

General Microbiology, Immunology and Healthcare-associated Infections (HAIs)

SECTION 1

General Microbiology

Overview of General Microbiology

1. Introduction and History of Microbiology
2. Taxonomy of Microorganisms
3. Epidemiology of Infections
4. Normal Microbial Flora of Human Body
5. Miscellaneous Topics: World Days and Healthcare-related Symbols

General Bacteriology

6. Morphology of Bacteria
7. Physiology of Bacteria
8. Genetics of Bacteria
9. Precipitating Factors of Bacterial Infections
10. Bacterial Growth Products

General Virology

11. General Properties of Viruses
12. Virus–Host Interactions (Viral Infections)

General Mycology

13. General Properties of Fungi

General Parasitology

14. Introduction and Classification of Parasites
15. General Properties of Parasites

OVERVIEW OF GENERAL MICROBIOLOGY

CHAPTER 1

Introduction and History of Microbiology

Chapter Outline

- Introduction
- Scientists and their Role (History)

INTRODUCTION

Microbiology

It includes the study of microorganisms like bacteria, viruses, fungi and parasites.

Branches of Microbiology

Medical microbiology: It is the branch of medical science which is related with study of microorganisms and disease produced by them (called infectious diseases) in humans. It includes diagnosis, prevention and treatment of disease and host response against microorganisms and or their products. Various branches of medical microbiology include:

- General microbiology: It includes study on general properties of microorganisms like taxonomy, morphology, physiology, etc.
- Immunology: It deals with the physiological functioning of the immune system in states of both health and diseases.
- Healthcare-associated infection (HAI): It deals with study on diagnosis, treatment and prevention of healthcare-associated infections.
- Systemic microbiology: It includes study on infections to various human body systems like
 - CVS and bloodstream infections
 - Gastrointestinal and hepatobiliary system infections
 - Skin, subcutaneous and musculoskeletal system infections
 - Central nervous system infections
 - Respiratory system infections
 - Urinary system, genital system and sexually transmitted infections
 - Miscellaneous infections like infections to eyes, ears, etc.

Food microbiology: The branch of microbiology that is involved in the study of the microorganisms that inhabit, create, enhance the flavour or contaminate the food.

Industrial microbiology: The branch of microbiology which is applied to create industrial products in mass quantities.

Soil microbiology: The study of microorganisms in soil, their functions and how they affect properties of soil or environment.

Plant microbiology: It is the study of association of plants with microorganisms.

Abiogenesis (Theory of Spontaneous Generation)

In an earliest time, people had believed that living organisms could develop from nonliving things like soil, elements, etc., called **spontaneous generation or abiogenesis**. In later part this theory was challenged by many scientists.

SCIENTISTS AND THEIR ROLE (HISTORY)

Antony Philips van Leeuwenhoek

Birth to death (1632–1723, Fig. 1.1a): He was born in Delft, Holland.



(a) Leeuwenhoek



(b) Jenner

Fig. 1.1: (a) Antony van Leeuwenhoek and (b) Edward Jenner

4 Contribution in Microbiology

1. The Dutchman was draper, who 1st prepared the single lens microscope (by own) and observed the diverse materials through it. He observed the minute organisms and other materials in rain water through an instrument (single lens microscope) with 40–300 magnification power and designated them as **animalcules**. He communicated his observation to the Royal Society of London in 1676. However, he did not realize the importance of these **animalcules**. As he worked on inventing different types of microscopes and improving the existing ones. Sure, his work helped to microbiology to achieve new heights because without a microscope, microbiology cannot exist, so he can be **called as father of microscopy**.
2. In 1678, **Robert Koch** developed a compound microscope and confirmed Leeuwenhoek's observation.
3. Almost after a century and after research of many people it was accepted that **animalcules** were the causes of many contagious disease.
4. He observed the *Giardia lamblia* in his own stool in 1681.
5. He defined the shape of bacteria as cocci, bacilli and spirochetes.

Edward Jenner

Birth to death (1749–1823, Fig. 1.1b): He was born in England.

Contribution in Microbiology

1. **Smallpox vaccine:** He discovered the prophylactic preparation for smallpox from cow lesion or cowpox. Such prophylactic preparation was labeled as **Vaccine (in Latin cow means Vacca)** by Pasteur.
2. **Father of immunology:** He is awarded as **father of immunology** for his contribution in the field of immunology.

Louis Pasteur

Birth to death (27th December 1822–28th September 1895, Fig. 1.2a): He was born in the village Dole, Jura, France on 27th December 1822. His father was a tanner.

Profession: He was trained as chemist, but his studies on fermentation led him to take interest in micro-organisms.



(a) Pasteur



(b) Lister

Fig. 1.2: (a) Louis Pasteur and (b) Joseph Lister

Contribution in Microbiology

1. **Germ theory of disease (biogenesis) (1857):** He established that putrefaction and fermentation were the result of microorganisms and their activities (Biogenesis).
2. **Disapproved abiogenesis (1860–61):** With series of classical experiments, he proved that all forms of life, even microbes, arouse only from their like and not *de novo* and disapproved abiogenesis.
3. **Contribution in sterilization technique:** He developed the steam sterilizer, hot air oven and autoclave. He invented the pasteurization (1863–65) which is useful for milk sterilization.
4. **Contribution in discovery of Pasteur pipette:** The Pasteur pipette name is given from Louis Pasteur, who used a variant of it extensively during his research. It is used to transfer small quantities of liquids in the laboratory and also to dispense small amounts of liquid medicines like eye drops.
5. **Contribution in cultivation technique:** He showed that growth medium, temperature, acidity, alkalinity and O₂ are required for successful cultivation.
6. **Studies on different diseases:** He identified the Microspora (*Nosema bombycis*) as a causative agent of **pebrine (silkworm disease)** in 1863 in France. He also studied on **anthrax, chicken cholera and hydrophobia (rabies)**. **Pneumococci** were 1st noticed by Pasteur and Stenberg.

Mnemonic

- **Vaccines discovered by Louis Pasteur:** CAR → Cholera, Anthrax and Rabies
- **Causative agents discovered by Robert Koch:** CAT → Cholera, Anthrax and Tuberculosis

7. **Coined the term vaccine:** Edward Jenner developed the prophylactic preparation for smallpox from cow (in Latin cow means **Vacca**) lesion. Pasteur termed the word **vaccine** for such prophylactic preparation.
8. **Discovery of theory of attenuation and chicken cholera vaccine:** When chicken cholera culture left on the bench for several weeks it lost its pathogenicity, but retains its ability to protect the bird against subsequent infection by it, led the discovery of theory of attenuation and live chicken cholera vaccine.
9. **Discovered live attenuated anthrax vaccine:** He attenuated the *Anthrax bacillus* by incubating at 42–43°C and proved that inoculation of such culture in animals induced specific protection against anthrax. The success of such immunization was dramatically demonstrated by an experiment on a farm at Pouilly-le-Fort in 1881 during which vaccinated sheep, cows and goats were challenged with a virulent *Anthrax bacillus* culture. All the vaccinated animals were survived while simultaneously unvaccinated animals died.
10. **Development of rabies vaccine:** He developed rabies vaccine (hydrophobia in human) in 1885.

Honor

1. **Father of microbiology:** His study on microorganisms leads the development of microbiology and he is awarded as **father of microbiology (medical microbiology, modern microbiology)**.
2. **Pasteur Institute, Paris:** It is built in honour of Louis Pasteur in Paris by public contribution. Similar institutes were established in other regions for vaccine preparation and diagnosis of infectious diseases.

Note: Who is the actual father of microbiology, A. Leeuwenhoek or Louis Pasteur? (link: <https://www.quora.com/Who-is-the-actual-father-of-microbiology-A-Leeuwenhoek-or-Louis-Pasteur>)

Antony van Leeuwenhoek can be considered more specifically as the “Father of Microscopy” because of his contribution as mentioned earlier. But had said that the contribution of Louis Pasteur was far more than A. Leeuwenhoek. He single handedly explored all the fields of Microbiology; right from proving that life arises from a preexisting life to industrial microbiology. His studies and sincere work is what makes microbiology one of the most interesting subjects to study. So, Louis Pasteur can rightly be called the “Father of Microbiology”.

Joseph Lister

Birth to death (1827–1912, Fig. 1.2b): He was born in Scotland.

Contribution in Microbiology

1. Pasteur’s work was immediately followed by Joseph Lister in 1867 with introduction of antiseptic techniques in surgery resulting decreased morbidity and mortality due to surgical sepsis.
2. He 1st used the carbolic acid as an antiseptic agent in surgery (1865).
3. He was awarded as **father of antiseptic surgery**.
4. **Lister Institute, London:** Was built in honour of Joseph Lister in London in 1891. It works for vaccine preparation and diagnosis of infectious diseases.

Robert Koch

Birth to death (11th December 1843–27th May 1910, Fig. 1.3a): He was born in Clausthal village, Hanover, Germany, on 11th December 1843.

Profession: He was German physician and pioneering Microbiologist.



(a) Koch



(b) Ehrlich

Fig. 1.3: (a) Robert Koch and (b) Paul Ehrlich

Contribution in Microbiology

1. **Contribution in microscopy:** In 1678, Robert Koch developed a compound microscope and confirmed Leeuwenhoek’s observation.
2. **Contribution in staining technique:** He described the staining method for identification of bacteria in dried fixed films stained with aniline dyes.
3. **Contribution in cultivation technique:** Robert Koch invented the culture method for pure isolation of bacteria over solid media. Earliest solid medium was **cooked cut potato** used by him, later he used gelatin for solidification of media, but gelatin is not satisfactory as it has tendency to get liquefy at 24°C and also by proteolytic bacteria. **Use of agar** in solidification of media was suggested by Frau Hesse, the wife of one investigator in Koch’s laboratory, who had seen her mother using agar to make jellies.
4. **Hanging drop technique:** He was the 1st to use hanging drop method to detect bacterial motility.
5. **Koch’s phenomenon (1890):** Koch observed that a guinea pig already infected with tubercle bacilli gives exaggerated response when injected with tubercle bacilli or tuberculin protein. This hypersensitivity or allergic reaction called **Koch’s phenomenon**.

Note: Molecular Koch’s postulates

Postulates: Gene presents in microbes should encode for disease production. Gene that satisfies molecular Koch’s postulates is often referred as virulence factor. The postulates were formulated by the microbiologist Stanley Falkow in 1988 and are based on Koch’s postulates as follow:

- The gene should be associated more with pathogenic species/strains than with nonpathogenic species/strains.
- Specific inactivation of the gene associated with the suspected virulence should lead a loss in pathogenicity or virulence.
- Replacement of the mutated gene should restore pathogenicity or virulence.
- The gene should be expressed at some point during infection or disease process.
- Antibodies or immune cells direct against the gene products should protect the host.

Limitation: For many pathogenic microorganisms, it is not currently possible to apply molecular Koch’s postulates, because of lack of suitable animal model for many important human diseases. Additionally, many pathogens cannot be manipulated genetically.

Difference with Koch’s original postulates: Instead of the presence or absence of a particular microorganism, they consider whether a particular virulence gene is present and active.

6. Koch’s postulates (1876)

- **Principles:** Any organism will be accepted as causative agent of disease if it satisfied following four generalized principles called **Koch’s postulates**.
 - The organism must be **present** in disease.
 - The organism must be **isolated** from the disease in pure culture.
 - Samples of the organism taken from pure culture must cause the same disease when inoculated into a healthy and susceptible **animal** in the laboratory.

- The organism must be **re-isolated** from the inoculated animal.
- **Additional criterion in Koch's postulates:** Later additional or fifth criterion was added, which is the **detection of antibody (Ab)** from patient's serum.
- **Limitations (clinical applications) of Koch's postulates:** Following bacteria are not satisfying the Koch's postulates.
 - *M. leprae*: Not growing over artificial media.
 - *T. pallidum*: Pathogenic strains are not growing over artificial media, but non-pathogenic can grow.
 - *N. gonorrhoeae*: No animal model.
- **Molecular Koch's postulates:** Follow box.

Honour

1. He was the founder of role of bacteria in production of diseases.
2. He identified the causative agents of cholera (*Vibrio cholerae* in 1883), anthrax (*Bacillus anthracis* in 1876) and tuberculosis (*M. tuberculosis* in 1882).
3. Winner of **Nobel Prize** in 1905 and known as **father of Bacteriology**.

Paul Ehrlich

Birth to death (1854–1915, Fig. 1.3b): He was born in Germany.

Profession: He was German scientist.

Contribution in Microbiology

1. He **stained** the cells and tissues to reveal their functions.
2. He reported the **acid fastness of tubercle bacilli**.
3. He introduced the method for **standardization of toxin and antitoxin** and coined the term **minimum lethal dose (MLD)**.
4. **Father of chemotherapy:** He used **salvarsan** (an arsenical compound) sometimes called '**magic bullet**' to kill spirochetes of syphilis with moderate toxic effect. He continued with his experiments till 1912 and discovered **neosalvarsan** and new branch in medicine **called chemotherapy**. Because of his extraordinary activities in medicine he is **called father of chemotherapy**.

Other Important Scientists and their Contribution in the Field of Microbiology

- Hans Christian Gram: A histologist developed the technique to identify the bacteria in tissue in 1884.
- Ziehl and Neelsen: Initially Ehrlich developed the acid fast stain in 1882 and later modified by Ziehl and Neelsen in 1882.
- Ernst Ruska and colleagues: Invented the electron microscope in 1931 for which Ernst Ruska won the Nobel Prize in Physics in 1986.
- Alexander Fleming: He discovered the penicillin from the fungus *Penicillium notatum* in 1928 and got Nobel Prize in 1945.

- von Behring Kitasato: He described antibody.
- Good Pasture: He developed the viral culture method in chick embryo in 1931.
- Kleinberger: He described the cell wall deficient form of bacteria in 1935, while studying the culture of *Streptobacillus moniliformis* in the Lister Institute, London called L-forms after the Lister Institute. He won Nobel Prize in 1941.
- Karry B Mullis: Invented the PCR technique in 1993.

Nobel Prizes

A number of scientists have been awarded with Nobel Prizes for their significant contribution and research work in the field of microbiology and are shown in Table 1.1.

Note: Year in Table 1.1 indicates the time of Nobel Prize given.

TABLE 1.1: Nobel Laureates

Nobel Laureate	Year	Contribution
Emil A Behring	1901	Developed antitoxin of Diphtheria
Sir Ronald Ross	1902	Studied life cycle of <i>Plasmodium</i> in Mosquito
Robert Koch	1905	Invented the <i>M. tuberculosis</i>
Charles LA Laveran	1907	Studied the <i>Plasmodium</i> in unstained slide of blood
Paul Ehrlich and Elie Metchnikoff	1908	Discovered the selective theory of antibody formation
Charles Richet	1913	Discovered the anaphylaxis
Jules Bordet	1919	Role in complement and CFT
Kleinberger	1941	Defined the L-Forms
Alexander Fleming	1945	Discovered the penicillin from the <i>P. notatum</i>
F Enders, FC Robbins, TH Weller	1954	Developed the tissue culture of polio virus
JL Lederberg and EL Tatum	1958	Discovered conjugation theory in bacteria
Sir M Burnet and Sir PB Medawar	1960	Immunological tolerance
Watson and Crick	1960	Discovered the double helix DNA structure
Peyton Rous	1966	Searched viral oncogenesis
Holley, Khurana and Nirenberg	1968	Invented the genetic model
BS Blumberg	1976	Discovered the HBsAg
Barbara Mc Clintock	1983	Discovered the transposon
George Kohler	1984	Discovered the hybridoma technique for monoclonal antibodies production
Stanley B Prusiner	1997	Discovered Prions
J Robin Warren and Barry J Marshal	2005	Discovered the <i>H. pylori</i> and its role in peptic ulcer
Luc Montagnier and F Barre Sinoussi	2008	Discovery of HIV

(Contd...)

TABLE 1.1: Nobel Laureates (Contd...)

Nobel Laureate	Year	Contribution
Bruce A Beutler and Jules A Hoffmann	2011	Discovery of the theory of innate immunity
Ralph M Steinman	2011	Searched dendritic cell and its role in adaptive immunity
Sir John B Gurdon and Shin-ya Yamanaka	2012	Mature cell can be reprogrammed to become pluripotent

Common Name of the Microorganisms from the Name of Scientists

Follow Table 1.2.

TABLE 1.2: Common name of the microorganisms from the name of scientists

Scientists	Common name	Scientific name
Victor Morax and Theodor Axenfeld	Morax-Axenfeld bacillus	<i>Moraxella lacunata</i>
Klebs and Loeffler	Klebs-Loeffler Bacillus (KLB)	<i>Corynebacterium diphtheriae</i>
Preisiz and Nocard	Preisiz-Nocard Bacillus	<i>Corynebacterium pseudotuberculosis</i>
Arthur Nicolair	Nicolaier's bacillus	<i>Clostridium tetani</i>
Robert Koch	Koch's bacillus	<i>Mycobacterium tuberculosis</i>
Heinrich A Johne	Johne's bacillus	<i>Mycobacterium paratuberculosis</i>
Gerard HA Hansen	Hansen's bacillus	<i>Mycobacterium leprae</i>
George Hoyt Whipple	Whipple's bacillus	<i>Tropheryma whipplei</i>
Carl Friedlander	Friedlander's bacillus	<i>Klebsiella pneumoniae</i>
Abel Rudolf	Abel's bacillus	<i>K. pneumoniae</i> subsp. <i>ozaenae</i>
Anton Von Frisch	Frisch's bacillus	<i>K. pneumoniae</i> subsp. <i>rhinoscleromatis</i>
Gaffky and Eberth	Gaffky-Eberth bacillus	<i>Salmonella Typhi</i>
Alexander Yersin	Yersin bacillus (plague bacillus)	<i>Yersinia pestis</i>
Whitmore	Whitmore's bacillus	<i>Burkholderia pseudomallei</i>
Pfeiffer	Pfeiffer's bacillus	<i>Haemophilus influenzae</i>
Koch and Weeks	Koch Weeks bacillus	<i>Haemophilus aegypticus</i>
Jules Bordet and Octave Gengou	Bordet-Gengou bacillus	<i>Bordetella pertussis</i>
Albert Doderlein	Doderlein's bacillus	<i>Lactobacillus acidophilus</i>
Eaton	Eaton agent	<i>Mycoplasma pneumoniae</i>

Scientific Name of the Microorganisms from the Name of Scientists

Follow Table 1.3.

TABLE 1.3: Scientific name of the microorganisms from the name of scientists

Scientists	Scientific name
Bacteria	
Shiga and Flexner	<i>Shigella flexneri</i>
Shiga and Boyd	<i>Shigella boydii</i>
Shiga and Sonne	<i>Shigella sonnei</i>
Amedee Borrel and Bergdorfer	<i>Borrelia burgdorferi</i>
Cox and Burnet	<i>Coxiella burnetii</i>
Parasites	
Leishman and Donovan	<i>Leishmania donovani</i>
Wucherer and Bancroft	<i>Wuchereria bancrofti</i>

Special Honor to Scientists

- Father of Microscopy: Antony van Leeuwenhoek.
- Father of Microbiology (Medical Microbiology, Modern Microbiology): Louis Pasteur.
- Father of Immunology: Edward Jenner.
- Father of Antiseptic Surgery: Joseph Lister.
- Father of Bacteriology: Robert Koch.
- Father of Virology: WM Stanely.
- Father of Tumor Virology: Peyton Rous.
- Father of Mycology: Raymond Jacques Sabouraud.
- Father of Chemotherapy: Paul Ehrlich.

Causative Agents of Infectious Diseases, Methods of Detection and their Role in Health and Diseases

These include bacteria, viruses, fungi and parasites. Their detection methods and their role in health and diseases are described in respective chapter.

ACCESS YOURSELF

Short Notes

1. Robert Koch.
2. Louis Pasteur.

Short Questions for Theory/Viva Questions

1. What are Koch's postulates?
2. What are molecular Koch's postulates?
3. Name the bacteria which are not satisfying the criteria of Koch's postulates.
4. Write the common name for following bacteria.
 - *C. diphtheriae*
 - *M. tuberculosis*
 - *S. Typhi*
 - *L. acidophilus*
5. Name the following.
 - Father of microbiology
 - Father of immunology
 - Father of antiseptic surgery
 - Father of bacteriology

1. *Mycobacterium leprae* is not satisfying the Koch's postulates.

MCQs for Chapter Review

Antony Philips Van Leeuwenhoek

1. Antony Van Leeuwenhoek is associated with:
 - a. Telescope
 - b. Microscope
 - c. Stains
 - d. Immunization

Louis Pasteur

2. Louis Pasteur is not associated with:
 - a. Introduction of complex media
 - b. Discovery of rabies vaccine
 - c. Discovery of *M. tuberculosis*
 - d. Discovery of spontaneous generation theory
3. Louis Pasteur is associated with:
 - a. Discovery of the bacillus of tuberculosis
 - b. The cellular concept of immunity
 - c. Introduction of anthrax vaccine
 - d. Discovery of penicillin
4. Vaccine of rabies was first discovered by:
 - a. Louis Pasteur
 - b. Robert Koch
 - c. Edward Jener
 - d. Landsteiner
5. Pasteur developed vaccine for:
 - a. Anthrax
 - b. Rabies
 - c. Chicken cholera
 - d. All of the above

Robert Koch

6. Microorganism that does not obey Koch's postulates:
 - a. *M. tuberculosis*
 - b. Polio virus
 - c. *M. leprae*
 - d. *Streptococcus*
7. *Vibrio cholerae* was discovered by:
 - a. Koch
 - b. Metchnikoff
 - c. John Snow
 - d. Virchow

Other Important Scientists and their Contribution in the Field of Microbiology

8. Electronic microscope was invented by:
 - a. Ruska
 - b. Robert Koch
 - c. Antony van Leeuwenhoek
 - d. Louis Pasteur
9. Egg inoculation technique for cultivation of viruses was first reported by:
 - a. Louis Pasteur
 - b. Ellermann and Bang
 - c. Good Pasteur
 - d. Lord Lister

Common Name of the Microorganisms from the Name of Scientists

10. *C. diphtheriae* is also called as:
 - a. Klebs-Loeffler Bacilli (KLB)
 - b. Roux bacilli
 - c. Koch's bacilli
 - d. Yersin bacilli
11. Which of the following is called Preisz-Nocard bacillus?
 - a. *C. diphtheriae*
 - b. *C. pseudotuberculosis*
 - c. *M. tuberculosis*
 - d. *Mycoplasma*
12. Eaton agent is:
 - a. *Chlamydia*
 - b. *Mycoplasma pneumoniae*
 - c. *Klebsiella*
 - d. *H. influenzae*

Answers and Explanation of MCQs

1. b
 - Follow section, **Antony Philips van Leeuwenhoek** for explanation.
 2. a and c
 - *M. tuberculosis* was discovered by Robert Koch.
 - Follow section, **Louis Pasteur** for explanation of other options.
 3. c
 4. a
 5. d
 6. c
 - Follow section, **Robert Koch (limitation of Koch's postulates)** for explanation.
 7. a
 - **Robert Koch** discovered the causative agent of Cholera, Anthrax and Tuberculosis (**Mnemonic CAT**).
 8. a
 - Ernst Ruska and colleagues: Invented the electron microscope in 1931 for which Ernst Ruska won the Nobel Prize in Physics in 1986.
 9. c
 - Good Pasture developed the viral culture method in chick embryo in 1931.
 10. a
 - *C. diphtheriae* was 1st identified by **Klebs** in 1883 but 1st cultivated by **Loeffler** in 1884 hence commonly called **Klebs-Loeffler Bacillus (KLB)**.
 - Emile Roux contributed in the discovery of diphtheria toxin along with Alexander Yersin.
 - Koch's bacilli is the common name given to *Mycobacterium tuberculosis* from the name of Robert Koch.
 - Alexander Yersin who discovered the bacilli along with Kitasato in 1894 from Hong Kong at the beginning of last epidemic, from his contribution genus called **Yersinia**; however Yersin bacilli word is not used for all species of genus, but only for *Yersinia pestis* which also called **plague bacillus**.
 11. b
 12. b
- Follow **Table 1.2** for explanation.

Taxonomy of Microorganisms

Chapter Outline

- Definition and Components

DEFINITION AND COMPONENTS

Definition

Taxonomy is defined as description, identification, nomenclature and ordered classification of organisms according to their presumed natural relationships.

Components

Taxonomy includes following three components:

- Classification/orderly arrangements.
- Identification of unknown with known unit.
- Nomenclature/naming of unit (bacteria).

A. Classification/orderly arrangements

Five kingdom system of classification: In 1969, RH Whittaker placed all organisms in five groups called **five kingdom system of classification**.

- Monera:** Prokaryote and unicellular, e.g., bacteria, blue green algae and archaebacteria.
- Protista:** Eukaryote and unicellular, e.g., protozoa
- Fungi:** Eukaryote and uni- or multicellular, e.g., fungi
- Plantae:** Eukaryote and multicellular, e.g., plants
- Animalia:** Eukaryote and multicellular, e.g., metazoa (helminths/worms), birds, animals, human, reptiles, arthropod, molluscs and coelenterates.

Modification of five kingdoms system of classification:

It is modified by Margulis and Schwartz, which includes two kingdoms like **Prokaryotes** and **Eukaryotes** as shown in **Table 2.1** with differences.

General scheme of classification: Kingdom → Phylum (Division) → Class → Order → Family (Tribe) → Genus → Species (Specific Epithet) → Subspecies (Strain/type).

Phylogenetic classification: This hierarchical classification represents a branching tree like arrangement based on evolutionary arrangement of species.

Adsonian classification: It based on all features expressed at the time of study.

Molecular or genetic classification: Based on genetic relatedness.

Abbreviation of species: Species word is common for both singular and plural form, but abbreviation may be used as sp., and spp., for singular and plural forms, respectively.

Intraspecies classification: It is useful for epidemiological purposes. It classifies species/unit up to subspecies/strain/type by using following methods.

- **Biotypes:** Based on biochemical properties.
- **Serotypes:** Based on serological properties.
- **Bacteriophage types:** Based on susceptibility to Bacteriophage.
- **Colicin types:** Based on production of bacteriocin.

B. Identification of unknown with known unit

It is done by using morphological, biochemical, genetic and other properties.

C. Nomenclature/naming of unit/species of bacteria

- **Order:** It is labeled with suffix “ales”.
- **Family:** It is labeled with suffix “aceae”.
- **Genus name:** It is the Latin noun and starts with capital letter. Scientific name includes genus and epithet/species with Italic pattern.
- **Species/epithet name:** It starts with small letter and in Italic pattern irrespective of person or place name. It based on different properties of unit like
 - *albus* meaning white.
 - *suis* meaning pig origin.
 - *pyogenes* meaning pus.
 - *welchii* meaning person who discovered it.
 - *tetani* meaning disease produced.
 - *australis* meaning place of origin.

TABLE 2.1: Differences between prokaryotes and eukaryotes

Features	Prokaryotes	Eukaryotes
General features		
Meaning	Pro = primitive/immature + Karyotes = nucleus (contains immature nucleus)	Eu = true/mature + Karyotes = nucleus (contains immature nucleus)
Organism's examples	Monera (bacteria, blue green algae)	Protista, fungi, plantae and animalia
Evolutionary ancient	Yes	No
Anatomy		
Number(s) of cell	Unicellular	Uni- and multicellular
Nucleus		
• Nuclear membrane	–	+
• Nucleolus	–	+
• Chromosome	Single-circular	Multiple-linear
Cytoplasm		
• Ribosomes	+ 70S	+ 80S
• Plasmid, episomes, transposon	+	–
• Golgi complex	–	+
• Endoplasmic reticulum	–	+
• Triglyceride fats	–	+
• Mitochondria and lysosomes	–	+
Physiology		
Site of respiration	Mesososome/chondroid (It is the invagination or infolding of cytoplasmic membrane contains respiratory enzymes)	Mitochondria
Reproduction	Mostly by asexual method (binary fission)	By asexual method (budding) and/or sexual method (meiosis or mitosis)
Pinocytosis	–	+
Protoplasmic streaming	–	+
Biochemistry		
Plasma membrane		
• Sterol	– (Except in <i>Mycoplasma</i> and <i>Ureaplasma</i>)	+
• Phospholipid	+	+
Cell wall		
• Muramic acid/peptidoglycan	+	–
• Diaminopimelic acid	Present in few GNB in pentapeptide bridge	Absent
• Others	Lipid and protein	Chitin, mannan, cellulose (green plants)

– (Absent), + (Present)

Notes:

Ancient: Prokaryotes are evolutionary ancient. Probably they are 1st organisms to evolve and eukaryotes evolving from prokaryotes like predecessors.

Viruses: Viruses are neither classified as prokaryotes nor eukaryotes.

Phospholipid: Present in both.

ACCESS YOURSELF**Short Notes**

1. Differences between prokaryotes and eukaryotes.

Short Questions for Theory/Viva Questions

1. What is protista?

MCQs for Chapter Review**Components (Classification/Orderly Arrangements)****1. Prokaryotes are:**

- a. Bacteria
- b. *Mycoplasma*
- c. Fungi
- d. Blue green algae
- e. Protozoa

2. Which is an eukaryote?

- a. *Mycoplasma*
- b. Bacteria
- c. Fungus
- d. *Chlamydia*

3. Fungi are:

- a. Prokaryotes
- b. Eukaryotes
- c. Plants
- d. Animals

4. Site of respiration in prokaryote is:

- a. Mitochondria
- b. Mesosome
- c. Endoplasmic reticulum
- d. All of above

5. Mesosomes are:
- Respiratory enzymes in bacteria
 - Cytoplasmic membrane invaginations
 - Destructive bodies
 - Protein-forming bodies
6. Prokaryotes are characterized by:
- Absence of nuclear membrane
 - Presence of microvilli on its surface
 - Presence of smooth endoplasmic reticulum
 - All of above
7. Which of the following is protista?
- Algae
 - Fungi
 - Protozoa
 - Bacteria
8. Which of the following is/are bacterial taxonomy?
- Chlamydia*
 - Rickettsia*
 - Mycoplasma*
 - Prion
 - Bacteriophage
9. True about bacteria:
- Mitochondria always absent
 - Sterols always present in cell wall
 - Divide by binary fission
 - Can be seen only under electron microscope
10. Eukaryotes are different in causing infection because:
- Divide by binary fission
 - Highly structure cell with organized cell organelles
 - Do not have all organelles
 - Evolutionary ancient

- a, b, d
 - c
 - b
 - b
 - a, b
 - a
 - c
- Follow section, **classification/orderly arrangements (five kingdom system of classification and Table 2.1)** for explanation of answers of MCQs 1–7.
8. a, b and c
- Chlamydia*, *Rickettsia* and *Mycoplasma* are bacteria. Prion is proteinaceous infectious virus like-particle without nucleic acid. Bacteriophage is a virus which eats bacteria.
9. a and c
- Mitochondria always absent in bacteria and respiration is possible by presence of mesosome/chondroid. Sterol is not present in all bacteria but only in *Mycoplasma*. and *Ureaplasma*. Bacteria divide by binary fission and can be seen under all types of microscopy.
10. b
- Eukaryotes are divided by meiosis or mitosis. Eukaryotes have highly structured cell with all organelles. Prokaryotes are evolutionary ancients (**Table 2.1**).

Epidemiology of Infections

Chapter Outline

- Definitions and Types of Infections
- Reservoirs, Sources and Modes of Transmission

DEFINITIONS AND TYPES OF INFECTIONS

Definitions

Infestation

- The lodgment and multiplication of infectious agents on the body surfaces or on the cloths of a host constitute infestation
- It is the lodgment and multiplication over the surfaces but no entry in body.

Contamination

- Only presence of infectious agents on surface or inside the body or inanimate object like fluid, food, clothes or soil but no multiplication
- It is the lodgment over surfaces or entry in body but no multiplication.
- Example: Bacteremia means entry of bacteria in blood and no multiplication (only detectable bacteria in blood).

Infection

- The entry and multiplication of infectious agents in the body of a host constitute infection
- It is the entry in body and multiplication but no sign-symptoms.
- Example: **Bacteremia** means entry of bacteria in blood (transient presence) and no multiplication.
- Infection is an interaction between microbes and host.

Infectious disease

- The entry, multiplication and production of clinical sign-symptoms **called infectious disease**
- It is the entry in body, multiplication and sign and symptoms.
- Examples:
 1. Septicemia: Means entry of bacteria in blood, multiplication and clinical sign-symptoms
 2. Toxemia: Means entry of bacteria in blood, multiplication and clinical sign-symptoms are

due to toxin production. It is one type of septicemia.

3. Pyemia: Septicemia and abscess in organs like liver, spleen and other tissues.

Contagious disease: A disease that can be transmitted from one host to another by direct contact.

Communicable disease: A disease that can be transmitted from one host to another either direct or indirect mode of transmission.

Non-communicable disease: A disease that is not transmitted from one host to another.

Types of Infections

A. According to habitat/involvement of systems

Following systemic infections are described in details in respective chapters.

1. Cardio vascular system infections
2. Bloodstream infections
3. Lymphatic system infections
4. Gastrointestinal tract infections
5. Infections of abdominal viscera
6. Skin and subcutaneous tissues infections
7. Musculoskeletal infections
8. Central nervous system infections
9. Respiratory tract infections
10. Urinary tract infections (UTIs)
11. Genital tract infection
12. Eye and ear infections
13. Oral cavity, dental, periodontal and salivary gland infections.

B. According to causative agents

1. Bacterial infection
2. Viral infection
3. Fungal infection
4. Parasitic infection

5. Mixed infection: It means more than one organisms cause infection simultaneously. Following are examples:

- Gas gangrene: **Ch. 55**
- Fournier gangrene: **Ch. 39**

6. Co-infection or symbiotic infection: Concomitant infection by combination of microbes called co-infection. Following are examples:

- Vincent's angina or fusospirochetosis: **Ch. 73**.
- Adeno Associated Virus (AAV) and adeno virus: Exact infection by AAV is not known but it always required adenovirus as a helper virus to produce the infection.
- HDV and HBV: **Ch. 88**.

C. According to nature of onset and progress

1. Acute infection: Infection characterized by sudden onset, rapid progression and often with severe symptoms.
2. Chronic infection: Infection characterized by gradual onset and slow progression.

D. According to sequence of involvement of host

1. Primary infection: Initial infection in a host by organisms.
2. Re-infection: Subsequent infection in a same host by same organisms.
3. Opportunistic infection: Infection in an immunodeficient host by organisms.

E. According to sites/locations

1. Local or focal infection (focal sepsis): Infection that is restricted to a specific location within the body of the host.
2. Systemic or generalized infection: Infection that has been spread to several organs or areas in the body of the host.

F. According to sources of infection

1. If source is human body itself or outer source

- **Endogenous infection:** Organisms are originated from host itself. These are mostly normal flora from different parts of body. For example meningitis by *N. meningitidis* from nasopharynx.
- **Exogenous infection:** Organisms are originated from outside, mostly from environment, soil, water, human, etc.

2. If source is hospital itself or outer source

- **Nosocomial (hospital-acquired or healthcare associated) infection:** **Ch. 29**.
- **Community acquired infection:** Infection originated from outside the hospital environment.
- **Iatrogenic (physician induced) infection:** Infection acquired by patients from physician during diagnosis, treatment or prevention of disease.

3. If source is animal/animal products, human body or non-living objects

- **Anthroponoses/cross infections:** It means entry of infection from one human host to another human

host. Word derived from *anthrōpos* (Greek) means man and *nosos* (Greek) means disease. Examples include rubella, smallpox, diphtheria, gonorrhea, ringworm (*Trichophyton rubrum*), and trichomoniasis.

- **Anthropozoonoses/zoonotic infections/zoonotic diseases/zoonosis:** It means infection of human from animal or animal products. Word derived from *anthrōpos* (Greek) means man, *zoon* means animal and *nosos* (Greek) means disease. For examples and more details follow **Ch. 47**.

- **Zooanthroponoses/reverse zoonosis:** This word is abandoned and not of much significance. It means infection of animal from human. Following are examples:

- Tuberculosis: Both zoonotic and reverse zoonotic, with birds, cows, elephants, meerkats, mongooses, monkeys, and pigs known to have been affected.
- Leishmaniasis: Both zoonotic and reverse zoonotic.
- Influenza, measles, pneumonia and other pathogens are reverse zoonotic for many type of primates.

- **Sapronoses:** It means entry of infection from abiotic/nonliving substrate like soil, water, decaying plants, or animal corpses, excreta, and others. Word derived from *sapros* (Greek) means decaying and *nosos* (Greek) means disease. Following are examples:

- Dimorphic fungi like coccidioido-mycosis and histoplasmosis
- Monomorphic fungi like aspergillosis and cryptococcosis
- Certain superficial mycoses like *Microsporum gypsum*, some bacterial diseases like legionellosis, and protozoan like primary amoebic meningo-encephalitis.

G. According to clinical presentation

1. **Overt infection:** Presence of clinical features of disease.

2. **Inapparent/subclinical infection:** Clinical features (C/Fs) are not apparent. It includes inapparent, missed or abortive cases. It can be detected by laboratory tests.

3. **Atypical infection:** Infection in which the typical clinical manifestation of disease are not present.

4. **Relapse:** It is the primary infection with on-off features. Following are examples:

- Relapsing fever: It is caused by bacteria from *Borrelia* spp. Sudden onset of fever (bacteria are in blood) which lasts for 3–5 days followed by afebrile period (bacteria are in brain). After afebrile period of 3–5 days another bout of fever will occur.
- Relapses in malaria: In the initial infection patient feels fever during erythrocytic phase and another episode of fever during exo-erythrocytic phase in case of *P. vivax*, *P. malariae* and *P. ovale*, but in *P. falciparum* there is no exo-erythrocytic phase, so there is no relapse or subsequent attack of fever.

- 14 5. **Latent (recrudescence):** Following primary infection parasites remain in the body in silent/latent form for a very long period and again proliferating and producing the clinical disease in a later part of life when the host resistance is lowered or by specific stimulation called latent infection. For example zoster/zona/shingles by varicella zoster virus (VZV).

H. Epidemiological types

1. **Sporadic:** It means scattered about. Few cases occur irregularly, haphazardly from time to time and separated widely in space neither identifiable common sources of infection nor connected with each other. It is a starting point of epidemic when conditions are favorable for spread of infection.
2. **Endemic disease:** 'En' means in and 'demos' mean people. It defined as constant presence of a disease within a given geographic area or population group without importation from outside.
3. **Epidemic disease:** Epi means upon and demos mean people. It defined as sudden onset of disease clearly in excess of "expected occurrence."
4. **Outbreak:** Small, usually localized epidemic in the interest of minimising public alarm, unless the numbers of cases are indeed large.
5. **Pandemic:** Epidemic that spreads in many areas of the world and affecting large population.
6. **Prosodemic diseases:** Some pandemic disease (like water or air borne) spread very rapidly while some spread very slowly by person to person contact called prosodemic diseases, e.g., cerebrospinal fever.
7. **Exotic:** Disease is imported in to country in which, it do not occur otherwise.

RESERVOIRS, SOURCES AND MODES OF TRANSMISSION

Reservoirs of Infections

Definition: Persons, animals, insects, plants or inanimate objects like water, soil, etc., (or combination of all these) in which infectious agents can live, multiply and pass to susceptible host called reservoirs of infections. It includes survival, multiplication and transmission of organism.

Types of reservoirs: Reservoirs for different agents are described in respective chapters. Few are described below in two different categories.

A. According to living or non living objects which act as reservoirs

- **Living reservoirs:** (1) **Human reservoir:** It includes human case or carrier. For example *M. tuberculosis* (tuberculosis), *S. Typhi* (typhoid), *V. cholerae* (cholera), *B. recurrentis* (louse borne relapsing fever), *N. gonorrhoeae* (gonorrhea), *Cl tetani* (tetanus, it also has non living reservoir), *T. pallidum* (syphilis), measles, etc., (2) **Animal reservoir:** It includes zoonotic disease. For example *M. bovis* (bovine tuberculosis), rabies, yellow fever, swine flu, bird flu, etc.

- **Nonliving reservoirs:** Soil and inanimate matter can also act as reservoir. For example soil is reservoir for *Cl. tetani* (tetanus), *B. anthracis* (anthrax), *C. immitis* (coccidioido-mycosis), agents causing mycetoma, etc.

B. Other types: (1) **Homologous reservoir:** It means reservoir and susceptible host are from same species. For example *V. cholerae* where reservoir is man and susceptible host is man. (2) **Heterologous reservoir:** It means reservoir and susceptible host are from different species. For example, typhoid where reservoir is man, and susceptible host is bird/animal.

Note: Multiple reservoirs

Cl. tetani (tetanus): Human and soil reservoirs.

E. coli: Human and animal reservoirs.

Sources of Infections

Definition: Persons, animals, insects, plants or inanimate objects like water or soil (or combination of all these) from which infectious agents can pass to susceptible host. It includes survival and transmission only but no multiplication.

Confusion with reservoir of infection: Sometimes it seems that reservoir and source of infection are synonyms but in fact both are different. For example;

- **Hookworm:** Reservoir of infection is man but source of infection is soil.
- **Tetanus:** Reservoir of infection and source of infection are soil.

Types of sources: Sources for different agents are described in respective chapters. Following are types (also reservoir).

A. Humans: Human is the commonest source of infections. Human may be case (patient) or carrier.

1. **Case (patient):** It is a person who harbors the pathogens with sign-symptoms of disease and serves as potential source of infection for others.
2. **Carrier:** It is a person who harbors the pathogens without any sign-symptoms of disease and serves as potential source of infection for others. Following are different types of carrier:
 - **According to clinical status:** (1) **Healthy carrier:** It harbors the pathogens but never suffered from the disease, e.g., polio, meningococcal meningitis, cholera, diphtheria, etc. (2) **Convalescent carrier:** It is a carrier who recovered from the disease and continue to harbors the pathogens in his body, e.g., typhoid fever, bacillary and amoebic dysentery, cholera, diphtheria, etc. (3) **Incubatory carrier:** It sheds pathogens during the incubation period of infection, e.g., measles, mumps, HBV, polio, pertussis, etc.
 - **According to duration:** (1) **Temporary carrier:** Carrier state lasts for <6 months, e.g., it may be healthy or convalescent or incubatory carrier. (2) **Chronic carrier:** Carrier state lasts for several

years or may for rest of life, e.g., typhoid fever, HBV, gonorrhea, etc.

- **According to portal of exit:** (1) Urinary, e.g., typhoid fever. (2) Intestinal (fecal), e.g., typhoid fever. (3) Respiratory, e.g., Influenza. (4) Nasal, e.g., Influenza. (5) Skin eruption, open wound and blood are also carriers.
- **Other types of carrier:** (1) **Contact carrier:** Person who acquires the pathogen from carrier. (2) **Paradoxical carrier:** Carrier who acquires pathogen from another carrier.

B. Animals and birds: These may act as case or carrier. Infectious diseases transmitted from animals to humans are called zoonoses. (1) **Cattle:** Anthrax, brucellosis and bovine tuberculosis. (2) **Goats:** Brucellosis. (3) **Sheeps:** Anthrax. (4) **Dogs:** Rabies and hydatid disease. (5) **Horses:** Glanders. (6) **Rats:** Rat bite fever, Weil's disease and plague. (7) **Pigs:** Swine flu. (8) **Birds:** Bird flu and ornithosis (psittacosis from parrot).

C. Insects: Diseases transmitted by insects are called arthropod-borne disease and that insects are called vectors. They may also act as sources of infections.

D. Soil: Spores of tetanus bacilli, fungi (*Histoplasma capsulatum*), *Nocardia asteroides*, larvae of round worm and hookworm are found on soil.

E. Water: *Vibrio cholerae*, infective hepatitis viruses (HAV) are found in water.

F. Food: Food may contain either preformed toxin of microorganisms (like *Staphylococcus aureus*, *B. cereus*, *Salmonella* species, *Cl. botulinum*, *Cl. perfringens*, etc.) causing food poisoning or preexisting infectious agents (like beef tape worm in beef, pork tape worm in pork, etc.) causing disease.

Modes of Transmission of Infections

Two categories modes of transmission of infections are as follows:

A. Direct transmission: Here mediators/vehicles for transmission of infections are not required.

1. **Droplet infections:** Droplet nuclei/particles of saliva or nasopharyngeal secretions arise during coughing, sneezing, speaking, talking or invasive procedures (bronchoscopy) are entering in to other host directly who is in close contact. Such particles are 5 µm in diameter and spread to short distance (<3 feet) can directly enter in other host. However, such larger particles can be filtered by nose. Particles 5 µm in diameter traverse to long distance and produce the air borne (indirect) infection described later in this chapter. Infection by droplet nuclei is increased in close contact, overcrowding and lack of ventilation. **Bacteria** transmitted by droplet nuclei are *Strept. pyogenes*, *N. meningitidis*, *C. diphtheriae*, *H. influenzae* type b, *B. pertussis*, *Y. pestis* and *M. pneumoniae*. **Viruses** transmitted by droplet nuclei are influenza

virus, rubella virus, mumps virus, adeno virus and parvo virus B19.

2. **Infections by inoculation/injection under skin or mucosa:** Such infections are transmitted by contaminated needles or syringes like, HBV, HCV, HIV, etc., mainly in healthcare workers.

3. **Infections by contact with skin/mucosa:** Following are examples:

- Sexually transmitted infections/diseases (STIs or STDs): **Ch. 44.**
- Direct skin-to-skin contact transmits diseases like scabies, fungal and viral infections such as HHV-1 and 2 (HSV-1 and 2, respectively), HHV-3 (VZV) pox virus, molluscum contagiosum, etc.

4. **Infections by contact with soil**

- **Bacteria**
 - *Cl. tetani* spores are present on soil and deposition of such soil on wounded skin allows the transmission.
 - *Bacillus anthracis* spores are present in soil and in dead animal products. Animal acquired the infection by ingestion of spores present in soil, but human infection direct from soil spore is query. Human infection of anthrax occurs from animal products.

- **Parasites:** Infection occurs by walking with bare foot on contaminated soil like in *Ancylostoma duodenale* and *Strongyloides stercoralis*.

5. **Vertical infections:** Follow **Table 3.1.**

B. Indirect transmission: Here mediators/vehicles for transmission of infections are required.

1. **Inhalation (air-borne) infections:** Particles arise during coughing, sneezing, talking or invasive procedure from patients, which are ≤5 µm in diameter and traverse to long distance can produce the air borne infection. Some droplet nuclei settle over different objects and become part of dust and cause air borne infections. **Bacterium** transmitted by air-borne route is *M. tuberculosis* (open/active pulmonary tuberculosis). **Viruses** transmitted by air borne route are measles virus, VZV, influenza virus and hemorrhagic fever viruses with pneumonia. Spores of many systemic (dimorphic) **fungi** are present in soil and they are transmitted to man by air like *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*.
2. **Ingestion (food- and water-borne) infections:** Like *E. coli*, *V. cholerae*, *Salmonella* spp., hepatitis A and E, poliovirus, Rotavirus, etc.
3. **Vector-borne infections:** For more details follow **Ch. 47.**
4. **Blood-borne infections:** By transfusion of blood or blood products following organisms are transmitted:
 - **Bacteria:** *T. pallidum*.
 - **Viruses:** HHV-4 (EBV), HHV-5 (CMV), HHV-8 (KSHV), HBV, HCV, HDV, HGV (GB/G Baker virus

TABLE 3.1: Vertical infections

Time	Infections
Antenatal/before birth/congenital/transplacental/teratogenic Commonly called TORCH agents	• T: Toxoplasmosis (<i>Toxoplasma gondii</i>) • O: Others – Congenital listeriosis (<i>Listeria monocytogenes</i>) – Congenital syphilis (<i>Treponema pallidum</i>) – Zika virus – Parvo virus B19 – Chikungunya virus – Malaria in pregnancy (<i>Plasmodium falciparum</i>) – Congenital trypanosomiasis (<i>Trypanosoma cruzi</i>) • R: Congenital rubella syndrome (<i>Rubella virus</i>) • C: Congenital cytomegalovirus infection (<i>CMV</i>) • H: – Herpesviruses like (1) congenital herpes simplex (HSV) (2) Fetal varicella syndrome and congenital/neonatal varicella by VZV – HBV – HIV-AIDS
Intranatal/during birth (transcervical)	Candidiasis, gonorrhea (ophthalmia neonatorum), listeriosis (neonatal listeriosis), <i>Strept. agalactiae</i> (neonatal meningitis), HSV, CMV and HPV
Postnatal/after birth by breast feeding	• Tuberculosis • CMV • HIV

C) HTLV-I, HTLV-II, HTLV-III (HIV), parvo virus B19, zika virus, etc.

- **Parasites:** *Trypanosoma cruzi*, *Leishmania donovani*, *Plasmodium* spp., *Babesia* spp., *Toxoplasma gondii*, etc.

5. **Saliva-borne infections:** Many microbes are present in saliva of humans and animals, which are deposited in tissues following bites. Following are the different examples of saliva borne infections:

- **From human saliva (human bites):** Viruses transmitted by human saliva are HHV-4 (EBV), HHV-6 (HBLV), HHV-7 (RK virus), ECHO virus, mumps virus, etc. **Aerobic bacteria** transmitted by human bites are *Streptococcus*, *Staphylococcus* and *Eikenella corrodens*. **Anaerobic bacteria** transmitted by human bites are anaerobic streptococci, *Fusobacterium* and *Prevotella*. Bites by children rarely get infected while bites from adults get infected in 15–20% cases. Human bites carry aerobes (44%) and anaerobes (55%).
- **From monkey bites:** Herpes virus simiae or B virus.

- **From dog bites:** Like rabies virus.
- **From cat bites:** Like *Bartonella henselae*.
- **Both by dog and cat bites:** *Pasteurella multocida*.
- **From rat bites (rat bite fever):** Like *Streptobacillus moniliformis* and *Spirillum minus*.

