Part 1. General Microbiology

SECTION OUTLINE

1. Introduction, History and Microscopy
2. General Bacteriology
    - 2.1. Morphology and Physiology of Bacteria
    - 2.2. Laboratory Diagnosis of Bacterial Infections
    - 2.3. Bacterial Genetics
    - 2.4. Antimicrobial Agents and Antimicrobial Resistance
    - 2.5. Normal Flora and Bacterial Pathogenesis
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Chapter 1. Introduction, History and Microscopy

CHAPTER PREVIEW

- Classification of Microorganisms
- History
- Microscopy

Medical microbiology is a branch of medicine that deals with the study of microorganisms and their role in human health and disease. It also concerns with the diagnosis, treatment and prevention of various infectious diseases.

The branches of medical microbiology are as follows:

- **General microbiology:** It deals with the study of general properties of microorganisms—taxonomy, morphology, pathogenesis, laboratory diagnosis, and treatment for their effective killing
- **Immunology**: It deals with the study of the immune system and various immunological methods for the diagnosis of infectious diseases
- **Systemic microbiology:** Microorganisms infect various organ systems of our body. There are four kinds of microorganisms that cause infectious disease: bacteria, fungi, parasites, and viruses
  - *Bacteriology:* The study of bacteria
  - *Virology:* The study of viruses
  - *Mycology:* The study of fungi
  - *Parasitology:* The study of parasites; has two arms:
    - Protozoology: The study of protozoa
    - Helminthology: The study of helminths.
- **Hospital infection control:** It deals with the study of various control measures to prevent the transmission of healthcare- associated infections.

CLASSIFICATION OF MICROORGANISMS

Microorganisms are grouped under both prokaryotes and eukaryotes.

- Bacteria are placed under prokaryotes. They have a primitive nucleus and other properties of a prokaryotic cell **(Table 1.1)**
- Whereas fungi and parasites (protozoa and helminths) belong to eukaryotes; having a well-defined nucleus and various eukaryotic cellular organelles

Table 1.1. Characteristics of prokaryotes and eukaryotes.

Characteristics	***Prokaryotes***	***Eukaryotes***
Major groups	Bacteria	Fungi, parasites, plants, animals
Nucleus	Diffuse	Well-defined
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
Cell division	Binary fission	Mitosis, meiosis
Plasmid	Present	Absent
Cell membrane	No sterols	Contains sterols
Cellular organelles	Absent (except ribosome)	Present
Ribosome	70S	80S
Abbreviation: S, Svedberg unit.		

- Viruses are neither considered prokaryotes nor eukaryotes because they lack the characteristics of living things, except the ability to replicate.

HISTORY

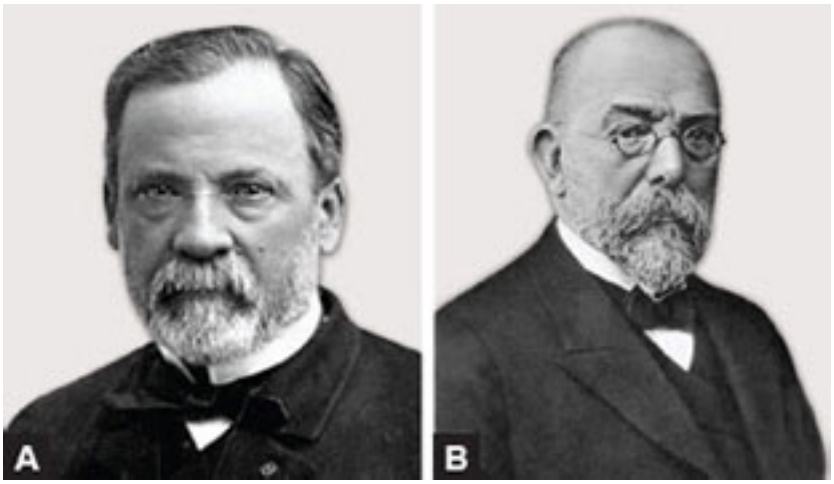
There were several eminent personalities in the field of Microbiology, whose important contributions have been described below.

