur

Ref: Ananthanarayan and Paniker's Textbook of Microbiology, 10th ed, Page 3

- Louis Pasteur - Disproved the theory of spontaneous generation and postulated germ theory of disease.

2. Ans. (c) Louis Pasteur

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 3

- Pasteur is the one who introduced techniques of sterilisation like steam steriliser, hot air oven and autoclave

3. Ans. (b) Robert Koch

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 4

- Robert Koch discovered Mycobacterium tuberculosis and Vibrio cholerae

4. Ans. (c) Antonie van Leeuwenhoek

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 3

- Antonie Van Leeuwenhoek - first time observed and reported bacteria with his simple hand made microscope

5. Ans. (d) Ernst Ruska

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 4

- Ernst Ruska in 1934 developed the electron microscope - to visualise viruses

6. Ans. (a) Ocular lens: objective lens: condenser lens

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 10

- Arrangement of lenses in bright field or light microscope is ocular lens in eye piece which is of 5X or 10X
- Objective lens which has low power (10X), high power (45X) and oil immersion (100X) lenses
- Condenser lens is located in the diaphragm which helps in focussing of lights from light rays
- Ocular lens → Objective lenses → Condenser lens

7. Ans. (d) 300 nm

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 11

- Resolving power of a microscope is the ability to differentiate two different objects as different
- It is limited by the wavelength of the light
- Limit of resolution of a light microscope is 300 nm

8. Ans. (b) Flagella

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 11

- Dark-field microscope is used to see very slender organisms like spirochaetes and to see the flagella
- While fimbriae can be demonstrated by hemagglutination

9. Ans. (c) Rhodamine

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 11

- Fluorescent dyes used in immunofluorescence are
 - Auramine
 - Rhodamine
 - Lissamine
 - FITC (M/c used) - Fluorescein isothiocyanate

10. Ans. (b) Electron microscopy

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 12

- To visualize the organism under electron microscope - two types of techniques are followed
 - Shadow casting
 - Negative staining with phosphotungstic acid

11. Ans. (b) Intracellular structures

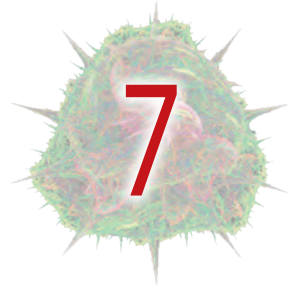
Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 12

- Polarization microscope: To study the intracellular structures using *differences in birefringence*

12. Ans. (b) Paul Ehrlich

Ref: Essentials of Medical Microbiology - Apurba Sastry - Page 3

- **Father of microbiology** → Louis Pasteur
- Germ theory of disease, Nutrient broth, pasteurization of milk, autoclaving, hot air oven and steam sterilizer Louis Pasteur
- **Father of antiseptic surgery** → Joseph Lister
- Introduction of solid media, pure culture techniques, anthrax bacilli, tubercle bacilli, cholera bacilli, Koch's phenomenon → Robert Koch
- **Father of chemotherapy** → Paul Ehrlich
- Acid fast nature of tubercle bacillus, toxin-antitoxin interaction called as Ehrlich phenomenon, side chain theory for antibody production
- Phagocytosis → Elie Metchnikoff



Streptococci

- Many species comes under family of Streptococci
- The species are classified based on certain properties as given below.

Table 1: Classification of Streptococci

Based on hemolysis in blood agar	<ul style="list-style-type: none"> • Alpha hemolytic - Viridans group • Beta hemolytic • Gamma (no hemolysis) – Enterococcus group
Beta hemolytic is classified based on the carbohydrate antigen (Lancefield grouping)	A to V (Except I and J) Group A – Eg: <i>Streptococcus pyogenes</i>
Group A Streptococci – classified based on M protein (Griffith typing)	80 types

Table 2: Different species of streptococci

Species	Lancefield group	Type of hemolysis	Laboratory tests
<i>S. pyogenes</i>	A	Beta	Bacitracin sensitive; PYR test positive; Ribose not fermented
<i>S. agalactiae</i>	B	Beta	CAMP test positive; hippurate hydrolysis
Viridans – <i>S. mitis</i> <i>S. mutans</i> <i>S. salivarius</i> <i>S. sanguinis</i>	Not typed	Alpha	Optochin resistant
Enterococcus sp	D	No hemolysis-gamma	PYR positive; Growth in 6.5% NaCl
Non-enterococcus sp	D	No hemolysis-gamma	No growth in 6.5% NaCl

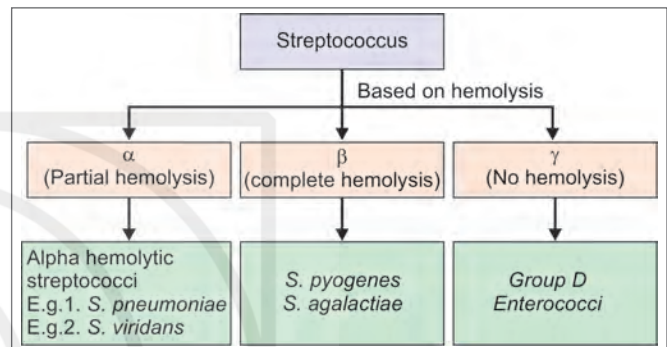


Figure 1: Classification of streptococcus based on hemolysis

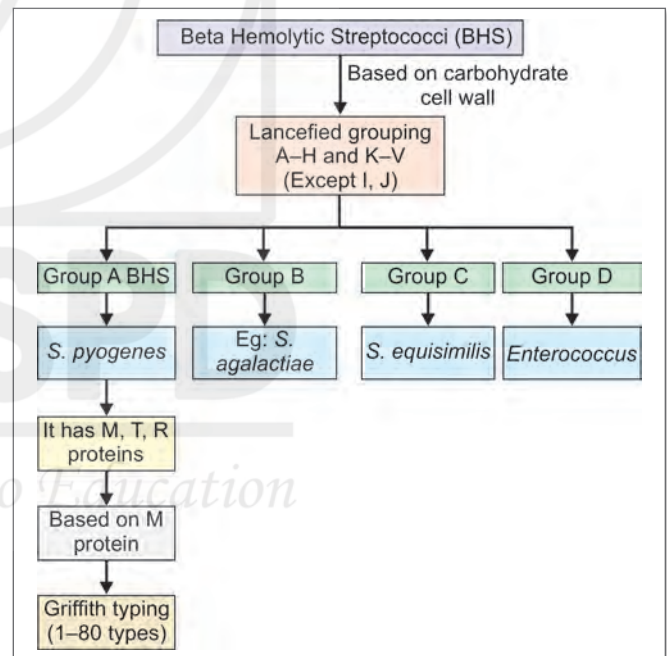
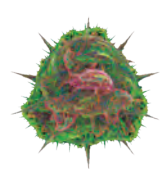


Figure 2: Strains of β-hemolytic streptococci



STREPTOCOCCUS PYOGENES (GAS)

- Gram-positive cocci
- Arrangement: In pairs and chains
- Key test for identification: Catalase test - negative, Beta type of hemolysis, Bacitracin test – sensitive.

Virulence Factors

- Capsule: Helps in the inhibition of phagocytosis
- Carbohydrate antigen: This antigen is responsible for cross reactivity leading to autoimmunity
- Protein antigens: (MTR)
 - M proteins – Griffith typing; helpful in virulence
 - T proteins – not associated with virulence
 - R proteins – not associated with virulence
- Pili – made up of M proteins and lipoteichoic acid

Toxin Secreted by Streptococcus Pyogenes

- Erythrogenic, Dick or scarlatinal toxin:
 - Intradermal injection into susceptible individuals
 - Produce an erythematous reaction (**Dick test**)
 - Effect of toxin is seen by induction of fever
 - Three types are there– A, B, C
 - A and C – Phage coded; B –Chromosomal
- Hemolysins – Streptolysin O and S.
 - **Streptolysin O**: Antibody to this appears post infections; **ASO titer** helps to diagnose; oxygen labile
 - **Streptolysin S**: Responsible for **hemolysis** seen around streptococcal colonies on the surface of blood agar plates; oxygen stable
- Streptokinase – Fibrinolysin – It is responsible for breaking down of fibrin barrier and spread of infections
- Deoxyribonucleases (Streptodornase, DNase) – depolymerization of DNA; helps to liquefy the thick pus; four types A, B, C, D; type B is the most antigenic; Demonstration of **anti DNase B antibody – retrospective diagnosis of S.pyogenes infection – especially in skin infections**
- Hyaluronidase helps in the spread of infection along the intercellular spaces

Table 3: Diseases caused by Streptococcus pyogenes

Suppurative diseases	Nonsuppurative diseases
<ul style="list-style-type: none"> • Pharyngitis – most common cause is <i>S. pyogenes</i> in children • Scarlet fever • Skin and soft tissue infections: <ul style="list-style-type: none"> ■ Impetigo ■ Pyoderma ■ Cellulitis ■ Necrotizing fasciitis • Toxic shock syndrome • Puerperal sepsis • Abscesses 	<ul style="list-style-type: none"> • Acute rheumatic fever • Poststreptococcal glomerulonephritis

Diseases caused by Streptococcus Pyogenes

Necrotizing Fasciitis

- Also called as hemolytic streptococcal gangrene
- Caused by M types 1 and 3 forming pyrogenic exotoxin A
- **Because of extensive necroses:** it is called as flesh eating bacteria
- Vancomycin is the DOC