6. **Fomites-borne infections:** Contamination of fomites like towels, handkerchiefs, pens, pencils, clothes, cups, spoons, keys, etc., may transmit the infections like eye disease (trachoma), ear infection, diphtheria, dysentery, hepatitis A, etc.

7. **Infections by unclean hands and fingers:** It may transmit the infections like dysentery, trachoma, etc.

ACCESS YOURSELF

Essay/Full Question

1. Describe the epidemiological basis of common infectious diseases.

Short Notes

1. Congenital infections.

Short Questions for Theory/Viva Questions

1. Define infestation and infection.
2. What are contact carrier and paradoxical carrier?
3. Define case and carrier.
4. What are TORCH agents?

MCQs for Chapter Review

Definitions and Types

1. **Septicemia is:**
 - a. Bacteria in blood
 - b. Toxin in blood
 - c. Pus in blood
 - d. Multiplication of bacteria and toxin in blood

Reservoirs, Sources and Modes of Transmission

2. Which of the following does not have non human reservoir
 - a. *Salmonella* Typhi
 - b. *N. gonorrhoeae*
 - c. *E. coli*
 - d. *Clostridium tetani*
 - e. *Treponema pallidum*
3. Man is the only reservoir of:
 - a. Rabies
 - b. Measles
 - c. Typhoid
 - d. Japanese encephalitis
4. Which of the following is not transmitted by soil?
 - a. Coccidioidomycosis
 - b. Tetanus
 - c. *Brucella*
 - d. Anthrax
5. Vertically transmitted disease caused by all except:
 - a. *Toxoplasma*
 - b. CMV
 - c. HIV
 - d. *Treponema pertenu*
6. Which of the followings are transmitted by blood?
 - a. *Toxoplasma*
 - b. Syphilis
 - c. CMV
 - d. Hepatitis B and C
 - e. Hepatitis A
7. All of the following infections may be transmitted via blood transfusion except:
 - a. Parvo B-19
 - b. Dengue virus
 - c. CMV
 - d. Hepatitis G virus

8. Most common agents responsible for human bite infections are:

- a. Gram-negative bacilli
- b. Gram-positive bacilli
- c. Sporochetes
- d. Anaerobic streptococci

Answers and Explanation of MCQs

1. d

- Multiplication of bacteria and toxin in blood called **toxemia**, which is one type septicemia.

2. c

3. b, c

- Follow section, **reservoirs of infection** for explanation of answers of MCQs 2–3. Also follow respective chapters for more explanation.

4. c and d

- Spores of coccidioidomycosis are present in soil and transmitted by air borne route. Follow section, **modes of transmission [indirect → inhalation (air borne)]** for more explanation.
- Option b and d: Follow section, **modes of transmission (direct → contact with soil)** for explanation.
- No role of soil transmission of brucella.

5. d

- Follow section, **Table 3.1 and respective chapters** for more explanation.

6. a, b, c, d

7. b

- Follow section, **modes of transmission (indirect → blood borne)** for explanation of answers of MCQs 6–7.

8. d

- Follow section, **modes of transmission (indirect → saliva borne)** for explanation.

Normal Microbial Flora of Human Body

Chapter Outline

- Introduction
- Normal Flora
- Probiotics
- Prebiotics

INTRODUCTION

Types of Microbes as per Habitats

Saprophytes

- **Meaning:** Sapro (Greek) means decayed and phyton (Greek) means plant.
- **Definition:** These are free living microbes in/on dead or decaying organic matter such as soil and water. They are incapable to multiply in living tissues except *B. subtilis*.

Commensals: (Mostly Normal Flora)

- **Meaning:** Com (Greek) means with and mensa (Greek) means table (eating at the same table).
- **Definition:** These are microbes which live in complete harmony with the host without causing any damage (living together).

Pathogens

- **Meaning:** Pathos (Greek) means suffering and gen (Greek) means to produce.
- **Definition:** These are microorganisms capable of producing disease in the host.

Host and Microbes Relationships

Normal floras are found in association with animals and also in the environment. Three types relationship between the host and microbes as follows.

Symbiosis

- **History:** This theory was given by Lynn Margulis and widely accepted.
- **Synonym:** It also called endosymbiosis or mutualism.
- **Definition:** Both microbes are dependent on each other, and both are getting benefit from each other without producing any harm to either partner.

- **Example:** Intestinal flora survive in gut produce vitamins for host.

Commensalism

- **Definition:** Association in which one partner gets benefit and other remains unharmed.
- **Example:** Flora living on skin.

Parasitism

- **Definition:** Association in which one partner (usually smaller called parasite) gets benefit with production of injuries to other partner (usually larger called host).
- **Example:** Microbes get benefits with production of infection to host.

Note: Predation

Association in which one animal (usually larger like tiger, lion, etc.), consumes or kills the other (usually smaller like deer, rabbit, etc.), for food. Larger animal is carnivorous called predator. Smaller is herbivorous called prey.

NORMAL FLORA

Synonym

Microbiota, microflora, microbial flora and indigenous microbial population.

Definition

Mixture of microorganisms regularly found at any anatomical site on or in the body of a healthy persons.

Advantages

1. **Immunostimulation:** They raise the immunity of body by sharing the common Ags.
2. **Endotoxin:** They liberate the endotoxin which triggers the alternative complement pathway as long as they are not produced in excessive amounts.

3. **Protection from external invaders:** Normal flora occupies body's epithelial surfaces. They prevent or interfere with colonization or invasion by other bacteria by blocking receptors (attachment) sites, competing for essential nutrients and producing anti-bacterial substances like peroxides bacteriocins, etc.
4. **Vitamins production:** Some of the intestinal flora like *E. coli* and *Bacteroides* are producing vitamin K, vitamin E and vitamin B in the gut which are available for use by host.
5. **Production of organic acids:** Like butyric acid and acetic acid which contribute in nutrition of host
6. **Digestion:** Intestinal flora degrades mucins, epithelial cells, carbohydrates and other dietary fibers and help in digestion.

Disadvantages

1. **Source of opportunistic infections,** e.g., they cause infection in immunodeficient persons.
2. **Entry in unusual sites cause infection,** e.g., intestinal flora may cause UTI.
3. **Interference with antibiotics:** By producing some enzymes like penicillinase which inactivates the penicillin and aggravates the infection.
4. **Confusion in diagnosis:** It is difficult to differentiate between normal flora and pathogens by laboratory tests.
5. **Supra-infection:** Ch. 47.

Types

Two types like transient and resident as mentioned below.

Transient flora

- **Properties:** They present for short (temporary) period in body not always. These include non-pathogenic and pathogenic groups. Among the pathogenic group some examples are like meningococcus and pneumococcus from nasopharynx. In hospital, patient may acquire the drug-resistance organisms as transient flora like MRSA from nose or skin, multi-drug resistance gram-negative bacteria like *E. coli*, *Klebsiella*, *Pseudomonas* and *Acinetobacter*, etc. Transient flora can be easily removed by improving the hygiene and taking infection control measures.
- **Transient flora in different organs:** Follow Table 4.1.

Resident flora

- **Properties:** They are constant flora of body or lifelong members. These include non-pathogenic groups which are harmless; infect beneficiary role.
- **Resident flora in different organs:** Described below in details.

Resident Flora of Skin

Human adult has two square meters of skin. Human skin is constantly attacked by organisms present in environment and objects come in contact, which are

TABLE 4.1: Transient flora in different organs

Organ	Flora
Skin	• Bacteria: <i>Staph. aureus</i>, <i>Strept. pyogenes</i>, <i>Strept. viridans</i>, <i>Enterococcus</i>, <i>E. coli</i>, <i>Proteus</i>, <i>Klebsiella</i> • Fungi: <i>Candida</i> spp., <i>M. furfur</i>, <i>Trychophyton</i>
Eyes	• Bacteria: <i>Staph. aureus</i>, <i>Strept. pneumoniae</i>, <i>Strept. viridans</i>, <i>Bacillus</i>, <i>Priopionibacterium</i>
Respiratory tract	• Bacteria: <i>Strept. pneumoniae</i>, <i>Staph. aureus</i>, <i>N. meningitidis</i>, <i>Moraxella</i>
Oral cavity	• Bacteria: <i>Strept. pyogenes</i>, <i>Lactobacillus</i>, <i>N. meningitidis</i>, <i>Moraxella</i>, <i>E. corrodens</i>, • Fungi: <i>Candida</i> spp. • Viruses: CMV, HSV
GIT	Stomach • Bacteria: <i>H. pylori</i>, <i>Streptococcus</i> spp., <i>Lactobacillus</i> spp. Small intestine • Fungi: <i>Candida</i> spp. • Parasites: <i>Entamoeba coli</i>, <i>Endolimax nana</i>, <i>Trichomonas hominis</i>, <i>Blastocystis hominis</i> Large intestine • Bacteria: <i>Corynebacterium</i>, <i>MAC</i>, <i>Pseudomonas</i> • Fungi: <i>Candida</i> spp. • Parasites: Same as small intestine
Urinary tract	• Bacteria: <i>Enterococcus</i>, <i>Mycoplasma</i>, <i>M. smegmatism</i>, <i>Bacteroides</i>, <i>Fusobacterium</i>
Genital tract	• Bacteria: <i>Strept. pyogenes</i>, <i>Enterococcus</i> • Fungi: <i>Candida</i> spp. • Parasites: <i>Trichomonas vaginalis</i>

loaded with microbes. Skin floras are mostly transient in nature. The populations of normal flora over the skin depend on many **factors** like differences in pH, oxygen, water, body secretions, wearing clothes, occupations, environmental conditions, etc. Following are the different **resident flora of skin**.

1. Bacteria

- *Propionibacterium acne*: It is an anaerobic *Corynebacterium*. Children younger than 10 years are rarely colonized with it. It presents in areas rich in sebaceous gland.
- Diphtheroids: These are non-pathogenic corynebacteria.
- Anaerobic cocci.
- *Staph. epidermidis*: It is the major inhabitants making up >90% of the flora.
- *Staph. aureus*: It presents in nose, perineum and vulval skin. Its occurrence in nasal passages varies with age being greatest in newborns and less in adults. It also presents in hair follicles. Penicillin resistant *Staph. aureus* presents in hospital staff members.
- Others: *Micrococcus*, hemolytic streptococci, *Enterococcus* spp., and non enterococci, GNB like *E. coli*, *Proteus* spp., etc., and nonpathogenic mycobacteria.

- 20 2. Fungi:** *Candida* spp., *Pityrosporum ovale* and *Cryptococcus* spp.

Resident Flora of Eyes

Conjunctiva is normally free from microbes due to constant flushing action of tears which contain lysozyme. Conjunctival floras are scanty and occasionally present. Predominant organisms of the conjunctiva are *Corynebacterium xerosis*, *Staphylococcus* spp., non-hemolytic *Streptococcus* spp., *Haemophilus* spp., and *Moraxella catarrhalis*.

Resident Flora of Respiratory Tract

Upper respiratory tract: It includes nose, ears, sinuses, pharynx and throat.

1. **Nose:** It includes the *Staphylococcus*, *Streptococcus* (*Strep. agalactiae*), *Corynebacterium*, *Haemophilus* and *Moraxella*.
2. **Nasopharynx:** Nasopharynx is often sterile at birth but may be contaminated within 2–3 days after birth by passage through the birth canal and by attendants. It includes the *Strept. viridans*, which established as the most prominent members of the resident flora and remain so for life. Others flora are aerobic and anaerobic bacteria such as staphylococci, pneumococci, GNC (*N. meningitidis* and *M. catarrhalis*), diphtheroids, lactobacilli, etc.
3. **Throat:** It includes the *Strept. viridans*, *Moraxella catarrhalis*, pneumococci and certain GNB from intestine like *E. coli*, *P. aeruginosa*, paracolons and *Proteus* spp.

Lower respiratory tract: It includes trachea, bronchi and lungs. Trachea contains organisms from pharynx or oral cavity. Bronchi and alveoli are normally sterile.

Resident Flora of Oral Cavity

Mouth is normally not sterile at birth. It contains same organisms as present in birth canal as mentioned below. It includes **bacteria** such as *Streptococcus mutans*, *Strept. sanguis*, *Strept. salivarius*, *Strept. mitior*, *Strept. milleri*, *Staphylococcus* spp., *Enterococcus* spp., *Micrococcus* spp., *Lactobacillus acidophilus*, *Corynebacterium* spp., *Actinomyces* spp., *Propionibacterium* spp., and *Bifidobacterium* spp., *Veillonella* spp., *Neisseria* spp., *Branhamella* (*Moraxella*) spp., *Bacteroides* spp., *Treponema* spp., *Fusiform bacillus* (*Leptotricha buccalis*), *Actinobacillus* spp., *Capnocytophaga* spp., *Haemophilus* spp., *Eikenella* spp., *Wollinella* spp., *Selenomonas* spp., etc., **fungi** such as *Candida* spp., and *Geotrichum candidum* and **parasites** such as *Trichomonas tenax* and *Entamoeba gingivalis*.

Resident Flora of Gastrointestinal Tract (GIT)

In children: At birth the intestine is sterile but organisms are soon introduced with food. Bowels of newborns in intensive care nurseries tend to be colonized by Enterobacteriaceae like *Klebsiella*, *Citrobacter* and *Enterobacter*. In breast-fed children the intestine contains large numbers of streptococci and lactobacilli

(*Lactobacillus bifidus* constitutes 99% of total organisms in feces). In bottle-fed children a more mixed flora exists in the bowel and lactobacilli are less prominent.

In normal adults

- **Effective factors:** **Diet** has a marked influence on the relative composition of the intestinal and fecal flora. Dietary habit develops toward the adult pattern which changes the bowel flora. **pH** changes also affects the intestinal flora. Esophagus contains microorganisms arriving with saliva and food. Acidic pH of stomach keeps the number of microbes at a minimum (103–105/g of contents) unless obstruction at the pylorus favors the proliferation of gram-positive cocci and bacilli. The normal acidic pH of the stomach markedly protects against infection with some enteric pathogens such as *V. cholerae*. Administration of cimetidine for peptic ulcer leads to a great increase in microbial flora of the stomach. Alkaline pH of intestine gradually allows the increment of resident flora. **Oral administration of antibiotics** can temporarily suppress the fecal flora. Neomycin plus erythromycin can suppress aerobes in 1–2 days. Metronidazole can suppress the anaerobes. The drug-susceptible microorganisms are replaced by drug-resistant ones, particularly *Staphylococcus*, *Enterobacter*, *Enterococcus*, *Clostridium difficile*, *Pseudomonas*, *Proteus* spp., and yeasts. The feeding of large quantities of *Lactobacillus acidophilus* may result in the temporary establishment of this organism in the gut and the concomitant partial suppression of other gut flora. **Minor traumas** during sigmoidoscopy, barium enema may induce transient bacteraemia in about 10% of procedures.
- **List of intestinal flora in adults:** More than 100 distinct types of organisms occur regularly as normal fecal floras are mentioned below.
 1. **Anaerobes:** They are mostly present in lower intestine. Anaerobes outnumber facultative aerobes organisms by 1000-fold. (1000:1). About 96–99% of the resident flora consist anaerobes like *B. fragilis* (most common), *Fusobacterium* spp., *Lactobacillus* (*L. bifidus* or *bifidobacteria*), *Cl. perfringens*, *Cl. difficile* and anaerobic gram-positive cocci (*Peptostreptococcus* spp.).
 2. **Facultative aerobes:** They are mostly present in upper intestine. About 1–4% of resident flora consist facultative aerobes like gram-negative coliform bacteria (*E. coli*), *Enterococcus*, *Proteus* spp., *Pseudomonas*, *Lactobacillus*, etc.
 3. **Fungi:** *Candida* spp.
- **Density of flora in different intestinal parts in adults:** Follow Table 4.2.
- **Clinical significances**
 1. Intestinal bacteria **synthesizes** vitamin K.
 2. They have role in **conversion** of bile pigments and bile acids.

TABLE 4.2: Density of flora in different intestinal parts

Location (adult)	Bacteria/gram contents
Stomach	10^3 – 10^5
Duodenum	10^3 – 10^6
Jejunum and ileum	10^5 – 10^8
Cecum and transverse colon	10^8 – 10^{10}
Sigmoid colon and rectum	10^{11} (10–30% of fecal flora)

3. They have role in **absorption** of nutrients and breakdown products.
4. They have role in **antagonism** to microbial pathogens.
5. The intestinal flora **produce ammonia** and other breakdown products that are absorbed and can contribute to hepatic coma.
6. **Abscess formation:** The anaerobic flora of the colon, including *B. fragilis*, *Clostridium* spp., and *Peptostreptococcus* play a main role in abscess formation after bowel perforation. *Prevotella bivia* and *P. disiens* are important in abscesses of the pelvis originating in the female genital organs.
7. **Drug resistant:** *B. fragilis* are penicillin-resistant; therefore, another drug should be used.
8. Role in **superinfection or suprainfection:** Ch. 47.

Resident Flora of Urinary Tract

Upper urinary tract includes kidneys and ureters which are usually sterile.

Lower urinary tract includes bladder and urethra. **Urethra** is usually sterile or may contain same flora as skin and intestinal flora like α -hemolytic *Streptococcus*, *Enterococcus*, *Lactobacillus*, diphtheroides *Bacteroides*, *G. vaginalis*, *U. urealyticum* and *C. albicans*. These may enter in bladder can result in UTI. The urine of a healthy individual is sterile but can become contaminated due to transfer of microbes from the GIT or genital tract. Normal floras in urine are 102–104/ml.

Resident Flora of Genital Tract

Male genital organs: *M. smegmatis* can be found on the penis or genital secretion of both sexes. Its presence in urine creates confusion in diagnosis of renal tuberculosis.

Female genital organs: Normal vaginal secretion contains 10^8 bacteria/ml and also showing presence of *M. smegmatis*. Vagina has complex microbiota at different time as mentioned below.

- **At birth:** Sterile at birth, but after 24 hours acquires microbes from vagina, skin and intestine.
- **In 24 hours:** Invaded by *Micrococcus*, *Enterococcus* and diphtheroides.
- **In 2–3 days:** Maternal estrin induces deposition of glycogen in vaginal epithelium. This favors the growth of lactobacilli (also called Doderlein's bacilli) and flora similar to adult stage. Lactobacilli convert glycogen in to acid. Acidic pH prevents the vaginal

colonization by foreign bacteria. After that estrin and lactobacilli are disappearing, pH becomes alkaline and vagina returns to normal flora.

- **At prepuberty:** Flora includes anaerobic cocci, *Listeria*, *Streptococcus*, *Mimeae*, *Mycoplasma*, *G. vaginalis*, *Neisseria*, spirochetes and *Candida* spp.
- **At puberty/adult:** *Lactobacillus* reappears, pH becomes acidic. Normal flora includes anaerobes like *Gardnerella vaginalis*, *Bacteroides*, *Mobilincus*, *Prevotella* and rare like *M. hominis*
- **At pregnancy:** Increase *Staph. epidermidis*, Doderlein's bacilli and *Candida*.
- **At menopause:** Return to prepuberty flora.

Resident Flora of Blood and Tissues

Microbes invade in blood and tissues from GIT, mouth, nasopharynx and some other body parts, but they are eliminated by defense systems of body.

PROBIOTICS

Definition: These are living microorganisms which are administered for treatment or prevention of diseases.

Properties: Probiotics are the part of normal flora of body. They are useful when normal floras are suppressed. They are available commercially in the form of capsules or sachets. Capsules or sachets contain *Bacillus coagulans*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Saccharomyces boulardii*, etc.

Uses: Probiotics are used in following clinical conditions.

1. **Infectious diseases:** In *H. pylori* infection, in bacterial vaginosis (to restore the vaginal pH by using lactobacilli which produce the acidic pH) and in antibiotic associated diarrhea.
2. **Inflammatory diseases:** Gastroenteritis, colitis, necrotizing enteritis, irritable bowel syndromes and modulatory response in inflammation.
3. **Hypersensitivity reactions:** Modulatory response in eczema, dermatitis and other allergic disorders.
4. **Immunodeficiency diseases:** To restore the immunity
5. **High lipid profile:** Probiotics reduce the serum cholesterol by breaking the bile in the gut, thus inhibiting the reabsorption
6. **Hypertension:** Probiotics reduce the high blood pressure by producing the acetyl choline esterase (ACE) inhibitors like peptides during fermentation.

Limitations: Live organisms present in probiotics produce their actions only after their establishment in intestine, for that they have to compete with normal flora of intestine to establish them self. So, nowadays another similar preparation called prebiotics are more useful than probiotics.

PREBIOTICS

These are the preparations contain dietary fibers which when administered stimulate the growth and activity of normal flora.

Essay/Full Question

1. Normal flora of human body.

Short Notes

1. Normal flora of skin/GIT/genital tract.
2. Probiotics.

Short Questions for Theory/Viva Questions

1. Define: Saprophytes and commensals.
2. Mention the two differences between resident and transient flora of human body.
3. Mention the different types of relationship between host and microbes.
4. Write two advantages and two disadvantages of normal flora.
5. What are Doderlein's bacilli?
6. How probiotics are differentiated from prebiotics.

Comments on

1. Normal floras are the useful properties of body.
2. Lactobacilli have protective role in adult vagina.
3. Prebiotics are more useful than probiotics.

MCQs for Chapter Review**Normal Flora**

1. **Transient colonization is caused by:**
 - a. HSV
 - b. *Trichomonas vaginalis*
 - c. *H. influenzae*
 - d. *N. gonorrhoeae*
 - e. *Staphylococcus aureus*
2. **It is true regarding the normal microbial flora present on the skin and mucous membrane that:**
 - a. It cannot be eradicated by antimicrobial agents
 - b. It is absent in the stomach due to the acidic pH
 - c. It establishes in the body only after the neonatal period
 - d. The flora in the small bronchi is similar to that of trachea
3. **Common natural floras of skin are:**
 - a. *Streptococcus*
 - b. *Staphylococcus aureus*
 - c. *Candida albicans*
 - d. *Bacteroides fragilis*
 - e. *Propionibacterium acne*
4. **Normal commensal of skin:**
 - a. *Staphylococcus aureus*
 - b. *Candida albicans*
 - c. *Bacteroides fragilis*
 - d. *Propionibacterium acne*
 - e. *Corynebacterium*
5. **Which of the following is the main colonizer of sebaceous gland?**
 - a. *Propionibacterium acne*
 - b. *Corynebacterium diphtheriae*
 - c. *Strept. pyogenes*
 - d. *Staph. aureus*
 - e. *Candida*
6. **In the gut, anaerobic bacteria outnumber the aerobes by ratio of:**
 - a. 10:1
 - b. 100:1
 - c. 1,000:1
 - d. 10,000:1

7. Most common commensals gut flora in adult:

- a. Lactobacilli
- b. *Bacteroides*
- c. *E. coli*
- d. *Klebsiella*

8. The predominant colonic bacteria are:

- a. Largely aerobic
- b. Largely anaerobic
- c. *Bacteroides*
- d. Staphylococci

9. Bacterial count in duodenum:

- a. 10^5 per gram
- b. 10^8 per gram
- c. 10^{10} per gram
- d. 10^{12} per gram

10. Doderlein's bacilli word associated with:

- a. Lactobacilli
- b. Mimeae
- c. *Listeria*
- d. *Gardnella*

11. Normal commensal in female genital tract:

- a. *Gardnerella vaginalis*
- b. *Bifidobacterium*
- c. *Proteus*
- d. *Neisseria*

Probiotics and Prebiotics**12. Probiotics are useful for:**

- a. Necrotizing enterocolitis
- b. Breast milk jaundice
- c. Hospital acquired pneumonia
- d. Neonatal seizures

13. Probiotics are differentiated from prebiotics:

- a. Probiotics contain microbes while prebiotics contains dietary fibers
- b. Probiotics contain dietary fibers while prebiotics contains microbes
- c. Probiotics are prepared from animal products while prebiotics are prepared from human products
- d. None

Answers and Explanation of MCQs**1. a, b and e**

- Follow section, **transient flora** → Table 4.1 for explanation.

2. a

- Resident floras are permanent and cannot be eradicated by antimicrobial agents.

3. a, b, c, e**4. a, b, d, e****5. a**

- Follow section, **resident flora of skin** for explanation of answers of MCQs 3–5.

6. c**7. b****8. b****9. a**

- Follow section, **resident flora of GIT** and Table 4.2 for explanation of answers of MCQs 6–9.

10. a**11. a**

- Follow section, **resident flora of genital tract** → Female genital organs for explanation of answers of MCQs 10–11.

12. a

- Follow sections, **probiotics (uses)** for explanation.

13. a

- Follow sections, **probiotics and prebiotics (definition)** for explanation.

Miscellaneous Topics: World Days and Healthcare-related Symbols

Chapter Outline

- World Days
- Healthcare-related Symbols

WORLD DAYS

Follow **Table 5.1**.

Diseases	World day	Comment
Tuberculosis	24th March of every year	<i>M. tuberculosis</i> was identified and described on 24th March 1882 by Robert Koch
Leprosy	Last Sunday of January in each year	This day was chosen in commemoration of the death of Gandhi, the leader of India who understood the importance of leprosy
Polio	24th Oct. of every year	It was fixed by Rotary International over a decade ago to commemorate the birth of Jonas Salk, who led the first team to develop a vaccine against poliomyelitis.
HIV-AIDS	1st Dec. of every year	Since 1988, 1st Dec is celebrated as an AIDS day to raising awareness of the AIDS pandemic caused by HIV by Josh Lowe and mourning those who have died of the disease
Rabies	28th Sept of every year	It is the anniversary of the death of Louis Pasteur who, with his colleagues, developed the 1st effective rabies vaccine
Hepatitis	28th July of every year	It aims to raise global awareness and to encourage the prevention, diagnosis and treatment of hepatitis A, B, C, D and E
Malaria	25th April of every year	It aims the control of malaria
Health	7th April of every year	It is celebrated under the sponsorship of the WHO, as well as other related organizations

Note: World health day is one of eight official global health campaigns marked by WHO, along with World tuberculosis day, World immunization week, World malaria day, World no tobacco day, World AIDS day, World blood donor day, and World hepatitis day.

HEALTHCARE-RELATED SYMBOLS

Follow **Figs 5.1 to 5.7**.

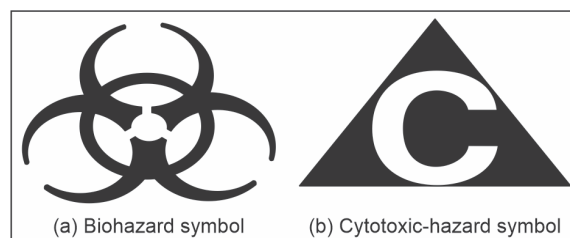


Fig. 5.1: Biohazard symbol and cytotoxic-hazard symbol

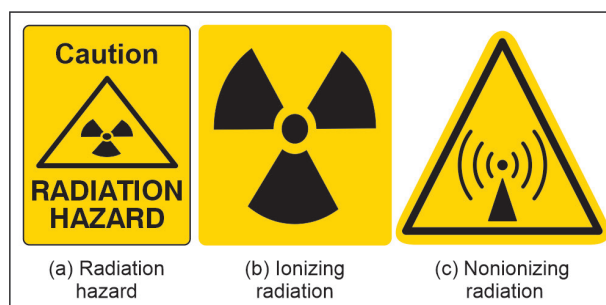


Fig. 5.2: Radiation hazard symbols

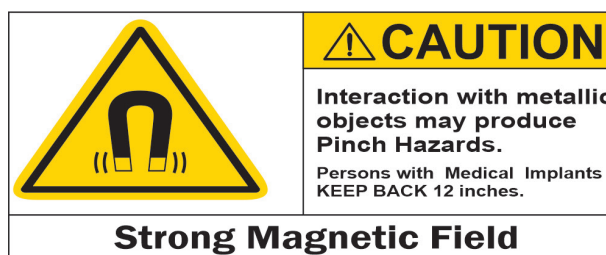


Fig. 5.3: Magnetic field symbol



Fig. 5.4: Gas hazard symbols



Fig. 5.5: Fumigation symbol

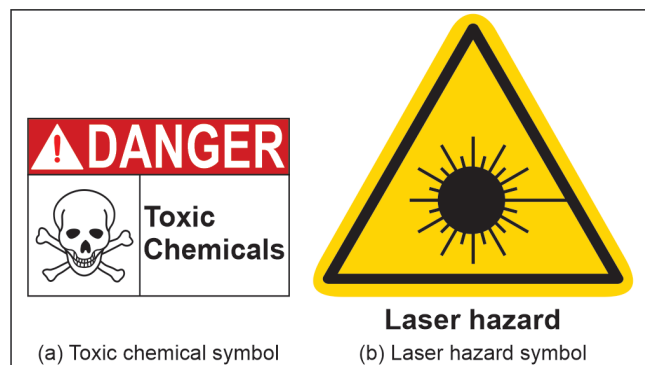


Fig. 5.6: Toxic chemical and laser hazard symbols



Fig. 5.7: Red ribbon symbol of HIV-AIDS

ACCESS YOURSELF

Short Questions for Theory/Viva Questions

1. Mention the day/date on which following diseases are celebrated as world day.
 - Tuberculosis
 - Rabies
 - Leprosy
 - Malaria

MCQs for Chapter Review

World Days

1. 24th March of every year is celebrated as:
 - a. World tuberculosis day
 - b. World leprosy day
 - c. World rabies day
 - d. World malaria day

Healthcare-related Symbols

2. Symbol shown in below image represent:
 - a. a: radiation hazard and b: cytotoxic hazard
 - b. a: radiation hazard and b: biohazard
 - c. a: bio medical waste and b: cytotoxic hazard
 - d. a: cytotoxic and b: bio medical waste



Answers and Explanation of MCQs

1. a
 - Follow section, world days (Table 5.1) for explanation.
2. c
 - Follow section, healthcare-related symbols (Fig. 5.1 to 5.7) for explanation.

Morphology of Bacteria

Chapter Outline

- Shapes of Bacteria
- Size of Bacteria
- Arrangement of Bacteria
- Morphological Parts (Anatomy) of Bacteria

SHAPES OF BACTERIA

Follow **Fig. 6.1** for shapes of different bacteria.

1. Coccus: From kokkos (Greek) means berry, indicating spherical shape.
2. Bacillus: From baculus (Latin) means rod, indicating straight rod shape.
3. Coccobacillus: Indicating oval shape (intermediate between coccus and bacillus).
4. *Vibrio comma*: Vibrio from vibrare means vibrating nature and comma means curved rod like comma indicates comma shaped and motile bacillus.
5. Spirillum: Rigid spiral form.
6. Spirochete: From speira means coil and chaite means hair, flexuous spiral form.
7. *Leptospira interrogans*: Shape like interrogation mark or umbrella hook.
8. Actinomycete: Actino from actis means rays and mycete from mykes (Greek) means branching

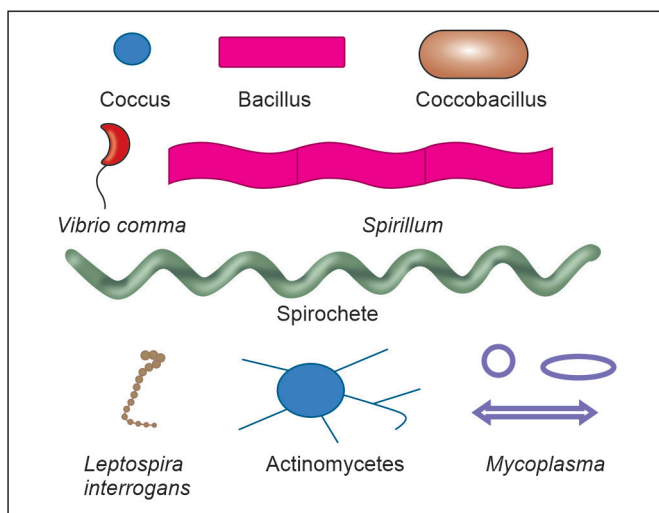


Fig. 6.1: Shape of bacteria

like fungi. It indicates the characteristic sun ray appearance and branching nature of bacterium.

9. *Mycoplasma*: Myco from Mykes (Greek) means branching/filamentous form and plasma means plasticity of nature. It is cell wall deficient bacteria and because of plasticity of nature assume any shape like spherical, oval, rod, balloon, filamentous, etc.

SIZES OF BACTERIA

Method to Measure the Size of Bacteria

Method to measure the size of bacteria called micro-metry. Measuring unit called micrometer or micron (μm or μ).

Size of Different Bacteria

1. Coccus: $0.5\text{--}1\ \mu\text{m}$.
2. Bacillus: $0.2\text{--}0.5\ \mu\text{m}$ in breadth $\times$ $1\text{--}6\ \mu\text{m}$ in length.
3. Coccobacillus: $0.2\text{--}0.5\ \mu\text{m}$ in breadth $\times$ $0.5\text{--}1\ \mu\text{m}$ in length.
4. Spirochetes: Very large up to $10\text{--}20\ \mu\text{m}$ in length.
5. Pleomorphic bacteria: Variable in size and shape.

ARRANGEMENT OF BACTERIA

Arrangement of Cocci

Follow **Fig. 6.2**.

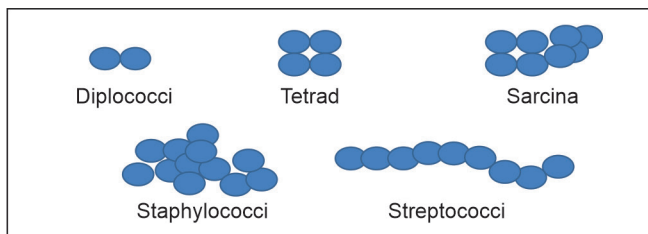


Fig. 6.2: Arrangement of cocci

- 26**
1. Diplococci: Arranged in pair/group of two like pneumococcus, meningococcus, gonococcus, etc.
 2. Tetrad: Arranged in group of four.
 3. Sarcina: Arranged in group of eight.
 4. Staphylococci: Arranged in grapes like cluster.
 5. Streptococci: Arranged in chains.

Arrangement of Bacilli

Follow **Fig. 6.3**.

1. Singly: Like *E. coli*.
2. Diplobacilli: Arranged in pair like *Klebsiella pneumoniae* and *Moraxella lacunata*.
3. Streptobacilli: Arranged in chain.
4. Arranged in group or in cluster: Like *E. coli*, *S. Typhi*, etc.
5. S-shape: Two comma shape bacilli arranged end-to end gives S-shape appearance like in *V. cholerae* (*V comma*).
6. Spiral shape: Many comma shape bacilli arranged end-to end gives spiral-shape appearance like in *V. cholerae* (*V comma*).
7. Chinese latter or cuneiform pattern: Bacilli arranged by making an angle to each other like Chinese letter V or L in *C. diphtheriae*.
8. Palisades: Like stakes of fence in *C. diphtheriae*.
9. Fish in stream/school of fish: Bacilli arranged parallel to each other called fish in stream like in *V. cholerae* and school of fish in *H. ducreyi*.

MORPHOLOGICAL PARTS (ANATOMY) OF BACTERIA

It includes organs (**Fig. 6.4**) like bacterial cell wall, cytoplasmic (plasma) membrane, protoplasm, capsule-slime layer, flagella, fimbriae, spores, pleomorphism and involution forms. Bacterial cell wall and cytoplasmic (plasma) membrane are collectively known as cell envelop or outer layer. Capsule-slime layer, flagella and fimbriae are considered as appendages of cell wall.

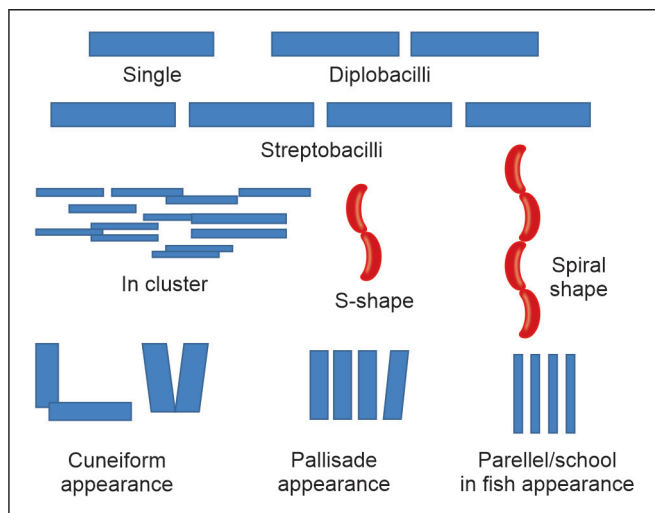


Fig. 6.3: Arrangement of bacilli

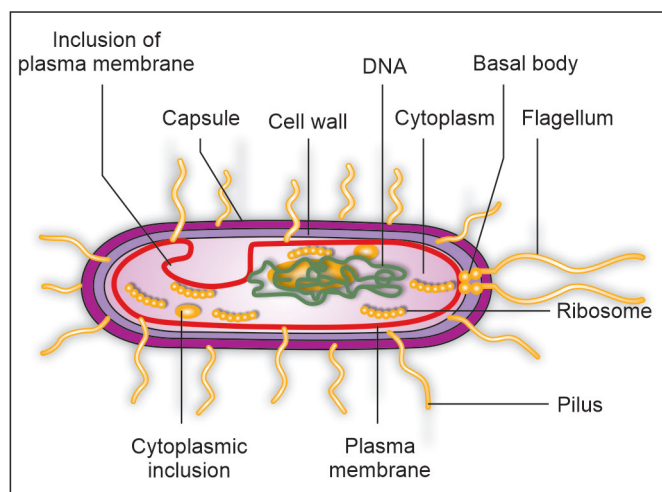


Fig. 6.4: Anatomy of bacteria

Bacterial Cell Wall

Properties: Cell wall is tough and rigid, surrounding the bacterium as like a shell. It is 10–25 nm thick and holds the 20–30% of the dry weight of the cell. It is absent in some bacteria discussed later in this chapter.

Cell wall antigen: It called somatic antigen. It is abbreviated as O-Ag, from ohne hauch (German) means without film of breath. It is opposite to hauch (German) means with film of breath for flagellar antigen (H-antigen).

Functions of cell wall

1. It provides rigidity to cell.
2. It maintains the bacterial shape.
3. It plays fundamental role in vital activity of bacteria.
4. It carries the bacterial antigen which play role in virulence and immunity.
5. It provides mechanical support to cell membrane.
6. It contains proteins (called porin proteins) that make the porin channels for diffusion of material to and fro by bacteria.
7. It helps to maintain osmotic pressure and protects cell against osmotic damages.
8. It provides site for the phage adsorption.
9. It takes part in cell division.

Biochemistry of cell wall

A. Cell wall of gram-positive bacteria: It is 80 nm thick with following structural details.

1. **Peptidoglycan layer:** It also called mucopeptide or mucoprotein or murein. Thick peptidoglycan is composed of alternating unit of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) linked together by beta-1-4 linkage and a set of tetra peptide side chain attached to N-acetylene muramic acid. Tetrapeptide chain attaches together by penta-peptide bridges as shown in **Fig. 6.5**.

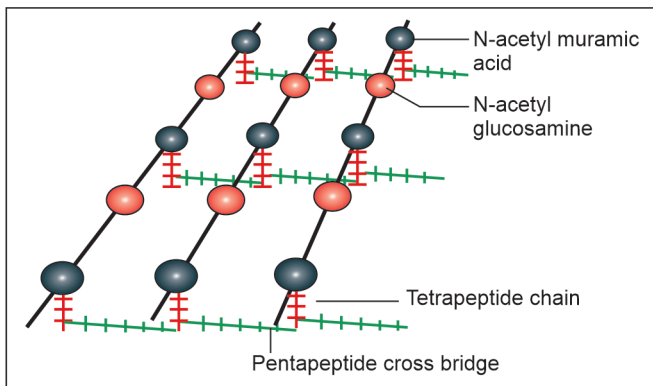


Fig. 6.5: Peptidoglycan/murein

2. Teichoic acids

- **Types of teichoic acids:** Two types.
 - **Cell wall teichoic acid:** Cell wall teichoic acid is polymer of ribitol (5-carbon) and covalently linked to peptidoglycan of cell wall as shown in Fig. 6.6.
 - **Membrane teichoic acid:** Membrane teichoic acid is polymer of glycerol (3-carbon) and linked to glycolipid of cytoplasmic membrane, so also called lipoteichoic acid as shown in Fig. 6.6.
- **Functions of teichoic acids**
 - Teichoic acid helps to synthesize the peptidoglycan
 - Organ of adhesion: It helps bacteria to adhere with host cells.

3. **Surface proteins:** Like M, T and R proteins are useful for grouping the bacteria (in streptococci). Surface proteins are also useful for virulence and as organs of adhesion.

B. Cell wall of gram-negative bacteria: It is 2 nm thick. It has four layers from inner to outer are periplasmic space, thin peptidoglycan layer, outer membrane protein (OMP) and lipopolysaccharide (LPS) as shown in Fig. 6.7 with following structural details.

1. **Periplasmic space:** It presents outer to plasma membrane contains enzymes.
2. **Peptidoglycan layer:** It is very thin layer.
3. **Outer Membrane Protein (OMP):** It is made up by phospholipids, lipoproteins and surface proteins.
 - **Phospholipids:** These are similar to plasma membrane.
 - **Lipoproteins:** OMP attaches to peptidoglycan by lipoproteins.
 - **Surface proteins:** Also called major membrane proteins or principal membrane proteins. There are following two types of surface proteins.
 - **Porin protein:** It makes porin channel for diffusion of material to and fro by bacteria.
 - **Non-porin protein:** It acts as an organ of adhesion, helps in production of exo-enzymes and also provides receptors for antibiotic agents. For example Penicillin Binding Proteins (PBPs).
4. **Lipopolysaccharide (LPS):** It has three regions as shown in Fig. 6.8.

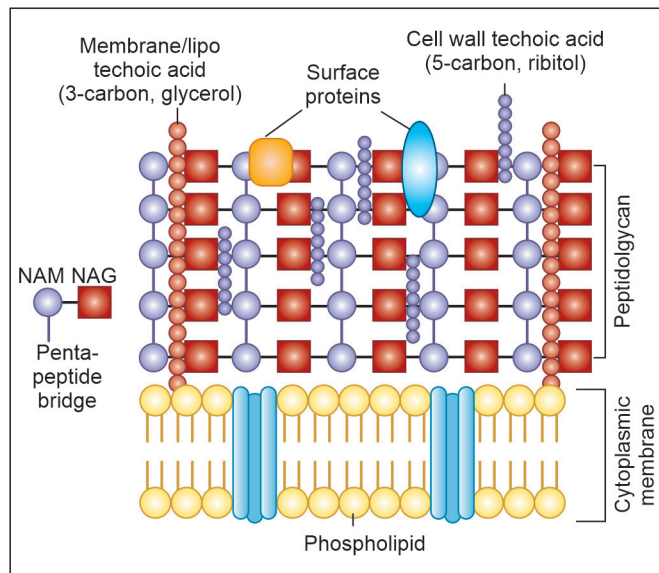


Fig. 6.6: Gram-positive cell wall

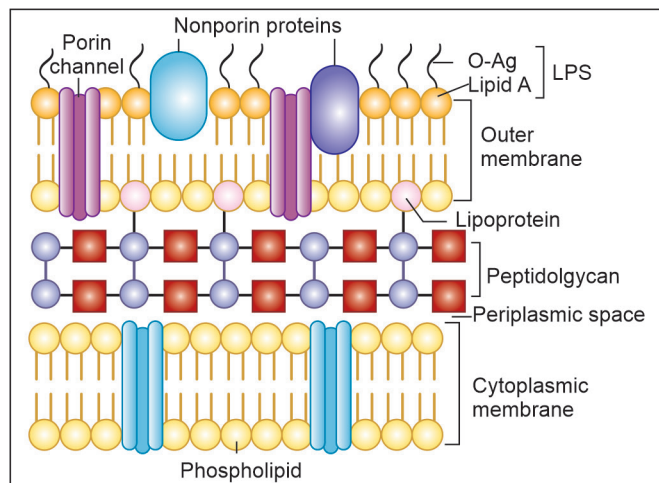


Fig. 6.7: Gram-negative cell wall

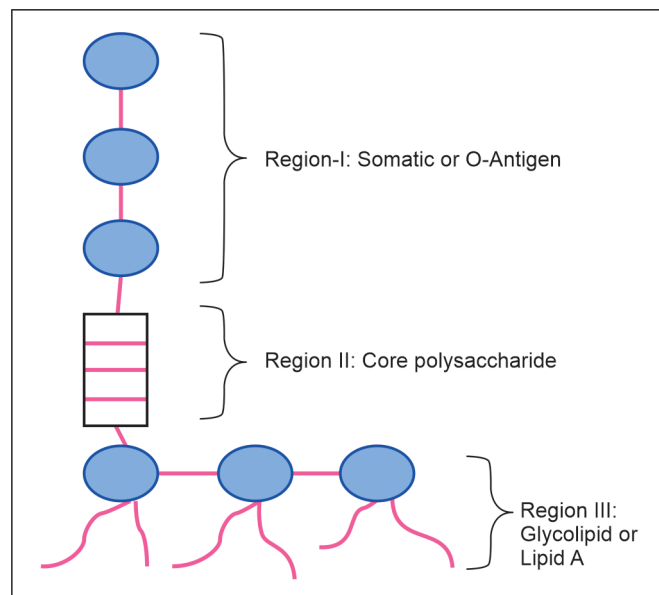


Fig. 6.8: LPS structure

- **Region-I:** It is the somatic/O-antigen. Formerly called Bovine-Ag. It is responsible for O-antigen specificity and typing.
- **Region-II:** It is the core polysaccharide.
- **Region-III:** It is the glycolipid or lipid A responsible for endotoxic activity like fever, shock, collapse, hemorrhage, necrosis, lethal effect, anticomplementary effect, B cell mitogenicity, antitumor activity, etc.

C. Cell wall of acid-fast bacteria: Acid fast bacteria (*Mycobacterium* spp., *Nocardia* spp., etc.) contain three layers from inner to outer are peptidoglycan, polysaccharide and fatty acids (glycolipid).

1. **Peptidoglycan:** It is similar to gram-negative bacterial cell wall and linked to polysaccharide layer by phosphodiester bond.
2. **Polysaccharide:** Layer called **arabinogalactan** and linked to mycolic acids.
3. **Fatty acid (glycolipid)**
 - It is responsible for acid fastness.
 - Glycolipid is the major part in cell wall which accounts 60% of the dry weight of cell wall.
 - It called mycolic acid in *Mycobacterium* and nocardic acid in *Nocardia* (unique mycolic/nocardic acid 6, 6' dimycolyltrehalose called cord factor).
 - Mycolic acid or cord factor in *Mycobacterium* acts as virulence factor and responsible for cell membrane cytotoxicity, inhibition of PMNs migration, granuloma formation and activation of complement pathway.
 - Peptidoglycan-polysaccharide-mycolic acid complex forms the skeleton in cell wall of mycobacteria, so acid fastness in mycobacteria is not the property of mycolic acid alone, but depends also on the integrity of cell wall.

Notes: Cell wall of *Corynebacterium*

Some workers noticed the presence of acid fast cell wall in *Corynebacterium* with three layers as mentioned above where the glycolipid called corynemycolenic acid.

Differences between gram-positive and gram-negative bacterial cell wall: Follow Table 6.1.

Demonstration of cell wall: Cell wall is demonstrated by following methods.

- **Plasmolysis:** When bacteria placed in hypertonic solution they lose the cytoplasm called plasmolysis

TABLE 6.1: Differences between gram positive and negative bacterial cell wall

Characteristics	Gram +Ve	Gram -Ve
Thickness	Thicker (80 nm)	Thin (2 nm)
Aminoacids (AA)	Few	Several
Aromatic and sulphur containing AA	–	+
Lipids	–, Or scanty	+
Techoic acid	+	–

but cell wall retains its size and shape such cell called bacterial ghost.

- Micro dissection.
- By mechanical rupture of cell wall.
- Differential staining.
- Reaction with antibody.
- Electron microscopy.

Inhibition of cell wall synthesis: Bacteria without cell wall are called cell wall deficient bacteria.

- **Causes:** Cell wall synthesis is inhibited naturally (like in *Mycoplasma* spp., cell wall is absent naturally) or by antibiotics like penicillin or by viruses like bacteriophage or by enzymes like lysozyme or spontaneously (sudden loss of cell wall).
- **Clinical significances:** Absence of cell wall created following issues in bacterial infections.
 - Difficulty in diagnosis: Cell wall deficient bacteria are difficult to cultivate and require agar contains solid medium having right osmotic strength.
 - Difficulty in treatment: Cell wall deficient form of bacteria is ineffective to antibiotics. Sometimes they appear after treatment with penicillin.
 - Persistence of infection: Cell wall deficient bacteria are not causing the disease but causing persistence of certain chronic infection such as pyelonephritis.
 - Recurrence: They cause recurrence of infection.
- **Types of cell wall deficient bacteria:** Four types of cell wall deficient bacteria as follows:

1. **Protoplast:** It is produced artificially by allowing lysozyme action on gram-positive bacteria in a hypertonic medium. It is spherical in shape with total loss of cell wall and cytoplasmic membrane holds the contents of cell.
2. **Spheroplast:** It is produced artificially by allowing lysozyme action on gram-negative bacteria in a hypertonic medium. It is spherical in shape with partial loss of cell wall and some cell wall structures are retained.
3. **L-forms:** L forms word derived from Lister institute, London. Kleinberger (Nobel Prize) while studying the culture of *Streptobacillus moniliformis* in the Lister institute, London, observed cell wall deficient, swollen, morphologically abnormal forms of bacteria and named them L-forms after the Lister institute in 1941. L forms have different types such as spontaneous (sudden development), induced (induced by some agents like penicillin), unstable (revert to original forms) and stable (irreversible). Examples of bacteria showing L-forms are *Staph. aureus*, *N. gonorrhoeae*, *M. tuberculosis* and *Streptobacillus moniliformis*.