Louis Pasteur

Louis Pasteur (1822–1895), also known as '**father of microbiology'** has made several remarkable contributions **(Fig. 1.1A)**.

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

Figs. 1.1A and B. Eminent microbiologists: A. Louis Pasteur; B. Robert Koch.



Source: Wikipedia *(with permission).*

Joseph Lister

Joseph Lister (1867) is considered to be the '**father of antiseptic surgery**'. He postulated that postoperative infections can greatly be reduced by using disinfectants to sterilize the surgical instruments and to clean the wounds.

Robert Koch

Robert Koch (1843–1910), made notable contributions to the field of microbiology **(Fig. 1.1B)**. His contributions were as follows:

- He introduced **solid media** for the culture of bacteria
- He introduced methods for isolation of bacteria in pure culture
- He described **hanging drop** method for testing motility
- He **discovered bacteria** such as the anthrax bacilli, tubercle bacilli and cholera bacilli
- **Koch's postulates:** Robert Koch had postulated that a microorganism can be accepted as the causative agent of an infectious disease only if four criteria are fulfilled. These criteria are as follows:

  1. The microorganism should be constantly associated with the lesions of the disease
  2. It should be possible to isolate the organism in pure culture from the lesions of the disease
  3. The same disease must result when the isolated microorganism is inoculated into a suitable laboratory animal
  4. It should be possible to re-isolate the organism in pure culture from the lesions produced in the experimental animals.

Exceptions to Koch's postulates: There are some bacteria that do not satisfy one or more of the four criteria of Koch's postulates. *Mycobacterium leprae* and *Treponema pallidum* cannot be grown in culture; whereas *Neisseria gonorrhoeae* has no animal model.

Other Important Contributors

- **Antonie van Leeuwenhoek** (1676): He was the first scientist who observed bacteria and other microorganisms, using a single-lens microscope constructed by him and he named those small organisms as '*Little animalcules'*
- **Edward Jenner** (1796): He, developed the first vaccine of the world, the smallpox vaccine. He used the cowpox virus *(Variolae vaccinae)* to immunize children against smallpox from which the term **'vaccine'** has been derived. The same principles are even used today for developing vaccines
- **Paul Ehrlich** (1854–1915): He is known as *'father of chemotherapy'*. He was also the first to report the *acid-fast nature* of tubercle bacillus
- **Hans Christian Gram (in 1884):** He developed a method of staining bacteria which was named as 'Gram stain' to make them more visible and differentiable under a microscope
- **Ernst Ruska:** He was the *founder of electron microscope* (1931)
- **Alexander Fleming (in 1929):** He discovered the most commonly used antibiotic substance of the last century, i.e., penicillin
- **Karry B Mullis:** Discovered polymerase chain reaction (PCR) and was awarded Noble Prize in 1993
- **Ignaz Semmelweis (1846):** He introduced the importance of hand hygiene in healthcare facilities. He proposed that improper hand hygiene practice during delivery led to the transmission of infection causing outbreak of puerperal fever.

Nobel Laureates

A number of scientists in the field of medicine or physiology have been awarded Nobel Prizes for their contributions in microbiology **(Table 1.2)**.

MICROSCOPY

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

Table 1.2. Nobel laureates in medicine or physiology for their contributions in microbiology.