Acute Rheumatic Fever

- It is a complication or post sequel of acute pharyngitis
- Occurs after 2-3 weeks of infection
- This is mainly an immunological mediated injury because of cross reactivity
- M protein present in *S. pyogenes* cross reacts with the antigens in the heart and joint tissues – leading to injury
- **Most important strains that cause RF are M types 5,18 and 24**

Acute Glomerulonephritis

- It is a sequela of impetigo or sometimes pharyngitis
- **Post-impetigo – nephritogenic strains commonly involved are M types 49, 52, 53, 57, 58, 59, 60, 61**
- **Postpharyngitis – nephritogenic strains are M types 3,4,12,21,25**

Table 4: Antigenic cross reactivity

<i>S.pyogenes</i>	Human
Hyaluronic acid in capsule	Human synovial fluid
Cell wall proteins	Myocardium
Group A carbohydrates	Cardiac valves
Cytoplasmic membrane antigens	Vascular intima
Peptidoglycans	Skin antigens

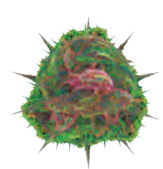
Lab Diagnosis of Streptococcus Pyogenes (GAS) Infection

- Gram staining – shows Gram-positive cocci in pairs and chains



Figure 3: Gram-positive cocci in chains (Courtesy: CDC)

- Culture needs to be done in blood agar for identification of type of hemolysis – *Streptococcus pyogenes* belongs to beta type of hemolysis;
- Key test is Bacitracin disk (0.04U) test – **all strains of S.pyogenes are sensitive to bacitracin.**



Intrinsic Resistance to Enterococci:

- β -lactams (particularly cephalosporins and penicillinase resistant penicillins)
- Low concentrations of aminoglycosides
- Clindamycin
- Fluoroquinolones
- Cotrimaxazole



Acquired Resistance to Enterococci:

- High concentration of β -lactams (alteration of PBPs of production of β -lactamase)
- Glycopeptides like Vancomycin and teicoplanin
- High concentration of aminoglycosides
- Tetracycline
- Erythromycin
- Fluoroquinolones
- Rifampin
- Chloramphenicol
- Fusidic acid

Table 6: Vancomycin resistant enterococci

Characteristics	Van A	Van B	Van C	Van D	Van E
Vancomycin MIC $\mu\text{g/mL}$	64 to >1000	4 to 1024	2 to 32	128	16
Teicoplanin MIC $\mu\text{g/mL}$	16–512	<0.5	<0.5	4	0.5
Most frequent species	<i>E. faecalis</i> <i>E. faecium</i>	<i>E. faecalis</i> <i>E. faecium</i>	<i>E. casseliflavus</i> <i>E. gallinarum</i>	<i>E. faecium</i>	<i>E. faecalis</i>
Genetic determinant	Acquired	Acquired	Intrinsic	Acquired	Acquired
Transferable	Yes	Yes	No	No	No

Treatment of Enterococcal Infections

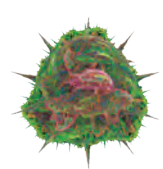
- Ideal management needs mandatory antimicrobial susceptibility testing
- Combination group of antimicrobials should be started

Table 7: Treatment of Enterococcal infections

Endovascular infections	Ampicillin + Aminoglycoside (or) Ampicillin + Ceftriaxone
Non-endovascular bacteremia	Ampicillin alone or with aminoglycosides
Meningitis	Ampicillin + aminoglycoside/ceftriaxone
UTI	Fosfomycin
VRE	High dose daptomycin +/- aminoglycoside (or) Linezolid (or) Quinupristin/Dalfopristin

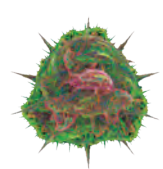
MISCELLANEOUS STREPTOCOCCI

Group C Streptococci	<ul style="list-style-type: none"> • Mostly it causes infections in animals • <i>S. equisimilis</i> is the most common human infection causing agent • Causes pharyngitis and tonsillitis; also causes deep infections • <i>S. equisimilis</i> is the source of Streptokinase – which is helpful for thrombolysis in Myocardial infarction
Nonenterococcal Group D Streptococci	<ul style="list-style-type: none"> • Most common organism is <i>S. bovis</i> • Normal commensal in fecal flora • Also causes UTI, SABC • DOC – Penicillin
Group F Streptococci	<ul style="list-style-type: none"> • This group of Streptococci poorly grows in blood agar; it needs special incubation • Proper incubation with CO_2 is needed – which gives pinpoint colonies in the agar media • Hence it is called as 'minute streptococci'



MULTIPLE CHOICE QUESTIONS

1. C-carbohydrate in *Streptococcus haemolyticus* is important for- (Recent Pattern Dec 2014)
a. Lancefield classification
b. Phagocytic inhibition
c. Toxin production
d. Hemolysis
2. All are medically important streptococci except- (Recent Pattern Nov 2013)
a. *Strep pyogenes*
b. *Strep agalactiae*
c. *Strep equisimilis*
d. *Strep salivarius*
3. CAMP test is positive for- (Recent Pattern July 2015)
a. Group A streptococcus
b. Group B streptococcus
c. Group C streptococcus
d. Group D streptococcus
4. Griffith typing is done for- (Recent Pattern July 2016)
a. *Staphylococcus*
b. *Streptococcus*
c. *Meningococcus*
d. *Gonococcus*
5. Group B beta hemolytic streptococci is- (Recent Pattern Dec 2012)
a. *Strep pneumonia*
b. *Strep pyogenes*
c. *Strep agalactiae*
d. *Enterococcus*
6. Causative agent of acute rheumatic fever- (Recent Pattern July 2016)
a. Group-A β -hemolytic streptococcus
b. Group-B β -hemolytic streptococcus
c. Group-C β -hemolytic streptococcus
d. Group-D β -hemolytic streptococcus
7. A child presents with sepsis. Bacteria isolated showed beta hemolysis on blood agar, resistance to bacitracin, and a positive CAMP test. The most probable organism causing infection is- (All India 2010)
a. *Streptococcus pyogenes*
b. *Streptococcus agalactiae*
c. *Enterococcus*
d. *Streptococcus pneumonia*
8. Bacitracin sensitivity is used to differentiate (Recent Pattern 2015)
a. Group A *Streptococcus* from staphylococcus
b. Group A *Streptococcus* from other beta hemolytic streptococci
c. Group A *Streptococcus* from gamma hemolytic streptococci
d. Group A *Streptococcus* from alpha hemolytic streptococci
9. Thick pus of Streptococci is converted thin by enzyme- (Recent Pattern July 2016)
a. DNase
b. Streptokinase
c. RNase
d. C5a peptidase
10. Which streptodornase is most antigenic in human beings (Recent Pattern Dec 2016)
a. A
b. B
c. C
d. D
11. *Streptococcus pyogenes* shows pathogenicity by all except- (Recent Pattern July 2015)
a. M protein
b. Pyrotoxin
c. Pili
d. Streptolysin O
12. Which of the following factor is mainly responsible for virulence in *Streptococcus*- (Recent Pattern Nov 2014)
a. Carbohydrate
b. Streptokinase
c. Streptodornase
d. M protein
13. Crystal violet blood agar is used for which bacteria- (Recent Pattern Dec 2016)
a. *Corynebacterium diphtheriae*
b. *Staphylococcus aureus*
c. β -hemolytic streptococcus
d. Meningococcus
14. Which toxin of *Streptococcus* causes hemolysis- (PGI Nov 2014)
a. Streptolysin O
b. Streptolysin S
c. Streptodornase
d. Hyaluronidase
15. Streptococcal cell wall polysaccharide cross reacts with- (Recent Pattern Dec 2016)
a. Myocardial muscle
b. Cardiac valve
c. Endocardium
d. Synovial fluid
16. Streptococcal toxic shock syndrome is due to the following virulence factor- (Recent Pattern Dec 2014)
a. M protein
b. Pyrogenic exotoxin
c. Streptolysin O
d. Carbohydrate cell wall
17. Streptolysin O functionally and structurally related to- (PGI Nov 2014)
a. Tetanolysin
b. Pneumolysin
c. Streptolysin S
d. Clostridium perfringens toxin
e. Listeriolysin
18. All are true about *Streptococcus*, except- (AIIMS May 2010, 2011)
a. Streptodornase cleaves DNA
b. Streptolysin O is active in reduced state
c. Streptokinase is produced from serotype A, C, K
d. Pyrogenic toxin A is plasmid mediated
19. Virulence factor of Group A beta hemolytic Streptococci- (Recent Pattern Dec 2014)
a. Protein M
b. Protein T
c. Protein R
d. Lipoteichoic acid
20. Which of the following Streptococcal antigen cross reacts with synovial fluid? (Recent Pattern 2008)
a. Carbohydrate [Group A]
b. Cell wall protein
c. Capsular hyaluronic acid
d. Peptidoglycan
21. Scarlet fever is caused due to- (Recent Pattern August 2013)
a. Streptococci
b. Staphylococci
c. *Klebsiella*
d. Proteus
22. Most common age group affected by *Streptococcus pyogenes*- (Recent Pattern Dec 2016)
a. <5 years
b. 5-15 years
c. 20-25 years
d. 30-40 years