4. **Mycoplasma:** Follow Ch. 74 for more details.

Cytoplasmic/Plasma Membrane

Properties: It presents inner to cell wall. It is elastic in nature and about 5–10 nm in size. It always presents in bacteria.

Functions

1. It is semipermeable layer acts as an osmotic barrier. It allows inflow and outflow of selective ions and molecules of the cell (called selective permeability) and prevents the others.
2. It participates in active transport of selective nutrients by enzyme permease.
3. It contains enzymes required for respiratory activity to generate energy (ATP) by electron transport and oxidative phosphorylation.
4. It participates in synthesis of cell wall components like peptidoglycan and OMP.
5. It participates in synthesis of bacterial toxins and other bacterial enzymes.
6. It provides energy for flagellar movement and chromosomal mobilization.

Biochemistry: It contains phospholipids (30–60%), protein (50–70%) and carbohydrates. Sterol (cholesterol or ergosterol) is absent in bacteria except in *Mycoplasma* spp., and *Ureaplasma urealyticum*.

Demonstration: Cytoplasmic membrane is demonstrated by Electron Microscopy (EM).

Mesosomes or chondroids (Fig. 6.4)

- **Definition:** Invaginations or infolding of the plasma membrane in to the cytoplasm called chondroids or mesosomes. They are more prominent in gram-positive bacteria.
- **Functions of mesosomes**
 1. They provide more surface area for anabolic and catabolic activities.
 2. They are the centers for respiratory enzymes and help in respiration.
 3. They act as analogs for mitochondria in cell.
 4. They take part in DNA replication and cell division or binary division.

Cytoplasm

Mixture of plasma membrane, cytoplasm, nucleus and nucleoplasm called **protoplasm**. Jelly like substance presents inner to plasma membrane but outer to nucleus called **cytoplasm**. Jelly like substance presents inner to nucleus called **nucleoplasm**. In eukaryotes nucleus is present but in prokaryotes like bacteria nucleus and nucleoplasm are absent, so we can say that cytoplasm acts as nucleoplasm which contains chromatin (chromosome, plasmid, episome and transposon). Plasma membrane is described above while cytoplasm is discussed below.

Properties of cytoplasm

- It is a colloidal system contains organic and inorganic solutes in a viscous watery solution.
- Organelles like endoplasmic reticulum, mitochondria, etc., are absent in bacteria.
- Cytoplasmic motility called **cytoplasmic (protoplasmic) streaming** is absent in bacteria unlike eukaryotic cells.

- Cytoplasm stains uniformly with basic dyes in young culture.

Intracytoplasmic inclusion/cytoplasmic matrix

1. **Ribosomes:** These are tiny granules scattered in cytoplasm. They are made up of two subunits larger (50s) and smaller (30s). They are sites for protein synthesis.
2. **Polar bodies:** They are situated at both poles of bacilli hence called **polar bodies**. They are also known as **metachromatic granules** because they had the property of changing the color of basic blue dyes used in light microscopy or **volutin granules** because they were 1st identified from *Spirillum volutans* by Meyer (1904) or **Babes-Ernst granules** because they are discovered and described by Romanian bacteriologist Babès Victor and German pathologist Paul Ernst, 1888. Biochemically they are composed of polymetaphosphate and strongly basophilic in nature. They act as energy and phosphate store house required in conditions with nutritional deficiency. They disappear when deficient nutrients are supplied. They are demonstrated by Albert stain, Loeffler's methylene blue (stain reddish violet), Neisser's stain and Ponder's stain. Bacteria contain polar bodies are *Corynebacterium diphtheriae* (causing diphtheria), *Corynebacterium xerosis*, *Mycobacterium leprae* (may be), *Bordetella pertussis*, *Gardnerella vaginalis*, and *Spirillum volutans*.
3. **Polysaccharide granules:** They stain by iodine. They act as storage products.
4. **Fat globules/lipid globules:** These are energy storage products. They contain lipid, polymetaphosphate (volutin granules) and crystalline protein. They present only in vegetative stage and make confusion with spores. Stains used to differentiate spores from fat globules are mentioned in **Table 6.2**.
5. **Chromosome:** It appears as oval or elongated body. Generally it is one per cell or two or more. It is demonstrated by acid hydrolysis or by EM. It contains single-circular ds-DNA. It controls the growth, multiplication and metabolism of cell. It also controls the hereditary transmission of genetic information.
6. **Plasmid:** Extrachromosomal intracytoplasmic DNA containing material called plasmid. It serves for toxin

TABLE 6.2: Stains to differentiate spores and fat globules

Staining method	Reagents	Spores	Fat globules
Ashby's method	Malachite green	Green spores and red bacilli	Unstained
Burdon's method	Sudan black B	Unstained	Blue-black
Holbrook and Anderson method	Malachite green + Sudan black B	Green spores and red bacilli	Blue-black
Modified acid fast	0.25% H ₂ SO ₄	Red spores and colorless bacilli	Red

- production, drug resistance and other virulence purposes.
- Episomes:** It is a type of plasmid which is linked with chromosome.
 - Jumping gene or transposon:** Plasmid jumps in-between chromosomal and extrachromosomal part called jumping gene or transposon.

Capsule and Slime Layer

Definitions

- Capsule:** Many bacteria secrete the viscid materials around them that organize in a sharply defined structure called capsule (**Fig. 6.9**).
- Microcapsule:** Capsule is too thin to be seen under microscope called microcapsule.
- Slime layer:** Many bacteria secrete the viscid materials around them that remains loose, unorganized as ill-defined structure called slime layer.

Capsular antigen: It abbreviated as K from Kapsel. (German language for capsule).

Examples of microorganisms containing capsule and slime layer

A. Capsule containing organisms

- Capsulated fungus:** *Cryptococcus neoformans*.
- Capsulated bacteria**
 - Staph. aureus* (in some strains).
 - Streptococcus* species: These are α -Hemolytic (Viridans group) streptococci such as *Strept. mutans* (no Lancefield grouping) and *Strept. pneumoniae*, β -Hemolytic streptococci such as *Strept. pyogenes* (Lancefield Group A), *Strept. agalactiae* (Lancefield Group B) and *Strept. equisimilis* (Lancefield Group C) and *Enterococcus* spp (Lancefield Group D).
 - Anthrax bacillus*.
 - Clostridium welchii*.
 - Neisseria meningitidis*.
 - E. coli*: In some strain.
 - Klebsiella pneumoniae*, *K. granulomatis* and other species.
 - Enterobacter* spp.
 - Yersinia enterocolitica*: Capsule occurs *in vivo* but not *in vitro*/culture.
 - Aeromonas hydrophila*.
 - Vibrio cholerae* O139.
 - Haemophilus influenzae* and other species.

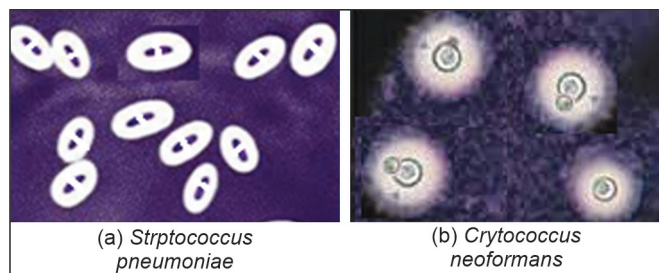


Fig. 6.9: Capsule under Indian ink

- Pasteurella multocida*.
- Bordetella pertussis* (no role in virulence).
- Bacteroides fragilis*.

B. Slime layer containing bacteria

- Staph. albus* (*Staph. epidermidis*).
- Leuconostoc*.
- Pseudomonas aeruginosa*.
- Yersinia pestis*.
- Rickettsia* spp.

C. Both capsule and slime layer containing bacteria

- Strept. salivarius*.

Biochemistry

- Polysaccharide:** It is polysaccharide (glucan, dextran, levans) in nature in almost all bacteria.
- Polypeptide:** In *Anthrax bacillus* capsule is polypeptide (d-glutamic acid) in nature.
- Protein:** In *Yersinia pestis* it is protein in nature.
- Hyaluronic acid:** In some strains of *Strept. pyogenes* (Lancefield Group A) and some strains of *Strept. equisimilis* (Lancefield Group C) capsule is hyaluronic acid in nature.
- Glycocalyx:** Slime layer is made of glycocalyx which is the mixture of sugars (oligosaccharides) and proteins. It presents in *Staph. albus* (*Staph. epidermidis*), *P. aeruginosa*, etc.

Functions of capsule

- Inhibition of phagocytosis:** It enhances bacterial virulence by inhibiting phagocytosis; however capsule of *Bordetella pertussis* does not contribute in virulence.
- Protection:** It acts as protecting covering and protects bacteria against lysozyme, bacteriophages, colicins, antibodies, antibiotics, heavy metals, free radicals, complement action and also against desiccation.
- Breakdown of dietary fibers:** Refer to **Flowchart 50.2**.
- Abscess formation:** Capsule of certain bacteria like *Bacteroides fragilis* is toxic in nature and responsible for abscess formation.
- Adhesion:** It is the function of slime layer. Slime layer made up of glycocalyx has ability to binds bacteria with damaged tissues. It also helps bacteria to bind with plastic material of medical devices like catheters, suture materials, pacemakers, implants, Ryle's tube, etc., and forms the growth on such devices called **biofilm**. Uses of such devices cause diseases production. Biofilm is formed by few bacteria like *Staph. albus*, *P. aeruginosa*, etc.
- Contribution in energy and nutrition:** It attracts the nutritional materials at cell surface because of polyanionic nature.
- Desiccation (drying) prevention:** Capsule contains water which prevents the bacterial desiccation.
- Vaccine preparation:** Capsule is antigenic in nature and produces protective antibodies. This principle

helps to prepare the vaccine for pneumococci, meningococci and *H. influenzae* serotype-b.

9. **Diagnosis:** It is antigenic in nature and helps in identification and typing of bacteria.

Demonstration: Following methods are used to demonstrate the capsule.

1. Microscopy

- **Negative stains:** Like Indian ink (Fig. 6.9), nigrosin and Manveal stain.
- **Gram's stain:** Slime layer (even capsule also) has little affinity for basic dyes, so not visible under Gram's stain. Capsule is sometimes demonstrated under Gram's stain.
- **Special stains:** Following are the special stains used for demonstration of capsule especially for capsule of *Anthrax bacillus*.
 - Giemsa stain: Capsule appears in red color.
 - Indian ink: Capsule appears as clear halo around the bacilli.
 - Methylene blue: Capsule appears as purplish materials around the bacilli called M'Fadyean's reaction.

2. **Culture:** Mucoid colonies produced by capsulated bacteria.

3. **Serological test:** Test known as Quellung reaction or Capsular swelling, developed by Neufeld in 1902. Mix capsulated strain with specific anticapsular serum on slide and examine under microscope. Capsule becomes prominent and gets swell due to increase in refractivity.

Enhancement of capsule production

- In *A. bacillus* capsule production is increased in presence of 10–25% CO₂ when medium is enriched with biocarbonate and in absence of CO₂ when medium is enriched with serum, albumin, charcoal or starch.
- In *C. neoformans* capsule production is increased by growing the fungus on chocolate agar at 37°C in a CO₂ incubator.

Inhibition of capsule synthesis (loss of capsule)

- **Methods:** Capsule synthesis is inhibited by mutation or by repeated subculture.
- **Clinical significance:** Capsule has virulent property and loss of capsule changes virulent strain to avirulent strain.

Flagella

Definition: One or more, long, unbranched, wavy, filamentous structure for movement of organism called flagella (singular → flagellum).

Flagellar antigen: It is abbreviated as H-Ag from Hauch (German) means film of breath, as growth of motile bacteria looks like film of breath on glass surface.

Properties: It is 3–20 µm long and uniform in diameter (0.1–0.013 µm). It terminates in square tip.

Types of bacteria according to location of flagella: Follow Flowchart 6.1 and Fig. 6.10.

Parts: Flagella have three parts (Fig. 6.11).

1. **Basal body:** It is a circular structure embedded in the cell envelope consist a central rod with following four rings:
 - i. M-ring: Embedded in the cell membrane.
 - ii. S-ring: Embedded in the periplasmic space.
 - iii. P-ring: Embedded in the peptidoglycan.
 - iv. L-ring: Embedded in the lipopolysaccharide.
2. **Hook:** It is short curved structure connecting the filament and basal body. It is broader than the filament, protein in nature (different from flagellin) and embedded in cell envelop.
3. **Filament:** It is external to the cell and made up of flagellin.

Biochemistry: Chemically flagella are composed of protein called flagellin, alike keratin and myosin.

Functions of flagella

1. **Organ of locomotion:** Flagella help in different type's movement of bacteria such as movement towards nutrients called chemotaxis, movement toward air called aerotaxis and movement toward light called phototaxis.
2. **Constitute the flagellar (H) antigen:** H antigen produces the antibody which is useful for diagnosis and typing of bacteria.

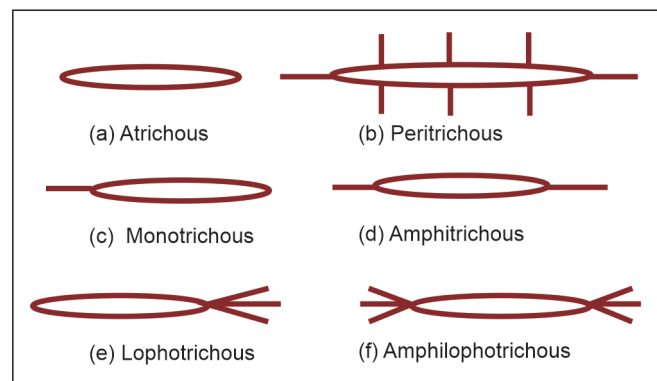


Fig. 6.10: Types of bacteria according to location of flagella

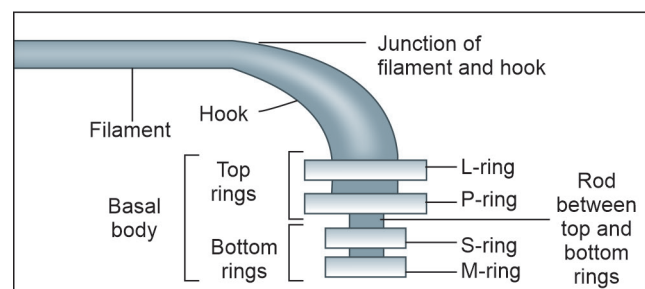
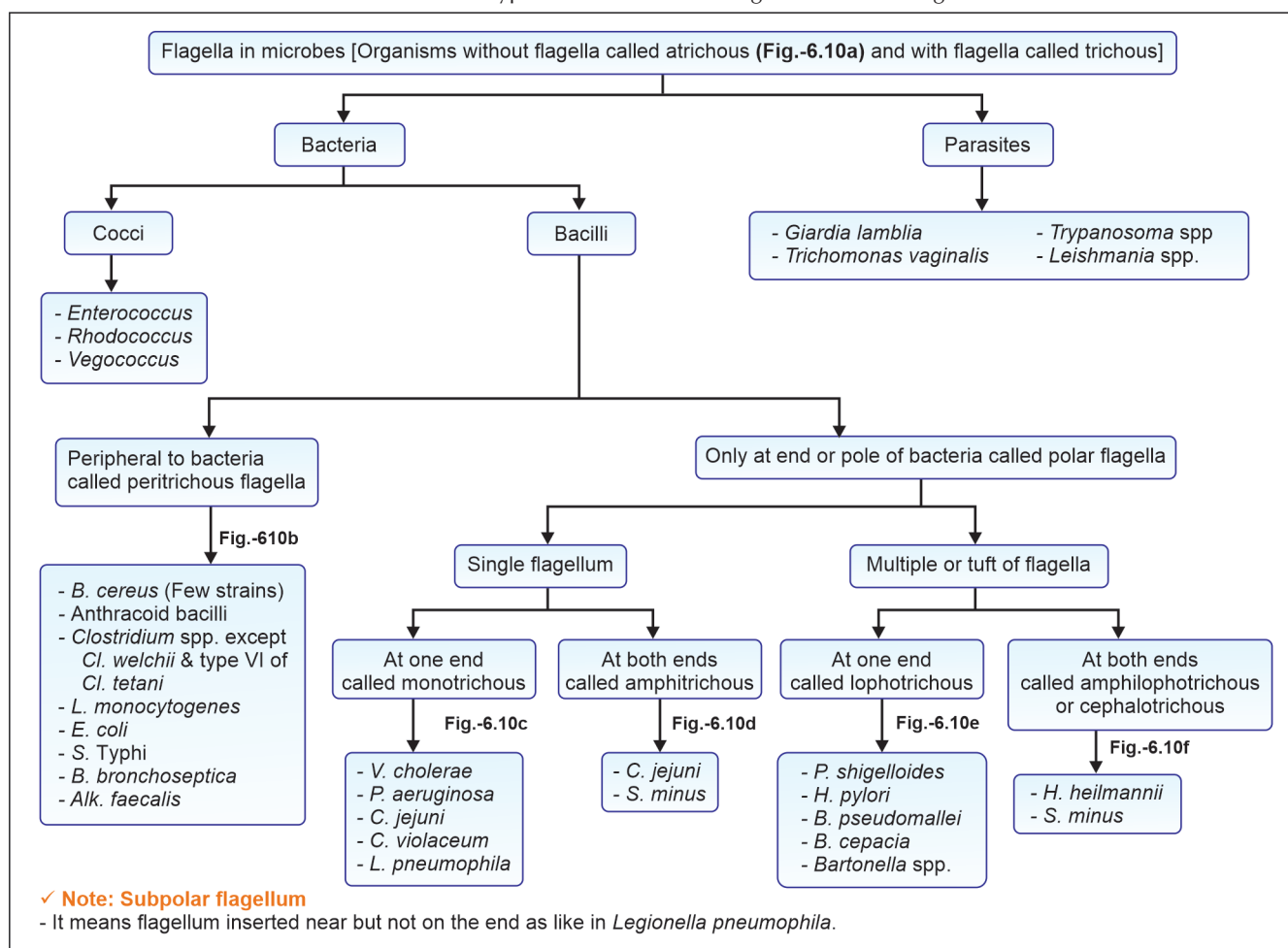


Fig. 6.11: Parts of flagella

Flowchart 6.1: Types of bacteria according to location of flagella



Demonstration of flagella

1. Microscopy and staining methods

• Light microscopy

- Wet mount: It includes hanging drop preparation and Ryu's stain (crystal violet and tannic acid as mordant).
- Impregnation technique: Thickness of the flagella is increased by coating them with mordants like tannic acid and potassium alum and staining them with basic fuchsin (called Gray method) or pararosaniline (called Leifson method) or silver nitrate (called West method) or crystal violet (called Difco's method). Although flagellar staining procedures are difficult to carry out, they often provide information about the presence and location of flagella which have great value in bacterial identification.

• Other microscopical methods: DGIM and EM.

2. Culture methods

- **By using semisolid media:** Media contain 0.2–0.5% agar. Different methods include semisolid stab agar method (Ch. 112), Craigie's tube technique (Ch. 115) and U-tube technique (Ch. 115).
- **By using solid media:** Media contain 1–2% agar and motile bacteria produce spreading/swarming growth on solid agar.

- **Serological test:** These are done by using specific antiserum.

Types of motility

1. **Stately motility:** It occurs in *Clostridium* spp., except *Cl. welchii* and *Cl. tetani* type VI.
2. **Active motility:** It occurs in *E. coli*, *Proteus* spp., *S. Typhi* and *L. interrogans*.
3. **Tumbling motility:** It occurs in *L. monocytogenes* at 25°C but not at 37°C (called differential motility due to temperature dependent flagellar expression) and *C. jejuni*.
4. **Darting motility:** It occurs in *V. cholerae*, *C. jejuni* and *S. minus*.
5. **Oscillatory motility:** It occurs in *Cl. difficile*.
6. **Twitching motility:** It occurs in *Bartonella* spp.
7. **Swarming growth or swarming motility:** It occurs in *Proteus vulgaris*, *Proteus mirabilis* and *Cl. tetani*.

Phase variation

- **Definition:** Flagellar antigen (H-Ag) undergoes frequent antigenic variation called phase variation. It was 1st observed in *Salmonella* and later in other GNB.
- **Clinical significance:** It helps in bacterial typing.

Notes: Endo-flagella and bacteria showing motility without flagella

Endo-flagella: They are present in spirochetes like *T. pallidum*, *Borrelia* spp., *Leptospira* spp., etc., but not useful for motility because not protruding outside and remain in periplasmic space between peptidoglycan and plasma membrane. These bacteria show following types motility.

1. *T. pallidum*: Three types of motility like flexion—extension, translatory and cork screw.
2. *Borrelia* spp.: Lashing motility.
3. *Leptospira* spp.: Active motility with spinning or translation type of motility.

Bacteria showing motility without flagella

1. Brownian motility / movement: It is the passive movement of bacteria not due to flagella but due to air current or by fluid movement.
2. Sluggish motility: *Moraxella lacunata* which is nonflagellated but it is sluggishly motile.
3. Jerking or twitching motility: It occurs in *Eikenella corrodens*, not due to flagella but by contractile-fimbriae like filamentous appendages.
4. Gliding motility: *Capnocytophaga canimorsus* and other species of genus are lacking flagella but show gliding motility, which is very difficult to observe. *Mycoplasma pneumoniae* is without flagella but showing gliding motility.

Fimbriae

Definition: Very fine hair like surface appendages in gram-negative bacteria called fimbriae (singular → fimbria).

Synonym: Also called pili (singular → pilus) or fibrillae. The word sex pili are used only for fimbriae concerned with conjugation.

Fimbrial antigen: It is abbreviated as FiAg.

Properties: Fimbriae are shorter (0.5 µm long), thinner (0.001 µm or 10 nm) and more in numbers than flagella as shown in Fig. 6.4. They are unrelated to motility and are found on motile and nonmotile bacteria. They are originated from cell membrane. They are best developed in fresh culture and also in liquid media. Bacteria lose the fimbriae following subculture in solid culture.

Classification

1. **According to bacteriophage sensitivity:** Type F, type I, etc.
2. **According to their functions:** Fimbriae are named on basis of their function.
 - **Sex pili:** They found in male bacterium and help to attach with female bacterium by forming a tube like structure called conjugation tube. Transfer of genetic information from one cell to other via conjugation tube called conjugation. Fimbriae help to make conjugation tube called sex pili and protein called pilin.
 - **Fimbrial adhesin or pilus adhesin:** It is one type of adhesin which helps the bacterium to attach with host cells and helps in virulence. Following are different subtypes with special name.

- **Colonization Factor Antigen:** It presents in ETEC and helps to adhere with intestinal epithelial cells.
- **Mannose resistant fimbriae:** It presents in EHEC, especially in nephritogenic strain and helps in attachment with uroepithelial cells.
- **Toxin Co-regulated Pilus (TCP):** It presents in *Vibrio cholerae* and constantly binds the *Vibrio* with intestinal epithelial cells.
- **Surface agglutinin:** It favors the adhesion of *Bordetella pertussis* with respiratory epithelium.
- **Hemagglutinin adhesin (160 KDa):** It favors the adhesion of *Staph. saprophyticus* with uroepithelial cells.
- **Contractile fimbriae:** These are filamentous appendages found in *Eikenella corrodens*. They are useful for unusual jerking or twitching motility which helps bacilli to spread and to produce corrosive effect (corroding appearance) over blood agar.
- **Hemagglutinating pili (HA-pili):** They participate in hemagglutination reaction. They serve as organ of adhesion as mentioned above. Hemagglutination is affected by mannose, and it identified two subtypes.
 - Type-I called mannose sensitive: Inhibition of hemagglutination by bacteria after incubation with mannose.
 - Type-II called mannose resistant: No Inhibition of hemagglutination by bacteria after incubation with mannose, e.g., gonococci.

Differences between flagella and fimbriae: Follow Table 6.3.

Biochemistry: Fimbriae are made up by a protein called fimbrilin (*Neisseria gonorrhoeae*) or by teichoic acid (*Staphylococcus*) or by lipoteichoic acid (*Enterococcus*) or by lipoteichoic acid mixed with M. protein (*Streptococcus*).

Functions: Follow classification.

Demonstration of fimbriae: Fimbriae are demonstrated by EM, liquid culture showing pellicle formation and

TABLE 6.3: Differences between flagella and fimbriae

Features	Flagella	Fimbriae
Length	Long (3–20 µm)	Short (0.5 µm)
Thickness	Thick (0.1–0.013 µm)	Thin (0.001 µm or 10 nm)
Made up by	Protein-flagellin	Protein-fimbrilin
Numbers	Less	More
Organ of adhesion	No	Yes
Organ of conjugation	No	Yes
Organ of locomotion	Yes	No

34 hemagglutination test which is used for demonstration of pili in *E. coli*, *K. pneumoniae*, etc.

Clinical significances

- Immune interference: Fimbriae are antigenic in nature and cross react with many related bacteria.
- Diagnostic: They help to form pellicle in liquid media by adhering the bacteria on surface. Pellicle is diagnostically useful.
- Vaccine preparation: *E. coli* diarrhea in calves and piglets and gonorrhea in humans are prevented by using vaccine prepared from fimbrial antigens.

Spores

Definition: Highly resistance, resting and metabolically inactive stage of bacterium in unfavorable conditions called spore.

Properties: Spores are resistant to heat, chemicals, cold, desiccation and other abnormal environmental conditions. Spores are formed inside the bacterial cell called endospores. Each bacterium forms one spore which on germination forms a single vegetative cell; hence it is not a method of reproduction.

Classification: Following are different spore bearing or sporing bacteria.

1. According to O₂ requirement of sporing bacteria

- Aerobic spore bearer: *Sporosarcina* spp., (GPC), *Bacillus* spp., (GPB) and *Micromonosporum* spp., (GPB).
- Anaerobic spore bearer: *Clostridium* spp., (GPB) and *Sporolactobacillus* spp., (GPB).

2. According to shape and location of spore (Fig. 6.12)

- **Central/equatorial spore:** It gives spindle shape appearance to bacilli like *Cl. bifermentans*, *Cl. sordellii* and *Bacillus* spp. Following are two different forms of central spore.
 - Spherical (non-bulging) spore: It is same in size of bacillary body like in *Bacillus* spp.
 - Oval (bulging) spore: It is larger than the size of bacillary body.
- **Subterminal spore:** It gives club shape appearance to bacilli like *Cl. perfringens* (*Cl. welchii*), *Cl. novyi*, *Cl. septicum*, *Cl. sordellii*, *Cl. bifermentans*, *Cl. botulinum*, *Cl. sporogenes*, *Cl. histolyticum* and *Bacillus* spp. Following are two different forms of subterminal spore.

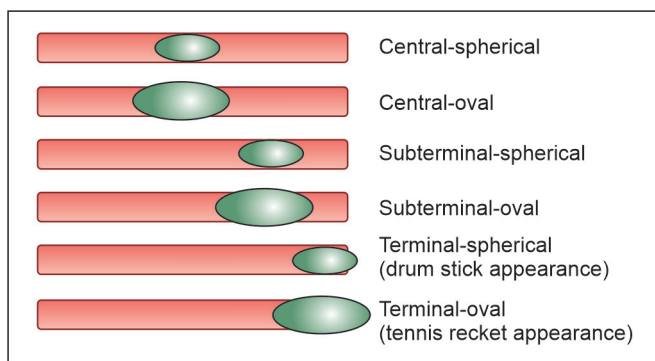


Fig. 6.12: Shape and location of spores

- Spherical (non-bulging) spore: It is same in size of bacillary body like in *Bacillus* spp.
- Oval (bulging) spore: It is larger than the size of bacillary body.
- **Terminal spore:** Two subtypes:
 - Spherical (non-bulging) spore: It gives drum stick appearance to bacilli like *Cl. sphenoides*, *Cl. tetani* and *Cl. tetanomorphum*.
 - Oval (bulging) spore: It gives tennis racket appearance to bacilli like *Cl. difficile*, *Cl. tertium* and *Cl. cochlearum*.

Notes: Mixed spores

- *Cl. bifermentans* and *Cl. sordellii* produce both central and subterminal spores.
- *Bacillus* spp., produce both central or subterminal, oval or elliptical spores and same in size of bacillary body (non-bulging).

Function: Spores protect bacteria in unfavorable condition.

Sporicidal methods/agents: Methods/agents which kill the spores are called sporicidal methods/agents like

1. Physical methods (sterilization)

- Autoclave (121°C at 15 lbs for 15 min.)
- Hydroclaving
- Hot air oven

2. Chemical methods (disinfection)

- Formaldehyde
- Glutaraldehyde (2% → cidex): Slow action in 3–10 hours
- Iodine (1%): At higher concentration
- Chlorine tablets and sodium hypochlorite (at higher concentration)
- Ethylene oxide (EO)
- H₂O₂
- KMnO₄ (4%)
- O-phthalic acid
- Paracetic acid
- Beta propiolactone
- Ozone

3. Physical + chemical methods (Chemosterilization): Plasma sterilization.

Sporulation: Conversion of vegetative bacterium in to spore in unfavorable condition called sporulation. It begins with appearance of clear area in the protoplasm of the cell that gradually opaque with condensation of nuclear material called forespore or core. The cell membrane grows to form spore membrane or spore wall. The cell wall grow around spore wall to form cortex and multilayered tough spore coat. Some spores have extra outer covering called exosporium. Spore structure is shown in Fig. 6.13.

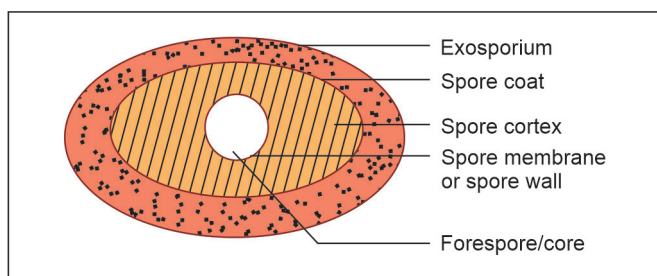


Fig. 6.13: Spore structure

Germination: It occurs in favorable conditions. Three stages are involved:

1. **Activation:** It occurs in nutritionally rich medium. Damage to spore coat is produced by heat, abrasion, acidity, etc.
2. **Initiation:** During this stage a cortex peptidoglycan and a variety of other components are degraded, water is taking up and calcium dipicolonic acid is released.
3. **Outgrowth:** A new vegetative cell with spore protoplast emerges out a period of active synthesis that terminates in cell division.

Uses of spores

1. **Biological indicator:** Spores of certain species of bacteria are used as biological indicator for different methods of sterilization as mentioned below.
 - *Bacillus (Geobacillus) stearothermophilus*: For autoclave.
 - *Cl. tetani* (non-toxigenic strain): For hot air oven.
 - *Bacillus subtilis* subsp. *niger*: For hot air oven.
2. **Bi terrorism:** Endospores of *Bacillus anthracis* were used in 2001 in anthrax bioterrorism attack on USA.

Demonstration of spores

1. Staining and microscopy

- Light microscopy: By using Gram's stain (colored bacillary body with unstained spores) and modified ZN stain (pink colored spore).
- Phase-contrast microscopy: By using wet mount
- Other staining and differential methods of spores with fat globules: Follow **Table 6.2**.

2. Culture

- Culture methods for spores of *A. bacillus*: In *A. bacillus* spore production occurs naturally only in soil but never in body. Spore production is promoted in oxalated agar by adding distilled water and 2% NaCl followed by incubation at 25–30°C in presence of O₂. Spore production is inhibited in presence of calcium chloride.
- Culture methods for spores of *Cl. welchii*: Spores of *Cl. welchii* may develop in animal body. Media used for spore production are Phillips medium, Ellner's medium, Duncan and Strong's medium and alkaline egg medium.

Notes: Fungal spores

Spores are also present in fungi called fungal spores. They are exogenous called conidia/conidiospores or endogenous called endospores/sporangiospores.

Pleomorphism and Involution Forms

Definitions

- **Pleomorphism:** Certain bacteria show variation in size and shape called pleomorphism, e.g., *Cl. welchii*, *V. cholerae*, *Y. pestis*, *H. influenzae*, *B. pertussis*, *Mycoplasma* spp., etc.
- **Involution forms:** When variation in size and shape is present in high salt concentration medium called involution forms, e.g., *N. gonorrhoeae*, *Y. pestis*, *Cl. welchii*, etc.

Causes: Involution forms occur in aging culture especially due to high salt concentration. However, pleomorphism and involution forms also occur due to defective cell wall synthesis or by actions of autolytic enzymes.

ACCESS YOURSELF

Essay/Full Question

1. Morphology of bacteria.

Short Notes

1. Arrangement of bacteria
2. Bacterial cell wall/Cell wall appendages
3. Polar bodies
4. L-forms
5. Capsule/bacterial capsule (Answer of capsule contains bacterial portion and fungal portion)
6. Flagella/bacterial flagella (Answer of flagella contains bacterial portion and parasitic portion)
7. Spores/bacterial spores/fungal spores (Answer of spores contains bacterial portion and fungal portion)
8. Fimbriae

Short Questions for Theory/Viva Questions

1. Write the four functions of bacterial cell wall.
2. Write the four differences between gram-positive and gram-negative bacterial cell wall.
3. What is Bovine antigen?
4. What are protoplast and spheroplast?
5. Name the four bacteria showing L-forms.
6. What is selective permeability of plasma membrane?
7. Write the four functions of cytoplasmic membrane.
8. What are mesosomes or chondrioides?
9. Define: Protoplasm and cytoplasm.
10. What are volutin granules?
11. Name the four stains for volutin granules.
12. Name the four bacteria contains volutin granules.
13. Write four names of cytoplasmic inclusions.
14. What are fat globules? Name the methods used to differentiate it from bacterial spores.
15. Write the four functions of capsule.
16. Write the four examples of capsulated bacteria.
17. What is Quellung reaction or capsular swelling?

18. Name the methods to increase and to decrease the capsule production in bacteria.
19. Write the four examples of bacteria with peritrichous flagella.
20. What is subpolar flagellum? Write one example.
21. Write the four examples of flagellar parasites.
22. What are endoflagella?
23. Define flagella and bacterial spores.
24. Write the four examples of sporing bacteria.
25. Write the four examples of sporocidal methods.
26. What are pleomorphism and involution forms?

Comments on

1. Endoflagella are not useful in motility.

MCQs for Chapter Review

Cell Wall

1. Peptidoglycans are present in:
 - a. Gram-positive bacteria
 - b. Gram-negative bacteria
 - c. Fungi
 - d. Protozoa
2. Teichoic acids:
 - a. Found in gram-positive bacteria
 - b. Make the outer wall of bacteria
 - c. Provide receptor for phage
 - d. Influence the permeability of phage
3. Bacterial cell wall is composed of all:
 - a. Muramic acid
 - b. Teichoic acid
 - c. Glucosamine
 - d. Mucopolysaccharide
4. The difference between gram-positive and gram-negative organisms is that gram-positive organism contains:
 - a. Teichoic acid
 - b. Muramic acid
 - c. N-acetyl neuramic acid
 - d. Aromatic amino acids
5. Acid fastness of tubercle bacilli is attributed to:
 - a. Presence of mycolic acid
 - b. Integrity of cell wall
 - c. Both of the above
 - d. None of the above
6. Cell wall deficient organisms are:
 - a. Chlamydia
 - b. Mycoplasma
 - c. Streptococcus
 - d. Anaerobes
7. Cell wall structure found in all except:
 - a. *Staph. aureus*
 - b. *Pseudomonas aeruginosa*
 - c. *Mycoplasma pneumoniae*
 - d. *Corynebacterium diphtheriae*

Cytoplasmic/Plasma Membrane

8. The cytoplasmic membrane of bacteria is responsible for:
 - a. Selective permeability
 - b. Motility
 - c. Cell division
 - d. Conjugation
9. Which is always present in bacteria?
 - a. Cell wall
 - b. Cytoplasmic membrane
 - c. Mitochondria
 - d. Nucleoli

Protoplasm

10. Metachromatic granules are found in:
 - a. Diphtheria
 - b. *Mycoplasma*
 - c. *Gardnerella vaginalis*
 - d. *Staphylococcus*

11. Metachromatic granules are stained by:
 - a. Ponder's stain
 - b. Negative stain
 - c. Gram's stain
 - d. Leishman's stain

Capsule and Slime Layer

12. All are capsulated bacteria except:
 - a. *Neisseria*
 - b. *Corynebacterium*
 - c. *Haemophilus*
 - d. *Streptococcus salivarius*
13. In which of the following organism capsule does not act as a virulence factor?
 - a. *H. influenzae*
 - b. *Strept. pneumoniae*
 - c. *N. meningitidis*
 - d. *Bordetella pertussis*
14. Capsulated organism:
 - a. *Candida*
 - b. *Klebsiella*
 - c. *Proteus*
 - d. *Cryptococcus*
 - e. *Histoplasma*
15. True about bacterial capsule is following except:
 - a. Prevents phagocytosis
 - b. Stained by Gram's stain
 - c. Protects bacteria from lytic enzymes
 - d. Lost by repeated subculture
16. Bacterial capsule is made up of:
 - a. Monosaccharide
 - b. Polysaccharide
 - c. Long-chain fatty acid
 - d. Small-chain fatty acid
17. Which of these bacteria has a capsule with polypeptide structure?
 - a. *Bacillus anthracis*
 - b. *Streptococcus pneumoniae*
 - c. *Neisseria gonorrhoeae*
 - d. *Neisseria meningitidis*
18. Quellung reaction is useful to detect:
 - a. Capsule
 - b. Flagella
 - c. Spores
 - d. Fimbriae

Flagella

19. Bacteria with tuft of flagella at one end called:
 - a. Monotrichate
 - b. Peritrichate
 - c. Bipolar
 - d. Lophotrichate
20. Darting motility is shown by:
 - a. *L. monocytogenes*
 - b. *Proteus vulgaris*
 - c. *Borrelia*
 - d. *V. cholerae*
21. Darting motility occurs in *V. cholerae* also found in:
 - a. *Shigella*
 - b. *Campylobacter jejuni*
 - c. *Pneumococcus*
 - d. *Bacillus anthracis*
 - e. *Aeromonas*
22. Following are motile organism except:
 - a. *Proteus*
 - b. *C. diphtheriae*
 - c. *T. pallidum*
 - d. *Clostridium welchii*
23. Tumbling motility is shown by:
 - a. *L. monocytogenes*
 - b. *Proteus vulgaris*
 - c. *Borrelia*
 - d. *Clostridia*
24. Bacteria are motile due to:
 - a. Flagella
 - b. Fimbriae
 - c. Both
 - d. None
25. Flagella not true:
 - a. Locomotion
 - b. Attachment
 - c. Protein in nature
 - d. Antigenic
26. Bacterium with endoflagella is:
 - a. *S. Typhi*
 - b. *E. coli*
 - c. *T. pallidum*
 - d. *S. minus*
27. Gliding motility is seen in:
 - a. *Clostridium*
 - b. *Listeria*
 - c. *Mycoplasma*
 - d. *Campylobacter*

Bacterial Spores

28. Example of bacterium with central spore is:

- a. *Cl. tetani* b. *Cl. bifermentans*
- c. *Cl. welchii* d. *Cl. tertium*

29. Subterminal spores are found in:

- a. *Clostridium sordelii* b. *Clostridium sporogenes*
- c. *Clostridium difficile* d. *Clostridium tertium*
- e. *Clostridium botulinum*

30. Drum-stick appearance is seen in:

- a. *Cl. sphenoides* b. *Cl. tetani*
- b. *Cl. tetanomorphum* d. *Clostridium tertium*

31. Spores are formed by all except:

- a. *E. coli* b. *B. anthrax*
- c. *B. cereus* d. None

32. Bacterial spores are best destroyed by:

- a. UV rays b. Autoclaving at 121°C for 20 min.
- c. Hot air oven d. Infrared rays

33. Sporocidal agents are:

- a. Glutaraldehyde b. Ethylene oxide
- c. Formaldehyde d. Bezalkonium chloride
- e. Chlorine

Answers and Explanation of MCQs

1. a and b

- Peptidoglycan is present in gram-positive (thick) and gram-negative bacteria (thin), but not in fungi and protozoa.

2. a

3. a, c, d

- Follow section, **bacterial cell wall (cell wall of gram-positive bacteria and Table 6.1)** for explanation of answers of MCQs 2–3.

4. a

- Follow **Table 6.1** for explanation.

5. c

- Peptidoglycan-polysaccharide-mycolic acids complex forms the skeleton in cell wall of mycobacteria, so acid fastness in mycobacteria is not the property of mycolic acid alone, but depends also on the integrity of cell wall.
- For more explanation follow the section bacterial cell wall (cell wall of acid-fast bacteria).

6. b

7. c

- Follow section, **bacterial cell wall (inhibition of cell wall synthesis)** for explanation of answers of MCQs 6–7.

8. a

- Motility is a function of flagella, cell division is a function of reproductive system mostly by binary fission and conjugation is due to sex pili.

9. b

- Follow section, **cytoplasmic/plasma membrane (properties)** for explanation.

10. a, c

11. a

- Follow section, **cytoplasm (polar bodies)** for explanation of answers of MCQs 10–11.

12. b

13. d

14. b, d

15. b

16. b

17. a

18. a

- Follow section, **capsule and slime layer** for explanation of answers of MCQs 12–18.

19. d

- Follow **Flowchart 6.1** for explanation.

20. d

- *Vibrio cholerae* is actively motile with single sheathed polar flagellum called darting motility.

21. b

- *Shigella*, pneumococcus and *Bacillus anthracis* are nonmotile while *Aeromonas* is motile in nature but showing motility other than darting.

22. b and d

- Follow section, **flagella (Flowchart 6.1) and notes** for explanation.

23. a

24. a

25. b

- Flagella is an organ of locomotion, it is made up by protein called flagellin and antigenic in nature. Fimbria is the organs of attachment.

26. c

27. c

- Follow section, **flagella (notes)** for explanation of answers of MCQs 26–27.

28. b

29. a, b, e

30. a, b, c

31. a

- Follow section, **bacterial spores (shape and location of spores)** for explanation of answers of MCQs 28–31.

32. b

33. a, b, c, e

- Follow section, **bacterial spores (sporicidal agents)** for explanation of answers of MCQs 32–33.

Physiology of Bacteria

Chapter Outline

- Bacterial Nutrition and Growth Factors
- Environmental Factors

- Reproduction (Multiplication)
- Metabolism

BACTERIAL NUTRITION AND GROWTH FACTORS

Definition

Nutrition is the process by which the organic and inorganic substances are obtained from surrounding environment and used for growth, metabolism and multiplication.

Nutrients or Growth Factors

Definition: Organic and inorganic substances used for nutrition are called nutrients or growth factors.

Types: Two types of nutrients:

- **Macronutrients:** These are required in relatively large quantities and play important role in cell structure and metabolism.
- **Micronutrients:** These are required in small quantities for functioning of certain enzyme systems.

Examples of nutrients required for bacteria

- **Water:** It holds 80% weight of total bacterial weight. Remaining is dry weight. It acts as vehicle to carry the nutrients to and fro from bacterial cell.
- **Carbon, nitrogen and energy:** Bacteria classified into two groups:
 1. **Lithotrophs (autotrophs):** They are able to utilize atmospheric CO_2 and N_2 for growth. They are medically less important because they survive independently in soil and water (called saprophytes). They are concerned with agriculture field and with soil fertility such as nitrogen fixing bacteria in soil. They require water, CO_2 and N_2 for growth. They have two subgroups based on energy requirement:
 - Photolithotrophs: They got energy from light.
 - Chemolithotrophs: They got energy from oxidation and reduction of chemicals like

inorganic substances (Anions → phosphate, sulfate, Cations → sodium, potassium, magnesium, iron, manganese, calcium).

2. **Organotrophs (heterotrophs):** They are not able to utilize atmospheric CO_2 and N_2 for growth. These are bacteria which are unable to synthesize their own metabolites and depend on preformed organic compounds such as carbohydrates, amino acids, nucleotides, lipids, vitamins (B_1 , B_2 , B_4 , B_6 , folic acid, B_{12}) and coenzymes for growth. Organic compound may be essential (no growth in their absence) or accessory (when they enhance growth). They are medically important to cause diseases. They have two further groups based on energy requirement:
 - Photo-organotrophs: They got energy from light.
 - Chemo-organotrophs: They got energy from oxidation and reduction of chemicals like inorganic substances.
- **Bacterial vitamins:** Certain fastidious bacteria required essential nutrients for growth in media called bacterial vitamins as shown in **Table 7.1**. They are similar to mammalian vitamins.

TABLE 7.1: Bacterial vitamins

Vitamins	Bacteria
Biotin	<i>Leuconostoc</i> spp.
B_{12}	<i>Lactobacillus</i> spp.
Folic acid	<i>Enterococcus faecalis</i>
Pantothenic acid	<i>Morgnella morganii</i>
B_6	<i>Lactobacillus</i> spp.
Niacin (nicotinic acid)	<i>B. abortus</i> and <i>H. influenzae</i>
B_2	<i>B. anthracis</i>

Carbon Dioxide

All bacteria require small amounts (1–2%) of carbon dioxide for growth. This requirement is usually met by the carbon dioxide present in the atmosphere, or produced endogenously by cellular metabolism. Energy required for utilization of CO₂ may come from light or from oxidation and reduction of chemicals like inorganic substances. However, some bacteria require higher concentration (5–10%) of CO₂ called **capnophilic bacteria** like *Brucella abortus*, *Neisseria* spp., *Strept. pneumoniae*, *Listeria monocytogenes*, *Helicobacter pylori*, *Campylobacter jejuni*, *Gardnerella vaginalis*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *Eikenella corrodens*, *Capnocytophage* spp., *Legionella pneumophila*, etc.

Oxygen

Based on oxygen requirement, bacteria can be classified into six types:

1. Obligate (strict) aerobes

- **Definition:** They grow only in presence of O₂.
- **Examples:** *Micrococcus*, *Neisseria meningitidis*, *Moraxella lacunata*, *Acinetobacter baumannii*, *Corynebacterium jeikeium*, *M. tuberculosis*, *Nocardia* spp., *Listeria monocytogenes*, *Serratia merscenae*, *V. cholerae*, *P. aeruginosa*, *Bukholderia pseudomallei*, *Bordetella pertussis*, *Brucella abortus*, *Francisella tularensis*, *Alkaligenes faecalis*, etc.

2. Obligate (strict) anaerobes

- **Definition:** They grow only in absence of O₂ (they die in presence of oxygen).
- **Subtypes and examples:** Two subtypes:
 - Sporing anaerobes: *Cl. botulinum*, *Cl. tetani* and *Cl. novyi*.
 - Nonsporing anaerobes: For examples follow Ch. 72.
- **Reasons of oxygen toxicity in anaerobes:** In presence of oxygen, hydrogen peroxide and other toxic peroxides accumulate. The enzyme catalase which splits hydrogen peroxide is present in most aerobic bacteria but absent in anaerobes. Some other enzymes like peroxidase and superoxide dismutase are also required to inactivate the oxygen products, but their deficiency in anaerobic bacteria make the oxygen toxic for anaerobes. Another reason is that obligate anaerobes possess essential enzymes that are active only in reduced state.

3. Facultative anaerobes

- **Definition:** They are aerobes but can grow in absence of O₂.
- **Examples:** *Strept. pyogenes*, *Staph. aureus*, *Strept. pneumoniae*, *N. gonorrhoeae*, *E. coli*, *H. influenzae*, etc.

4. Facultative aerobes

- **Definition:** They are anaerobes, but can grow in presence of O₂.
- **Examples:** *Lactobacillus* spp., etc.

5. Aerotolerant

- **Definition:** They can tolerate oxygen for some times, but cannot grow in presence of O₂.
- **Examples:** *Cl. histolyticum*, *Propionibacterium acne*, *P. avidum*, *P. granulosum* and *Arcobacterium* spp.

6. Microaerophilic

- **Definition:** They require O₂ less than the environmental O₂ (about 5–10%).
- **Examples:** *Clostridium welchii*, *Mycobacterium bovis*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Campylobacter jejuni*, *Helicobacter pylori*, *Streptobacillus moniliformis*, *Spirillum minus*, *Aggregatibacter actinomycetemcomitans*, *Borrelia recurrentis*, *Borrelia burgdorferi*, *Leptospira interrogans*, etc.

Temperature

Temperature affects the growth and viability of bacteria.

Effect on growth

- **Definition:** For each species, there is a temperature range and growth does not occur above the maximum temperature or below the minimum temperature. The temperature at which growth occurs best is called the optimum temperature.
- **Types of bacteria:** Bacteria are classified as follows as per temperature effect on growth:
 1. Psychrophilic (cryophilic) bacteria: Bacteria which can grow best at temperature below 20°C are called psychrophilic (cryophilic) bacteria, some of them even growing at temperature as low as –7°C. For example saprophytes. They may cause spoilage of refrigerated food.
 2. Mesophilic bacteria: Bacteria which grow best at temperature of 25–40°C are called mesophilic bacteria. For example, all pathogenic bacteria. Optimum temperature for this group is 37°C.
 3. Thermophilic bacteria: Bacteria which grow best at high temperature around 55–80°C are called thermophilic bacteria. For example, *Bacillus steareothermophilus* and *Campylobacter* spp. They may cause spoilage of under processed canned food.
 4. Extremely thermophilic bacteria: Bacteria which grow at higher temperature at 250°C are called extremely thermophilic bacteria. For example, bacterial spores.

Effect on viability: Heat is an important method for destruction of microorganisms. Moist heat causes coagulation and denaturation of proteins and dry heat causes oxidation and charring. Moist heat is more lethal than dry heat. The lowest temperature that kills a bacterium under standard conditions in a given time is called the thermal death point. Under moist condition, most vegetative and hemophilic bacteria have a thermal death point is between 50°–65°C and for spores is between 100°–120°C. At low temperature

- 40 some species die rapidly but most survive well. Storage in the refrigerator (3°–5°C) or deep freeze cabinet (–30° to –70°C) is used for preservation of cultures. Rapid freezing as with solid carbon dioxide or the use of a stabilizer such as glycerol, minimize the death of cells on freezing.

Notes: Thermotolerant bacteria

Definition: Bacteria which can survive, but do not grow to varying extents of the pasteurization process or pasteurization temperature called thermotolerant bacteria.

Examples: *Bacillus*, *Clostridium*, *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Enterococcus* and few gram-negative rods.

Clinical significances: The sources of contamination are poorly cleaned and sanitized utensils and equipment on farm and processing plants. These bacteria contribute to significantly higher standard plate count on pasteurized milk.

Thermotolerant count: It has been used in the dairy industry primarily as a test of care employed in utensil sanitation and as a means of detecting sources of organisms responsible for high counts in the final product.

H-ion Concentration (pH)

Range of pH: Each species has a pH range, above and below which, it cannot survive. Bacteria are sensitive to variations in pH. Strong solutions of acid or alkali (5% HCl) readily kill most bacteria, though mycobacteria are exceptionally resistant to them. pH at which, bacteria can grow best is called optimum pH.

Pathogenic bacteria: Following are different types of pathogenic bacteria as per pH effect:

1. Acidic pH: Some bacteria grow at acidic pH (<6.0) are called acidophilic bacteria. For example, *Lactobacillus* spp.
2. Neutral pH: Most of the pathogenic bacteria grow at neutral pH (7.2–7.6).
3. Alkaline pH: Some bacteria grow at alkaline pH (>7.8) like *V. cholerae*.

Moisture and Drying

Moisture is absolute requirement for growth of bacteria. The capacity to survive in dry environment varies from organism to organism. *N. gonorrhoeae* and *T. pallidum* die quickly in dry conditions. *Staph. aureus* and *M. tuberculosis* can survive drying for weeks and months. Bacterial spores can survive in drying for decades. Drying in vacuum in the cold condition is a method for the preservation of bacteria, viruses and many labile biological materials called freeze drying or lyophilization.

Light

Some bacteria required light for energy like photolithotrophs/photo-organotrophs. Light also required for certain bacterial functions like pigment production. Following are different types of bacteria as per requirement of light:

1. Photochromogenic bacteria: Bacteria produce the pigment only in light, not in the dark called photochromogenic bacteria. For examples *M. kansasii*, *M. marinum*, *M. simiae*, *M. asiaticum*, etc.
2. Scotochromogenic bacteria: Bacteria produce the pigment in light and in dark called scotochromogenic bacteria. For examples, *M. scrofulaceum*, *M. gordonae* and *M. szulgai* (scotochromogen at 37°C and photochromogen at 25°C).
3. Nonphotochromogenic (nonchromogenic) bacteria: Bacteria which do not produce pigment in presence or absence of light called nonphotochromogenic (nonchromogenic) bacteria. For examples, *M. avium*, *M. intracellulare*, *M. xenopi* and *M. ulcerans*.

Osmotic Effect

Bacteria more tolerate osmotic variation than most other cells due to the mechanical strength of their cell walls. Sudden exposure to hypertonic solutions may cause loss of water and shrinkage of cell called plasmolysis. It occurs more in gram-negative than in gram-positive bacteria. Sudden transfer to distilled water or any other hypotonic solution may cause water flow inside the cell and swelling and rupture of cell called plasmolysis.

Mechanical and Sonic Stress

Though bacteria have tough cell walls, they may be ruptured by mechanical stress such as grinding, vigorous shaking with glass beads or ultrasonic vibration.

REPRODUCTION (MULTIPLICATION)

Methods of Reproduction

Following are different methods of reproduction.

1. **Binary fission:** Bacteria multiply by binary fission after attaining certain size and one mother cell gives two daughter cells. Nuclear division occurs at 1st followed by cell division. Cell division initiated with formation of transverse septum across the cell. Normally daughter cell completely gets separated from mother cell; however in some it attaches to mother cell due to incomplete binary fission like in *C. diphtheriae*.
2. **Budding:** Some bacteria also reproduced by budding in which a small bud (small pouch like structure) develops from the mother bacterium, which later gets separate and grows further to form a new bacterium. For example, *F. tularensis*, *M. pneumoniae*, etc.
3. **Filamentous formation (?), e.g., *F. tularensis*.**

Reproduction Time

Definition: The interval of time between two cell divisions or the time required for a bacterium to give rise to two daughter cells under optimum conditions is called generation time or population doubling time. One bacterium gives 10²¹ progeny in 24 hours.

Generation time of few bacteria: Follow Table 7.2.

TABLE 7.2: Generation time of different bacteria

Organisms	Generation time
<i>Cl. welchii</i> (GPB)	10 minutes
Coliform bacilli (lactose fermenters) like <i>E. coli</i> and <i>K. pneumoniae</i> (GNB)	20 minutes
<i>Leptospira</i> spp., (GNB)	4–8 hours in animals and 12–16 hours in media
<i>M. tuberculosis</i> (GPB)	20 hours
<i>T. pallidum</i> (GNB)	30–33 hours
<i>M. leprae</i> (GPB)	13–15 days

Effective Factors on Reproduction

1. Nutrients and toxic products

- **Depletion of nutrients and accumulation of toxic products:** When bacteria grow in artificial prepared nutritional media, multiplication is arrested after few cell divisions due to depletion of nutrients or accumulation of toxic products. Such culture called batch culture.
- **Replacement of nutrients and removal of toxic products**
 - By using special device: A special device is used which replaces the nutrients and removes toxic products during cell division. Such culture called continuous culture. It used to maintain bacteria for industrial or research purposes.
 - In host tissue: Tissue replaces the nutrients and removes toxic products during cell division, but bacteria have to compete with host defense. It is intermediate between batch culture and continuous culture.

2. Inhibition of cell division:

By certain drugs like penicillin.

Bacterial Count

Definition: Bacterial multiplication in number called bacterial count.

Methods: Two types of bacterial counts like total count and viable count.

1. Total count:

Total number of cells in the sample irrespective of whether they are living or dead called total count. Total counting can be obtained by following methods:

- Direct counting under the microscope using counting chamber
- Counting in an electronic device as in the coulter counter
- Using stained smear prepared by spreading a known volume of the culture over a measured area of a slide
- Comparing relative number, in a smears of the culture mixed with known numbers of other cells

- Opacity measurement using an absorptiometer or nephelometer
- Separation of cells by centrifugation or filtration and measuring their wet or dry weight
- Chemical assay of cell components like nitrogen.

2. Viable count:

Number of living cells which are capable to multiply called viable count. Viable counting can be obtained by following methods:

- **Dilution method:** It is done by using liquid media. The suspension is diluted to a point beyond which unit quantities do not yield growth when inoculated in to suitable liquid media. Several tubes are incubated with varying dilutions and the viable count calculated statistically from the number of tubes showing growth. It do not give accurate value is the drawback of this method. It is used for water culture to estimate the presumptive coliform count in drinking water.
- **Plating method:** It is done by using solid media. Appropriate dilutions are inoculated on solid media, either on the surface of plates or as pour plates, followed by incubation. The numbers of colonies develop can give an estimate of the viable count. The method was employed and is described by Miles and Misra (1938).

Bacterial Growth Curve

Definition: When a bacterium is inoculated into a suitable liquid medium and incubated, it under goes the multiplication. Count the bacteria at regular interval and plotted it against the time, a growth curve is obtained called bacterial growth curve or kinetic of bacterial growth.

Phases: The curve shows the four phases during which morphological and physiological changes occur in cells are shown in Fig. 7.1.

1. Lag phase

- It is the initial period.
- It required to adapt the new environment, to synthesize the necessary enzymes and metabolites for multiplication.

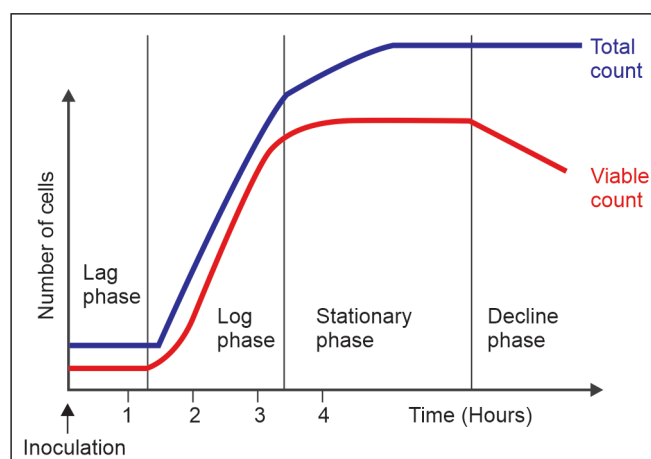


Fig. 7.1: Bacterial growth curve

- Immediately following the seeding of a culture medium there is no increase in numbers, though there may be an increase the size of a cell.
 - The duration of the lag phase varies from 1–4 hours with the type of species, size of the inoculum, nature of the medium, presence of growth factors and environmental factors like temperature, O₂, CO₂, etc.
- 2. Log phase/logarithmic phase/exponential phase**
- As the time pass, cells start to divide and their numbers increase.
 - If the logarithm of the viable count is plotted against time a straight-ascending line will be obtained.
 - Smaller size and uniform staining occurs in this phase.
- 3. Stationary phase**
- After a varying period of exponential growth, cell division stops due to depletion of nutrients and accumulation of toxic products (batch culture).
 - Progeny cells formed are enough to replace the number of cells that die.
 - Viable count remains stationary as equilibrium exists between the dying and new cells.
 - Variable gram reaction, irregular staining due to storage granules and sporulation.
- 4. Decline phase**
- During this phase viable count decreases due to cell death.
 - Cell death is due to nutritional exhaustion, accumulation of toxic products (secondary metabolic products) and by autolytic enzymes.
 - Total count is runs parallel to viable count up to stationary phase, after that it continues steadily without any decline till autolysis will start.
 - After autolysis, total count also decreases.
 - Involution forms occur due to actions of autolytic enzymes, ageing culture or by defective cell wall synthesis.

Summary of bacterial growth curve: Follow Table 7.3.

TABLE 7.3: Summary of bacterial growth curve				
Features	Lag	Log	Stationary	Decline
Bacterial division	No	Yes and high	Yes but low	Yes
Bacterial death	No	No	Yes	Yes
Total count	Flat	Increases	Increases	Flat
Viable count	Flat	Increases	Flat	Decreases
Key features	• Synthesis of enzymes and metabolites • Size: Maximum	• Size: Small • Stain:	• Stain: Gram variable • Granules, toxin and spores	• Auto-lysis • Involution forms

Mnemonic

- SS: Stationary phase and Sporulation
- In-In: Decline phase and Involution forms

METABOLISM

Steps

- Absorption of food material.
- Breakdown and utilization.
- Elimination of metabolic end products.

Metabolic Pathway

Bacteria are differentiated in their ways of metabolism.

- 1. Aerobic bacteria:** They obtain their energy by oxidation (aerobic respiration). Oxygen as the ultimate hydrogen (electron) acceptor. O₂ accepts the carbon and energy to form CO₂ and H₂O. During this process energy rich phosphate bonds are released which convert ADP to ATP called oxidative phosphorylation.
- 2. Anaerobic bacteria:** They obtain their energy by fermentation (anaerobic respiration). Nitrite or sulfite is hydrogen (electron) acceptor. Fermentation is an anaerobic utilization of sugar to produce acids (like lactic acid, formic acid, pyruvic acid, etc.), alcohols and gases (CO₂, H₂, etc.). During this process, energy rich phosphate bonds are released which convert ADP to ATP called substrate level phosphorylation.
- 3. Facultative anaerobes:** They utilize both pathways.

Redox Potential (Oxidation–Reduction Potential or Eh)

Oxidizing agents are substances, which are capable of accepting electrons. Reducing agents are substances that are able to lose electrons. The capability of substance to accept or to lose the electron is called oxidation-reduction potential. It is abbreviated as *Eh* and measured in millivolts. It is higher in oxidized substances and lower in reducing agents. The strict anaerobes require low redox potential—less than 0.2 volts; however, the redox potential of most of the media exposed to air is +0.2 to +0.4 volts.

ACCESS YOURSELF

Essay/Full Question

1. Physiology of bacteria.
2. Environmental factors affecting growth of bacteria.

Short Notes

1. Effect of oxygen on bacteria.
2. Effect of temperature on bacteria.
3. Bacterial count.
4. Bacterial growth curve.

Short Questions for Theory/Viva Questions

1. What is a bacterial vitamin?
2. What are capnophilic bacteria?
3. Write four examples of capnophilic bacteria.

4. Write four examples of strict aerobic bacteria.
5. Write four examples of strict anaerobic bacteria.
6. Write four examples of microaerophilic bacteria.
7. What are plasmolysis and plasmoptysis?
8. What is generation time?
9. Write the generation time for following bacteria: *E. coli*, *M. leprae*, *M. tuberculosis* and *T. pallidum*.
10. What are thermophilic bacteria? Write two examples.
11. What are batch culture and continuous culture?
12. Name the four phases of bacterial growth curve.
13. Name the phases of bacterial growth where sporulation and involution forms can develop.
14. Name the two methods for multiplication (reproduction) and two methods for metabolism in bacteria.
15. What is Eh or Redox potential?

Comments on

1. Oxygen acts as toxin for strict anaerobic bacteria.
2. Bacteria tolerate osmotic variation more than any other cells.
3. Bacterial multiplication is arrested after certain divisions in nutritional media.
4. Cell division stops in stationary phase of bacterial growth curve.

MCQs for Chapter Review

Bacterial Nutrition and Growth Factors

1. What percentage of mass of bacterial cell is its dry weight?
 - a. 10–15%
 - b. 20–25%
 - c. 30–35%
 - d. 40–45%

Environmental Factors

2. Obligate anaerobes cannot withstand oxygen because of absence of:
 - a. Superoxide dismutase
 - b. Catalase
 - c. Peroxidase
 - d. Cytochrome oxidase
3. Which of the following bacterium is classified as facultative anaerobe?
 - a. *Pseudomonas*
 - b. *Bacteroides*
 - c. *Escherichia*
 - d. *Clostridia*
4. Strict anaerobe(s) is/are:
 - a. *Cl. novyi*
 - b. *Cl. botulinum*
 - c. a + b
 - d. None of above
5. Obligate anaerobes are all except:
 - a. *Cl. botulinum*
 - b. *Eikenella corrodens*
 - c. *Bacteroides*
 - d. *H. pylori*
6. Which of the following is microaerophilic?
 - a. *Campylobacter*
 - b. *Vibrio*
 - c. *Bacteroides*
 - d. *Pseudomonas*
7. Mesophilic organisms are those that grow best at temperature of:
 - a. –20°C to –7°C
 - b. –7°C to +20°C
 - c. 25°C to 40°C
 - d. 55°C to 80°C
 - e. 90°C to 120°C

Reproduction (Multiplication)

8. Generation time for *M. tuberculosis* is:
 - a. 12 days
 - b. 5 minutes
 - c. 20 hours
 - d. 4 hours
9. Generation time for lepra bacilli is:
 - a. 12 days
 - b. 5 minutes
 - c. 10 hours
 - d. 24 hours
10. Correct sequence of bacterial growth curve:
 - a. Log phase → Lag phase → Stationary phase → Decline phase
 - b. Lag phase → Log phase → Stationary phase → Decline phase
 - c. Stationary phase → Lag phase → Log phase → Decline phase
 - d. Lag phase → Exponential phase → Log phase → Death phase
 - e. Exponential phase → Lag phase → Death phase → Stationary phase
11. True regarding lag phase:
 - a. Time taken to adopt in the new environment
 - b. Growth occurs exponentially
 - c. The plateau in lag phase is due to cell death
 - d. It is the 2nd phase in bacterial growth curve
12. Sporulation occurs in:
 - a. Lag phase
 - b. Log phase
 - c. Stationary phase
 - d. Decline phase

Answers and Explanation of MCQs

1. b
 - Follow section, **bacterial nutrition and growth factors (nutrients or growth factors → water)** for explanation.
2. a, b, c
 - Follow section, **environmental factors (oxygen → Reasons of oxygen toxicity in anaerobic bacteria)** for explanation.
3. c
 - *Pseudomonas* is strict aerobe while *Bacteroides* and *Clostridium* are strict anaerobes.
4. c
5. b, d
 - Follow section, **environmental factors (oxygen → obligate or strict anaerobes)** for explanation of answers of MCQs 4–5.
6. a
 - *Vibrio* and *Pseudomonas* are strict aerobes while *Bacteroides* is strict anaerobe.
7. c
 - Follow section, **environmental factors (temperature)** for explanation.
8. c
9. a
 - Generation time of different bacteria is given Table 7.2.
10. b
11. a
12. c
 - Follow section, **reproduction (multiplication) → Bacterial growth curve** for explanation of answers of MCQs 10–12.

Genetics of Bacteria

Chapter Outline

- Genetic Structure
 - Chromosomal part
 - Extrachromosomal part
- Bacterial Variation
 - Phenotypic variation
 - Genotypic variation

GENETIC STRUCTURE

The unit or segment of DNA for heredity is called gene. Total component of genes in the cell called genome. Study of heredity and variation called genetics.

Bacterial genome (**Fig. 8.1**) has two parts like chromosomal part and extrachromosomal part. Chromosomal part is a single (haploid), circular coiled body contains two types of nucleic acids such as DNA and RNA. Extrachromosomal part contains plasmid, episome and transposon (jumping gene).

Note: Viral nucleic acid and meaning of haploid and diploid

Viral nucleic acid: Viruses contain either DNA or RNA, but not both.

Meaning haploid and diploid: Haploid indicates single (n) set of chromosome and diploid indicates double ($2n$) set of chromosome.

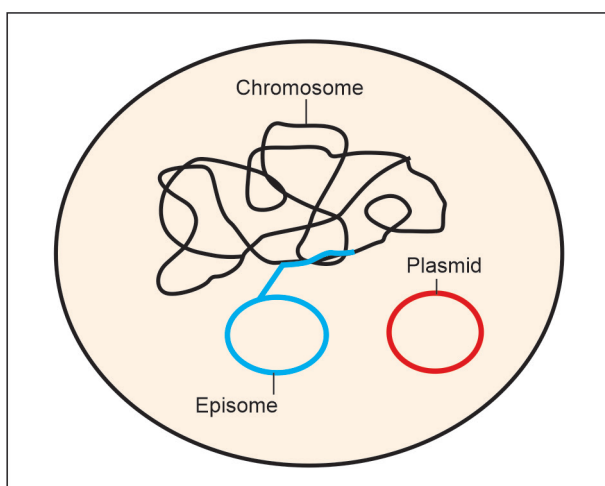


Fig. 8.1: Bacterial genome

Chromosomal Part

Bacteria have two types of nucleic acids like DNA and RNA from chromosomal part as described below.

DNA

Full form: Deoxyribo Nucleic Acid.

History: DNA model was described by James Watson and Francis Crick (Watson and Crick) in 1953.

DNA structure

- **DNA model:** It is shown in **Fig. 8.2a**. DNA is single, spiral shape and about 1000 μm in length when straightened. It is composed of two strands (double helix) with complementary nucleotides. Length is expressed in kilobase (1 kb = 1000 base pairs). Bacterial DNA is about 4000 kb long while human DNA is about 3 million kb long. For the sake of simplicity, it

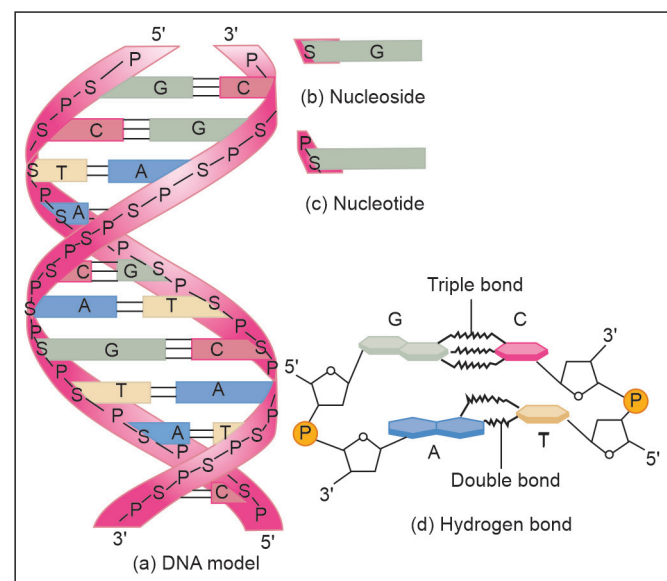


Fig. 8.2: DNA structure

is denoted as a ladder. Rungs of ladder are formed by nitrogen base united with phosphate bond. Side of ladder formed by deoxyribose sugar and phosphate bond. Bacterial DNA is available in circular/loop form without ends (closed form).

- **Nucleoside and nucleotide:** Term nucleoside and nucleotide are frequently used in the DNA model. Nucleoside (**Fig. 8.2b**) denotes sugar (s) plus nitrogen base. Nucleotide (**Fig. 8.2c**) denotes nucleoside plus phosphate bond (P).
- **Nitrogen bases:** Two types of nitrogen bases such as purine includes adenine (A) plus guanine (G) and pyrimidine includes thymine (T) plus Cytosine (C). Numbers of A are equal to that of T and also numbers of G are equal to that of C. Ratio of A + T and G + C are constant for each species but vary widely from one species to another.
- **Hydrogen bond:** Both strands of DNA held together by the hydrogen bonds between the nitrogen base of opposite strand
- **Rule of bonding (Fig. 8.2d)**
 - A-T bond: Adenine (A) of one strand bound to the Thymine (T) of opposite strand by double hydrogen bonds.
 - C-G bond: Cytosine (C) of one strand bound to the Guanine (G) of opposite strand by triple hydrogen bonds.

Functions of DNA

- **Replication:** Copying of DNA.
- **Transcription:** Synthesis of RNA from DNA.

Segment of DNA (gene): Segment of DNA called codon which is specifying for particular polypeptide chain called gene. Bacteria possess 1000–3000 genes which are coded for 1000–3000 polypeptide chains, as each gene is specify the particular polypeptide chain. Each polypeptide chain made up of 1200 nucleotides and each nucleotide consists of 400 amino acids. Chromosome contains 5×10^4 nucleotide pairs.

Introns and exons in DNA: In DNA there are several codons which do not function as gene called introns while codon which codes for particular gene are called exons. During transcription the genome is copied entirely with introns and exons. During translation the introns are excised from RNA before being translated in to the proteins.

RNA

Full form: Ribo nucleic acid.

RNA structure: Similar to DNA with minor differences as shown in **Table 8.1**.

Types: Three types:

1. mRNA: messenger RNA.
2. rRNA: ribosomal RNA.
3. tRNA: transfer RNA.

TABLE 8.1: Similarities and differences between DNA and RNA

Features	DNA	RNA
Acid	Phosphoric acid	Phosphoric acid
Purine bases	A and G	A and G
Pyrimidine bases	T and C	U and C
Sugar	D-2-Deoxyribose	D-Ribose

Function: It synthesizes the protein called translation.

Genetic Codons

Sequence of genetic information stored in the DNA called genetic codon. It was described by Nirenberg and Khorana in 1968. Each codon consists of a sequence of three nitrogen bases, so called triplet codon or triplet. Each triplet codon codes for single amino acid. For example, ACG codes for threonine. Codon is the structural terminology while code is the functional terminology. Following are the different types of codons:

1. **Sense codons:** There are total 64 codons and out of which 61 codons are code for 20 essential amino acids called sense codons.
2. **Nonsense codons (stop codons):** Out of 64 codons, 3 codons do not code for any amino acid called nonsense codons. They terminate the elongation of polypeptide chain so called stop codons. For examples UAA, UAG and UGA.
3. **Start codon:** First codon which starts the amino acid synthesis called start codon. For example, AUG which codes for methionine in eukaryotes and modified methionine [N-Formyl methionine (fMet)] in prokaryotes.
4. **Anticodon:** It is a set of three nucleotide bases present on tRNA that is complementary to the nucleotide bases of codon on mRNA.

Extrachromosomal Part

Bacteria have following three types of nucleic acids from extrachromosomal part like plasmid, episome and transposon.

Plasmid

Definition: It is an extrachromosomal intracytoplasmic DNA material (**Fig. 8.1**).

Properties

- **Numbers:** A bacterium can have no plasmids at all or have many plasmids (20–30) or multiple copies of a plasmid.
- **Shape:** Usually, it is closed circular molecules; however it occurs as linear molecule in *Borrelia burgdorferi*.
- **Size:** Varies from 1 kb to 400 kb.
- **Replication:** Autonomous (independent) replication.
- **Present:** It presents in bacteria and also in yeast.

46 Types of plasmid

1. According to transmission

- **Self transmissible/conjugative plasmid:** Contains information for self transfer to other cell via conjugation, e.g., F factor
- **Non-transmissible/non-conjugative plasmid:** Does not contain information for self transfer via conjugation (can be transduced).

2. According to functions

- **Fertility (F)/sex factor or sex pili:** F factor is a plasmid codes for special fimbriae or sex pili which makes a conjugation tube between two cells and for necessary enzyme of conjugation. Those bacteria that possess F factor are called F⁺, such bacteria have sex pili on their surface and those are lacking called F⁻. The F factor plasmid is transferred to other cells through conjugation tube. F⁻ cell will become F⁺ when it receives the fertility factor from another F⁺ cell.
- **Resistance (R) Factor:** It is responsible for drug resistance.
- **Colicinogenic (Col) factor:** It is responsible for bacteriocins production.

3. According to restriction endonuclease enzyme fingerprinting.

- **Closely related plasmids:** They produce same or very similar fingerprinting.
- **Unrelated plasmids:** They produce different fingerprinting.

4. Incompatibility typing

- **Closely related plasmids:** They do not coexist stably in same bacterium.
- **Unrelated plasmids:** They coexist stably in same bacterium.

Functions of plasmid: It transfers the genetic information from one cell to another cell and enables virulence properties to bacteria as mentioned below:

1. Virulence determinant

- **Conjugation:** Fertility (F) factor is a plasmid codes for special fimbriae or sex pili. Fimbriae or sex pili make a conjugation tube between two cells and allow the transfer of genetic information from one cell to other called conjugation.
- **Iron sequestering:** Plasmid carries virulence determinant genes like the Col V plasmid of *E. coli* contains genes for iron sequestering compounds.
- **Capsule and toxin in *B anthracis*** are plasmid encoded. Removal of such plasmid makes the strain avirulent called Sterne strain, which used for vaccine production.
- **Invasive property in *Shigella* and EIEC** is due to a special type of OMP called VMA (virulence marker antigen) which is plasmid encoded, responsible for invasion, multiplication of bacilli and destruction of epithelial cells.

2. Drug resistance: It codes for drug resistance to several antibiotics called resistance transfer factor (RTF). Gram-negative bacteria carry plasmids that give resistance to antibiotics such as neomycin, kanamycin, streptomycin, tetracycline, chloramphenicol, penicillins and sulfonamides.

3. Bacteriocins production: It codes for the production of bacteriocins called col factor.

4. Toxins production: It codes for the toxins production in following bacteria:

- Enterotoxin production by *Staph. aureus*.
- Anthrax toxin by *B anthracis*.
- Tetanospasmin by *Cl. tetani*.
- Labile toxin (LT) of *E. coli*.
- Stable toxin (ST) of *E. coli*.

5. Resistance to heavy metals: It codes for resistance to heavy metals like Hg, Ag, Cd, Pb, etc.

6. Resistance to UV light: It codes for resistance to UV light (DNA repair enzymes are coded in the plasmid).

7. Coding for enzymes: It contains genes coded for enzymes that allow bacteria unique or unusual materials for carbon or energy sources, e.g., urease synthesis in bacteria.

8. Metabolic plasmid: It enables the bacteria in various metabolic activities such as nitrogen fixation, digestion of unusual substances like toluene, salicylate, camphor, etc.

Clinical applications of plasmid

1. Used in genetic engineering as vector: Plasmid contains many sites for artificial insertion of genes by recombinant technology. Such plasmid can be used for various purposes such as protein production, gene therapy, etc.

2. Plasmid profiling is a useful genotyping method: It is useful tool for epidemiological study of microbes.

Episome

Plasmid sometimes integrated with chromosomal part called 'episome' (Fig. 8.1) and such bacterial cells are called 'Hfr cells' (High frequency of recombination). Previously, it was considered as synonymous with plasmid. Jacob and Wollman coined the term episome. When it detaches with part of chromosome, it becomes free called F prime (F[']) factor.

Transposon (Jumping Gene)

Gene that jumps between chromosomal and extra-chromosomal part called transposon or jumping gene. It called jumping gene because it jumps between chromosomal and extrachromosomal part. It also called transposable genetic element.

BACTERIAL VARIATION

Bacteria have two types of variation like phenotypic variation and genotypic variation, as mentioned below. Differences between two types are mentioned in Table 8.2.

TABLE 8.2: Differences between phenotypic and genotypic variation

Phenotypic variation	Genotypic variation
Reversible	Irreversible
Temporary (unstable)	Permanent (stable)
Not heritable	Heritable
Environment effect	No environment effect

Phenotypic Variation

Meaning: Phaeno means display.

Definition: It is a physical expression of bacterial characteristics in given environment.

Examples of phenotypic variation: Such as synthesis of flagella and synthesis of enzyme described below:

- **Synthesis of flagella:** *S. Typhi* is normally flagellated, but flagella are not synthesized when it grows in phenol agar. It reversed when subcultured in broth.
- **Synthesis of enzyme:** Galactosidase is the enzymes required for *E. coli* for lactose fermentation, but it produced when *E. coli* grows in a lactose containing medium. It is not synthesized when *E. coli* grows in lactose free medium. Such enzyme which requires the presence of substance called induced enzyme and opposite which does not require the presence of substance called constitutive enzyme.

Genotypic Variation

Meaning: Geno means related to gene.

Definition: It is a genetic expression of bacterial characteristics in given environment.

Examples of genotypic variation: Such as mutation and gene transfer mechanisms/methods described below.

Mutation

History: Word “mutation (Latin)” was coined by Hugo de Vries, which means “to change”.

Definitions

- **Mutation:** Change in the nucleotide sequence of DNA called mutation.
- **Wild type:** Organisms selected as reference (Normal) strains are called wild type.
- **Mutants:** Yield of mutation called mutants.
- **Mutagenesis:** The process of mutation called mutagenesis.
- **Mutagens:** Agents inducing mutations are called mutagens.

Types of mutations: Two main types:

A. According to induction

1. **Spontaneous mutation:** It occurs naturally about one in every million to one in every billion divisions. It occurs during DNA replication.
2. **Induced mutation:** It is induced by following agents:

- **Chemical agents:** Such as alkylating agents, acridine dyes (nucleoside analogs that are similar in structure to nitrogenous bases), aflatoxin, 2-amino purine, 5-bromouracil, benzpyrene (from smoke and soot) and nitrous acid (alters adenine to pair with cytosine instead of thymine).
- **Physical agents:** X-rays and gamma rays have been shown to damage DNA. UV rays are responsible for the formation of thymine dimers in which covalent links are established between the thymine molecules which change the physical shape of the DNA preventing transcription and replication.
- **Biological agents:** Such as viruses.

B. According to mechanisms: Follow Fig. 8.3.

1. **Point mutation:** Due to addition, deletion or substitution of single base.
 - **Substitution of a nucleotide:** It involves the changing of single base in the DNA sequence. This mistake is copied during replication to produce a permanent change. This is the most common mechanism of mutation. If a purine is replaced by a pyrimidine or *vice versa*, the substitution is called **transversion**. If one purine is replaced by the other purine or one pyrimidine is replaced by other pyrimidine, the substitution is called **transition**.
 - **Deletion or addition of a nucleotide:** Deletion or addition of a nucleotide occurs during DNA replication. When a transposon (jumping gene) inserts itself into a gene, it leads the disruption of gene called **insertional mutation**.
2. **Frame shift mutation:** Deletion or addition of a numbers of nucleotide.
3. **Multiple mutation:** It causes extensive chromosomal rearrangement.
4. **Other types**
 - **Missense mutation:** Changes in the amino acid sequence due to change in triplet codon of the particular protein called missense mutation. This could be caused by a single point mutation or a series of mutations.
 - **Nonsense mutation:** Termination of polypeptide or protein synthesis due to formation of stop codon is called a nonsense mutation. It leads incomplete or immature protein production.

<u>A B C D E F</u>	Normal (wild type)
<u>A B X C D E F</u>	Addition
<u>A B D E F</u>	Deletion
<u>A B B C D E F</u>	Duplication
<u>A B X D E F</u>	Substitution
<u>A B C F E D</u>	Inversion

Fig. 8.3: Mutation according to mechanism

- **Suppressor mutation:** It is a reversal of a mutant phenotype by another mutation at a position on the DNA distinct from that of original mutation. True reversion or back mutation results in reversion of a mutant to original form, which occurs as a result of mutation occurring at the same spot once again.
- **Lethal mutation:** Sometimes some mutations affect vital functions and the bacterial cell become nonviable. Hence, those mutations that can kill the cell are called lethal mutations.
- **Conditional lethal mutation:** Sometimes a mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition called conditional lethal mutation. For example, a temperature sensitive mutant (ts mutant) can survive at permissive temperature of 35°C but not at restrictive temperature of 39°C.
- **Inversion mutation:** If a segment of DNA is removed and reinserted in a reverse direction, it is called inversion mutation.
- **Silent mutation:** Sometimes a single substitution mutation change in the DNA base sequence results in a new codon still coding for the same amino acid. Since there is no change in the product, such mutation is called silent mutation.

Clinical significance of mutation

1. **Identification of the function of gene:** Discovery of a mutation in a gene can help in identifying the function of that gene.
2. **Vaccine production:** Mutations can be induced at a desired region to create a suitable mutant, especially to produce vaccine.
3. **Drug resistant:** Spontaneous mutation can result in emergence of antibiotic resistance in bacteria like drug resistance in *M. tuberculosis*.
4. **Functional changes in bacteria:** Mutation leads functional changes like *E. coli* mutant loses the ability to ferment lactose. It can be detected on MacConkey's medium, but not in nutrient agar.
5. **Survival advantage:** Mutation affects the vital function and confers survival advantage. For example, streptomycin resistant mutant to *M. tuberculosis* develops in a patient taking treatment. It multiplies and replaces initial drug sensitive bacteria and causes survival advantage, but a patient who is not under treatment, it does not cause any survival advantage.
6. **Change in phenotypes:** Mutations can result in change in phenotypes such as
 - Appearance of novel surface antigen
 - Alteration in physiological properties
 - Change in colony morphology
 - Nutritional requirements
 - Biochemical reactions
 - Growth characteristics
 - Virulence
 - Host range

Tests to detect the mutations

1. Fluctuation test

- **History:** It was developed by Luria and Delbruck in 1943.
- **Principle:** Bacteria undergoes to spontaneous mutation when they are challenged on agar plate contains growth limiting substances like streptomycin or bacteriophage. However, the rate of mutation is wide, some bacteria mutate early while some late which leads fluctuations. Fluctuations are wide from small volume subculture (more frequent mutation) than large volume subculture (less frequent mutation).
- **Disadvantages:** It is not widely accepted due to complicated statistical evaluation.
- **Method:** Luria and Delbruck seeded the *E. coli* from small volume culture and single large volume culture on media contain bacteriophages. The mutant bacteria formed the colonies. Colony count was compared. There was wide fluctuation in the numbers of resistant variant in small volume culture as compare to single large volume culture.

2. Replica plating method

- **History:** It was developed by Lederberg in 1952.
- **Principle:** It based on differentiation between normal strain and auxotrophic mutant in which auxotrophic mutant cannot grow in absence of particular nutrient, e.g., lysin auxotroph can grow only on media contain lysin but not on lysin deficient media.
- **Method (Fig. 8.4):** Using velvet template, mixture of colonies (normal strain and auxotrophic mutants) of bacteria transferred from a master plate, on to two subculture plates where one plate is lacking with lysin. After incubation same colonies as master plate are obtained in subculture plates, except lysin auxotroph which do not grow on the media lacking lysin.

3. Ames test (carcinogenicity testing)

- **History:** It was developed by Bruce Ames in 1970.
- **Aim:** It is used to identify the environmental carcinogens.

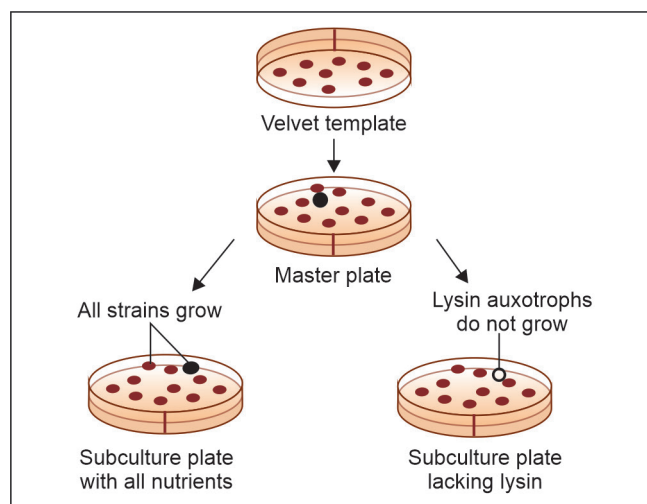


Fig. 8.4: Replica plating method

- **Principle:** It based on mutational reversion by carcinogen.
- **Method (Fig. 8.5):** Mutant strains (histidine auxotroph) of *Salmonella* which are subscribed on two agar plates contain small amount of histidine; one of the plate is added with the test mutagen. Incubate the plate at 37°C for 2–3 days. All of the histidine auxotroph will grow for the first few hours until the histidine is depleted. Once the histidine source is exhausted, mutation revertant strain can grow by their ability to synthesize the histidine. Reversed mutation may be induced by carcinogen (affect large numbers of strains) or occur spontaneously (affect only few strains). The relative mutagenicity of the carcinogen can be estimated by counting the colonies. More the numbers higher the carcinogenicity.

4. Penicilin enrichment

Gene Transfer Mechanisms/Methods

Definition: Transfer of genetic materials or genetic information from one cell to other cell called genetic recombination. Cell donating the genetic materials or genetic information called donor cell to another cell called recipient cell.

Methods of gene transfer: It includes vertical gene transfer method and horizontal gene transfer method. In vertical gene transfer method, transmission of genes occurs from parents to offsprings which is not much important. In horizontal gene transfer method transmission of genes occurs from one bacterium to other by different mechanisms such as transformation, transduction, lysogenic conversion, conjugation and transposition. Horizontal gene transfer methods are described below.

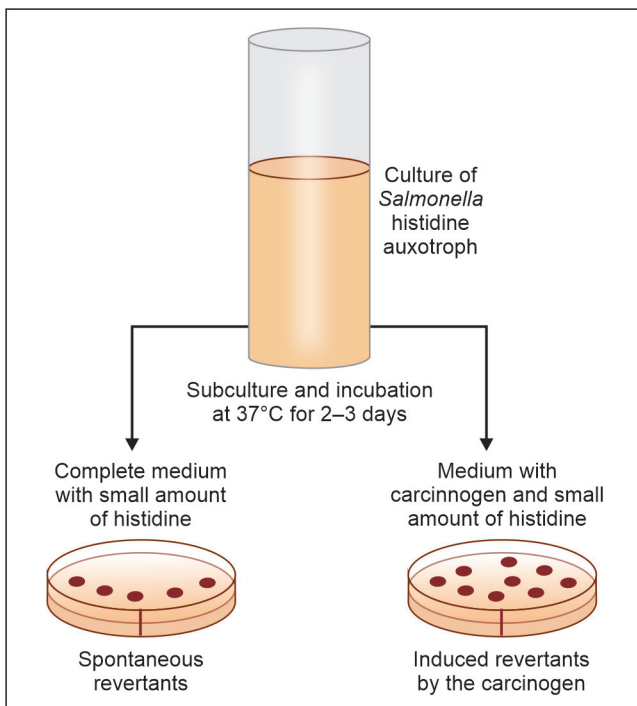


Fig. 8.5: Ames test

A. Transformation

Definition: Transfer of genetic information from one cell to other cell through agency of free/naked DNA called transformation.

Mechanism (Fig. 8.6): Transformation occurs in less than 1% of bacteria like *Bacillus*, *Haemophilus*, *Neisseria* and *Streptococcus*. When bacteria die, their DNA is released; such free DNA is referred as naked DNA. Fragments of the naked DNA are taken up by the cell wall of another bacterium and incorporated into its chromosome.

History (Fig. 8.7): This was first demonstrated in an experiment conducted by Griffith in 1928. Capsulated pneumococci give glistening, smooth (S) colonies while noncapsulated strains give rough (R) colonies. Pneumococci with a capsule (type I) are virulent and can kill a mouse while strains lacking it (type II) are harmless. Griffith found that mice died when they were injected with a mixture of live noncapsulated (R, type II) strains and heat killed capsulated (S, type I) strains. Neither of these two when injected alone could kill the mice, only the mixture of two proved fatal. Live S strains with capsule were isolated from the blood of the animal suggesting that some factor from the dead S cells converted the R strains into S type. The factor that transformed the other strain was found to be DNA by Avery, McLeod and McCarty in 1944.

Uptake of free DNA by bacteria: Some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA at particular time in their growth cycle (log phase) when they produce a specific protein called a competence factor. Gram-positive bacteria take up single stranded DNA and the complementary strand is made in the recipient. Gram-negative bacteria take up double stranded DNA.

Clinical significances: Transformation occurs in nature, and it increases the virulence.

Use: It used in recombinant DNA technology.

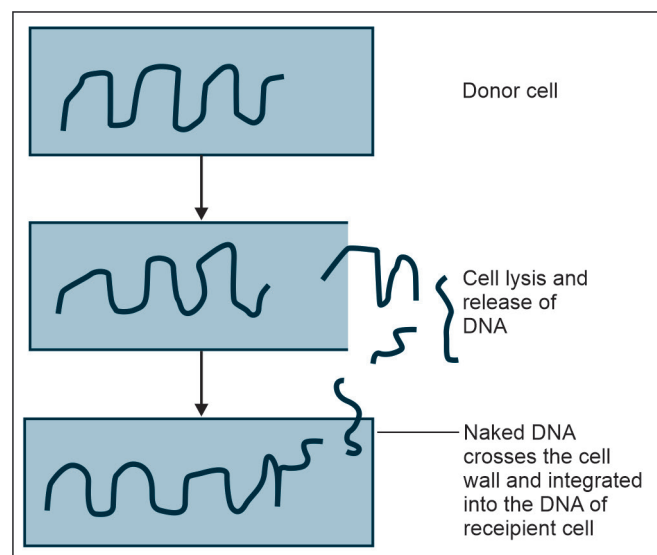


Fig. 8.6: Mechanism of transformation

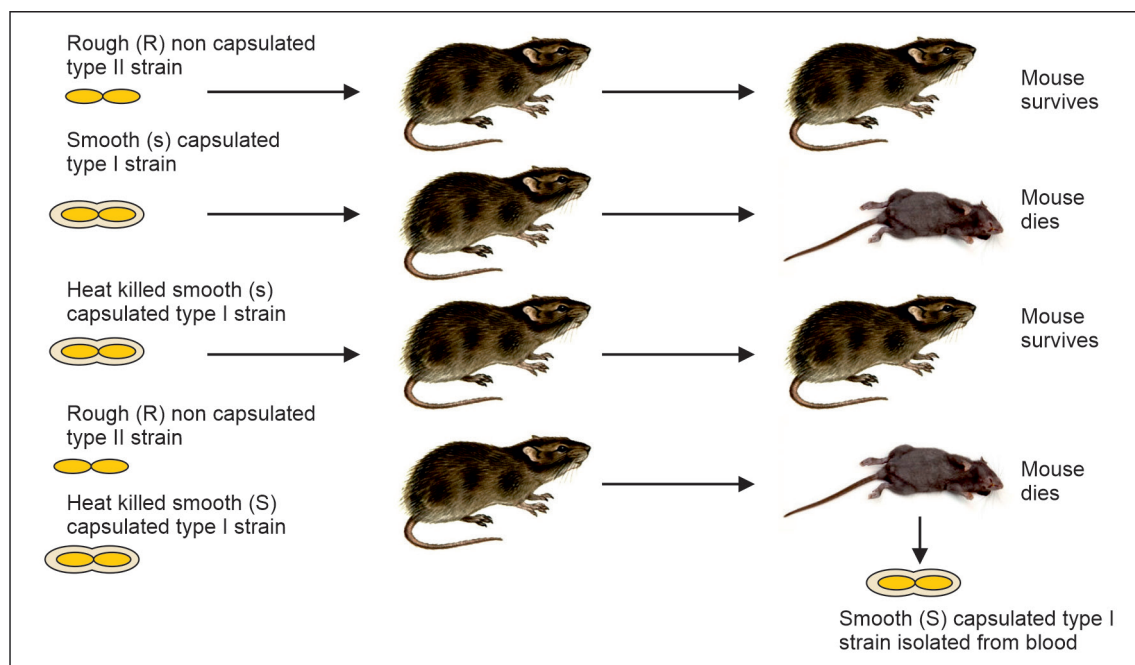


Fig. 8.7: Transformation of Griffith

B. Transduction

Definition: Transfer of genetic information from one cell to other cell through bacteriophage called transduction.

Bacteriophage: Bacteriophage is a virus that eats bacterium and uses its machinery for its own replication. To understand the mechanism of transduction it is must to study the life cycle of bacteriophage as described below:

1. Lytic or virulent cycle (Fig. 8.8A to G): Bacterial cell is killed with the release of mature phages called lytic cycle. Following are the stages of transduction involving a lytic cycle:

- **Adsorption:** Bacteriophage adsorbs to a bacterium surface by its tail. It is a complementary process between phage base plate and bacterial cell wall receptors. Cell wall receptors are present at different places like Vi Ag of *S. Typhi*, on flagella or sex pili. Cell wall deficient bacteria cannot adsorb the phage.
- **Penetration:** It includes release of phage DNA and entry in to the bacterium. It is possible by formation of hollow core in cell wall by contractile tail sheath which derives energy from phage tail. Hollow core formation facilitated by phage tail lysozyme. After penetration of phage DNA, head and tail sheath remain as ghost on surface. Penetration is also possible practically by direct injection of phage DNA. Infection of bacterium by naked phage DNA called transfection. Sometimes bacterium is infected by multiple phages resulting multiple holes in cell wall and cell lysis without viral multiplication called lysis from without.
- **Biosynthesis:** Phage DNA directs the bacterium's metabolic machinery to manufacture bacteriophage components like head, tail and enzymes.

- **Maturation and assembly:** Phage components synthesized in bacterium undergo maturation and assembly.

- **Release:** After maturation bacterial cell wall gets weakened. Phage enzyme acts on weakened site and ruptures the wall with the lysis of bacterium and release of progenies of bacteriophage. The interval between entry of phage DNA and appearance of 1st intracellular phage particles called eclipse phase. It is a time required for synthesis and assembly of phage particles. The interval between infection of bacterium and 1st release of phage progenies called latent period. Immediately following latent period numbers of phage particles released are rise till it becomes maximum called rise period. Average yield of progeny phage per infected bacterium called burst size. It is estimated practically by infecting one bacterium with phage and counting the release of phage particles over a period of time and plotting on a graph called one step growth curve.

2. Lysogenic or temperate cycle (Fig. 8.8J to L): Phage DNA gets incorporated into the bacterial chromosome called prophage, behaves like additional segment of bacterial chromosome and multiplies along with it called lysogenic or temperate cycle. Bacterium undergoes to this cycle called lysogenic bacterium. This process or cycle also called lysogenic conversion or phage conversion or lysogenization or lysogeny. Prophage confers certain new properties in lysogenic bacterium due to synthesis of new protein that are coded by prophage like

- *C. diphtheriae* that had been lysogenized with β -prophage produces the diphtheria toxin. Elimination of β -prophage makes the strain nontoxic.

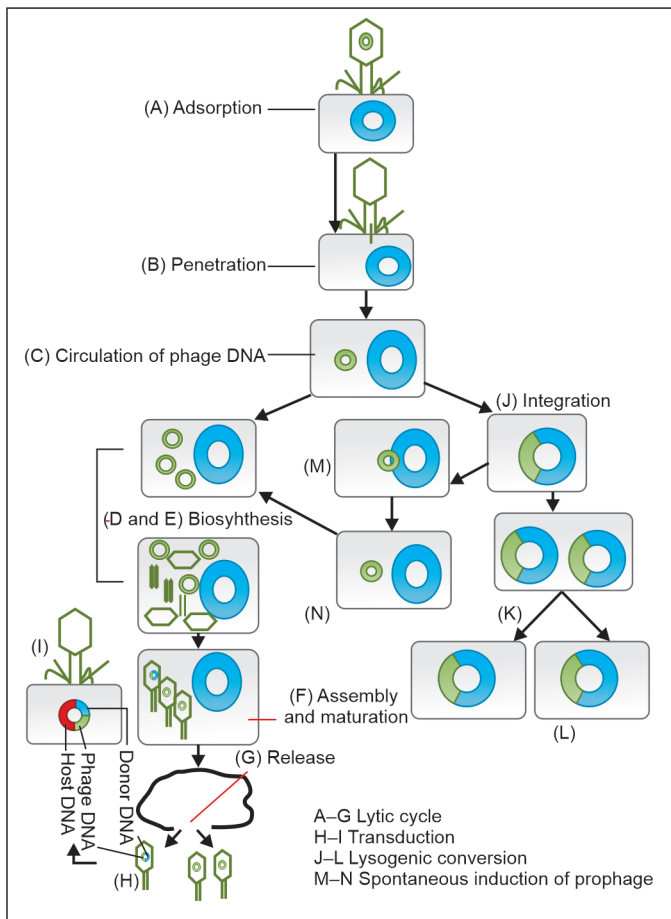


Fig. 8.8: Life cycle of bacteriophage

- Lysogenic bacteria are resist to infection by same or related phages called superinfection immunity
 - It influences antigenic properties of bacterium.
3. **Variation in life cycle stage**
- **Spontaneous induction of prophage (Fig. 8.8M and N):** The prophage sometimes excised from the host chromosome during multiplication of lysogenic bacteria and starts lytic cycle with release of daughter phages called spontaneous induction of prophage. It sometimes carries along with itself a fragment of bacterial chromosome; the subsequent phage progeny may have a piece of chromosomal DNA. When such phage infects another bacterium, newer characteristics coded by that chromosomal gene are conferred.
 - **Pseudolysogeny:** In certain bacteria prophage is not incorporated in host DNA but instead remains free as like plasmid called pseudolysogeny, e.g., *M. tuberculosis*.
 - **Shifting of cycle:** Lysogenic cycle can be shift to lytic cycle by exposure to UV light, H_2O_2 and nitrogen mustard.
 - **Infection by two phages DNA:** If a bacterium simultaneously infected by two same or related phages, it releases two types of progenies. When this occurs many of the progenies are recombinants.