**42. False about Gram-positive cocci is-***(Recent Pattern 2008)*

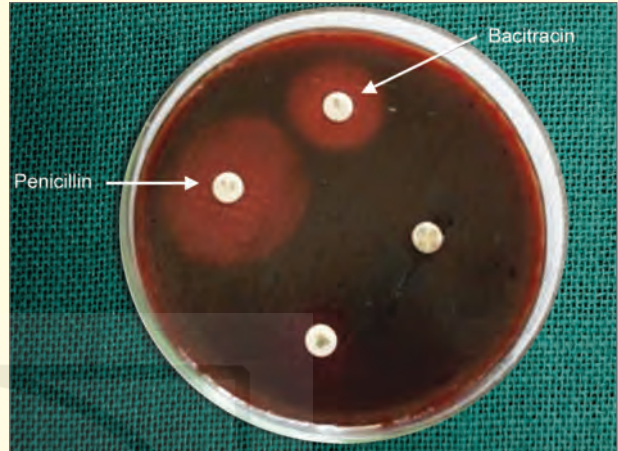
- a. Staphylococcus saprophyticus causes UTI in females
- b. Most enterococci are sensitive to penicillin
- c. Nonpathogenic strains are coagulase negative
- d. Neonatal meningitis causing streptococci hydrolyses hippurate

43. Heating at 60°C for 30 minutes would isolate-*(Recent Pattern Dec 2014)*

- a. Staphylococci
- b. Enterococci
- c. Micrococci
- d. Streptococci

44. Which of the following is/are feature of streptococcus agalactiae rather than staphylococcus aureus?*(PGI May 2018)*

- a. Catalase positive
- b. Bacitracin resistant
- c. Coagulase negative
- d. α hemolysis
- e. β hemolysis

45. The following test is helpful in identification of:*(AIIMS May 2018)*

- a. *Staph aureus*
- b. *Strep pyogenes*
- c. *Clostridium botulinum*
- d. *Bacillus*

ANSWERS AND EXPLANATIONS**1. Ans. (a) Lancefield classification**

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 211

- Hemolytic Streptococci were classified by Lancefield serologically into groups based on the nature of a carbohydrate (C) antigen on their cell wall.
- These are known as Lancefield groups. A-H and K-V are twenty of them that have been identified and named so far.

2. Ans. (d) Strep salivarius

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 211 and 220.

- *S. salivarius* belongs to viridians group Streptococci. They are normal residents of the mouth.
- *S. pyogenes*, *S. agalactiae* and *S. equisimilis* are medically important Streptococci.

3. Ans. (b) Group B streptococcus

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 219

- CAMP test differentiates Group B Streptococci (GBS) (CAMP test positive) from other beta hemolytic Streptococci (Negative).
- An accentuated zone of hemolysis when *S. agalactiae* is inoculated perpendicular to a streak of *S. aureus* (Staphylococcus plizens streak producing beta lysin) grown on blood agar.
- *Listeria monocytogenes* is also CAMP test positive.

4. Ans. (b) Streptococcus

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 211

- Griffith typing is used for further classification of *Streptococcus pyogenes*, belonging to Lancefield group A.
- Based on M proteins on cell surface, they are subdivided into M types. Till now, about 80 types of *S. pyogenes* have been identified.

5. Ans. (c) Strep agalactiae

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 211

- *S. agalactiae* is group B beta hemolytic Streptococci.
- *S. pyogenes*, which is also beta hemolytic belongs to group A.

6. Ans. (a) Group-A β -hemolytic streptococcus

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 215

S. pyogenes is group A beta hemolytic Streptococcus which causes acute rheumatic fever and poststreptococcal glomerulonephritis as nonsuppurative diseases.

7. Ans. (b) Streptococcus agalactiae

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 211

- Beta hemolysis is produced both by *S. pyogenes* and *S. agalactiae*. *S. pyogenes* is sensitive to bacitracin

Haemophilus, Francisella and Pasteurella



HAEMOPHILUS

Haemophilus Influenzae

- Small, Gram negative, nonmotile bacilli
- It has characteristic growth requirements
- It needs two accessory growth factors called X and V
- It is aerobic and grows only in chocolate agar.

Table 1: Differences between factor V and factor X

FACTOR V	FACTOR X
It is not clotting factor five	It is not factor ten
Factor V – codes for NAD	Factor X codes for hemin
Heat labile	Heat stable

Satellitism

- *H. influenzae* depends on factor V and X for its growth
- When *S. aureus* is streaked across a plate in a straight line on blood agar that was inoculated with *H. influenzae* culture or specimen – colonies grow well near the midline and fades toward the periphery – due to availability of factor V that is produced in staphylococcus growth
- This phenomenon is called a satellitism.

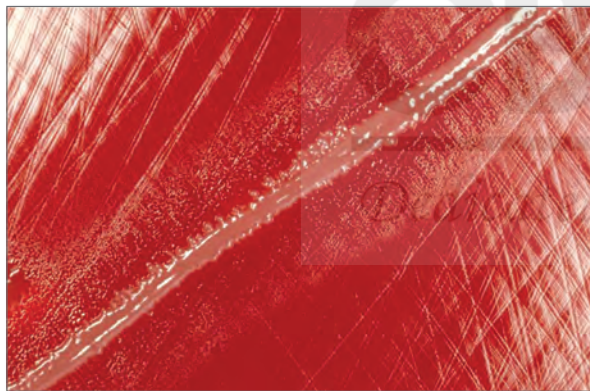


Figure 1: Satellitism (Courtesy: CDC/ Dr. Mike Miller)

Antigenic Nature

- Three major antigens are:
 - Capsular polysaccharide
 - Outer membrane proteins
 - Lipooligosaccharides

- Capsular polysaccharide is the most important virulence factor
- **Based on the capsule:** Pitman classified *H. influenzae* into six types: a to f
- **Type b *H. influenzae*** is unique in chemical structure by having pentose sugars while others have hexose sugars.
- **Hib antigen induces IgM, IgG and IgA** antibodies in human body; hence this antigen is being used for vaccination.
- Other strains who do not have any capsule antigens are called as nontypable strains

Pathogenicity

Table 2: Invasive and noninvasive infections

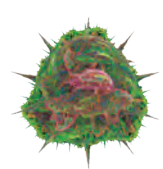
Invasive infections	Noninvasive infections
<ul style="list-style-type: none"> • <i>H. influenzae</i> acts a primary pathogen and causes invasive infections • Mostly seen in children • Caused by capsulated strains • The most important is meningitis • It also causes laryngo epiglottitis, conjunctivitis, bacteremia, pneumonia, arthritis, endocarditis and pericarditis 	<ul style="list-style-type: none"> • Infections are caused secondary to some other infections • Usually seen in adults • Caused by noncapsulated or nontypable strains • Causes otitis media, sinusitis, exacerbations of chronic bronchitis

Clinical Features

- Meningitis is the most-common manifestation seen in children less than 2 years of age
- Fevers and altered CNS manifestations are seen
- The most common complication is subdural effusion.
- Epiglottitis is a life-threatening infections that is caused in 2–7 years old
- Cellulitis is also caused especially in head and neck
- It causes pneumonia in infants.

Table 3: Diseases caused by *H. influenzae* in specific age group

<i>H. influenzae</i> in age group	Disease
Infants	Pneumonia
< 2years	Meningitis
2-7 years	Epiglottitis



Laboratory Diagnosis

- Specimens are usually CSF in meningitis and sputum in pneumonia
- **Culture is done in:**
 - Chocolate agar
 - Satellitism demonstration in blood agar
 - Levinthal's medium shows clear transparent medium with translucent colonies
 - Fildes's peptic digest agar
- Serological tests: Latex agglutination test in CSF and urine.

Treatment

- DOC: Ceftriaxone or cefotaxime
- Administration of glucocorticoids to patients with Hib meningitis reduces the incidence of neurological sequelae
- Immunoprophylaxis:
 - Two conjugate vaccines are available
 - ◆ All children should be immunized with Hib conjugate with first dose within 2 months of age (HibPRP vaccine)
 - ◆ There is no vaccine available for nontypable strains
 - ◆ Chemoprophylaxis - for contacts - Rifampicin



Remember

Childhood otitis media:

- Nontypable *H. influenzae* (Most common)
- *Streptococcus pneumoniae*
- *Moraxella catarrhalis*

Haemophilus Ducreyi

- It is the causative agent for chancroid
- Gram-negative bacilli that needs only Factor X
- Infection is acquired through sexual route
- Incubation period is 4-7 days
- An initial papular lesion develops which then becomes pustule and ruptures formed ulcers; these ulcers are painful



Remember

Painful ulcers – Chancroid	<i>Haemophilus ducreyi</i>
Painless ulcers - Chancre	<i>Treponema pallidum</i>



High Yield

- Probable diagnosis of chancroid are made under following conditions:
 - One or more painful genital ulcers
 - No evidence of *Treponema pallidum* as seen by dark field examination
 - No evidence of syphilis done by serological tests (7 days after ulcer onset)
 - Clinical evidence of chancroid
 - Negative test for Herpes

Treatment

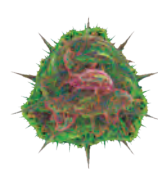
- Any one can be taken from the following drugs:
 - Single dose of 1 g of azithromycin
 - Ceftriaxone IM single dose
 - Ciprofloxacin bd for 3 days