Mechanism of transduction (Fig. 8.8H and I):

Occasionally during the assembly of bacteriophage's components inside the host bacterium, an error occurs called packaging error that resulting the incorporation of host DNA in to bacteriophage genome. The bacteriophage carrying the donor bacterium's DNA adhere on another bacterium and inserts the donor bacterium's DNA into the recipient cell. Transduction is not confirmed to transfer the chromosomal DNA only but episome and plasmid may also be transduced.

History: Bacterial transduction was discovered by Norton Zinder and Joshua Lederberg in 1952 at the University of Wisconsin-Madison in *Salmonella*.

Types of transduction: Two types:

1. **Restricted (specialized) transduction:** It can transfer only those genes that lie adjacent to the prophage. It is best studied by lambda phage. The lambda phage that infects *E. coli* always transfers *gal⁺* gene which is responsible for galactose fermentation.
2. **Generalized transduction:** It can transfer any bacterial gene. This process may occur with phages (lytic phages) that degrade their host DNA into pieces the size of viral genomes. If these pieces are erroneously packaged into phage particles, they can be delivered to another bacterium. Phage P22 of *S. Typhimurium* and P1 and μ of *E. coli* carry out generalized transduction.

Clinical significances of transduction

1. **Transfer of extrachromosomal nucleic acid:** Plasmid (R factor) and episome are also transferred by transduction. For example plasmid mediated penicillin resistant in *Staphylococcus* is transfer from cell to cell by transduction.
2. **Genetic mapping:** Most useful mechanism in prokaryotes for gene transfers and provides an excellent tool for genetic mapping in bacteria.
3. **Treatment of metabolic disease:** It affects the eukaryotic cell and proposed method of genetic engineering in treatment of certain inborn error of metabolism. For example, metabolic defect in fibroblast from galactosemic patients can be corrected by transduction using the lambda phage carrying the *gal* gene.

Differences between lytic/transduction and temperate cycle/lysogenic conversion: Follow Table 8.3.

TABLE 8.3: Differences between lytic and temperate cycle	
Lytic cycle	Temperate cycle
Lysis of bacterium	Host bacterium is unharmed
Transfer of donor bacterium's DNA	Transfer of phage DNA
Phage acts like vehicle or carrier between donor and recipient cells	Phage DNA behaves like additional segment of bacterial chromosome and multiplies along with it and confers new properties

52 C. Lysogenic conversion

Follow lysogenic/temperate cycle of bacteriophage.

D. Conjugation

Definition: Transfer of genetic information from one cell to other cell by formation of tube like structure between two cells called conjugation and such tube called conjugation tube.

History: Conjugation was 1st discovered by Joshua Lederberg and Tatum in 1946 in *E. coli* K 12 strain.

Properties of conjugation: Conjugation is a process where male/donor bacterium mates or makes physical contact with female/recipient bacterium and transfers genetic information. It is like matting in higher organisms but following conjugation female bacterium is converted in to male bacterium.

Mechanisms of conjugation: It is due to formation of conjugation tube between two cells by specialized fimbriae or sex pili present on cell surface. Such fimbriae are encoded by a plasmid called sex or fertility (F) factor. Such plasmid multiplies and acts as donor (copy of it passes to recipient cell). Such plasmid is self transmissible or conjugative. Several such plasmids were discovered and act as donors are collectively called transfer factors.

Clinical significances of conjugation: Conjugation allows transfer of genes (like plasmid, episome) or genetic information from one cell to other as below:

1. **Fertility (F)/sex factor conjugation (Fig. 8.9):** F factor is a plasmid codes for special fimbriae or sex pili and for necessary enzyme of conjugation. Fimbriae or sex pili make a conjugation tube between two cells for gene transfer. Those bacteria that possess F factor are called F^+ , such bacteria have sex pili on their surface and those are lacking called F^- . It mediates its own transfer. Vertical (inheritance) or horizontal (transfer) transmission maintains plasmid. This results in the transfer of an F^+ plasmid (coded only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. A F^- female cell will become F^+ male when it receives the fertility factor from another F^+ cell and can make a sex pilus. During conjugation, no cytoplasm or cell material except plasmid passes from donor to recipient. Two cells mate and form conjugation tube through which F factor can transferred. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time. After conjugation, the cells break apart. Following successful conjugation the recipient becomes F^+ and the donor remains F^+ .
2. **R factor conjugation (Fig. 8.10):** R factor is a plasmid, which is responsible for drug resistance. It presents in gram-negative bacteria. It has two components such as RTF (resistance transfer factor) and 'r' determinant. RTF is the first part that codes for self transfer (like

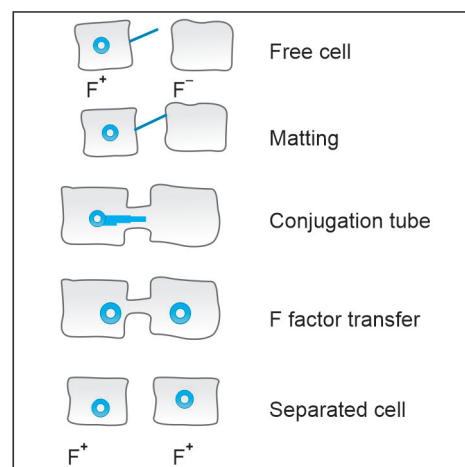


Fig. 8.9: F/Sex factor conjugation

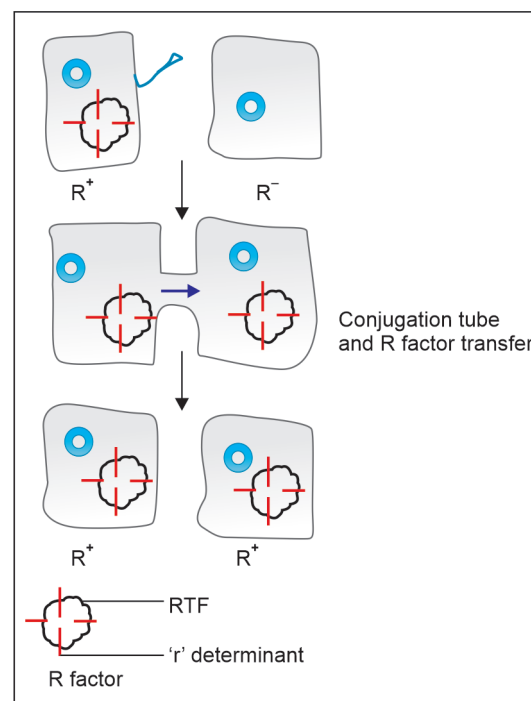


Fig. 8.10: R factor conjugation

F factor) and hemolysin-enterotoxin production in *E. coli* while r determinant is the second part that codes for antibiotic resistance. Transfer of drug resistance by R factor called transferable or episomal or infectious drug resistance. R plasmid may confer resistance to as many as eight different antibiotics at once upon the cell and by conjugation it can be rapidly transfer to bacterial population. Both F factor and R factor are self transferable but the differences are that the latter has additional genes coded for drug resistance and hemolysin-enterotoxin production. During conjugation there is transfer of resistance (R) factor from a donor bacterium to a recipient. The recipient becomes multiple antibiotic resistant and male, and is now able to transfer R-plasmid to other bacteria. When the recipient cell acquires entire R factor, it too expresses antibiotic resistance. Sometimes, RTF may disassociate from the r determinant and the two

components may exist as separate entities. In such cases the host cell remains resistant to antibiotics; it cannot transfer this resistance to other cells. Transfer of multiple antibiotic resistances by conjugation has become a major problem in the treatment of certain bacterial diseases.

3. **Colicinogenic (Col) factor conjugation:** Col factor is a plasmid which is responsible for bacteriocin production. It can also transfer via conjugation.
4. **Episome conjugation/sexduction (Fig. 8.11):** It includes the transfer of episome (plasmid integrated with chromosome). Cell carrying episome can transfer gene with high frequency hence called Hfr cells. Sometimes episome detached from chromosome along with some part of chromosome and becomes free called F prime (F') factor. When F prime (F') factor cell mates to recipient cell (F⁻), it transfers F prime (F') factor and host chromosome linked with it called sexduction. Following successful conjugation both cells become F'.

E. Transposition

Definitions: Transfer of genetic information from one cell to other cell via transposon called transposition.

History: It was discovered by Barbara Mc Clintock in plants during work in the 1940s and 1950s, for which she was awarded with Nobel Prize in 1983.

Properties of transposition

- **Random movement:** This mobile gene can move from one DNA to any DNA or even to another location on

the same DNA. The movement is not totally random; there are preferred sites in a DNA molecule at which the transposable genetic element will be inserted.

- **Not capable of self replication:** It is not self replicating and depends on plasmid or chromosomal DNA for replication.
- **Transposition can be accompanied by duplication:** In many instances transposition results in removal of the element from the original site and insertion at a new site. However, in some cases the transposition event is accompanied by the duplication of the transposable genetic element. One copy remains at the original site and the other is transposed to the new site.

Structural types and significances of transposition

1. Insertion sequences (IS)

- **Structure:** It is the small segment of DNA about 1–2 kb without any essential genes called IS. Such DNA encodes for transposition.
- **Significances**
 - Mutation: The introduction of an insertion sequence into a bacterial gene will result in inactivation of gene.
 - Selection of site for plasmid insertion in chromosome: The sites at which plasmid inserted into the bacterial chromosome are at or near insertion sequence in the chromosome.
 - Phase variation: In *Salmonella* there are two genes, which code for two antigenically different flagellar antigens. The expression of these genes is regulated by an insertion sequence.

2. Transposons

- **Structure:** It is the large segment of DNA about 4–25 kb with essential genes. It carries one or more genes in the center and the two ends carrying inverted repeat sequences complementary to each other but in reverse order. Because of these, two ends contain single stranded loop and center contains double stranded stem formed by H₂ bonding between inverted repeat sequences.

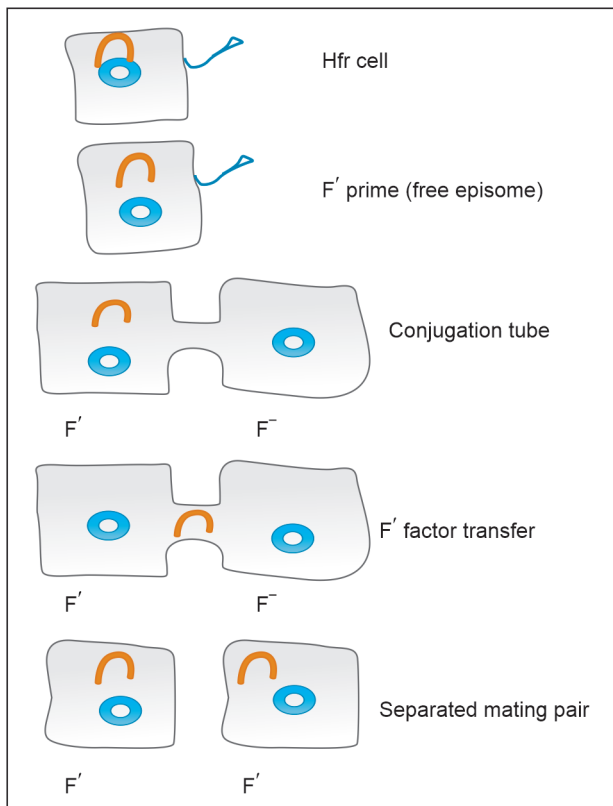


Fig. 8.11: F' factor conjugation (sexduction)

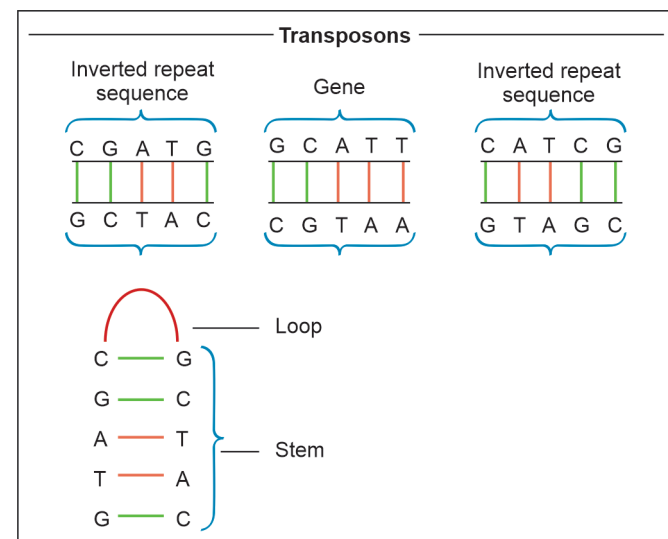


Fig. 8.12: Structure of transposon

• Significances

- Drug resistance: Many antibiotic resistance genes are located on transposon. When it jumps on a transferable plasmid it carries multiple drug resistance in a bacterium and causes major medical problem.
- Genetic engineering: It is useful in laboratory for gene manipulation.

ACCESS YOURSELF

Essay/Full Question

1. Genetic structure of bacteria.
2. Genotypic and phenotypic variation of bacteria.

Short Notes

1. Bacterial DNA
2. Plasmid
3. Mutation
4. Gene transfer mechanisms
5. Transduction
6. Bacterial conjugation.

Short Questions for Theory/Viva Questions

1. What are sense codons and non-sense codons?
2. What are introns and exons?
3. What is plasmid?
4. Name the two chromosomal and two extrachromosomal nucleic acids of bacteria.
5. What are episome and transposons?
6. Write the four differences between phenotypic and genotypic variation.

Comments on

1. Transposon is also called jumping gene.

MCQs for Chapter Review

Genetic Structure

1. Bacterial chromosome consists of double stranded DNA, in a circular configuration the length of which is about:
 - a. 100 μm
 - b. 1000 μm
 - c. 10000 μm
 - d. 100000 μm
2. Plasmid is:
 - a. Intracytoplasmic
 - b. Extrachromosomal
 - c. a + b
 - d. None
3. Plasmid:
 - a. Involved in multiple drug resistance transfer
 - b. Involved in conjugation
 - c. Imparts capsule formation
 - d. Imparts pili formation
4. Jumping gene:
 - a. Transposon
 - b. Episome
 - c. Cosmid
 - d. Plasmid

Genotypic Variation

5. Natural method of horizontal gene transfer among bacteria includes:
 - a. Electroporation
 - b. Transduction
 - c. Transformation
 - d. Conjugation
 - e. Mutation

6. Mechanism of direct transfer of free DNA:

- a. Transformation
- b. Conjugation
- c. Transduction
- d. None

7. Virus mediated transfer of host DNA from one cell to another is known as:

- a. Transduction
- b. Transformation
- c. Transcription
- d. Integration

8. Lysogenic conversion is:

- a. New properties in a bacterium due to integration of phage genome
- b. Transfer of DNA from one bacterium to another by a bacteriophage
- c. Transfer of free DNA
- d. Transfer of genome during physical contact

9. The discovery of "gene transformation" came from the study of one of the following bacteria:

- a. *Bacillus subtilis*
- b. *Streptococcus pyogenes*
- c. *Streptococcus pneumoniae*
- d. *Escherichia coli*

10. F factor integrated with bacterial chromosome to form:

- a. HFr
- b. RTF+r
- c. F⁻
- d. RTF

11. Conjugation does not involve:

- a. Bacteriophage
- b. HFr
- c. F factor
- d. Plasmid

12. The role of plasmid in conjugation was first described by Lenderberg and Tatum in:

- a. *H. influenzae*
- b. *Corynebacterium*
- c. *Pseudomonas*
- d. *Escherichia coli*

13. Bacteria may acquire characteristics by all of the following except:

- a. Taking up soluble DNA fragments across their cell wall from other species
- b. Incorporating part of host DNA
- c. Through bacteriophage
- d. Through conjugation

14. Insertion sequences are _____ in size:

- a. 1–2 kb
- b. 2–3 kb
- c. 3–4 kb
- d. 4–5 kb

Answers and Explanation of MCQs

1. b
 - Follow section, **genetic structure** → **DNA** for explanation.
2. c
 - Plasmid is an extrachromosomal and intracytoplasmic DNA material.
3. a, b, c and d
 - Follow section, **genetic structure** (extra-chromosomal part → **plasmid**) for more details.
4. a
 - Follow section, **genetic structure** (extra-chromosomal part → **transposon**) for explanation

5. b, c and d

- Follow section, **bacterial variation (genotypic variation → gene transfer mechanisms/methods)** for more details.

6. a

7. a

8. a

9. c

- Follow section, **bacterial variation (genotypic variation → gene transfer mechanisms/methods → lysogenic conversion)** for explanation of answers of MCQs 6–9. Gene transformation came from the study of *Streptococcus pneumoniae* by Griffith.

10. a

- F factor is plasmid but in question its written as ‘integrated with bacterial chromosome’ so it considered as episome. Cells contain episome are transferring it with high frequency called HFr cells (high frequency of recombination), so option a is right answer.

11. a

- Bacteriophage is involved in transduction.

12. d

- Conjugation was 1st discovered by Joshua Lederberg and Tatum in 1946 in *E. coli* K 12 strain.

13. b

- Bacteria are not acquiring any properties from host cell DNA but they may acquire characteristics by
 - taking up soluble DNA fragments across their cell wall from other species called transformation.
 - through bacteriophage called transduction.
 - through conjugation tube called conjugation.

14. a

- Follow section, **bacterial variation [genotypic variation → gene transfer mechanisms → transposition → insertion sequence (IS)]** for more details.

Precipitating Factors of Bacterial Infections

Chapter Outline

- Definitions
- Precipitating Factors
 - Agent factors (virulence factors or determinants of virulence)
 - Host factors
 - Environmental factors

DEFINITIONS

Pathogenicity: Ability of a microbial species to produce the disease called pathogenicity.

Virulence: Ability of microbial strain to produce the disease called virulence.

Exaltation: Enhancement of virulence called exaltation. It is demonstrated by serial passage in susceptible individual.

Attenuation: Reduction of virulence called attenuation. It is demonstrated by serial passage in unfavorable host, repeated subculture, growth in high temperature, desiccation, long storage of culture or by using anti-septics like formalin.

PRECIPITATING FACTORS

Precipitating factors are also known as epidemiological determinants. There are three types of epidemiological determinants affecting the virulence of bacteria such as agent factors (virulence factors or determinants of virulence), host factors and environmental factors. All are described below.

Agent Factors (Virulence Factors or Determinants of Virulence)

Agent factors are following three types.

Intracellular or Cell Associated Factors

Bacterial adhesins: The process of attachment of pathogens with host cells called adhesion. Organs used for adhesion are called adhesins. Adhesins may account for tissue tropism and host specificity. As they used for virulence, loss of adhesins make the strain avirulent. Adhesins are usually protein and antigenic. They produce antibodies which are protective; hence,

they can be used as method of prophylaxis in certain bacteria like *E. coli* diarrhea in calves and piglets and gonorrhea in humans are prevented by using vaccine prepared from fimbrial antigens. Following are the different types of adhesins:

1. Fimbrial adhesins or pilus adhesins: Follow Ch. 6 for more details.

2. Nonfimbrial adhesins or non pilus adhesins:

- Protein receptors in staphylococci: *Staph. aureus* has receptors for many mammalian proteins like fibronectin, fibrinogen, IgG and C1q which help in adhesion with host cells.
- Protein F in *Streptococcus pyogenes*: It helps in attachment with pharyngeal wall.
- Extracellular surface protein in *Enterococcus*: It helps in adhesion.
- Slime layer (glycocalyx): It inhibits phagocytosis. Slime layer made up of glycocalyx has ability to bind bacteria with damaged tissues. It also helps bacteria to bind with plastic material of medical devices like catheters, suture materials, pacemakers, implants, Ryle's tube, etc., and forms the growth on such devices called biofilm. Use of such devices cause diseases. Biofilm is formed by few bacteria like *Staph. albus* (*Staph. epidermidis*), *P. aeruginosa*, etc.
- Outer membrane protein (OMP) Ag: It presents in *N. gonorrhoeae*, *H. influenzae*, *Chlamydia* spp., *Rickettsia* spp. (spotted fever group), etc., which contributes in adhesion and invasion.
- Fragment B of diphtheria toxin: Diphtheria toxin has two fragments like A and B. A is active fragment while, fragment B is for binding of toxin to cells. Antibody to fragment B protects binding of toxin to cells.

- **D-galactose:** It presents in *Listeria monocytogenes* and helps to bound with D-galactose receptors on macrophage's polysaccharides of Peyer's patches and epithelial cells.
- **Internalins (A and B are subtypes):** They present in *L. monocytogenes* and help to attach with phagocytic cells.
- **Adhesin proteins in *Yersinia* spp.:** Different types like MyF Ag, pH6 Ag, inv (invasive) protein, ail (attachment and invasive locus) protein and Yad A (*Yersinia* adhesin A → it binds with collagen and fibronectin to aid the invasion of tissue by organism and also it inactivates the complement).
- **Filamentous hemagglutinin (FHA):** It appears as filamentous structure under electron microscope, hence the name. It adheres the *B. pertussis* with cilia of respiratory epithelium. It also favors adhesion of other bacteria like *H. influenzae* and *Strept. pneumoniae* to respiratory epithelium called piracy of adhesion.
- **Adhesins in *Mycoplasma pneumoniae*:** Different adhesins like P1 (cytadhesin/protein Ag), bulbous enlargement and glycolipid Ag help to adhere the bacteria with RBCs (called hemadsorption), respiratory epithelium and with other cells.
- **Hook of *Leptospira*:** Hooks on the ends of *Leptospira* allow it to attach and latch on to host tissues.
- **Cell surface lectin in *Chlamydia*:** It also acts as an adhesin.

Peptidoglycan: It provides rigidity and structural integrity to cell.

Protein antigen: M protein in streptococci acts as virulence factor and inhibits the phagocytosis.

Somatic (carbohydrate) Ag: It plays role in non-suppurative lesions in streptococci.

Endotoxins: Following are different types of endotoxins as per biochemical nature.

- **Lipopolysaccharides (LPSs):** LPSs are present in gram-negative not in gram-positive bacteria and responsible for endotoxic activities as mentioned below. However, LPSs of *V. cholerae* have no role in the pathogenesis of disease (called cholera) in human, but intraperitoneal inoculation in mouse causes fatal effects. LPSs have different biological activities such as pyrogenicity, activation of complement, leucocytosis, macrophage inhibition, lethal action, inhibition of glucose and glycogen synthesis in liver, interferon release, depression of blood pressure, leucopenia, stimulation of B lymphocytes, induction of prostaglandin synthesis, protection of bacilli from phagocytosis (in *E. coli*), protection of bacilli from serum complement (in *Klebsiella* spp.), DIC, septic shock (endotoxic shock) and possible death.

- **Lipooligosaccharide:** It presents in *H. influenzae* and acts as endotoxin like LPS as described above.
- **Endotoxin in *Listeria*:** An early study suggested that *L. monocytogenes* is unique among gram-positive bacteria that might possess LPS, which serves as an endotoxin. Later, LPS was found not to be true endotoxin. *Listeria* cell wall consistently contains lipoteichoic acid resembles the LPS of gram-negative bacteria in both structure and function.

Other components related to bacterial cell

- **Capsular (K) Ag:** Capsular (K) antigen presents in different bacteria (like *K. pneumoniae*, *Strept. pneumoniae*, etc.), and fungus (like *C. neoformans*) inhibits the phagocytosis; however capsule of *Bordetella pertussis* does not contribute in virulence.
- **Slime layer:** It inhibits the phagocytosis.
- **Vi antigen:** It presents in *S. Typhi*. It inhibits the phagocytosis and resists complement activity. It resists bacterial lysis by alternative pathway and by peroxidase killing. Bacilli with Vi Ag are causing more consistent disease than those are lacking.
- **Antigenic cross reactivity:** Like cytoplasmic membrane and vascular intima in *Streptococcus*.
- **Virulence marker antigen (VMA):** Invasive property in EIEC and *Shigella* is due to a special type of OMP called VMA (virulence marker antigen) which is plasmid encoded, responsible for invasion, multiplication of bacilli and destruction of epithelial cells. It is detected by VMA ELISA or by culture cells like Hela or Hep-2 cells.

Genetic factors

- **Plasmid:** It codes for many virulence properties of bacteria. Follow **Ch. 8** for more details.
- **Tox⁺ gene:** It presents in diphtheria bacilli responsible for toxin production.

Extracellular Factors

Enzymes

- **Coagulase:** It is produced by staphylococci. It forms fibrin barrier around bacteria and produces localized lesions.
- **Hyaluronidase:** It splits the components of intracellular connective tissues.
- **Fibrinolysin, proteases and nucleases (DNase):** They help in initiation and spread of infection by breaking the fibrin barrier.
- **Lipase:** It helps to infect skin and subcutaneous tissues.
- **Enzymatic inactivation of the antibiotics:** Follow **Ch. 116**, for more details.
- **IgA protease:** It is produced by *N. meningitidis*, *N. gonorrhoeae*, *H. influenzae* and *Strept pneumoniae*. It destroys the IgA and reduces the local immunity.
- **Amidase:** In pneumococci, this autolytic amidase activated by surface-active agents such as bile or bile salts, cleaves bond between alanine and muramic

acid of peptidoglycan of cell wall, resulting in lysis of organisms and release of bacterial products in medium.

- **Neuraminidase (receptor destroying enzyme) in *V. cholerae*:** Formerly called 'cholera lectin'. It cleaves mucus and fibronectin and releases vibrios which are bounded to intestinal epithelium and favors their spread to other intestinal parts.
- **Other diagnostically useful enzymes are:** Catalase, phosphatase, urease, oxidase, etc.

Exotoxins: Bacteria produce two types of toxins like endotoxin (described above) and exotoxin. Exotoxins are described below:

- **Properties of exotoxins**
 - In contrast to endotoxins, which are integral part of bacteria; exotoxins are actively synthesized and released free in medium.
 - Exotoxins are produced by a variety of bacteria including gram-positive and gram-negative bacilli
 - They are antigenic and can be toxoided.
 - Their activity can be neutralized by antitoxins.
 - Most of the toxins have enzymatic activity.
 - Many toxins are extraordinarily powerful, small amount can be lethal.
 - Toxins can be separated from the culture broth by filtration.
 - Exotoxins are heat labile or heat stable.
- 1. **Heat labile toxin producing bacteria:** *Cl. perfringens* (*Cl. welchii*), LT (labile) of ETEC, *V. cholerae*, diarrheal illness producing toxin of *B. cereus*.
- 2. **Heat stable toxin producing bacteria:** *Staph. aureus*, ST (stable) of ETEC, *Y. enterocolitica*, emetic illness producing toxin of *B. cereus*.
- **Exotoxins producing bacteria:** Exotoxins are mostly produced by gram-positive bacteria and rarely by gram-negative bacteria.
 1. **Exotoxins producing gram-positive bacteria:** *Staph. aureus*, *Strept. pyogenes*, *Strept. pneumoniae*, *B. anthracis*, *B. cereus*, *Cl. welchii*, *Cl. tetani*, *Cl. botulinum*, *C. diphtheriae*, *M. ulcerans* (*M. buruli*), etc.
 2. **Exotoxins producing gram-negative bacilli:** ETEC, EIEC, EHEC, *Shigella* spp., *Y. enterocolitica*, *V. cholerae*, *P. aeruginosa*, *C. jejuni*, *H. pylori*, *B. pertussis*, *F. fusiforme*, etc.
- **Nomenclature of exotoxins**
 1. **According to targeted cells/organs:** Exotoxins which attack a variety of cells are generally called cytotoxins. Further naming is done as per cell like hemolysin for RBCs, leucocidin for leucocytes, neurotoxin for nerve cells, enterotoxin for intestinal cells, etc.
 2. **According to the species, which produces them and from the disease with which they are associated:** Like cholera toxin from *Vibrio cholerae*, causes cholera and tetanus toxin from *Clostridium tetani*, causes tetanus.

3. **According to activities:** Like adenylate cyclase or exotoxin A of *Pseudomonas aeruginosa*.

- **Biological actions of exotoxins**

1. **Cytotoxicity:** Following toxins are causing cell lysis.
 - Hemolysin: For example, lysis of RBCs in *Staph. aureus*.
 - Leucocidin: For example, damage to polymorphonuclear leucocytes in *Staph. aureus*.
 - Verotoxin/verocytotoxin (also have enterotoxic and neurotoxic properties): It includes Shiga toxin and shiga-like toxin (SLT). Shiga toxin produced by *Shigella dysenteriae*. SLT produced by enterohemorrhagic *E. coli*, *V. cholerae*, *Aeromonas hydrophila* and *Campylobacter jejuni*. Both toxins have *in vitro* effect and *in vivo* effect. *In vitro* effect includes cytotoxicity to the cultured Vero cells (African monkey kidney cells used for cell culture are called Vero cells). For *in vivo* effect toxins have two subunits like binding (B) and active (A). Subunit A divided in to two fragments like A1 and A2. B helps in adhesion with host cells. A2 links A1 to B. A1 inactivates the host cell 60S ribosome and inhibit the protein synthesis leading to cell death. Cell death resulting discontinuity of mucosa and hemorrhage (bloody diarrhea).
 - Diphtheria toxin: It is produced by *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. It inhibits protein synthesis by inactivating elongation factor (EF-2) and causing cell death.
 - Botulinum C2: It is cytotoxic in nature while other types like A, B, C1 and D-G are neurotoxins.
 - Toxin A and B of *Clostridium difficile*.
 - Vacuolating cytotoxin produced by *H. pylori* produces injury to host cells.
 - Exotoxin A of *P. aeruginosa*: It has same actions as like diphtheria toxin.
2. **Enterotoxicity:** Following toxins are causing outpouring of electrolytes and fluid in to intestinal lumen responsible for watery diarrhea.
 - Toxin causing diarrheal illness of *Bacillus cereus*.
 - LT and ST produced by enterotoxigenic *E. coli*.
 - Verotoxin/verocytotoxin (shiga toxin and SLT).
 - Enterotoxin of *Y. enterocolitica*.
 - Cholera toxin produced by *Vibrio cholerae*.
 - Enterotoxin of *P. aeruginosa*.
 - Enterotoxin of *C. jejuni*.
3. **Neurotoxicity:** Following toxins act on nerve system.
 - Enterotoxin of *Staph. aureus*: It causes food poisoning features by vagal stimulation.
 - Toxin of *Bacillus cereus*: It causes emetic illness.
 - Tetanospasmin from *Cl. tetani*: It locks synaptic inhibition (presynaptic) in spinal cord by blocking the release of inhibitory neurotransmitters like glycine and gamma amino butyric acid (GABA)

- Resulting in uncontrolled spread of impulses
- Tonic muscle rigidity and spasm.
- Botulinum (A, B, C1 and D-G except C2) from *Cl. botulinum*: It blocks the production or release of acetylcholine and causes descending flaccid paralysis (arflexia).
- Verotoxin/verocytotoxin (shiga toxin and SLT): Vero toxin causes paralysis and death on injection in mice/rabbit. It does not act directly on CNS but on the blood vessels of CNS, neurotoxic effect is secondary.
- 4. **Enzyme-based actions:** Exotoxins produced by *Cl. welchii* have enzyme based actions.
- 5. **Connective tissues action:** Following toxins act on the extracellular matrix of connective tissue and aid in spreading the infection by breaking down extracellular matrix of connective tissue.
 - Kappa toxin (collagenase) produced by *Cl. welchii*.
 - Exfoliative toxin produced by *Staph. aureus*.
- 6. **Hormonal actions:** Pertussis toxin of *B. pertussis* activates the intracellular cAMP in pancreatic islets and increases the insulin secretion in animals not in humans.
- 7. **Immune-mediated actions**
 - Lymphocytosis produced by pertussis toxin of *B. pertussis*.
 - Superantigens: Staphylococcal enterotoxin, staphylococcal toxic shock syndrome toxin (tsst-1) and streptococcal pyrogenic exotoxin (exotoxin A and exotoxin B) are superantigens. They stimulate large numbers of T cells. Follow Ch. 18 for more details.
- **Regulatory genes of exotoxins:** Three types as below:
 1. **Plasmid mediated exotoxins**
 - Enterotoxin production by *Staph. aureus*
 - Exfoliative toxin-B (heat labile) by *Staph. aureus*
 - Anthrax toxin by *B. anthracis*
 - Tetanospasmin by *Cl. tetani*
 - Labile toxin (LT) of *E. coli*
 - Stable toxin (ST) of *E. coli*.
 2. **Chromosome mediated exotoxins**
 - Staphylococcal exfoliative toxin A (heat stable)
 - Streptococcal pyrogenic exotoxins B
 - Shiga toxin.
 3. **Bacteriophage mediated exotoxins**
 - Staphylococcal toxic shock syndrome toxin
 - Streptococcal pyrogenic exotoxins A and C
 - Botulinum type C and D
 - Diphtheria toxin (Tox+ gene/ β -prophage)
 - Shiga like toxin (SLT)
 - Labile toxin (LT) of *V. cholerae*.
- **Detection of exotoxins**
 1. **Culture methods**
 - *In vivo*: By using laboratory animals.

- *In vitro*: By using cell/tissue culture, like Vero cell culture for verotoxin.

2. **Serological tests:** Precipitation (like Elek's gel precipitation test for diphtheria toxin), agglutination, ELISA, RIA, etc., can be used.

3. **Molecular tests:** DNA probe.

- **Clinical uses:** Uses and other details of different toxins are described in respective chapters.
- **Differences between two toxins:** Follow Table 9.1.

Biological active substances: They are released by *Cl. perfringens* in gas gangrene as listed below:

- Hemagglutinin: Active against RBCs of humans and animals in gas gangrene.
- Bursting factor: For muscle lesion in gas gangrene.
- Circulating factor: It increases sensitivity to capillary bed and also inhibits phagocytosis.
- Histamine.

Pigments: Follow Ch. 10 for more details.

Others Factors

Invasiveness: It is defined as an ability of a pathogen to spread in the host tissues, e.g., *Streptococcus* is highly invasive producing septicemia whereas *Staphylococcus* is less invasive producing localized lesions. However, some pathogens are less invasive although produce fatal diseases, e.g., *Cl. tetani* confined to site of entry and produce fatal disease by elaborating the toxin.

TABLE 9.1: Differences between two exotoxin and endotoxin

Exotoxin	Endotoxin
Produced by gram-positive bacteria and also by GNB	Produced by GNB
Converted in toxoid with formalin treatment	Not converted in toxoid
Proteins	LPSs (lipooligosaccharide in <i>H. influenzae</i>)
Heat labile (gets denatured on boiling) and few are heat stable	Heat stable (Not denatured on boiling)
MW: 50–1000 kDa	MW: 10 kDa
Actively secreted by cells and diffuses into surrounding medium	Part of cell wall and do not diffuses into surrounding medium
Separated by filtration	Separated by cell lysis
Enzymatic	Nonenzymatic
Specific pharmacological effect for each toxin	Nonspecific
Specific tissue affinity	No specific tissue affinity
Active in very minute dose (<1 μ g)	Active only in very large dose (>100 μ g)
Highly antigenic	Weakly antigenic
Action specifically neutralized by antibody	Neutralization by antibody is ineffective
Detected by different tests as described earlier in text	Detected by limulus lysate assay
Causes exotoxemia	Causes endotoxemia

60 Communicability: It is defined as an ability of micro-organism to spread from one host to another host. It plays major role in development of epidemic or pandemic.

Infecting dose and lethal dose

• Definitions

- Infectious dose (ID): It is the amount of pathogens (measured in numbers of microorganisms) required to cause an infection in the host.
- Lethal dose: It is the amount of pathogens (measured in numbers of microorganisms) required to cause death in the host.
- MID (minimum infecting dose) or MLD (minimum lethal dose): It is the minimum numbers of microorganisms required to produce clinical evidence of infection or death respectively.
- ID₅₀ or LD₅₀: It is the dose required to infect or to kill 50% animal under standard condition.

• Effective factors over infective dose

- Age: Low dose requires in lower age.
- Gastric acidity: Many microbes are susceptible to gastric acidity (like *Salmonella*, *Vibrio cholerae*, etc.), require high dose while many resist gastric acidity (like *Shigella*, etc.), require low dose. Factors like use of antacids, presence of local diseases like hypochlorhydria, achlorhydria, etc., can alter the gastric acidity are also effective over ID.
- Use of antibiotics: Antibiotics reduce the competition between pathogens and normal flora, so they decrease the ID.
- Others: Like local surgery, IDs, etc., are also effective over ID.

• Infective dose by different microbes: Follow Table 9.2.

Route of infection: Initiation of infection depends on route of entry, e.g., *V. cholerae* is infective orally only while *Streptococcus* is infective by any mode.

TABLE 9.2: Infective dose by different microbes

Bacteria	Infective dose (ID)
Low infective dose	
<i>Mycobacterium tuberculosis</i>	<10 bacilli
<i>Escherichia coli</i> O157:H7	<10 bacilli
<i>Francisella tularensis</i>	10–50 bacilli
<i>Shigella</i>	10–100 bacilli
<i>Campylobacter jejuni</i>	500–10 ³ bacilli
<i>Cryptosporidium parvum</i>	10–30 oocysts
<i>Entamoeba coli</i>	1 cyst
<i>Giardia lamblia</i>	Few cysts
High infective dose	
<i>Bacillus anthracis</i>	10 ⁴ spores
<i>Escherichia coli</i>	10 ⁶ –10 ⁸ bacilli
<i>Salmonella</i> Typhi	10 ³ –10 ⁶ bacilli
<i>Vibrio cholerae</i>	10 ⁴ –10 ⁶ bacilli

Site of infection: Bacteria differ in their site of selection in host and their ability to damage different organs, e.g., *M. tuberculosis* injected in rabbits damages kidneys and infrequently to liver and spleen while in guinea pigs lesions are mainly in liver and spleen and sparing the kidneys.

Intracellular location of microbes: Intracellular location of the organisms helps to escape the host defense and the effect of antibiotics. There are two types of microbes as per intracellular location like obligate and facultative as shown in Table 9.3.

Bacterial secretory system: Bacteria utilize particular strategies to release the virulence factors called bacterial secretory system. It is the important mechanism in bacterial survival and pathogenesis. There are six types of secretory system from type I to type VI.

Regulatory genes: Above all virulence factors of bacteria, responsible for pathogenicity are under control of specific genes located on the chromosome called pathogenicity islands. Removal of such genes make the bacterium avirulent. Pathogenicity islands have been detected in many bacteria like *Staphylococcus aureus*, *Escherichia*, *Shigella*, *Salmonella*, *Vibrio cholerae* *Helicobacter*, etc.

Host Factors

1. **Age:** Certain infections are common at particular age like *H. influenzae* B infection is common in pediatrics.
2. **Gender:** UTI is more common in female due to close proximity of genitals to anus allows the fecal contamination.

TABLE 9.3: Intracellular location of microbes

Obligate intracellular	Facultative intracellular
Definition	
These microbes are not able to synthesize their own ATP and remain dependent on host cells	These microbes are able to synthesize their own ATP and remain independent on host cells and can live extracellularly
Bacteria	
<i>M. leprae</i> <i>Chlamydia</i> spp. <i>Rickettsia</i> spp. <i>C. burnetii</i>	<i>M. tuberculosis</i> , <i>S. Typhi</i> <i>Y. pestis</i> , <i>N. meningitidis</i> <i>Nocardia</i> spp., <i>Brucella</i> spp., <i>Francisella tularensis</i> , <i>L. monocytogenes</i> , etc.
Viruses	
All viruses	
Fungi	
<i>P. jirovecii</i>	<i>H. capsulatum</i> and <i>C. neoformans</i>
Parasites	
<i>T. gondii</i> , <i>C. parvum</i> , <i>Plasmodium</i> spp., <i>Leishmania</i> spp., <i>Babesia</i> spp., <i>Trypanosoma</i> spp.	

3. **Immune status:** Immunodeficiency status favors certain infections like *S. Typhi*, *M. tuberculosis*, etc.
4. **Blood groups:** *N. gonorrhoeae* infection is common in group O. *V. cholerae* infections is common in blood group B and least in blood group AB. Exact reasons for all these are not known.
5. **Deficiency of complement components:** Deficiency of C₅–C₉ components favor the meningococcal infection.
6. **Occupation:** Like laboratory infection in laboratory workers.
7. **Gastric acidity:** It affects over the infective dose of bacteria.

Environmental Factors

These include overcrowding, humidity and presence of reservoirs or sources or transmitting agents of infection (e.g., vectors) in environment.

ACCESS YOURSELF

Essay/Full Question

1. Epidemiological determinants of virulence of bacteria.

Short Notes

1. Bacterial adhesions.
2. Bacterial exotoxin.

Short Questions for Theory/Viva Questions

1. Write four examples of bacterial adhesions.
2. What are attenuation and exaltation?
3. Name the four bacteria producing IgA protease.
4. Write the four functions of endotoxin.
5. Name the four GNB producing the exotoxin.
6. Name the four bacteria producing the neurotoxin.
7. How following toxins are showing neurotoxicity?
8. Tetanospasmin, botulinum, shiga toxin and shiga-like toxin.
9. What is biofilm?
10. What is pathogenicity Island?

Comments on

1. IgA protease is known to reduce local immunity.
2. Removal of pathogenicity islands make the bacteria (microorganisms) avirulent.
3. UTI is more common in female.

MCQs for Chapter Review

Definitions

1. **Exaltation is:**
 - a. Decreased virulence
 - b. Increased virulence
 - c. No change
 - d. None

Precipitating Factors

2. **Adhesin is useful in:**
 - a. Motility
 - b. Bacterial attachment
 - c. Toxigenicity
 - d. Bacterial division
3. **The endotoxin which leads to endotoxic shock is actually:**
 - a. Lipoprotein
 - b. Lipopolysaccharide
 - c. Polysaccharide
 - d. Polyamide

4. All are true except:

- a. Exotoxin has enzymatic action
- b. Endotoxin has enzymatic action
- c. Exotoxin is highly antigenic
- d. Endotoxin is weakly antigenic

5. Endotoxin from gram-negative organism is:

- a. Polysaccharide
- b. Glycoprotein
- c. Lipoprotein
- d. Lipopolysaccharide

6. True about exotoxins:

- a. Lipopolysaccharide
- b. Not antigenic
- c. Can be toxoided
- d. Heat stable

7. Exotoxins are:

- a. Lipopolysaccharide in nature
- b. Produced by gram-negative bacilli
- c. Highly antigenic
- d. Very stable and resistant to chemical agents

8. Septic shock is due to:

- a. Protein
- b. Lipopolysaccharide
- c. Techoic acid
- d. Peptidoglycan

9. Heat stable enterotoxin causing food poisoning is caused by all the following except:

- a. *Bacillus cereus*
- b. *Yersinia enterocolitica*
- c. *Staphylococcus*
- d. *Clostridium perfringens*

10. Gram-negative bacterium producing exotoxin is:

- a. *V. cholerae*
- b. *Sh. dysenteriae*
- c. *C. jejuni*
- d. All of above

11. True about mechanism of bacterial toxins:

- a. Cholera toxin acts by inhibition of guanyl cyclase
- b. Botulinum toxin inhibits Ach release
- c. Shiga toxin of *Shigella dysenteriae* acts by inhibiting protein synthesis
- d. Diphtheria toxin acts by inhibiting protein synthesis

12. Endotoxin of following gram-negative bacteria does not play any part in the pathogenesis of the natural disease:

- a. *E. coli*
- b. *Klebsiella*
- c. *Vibrio cholerae*
- d. *Pseudomonas*

13. Enterotoxin is produced by all except:

- a. *Clostridium perfringens*
- b. *Staphylococcus aureus*
- c. *Streptococcus pyogenes*
- d. *Bacillus cereus*

14. *Salmonella Typhi* is the causative agent of typhoid fever. The infective dose of *S. Typhi*:

- a. One bacillus
- b. 108–1010 bacilli
- c. 102–105 bacilli
- d. 1–10 bacillus

15. All of the following organisms are known to survive intracellularly except:

- a. *Neisseria meningitidis*
- b. *Salmonella Typhi*
- c. *Streptococcus pyogenes*
- d. *Legionella pneumophila*

16. Which of the following are intracellular?

- a. Viruses
- b. *Chlamydiae*
- c. *Mycoplasma*
- d. *Rickettsia*

17. Obligate intracellularly organisms is:

- a. *Mycoplasma*
- b. *Chlamydia*
- c. *Cryptococcus*
- d. *H. pylori*

1. b

- Decreased virulence called attenuation and increased virulence called exaltation.

2. b

- Adhesin is the organ of adhesion and useful in bacterial attachment with host cells.

3. b

- Endotoxic shock is due to endotoxin which is lipopolysaccharide in nature while exotoxin is protein in nature.

4. b

5. d

6. c, d

7. b, c

- Follow **Table 9.1** for explanation of answers of MCQs 4–7.

8. b

- Septic shock is also called endotoxic shock, which is due to endotoxin which is lipopolysaccharide in nature.

9. d

- Follow section, **exotoxins (properties)** for explanation.

10. d

- Follow section, “**exotoxins (exotoxins producing Gram negative bacilli)**” for explanation.

11. b, c and d

- Follow section, **exotoxins (biological actions of exotoxins)** for explanation.

12. c

- Follow section, **endotoxins** (Lipopolysaccharide/LPS) for explanation.

13. c

- *Streptococcus pyogenes* produces the pyrogenic exotoxin and hemolysin (called streptolysin) but not the enterotoxin.

14. c

- Infective dose of different bacilli is mentioned in **Table 9.2**.

15. c

16. a, b, d

17. b

- Follow **Table 9.3** for explanation of answers of MCQs 15–17.

Bacterial Growth Products

Chapter Outline

- Definition and Types

DEFINITION AND TYPES

Definition

During the growth, bacteria secrete the several products called bacterial growth products.

Types

Following are the different types of bacterial growth products.

Vitamins: Vitamin K, E and B are produced by intestinal flora like *Bacteroides* spp., and *E. coli*.

Chemicals/drugs

- 1. Chemicals:** Acetone and butanol from *Cl. acetobutylicum*.
- 2. Toxins:** Intramuscular injection of *Cl. botulinum* toxin type A was 1st used for strabismus, is now recognized as safe and effective for many neuromuscular diseases.
- 3. Enzymes**
 - **Streptokinase:** It is useful for myocardial infarction and other thromboembolic diseases.
 - **Streptodornase:** It is useful for empyema to liquefy thick pus.
- 4. Antibiotics:** Following are the examples.
 - **Chloramphenicol:** Initially it was prepared from *Streptomyces venezuelae* in 1947. But nowadays, all the commercial products of chloramphenicol are prepared synthetically.
 - **Aminoglycosides:** Two categories are as follows.
 - Obtained from *Streptomyces* spp.: These drugs are labeled with suffix “mycin” such as streptomycin (1944) from *Streptomyces griseus*, kanamycin (1957) from *Streptomyces kanamyceticus*, tobramycin (1970) from *Streptomyces tenebrarius*, neomycin from *Streptomyces fradiae* and framycetin from *Streptomyces lavendulae*.
 - Obtained from *Micromonosporum* spp.: These drugs are labeled with suffix “micin” such

as gentamicin (1964) from *Micromonosporum purpurea* and sisomicin (1980) from *Micromonosporum inoyoensis*.

• Others

- Erythromycin (1952) from *Streptomyces erythreus*.
- Mupirocin from *Pseudomonas* spp.
- Polymyxin B (1940) from *Bacillus polymexa*.
- Colistin (1940) from *Bacillus colistinus*.
- Bacitracin from *Bacillus subtilis*.
- Tyrothricin from *Bacillus brevis*.
- Rifampicin (rifampin) from *Streptomyces mediterranei* (suffix “micin”).

- 5. Insecticides:** Prepared from *B. thuringensis* and useful to prevent the food crops from diseases.

Bacteriocins

- **Definition:** Specific antibacterial substances produced by bacteria called bacteriocins.
- **History:** Bacteriocin production was 1st observed by Gratia in 1952 from *E. coli*.
- **Names of bacteriocins with producing bacteria**
 - Colicins: *E. coli* and *Sh. sonnei*
 - Diphthericin: *C. diphtheriae*
 - Megacin: *B. megaterium*
 - Proticin: *Proteus* spp.
 - Aeroginosin (pyocins): *P. aeruginosa* (*P. pyocyanea*)
 - Pesticins: *Y. pestis*.
- **Synthesis:** Bacteriocin is determined by specific plasmid called col factor. Col factor is transferred from one cell to other cell by conjugation or by transduction. Its production is stimulated by physical (UV rays) and chemical agents (nitrogen mustard).
- **Properties**
 - Bacteriocins are proteins while some are LPSs in nature.
 - They resemble like phages, e.g., pyocin appears like tail of phage under electron microscopy.

- Bacterium produces bacteriocin is immune to it, but it is susceptible to other bacteriocin.
- They adsorb on surface of susceptible cells as like phages.
- **Bacteriocin typing:** It based on ability of bacteriocin producing strain to kill standard indicator strain. Typing is useful in epidemiological typing of bacterial strain. Typing is done by plate diffusion technique as mentioned below:
 - Steps: Inoculate the test bacterium as broad streak in center of culture plates. Standard indicator strain is inoculated at right angle to original inoculum. Incubate the plate at required temperature and time.
 - Result: Pattern of inhibition of standard indicator strain represents the type of bacteriocin.

Pigments

- **Definition:** These are colored substances produced by bacteria.
- **Role of pigment in virulence:** Exact role of pigment is not known but may produce the following effects which increase the virulence of bacterium.
 - Pigment stops ciliary movement of respiratory epithelium and protects the bacterium from host defense.
 - It inhibits the growth of other bacteria and makes the bacterium dominant in mixed infections.
 - It catalyzes the production of superoxide and H_2O_2 .
- **List of bacteria and pigment produced by them**
 1. ***Staphylococcus* spp.:** Types and properties of pigments of staphylococci are described in **Ch. 49.**
 2. ***Streptococcus agalactiae* (Group B, β -hemolytic *Streptococcus*):** **Ch. 50.**
 3. ***Neisseria* spp.:** Commensal *Neisseria* like *N. flavescens* (yellow pigment) and *N. flava* produce the pigment.
 4. **Photochromogens:** They produce the yellow-orange pigment in light only.
 5. **Scotochromogens:** They produce the yellow-orange-red pigment in light and in dark.
 6. ***Serratia marcescens* (*S. prodigiosus*):** It produces red color pigment called prodigiosin. It is best produced at room/at $20^\circ C$. Its presence in sputum simulating presence of blood called pseudohemoptysis.
 7. ***Erwinia* spp.:** It produces the yellow pigment.
 8. ***Yersinia pestis*:** It absorbs the hemin and produces the dark brown pigmented colonies in blood agar and other hemin-containing media. Pigment production is essential for biofilm formation and flea blocking.
 9. ***Elizabethkingia* (*Flavobacterium*) *meningosepticum*:** **Ch. 67.**
 10. ***Pseudomonas aeruginosa*:** **Ch. 67.**
 11. ***Bordetella parapertussis*:** It produces the brown diffusible pigment on nutrient agar after two days of incubation.
 12. ***Chromobacterium violaceum*:** **Ch. 71.**

13. *Capnocytophaga canimorsus* and other species: Believed to produce yellow or orange pigments on blood agar.

14. *Legionella pneumophila*: It produces diffusible brown pigment on Feeley Gorman (FG) agar, which fluoresces in dull yellow color on UV light exposure. This may be enhanced by addition of tyrosine in medium.

15. *Prevotella melaninogenica*: It produces the hemin derived black or brown pigment.

16. *Nocardia* spp., and *Porphyromonas* spp.: These are also the pigment producing bacteria.

Exotoxins and enzymes: Follow **Ch. 9.**

Cell wall components: Like antigens, proteins, etc., are released free in medium during infection or growth of bacteria.

Others

- Gases: Like NH_3 , H_2S , etc.
- Acids (by fermentation) and alkalis.

ACCESS YOURSELF

Short Notes

1. Bacterial growth products
2. Bacteriocin
3. Pigment producing bacteria.

Short Questions for Theory/Viva Questions

1. Name the four bacteria producing the bacteriocins.
2. Name the four pigment-producing bacteria.
3. Name the bacteria producing following pigments: Staphyloxanthine, pyocyanin, prodigiosin and violacein

MCQs for Chapter Review

1. Bacterial pigment also considered as a virulence factor.

MCQs for Chapter Review

1. Following is/are the bacteriocin(s) producing bacterium / bacteria:

a. <i>E. coli</i>	b. <i>Sh. sonnei</i>
c. <i>C. diphtheriae</i>	d. <i>B. megaterium</i>
2. Pigment-producing bacterium is:

a. <i>Streptococcus pyogenes</i>
b. <i>Streptococcus agalactiae</i>
c. <i>Streptococcus mutans</i>
d. <i>Streptococcus pneumoniae</i>
3. Pigment-producing colonies are seen in

a. <i>Pseudomonas</i>	b. Atypical <i>mycobacteria</i>
c. <i>Serratia marcescens</i>	d. All of the above

Answers and Explanation of MCQs

1. a, b, c and d
 - Follow section, types (bacteriocins → names of bacteriocins and producing bacteria) for explanation.
2. b
3. d
 - Follow section, types (pigments → list of bacteria and pigments produced by them) for explanation of answers of MCQs 2–3.

General Properties of Viruses

Chapter Outline

- Introduction
- Morphology of Viruses
- Resistance
- Viral Multiplication
- Viral Genetics
- Viral Taxonomy
- Subviral Agents
- Precipitating Factors of Viral Infections

INTRODUCTION

Meaning of virus: Virus is a Greek word means poison. Term virus was coined by Edward Jenner in 1798.

Definitions

- **Virion:** Extracellular infectious virus particle called virion.
- **Viroid:** Virus particle without extracellular phase called viroid.

Differences between bacteria and viruses: Follow Table 11.1.

MORPHOLOGY OF VIRUSES

Sizes of Viruses

Viruses are very smaller than bacteria and they are determined by electron microscopy. Some larger viruses can be examined by light microscope like pox virus. Size of virus is measured in nm. They are very small, and they can pass through filter so called filterable viruses. Sizes of different viruses are mentioned in Fig. 11.1. Variation in size of viruses is described below:

1. Smallest (also smallest DNA) virus: *Parvovirus* [Parvo (Latin) means small] about 20 nm in size. It is as small as largest protein particle like hemocyanin.
2. Largest (also largest DNA) virus: Pox virus about 300 nm in size. It is as large as smallest bacterium like *Mycoplasma*.
3. Smallest RNA virus: Picornaviridae about 27–30 nm in size.
4. Largest RNA virus: Paramyxoviridae about 100–300 nm in size.

TABLE 11.1: Differences between bacteria and viruses

Bacteria	Viruses
Prokaryotes category	Neither prokaryotes nor eukaryotes
Larger, measured in μm	Smaller, measured in nm
Contain both DNA and RNA	Contain either DNA or RNA, but never both
Nucleic acid surrounded by plasma membrane	Nucleic acid surrounded by a capsid
Thick cell wall contains peptidoglycan and LPS	Some viruses have additional outer lipoprotein envelope
Contain organelles like mitochondria, Golgi apparatus, ribosomes, etc.	No cellular organelles
Extracellular except mycobacteria, chlamydiae, etc.	Intracellular
Contain the enzymes necessary for reproduction	Lack the enzymes necessary for reproduction
Multiply by binary fission	Multiply by replication of NA and synthesis of the viral proteins
Growth occurs on cell-free media	No growth on cell-free media
Not pass through filter except few like <i>M. pneumoniae</i> , <i>F. tularensis</i> , <i>C. burnetii</i> , <i>Chlamydia</i> spp., etc.	Pass through filter
Sensitive to antibiotics	Not sensitive to antibiotics
Few bacteria are motile	Motility absent

Viruses are very variable in shapes. Most are spherical (like parvo virus, picorna virus, etc.), and few are irregular in shape. Shapes of different viruses are mentioned in Fig. 11.1. Few viruses with particular shape are described below:

1. Rabies virus: Bullet shape
2. Ebola virus: Filamentous in shape
3. Pox virus: Brick shape
4. Tobacco Mosaic Virus (TMV): Rod shape
5. Bacteriophage virus: Tad pole in shape.

Structure of Viruses

Virus consists of nucleic acid, capsid, envelope, fibrils and enzymes. All are described below (Fig. 11.2).

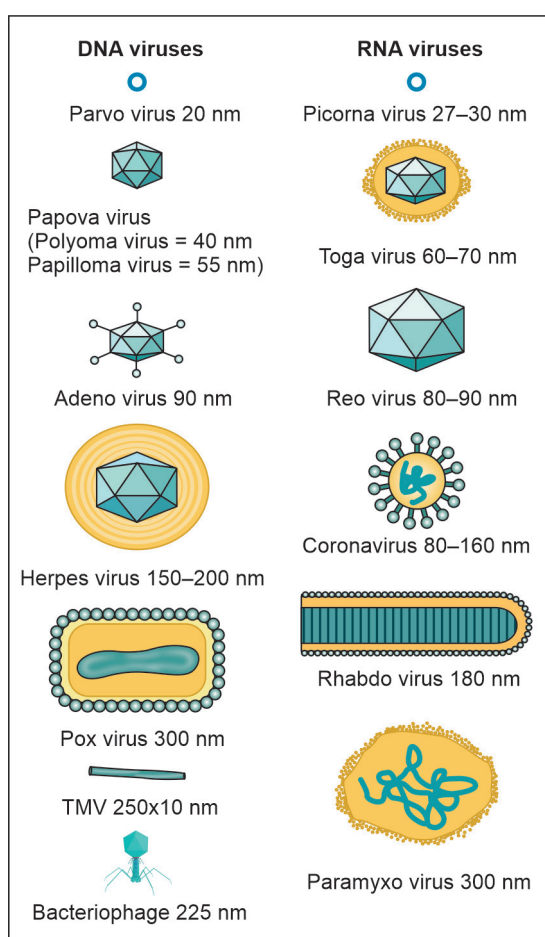


Fig. 11.1: Sizes and shapes of different viruses

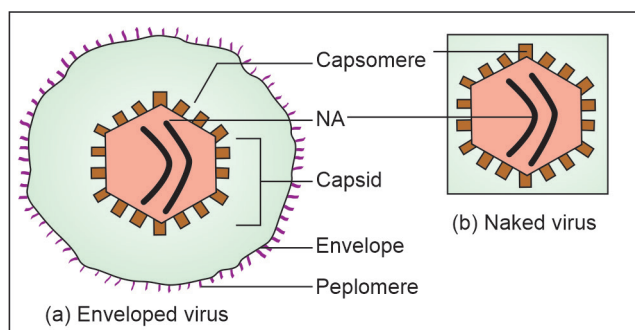


Fig. 11.2: Structure of virus

Nucleic acid (NA)/genome/core

Virus consists either DNA or RNA (never both). Protein range in NA is from about 1–50%. NA is extracted by treatment with detergent or phenol red. In some viruses like picorna virus and papova virus extracted NA is able to initiate the infection. It may be single stranded (ss) or double stranded (ds). It presents in different shapes like circular (nonsegmented) or linear (nonsegmented or segmented).

- **Circular (nonsegmented) genome:** It presents in coil form without ends in Papovaviridae and Poxviridae.
- **Linear genome**
 - Nonsegmented NA: It is available as single piece in following families of viruses
 1. Bacteriophages (in few)
 2. Rhabdoviridae
 3. Togaviridae
 4. Paramyxoviridae
 5. Pneumoviridae.
 - Segmented NA: It is available in multiple pieces in following families of viruses
 1. Retroviridae: It consists two segments of ss-RNA (+) in *Lentivirus* (Visna virus, SIV and HIV)
 2. Orthomyxoviridae: It consists 8 pieces of ss-RNA (–) in influenza virus A and B while 7 pieces of ss-RNA (–) in influenza virus C.
 3. Bunyaviridae: It consists three segments of ss-RNA (–) in *Hantavirus*, *Nairovirus* and *Phlebovirus*.
 4. Reoviridae: It consists 10–12 pieces of ds-RNA (+/–) in *Orthoreovirus* and *Orbivirus* while 11 pieces of ds-RNA (+/–) in *Rotavirus*.
 5. Arenaviridae: It consists two segments of ss-RNA (–) Mammarenavirus (LCM virus, junin virus and machupo virus).

Mnemonic

- Nonsegmented: BTRP₂
- Segmented: ROBRA

Capsid or shell

- **Definition:** NA is surrounded by a protein coat called capsid.
- **Biochemistry:** Structural unit of capsid called capsomere which is made up by protein.
- **Functions of capsid**
 1. It protects the NA from deleterious agents like nuclease or other environmental agents.
 2. It introduces the NA in to host cell by adsorbing to cell surface.
 3. It provides antigenic property to virus.
- **Three types (symmetry) of capsid**
 1. **Icosahedral (cubical) symmetry (Fig. 11.3):** NA is surrounded by equilateral triangle of capsomeres (like soccer ball). It presents in herpes viruses, adeno viruses, etc. It composed of 12 vertices/

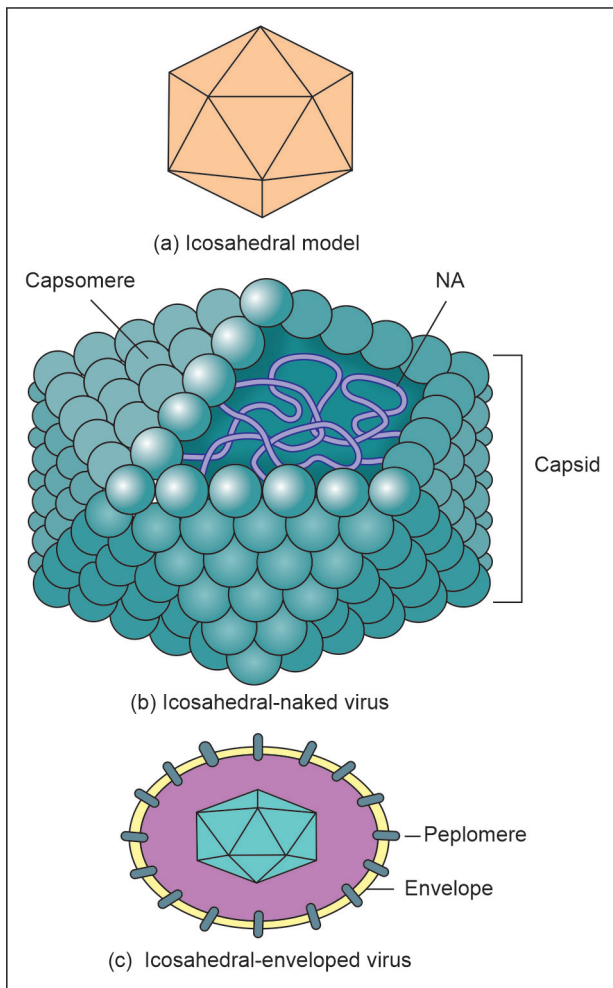


Fig. 11.3: Icosahedral symmetry of capsid

corners and 20 facets/sides. Each facet has an equilateral triangle. Two types of capsomere in this type of capsid.

- Pentons: Present at vertices, always 12 in numbers
- Hexons: Present at facets, in variable numbers.

- 2. Helical (spiral tube) symmetry (Fig. 11.4):** NA is surrounded by capsomeres which are arranged together in tube shape structure. The helix can be either rigid in TMV or flexible in influenza viruses.
- 3. Complex symmetry (Fig. 11.5):** These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. It presents in Poxviridae, Arenaviridae and Enterobacteriophage T4.

Envelope

- **Definitions:** Some viruses have an outer lipoprotein layer called envelope. Viruses with envelope are called enveloped viruses while some viruses without envelope are called non-enveloped (naked) viruses.
- **Biochemistry:** Envelope is made up by lipoprotein where lipid derived from the host cell plasma membrane by budding and proteins are viral encoded.

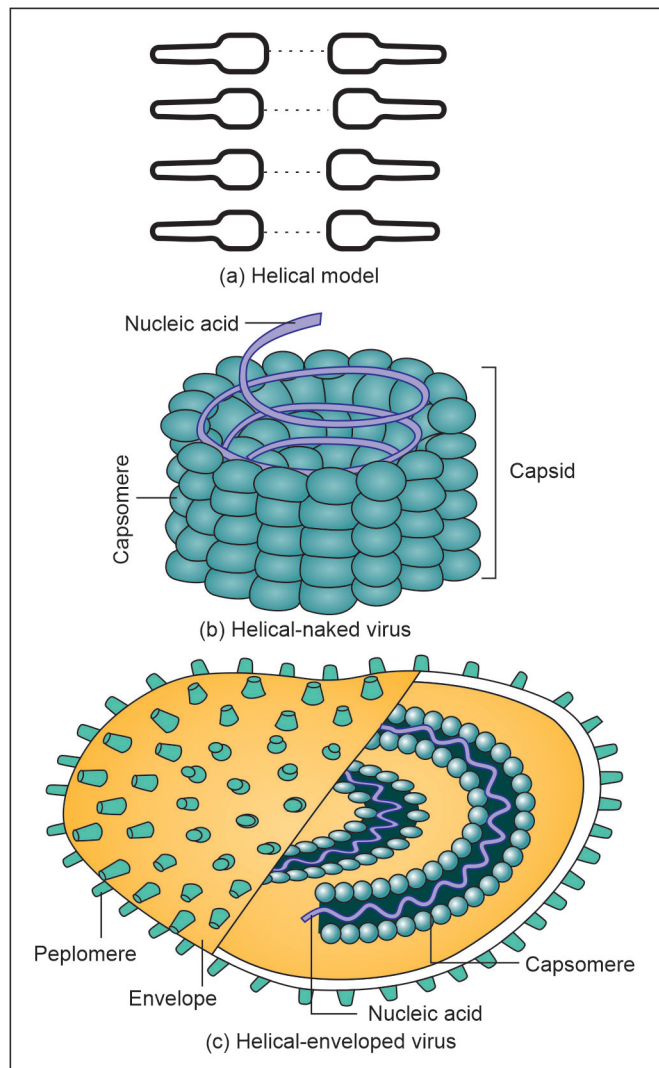


Fig. 11.4: Helical symmetry of capsid

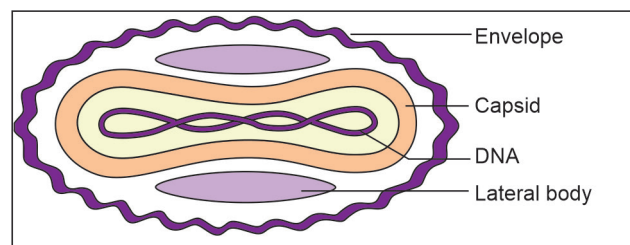


Fig. 11.5: Complex symmetry of capsid (pox virus)

- **Functions:** It confers following properties on viruses.
 - 1. Chemical:** Enveloped viruses are susceptible to lipid solvents like ether, chloroform and bile salts.
 - 2. Antigenic:** Viral neutralization by specific antibodies to envelop antigens.

Note:

Enveloped viruses (Fig. 11.2a): NA, capsid and envelope.

Non-enveloped (naked) viruses (Fig. 11.2b): NA and capsid (nucleocapsid).

- **Definitions:** Some envelope contains spikes like projections called peplomers, from peplos meaning envelop.
- **Biochemistry:** Peplomers are glycoproteins.
- **Types of peplomers with example of influenza virus:** Influenza virus has two kinds of peplomers.
 1. H (hemagglutinin): It allows the virus to attach to host cells (and red blood cells).
 2. N (neuraminidase): It is an enzyme that allows the mature viral particles to escape from the host cell.
- **Functions:** Peplomers confer following properties on viruses:
 1. Antigenic: Viral neutralization by specific antibodies to peplomere antigens.
 2. Biological: Follow H and N (**described above**) peplomers of influenza viruses.

Fibrils: It is an additional feature in some viruses such as adeno viruses. Fibrils are protruding from vertices.

Enzymes: Most viruses do not possess any enzymes for biosynthesis or energy production. They remain dependent on host cells; except influenza virus has neuraminidase and retro virus has reverse transcriptase which transcribes RNA to DNA.

RESISTANCE

Sterilization

1. **Heat and cold:** Viruses are heat labile and generally destroyed by heating at 50–60°C for 30 minute or at 4°C in a day. They are stable at low temperature. Viruses can be preserved at –70°C or for long storage. They are preserved by lyophilization (freeze drying).
2. **Radiation:** Ultraviolet, X-ray and high-energy particles inactivate the viruses.

Disinfection

1. **pH:** Viruses can be preserved at neutral pH (7.3). All viruses are killed at alkaline pH. Enteroviruses are resistance to acidic pH, while rhinoviruses are susceptible to acidic pH.
2. **Disinfectants:** Viruses are most resistant to disinfectants. Most common virucidal agents are KMnO_4 , H_2O_2 , hypochlorite, iodine and chlorine (but affected by organic matter present in water). Formaldehyde and BPL (beta propiolactone) are virucidal and used to prepare the killed vaccine. Enveloped viruses are susceptible to lipid solvents like ether, chloroform and bile salts. Susceptibility can be used to distinguish envelope viruses from non-envelope viruses. Naked viruses are most resistant to disinfectants. Many viruses can be stabilized by salt in concentrations of 1 mol/L, e.g., MgCl_2 , MgSO_4 and Na_2SO_4 .

Drug Resistance

Antibacterial agents have no effect on viruses.

VIRAL MULTIPLICATION

Steps

Total six steps (**Fig. 11.6**) described below.

Attachment or adsorption

- **Definition:** Specific binding between virus and specific receptors on the host cellular surface called adsorption.
- **Examples:**
 - HIV infects CD4 cells by specific receptors for gp120 spikes.
 - Influenza virus attaches to glycoprotein receptors on respiratory epithelium by hemagglutinin (H) spikes.
 - Polio virus can attach to lipoprotein receptors present on primate, not on rodent cell.
 - However, some viruses like picorna virus can directly inject the NA in to the host cells, which are resistant to infection by whole virus.

Penetration

- **Bacterial viruses:** Bacterial viruses called bacteriophages. They can enter in to bacteria by complex mechanisms due to tough bacterial cell wall. For more details follow **Ch. 87**.
- **Human viruses:** Human viruses can enter in host cells by mechanisms resembling phagocytosis called 'virophagocytosis.' Human host cells do not have tough wall, so whole naked virus can enter in cell, while in enveloped virus, viral envelope fuse with plasma membrane and only nucleocapsid can enter in to cell.

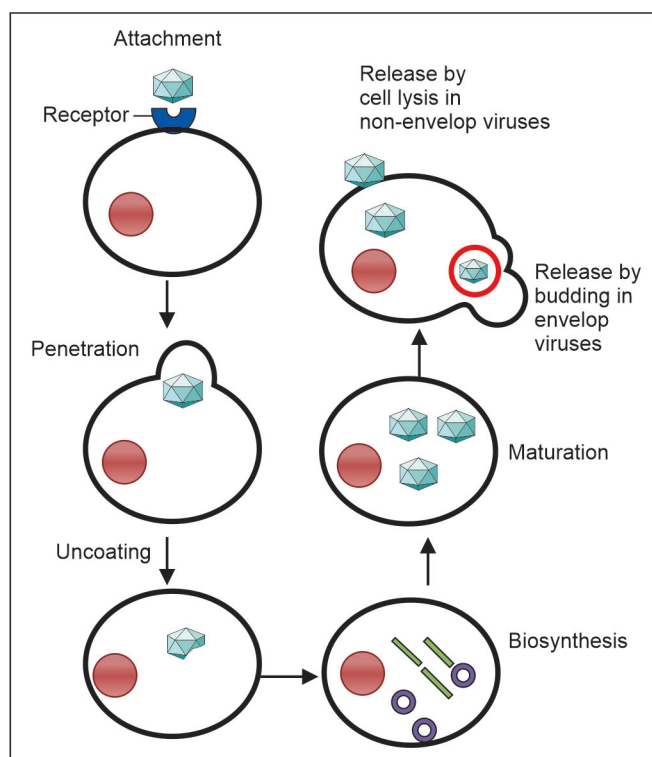


Fig. 11.6: Viral multiplication

Uncoating

- **Definition:** It is a process of stripping of envelop and capsid, so NA released in to the host cell.
- **Features:** Uncoating of virus is accomplished by lysosomal enzymes. Bacteriophage do not require uncoating because their nucleic acid is injected into the host cell. In pox virus uncoating is two steps process. In 1st step stripping of envelop by lysosomal enzymes and in 2nd step stripping of capsid by specific viral uncoating enzymes.

Biosynthesis

- **Biosynthesis of viral parts:** It includes the synthesis of NA, capsid, enzymes and regulatory proteins: Regulatory proteins help in biosynthesis by inhibiting the host cell metabolism.
- **Sites of biosynthesis:** It depends on types of particles to be synthesized and types of virus.

1. Biosynthesis of NA

- In DNA viruses: NA is synthesized in nucleus except pox virus in which it is synthesized in cytoplasm.
- In RNA viruses: NA is synthesized in cytoplasm except orthomyxo virus, paramyxo virus and retro virus in which it is synthesized in nucleus.

2. Biosynthesis of viral proteins: Take place only in cytoplasm.

• Steps of biosynthesis

1. Transcription: It is the process of synthesis of mRNA from viral NA. Six classes are described by Baltimore in 1970.

- **Class-1:** In ds-DNA viruses like adeno virus, herpes virus and papova virus, DNA enters in host cell nucleus and uses host cell enzymes for transcription. In partially ds-DNA virus like hepadna virus duplex is formed by DNA polymerase in cytoplasm, than mature NA enters in to host cell nucleus and uses host cell enzymes for transcription.
- **Class-2:** It occurs in ss-DNA virus like parvo virus. DNA enters in host cell nucleus, converted into duplex and than uses host cell enzymes for transcription.
- **Class-3:** It occurs in ds-RNA virus like reo virus where ds-RNA transcribed to mRNA by viral polymerase.

- **Class-4:** Plus (positive) strand ss-RNA viruses like picorna virus and toga virus. RNA itself acts as mRNA and translated to viral proteins as shown in **Flowchart 11.1a**.

- **Class-5:** Minus (negative) strand ss-RNA virus like rhabdo virus, orthomyxo virus and paramyxo virus. Viruses possess their own RNA polymerase (RNA dependent RNA polymerase) which transcribed RNA to mRNA which is translated to protein as shown in **Flowchart 11.1b**.

- **Class-6:** Like retro viruses. Conversions of ss-RNA to ds-DNA by enzyme reverse transcriptase (RNA-dependent DNA polymerase, DNA polymerase), DNA transcribed to mRNA which is translated to protein as shown in **Flowchart 11.1c**.

2. Translation: It is the process of synthesis of early or nonstructural proteins from mRNA. These are enzymes which induce and maintain the synthesis of viral components by inhibiting the host cell metabolism.

3. Replication: It includes the replication of viral NA.

4. Synthesis of late or structural proteins: Like capsid.

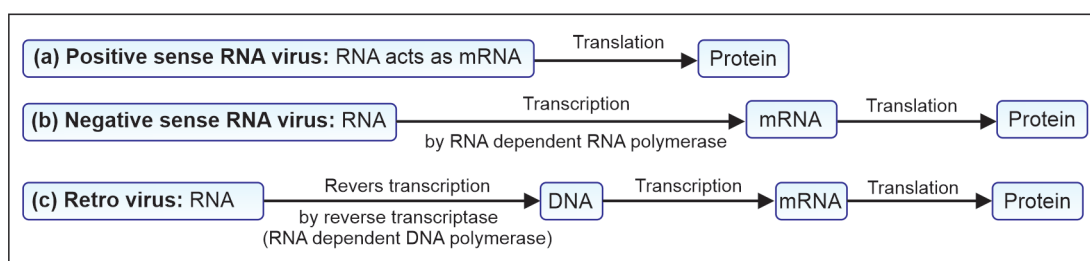
Maturation and assembly

- After synthesis of viral particles, they undergo to maturation and assembly to produce the complete virus. In adeno virus and herpes virus it takes place in nucleus. In picorna virus and pox virus it takes place in cytoplasm.
- At this stage non-enveloped virus are complete, while in enveloped virus only nucleocapsid is synthesized followed by envelope synthesis from host cell membrane by budding method.
- This envelop undergoes modification to introduce virus specific Ags like hemagglutinin (H) and neuraminidase (N) in influenza virus.

Release: Release of daughter virus is possible by following mechanisms.

- **Host cell lysis:** Viruses can be released from the host cell by lysis, a process that kills the host cell by bursting its membrane and cell wall if present. This is a feature of many viruses like bacteriophage and some animal viruses like polio virus.

Flowchart 11.1: Mechanisms of translation in RNA viruses



- 70** • **Budding:** Enveloped viruses (e.g., HIV) are typically released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other internal membrane.
- **Some viruses undergo a lysogenic cycle:** No release of progeny virus, but viral genome is incorporated by genetic recombination into the host's chromosome. Such viral genome "called provirus" or in the case of bacteriophage "called prophage". "Provirus" or "prophage" behaves like host genome and multiplies simultaneously with host cell's genome and confers certain new properties in host cell called lysogenic conversion. Following are examples of lysogenic conversion
 - Malignant transformation by oncogenic virus: Normal cells changed to malignant cells.
 - Conversion of nontoxic strain of bacteria to toxin producing strain: Such as in *C. diphtheriae*.

Eclipse Phase

From the stage of entry to synthesis of daughter virions, virus remained underground or disappeared and cannot be demonstrated in host cells called eclipse phase. It is about 15–30 minutes in bacteriophage and 15–30 hours in human virus.

Burst Size

Release of progenies of virus per infected cell called burst size. Burst size is estimated in bacteriophages and difficult to measure in human viruses.

Abnormal Replicative Cycle

Abortive infection: Due to defect in release, virus infection in some cells does not lead the production of progenies of virus called abortive infection.

Incomplete viruses: Due to defective assembly, progenies of virus released may not be infective called incomplete viruses.

von Magnus phenomenon: Such incomplete viruses are seen when cell infected with high dose of influenza virus, where progenies of virus released have high hemagglutinin titer but low infectivity called von Magnus phenomenon. It is due to formation of incomplete viral particles lacking NA.

Defective viruses: Some viruses when infect the cells are not able to give rise fully formed progenies called defective viruses. Defective viruses can give rise fully formed progenies, only when they are coinfecting by helper virus like

- Rous sarcoma virus is not able to synthesize the envelope by self. When it is coinfecting by helper virus like Avian Leukosis Virus (ALV), resulting progenies are with envelope. The envelope Ags of progenies of Rous sarcoma virus are therefore determined by the type of helper virus.

- HDV (defective virus) and HBV (helper virus)
- Adeno-associated satellite virus (defective virus) and adeno virus (helper virus).

VIRAL GENETICS

Two main mechanisms for genetic inheritance of virus like mutation and recombination. Additionally, virus also exhibits nonheritable mechanisms due to interaction between viral products.

Mutation

Some important points are highlighted at here. For more details follow **Ch. 8**.

Frequency of mutation: It is same as bacteria like 10^{-4} – 10^{-8} .

Change in antigenic properties: It confers certain new properties in virus like virulence, host range, antigenicity (antigenic drift and antigenic shift in influenza virus), pock or plaque morphology and drug resistance.

Conditional lethal mutation: A mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition called conditional lethal mutation. For example, a temperature sensitive mutant (ts mutant) can survive at permissive temperature of 35°C but not at restrictive temperature of 39°C. It is used in study of viral genetics and vaccine production (influenza vaccine).

Recombination

Definition: Transfer of genetic materials or genetic information from one cell to other cell in viruses (viruses are different but related) called genetic recombination.

It occurs between

- Two active (infectious) viruses.
- One active and one inactive virus.
- Two inactive (noninfectious) viruses.

Methods of viral gene transfer: Following types:

1. **Intramolecular recombination:** It occurs when two closely related viruses infect the host cell. Two viruses exchange the NA and form the hybrid that harbors the genes from both species. It occurs in ds-DNA and in some RNA viruses.
2. **Reassortment:** It occurs when different strains of same virus are growing together with different antigens. Such as one human strain, one avian strain and two pig (swine) strains of influenza virus (total four strains) growing together in pig with different H and N Ags. A hybrid may obtain H-Ag from one parent and N-Ag from other resulting a novel virus is formed like swine origin influenza virus (S-OIV) or H1N1 2009 pandemic strain.
3. **Cross reactivation/marker rescue/reactivation:** It occurs when active strain and related inactive strain infect the host cell together. A progeny virus may possess gene from active strain and one or more

genes from inactive strain. For example active strain of influenza virus (A2) grows very poorly in eggs. When A2 grows in eggs along with standard strain (A1) under activation by radiation, resulting progeny contains growth properties of A1 and antigenic properties of A2. This is useful in preparation of influenza vaccine.

4. **Multiplicity reactivation:** When cell is infected by larger dose of single virus (high MOI: Multiplicity of infection), inactivated by UV radiation, it produces changes in host cell gene and produces the live virus called multiplicity reactivation. This is useful in preparation of vaccine; however, vaccine inactivated by UV radiation is not safe.
5. **Lysogenic conversion:** Described above.
6. **Pseudovirion:** Normally viral NA is enclosed by viral capsid, but sometimes host NA is enclosed by viral capsid called pseudovirion. For example, in papova virus.
7. **Transduction:** Transfer of host NA from one cell to other cell by bacteriophage called transduction. This is useful to correct the inborn errors of metabolism.

Interaction between Viral Products/ Nonheritable Mechanisms

Phenotyping mixing: When two different viruses infect the host cell, NA of one virus is surrounded by capsid of other virus called transcapsidation. It is not a stable change; on subsequent passage original capsid will form.

Genotyping mixing: Incorporation of more than one complete genome into virus (heterozygosis). On subsequent passage, two types of viral progenies will form.

Complementation: It is the functional interaction between two viruses, in which one or both may be defective. One virus provides gene products to other virus which are defective and resulting other virus will replicate and progeny viruses are like parent virus. For example when rabbit is infected with heat inactivated virulent myxoma virus and active avirulent fibroma virus, it develops fatal myxomatosis. Heat inactivated myxoma virus is not able to produce infection due to deficiency of DNA dependent RNA polymerase. When coinfecting by fibroma virus, it provides the required enzymes to produce infection.

Interference: Presence of one virus may interfere the function of other virus. Interference is produced by interferon production, by destruction of receptors required for attachment or by autointerference. In auto-interference high MOI inhibits the production of own progeny virus. Interference is useful in controlling polio outbreak by administration of live attenuated polio virus vaccine (OPV), which interfere with wild polio virus. On other hand presence of preexisting virus will interfere the vaccine virus.

Enhancement: Presence of one virus will increase the effect of (CPE) other virus called enhancement.

VIRAL TAXONOMY

Definition

Viral taxonomy is the description, identification, nomenclature and ordered classification of viruses according to their presumed natural relationships.

Nomenclature

Order: It is labeled by adding the suffix 'virales'.

Family: Family is classified on the basis of morphology, genome structure and strategies of replication. Virus family is labeled by adding the suffix 'viridae'. Virus subfamily is labeled by adding the suffix 'virinae'.

Genus: Genus is classified on the basis of physicochemical/serological differences. Genus name is given by adding the suffix '*virus*' and it is written in *Italic* pattern.

Species: No exact system for species nomenclature.

Classification

Properties: All microbes are grouped either in prokaryotes or in eukaryotes. Viruses are not classified in any group. Viral classification system is proposed by an International Committee on Taxonomy of Viruses in 2000.

Bases for classification: Two main types of viruses on the bases of NA such as DNA viruses and RNA viruses as mentioned in **Table 11.2**. Further types are based on following features:

- **Morphological features:** Size, shape, type of symmetry, presence or absence of envelope.
- **Genetic features:** Size of genome, strandedness (ss or ds), linear or circular, positive (+) or negative (–) sense.
- **Other features:** Such as physicochemical or serological features.

SUBVIRAL AGENTS

Viroids

Definition: Virus particles without extracellular phase called viroids.

History: Term was 1st introduced by Diener in 1971.

Morphology: Viroids contain ss-RNA, which is smaller than normal virus. HDV may resemble to viroid. Capsid and envelope are absent. They contain enzyme RNA polymerase II which instead of synthesizing RNA from DNA, uses the rolling cycle to synthesize RNA by using viroid's RNA as template.

Resistant: They are resistant to heat and sensitive to nuclease.

Pathogenicity: First viroid identified was potato spindle tuber viroid. It is a plant pathogen causing potato spindle tuber disease. Viroids also cause human and animal diseases.

TABLE 11.2: Classification of viruses

Naked or enveloped	Virus family	Genus/species	Capsid symmetry	Nucleic acid type
DNA viruses				
Enveloped	<u>P</u> oxviridae	Smallpox virus, cowpox virus, sheep pox virus, orf virus, monkeypox virus and vaccinia virus	Complex	ds
	<u>H</u> erpesviridae	Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, etc.	Icosahedral	ds
	<u>H</u> epadnaviridae	Hepatitis B virus	Icosahedral	Circular, partially ds
Naked	<u>A</u> denoviridae	Adeno virus	Icosahedral	ds
	<u>P</u> apovaviridae	HPV, SV-40 virus, JC virus and BK virus	Icosahedral	ds circular
	<u>P</u> arvoviridae	Parvovirus B19	Icosahedral	ss
RNA viruses				
Enveloped	<u>T</u> ogaviridae	<i>Rubivirus</i> (Rubella virus), <i>Pestivirus</i> , <i>Alphavirus</i>	Icosahedral	ss (+)
	<u>F</u> laviviridae	Dengue virus, HCV, Yellow fever virus	Icosahedral	ss (+)
	<u>A</u> renaviridae	<i>Mammarenavirus</i> (LCV, Junin virus, Machupo virus)	Complex	ss (–)
	<u>O</u> rthomyxoviridae	<i>Alphainfluenzavirus</i> , <i>Betainfluenzavirus</i> , <i>Gammainfluenzavirus</i> , <i>Deltainfluenzavirus</i>	Helical	ss (–)
	<u>P</u> aramyxoviridae	Mumps virus, measles virus, <i>henipavirus</i> (Nipah virus)	Helical	ss (–)
	<u>P</u> neumoviridae	Human orthopneumovirus (RSV), human metapneumovirus	Helical	ss (–)
	<u>B</u> unyaviridae	<i>Hantavirus</i> , <i>Nairovirus</i> , <i>Phlebovirus</i> , <i>Ukuvirus</i>	Helical	ss (–)
	<u>R</u> habdoviridae	<i>Vesiculovirus</i> (Vesicular stomatitis virus, Chandipura virus), <i>Lyssavirus</i> (Rabies virus)	Helical	ss (–)
	<u>F</u> iloviridae	Ebola virus, Marburg virus	Helical	ss (–)
	<u>C</u> oronaviridae	SARS-CoV1, MERS-CoV and SARS-CoV2	Helical	ss (+)
	<u>R</u> etroviridae	<i>Lentivirus</i> (Visna virus, SIV and HIV)	Icosahedral	ss (+)
Naked	<u>R</u> eoviridae	<i>Rotavirus</i> , <i>Orbivirus</i> , <i>Coltivirus</i> , <i>Orthoreovirus</i>	Icosahedral (two capsid)	ds (+/–)
	<u>P</u> icornaviridae	<i>Enterovirus</i> , <i>Rhinovirus</i>	Icosahedral	ss (+)
	<u>C</u> aliciviridae	<i>Norovirus</i>	Icosahedral	ss (+)
	<u>H</u> epeviridae	HEV	Icosahedral	ss (+)
	<u>A</u> stroviridae	<i>Astrovirus</i>	Icosahedral	ss

Mnemonics

DNA viruses: All viruses have ds DNA except Parvoviridae.

- **Enveloped DNA viruses: PH2** → Poxviridae, Herpesviridae and Hepadnaviridae.
- **Naked DNA viruses: AP2** → Adenoviridae, Papovaviridae and Parvoviridae.
- **Icosahedral capsid:** All the DNA viruses whether enveloped or naked have icosahedral type of capsid except Poxviridae having complex type of capsid

RNA viruses: All viruses have ss RNA except Reoviridae

- **Enveloped RNA viruses: FABRha-FOT PPCR** → Flaviviridae, Arenaviridae, Bunyaviridae, Rhabdoviridae, Filoviridae, Orthomyxoviridae, Togaviridae, Paramyxoviridae, Pneumoviridae, Coronaviridae and Retroviridae.
- **Naked RNA viruses: Astro-H PCR** → Astroviridae, Hepeviridae, Picornaviridae, Caliciviridae and Reoviridae.
- **Icosahedral and helical capsid:** RNA viruses have icosahedral or helical type of capsid except Arenaviridae having complex variety of capsid.

Prions and Prion Diseases

Definition: Proteinaceous infectious virus like-particles without nucleic acid (NA) called prions.

History: Prion was identified by Stanley B Prusiner, awarded with Nobel Prize in 1997.

Morphology: Prions contain protein without NA. MW of protein is 50,000 and size is 4–6 nm.

Resistant: Prions are sensitive to protease. They are resistant to nuclease, dry heat (90°C for 3 min.) and UV-rays. Routine methods like autoclaving (120°C for 20 min.), boiling, radiation, etc., are not effective. Prions are killed by autoclaving at 134°C for 5 hours with prior treatment with acidic detergent or by treatment with 2N NaOH for several hours or by 0.5% sodium hypochlorite for 2 hours.

Pathogenicity

- **Disease name:** Disease called prion disease.
- **Incubation period:** Long incubation period may be up to 30 years, but once the disease sets, progress will be fast.
- **Sites:** CNS.
- **Pathogenesis:** Many theories are proposed for the pathogenesis of prion disease as follows:
 1. **Stanley B Prusiner theory:** It is mentioned in **Flowchart 11.2**. Because of his great work he was awarded with Noble Prize in 1997.
 2. **Abnormal protein folding:** α -helix structure of PrP^c changes to structure PrP^{sc} called misfold or unfold protein. This unfolded protein is dangerous because it unfolds many other folded prion proteins. Unfold protein undergoes replication and spreads throughout tissues without using DNA or RNA to produce the disease.
- **Pathology:** Prions produce progressive vacuolation in the dendritic plus axonal process of the neurons. They cause extensive astroglial hypertrophy plus proliferation, spongiform encephalopathy, neurodegeneration in the grey matter, amyloid plaque formation in brain and gliosis. There is no inflammation and absence of immune response (non-immunogenic).
- **Clinical types:** Two types of prion diseases:
 1. **Prion diseases in animals**
 - Scrapie: It is transmitted vertically from ewe to lamb.
 - Mink encephalopathy: It spreads to mink when it feeds on scrapie infected sheep's meat.
 - Mad cow disease [Bovine spongiform encephalopathy (BSE)]: It spreads to cattle when they fed on scrapie infected sheep's meat. Enzootic occurred in 1986 in Britain.

Flowchart 11.2: Pathogenesis of prion particle

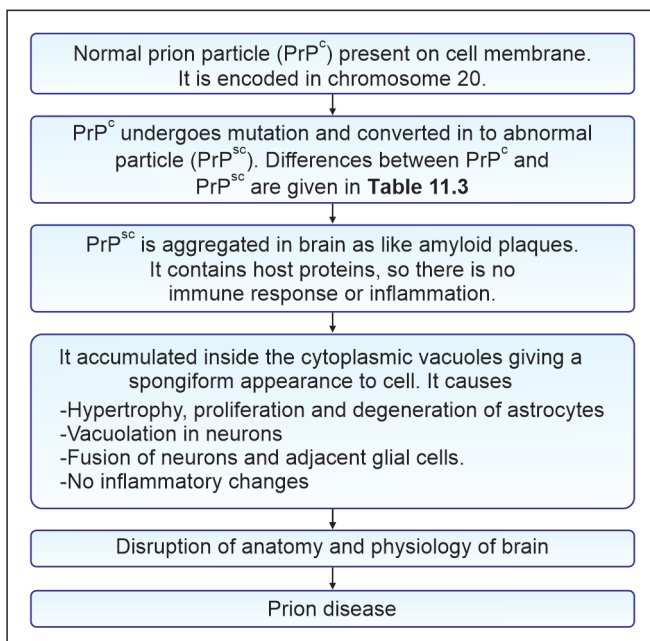


TABLE 11.3: Differences between PrP^c and PrP^{sc}

Features	PrP^c	PrP^{sc}
Full form	Prion particle cellular	Prion particle scrapie
Forms	Normal form	Active or pathogenic form
Structure	Elongated polypeptide with more α helix and less β structure	Globular polypeptide with less α helix and more β structure
Location	Cell membrane	Cytoplasm
Protease	Sensitive	Resistant

2. Prion diseases in humans

- **CJD (Creutzfeldt-Jacob Disease):** A variety of CJD (vCJD) was occurred in persons <45 years in Britain in 1996, due to consumption of beef, infected with BSE and many cattle were slaughtered before anxiety was allayed. It occurred in sporadic due to mutation in prion. It also occurred in inherited (familial) forms. It is **transmitted by** iatrogenic ways due to corneal transplantation, dura mater graft implantation (>160 cases have been reported), injection of pituitary growth hormone from infected person (>180 cases have been reported), electro encephalogram electrode implantation or by ingestion of beef, infected with BSE or by hereditary. **Clinical features** include senile encephalopathy, progressive incoordination, cerebellar gait, pyramidal signs, extrapyramidal dysfunctions, seizures, visual impairment, 90% myoclonus, and dementia. Death occurs within a year. It presents with defective higher cortical functions.
- **Kuru:** Kuru means tremor, a characteristic feature of disease. **Incubation period** is about 5–10 years. It is **transmitted** due to anthropophagy or cannibalism. It is an act or practice of humans, eating the flesh or internal organs of other human beings. Word came from Caníbales, the Spanish name for the Caribs, a West Indies (New Guinea) tribe that formerly practiced cannibalism after the death of relatives due to customs. It was identified in New Guinea and disease was stopped after stopping the cannibalism. **Clinical features** include cerebellar ataxia, tremor and death. **Nobel Prize** was given to Carlton Gajdusek in 1976 for his contribution in disease.
- **Other diseases:** Such as GSSS (Gerstmann Straussler Scheinker Syndrome) and fatal familial insomnia.

Laboratory diagnosis

- **Specimens:** Brain biopsy and CSF.
- **Testing methods**
 1. **Light microscopy:** It shows histopathological changes like hypertrophy, proliferation and

degeneration of astrocytes, vacuolation in neurons, fusion of neurons and adjacent glial cells. No inflammatory changes are present.

2. **Conformation-dependent assay:** It is specific diagnosis for measurement of PrP^{Sc}.
3. **Stress protein 14-3-3:** Elevated in CSF.
4. **Sequencing the gene:** It is important to detect the mutation to identify the familial CJD.
5. **Abnormal EEG:** In late stage of disease, high voltage, triphasic sharp discharges are observed.
6. **MRI:** Greater than 90% cases showing the increased intensity in the basal ganglia and cortical ribboning.

Prevention: No effective measures are available.

Treatment: Several measures using drug like quinacrine and anti-PrP antibodies eliminate the prion particles from the cultured cells but failed to do so *in vivo*.

PRECIPITATING FACTORS OF VIRAL INFECTIONS

Synonym

Epidemiological determinants.

Types

Virulence of viruses is affected by following three factors.

Agent factors (virulence factors or determinants of virulence)

1. **Intracellular location:** It provides protection against the host defense system.
2. **Viral capsid:** It protects the nucleic acid against the deleterious agents.
3. **Viral adhesins:** Glycoprotein spikes present on envelope of virus like gp120 in HIV, H (hemagglutinin) protein in influenza virus, S (spike) protein in SARS-CoV2, F (fusion) protein in paramyxoviruses, etc., help in attachment with host cells **called adhesins**.
4. **Neuraminidase in influenza virus:** It is a mushroom like glycoprotein enzyme which destroys the cell receptors by splitting of N-acetyl neuraminic acid from it. It destroys the hemagglutinin (H) receptors on RBCs hence called receptor destroying enzyme (RDE) and causes reversal of hemagglutination and releases the bounded viruses to infect the other RBCs called elution.
5. **Exotoxin-producing virus:** In *Rotavirus* NSP4 (non-structural protein 4) considered as enterotoxin which increases the vascular permeability and the secretion.

Host factors

1. **Age:** Certain infections are common at particular age like *Rotavirus* common in pediatrics.
2. **Smoking:** People who smoke do have more frequent infections by rhino viruses.

3. **Immune status:** Immunodeficiency status favors certain infections like SARS-CoV2, etc.

4. **Occupation:** Like HBV, SARS-CoV2, etc., are common in laboratory workers.

Environmental factors

1. **Seasonal:** *Rhinovirus* infection occurs year round with seasonal peaks of incidence in the early fall, usually September to November and again in the spring from March to May. During these periods increased incidence up to 80% of common cold. In India, most cases of polio were reported in rainy season during June–September.
2. **Overcrowding and poor sanitation:** They also increase risk of mosquito borne viral infections.

Note: Important terminology

- Equine: Horse
- Feline: Cat
- Bovine: Cattle (cow/buffalo)
- Canine: Dog
- Murine: Rat/mice
- Simian: Monkey
- Swine (Porcine): Pig
- Avian: Birds
- Caprine: Sheep/goat

ACCESS YOURSELF

Essay/Full Question

1. Morphology of viruses.

Short Notes

1. Structure of viruses
2. Viral multiplication
3. Classification of viruses or viral taxonomy
4. Prion particle.

Short Questions for Theory/Viva Questions

1. Write the four differences between viruses and bacteria.
2. What is capsid? Write its two functions.
3. What is peplomere? Write its two functions.
4. What is eclipse phase?
5. What is von Magnus phenomenon?
6. Define: Burst size, incomplete virus, von Magnus phenomenon and abortive viral infection.
7. What is defective virus? Write two examples.
8. Define: Provirus and prophage.
9. Write four examples of bacteria producing toxin after phage conversion.
10. Define: Virion and viroid.