Table 4: Important points in all species of Haemophilus

<i>Haemophilus influenzae</i> (Pfeiffer's bacillus)	<ul style="list-style-type: none"> • Gram negative, capsulated, pleomorphic bacilli • Required factor X and V for growth • Factor X – hemin/porphyrin • Factor V – NAD/NADP • Does not grow on blood agar; grows only in chocolate agar • Culture media – Levinthal's medium • Satellitism – due to high concentration of factor V in <i>Staph aureus</i> – released in the medium – helps in the growth of <i>H. influenzae</i> • Major virulence factor – Capsulated polysaccharide • Invasive – meningitis – Most commonly by capsulated stains of group b – (Hib) – in <2 years of age • Most common cause of acute epiglottitis • Noninvasive – Otitis media and sinusitis – caused by noncapsulated – nontypeable strains
<i>Haemophilus parainfluenzae</i>	<ul style="list-style-type: none"> • Requires only Factor V
<i>Haemophilus aegyptius</i>	<ul style="list-style-type: none"> • Also known as Koch's Weeks bacillus • Causes Brazilian purpuric fever • Pink eye – Conjunctivitis
<i>Haemophilus ducreyi</i>	<ul style="list-style-type: none"> • Bipolar staining • School of fish / Rail road track appearance • Chocolate agar enriched with isovital X and fetal calf serum with Vancomycin • Causes Chancroid or soft sore – painful and non indurated • DOC – single dose azithromycin

Table 5: Growth factors in Haemophilus species

Species	Growth factors
<i>Haemophilus influenzae</i> , <i>H. aegyptius</i> , <i>H. haemolyticus</i>	Factor X and V
<i>Haemophilus parainfluenzae</i> , <i>H. parahemolyticus</i> , <i>H. paraphrophilus</i>	Factor V
<i>H. ducreyi</i> , <i>H. aphrophilus</i>	Factor X



MULTIPLE CHOICE QUESTIONS

1. **About H. influenza all true, except:** (AIIMS Nov 2010)
 - a. Requires factor X and V for growth
 - b. Rarely presents as meningitis in children less than 2 months of age
 - c. Capsular polypeptide protein is responsible for virulence
 - d. M.C invasive disease of *H. influenza* is meningitis
2. **The major antigenic determinant of H. influenza is:** (Recent Pattern Dec 2014)
 - a. M protein
 - b. Capsular polysaccharide
 - c. Catalase
 - d. Coagulase
3. **Agar media used for H. influenza -** (Recent Pattern Dec 2015)
 - a. Blood agar
 - b. Chocolate agar
 - c. Tryptose agar
 - d. BYCE agar
4. **Satellitism is seen in cultures of:** (Recent Pattern Dec 2016)
 - a. Hemophilus
 - b. Streptococcus
 - c. Klebsiella
 - d. Proteus
5. **Not caused by non typhable Hib:** (AIIMS Nov 2014)
 - a. Meningitis
 - b. Otitis media
 - c. Puerperal sepsis
 - d. Exacerbation of COPD
6. **True about H. influenza:** (Recent Pattern Dec 2012)
 - a. Grown on sheep blood agar and CO₂
 - b. It is not capsulated
 - c. Invasive strain is most common
 - d. Gram positive
7. **Brazilian purpuric fever is caused by:** (Recent Pattern Dec 2016)
 - a. *Bordetella pertussis*
 - b. *Hemophilus aegypticus*
 - c. *Hemophilus ducreyi*
 - d. *Hemophilus parainfluenzae*
8. **Which of the following hemophilus doesn't require Factor V:** (Recent Pattern Dec 2015)
 - a. *H. parainfluenzae*
 - b. *H. influenza*
 - c. *H. ducreyi*
 - d. *H. aegypticus*
9. **Chancroid is caused by:** (Recent Pattern Dec 2014)
 - a. *H. ducreyi*
 - b. *T. pallidum*
 - c. Gonococcus
 - d. HSV
10. **School of fish appearance is characteristic of:** (Recent Pattern July 2016)
 - a. *Bordetella pertussis*
 - b. *Yersinia enterocolitica*
 - c. *Haemophilus ducreyi*
 - d. *Legionella*
11. **Pasteurella multocida is transferred by:** (Recent Pattern June 2014)
 - a. Aerosols
 - b. Animals
 - c. Sexual
 - d. Transplacental
12. **Most common mode of transmission of Pasteurella multocida is:** (Recent Pattern Dec 2016)
 - a. Animal bites or scratches
 - b. Aerosols or dust
 - c. Contaminated tissue
 - d. Human to human
13. **True statement about genital ulcer:** (PGI Nov 2017)
 - a. Donovanosis- Painful and multiple lesion
 - b. Granuloma inguinale is donovanosis
 - c. *Haemophilus ducreyi* causes chancroid
 - d. Systemic feature common in primary syphilis and *H. ducreyi* infection
 - e. Primary syphilis-Painful and indurated papule
14. **A patient is presenting with multiple painful ulcers and discharging inguinal lymphadenopathy. Which of the following finding is suggestive of this disease:** (PGI Nov 2017)
 - a. Demonstration of school of fish appearance of bacilli
 - b. Culture of *H. ducreyi* from an aspirate of suppurative lymph nodes
 - c. Microscopic identification of Donovan bodies in tissue smear
 - d. Evidence of *Treponema pallidum* infection by dark-field examination of ulcer exudate
 - e. Herpes simplex virus in the ulcer exudate

ANSWERS AND EXPLANATIONS

1. **Ans. (c) Capsular polypeptide protein is responsible for virulence**

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 334

- There are three main surface antigens: (a) The major antigenic determinant of capsulated strains is the capsular polysaccharides based on which *H. influenzae* strains have been classified into six capsular types - a to f. Type b contains pentose sugars ribose and ribitol instead of hexoses and hexosamines as in the other five serotypes. Its called Polyribosylribitol phosphate antigen. (b) Outer membrane protein antigens. (c) Lipooligosaccharides (LOS).

2. **Ans. (b) Capsular polysaccharide**

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 334

- Already explained in Q.1

3. **Ans. (b) Chocolate agar**

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 336

- Chocolate agar is made by heating blood agar due to which the V factor is released from within the erythrocytes and it becomes the best agar media for growing *H. influenzae*.

Bacteriophages

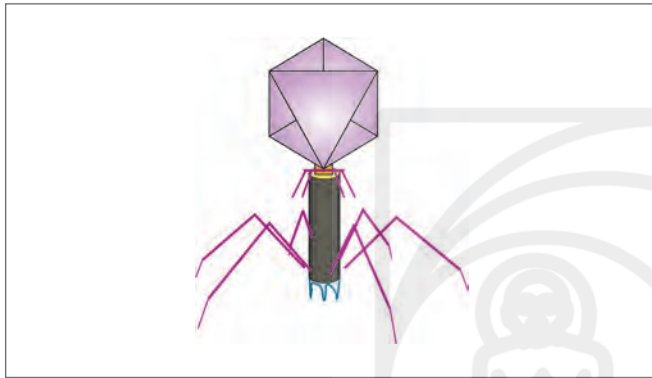


Figure 1: Bacteriophage

- Bacteriophages are the viruses that infect bacteria
- Discovered by Twort and d' Herelle

MORPHOLOGY

- Bacteriophage that infect *E. coli* is called as T even phages
- Phages are tadpole shaped with hexagonal head and cylindrical tail

- Head of the phage has core of nucleic acid – dsDNA which is surrounded by a protein coat called capsid
- Tail is made of core with contractile sheath and prongs and tail fibers.

LIFE CYCLE

1. Virulent or Lytic cycle
2. Temperate or Lysogeny cycle

Virulent or Lytic Cycle

A virulent phage uses the cellular apparatus of its bacterial host for multiplication, typically resulting in cell lysis (for obligatory lytic phages) and release of progeny virions

Temperate Cycle or Lysogeny Cycle

Temperate phages have alternative replication cycles: A productive, lytic infection or a reductive infection, in which the phage remains latent in the host, establishing lysogeny.

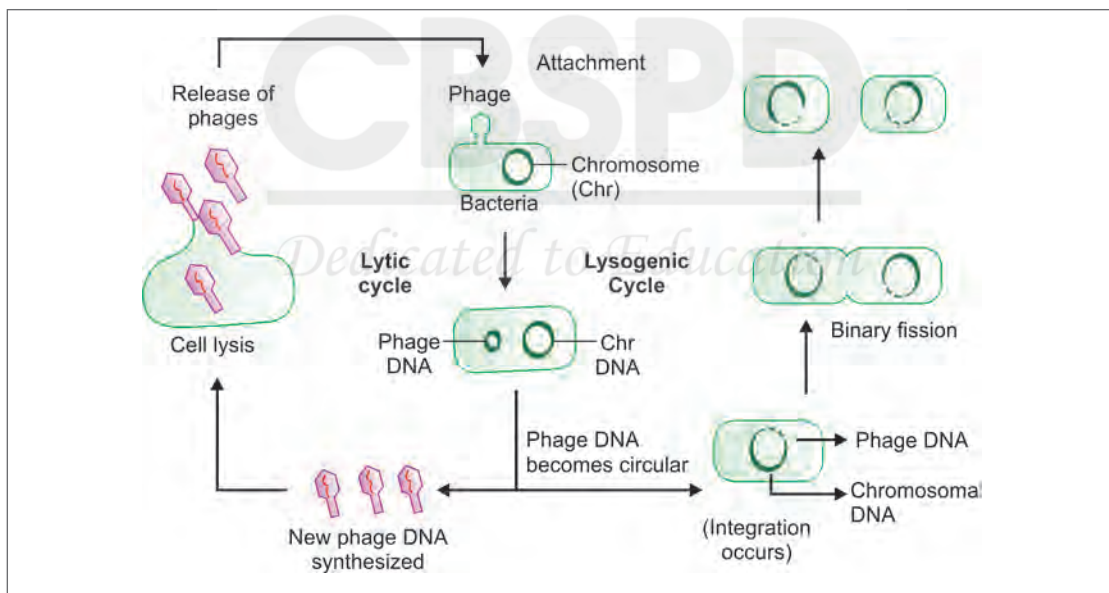


Figure 2: Lytic and lysogenic cycle of bacteriophage



Introduction to Parasitology

INTRODUCTION

- A parasite is an organism that lives on another organism for nutrition
- **Types of parasites:**
 - **Ectoparasites:** The Parasites that live on the outer surface of the host E.g. Lice—this condition is called as **infestation**.
 - **Endoparasites:** Parasites that live within the host,–e.g. Plasmodium— this condition is called as **infection**.