MCQs for Chapter Review

Introduction

1. **False about viruses is:**
 - a. Ribosome absent
 - b. Mitochondria absent
 - c. Motility absent
 - d. Nucleic acid absent

2. Following is not the property of virus:
 - a. Contains both DNA and RNA
 - b. Neither prokaryotes nor eukaryotes
 - c. No growth on media
 - d. Passes through filter
3. Which of the following does not possess both DNA and RNA?
 - a. Bacteria
 - b. Fungi
 - c. Viruses
 - d. Spirochetes
4. All of the following are general properties of viruses *except*:
 - a. May contain both DNA and RNA
 - b. Form extracellular infectious particles
 - c. Heat labile
 - d. Not affected by antibiotics

Morphology of Viruses

5. Brick-shaped virus is:
 - a. Chickenpox
 - b. Smallpox
 - c. CMV
 - d. EBV
6. Rabies virus is in:
 - a. Bullet shape
 - b. Spherical shape
 - c. Brick shape
 - d. Rod shape
7. Smallest DNA virus is:
 - a. Herpes virus
 - b. Adeno virus
 - c. Parvo virus
 - d. Pox virus
8. The virus with smallest genome:
 - a. Reo virus
 - b. Parvo virus
 - c. Picorna virus
 - d. HIV
9. Segmented genome is found in all *except*:
 - a. Influenza virus
 - b. Reo virus
 - c. Bunya virus
 - d. Rhabdo virus
10. Segmented genome is found in:
 - a. Retro virus
 - b. Rota virus
 - c. Polio virus
 - d. Rhabdo virus
11. Segmented RNA is found in:
 - a. Influenza virus
 - b. Rabies virus
 - c. Herpes virus
 - d. Molluscum contagiosum virus
12. Segmented double stranded RNA virus is seen in:
 - a. Reo virus
 - b. Myxo virus
 - c. Rabies
 - d. Parvo virus
13. DNA covering material in a virus is called as:
 - a. Capsomere
 - b. Capsid
 - c. Nucleocapsid
 - d. Envelope
14. Symmetric protein shell which encase the nucleic acid core of virus?
 - a. Capsomere
 - b. Capsid
 - c. *Basidiomycetes*
 - d. Fungi imperfecti

Viral Multiplication

15. von Magnus phenomenon:
 - a. Is a normal replicative cycle
 - b. Virus yield has low hemagglutination
 - c. Virus has high infectivity
 - d. Virus yield has high hemagglutination titer but low infectivity
16. Which of this/these is/are an example of a defective virus?
 - a. Hepatitis C virus
 - b. Delta virus
 - c. Rous sarcoma virus
 - d. Both b and c

Viral Genetics

17. One virus particle prevents multiplication of second virus. This phenomenon is:
 - a. Viral interference
 - b. Mutation
 - c. Supervision
 - d. Permutation

Viral Taxonomy

18. Adenovirus:
 - a. ds-DNA
 - b. Enveloped
 - c. Complex symmetry of capsid
 - d. None
19. Human papillomavirus contains:
 - a. ds-DNA
 - b. ss-DNA
 - c. ds-RNA
 - d. ss-RNA
20. Lipid envelope is found in which virus?
 - a. Reo
 - b. Herpes
 - c. Picorna
 - d. All of the above
21. In which of the following genome has double-stranded nucleic acid?
 - a. Orthomyxo viruses
 - b. Pox viruses
 - c. Papova viruses
 - d. Reo viruses
22. Which of the following DNA viruses possesses a capsid with icosahedral symmetry and no lipid envelope?
 - a. Herpes virus
 - b. Adeno virus
 - c. Pox virus
 - d. Papova virus
23. Which is not a DNA virus?
 - a. Parvo virus
 - b. Papova virus
 - c. Pox virus
 - d. Rhabdo virus
24. Which of the following is not a RNA virus?
 - a. Ebola
 - b. Simian-40
 - c. Rabies
 - d. Vesicular stomatitis virus
25. Which is enveloped virus?
 - a. Dengue virus
 - b. Norwalk virus
 - c. Hepatitis A virus
 - d. Adeno virus
26. Non enveloped ss RNA virus is:
 - a. Picorna virus
 - b. Pox virus
 - c. Retro virus
 - d. Bunya virus
27. Which of the following virus has negative sense RNA?
 - a. Rabies virus
 - b. Reo virus
 - c. Corona virus
 - d. Calci virus
28. Negative sense nucleic acid genome is found in:
 - a. Polio virus
 - b. Rabies virus
 - c. Measles virus
 - d. Picorna virus
 - e. Influenza virus

Subviral Agents

29. Which of the following infectious agent lacks RNA?
 - a. Virus
 - b. Staphylococci
 - c. Prions
 - d. *Cryptococcus*
30. True about viroid is:
 - a. Causes tumor in animals
 - b. Lacks envelope like covering
 - c. Has only genetic material
 - d. Visible on light microscope
31. True about prion protein disease is all *except*:
 - a. Myoclonus is seen in 10% of the patients
 - b. Caused by infectious protein
 - c. Brain biopsy is diagnostic
 - d. Commonly manifests as dementia

- 76 32. Prions are best killed by:**
 a. Autoclaving 121°C b. 5% formalin
 c. Sodium hydroxide d. Sodium hypochlorite
- 33. Prions are extremely resistant to dry heat and can survive dry heat at:**
 a. 180° for 1 hour b. 240° for 1 hour
 c. 360° for 1 hour d. 90° for 3 minutes
- 34. Which of the following is correct about prions?**
 a. Long incubation period
 b. Destroyed by autoclaving 121°C
 c. Nucleic acid present
 d. Immunogenic
- 35. Prions are:**
 a. Made up of bacterial and viral particles
 b. Immunogenic
 c. Infectious
 d. RNA particles
- 36. Prions consist of:**
 a. DNA and RNA b. DNA, RNA and protein
 c. RNA and protein d. Only proteins
- 37. Regarding prion protein which of the following statement is true:**
 a. It is a protein product coded in viral DNA
 b. It catalyses abnormal folding of other proteins
 c. It protects disulfide bond from oxidation
 d. It cleaves normal proteins
- 38. True about prion is:**
 a. Encoded by viral genome
 b. Associated with misfolding of protein
 c. Noninfectious
 d. Immunogenic
- 39. Which of the following is not a prion associated disease?**
 a. Scrapie
 b. Kuru
 c. Creutzfeldt-Jacob disease
 d. Alzheimer disease
- 40. Which of the following is not a prion disease?**
 a. Bovine spongiform encephalopathy
 b. Transmissible mink encephalopathy
 c. Scrapie
 d. Progressive multifocal leucoencephalopathy
- 41. Mad cow disease (Bovine spongiform encephalopathy) is similar to man in:**
 a. Alzheimer disease b. Creutzfeldt-Jacob disease
 c. Rabies d. Polio
- 42. Mad cow disease is due to:**
 a. Slow virus b. *Mycoplasma*
 c. Bacteria d. Fungi
- 43. Human cannibalism is associated with:**
 a. Q fever b. Sleeping sickness
 c. Trachoma d. Kuru
- 44. Fatal familial insomnia is associated with:**
 a. Prion disease b. Degeneration disease
 c. Neoplastic disease d. Vascular disease

Answers and Explanation of MCQs

1. d
 2. a
 3. c

• Follow Table 11.1 for explanation of answers of MCQs 1–3.

4. a
 • Follow section, **introduction, Table 11.1 and resistance (heat and cold)** for explanation.
5. b
6. a
7. c
8. b
 • Follow section, **morphology of viruses (size and shapes)** for explanation of answers of MCQs 5–8.
9. d
10. a, b
11. a
12. a
 • Follow section, **morphology of viruses [structure of viruses → nucleic acid (NA)/genome/core]** for explanation of answers of MCQs 9–12.
13. b
14. b
 • Follow section, **morphology of viruses (structure of viruses → capsid or shell)** for explanation of answers of MCQs 13–14.
15. d
 • Follow section, **viral multiplication (abnormal replicative cycle → von Magnus phenomenon)** for explanation.
16. d
 • Follow section, **viral multiplication (abnormal replicative cycle → defective viruses)** for explanation.
17. a
 • Follow section, **viral genetics (interaction between viral products/nonheritable mechanisms → viral interference)** for explanation.
18. a
19. a
20. b
21. b, c, d
22. b, d
23. d
24. b
25. a
26. a
27. a
28. b, c, e
 • Follow section, **viral taxonomy (Table 11.2)** for explanation of answers of MCQs 18–28.
29. c
 • Follow section, **subviral agents (viroids)** for explanation.
30. c
31. a
32. c, d
33. d
34. a
35. c
36. d
37. d
38. b
39. d
40. d
41. b
42. a
43. d
44. a
 • Follow section, **subviral agents (prions and prion disease)** for explanation of answers of MCQs 30–44.

Virus–Host Interactions (Viral Infections)

Chapter Outline

- Introduction
- Immune Response (Ir) to Viral Infections

INTRODUCTION

Effects on Cell by Virus

Sometimes virus causes no damage called steady-state infection or causes following types of damages.

Types of cellular damage

- Cytocidal: Cell death like in polio virus (virus causes cell degeneration which leads cell death).
- Cytolysis: Cell lysis like in bacteriophage virus (virus multiplies in cell to produce daughter viruses, so cell is not able to hold the weight and finally dies).
- Cell proliferation: For example, MCV
- Malignant transformation: For example, oncogenic virus
- Cytopathic effect (CPE): It occurs in tissue/cell culture.
- Morphological changes: For example, inclusion body formation by rabies virus, ballooning of cells in herpes virus.
- Fusion of adjacent infected cells lead polykaryocytosis or syncytium formation.
- Damage to chromosomes by measles, mumps, adeno virus, CMV, etc.
- Antigenic changes on surfaces: Such as hemagglutinin on surface of influenza infected cells, which cause hemadsorption of RBCs, tumor antigens on surface of cells infected by oncogenic virus.

Mechanisms of cellular damage

- Shutting down of protein (DNA) synthesis in cell
- Accumulation of toxic viral molecules causes distortion of cellular architecture.
- Alteration of plasma membrane permeability, which releases the lysosomal enzymes causes autolysis.

Modes of Transmission of Viruses

Direct modes: These routes do not required mediator/vehicle.

1. **Droplet nuclei:** Droplet nuclei/particles of saliva or nasopharyngeal secretion arise during coughing, sneezing, speaking, talking or invasive procedure (bronchoscopy) enter in to other host directly, who is in close contact. Such particles are $\geq 5 \mu\text{m}$ in diameter and spread to short distance (< 3 feet) can directly enter in other host. However, such larger particles can be filtered by nose. Particles $\leq 5 \mu\text{m}$ in diameter are traverse to long distance and produce the air borne (indirect) infection described later in this chapter. Infection by droplet nuclei is increased in close contact, overcrowding and lack of ventilation. **Viruses** transmitted by droplet nuclei are influenza virus, mumps virus, rubella virus, adeno virus and parvo virus B19.
2. **Inoculation/injection under skin or mucosa:** Viruses transmitted by contaminated needle/syringe are HIV, HBV, HCV, etc.
3. **Contact with skin/mucosa:** It includes sexually transmitted infections, which are described in **Ch. 44** and infections transmitted by direct skin-to-skin contact such as HHV-1 plus 2, HHV-3 (VZV), poxvirus, MCV, etc.
4. **Vertical:** Follow **Table 12.1**.

Indirect modes: These routes required mediator/vehicle.

1. **Inhalation (air borne):** Particles arise during coughing, sneezing, talking or invasive procedures from patients which are $\leq 5 \mu\text{m}$ in diameter and traverse to long distance can produce the air borne infection. Some droplet nuclei settle over different objects and become part of dust and cause air borne infection. **Viruses** transmitted by air-borne route are influenza virus, measles virus, VZV and hemorrhagic fever viruses causing pneumonia.
2. **Ingestion (food- and water-borne):** Polio virus, *Rotavirus*, HAV, HEV, adeno virus, reo virus, etc.

TABLE 12.1: Vertical viral infections

Time	Infections
Antenatal/before birth/ congenital/transplacental/ teratogenic	• Rubella (congenital rubella syndrome) • CMV (congenital cytomegalovirus infection) • Herpes viruses (HSV causing congenital herpes simplex while VZV causing fetal varicella syndrome and congenital/neonatal varicella) • HBV • HIV-AIDS • Zika virus • Parvovirus B19 • Chikungunya virus
Intranatal/during birth (transcervical)	• CMV • HSV • HPV
Postnatal/after birth by breastfeeding	• CMV • HIV

3. **Blood borne:** By transfusion of blood or blood products like HHV-4 (EBV), HHV-5 (CMV), HHV-8 (KSHV), HBV, HCV, HDV, HTLV-I, HTLV-II, HTLV-III (HIV), parvo virus B19, zika virus, etc.

4. **Saliva borne**

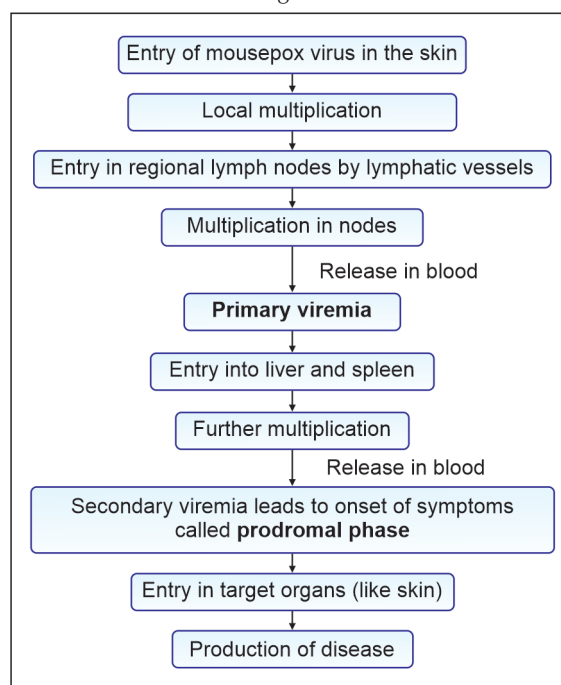
- From human saliva like HHV-4 (EBV), HHV-6 (HBLV), HHV-7 (RK virus), ECHO virus, mumps virus, etc.
- From monkey bite like herpes virus simiae or B virus.
- From dog bite like rabies virus.

5. **Vector borne**

- **Mosquitoes-borne viruses**
 - *Aedes*: Chikungunya virus, yellow fever virus, dengue virus, zika virus, Rift Valley fever virus (also by *Culex*), Orungo virus, etc.
 - *Anopheles*: O'nyong-nyong virus.
 - *Culex*: JEV, St. Louis encephalitis virus, Ilheus virus, West Nile virus, Murray Valley encephalitis virus, Oropouche virus, Rift Valley fever virus and African horse sickness virus.
- **Ticks-borne viruses:** RSSE virus, Central European encephalitis virus, Western Siberian encephalitis virus, Powassan encephalitis virus, Louping ill virus, Kyasanur forest disease virus, Omsk hemorrhagic fever virus, Nairobi sheep disease virus, CCHF virus and Kolarado tick fever virus.
- **Sand fly-borne viruses**
 - *Phlebotomus*: Sand fly fever virus or three-day fever virus, Chandipura virus (also by *Sergentomyia*).
 - *Lutzomyia shannoni*: Vesicular stomatitis virus.
 - *Sergentomyia*: Chandipura virus.

6. **Fomites borne:** Contamination of fomites like towel, handkerchief, pen, pencils, clothes, cups, spoon, keys, etc., may transmit the infections like HAV, swine flu, etc.

Flowchart 12.1: Pathogenesis of viral infections



7. **Unclean hands and fingers:** Swine flu, SARS-CoV, MERS-CoV, etc.

Pathogenesis of Virus or Spread of Virus in the Body

History: Spread of viral infection was 1st studied by Fenner in 1948 by using the mousepox virus as the experimental model.

Pathogenesis: Follow Flowchart 12.1.

Incubation period (IP): In viral infection it depends on following factors.

- **Sites of entry, multiplication and lesion:** (1) If sites of entry and sites of lesion are same then IP is short about 1–3 days such as in *Rotavirus*. (2) If sites of entry and sites of lesion are distant then IP is long about 10–20 days such as in polio virus which enters by GIT and produces lesion in CNS.
- **Direct deposition of virus in blood:** Short IP about 5–6 days like in dengue or yellow fever.
- **Nature of multiplication:** IP is long about months or years, if virus multiplies slowly such as in HBV, IP is 2–6 months and in HIV after primary infection AIDS will start approximately after 10 years.

IMMUNE RESPONSE (Ir) TO VIRAL INFECTIONS

Types of Ir of Viral Infections

Two main types of Ir like specific and nonspecific are described below.

Specific (Immunological) Ir

AMI (HI): Different classes of antibodies are formed in viral infections. IgM and IgG play major role in blood and tissue immunity. IgA provides local or mucosal

immunity. Antibodies neutralize the virus by different mechanisms such as prevention of adsorption of virus to cell, enhancement of viral degradation, prevention of release of viral progenies, etc. Along with complement antibody causes damage to viral envelop and cytolysis of viral infected cells. Not all antibodies neutralize the virus, but enhance the viral infectivity and contribute to viral pathogenesis by activation of complement system followed by lysis of infected cells or by immune complex type of injury.

CMI: Cytotoxic T cells (Tc) directly kill the viral infected cells. Sensitized T cells release the cytokines, which enhance phagocytosis. Release of interferon (IFN) from T cells, which inhibits the viral multiplication and limits the infection. CMI also takes part in DTH following viral vaccination.

Nonspecific Ir

Phagocytosis: Microphages have no role in viral clearing. **Macrophages** phagocytose the virus and important in clearing virus from bloodstream.

Interferon (IFN)

- **History:** IFN was discovered in 1975 by Issacs and Lindemann, who showed that when chick CAM is treated with live or inactivated influenza virus produces antiviral substances which rendered the cell resistant to viral infection.
- **Definition:** It is a protein produced by vertebrate cells due to viral or nonviral stimulation.
- **Mechanisms of actions:** IFN has no direct action on virus, but acts on other cells of same species, rendering them refractory to viral infection. It inhibits the viral transcription in infected cells. On exposure to IFN cell produces TIP (translation-inhibiting protein), this inhibits the viral translation without affecting the cell. TIP is actually a combination of three enzymes like protein kinase, oligonucleotide synthetase and RNAase.
- **Properties of IFN: (1) Synthesis:** IFN synthesis begins within about an hour of induction and reaches in high levels in 6–12 hours. It synthesized very rapidly than Ab; hence, play primary role in defense. It is produced from host cells, not from virus so it is a host protein. IFN production increased by increasing the temperature up to 40°C. IFN production decreased by increasing the O₂ tension and by steroid therapy. **(2) Species specific:** IFN produced by one species can protect the same or related species, but not other like IFN produced by human cells can protect human cells or related species such as monkey, but not other species like mouse or chick. **(3) Cell's factors:** Cellular transcription and protein synthesis necessary for IFN production. **(4) Viral factors:** IFN is not virus specific because IFN induced by one virus can protect against any viral infection. Viruses are varying in their susceptibility to IFN. They are also varying in their capacity to induce IFN production like virulent and RNA viruses are good inducers while avirulent and DNA viruses are poor inducers. **(5) Inactivation:** IFN is inactivated by proteolytic enzyme, but not by protease or lipase. **(6) Resistant:** IFN is resistant to protease, lipase and heat at 56–60°C for 30–60 minutes. It is stable to wide range of pH (2–10). IFN is labile at pH-2. **(7) Molecular weight:** It is LMW about 17,000. **(8) Antigenic property:** It is poorly antigenic, so serological tests are not useful for its detection. **(9) Other properties:** It is nondialyzable and nonsedimentable.
- **Nomenclature of IFN:** It is abbreviated as IFN and species of origin is indicated as prefix like HuIFN for human.
- **Types of IFN:** Three types as mentioned in Table 12.2.
- **Clinical uses of IFN: (1) Antiviral effect (IFN-α and β):** IFN applied systemically in immunocompromised host such as in HBV and HCV infection and locally in genital warts, herpes keratitis and upper respiratory tract infection. **(2) Antibacterial effects (IFN-γ):**

TABLE 12.2: Types of IFN

Features	IFN-α	IFN-β	IFN-γ
Earlier name	Leucocytic IFN	Fibroblast IFN	Immune IFN
Type	I	II	III
Origin	Virus-infected leukocytes	Infected fibroblasts or epithelial cells	T-cells
Inducer	Viruses; dsRNA	Viruses; dsRNA	Antigen or mitogen
Biochemical nature	Non-glycosylated protein	Glycoprotein	Glycoprotein
Actions	Antiviral, anti-proliferative, ↑ MHC-I expression and activates NK cells	Antiviral, anti-proliferative, ↑ MHC-I expression and activates NK cells	Anti-proliferative, ↑ MHC-I and II expression and immunomodulatory functions
Stability at pH 2.0	Stable	Stable	Labile
Location of gene	Chromosome 9	Chromosome 9	Chromosome 12
Receptors	α/β receptor	α/β receptor	γ receptor
Receptor gene	Located on chromosome 21	Located on chromosome 21	Located on chromosome 6
Antigenic types	16	—	—

It is used in chlamydial infection, LL, and chronic granulomatous disease. **(3) Antiparasitic effects (IFN- γ):** It is used in toxoplasmosis, leishmaniasis and malaria. **(4) Antioncogenic effects:** It is used in hairy cell leukemia, chronic myelocytic leukemia, T cell lymphoma, Kaposi's sarcoma, endocrine pancreatic neoplasm, non-Hodgkin's lymphomas, etc., **(5) Cellular effects:** It inhibits cell growth, cellular proliferation, DNA synthesis and protein synthesis. It increases expression of MHC-Ag on cell surfaces. **(6) Immunoregulatory effects:** It increases cytotoxic activity of NK, K and T cells. It activates macrophage cytotoxic activity and suppressor T cells. It modulates the Ab formation. It suppresses the DTH.

- **Advantages of IFN:** It is nontoxic, non-antigenic, diffuses freely in the body and has wide antiviral activity.
- **Disadvantages of IFN:** **(1) Species specific:** Non-human origin IFN is not useful in human. This drawback is corrected by preparing the IFN from human cells like buffy coat leucocytes from blood bank induced by Sendai virus or Norwalk disease virus (NDV). **(2) Side effects:** It causes fever, malaise, fatigue, muscle pains and toxicity to organs.
- **Contraindications of IFN:** These are history of major depressive illness, active alcohol use, cytopenia (more than one type of blood cell deficiency), hyperthyroidism, renal transplantation, autoimmune diseases, etc.
- **IFN assay:** Estimation of IFN level called IFN assay. It is measured in IU (International Unit) per ml. It is poorly antigenic, so assay by serological method is not useful. It is done by inhibition of plaque formation by particular virus by IFN.
- **Preparation of IFN**
 - From buffy coat leukocytes: As described earlier.
 - DNA recombinant technology: Best for human use.
 - Pegylated IFN: Interferon- α linked to polyethylene glycol. This linkage results in slow absorption, decreased clearance and more sustained concentration, hence it can be administered once in a week.

Effective Factors on Ir of Viral Infections

1. **Body temperature:** Fever acts as a natural defense against viral disease as most viruses are inhibited by temperature above 39°C. However, in few cases fever causes damages like herpes simplex is reactivated by fever and produces 'fever blisters' and herpes fibrilis which is accompany with fever and other bacteria like *Strept. pneumoniae*, *Strept. pyogenes*, *Haemophilus influenza* and parasite like malaria.
2. **Age:** Most viral infections are common and more dangerous at the extremes of age.
3. **Hormones:** Administration of steroids enhance most viral infections, because of depression of immune response and inhibition of IFN synthesis. Such as

coxsackie B1 not causes disease in adult mouse, but induces fatal infection in mouse treated with cortisone. Varicella and vaccinia are lethal in patients on cortisone.

4. **Malnutrition:** Some viral infections, such as measles, produces a much higher incidence of complications and a higher case fatality rate in malnourished children than in well-fed patients.
5. **Viral infections:** Several viruses suppress the Ir, such as measles causes temporary depression of DTH, leukemia or infection by lymphocytic chorio meningitis (LCM) virus reduces the Ab synthesis and HIV destroys the CD-4 cells and reduces the CMI.

ACCESS YOURSELF

Short Notes

1. IFN.

Short Questions for Theory/Viva Questions

1. Write four examples of viruses transmitted by sexual intercourse.
2. Write four examples of viruses transmitted by placenta.
3. Write four examples of mosquito-borne viruses.
4. Write four uses of IFN.
5. Write the four differences between IFN- β and IFN- γ .

Comments on

1. Administration of steroids enhance viral infections.
2. IFN is called species specific, but not virus specific.

MCQs for Chapter Review

Introduction

1. Which virus given below is not a teratogenic virus?
 - a. Rubella
 - b. Cytomegalo virus
 - c. Herpes simplex
 - d. Measles
2. Which of the following is/are transfusion transmitted viruses?
 - a. Hepatitis B
 - b. CMV
 - c. HTLV-1
 - d. Rubella
 - e. HHV-8

Immune Response (Ir) to Viral Infections

3. **True about interferon:**
 - a. It is virus specific
 - b. It is bacteria specific
 - c. Produced from bacteria
 - d. Effective against viral infection
 - e. It is species specific
4. **About interferon true is:**
 - a. It is synthetic viral agent
 - b. Inhibits viral replication in cells
 - c. It is specific for a particular virus
 - d. None
5. **Interferon:**
 - a. Species specific
 - b. Reacts directly with virus particles to inactivate them
 - c. Reacts with cells, and the affected cell becomes resistant to a number of different viruses
 - d. Constitutively produced at high levels in cells, but requires an inducer for activity

6. True about interferon is:

- a. Host protein
- b. Viral protein
- c. Inactivated by nuclease
- d. Virus specific

7. The temperature required for optimum production of interferon is:

- a. 37°C
- b. 30°C
- c. 40°C
- d. 50°C

8. Fibroblast in tissue culture form interferon of type:

- a. Alpha
- b. Beta
- c. Gamma
- d. All of above

9. Interferon in nature:

- a. Protein
- b. Lipid
- c. Polysaccharide
- d. All of above

Answers and Explanation of MCQs

1. d

- Follow section, **modes of transmission** and Table 12.1 for explanation.

2. a, b, c and e

- Follow section, **modes of transmission (blood borne)** for explanation.

3. d and e

- Interferon is species specific not virus or bacteria specific and effective against bacterial, viral, parasitic, malignant and other diseases.

- **Option 'c'**: Follow section, Table 12.2 (**origin**) for explanation.

4. b

5. a

- Follow section, **interferon (IFN → mechanism of actions)** for explanation of answers of MCQs 4–5.

6. a

7. c

- Follow section, **interferon (IFN → properties of IFN → synthesis)** for explanation of answers of MCQs 6–7.

8. b

9. a

- Follow Table 12.2 for explanation of answers of MCQs 8–9.

General Properties of Fungi

Chapter Outline

- Introduction
- Classification
- Hyphae, Pseudohyphae and Mycelium
- Precipitating Factors of Fungal Infections

INTRODUCTION

Meaning

Fungus (Latin) and Mykes (Greek) both have same meaning as mushroom (type of edible fungus), because it has mushroom like appearance.

Definition

Microorganism with mushroom like appearance is called fungus (plural; fungi). Branch of medical science related with study of fungus and disease produce by it is called Mycology.

History

Raymond Jacques Sabouraud (1864–1936) is called the father of Mycology.

Taxonomy

Fungi are eukaryotes (Eu means true and karyotes means nucleus), because they contain mature nucleus. Actually fungi are branching filamentous in nature and prefix Myco is used for some bacteria like *Mycobacterium* and *Mycoplasma*, because they have branching-filamentous form as like fungi.

Morphological Properties

- **Capsule:** *Cryptococcus neoformans* is the only capsulated fungus. Capsule aids in virulence and also helps in diagnosis of fungus.
- **Cell wall:** It contains polysaccharides like chitin, glucans, mannans, chitosan, cellulose, etc., mixed with polypeptide.
- **Plasmalemma (cytoplasmic membrane):** It presents inner to cell wall and contains glycoproteins, lipids and ergosterol.

- **Cytosol (cytoplasm):** Fungi are eukaryotic microbes and contain nucleus, nuclear membrane, mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, etc. They have both DNA and RNA.
- **Chlorophyll:** It is absent in fungi, so fungi are not able to do photosynthesis, and they remain dependent on host cell for nutrition.
- **Reproduction:** Fungi reproduced by following methods:
 - Asexual methods: These include hyphal extension, budding or fission.
 - Sexual methods: These include meiosis and mitosis. In many fungi sexual methods of reproduction are unknown. Such fungi are called fungi imperfecti.

Differences between Bacteria and Fungi

Follow Table 13.1.

Useful Properties of Fungi

Food industries: Fungi are used to alter the texture, flavor, digestibility and palatability of natural and processed food. Following are the examples:

1. Mushroom of basidiomycetes is used to prepare the food.

TABLE 13.1: Differences between bacteria and fungi

Features	Bacteria	Fungi
Taxonomy	Prokaryotes	Eukaryotes
Cell	Unicellular	Uni/multicellular
Cell wall/envelope	Thick and contains peptidoglycan and LPS	It contains chitin, manna and other polysaccharides
Sterol in plasma membrane	Absent (except in <i>Mycoplasma</i> and <i>Ureaplasma</i>)	Present (like cholesterol, ergosterol, etc.)

2. *Candida fukuyamaensis* is used to prepare the Russian manchurian tea. It also used as medicine in many diseases.
3. *Endomyces* is used to prepare the fat.
4. *Torulopsis* is used to prepare the protein.
5. *Saccharomyces cerevisiae*: Baker's yeast is used in baking bread and bakery products. Brewer's yeast is used to prepare the beer/alcohol. It is also the common name of *S. cerevisiae*, but of different strain. It also used as vector in HBV vaccine preparation.

Drugs preparation

- *Penicillium notatum*: Penicillin was discovered from this fungus by Alexander Fleming in 1928.
- *Penicillium chrysogenum*: Also useful to prepare the penicillin.
- *Penicillium griseofulvum*: Useful to prepare gresiofulvin.
- *Acremonium* (*Cephalosporium*) *chrysogenum*: Useful to prepare cephalosporin.
- *Aspergillus terreus*: Useful to prepare simvastatin (cholesterol-lowering agent).
- *Claviceps purpurea*: Useful to prepare ergot alkaloids like ergometrine or ergotamine.
- *Tolypocladium inflatum*: Cyclosporine an immuno-suppressive drug derived from this fungus.

Vaccine preparation: Certain fungi like *Saccharomyces cerevisiae* (Brewer's yeast), *Hansenula polymorpha* and *Pichia* spp., are used as a cloning vector to prepare recombinant vaccine of HBV.

Research model: *Neurospora crassa* acts as an ideal model to study host-pathogen relationship.

Vector control

1. *Culicinomyces clavosporus*: It is useful in malaria eradication.
2. *Coelomomyces*: It is able to kill the larvae of mosquitoes.

Agriculture industries

1. *Beauveria bassiana*: It is used to control the banana root borer, sugar cane borer, sweet potato weevil and rice water weevil.
2. *Verticillium lecani*: It is used to control the sweet potato white fly, most damaging pest in Cuba.
3. *Trichoderma*: It is used to control the soil-borne disease that attacks the tobacco, tomatoes and peppers.

Harmful Properties of Fungi

Diseases in humans

1. Mycosis (mycoses): Disease produced by fungus invasion in body called mycosis (Plural; mycoses).
2. Allergic reactions: Two types of allergic reactions like "id" reaction and systemic disease. Follow Ch. 92 for more details.

Fungal food poisoning: These are two types like mycetism (mycetismus, muscarinism) and mycotoxicoses. Follow Ch. 92 for more details.

Spoiling of stored food: Fungi spoil the stored grains, fruits, vegetables and foodstuff. Fungi present as bread molds in stored food. About 10% of world's foods are spoiled by fungal contamination.

Decaying of materials: Fungi cause decaying of leather, timber, fabrics, electric devices, etc.

Fungal growth on plasticize products: Fungi grow on plastic items like computer disks, videotapes, audiotapes, etc. Commonly encountered fungi are *Alternaria*, *Aspergillus*, *Epicossum*, *Paecilomyces*, *Penicillium* and *Trichoderma*.

Biological warfare

1. Yellow rain: Yellow rain was the subject of a 1981 political incident in which the US Secretary of State Alexander Haig accused the Soviet Union of supplying T-2 mycotoxin to the Communist states in Vietnam, Laos and Cambodia for use in counterinsurgency warfare. Refugees described many different forms of attacks, including a sticky yellow liquid falling from planes or helicopters, which was called yellow rain. Those exposed claimed neurological and physical symptoms including seizures, blindness and bleeding with over 10 thousand deaths. Sample analysis from the victims reported the presence of *Trichothecene* mycotoxins, including T-2 toxin, diacetoxyscirpenol (DAS) and deoxynivalenol (DON); however, presence of mycotoxin in yellow rain was challenged by later studies.
2. *Coccidioides immitis*: It is transmitted by inhalation and dangerous to work with it in laboratory enables its use in war.

CLASSIFICATION

Morphological Classification

It includes following four types.

Yeast (Fig. 13.1a): These fungi are unicellular and spherical. Cells without budding are called yeast cells or in budding form called budding yeast cells. They developed by asexual (budding) method. They are without hyphae or filamentous form. They produce the creamy or pasty colony on SDA. Examples include *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, etc.

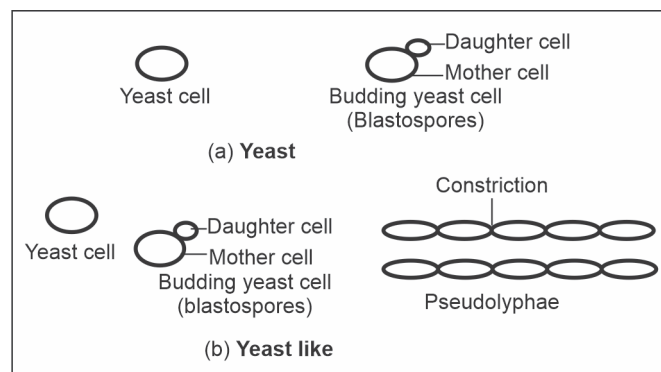


Fig. 13.1: Yeast, budding yeast cell and pseudohyphae

84 Yeast like (Fig. 13.1b): It is unicellular and spherical or oval. Cells without budding are called yeast cells or in budding forms called budding yeast cells. During budding process, daughter cell incompletely gets separated from mother cell and produces a chain of elongated yeast cells called pseudohyphae. It is developed by asexual (budding or binary fission) methods. It produces the creamy or pasty colony on SDA. Example includes *Candida* spp.

Mold/filamentous fungi: These fungi are multicellular. They are developed by asexual (budding/binary fission) or by sexual methods. They have filamentous hyphae (Fig. 13.2), which may be septate or nonseptate (coenocytic). They produce cottony, woolly, velvety, granular and pigmented colonies. Examples include dermatophytes, *Geotrichum candidum*, *Coccidioides immitis*, *Aspergillus* spp., *Zygomycetes*, etc.

Dimorphic fungi: They grow at two different temperatures, hence called dimorphic fungi. At 37°C they produce yeast or parasitic phase, and at 22°C they produce mold or mycelial phase. Examples include all systemic fungi like *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* plus *Sporothrix schenckii* and *Penicillium marneffei* (other species of *Penicillium* are not dimorphic, and they can grow at 25°C as mold).

Systemic (Taxonomical) Classification

It includes naming and typing of fungus.

Five kingdom system of classification: All organisms can be placed in five kingdoms like Monera, Protista, Fungi, Plantae and Animalia by RH Whittaker in 1969, called five kingdom system of classification. Fungi were initially classified with the plants, but in 1969 it is classified in a separate kingdom as “fungi” under the superkingdom “eukaryotes”. General scheme of classification includes Superkingdom → Kingdom → Phylum (Division was used initially now replaced by phylum) → Class → Order → Family (Tribe) → Genus → Species (specific epithet) → Subspecies (subsp.)/Variety (var.)/Forma (f.)/Forma specials (f. sp.) as mentioned in Table 13.2. The name of phylum, class, order and family ends by adding suffix “mycota”, “mycetes”, “ales” and “aceae”, respectively as shown in Table 13.2.

Binomial nomenclature: Binomial nomenclature (scientific name) system was proposed by Swedish

TABLE 13.2: General scheme of classification of fungi			
Superkingdom → Eukaryotes			
Kingdom → Fungi			
Taxon	Suffix	Teleomorph (sexual)	Anamorph (asexual)
Phylum	mycota	Ascomycota	Deuteromycetes
Class	mycetes	Ascomycetes	Hyphomycetes
Order	ales	Onygenales	Moniliales
Family	aceae	Onygenaceae	Moniliaceae
Genus	—	<i>Ajellomyces</i>	<i>Histoplasma</i>
Species	—	<i>capsulatus</i>	<i>capsulatus</i>
Variety	—	<i>capsulatus</i>	<i>capsulatus</i>

botanist in 1758 which includes genus name at 1st followed by species/epithet name in Italic pattern. In case of male scientist species name ends with suffix “ii”, and in case of female scientist species name ends with suffix “ae”. Both singular and plural are written same but in abbreviation sp., and spp., are used for singular and plural, respectively. Genus name is written in Latin noun and starts with capital letter with Italic pattern. Species/epithet name starts with small letter with Italic pattern irrespective of name of person or place. Species name is based on different properties of unit like *albicans* from white colony, *brisiliensis* from place of origin, *marneffei* from scientists, etc. For example, *Histoplasma capsulatum*.

Trinomial nomenclature: The word in the name (second epithet) might by subspecies (subsp.)/variety (var.)/forma (f.)/forma specials (f. sp.) and also italicized or underlined. For examples, *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii* and *Histoplasma capsulatum* var. *farcinosum*.

Taxonomical types of fungi: They are based on spore formation methods as mentioned below. This classification also used other supportive features like fatty acids analysis, zymogram pattern, DNA hybridization, RFLP, etc.

Mnemonic

- **Asexual spores:** ABC – CAS → Below explanation
- **Sexual spores:** ZABO → Table 13.3

1. Asexual spores: Fungi developed by budding, binary fission or by apical elongation.

- **Blastospores (Fig. 13.1):** They are developed by budding method. Budding part of yeast cell called blastospore. For example, *Cryptococcus neoformans*.
- **Chlamydospores:** They are developed by apical elongation method. Preexisting hyphal cell becomes thick, double wall and circular called chlamydospore. For examples, *Candida* spp., and *Paracoccidioides brasiliensis*. Chlamydospores produced on media like rice starch agar and cornmeal agar when incubated at 20°C. Three subtypes (Fig. 13.3) of chlamydospores are terminal, sessile and intercalary.

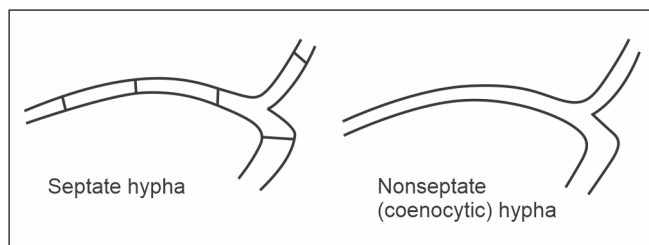


Fig. 13.2: Types of hyphae

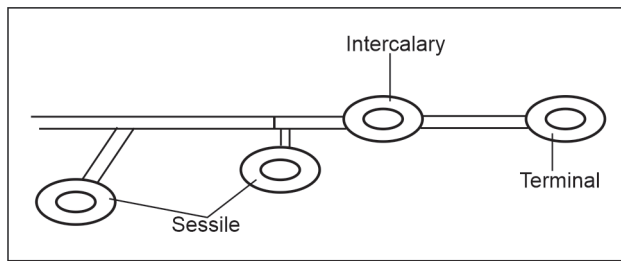


Fig. 13.3: Subtypes of chlamydospores

- **Arthrospores (Fig. 13.4):** They are developed by apical elongation method. Preexisting hyphal cells become thick, double-walled and cuboidal or rectangular or barrel-shaped called arthrospores. They detach after fracture of supporting cell. For examples, dermatophytes, *Geotrichum candidum*, *Coccidioides immitis*, *Hortaea werneckii* and *Trichosporon beigeli*.
- **Sporangiospores (Fig. 13.5):** They are developed by mitosis. These are endogenous spores. Spores are enclosed in a sac-like structure called sporangium and spores called sporangiospores. For example, Zygomycetes.
- **Conidiospores or conidia (Fig. 13.6):** They are developed by mitosis. These are exogenous spores. Spores are arranged in a chain-like structure and arise from a vase-like structure called phialides (conidia-producing cells). For example, *Aspergillus* spp. Following are two subtypes of conidia.

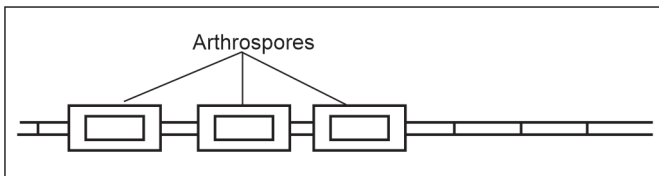


Fig. 13.4: Arthrospores

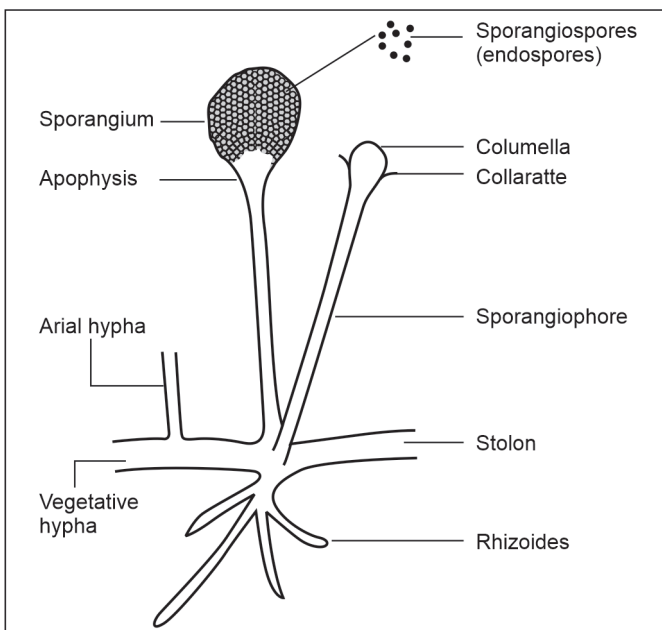


Fig. 13.5: Sporangiospores

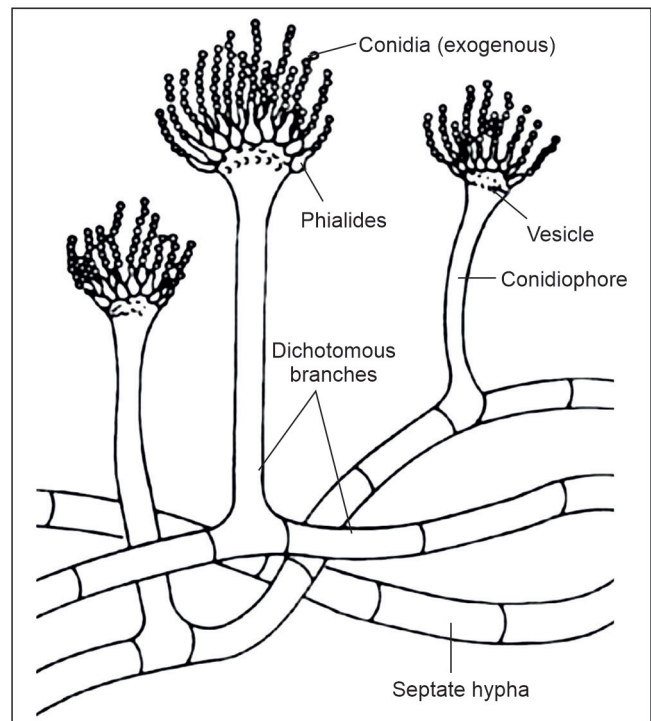


Fig. 13.6: Conidiospores

- **Microconidia:** They are further divided as basipetal and acropetal. Basipetal means youngest conidium is at base; for examples, *Penicillium*, *Aspergillus*, *Scopulariopsis brevicaulis*. Acropetal means youngest conidium is at top; for examples, *Alternaria* and *Cladosporium*.
- **Macroconidia:** They are further divided as dictyoconidia and phragmoconidia. Dictyoconidia means macroconidia with transverse and longitudinal septa; for examples, *Alternaria* and *Bipolaris*. Phragmoconidia means macroconidia with only transverse septa; for example, *Curvularia*.
- **Aleurospores (Fig. 13.7):** Macroconidia arranged the side of hyphae by a supporting cell called aleurospores. They are released after detachment from cell. For examples, *Epidermophyton* and *Microsporum canis*.

2. **Sexual spores:** They are developed by meiosis and mitosis. Sexual spores include zygospores, ascospores, basidiospores and oospores. All details are mentioned in Tables 13.3, 13.4 and Fig. 13.8.

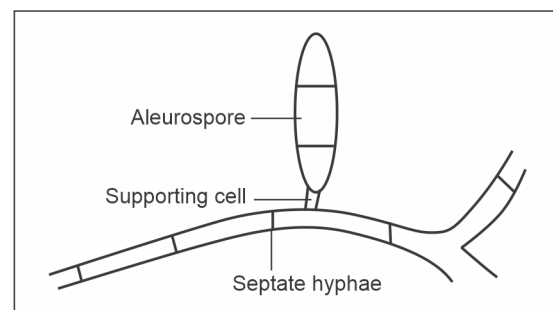


Fig. 13.7: Aleurospores

TABLE 13.3: Sexual spores with taxonomical types of fungi

Kingdom: Fungi						
Spores	Phylum	Class	Order	Family	Genus	Species
Nonseptate (aseptate/coenocytic) hyphae						
Zygospores (Fig. 13.8a)	Zygomycota	Zygomycetes (Phycomycetes)	Mucorales	Mucoraceae	<i>Mucor</i>	<i>M. racemosus</i>
					<i>Rhizomucor</i>	<i>R. pusillus</i>
					<i>Rhizopus</i>	<i>R. arrhizus</i>
						<i>R. microspores</i>
					<i>Absidia</i>	<i>A. corymbifera</i>
					<i>Apophysomyces</i>	<i>A. elegans</i>
				Cunninghamellaceae	<i>Cunninghamella</i>	<i>C. bertholletiae</i>
				Saksenaeaceae	<i>Saksenaea</i>	<i>S. vasiformis</i>
			Thamnidaceae	<i>Cokeromyces</i>	<i>C. recurvatus</i>	
			Synccephalastraceae	<i>Synccephalastrum</i>	<i>S. recemosum</i>	
Entomophorales	Ancylistaceae	<i>Conidiobolus</i>	<i>C. coronatus</i>			
	Basidiobolaceae	<i>Basidiobolus</i>	<i>B. ranarum</i>			
Septate hyphae						
Ascospores (Fig. 13.8b)	Ascomycota	Ascomycetes	Pneumocystidales	Pneumocystidaceae	<i>Pneumocystis*</i>	<i>P. jirovecii</i>
		Pyrenomycetes (Unitunicate)	Microascales	Microascaceae	<i>Pseudoallescheria</i>	<i>P. boydii</i>
			<i>Scopulariopsis</i>	<i>S. brevicaulis</i>		
				Ophistomatales	Ophistomataceae	<i>Sporothrix</i>
			Sordariales	Sordariaceae	<i>Neurospora</i>	<i>N. crasa</i>
			Hypocreales	Nectriaceae	<i>Fusarium</i>	<i>F. graminearum</i>
				Hypocreaceae	<i>Acremonium</i>	<i>A. falciforme</i>
		Pyrenomycetes (Bitunicate)	Dothideales	Dothideaceae	<i>Hortaea</i>	<i>H. werneckii</i>
			Plectomycetes	Piedraiaceae	<i>Piedraia</i>	<i>P. hortae</i>
		Eurotiales			Trichocomaceae	<i>Aspergillus</i>
				<i>Penicillium</i>	<i>P. marneffei</i>	
		Onygenales		Onygenaceae	<i>Chrysosporium</i>	<i>C. parvum</i>
<i>Ajellomyces</i>	<i>A. capsulatus</i>					
Basidiospores (Fig.13.8c)	Basidiomycota	Basidiomycetes	Tremellales	Cryptococcaceae	<i>Trichosporon</i>	<i>T. beigeli</i>
		Pucciniomycetes	Sporidiales	Sporidiobolaceae	<i>Rhodotorula</i>	<i>R. glutinis</i>

TABLE 13.4: Parafungal agents

Spores	Phylum	Class	Order	Family	Genus	Species
Nonseptate (aseptate/coenocytic) hyphae and sexual spores						
Oospores (Fig. 13.8d)	Oomycota	Oomycetes	Pythiales	Pythiaceae	<i>Pythium</i>	<i>P. insidiosum</i>
No hyphal structure, asexual spores						
Spores	—	Mesomycetozoea	Dermocytida	—	<i>Rhinosporidium</i>	<i>R. seeberi</i>
Sporangiospores	Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	<i>Prototheca</i>	<i>P. wickerhamii</i> <i>P. zopfii</i>

Notes:

- *Pneumocystis has no hyphae but taxonomically classified as fungus as Ascomycetes; however, still it is controversial to classify it as fungus and better to classify as atypical fungus under the new phylum called Protomycota.
- Tables do not include all fungi but only fungi important at undergraduate level.