Table 1: Categories of parasites

Obligate parasites	These parasites cannot exist without host e.g. <i>Toxoplasma</i>
Facultative parasites	These parasites can live freely also e.g. <i>Naegleria</i>
Accidental parasites	They attack human as unusual host, e.g. <i>Echinococcus</i>
Aberrant parasites	Some parasites when entering the host (called as paratenic host)—enters a site where they cannot live or develop further—called as aberrant, e.g. <i>Toxocara</i> causing larva migrans

- Organisms that gives shelter and nutrition to parasite are called as hosts
- **Types of hosts:**
 - **Definitive host:** Host that harbors adult parasite; Host where **sexual reproduction** occurs
 - **Intermediate host:** Host that has the **asexual forms** of parasites
- **Parasites are broadly classified as:**
 - Protozoa
 - Helminths

PROTOZOLOGY

- All protozoa are unicellular **eukaryotes**
- Single cell performs all the functions
- Most of the protozoa are < 50 µm size **except *Balantidium coli* which is >100 µm**
- All the protozoan nucleus are vesicular except *B. coli*
- Stages of protozoa are trophozoites and cyst.

Intestinal Amoebae

- Group of Amoeba that attacks the intestine with unique morphological features of naked cytoplasm and lobose pseudopodium comes under intestinal Amoeba
- **Based on the pathogenicity:** Amoebae are classified into pathogenic and nonpathogenic Amoebae

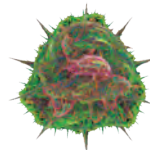


- **How to differentiate pathogenic and nonpathogenic Amoeba:**
 - **By zymodeme pattern**
 - **Zymodeme:** It is a group of Amoeba strains that share the same electrophoretic pattern and mobility for different enzymes
 - **Enzymes used are:**
 - L-malate
 - NADP⁺ oxidoreductase
 - Glucose phosphate isomerase
 - Hexokinase
 - Phosphoglucomutase
 - Based on these—Amoeba which come under seven populations namely 2, 6, 7, 11, 12, 13, 14 are pathogenic (total zymodemes = 24)

Pathogenic Amoeba	Entamoeba histolytica
Nonpathogenic Amoeba	E. dispar, E.coli, E.hartmanni, E. moshkovskii, E. gingivalis, E. polecki

ENTAMOEBA HISTOLYTICA

- Lives in **large intestine (Caecum)** of man
- Three morphological forms:
 - Trophozoite
 - Precyst
 - Cyst
- Definitive host: Human beings
- Infective form: Mature quadrinucleate cyst
- Source of infection: Contaminated food by ingestion (fecal oral route), anal sexual transmission, through **vectors like cockroaches** and flies.



Clinical Features

- Intestinal Amoebiasis— the **most common type is asymptomatic cyst passage**.
- Symptomatic amoebic colitis can present as lower abdominal pain, mild diarrhea, malaise, weight loss.
- Fecal material is full blown with bad odor; no blood or scanty blood is seen.
- Sometimes patients may go for **toxic megacolon** leading to bowel dilation.

Complications

- **The most common form of extra intestinal amoebiasis is Amoebic liver abscess (ALA)**; patients have characteristic fever with right upper quadrant pain. Some has pleural effusion. In case of endemicity and PUO are seen, Amoebic liver abscess must be ruled out.
- **The most common complication of ALA is pulmonary amoebiasis (Pleuropulmonary involvement)**. Manifestations seen are sterile effusions, or spread from liver due to rupture.
- Genital and cerebral involvement also can occur in extreme cases.

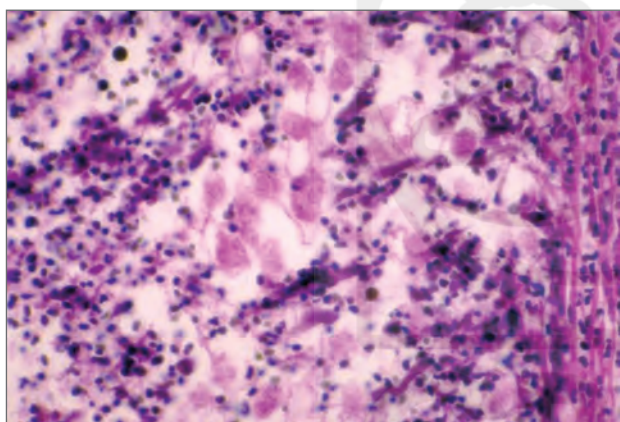


Figure 4: HPE in intestinal amoebiasis showing many trophozoites (Courtesy: CDC/Dr Mae Melvin)

Diagnosis by Wet Mount

- **Saline preparation:** Motile trophozoites seen
- **Iodine preparation:** Trophozoites (motility can not be seen) and cysts seen

Culture of E. Histolytica

- **Polyxenic culture:** Means culture is done in the presence of a bacteria—Boeck and Drbohlav egg serum medium (first medium used for culture of *E. histolytica*)
- **Axenic culture–bacteria free culture:** Its uses are
 - Pathogenicity of Amoeba can not be studied
 - In vitro anti Amoeba drug susceptibility
 - Antigen preparation

- **Medium used for isolation of E. histolytica are:**

- Boeck and Drbohlav medium
- Balamuth's medium
- Diamond's medium
- Philip's medium
- Jones medium

Invasive form	Trophozoite
Infective form	Cyst

Table 2: Identification of the morphological forms

Trophozoite	Cyst
<ul style="list-style-type: none"> • Invasive and growing stage • It is motile with the help of pseudopodium • Nucleus is eccentric in position • Karyosome is central in position • Nuclear membrane has fine chromatin granules • It has ingested RBCs (called as erythrophagocytosis) 	<ul style="list-style-type: none"> • Infective form • Immature cyst has glycogen mass and chromatoid bodies • Mature cyst is quadrinucleate

Serological Test

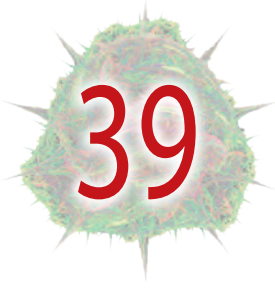
- Mainly used to diagnose invasive amoebiasis–extra intestinal
- Indirect hemagglutination test
- **ELISA–most sensitive and specific test:** Detects stool antigen (**Copro antigen**)-antigen positive means recent infection
- Serum showing antibody **titer of 1:128** is diagnostic of ALA
- But antibody detection is not helpful to diagnose recent or past infection

Additional Lab Features

- Helps to differentiate from bacillary dysentery
- **Stool microscopy:** Few pus cells, RBCs agglutinated, Charcot Leyden crystals are present
- **Blood smear:** No/mild leucocytosis
- **WHO recommendation** for diagnosis of intestinal amoebiasis–specific tests needs to be done; Hence **stool microscopy is not confirmative**.

Treatment

- **Amoebic dysentery:** Metronidazole, emetine hydrochloride
- **ALA:** Metronidazole, Dihydroemetine, chloroquine
- **Asymptomatic cyst passers:** Metronidazole, diloxanide furoate, tetracycline
- Metronidazole is the DOC for amoebic liver abscess.



Hemoflagellates

Table 1: Important Hemoflagellates

Leishmania	<ul style="list-style-type: none"> • <i>Leishmania donovani</i> • <i>Leishmania tropica</i> • <i>Leishmania mexicana</i> • <i>Leishmania braziliensis</i>
Trypanosoma	<ul style="list-style-type: none"> • <i>Trypanosoma cruzi</i> • <i>Trypanosoma brucei gambiense</i> • <i>Trypanosoma brucei rhodesiense</i>

FEATURES OF HEMOFLAGELLATES

- They all have a kinetoplast—which is a DNA carrying organelle
- They are pleomorphic—variety of stages are seen morphologically
- They live in blood and tissue
- Presence of undulating membrane

Table 2: Stages of Hemoflagellates

Amastigotes and Promastigotes	Leishmania
Amastigote	<i>Trypanosoma cruzi</i>
Epimastigotes and Trypomastigotes	<i>Trypanosoma brucei</i> and <i>cruzi</i>

TRYPANOSOMA

- Flagellate that infects humans and are found in blood and lymph nodes
- They are elongated in shape with a central nucleus and kinetoplast—which contains DNA and mitochondria
- An undulating membrane originates from the kinetoplast
- A single flagellum is seen at the anterior end—organism is motile

<i>Trypanosoma cruzi</i>	Chagas disease (American trypanosomiasis)
<i>Trypanosoma brucei</i>	Sleeping sickness (African trypanosomiasis)

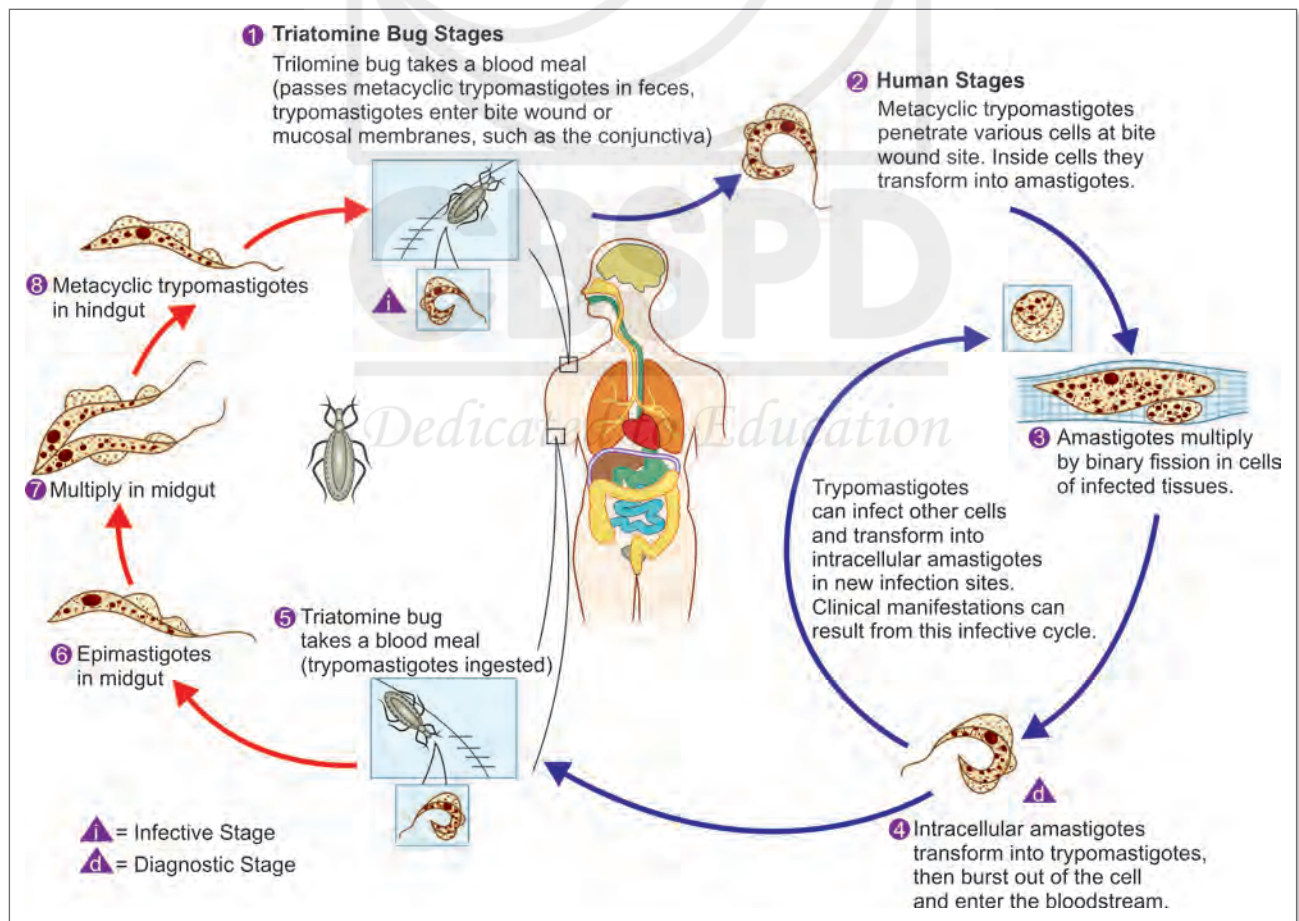
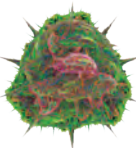


Figure 1: Life cycle of *Trypanosoma cruzi*

(Courtesy: CDC/ Alexander J. da Silva, PhD, Melanie Moser)



TRYPANOSOMA CRUZI

- Zoonotic disease—seen in South America
- Not seen in India
- *Three main developmental forms are:*
 - Amastigotes
 - Trypomastigotes
 - Epimastigotes
- *Definitive host:* Humans and other vertebrates
- *Intermediate host:* Reduviid bugs

Table 3: Location of morphological forms

Amastigotes	Reticulo endothelial system, muscles, mononuclear phagocytes
Non multiplying trypomastigotes	Peripheral blood
Multiplying trypomastigotes	Reduviid bug
Epimastigotes	Reduviid bug

- ***Infective form to man is metacyclic trypomastigotes***
- *Apart from vector transmission, Trypanosoma is transmitted by:*
 - **Blood transfusion**
 - Transplacental transfusion
 - Organ transplantation
 - Laboratory accidental inoculation

Clinical Features

- Chaga's disease is most commonly seen in infants and children
- At the bite area—a localized edema and erythema occurs named as **Chagoma**
- Followed by local lymphadenopathy
- In **Acute Chaga's disease**—when the site of inoculation is in conjunctiva—it leads to unilateral painless edema of the eye named as **Romana's sign**
- *Complications of acute disease are:*
 - **Myocarditis**
 - Meningoencephalitis
- In Chronic Chaga's disease—it causes: (**Mega disease**)
 - Cardiomyopathy—heart block
 - **Mega colon**
 - **Megaesophagus**
 - Colopathy
 - Sudden death occurs due to ventricular fibrillation

Diagnosis

- Wet mount of anticoagulated blood—shows motile trypomastigotes
- Thick or thin blood smear preparation is done—to see the morphology clearly
- Blood culture done in NNN medium
- **The only confirmatory method to diagnose chronic chagas disease is xenodiagnosis** (Using bugs); Serological methods also help to diagnose chronic chagas disease
- Animal inoculation—intraperitoneally into mice

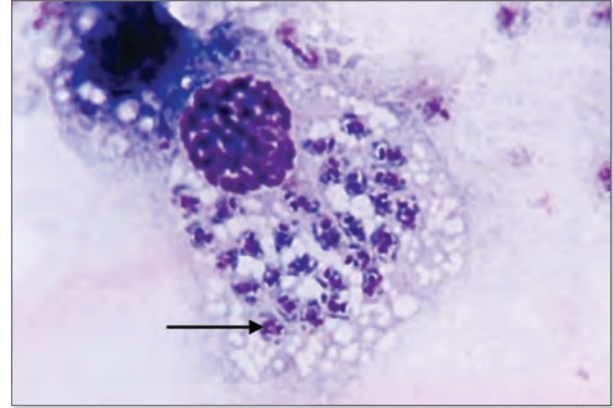


Figure 2: Amastigotes of *Trypanosoma cruzi* (Courtesy: CDC/Dr AJ Sulzer)

Treatment

- Nifurtimox
- Benznidazole
- Gentian violet

TRYPANOSOMA BRUCEI

- **Trypanosoma brucei has two subspecies:** gambiense and rhodesiense
- Seen in Africa; Not in India
- Morphological forms are trypomastigotes and epimastigotes (No amastigotes)

Trypomastigote	Humans and other vertebrates
Epimastigote	Tsetse fly

- *Definitive host:* Humans and other vertebrates
- **Intermediate host:** Tsetse fly (*Glossina*)
- **Infective form:** Metacyclic trypomastigote

Clinical Features

Table 4: Clinical Features of different subspecies of T.brucei

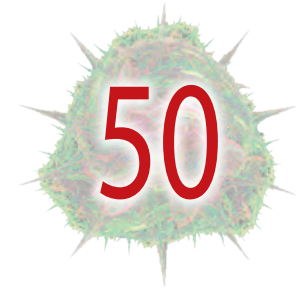
T.brucei gambiense	T.brucei rhodesiense
<ul style="list-style-type: none"> • Causes West African trypanosomiasis • Winterbottom sign is seen—cervical lymphadenopathy 	<ul style="list-style-type: none"> • Causes East African trypanosomiasis • Less common lymphadenopathy

- After the bite from the fly a chancre develops at the site—then leads to hemolymphatic spread followed by CNS spread
- CNS stage leads to sleeping sickness

Table 5: Differentiating features of West African and East African trypanosomiasis

Features	West African trypanosomiasis	East African trypanosomiasis
Causative agent	T. brucei.gambiense	T. brucei.rhodesiense
Vector	Tsetse flies	Tsetse flies
Reservoir	Humans	Antelope and cattle
Type of illness	Chronic CNS disease	Acute CNS disease
Lymphadenopathy	Prominent	Minimal
Parasitemia	Low	High

Endemic/Systemic Mycoses



CHARACTERISTICS

- Most of the infections are caused by soil fungi
- Systemic infections are caused by dimorphic fungi.