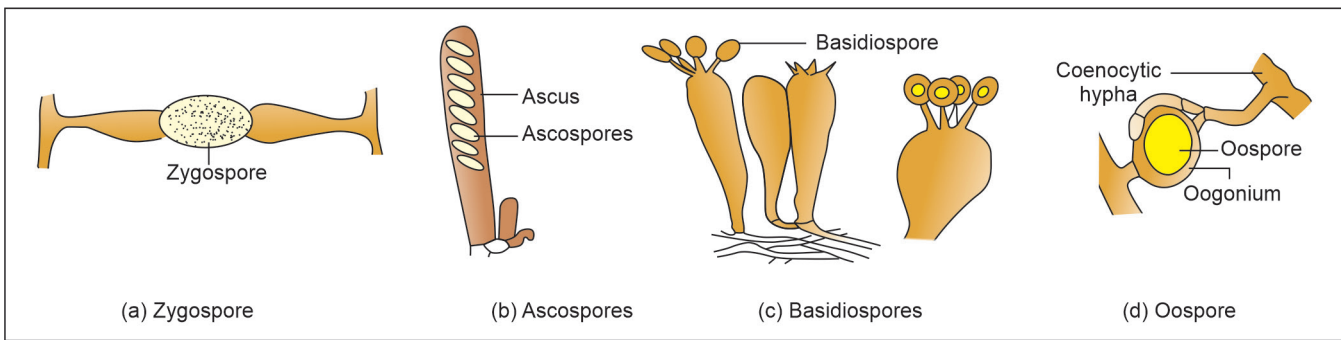


Fig. 13.8: Sexual spores

3. Unknown method (sexual spores): Exact method of sexual reproduction is unknown and such fungi are called miosporic fungi or mitosporic fungi or deuteromycetes or deuteromycota or hyphomycetes or fungi imperfecti.

Note: Parafungal agents

- Few pathogens like *Pythium insidiosum*, *Rhinosporidium seeberi* and *Prototheca* are producing disease similar to mycoses called parafungal agents.
- Sexual spores: Produced by *Pythium insidiosum*.
- Asexual spores: Produced by *Rhinosporidium seeberi* and *Prototheca*. In *Prototheca* spores are produced by internal septation and cleavage.
- They are studied in mycology but not classified in the kingdom fungi. Their classification is given in Table 13.4.

Clinical Classification

Nomenclature: Fungal disease called mycosis (singular)/mycoses (plural). Name of fungal disease are given by adding the suffix “sis” or “mycosis” to genus name; however, it is not satisfactory because same fungus causes many diseases and same disease is caused by many fungi. Some names of the fungal diseases are given by adding the suffix “i” to species name, like Histoplasmosis capsulati/duboisii/farciminosi.

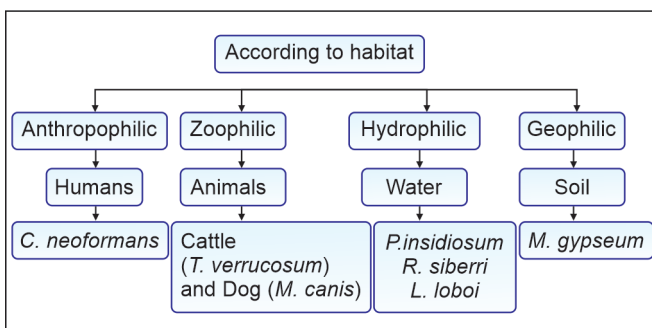
Clinical types of fungi: Two types:

- **Superficial mycoses:** Ch. 90.
- **Deep mycoses:** Ch. 91.

Ecological Classification

Follow Flowchart 13.1.

Flowchart 13.1: Ecological types of fungus



HYPHAE, PSEUDOHYPHAE AND MYCELIUM

Hypha

Meaning: Hypha derived from huphe (Greek) means Web.

Definition: It is an elongated, tubular and branching structure of fungus.

Types of hyphae

- 1. According to presence or absence of septa (Fig. 13.2)**
 - Septate hyphae
 - Nonseptate hyphae (coenocytic).
- 2. According to projection (Fig. 13.5)**
 - Aerial hyphae: Hyphae projected in air and containing spores called aerial hyphae. They are concerned with developmental function.
 - Vegetative hyphae: Hyphae submerged with surface or media called vegetative hyphae. They are concerned with nutritional function.
- 3. According to color**
 - Dark (brown-black) hyphae: Brown-black pigmented hyphae occur in some fungi and such fungi called phaeoid fungi or demateaceous fungi or black fungi.
 - Hyaline hyphae: Colorless hyphae occur in some fungi and such fungi called hyaline fungi.
- 4. According to morphology (Fig. 13.9)**
 - Racquet hyphae: Enlargement of hyphal cell in-between the length of hyphae with one broad end and other pointed end called racquet hyphae. For examples, *T. mentagrophytes* and *E. floccosum*.

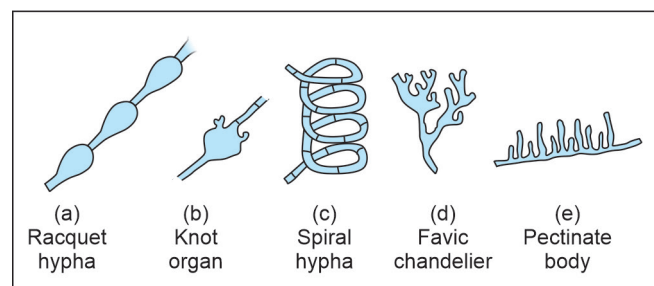


Fig. 13.9: Morphological types of hyphae

- Nodular hyphae or knot organ: In-between length of hyphae presence of swelling, like knot called nodular hyphae. For examples, *T. mentagrophytes*, *M. canis*, etc.
- Spiral hyphae: Hyphae present in spiral shape. For example, *T. mentagrophytes*.
- Favic chandelier: At the end of hyphae presence of multiple small projections like horn of reindeer or chandelier called favic chandelier. For examples, *T. violaceum* and *T. schoenleinii*.
- Pectinate body: One surface of hyphae is uniform while other shows some projections like broken comb called pectinate body. For example, *M. audouinii*.

Pseudohyphae

Definition is described above. Differences between hyphae and pseudohyphae are given in Table 13.5.

TABLE 13.5: Differences between hyphae and pseudohyphae

Features	Hyphae	Pseudohyphae
Reproduction	By apical elongation	By budding
Morphology	Fig. 13.2	Fig. 13.1
Hyphal cell	Cuboidal/rectangular	Oval
Constriction in between two cells	Absent 	Present 
Cell wall	Uniform due to absence of constriction	Not uniform due to presence constriction
Dividing septum	Straight	Oblique/curved

Mycelium

It is an entangled mass of hyphae (**Fig. 13.10**).

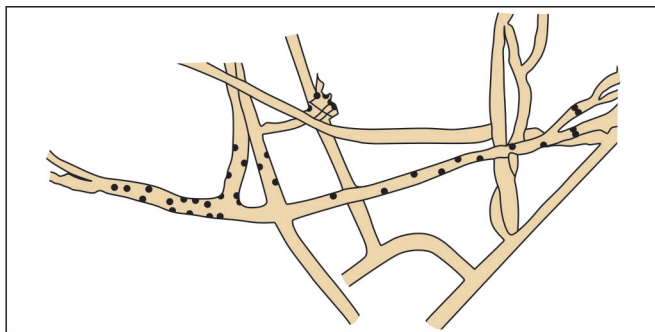


Fig. 13.10: Mycelium

PRECIPITATING FACTORS OF FUNGAL INFECTIONS

Synonym

They are also known as epidemiological determinants.

Types

Virulence of fungi is affected by following three factors.

Agent factors (virulence factors or determinants of virulence)

1. Intracellular or cell associated factors

- Fungal adhesins: Cell wall glycoprotein helps in attachment with host cells called adhesin.
- Polysaccharide and polypeptide complex: It provides rigidity and structural integrity to cell. It acts as a barrier against the effect of deleterious agents on fungi. Glucan is antiphagocytic.
- Capsular (K) Ag: Capsular (K) antigen presents in *Cryptococcus* inhibits the phagocytosis.
- Endotoxin: It is a glycoprotein presents in cell wall of *Candida*, *A. flavus* and *A. fumigatus*. It is responsible for tissue necrosis, pyogenic and anaphylactic reaction.

2. Extracellular factors

- Enzymes: Follow **Table 13.6**.
- Toxins: **Ch. 92** (section → fungal food poisoning).
- Antigens: They produce allergic reaction called id reaction (candidid in *Candida* and dermatophytid in dermatophytes).
- Pigment production: Melanin pigment produced by *Cryptococcus neoformans* protects fungus against the UV radiation and effects of immune cells.
- Mannitol production: It produced by *Cryptococcus neoformans* and protects the fungus from intracellular killing by phagocytes.

3. Other factors

- Fungal dimorphism: All the dimorphic fungi (mentioned above) have two forms like mycelium and yeast phase, possess different antigenic and surface features, and require different host mechanisms to contain them.
- Thermotolerance: It is an ability of fungus to survive at 37°C. It makes fungus a potent human

TABLE 13.6: Enzymes, sources and actions in fungi

Enzyme	Sources	Actions
Elastase	<i>A. flavus</i> , <i>A. fumigatus</i> and dermatophytes	Enhances the fungal invasion in elastin containing tissues like lungs, skin, blood vessels, etc., by degrading the elastin and scleroprotein
Alkaline protease	<i>A. flavus</i> , <i>A. fumigatus</i> and <i>Rhizopus</i> spp.	Enhances the fungal invasion in tissues like lungs, by degrading the elastin and collagen
Keratinase Collagenase	Dermatophytes	Enhances the fungal invasion in tissues like skin by degrading the scleroprotein
Acid protease	<i>A. fumigatus</i> and <i>Candida</i> spp.	Cleavage of IgA to reduce the local immunity
Urease	<i>C. neoformans</i>	Converts urea in to ammonia which acts as nitrogen source

pathogen. *Cryptococcus neoformans* can survive at higher temperature about 41–43°C.

- Resistance to antimicrobial products released by host cells: Like yeast, spherules, etc., are showing resistant to antimicrobial products like H_2O_2 released by host cells.
- Phenotypic switching: *C. albicans* has ability to adapt the different changing conditions of host, which helps to evade the host defense mechanism and survival of fungus.
- Survival in acidic pH: Fungus grows best at acidic pH and it may tolerate the gastric acidity.

Host factors

1. **Trauma:** Local trauma may precipitate the fungal infections.
2. **Immunosuppressive diseases or conditions:** Conditions like long-term steroid therapy, antibiotic therapy, immunosuppressive therapy, renal transplantation, malignancy, DM and AIDS favor the fungal infections.
3. **Occupation:** Fungal infections are common in wooden workers like carpenters, farmers, etc.
4. **Gender:** Fungal infections are common in female due to wet work.
5. **Wet areas:** Moisture may precipitate the fungal infection in areas like inguinal, axilla and foot due to constant wearing of shoes.

Environmental factors

1. **Economical status:** Fungal infections are common in developing countries.
2. **Other factors:** Like overcrowding and poor sanitation also increase risk of fungal infections.

ACCESS YOURSELF

Essay/Full Question

1. Classification of fungi.

Short Notes

1. Useful and harmful properties of fungi
2. Morphological classification of fungi
3. Taxonomical (systemic) classification of fungi
4. Fungal spores
5. Fungal hyphae
6. Virulence factors of fungi.

Short Questions for Theory/Viva Questions

1. Write four differences between bacteria and fungi.
2. Write four differences between hyphae and pseudohyphae.
3. Write four examples of asexual spores of fungus.
4. Write four examples of sexual spores of fungus.
5. Define vegetative hyphae and aerial hyphae.
6. What are pectinate body and favic chandelier?
7. What is mycelium (in fungus)?

Comments on

1. Fungus is not able to do photosynthesis.

Introduction

1. **Brewer's yeast is a common name for:**
 - a. *Pichia guilliermondii*
 - b. *Aspergillus niger*
 - c. *Saccharomyces cerevisiae*
 - d. *Penicillium notatum*
2. **Fungus useful for penicillin preparation is:**
 - a. *Penicillium notatum*
 - b. *Aspergillus tereus*
 - c. *Candida fukuyamaensis*
 - d. *Penicillium marneffeii*

Classification

3. **Which of the following is only yeast?**
 - a. *Candida*
 - b. *Mucor*
 - c. *Rhizopus*
 - d. *Cryptococcus*
4. **Fungi that possess capsule is:**
 - a. *Blastomyces dermatitidis*
 - b. *Histoplasma*
 - c. *Cryptococcus*
 - d. *Coccidioides immitis*
5. **Which of the following is false regarding dimorphic fungi?**
 - a. Occurs in two growth forms
 - b. Can cause systemic infection
 - c. *Cryptococcus* is an example.
 - d. *Coccidioides* is an example.
6. **Dimorphic fungi behave like yeast at:**
 - a. <10°C
 - b. Body temperature
 - c. >40°C
 - d. *In vitro*
7. **Dimorphic fungus/fungi:**
 - a. *Candida*
 - b. *Cryptococcus*
 - c. *Blastomycosis*
 - d. *Coccidioidomycosis*
 - e. *Sporothrichosis*
8. **All are dimorphic fungi except:**
 - a. *Blastomyces dermatitidis*
 - b. *Histoplasma*
 - c. *Penicillium marneffeii*
 - d. *Phialophora*
9. **Dimorphic fungus:**
 - a. *Candida*
 - b. *Histoplasma*
 - c. *Rhizopus*
 - d. *Mucor*
10. **The following fungi are thermally dimorphic except:**
 - a. *Sporothrix schenckii*
 - b. *Cryptococcus neoformans*
 - c. *Blastomyces dermatitidis*
 - d. *Histoplasma capsulatum*
11. **Budding reproduction in tissue is seen in:**
 - a. *Cryptococcus*, *Candida*
 - b. *Candida*, *Rhizopus*
 - c. *Rhizopus*, *Mucor*
 - d. *Histoplasma*, *Candida*
12. **The fungi which do not have sexual phase belong to which of the following groups?**
 - a. Phycomycetes
 - b. Fungi imperfecti
 - c. Basidiomycetes
 - d. Ascomycetes
13. **Barrel-shaped spores (arthrospores) are seen with:**
 - a. *Blastomyces*
 - b. *Histoplasma*
 - c. *Coccidioides*
 - d. *Candida*
14. **A sporangium contains:**
 - a. Spherules
 - b. Sporangiospores
 - c. Chlamydospores
 - d. Conidia

- 90** 15. Following is the asexual spore:
 a. Oospore b. Zygospor
 c. Ascospore d. Blastospore
16. Aseptate hyphae are seen in:
 a. Phycomycetes b. Ascomycetes
 c. Basidiomycetes d. Deuteromycetes
17. Ascospore is:
 a. Asexual spore b. Sexual spore
 c. Conidia d. None of above
18. Which of the following is produced sexually?
 a. Ascospore b. Conidium
 c. Odium d. Yeast bud
19. Fungal spores may be produced:
 a. Singly b. In chains
 c. In sporangium d. All of above
20. Human fungal infection is known as:
 a. Mycosis b. Mycoses
 c. a + b d. Fungosis

Answers and Explanation of MCQs

1. c
 • Common names for *Saccharomyces cerevisiae* as per uses are Brewer's yeast and Baker's yeast.
2. a
 • Penicillin was discovered from *Penicillium notatum* by Alexander Fleming in 1928.
3. d
 • *Candida* is yeast like.
 • *Mucor* and *Rhizopus* are mold (filamentous) type.
4. c
 • In *Histoplasma capsulatum*, species name indicating the capsulated property of fungus, but it is noncapsulated.
 • *Cryptococcus neoformans* is the only capsulated fungus.
5. c
6. b
7. c, d, e
8. d
9. b
10. b
11. a
 • Follow section, **morphological classification** for explanation of answers of MCQs 5–11.
12. b
 • Phycomycetes, Basidiomycetes and Ascomycetes are showing sexual method of reproduction, while in fungi imperfecti method of reproduction is unknown.
13. c
 • Follow section, **classification [systemic (taxonomical) classification → asexual spores → arthrospores]** and Fig. 13.4 for explanation.
14. b
 • Follow section, **classification [systemic (taxonomical) classification → asexual spores → sporangiospores]** and Fig. 13.5 for explanation.
15. d
 • Oospore, zygospor and ascospore are the sexual spores.
16. a
17. b
18. a
 • Follow **Table 13.3** for explanation for explanation of answers of MCQs 16–18.
19. d
 • Follow section, **classification [systemic (taxonomical) classification]** to know about all types of fungal spores, method of production and for other details.
20. c
 • Mycosis is singular form for fungal infection, while mycoses is the plural form for fungal infections.

Introduction and Classification of Parasites

Chapter Outline

- Parasite
- Host
- Life Cycle

PARASITE

Meaning

Parasite word derived from parasitos (Greek) means eater at the court (meat eater).

Definition

Living organism which receives nourishment and shelter from other organism is called parasite.

Nomenclature

Parasites have Latinized name, hence written in Italics. It consists of two parts: 1st part is genus name, starts with capital letter and 2nd part is species name, starts with small letter like *Giardia lamblia*.

Classification

Different types of parasites are as follows.

According to habitat: Two types:

1. Ectoparasites: Parasites living outside on the body surface of the host called ecto-parasites. Follow **Ch. 47** and **Ch. 110** for more details.

2. Endoparasites: Parasites living inside the body of the host called endo-parasites.

According to visit to host: Following are different types.

1. Temporary parasite: It visits the host for short time.
2. Permanent parasite: Whole life passes as a parasite.
3. Facultative parasite: It lives the parasitic life when the opportunity arises.
4. Obligatory parasite: It cannot exist without parasitic life.
5. Occasional (accidental) parasite: It attacks on unusual host.
6. Wandering (aberrant) parasite: Happen to reach a place where it cannot live.

Morphological classification: Based on their cellular structure, all medically importance parasites are fall in two broad categories like protozoa and metazoa (helminths or worms) as shown in **Flowchart 14.1**, **Tables 14.1** and **14.2**.

Taxonomical (systemic) classification: It based on general scheme of classification such as Kingdom (sub-

Flowchart 14.1: Morphological classification of parasites

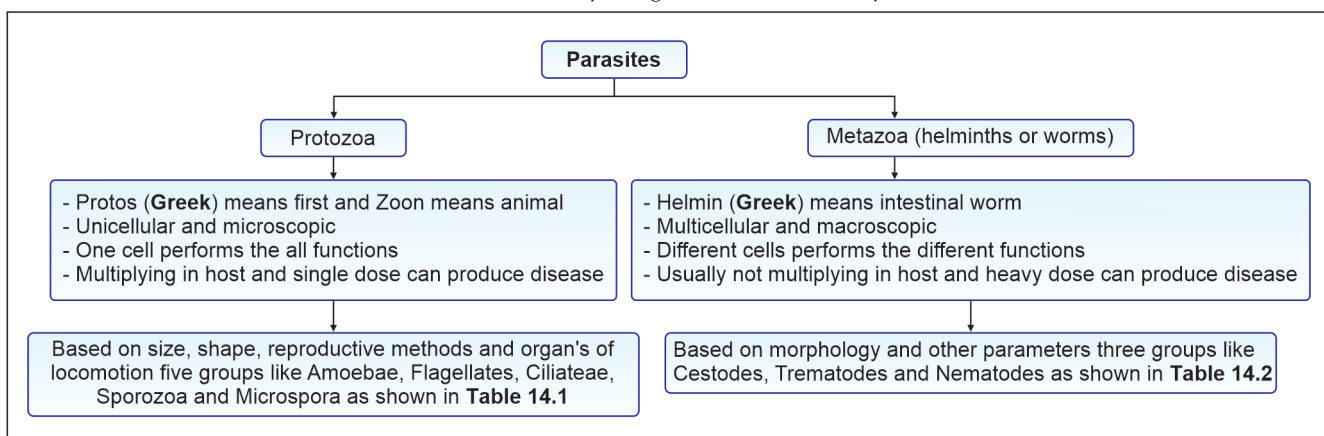


TABLE 14.1: Morphological classification of protozoa

Features	Amoebae	Flagellates	Ciliateae	Sporozoa	Microspora
Organ of locomotion	Pseudopodium	Flagella	Cilia	Non-motile	Non-motile
Examples	<i>E. histolytica</i> , <i>E. coli</i> , <i>N. fowleri</i> , etc.	(1) Oral/genital flagellates: <i>Giardia</i> , <i>Trichomonas</i> , etc. (2) Hemo-flagellates: <i>Leishmania</i> and <i>Trypanosoma</i>	<i>B. coli</i>	(1) Blood inhabiting: <i>Plasmodium</i> , <i>Babesia</i> (2) GIT inhabiting: <i>Isospora</i> , <i>Toxoplasma</i> , etc.	<i>Pleistophora</i> , <i>Nosema</i> , <i>Enterocytozoon</i> , <i>Encephalitozoon</i> , etc.

TABLE 14.2: Morphological classification of metazoa

Features	Cestoda	Trematoda	Nematoda
General features			
Common name	Tape worms or cestodes	Flukes or trematodes	Round worm or nematodes
Example (s)	<i>D. latum</i> , <i>T. solium</i> , etc.	<i>S. haematobium</i> , <i>F. hepatica</i> , etc.	<i>A. lumbricoides</i> , <i>W. bancrofti</i> , etc.
Adult stage			
Shape	Tape like	Leaf like	Cylindrical
Segments	Segmented	Unsegmented	Unsegmented
Sexes	Not separate, hermaphrodite (monoecious)	Not separate/hermaphrodite (monoecious) except <i>Schistosoma</i>	Separate (diecious)
Head	Suckers often with hooks	Suckers, no hooks	No suckers, no hooks but well developed buccal capsule for adhesion
GIT	Absent	Present but incomplete (no anus)	Present and complete with anus
Body cavity (Coelom)	Absent	Absent	Present
Female	Oviparous	Oviparous	Oviparous/vivi (larvi) parous/ovivivi parous
Egg stage			
Opeculum	Present in Pseudophylidea and absent in Cyclophylidea	Present in monoecious trematodes and absent in diecious trematodes	Absent
Larval stage			
Larval forms	Cysticercus, cyticercoid, hydatid cyst, coenurus, coracidium, proceroid and plerocercoid	Miracidium, redia, cercaria, metacercaria, redia and sporocyst	Rhabditiform larvae, filariform larvae and microfilariae

kingdom) → phylum (subphylum) → class (superclass/subclass) → order (suborder) → family (super family/subfamily) → genus → species (subspecies). Medical important parasites are fall in to two kingdoms.

1. Protista: Eukaryotic, microscopic and unicellular organisms called protozoa are classified as protista as shown in **Table 14.3**.
2. Animalia: Eukaryotic, macroscopic and multicellular organisms called metazoa are classified as animalia as shown in **Table 14.4**.

Pathogenic classification: Following two types on the basis of ability of species to produce diseases.

1. Pathogenic:
 2. Nonpathogenic:
- } Described in respective chapter

HOST

Definition

Living organism which harbors the parasite called host.

Classification

Following are different types.

Definitive (primary) host: Host which harbors the adult or sexual stage of parasite called definitive host. In majority of cases man is the definitive host.

Intermediate (secondary) host: Host which harbors the larval or asexual stage of parasite called intermediate host. Usually there is one intermediate host; however, in some parasitic infections two intermediate hosts are required called first and second intermediate host respectively like in *D. latum*. In majority of cases human is the definitive host; however, in following parasites, human acts as an intermediate host.

- *Plasmodium* spp., (malaria).
- *Babesia* spp., (babesiosis).
- *Echinococcus granulosus* (hydatid disease).
- *Toxoplasma gondii* (toxoplasmosis).
- *Sarcocystis lindemanni* (sarcocystosis).

TABLE 14.3: Systemic classification of protozoa

Kingdom: → Protista Subkingdom: → Protozoa							
Phylum	Class	Subclass	Order	Suborder	Family	Genus	Species
Sarcomas- tigophora	Subphylum (Sarcodina) Superclass (Rhizopoda) Class (Lobosea/ Amoebae)		Amoebida	Tubulina	Entamoebidae	Entamoeba	Pathogenic and non- pathogenic species are mentioned in respective chapters
						Endolimax	
				Acanthopodina	Hartmannellidae	Iodamoeba	
						Acanthamoeba	
	Class (Heterolo- bosea)		Schizopyrenida		Vahlkampfiidae	Naegleria	
	Subphylum (Mastigo- phora)		Trichomonadidae		Trichomonadidae	Trichomonas	
						Dientamoeba	
			Diplomonadida	Deploomonadida	Hexamitidae	Retortomonas	
						Giardia	
						Enteromonadida	
Class (Zoomastigo- phora)		Retortamonadida			Chilomastix		
		Kinetoplastida	Trypanosomatina	Trypanosomatidae	Trypanosoma		
					Leishmania		
Ciliophora	Kinetofrga- minophorea	Vesti- bulifferia	Trichostomatida	Trichostomatina		Balantidium	
Apicom- plexa	Sporozoa		Haemosporida	Haemosporina	Haemosporidae	Plasmodium	
						Laverania	
		Coccidia	Piropiasmida	Piropiasmina	Piropiasmidae	Babesia	
			Eucoccidia	Eimeriina	Eimeriidae	Cystoisospora	
						Cyclospora	
					Sarcocystidae	Toxoplasma	
						Sarcocystis	
					Cryptosporidae	Cryptosporidium	
Microspora		Microsporida			Enterocytozoon		
					Encephalitozoon		
					Microsporidium		
					Nosema		
					Pleistophora		

Paratenic host (carrier/transport host): Host where parasite remains viable without further development called paratenic host.

Accidental/incidental host: Host in which parasite is not found usually but enters accidentally and host will be considered as dead end host. Following are parasites where human acts as dead end host:

1. Free living amoebae: *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris*
2. Invasive or muscular *Sarcocystis*: *S. lindamanni*
3. *Spirometra* species
4. *Echinococcus granulosus* in human
5. *Multiceps multiceps*
6. *Trichina spiralis*
7. *Angiostrongylus* species
8. *Capillaria hepatica*
9. *Gnathostoma* spp.
10. Others: Normally cycle is continuing; but when parasite enters in certain tissues, human will be act

as dead end host like in *Taenia solium* when larvae enter beyond the intestine and in *E. histolytica* when trophozoites enter beyond the intestine (extra-intestinal amoebiasis).

LIFE CYCLE

Definition

Sequential change in growth, development and multiplication of parasite called life cycle.

Types

Following are the different types:

According to reproductive methods and types of host

1. Sexual cycle/cycle in definitive host: Development and multiplication occurs by sexual methods.
2. Asexual cycle/cycle in intermediate host: Development and multiplication occurs by asexual methods.

TABLE 14.4: Systemic classification of metazoan/helminths/worms

Kingdom: → Animalia								
Subkingdom: → Metazoa or Helminths								
Phylum	Class	Subclass	Order	Suborder	Family	Genus	Species	
Platyhelminths	Cestoda		Pseudophylidea		Diphylobothriidae	<i>Diphylobothrium</i>	Pathogenic and non-pathogenic species are mentioned in respective chapters	
			Cyclophylidea			Taeniidae		<i>Taenia</i>
						Hymenolepidae		<i>Hymenolepis</i>
								Dilepididae
	Trematoda	Digenea (So called because di (two) and genetic generation), means required two hosts for cycle		Prosostomata		Strigeata		Schistosomatidae
			Amphistomata		Fasciolidae	<i>Fasciolopsis</i>		
						<i>Fasciola</i>		
			Distomata		Heterophyidae	<i>Heterophyes</i>		
						<i>Metagonimus</i>		
					Echinostomatidae	<i>Echinostoma</i>		
						<i>Paryphostomum</i>		
					Paraphistomatidae	<i>Watsonius</i>		
						<i>Gastrodiscoides</i>		
					Opisthorchidae	<i>Clonorchis</i>		
						<i>Opisthorchis</i>		
			Triglotrematidae		<i>Paragonimus</i>			
Nemat-helminths	Nematoda	Aphasmidia (no caudal chemo-receptors)	Enoplida		Trichinellidae	<i>Trichinella</i>		
					Trichuridae	<i>Trichuris</i>		
						<i>Capillaria</i>		
					Phasmidia (Caudal Chemo-receptors)	Rhabditidia		Ascarididae
		Ancylostomatidae	<i>Ancylostoma</i>					
			<i>Necator</i>					
		Strogyloididae	<i>Strongyloides</i>					
		Oxyuridae	<i>Enterobius</i>					
		Angiostrogylidae	<i>Angiostrongylus</i>					
		Spirurida		Onchocercidae (Acantho-cheilonematidae)		<i>Wuchereria</i>		
						<i>Brugia</i>		
						<i>Onchocerca</i>		
						<i>Dipetalonema</i>		
						<i>Mansonella</i>		
						<i>Dirofilaria</i>		
		<i>Loa</i>						
Dracunculidae	<i>Dracunculus</i>							
Gnathostomatidae	<i>Gnathostoma</i>							

According to numbers of host

1. Direct (simple) cycle: Parasite requires only single host to complete the cycle called direct cycle. For examples follow **Table 14.5**.
2. Indirect (complex) cycle: Parasite requires two or more host to complete the cycle called indirect cycle. Following are subtypes:
 - Requires two hosts: In this cycle one host act as definitive while other acts as intermediate host. For examples follow **Table 14.6**.
 - Requires three hosts: Some parasites require three hosts, of this one act as definitive while other two acts as 1st intermediate host and 2nd intermediate host. For examples, follow **Table 14.7**.

Clinical Significances

Ideas of life cycle help to know the growth and development of parasite, to know the clinical disease produced by parasites, to diagnose the parasite in laboratory, to treat parasitic disease and to prevent the parasitic disease.

Role of Human in Parasitic Life Cycle

- **Normal (natural) life cycle:** Life cycles of many parasites normally continue between human and external environment or other host like animals, vectors, etc., all such life cycles are described with **green arrows (↓)** in respective chapters.

TABLE 14.5: Parasites with direct (simple) life cycle

Parasites	Host
Protozoa	
<i>E. histolytica</i>	Human
<i>G. lamblia</i>	Human
<i>T. vaginalis</i>	Human
<i>B. coli</i>	Pig and human
<i>C. belli</i>	Human
<i>C. parvum</i>	Human
<i>C. cayetanensis</i>	Human
<i>Microspora</i>	Human
Metazoa	
<i>A. lumbricoides</i>	Human
<i>A. duodenale</i>	Human
<i>N. americanus</i>	Human
<i>S. stercoralis</i>	Human
<i>T. trichuria</i>	Human

TABLE 14.6: Parasites requires two hosts

Parasites	Definitive host	Intermediate host
Protozoa		
<i>Plasmodium</i> spp.	Mosquito	Human
<i>Babesia</i> spp.	Hard tick	Human
<i>T. gondii</i>	Cat	Human/animals
<i>S. bovis</i>	Human	Cattle
<i>S. suis</i>	Human	Pig
<i>S. lindamanni</i>	Cat or dog	Human
Metazoa		
<i>T. saginata</i>	Human	Cattle (cow)
<i>T. solium</i>	Human	Pig
<i>E. granulosus</i>	Dog	Human
<i>H. nana</i> (direct cycle)	Human/rat	Human/rat
<i>H. nana</i> (indirect cycle)	Human	Flea
<i>D. caninum</i>	Human	Flea
<i>Schistosoma</i> spp	Human	Snail
<i>F. buski</i>	Human	Snail
<i>G. hominis</i>	Human	Snail
<i>F. hepatica</i>	Human	Snail
<i>A. costaricensis</i>	Human	Slug
<i>W. bancrofti</i>	Human	Mosquito
<i>B. malayi</i>	Human	Mosquito
<i>O. volvulus</i>	Human	Black fly
<i>L. loa</i>	Human	Deer fly
<i>Mansonella</i> , spp.	Human	Mosquito
<i>D. medinensis</i>	Human	Cyclops

- **Dead end life cycle:** Many parasites once enter in human, cannot continue their life cycle and human acts as **dead end**, all such life cycles are described with

TABLE 14.7: Parasites requires three hosts

Parasites	Definitive host	1st	2nd intermediate host
Metazoa			
<i>D. latum</i>	Human	Cyclops or diaptomus	Fish
<i>H. heterophyes</i>	Human	Snail	Fish
<i>M. yokogawai</i>	Human	Snail	Fish
<i>Echinostoma</i> spp.	Human	Snail	Mollusc
<i>C. sinensis</i>	Human	Snail	Fish
<i>O. felineus</i>	Human	Snail	Fish
<i>P. westermanii</i>	Human	Snail	Crab/cray fish
<i>G. spinigerum</i>	Animal	Cyclops	Fish

red arrows (↓) in respective chapters. **Red arrows** (↓) also given where parasites, (especially trophozoites) are released in external environment where they get disintegrate.

- **Life cycle with autoinfection in human:** Life cycles of parasites in which autoinfection in human is possible are described with **sky blue arrows** (↓) in respective chapters.
- **Other type of life cycles:** All other type life cycles in human like exoenteric cycle in *T. gondii*, retrograde cycle in *E. vermicularis*, etc., are described with **black arrows** (↓) in respective chapters. These cycles allow the continuation of life cycle but these are not occurring normally or naturally.
- In certain parasitic life cycle, either naturally or accidentally human acts as both definitive host (harbors adult/sexual stage) as well as intermediate host (harbors larval/asexual stage) like in *H. nana*, *T. solium* and *T. spiralis*.

ACCESS YOURSELF

Short Notes

1. Differences between cestodes and trematodes/cestodes and nematodes/trematodes and nematodes.

Short Questions for Theory/Viva Questions

1. Write two differences between protozoa and metazoa.
2. Name the four parasites in which human acts as an intermediate host.
3. Name the four parasites in which human acts as dead end host.
4. Define definitive host and intermediate host.
5. Name two parasites in which human acts as both definitive host and intermediate host.

MCQs for Chapter Review

Parasite

1. **Nematodes are differentiated from other worms by:**
 - a. Segmentation absent
 - b. Separate coelomic cavity
 - c. Sexes are separate
 - d. They are cylindrical
 - e. GIT is complete

2. Operculated eggs are seen in:
 - a. Nematodes
 - b. Cestodes
 - c. Trematodes
 - d. Protozoa
3. Alimentary canal is absent in:
 - a. Cestodes
 - b. Trematodes
 - c. Nematodes
 - d. None of above
4. Cylindrical worms are:
 - a. Tape worms
 - b. Flukes
 - c. Round worms
 - d. Cestodes
5. True about trematodes:
 - a. Two hosts required
 - b. Segmented
 - c. Anus present
 - d. Body cavity present

Host

6. Human acts as an intermediate host in:
 - a. *L. donovani*
 - b. *Plasmodium* spp.
 - c. *E. granulosus*
 - d. b + c

Life Cycle

7. Simple life cycle requires:
 - a. One host
 - b. Two host
 - c. Three host
 - d. Four host
8. Simple life cycle seen in:
 - a. *Ascaris*
 - b. *T. solium*
 - c. *Toxoplasma*
 - d. *Giardia*
 - e. *Schistosoma*
9. Two hosts required in:
 - a. *T. solium*
 - b. *E. histolytica*
 - c. *T. saginata*
 - d. *Giardia*
 - e. *Toxoplasma*
10. The intermediate host for *T. saginata*:
 - a. Man
 - b. Cow
 - c. Dog
 - d. Pig
11. Which of the following parasites passes through three hosts?
 - a. *Fasciola hepatica*
 - b. *Fasciola buski*

- c. *Schistosoma haematobium*
- d. *Clonorchis sinensis*

12. Crab is the intermediate host for:

- a. *Clonorchis sinensis*
- b. *Paragonimus westermanii*
- c. *Fasciola hepatica*
- d. *Schistosoma haematobium*

13. Fish acts as an intermediate host in:

- a. *D. latum*
- b. *Clonorchis sinensis*
- c. *H. diminuta*
- d. *H. nana*

Answers and Explanation of MCQs

1. a, b, c, d, e
2. b, c
3. a
4. c
 - Follow Table 14.2 for explanation of answers of MCQs 1–4.
5. a
 - Option a: Follow Table 14.4 for explanation (trematodes are classified under class Digenea).
 - Option b, c and d: Follow Table 14.2 for explanation.
6. d
 - Follow section, host (classification → intermediate host) for explanation.
7. a
8. a, d
 - Follow section, life cycle (Table 14.5) for explanation of answers of MCQs 7–8.
9. a, c, e
10. b
 - Follow section, life cycle (Table 14.6) for explanation of answers of MCQs 9–10.
11. d
12. b
13. a, b
 - Follow section, life cycle (Table 14.7) for explanation of answers of MCQs 11–13.

General Properties of Parasites

Chapter Outline

- Protozoa
- Metazoa
- Precipitating Factors of Parasitic Infections

PROTOZOA

Meaning

Protozoa word derived from Protos (Greek) means first and Zoon means animal.

Variation in Species

There are about 65,000 known species of protozoa, 10,000 are parasitic and 17 are medically important.

Morphology

Size of protozoa: Protozoa are microscopic in size and visible under high power microscope. Largest protozoan is *B. coli*.

Structure of protozoa: Protozoa are unicellular and single cell performs the all functions. Structure of protozoa consists cytoplasm, nucleus and other materials.

- **Cytoplasm:** It is divided in two portions:
 - **Endoplasm (Fig. 15.1):** It is an internal, granular portion of cytoplasm. It plays role in nutrition, excretion and reproduction. It contains nucleus, contractile vacuoles, food debris, endoplasmic reticulum, Golgi bodies and RBCs in some parasite.
 - **Ectoplasm (Fig. 15.1):** It is an external, clear, hyaline portion of cytoplasm. It plays role in protection, locomotion and sensory function. Following structures are developed from ectoplasm:

1. **Organs of locomotion:** Three types as follows:

- **Pseudopodium (Fig. 15.1):** It is the temporary prolongation of ectoplasmic process. It helps in ingestion of food. It occurs in Rhizopodea (*E. histolytica*). Speed of locomotion by pseudopodia is 0.2–0.3 μm per seconds.
- **Flagellum (Fig. 15.2):** It is a permanent, long, delicate and thread like filament. It occurs in Zoomastigophorea like *G. lamblia*, *Trichomonas*

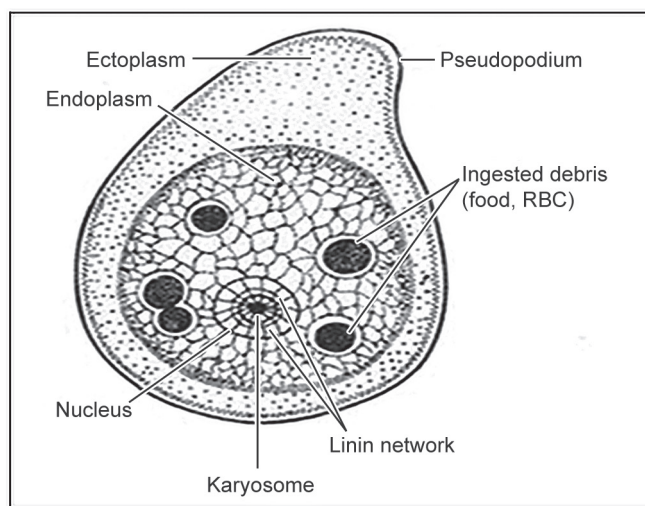


Fig. 15.1: Pseudopodium in *E. histolytica*

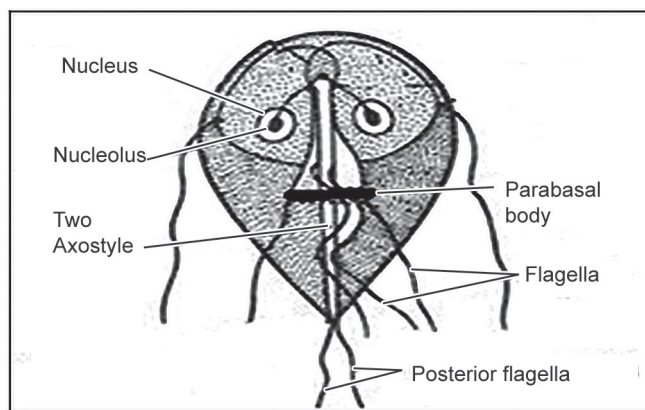


Fig. 15.2: Flagella in *G. lamblia*

spp., *Trypanosoma* spp., and *Leishmania* spp. Speed of locomotion by flagella is 15–30 μm per seconds.

- **Cilia (Fig. 15.3):** It is a permanent short, fine and needle like filament. It occurs in Ciliata like *B. coli*. Speed of locomotion by cilia is 400–2000 μm per seconds.
- 2. **Rudimentary digestive organs**
 - Cytostome (cell mouth) presents in *B. coli*.
 - Cytopharynx presents in *B. coli*.
 - Cytopyge (cell anus): To excrete the food wastes.
- 3. **Contractile vacuoles:** It developed from ectoplasm, but situated inside the endoplasm. It concerned with excretory function and maintains the osmotic pressure.
- 4. **Cyst wall:** Thick resistant wall seen in cystic stage.
- **Nucleus:** It is bounded by well-defined nuclear membrane. It has following features:
 - **Contains of nucleus:** Internally it contains following materials:
 - Nucleolus (karyosomes): It is a small dot like concentrated chromatin material located either centrally or peripherally.
 - Nuclear sap: It is the fluid present in nucleus.
 - Linin network: Space between karyosome and nuclear membrane is filled by radially (spoke like) arranged fine threads called linin network.
 - **Numbers of nucleus:** In most of the protozoa, there is one nucleus. Following protozoa consist two or more nuclei:
 - Both are in similar size in *G. lamblia*
 - Both are in dissimilar size in *B. coli*, where smaller nucleus known as micronucleus and larger nucleus known as macronucleus.
 - **Functions of nucleus**
 - It controls all activities of cell.
 - It is essential for cell division, growth and replication.
 - It takes part in fertilization and hereditary transmission of genetic information.

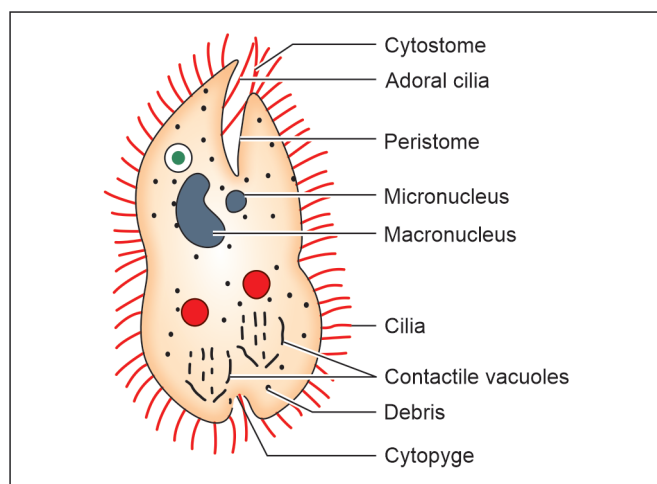


Fig. 15.3: Cilia in *B. coli*

• Other materials

1. **Kinetoplast:** Nonnuclear DNA containing body called kinetoplast. It has mitochondrial structure and acts as energy store house.
2. **Basal body:** It is a small body represents the origin of flagellum.
3. **Chromatoid bars (chromodia):** Extranuclear chromatin materials called chromatoid bars. They present in premature cyst of *E. histolytica* and disappear in mature cyst. They stain black with iron-hematoxylin stain.
4. **Glycogen mass:** It presents in premature cyst of *E. histolytica* and disappears in mature cyst. It stains brown with iodine.

Morphological stages of protozoa: In all protozoa mainly two morphological stages are present like trophozoite stage and cyst stage. In some protozoa intermediate stage called precyst stage also occurs.

1. **Trophozoite stage:** Word derived from tropos (Greek) means nourishment. It has following properties:
 - It is the active and feeding stage.
 - It is motile in nature.
 - Excretion of waste products from trophozoites occurs by contractile vacuoles or by osmosis.
 - Respiration takes place by osmosis.
 - It receives the nutrition from surrounding environment by different methods such as diffusion, active transport across the plasma membrane, phagocytosis through pseudopodia, mouth like structure called cytostomes or by pinocytosis for minute drop of food particles.
2. **Cyst stage:** Trophozoite transformed in to a tough wall structure called cyst. It has following properties:
 - It is an inactive, resting and resisting stage.
 - It is nonmotile in nature.
 - It is infective to human beings and transmitted by food-water, vectors or by other ways.

Methods of Reproduction

Methods of transformation: Two types:

1. Encystation: Transfer of trophozoite (active) to cyst (inactive) called encystation.
2. Excystation: Transfer of cyst (inactive) to trophozoite (active) called excystation.

Methods of multiplication: Multiplication occurs only in trophozoites stage. Following are the methods of reproduction and multiplication in protozoa:

1. Asexual multiplication in protozoa

- **Binary fission:** Parasites divide in to two equal parts. Before division all the structure are duplicated. It occurs longitudinally in flagellates (Fig. 15.4a) or transversely in ciliates (Fig. 15.4b) or in *E. histolytica*.
- **Multiple fissions (schizogony):** Parasites divides in to more than two cells. First nucleus of parent

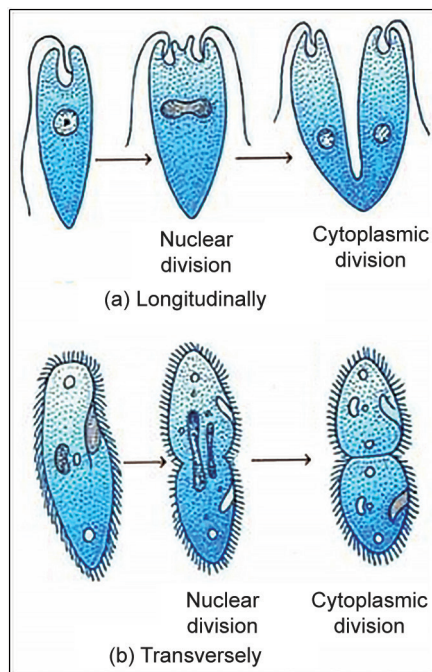


Fig. 15.4: Binary fission

cell undergoes repeated division, which are then surrounded by cytoplasm, resulting daughter cell known as merozoite. When multiplication is completed, the whole parasitic body called schizont ruptures and liberates these daughter cells, which repeat the life cycle later on. For example, *Plasmodium* spp., showing multiple fissions.

- **Endodyogeny:** Parasite undergoes the single internal budding, resulting two daughter cells are produced, e.g., *T. gondii*.

2. Sexual multiplication in protozoa

- **Conjugation:** In this process two cells are united for a temporary period, during which time interchange of nuclear materials takes place. Later on, two cells will be separated. For example, *B. coli*.
- **Gametogony (syngamy):** Two sexually differentiated cells like male and female gametocytes unite permanently to form a zygote. Which continue the life cycle later on; for example, *Plasmodium* spp.

METAZOA

Synonym

Term metazoan also called helminths or worms.

Meaning

Helminths is a Greek word derived from helmin means worm, originally referred to intestinal worms.

Morphology and Methods of Reproduction

Morphological stages, reproductive organs and reproductive methods for cestodes are described in Ch. 100, for trematodes in Ch. 103 and for nematodes in Ch. 106.

Synonym

These factors also called epidemiological determinants.

Types

Virulence of parasites is affected by following three factors:

Agent factors (virulence factors or determinants of virulence)

1. Intracellular or cell associated factors

- **Intracellular location:** It provides protection against the host defense system.
- **Tough cyst wall in protozoa:** It provides protection against gastric acidity.
- **Adhesins**
 - Lectin mediated adherence protein in *E histolytica*: It helps in contact with target cells.
 - Sucking disc *G lamblia*: It is the organ of adhesion. It does not invade the tissue but remains tightly adhere to the intestinal epithelium.
- **Motility:** It helps in deeper penetration and movement toward nutrition.
- **Spine or lateral knob in schistosomes:** It erodes the blood vessels to cause hemorrhagic manifestations.

2. Extracellular factors

- **Enzymes**
 - Enzymes in *E histolytica*: Histiocytin brings the destruction and necrosis of tissues and slough formation. Organisms receive the nourishment by absorption of dissolved tissue juice. Other enzymes in *E. histolytica* like hyaluronidase, trypsin, pepsin, amylase, etc., may induce tissues destruction.
 - Enzymes in *T vaginalis* like cysteine protease which contributes in pathogenesis.
 - Enzymes in *B. coli* like hyaluronidase, which degrades the intestinal mucosa and facilitates the penetration.
- **Toxins or metabolites production by parasites:** Liberated by trophozoites, eggs, larvae or adult stages of parasites are responsible for allergic manifestations.

Host factors

1. **Age:** Certain infections are common at particular age like thread worm in pediatrics while *T. vaginalis* infection in adults.
2. **Immune status:** Immunodeficiency status favors certain parasitic infections like *S. stercoralis*, *G. lamblia*, etc. Splenectomized persons are more susceptible to babesiosis.
3. **Blood group:** Group A is more prone to *G. lamblia*.
4. **Food habit:** *T. saginata* is common who eats beef while *T. solium* is common who eats pork.

- 100 5. Occupation:** *D. medinensis* is common in bhistis (person who carries bag of water on back).

Environmental factors

- Poor sanitation and overcrowding:** They favor the mosquito-borne diseases like malaria and filaria.
- Humidity:** *Naegleria fowleri* is common in air of air cooler and live symbiotically with *Legionella* or *Listeria* and transmitted by inhalation of air of air-cooler.

ACCESS YOURSELF

Short Questions for Theory/Viva Questions

- What are encystation and excystation?
- Write the functions ectoplasm and endoplasm in protozoa.

- Name the organ of locomotion in following parasites: *Entamoeba histolytica*, *Balantidium coli*, *Trypanosoma cruzi*, *Leishmania donovani* and *Giardia lamblia*.
- What are monoecious and diecious parasites?

MCQ for Chapter Review

Protozoa (Methods of Reproduction)

1. Encystation is:

- Trophozoite → cyst
- Cyst → trophozoite
- Egg → larvae
- Cyst → egg

Answer and Explanation of MCQ

1. a

- Follow section, **Protozoa methods of reproduction (methods of transformation → encystation)** for explanation.