<i>Blastomyces dermatitidis</i>
<i>Paracoccidioides brasiliensis</i>
<i>Coccidioides immitis</i>
<i>Histoplasma capsulatum</i>

BLASTOMYCOSIS

- **Causative agent:** *Blastomyces dermatitidis*
- **Teleomorph stage (sexual stage):** *Ajellomyces dermatitidis*
- **Region:** North America (**North American blastomycosis**)
- Source of infection is soil
- Mode of infection is inhalation fungal spores enters the body through inhalation from the soil and then goes to lungs. Alveolar macrophages and polymorphonuclear leukocytes are most needed for phagocytosis and killing of inhaled conidia.
- Conidia upon entering inside the body, at body temperature (37°C) – it gets converted into thick walled yeast which are difficult to kill by phagocytosis.
- This conidia to yeast phase conversion leads to expression of protein called as **BAD-1 (virulence factor)**
- It causes acute pulmonary infection (resembles that of TB/histoplasmosis)
- **Pulmonary** presentation – may be asymptomatic; sometimes consolidation and abscess can occur
- Fungus then spreads to the blood and to various organs (Disseminated blastomycosis)
- In **cutaneous blastomycosis**, a papule occurs followed by nodule develops – leading to ulcerative lesions; Site is usually skin of face or hands; Two types of skin diseases are seen:
 - Verrucous blastomycosis
 - Ulcerative blastomycosis
- It also causes **osteomyelitis**, which usually affects vertebrae, sacrum, pelvis, skull and ribs.
- CNS blastomycosis presents as **brain abscess**.

Laboratory Diagnosis

- Body tissue/Culture at 37°C shows Yeast phase – **single broad bud** with double contoured wall
- Culture at 25°C (Room temperature) shows Septate hyphae with round to oval conidia

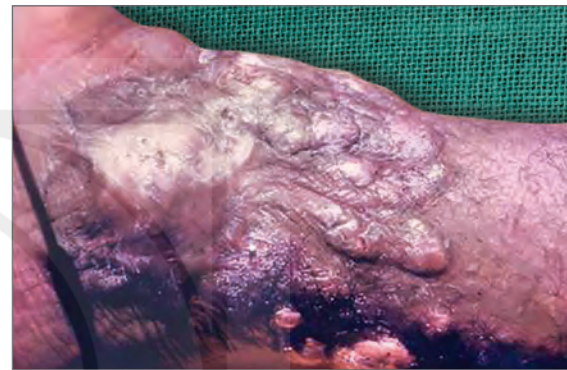


Figure 1: Cutaneous lesions of Blastomycosis
(Courtesy: CDC/ Dr. Lucille K. Georg)

- Antigen detection in urine is more sensitive than in serum.

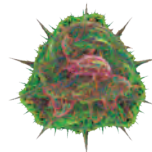
Treatment

Table 1: Treatment of blastomycosis

Patient condition	Type of disease	Treatment
Immunocompetent patient/life threatening disease	Pulmonary	Lipid AmB or Itraconazole
	Disseminated CNS	Lipid AmB or fluconazole
	Disseminated Non - CNS	Lipid AmB or itraconazole
Immunocompetent patient/ non-life threatening disease	Pulmonary or disseminated non CNS	Itraconazole or fluconazole or ketoconazole
Immuno-compromised patient	All infections	Lipid AmB or AmB deoxycholate

PARACOCCIDIOIDOMYCOSIS

- **Causative agent:** *Paracoccidioides brasiliensis*
- **Region:** South America (**South American blastomycosis**)
- Source of infection is soil
- Mode of infection is inhalation
- **Fungal spores are inhaled:** Primary pulmonary infection – spreads through blood – to **mucosa** of nose, mouth, GIT, skin, lymphatics



- **Region:** USA, Africa
- *H. capsulatum*.var.*duboisii* – responsible for **African histoplasmosis**
- Source of infection is soil, rotting trees, birds
- Mode of infection is inhalation of microconidia
- Inhalation of spores – pulmonary entry

Clinical Features

- In immunocompetent individuals with low level of exposure – infections are usually mild and self-limiting
- Most of the adults in endemic area have 50-80% positive skin tests suggest that they had mild infections.
- Heavy exposure leads to flu like illness with CXR showing pneumonitis with hilar or mediastinal lymphadenopathy.
- **Classical histoplasmosis** – asymptomatic – heal with just a miliary calcification
- DD: Tuberculosis
- **Progressive Disseminated histoplasmosis (PDH)** – occurs only in immunocompromised individuals which affects **Reticuloendothelial system** (intracellular infection) and it is highly fatal

Risk factors for PDH are:

- AIDS with CD4 counts less than 200/uL
- Extremes of age
- Immunosuppressive drug therapy
- Inflammatory diseases

- Lymphadenopathy, hepatosplenomegaly, fever, anaemia occurs
- **African histoplasmosis** – affects skin, subcutaneous tissues and bones; **lungs infection** and dissemination is **very rare**.
- Chronic cavitary histoplasmosis (resembles TB) is seen in smokers.
- In healed histoplasmosis – the calcified mediastinal nodes or lung parenchyma erodes the airways and causes hemoptysis. This condition is called as **broncholithiasis**.

Laboratory Diagnosis

- **Gold standard for diagnosis is fungal culture**
- Cultures are positive in 75% cases of PDH and chronic pulmonary histoplasmosis

- Specimens from tissues shows yeast cells which are seen within phagocytic cells (intracellular)
- Growth at 37°C produces yeast cells
- Room temperature – **Spores with tubercles** (finger like projections)
- Detection of histoplasma antigen in body fluids helps in diagnosis of PDH and acute diffuse pulmonary histoplasmosis.
- Histoplasmin skin test helps in endemic area to know the disease burden.

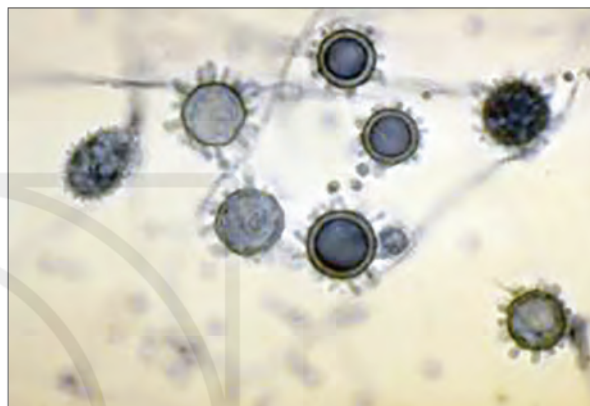


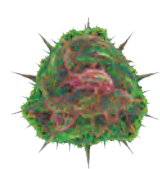
Figure 4: Tuberculate spores (Courtesy: CDC)

Treatment

Table 4: Treatment of histoplasmosis

Type of histoplasmosis	Treatment
Acute pulmonary illness with diffuse infiltrates	Lipid amphotericin B ± glucocorticoids for 1-2 weeks Followed by Itraconazole for 12 weeks
Chronic/Cavitary pulmonary	Itraconazole bd for at least 12 months
Progressive disseminated	Lipid amphotericin B for 1-2 weeks followed by Itraconazole for 12 weeks
Central nervous system involved	Lipid amphotericin B for 4-6 weeks followed by Itraconazole for 12 months

Dedicated to Education



MULTIPLE CHOICE QUESTIONS

1. Which of the following is not an endemic mycosis:

(Recent Pattern Dec 2013)

- | | |
|-------------------|------------------|
| a. Histoplasmosis | b. Blastomycosis |
| c. Cryptococcosis | d. Candidiasis |

2. Darling disease is caused by (Recent Pattern Dec 2012)

- | | |
|-----------------|-------------|
| a. Histoplasma | b. Candida |
| c. Cryptococcus | d. Rhizopus |

3. "Tuberculate spores" are characteristic feature of

(Recent Pattern Dec 2014)

- | | |
|-----------------------|-----------------|
| a. Candida | b. Histoplasma |
| c. Coccidioidomycosis | d. Cryptococcus |

4. Fungus that infects reticuloendothelial cells is:

(Recent Pattern July 2016)

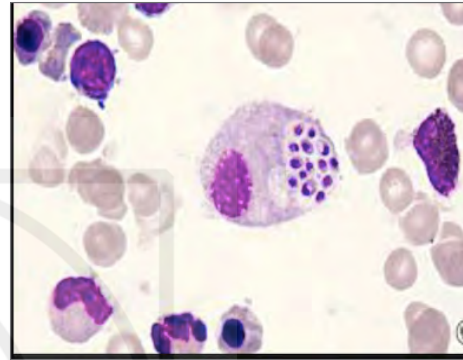
- | |
|-----------------|
| a. Cryptococcus |
| b. Candida |
| c. Aspergillus |
| d. Histoplasma |

5. Valley fever or desert rheumatism is caused by

(Recent Pattern Dec 2013)

- | |
|-----------------|
| a. Sporothrix |
| b. Coccidioides |
| c. Phialophora |
| d. Histoplasma |

6. A patient with HIV develops diarrhea and fecal examination shows *Isospora belli*. He was given treatment with TMP-SMX. Diarrhea subsided but fever persisted. Bone marrow examination showed the following picture with an intracellular fungi. Which of the following is wrong statement:



- | |
|--------------------------------------|
| a. It cannot be grown in SDA |
| b. Spores are infective form |
| c. It is intracellular budding yeast |
| d. It can cause systemic disease |

ANSWERS AND EXPLANATIONS

1. Ans. (d) Candidiasis

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 609

- *Blastomyces dermatitidis*
- *Paracoccidioides brasiliensis*
- *Coccidioides immitis*
- *Histoplasma capsulatum*
- *Cryptococcus sp*

2. Ans. (a) Histoplasma

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 609

- Histoplasmosis - Causative agent: *Histoplasma capsulatum*
 - Two types: *H.capsulatum.var.capsulatum*, *H.capsulatum.var.duboisii*
 - Disease identified by Darling - hence called as Darling's disease

3. Ans. (b) Histoplasma

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 610

- Lab diagnosis of Histoplasmosis:
 - Tissues - yeast cells occurs within phagocytic cells (intracellular)

- 37°C - yeast cells

- Room temperature - Spores with tubercles (finger like projections)

4. Ans. (d) Histoplasma

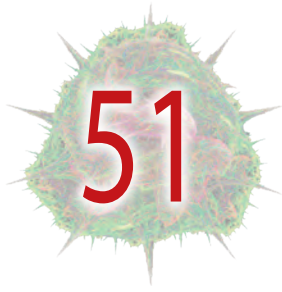
Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 610

- Classical histoplasmosis - asymptomatic - heal with just a miliary calcification
- Disseminated histoplasmosis - occurs in few - affects Reticuloendothelial system (intracellular infection) - highly fatal
- Lymphadenopathy, hepatosplenomegaly, fever, anemia occurs

5. Ans. (b) Coccidioides

Ref: Ananthanarayan and Paniker's Textbook of Microbiology - 10th ed - Page 611

- Coccidioidomycosis - Causative agent: *Coccidioides immitis*
- Region: USA - called as Rift valley fever or Desert Rheumatism



Opportunistic Mycoses

- Aspergillosis
- Penicilliosis
- Zygomycosis
- Candidiasis
- Cryptococcosis
- *Pneumocystis jirovecii*

ASPERGILLOSIS

- Aspergillosis is a clinical term used to describe all diseases that are caused by species of aspergillus;
- The species that grows at 37°C can cause invasive infections and others can cause only allergic manifestation.
- Most common species are: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus*.
- Aspergillus is seen ubiquitously in all the environments especially in decomposing plant materials and in bedding.
- Incubation period of invasive aspergillosis after inhalation is 2 to 90 days.
- Main important risk factors for aspergillosis are:
 - Profound neutropenia
 - Glucocorticoid usage

Clinical Features

- Clinical diseases caused by Aspergillus are:
 - **ABPA - Allergic Bronchopulmonary Aspergillosis** - Type I (M/c) and Type III hypersensitivity reactions that occurs after inhalation of spores - spores enters inside lungs and grows within the lumen of bronchioles - occludes them
 - **Aspergilloma** - Fungus grows within and occurs as a **fungus ball**
 - **Invasive aspergillosis** - Pneumonia occurs then dissemination starts to involve all organs - occurs in immunocompromised individuals, and those who are taking chronic steroids.
 - Otomycosis, mycotic keratitis, sinusitis
 - Cerebral aspergillosis
 - Cutaneous aspergillosis
 - Endocarditis

Laboratory Diagnosis

- Microscopy - Septate hyphae with acute angle branching at 45°C
- Morphology of each species differs and identification is mainly by the conidial arrangement

Table 1: Microscopic appearance of various species of Aspergillus

Species	Microscopic appearance
<i>Aspergillus fumigatus</i>	Uniseriate, Conidia covers only upper one third of vesicle,
<i>Aspergillus flavus</i>	Uniseriate and Biseriate, covers entire vesicle
<i>Aspergillus niger</i>	Biseriate, covers entire vesicle - Black colored

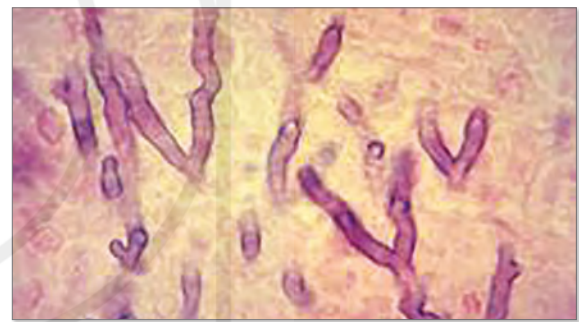
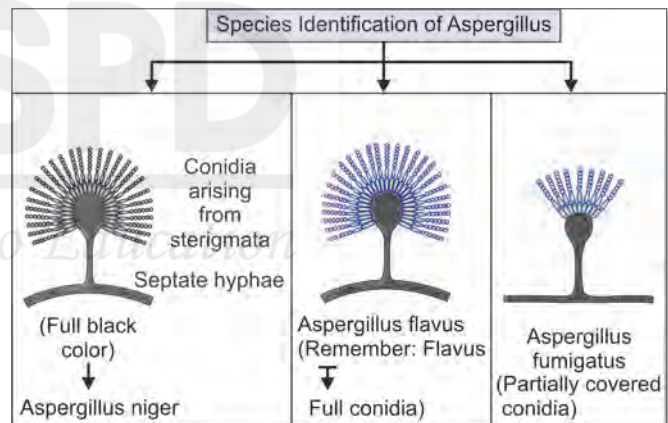
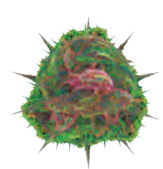


Figure 1: Acute angled hyphae of Aspergillus (Courtesy: CDC)



- **Serological test:** Antibodies can be demonstrated by ELISA, counter current immunoelectrophoresis and immunodiffusion
- Serological tests are most helpful in **allergic aspergillosis**
- Intradermal injection of Aspergillus antigen - Immediate reaction (Type I hypersensitivity) and Arthus type reaction (Type III reaction)
- Beta-D-glucan assay helps in invasive aspergillosis



HIGH YIELDING FACTS TO BE REMEMBERED IN MYCOLOGY

Examples for dimorphic fungi (BPH – CPS)	<ul style="list-style-type: none"> • Blastomyces dermatitidis • Paracoccidioides brasiliensis • Histoplasma capsulatum • Coccidioides immitis • Penicilliosis marneffi • Sporothrix schenckii
Darling disease	<ul style="list-style-type: none"> • Histoplasmosis
Tuberculate spores are seen in	<ul style="list-style-type: none"> • Histoplasmosis
Arthrospores seen in	<ul style="list-style-type: none"> • Coccidioides
Valley fever	<ul style="list-style-type: none"> • Coccidioidomycosis
Captain wheel formation seen in	<ul style="list-style-type: none"> • Paracoccidioidomycosis
Genus that comes under dermatophytes are	<ul style="list-style-type: none"> • Trichophyton • Microsporum • Epidermophyton
Only species in Epidermophyton is	<ul style="list-style-type: none"> • E.floccosum
Tinea versicolor is caused by	<ul style="list-style-type: none"> • It is not caused by dermatophytes; it is caused by Malassezia furfur
Germ tube formation seen in	<ul style="list-style-type: none"> • Candida albicans • Candida dublinensis
Pseudohyphae is absent in which candida species	<ul style="list-style-type: none"> • Candida glabrata
Chlamydo spores are seen in	<ul style="list-style-type: none"> • Candida albicans
Reynolds Braude phenomenon is seen in	<ul style="list-style-type: none"> • Candida albicans
CHROM agar is useful for	<ul style="list-style-type: none"> • Speciation of Candida
Acute angled dichotomous branching septate hyphae seen in	<ul style="list-style-type: none"> • Aspergillus sp
Broad aseptate hyphae seen in	<ul style="list-style-type: none"> • Mucor, Rhizopus
Capsulated fungus	<ul style="list-style-type: none"> • Cryptococcus neoformans
Rose Gardener's disease is caused by	<ul style="list-style-type: none"> • Sporothrix schenckii
Potassium iodide is used in the treatment of which fungi	<ul style="list-style-type: none"> • Sporothrix schenckii
Sclerotic bodies are seen in	<ul style="list-style-type: none"> • Chromoblastomycosis
Pigmented fungi are called as	<ul style="list-style-type: none"> • Dematiaceous fungi
Botryomycosis is caused by	<ul style="list-style-type: none"> • Not fungi ; it is caused by Staph aureus and other bacteria
Entomophthromycoses is caused by	<ul style="list-style-type: none"> • Conidiobolus coronatus • Basidiobolus ranarum
Non cultivable fungi	<ul style="list-style-type: none"> • Rhinosporidium seeberi
Dapsone is used in treatment of which fungi	<ul style="list-style-type: none"> • Rhinosporidium seeberi
Which kingdom does Rhinosporidium seeberi belongs to	<ul style="list-style-type: none"> • Aquatic protista – DRIP CLADE
Fungi that has trophozoites and cystic stage is	<ul style="list-style-type: none"> • Pneumocystis jirovecii
TMP-SMX drug is given for which fungi	<ul style="list-style-type: none"> • Pneumocystis jirovecii
ABPA is an example of	<ul style="list-style-type: none"> • Type I and III hypersensitivity
Pigmented penicillium species that causes infections in AIDS patients	<ul style="list-style-type: none"> • Penicillium marneffeii
Most common fungal infection in diabetics and steroid intake patients	<ul style="list-style-type: none"> • Zygomycosis
Which bird and Which tree is associated with Cryptococcal infection	<ul style="list-style-type: none"> • Bird – Pigeon • Tree - Eucalyptus

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She has presented many posters and has received best poster award in a national conference. There are around 15 publications to her credit in national and international journals. She has contributed to AIIMS-CBS Instant revision series, and is author of AIIMS, KONCPT-20 Authors, and editor of CBS Textbook of Microbiology for nursing students. Last but not least, she has contributed to a chapter in API Textbook of Medicine and many more chapters in other titles of repute.



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