Nobel laureate	*Year*	*Research done*
Sir Ronald Ross	1902	Life cycle of malarial parasite in mosquitoes
Robert Koch	1905	Discovery of the causative agent of tuberculosis
Charles LA Laveran	1907	Discovery of malarial parasite in unstained preparation of blood
Sir Alexander Fleming	1945	Discovery of penicillin
J Lederberg and EL Tatum	1958	Discovery of conjugation in bacteria
Watson and Crick	1962	Discovered double helix structure of DNA
Holley, Khurana and Nirenberg	1968	Discovered genetic code

Nobel laureate	***Year***	***Research done***
BS Blumberg	1976	Discovered Australia antigen (HBsAg)
Barbara McClintoch	1983	Discovered mobile genetic elements (transposon)
Georges Kohler	1984	Developed hybridoma technology for monoclonal antibodies
Kary B Mullis	1993	Invented polymerase chain reaction
Stanley B Prusiner	1997	Described Prions
Luc Montagnier and Barre-Sinoussi	2008	Discovery of human immunodeficiency virus (HIV)
William C Campbell and S. Omura	2015	For discovering ivermectin for the treatment of roundworm infections
Youyou Tu	2015	For discovering artemisinin, a novel drug used for malaria

Therefore organisms require specialized instrument— called ‘microscope’ to view objects and areas of objects that cannot be seen with the naked eye.

There are various types of microscopes that are used in diagnostic microbiology.

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

Bright-field or Light Microscope

The bright-field or light microscope forms a dark image against a brighter background.

Structure

The parts of a bright-field microscope are divided into three groups **(Fig. 1.2)**:

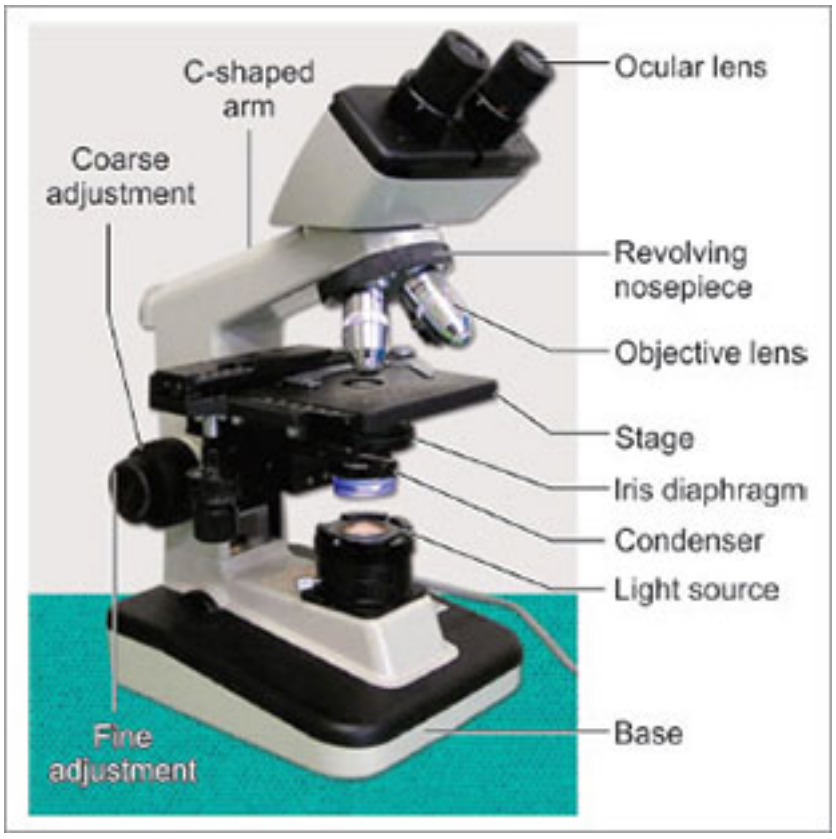
1. **Mechanical parts:**
    - *Base*: It holds various parts of the microscope, such as the light source, the fine and coarse adjustment knobs
    - *C-shaped arm*: It holds the microscope, and it connects the ocular lens to the objective lens
    - *Mechanical stage:* The arm bears a stage with stage clips to hold the slides and the stage control knobs to move the slide during viewing. It has an aperture at the center that permits light to reach the object from the bottom.
2. **Magnifying parts:**
    - *Ocular lens:* The arm contains an eyepiece that bears an ocular lens of 10x magnification power

- *Objective lens*: The arm also contains a revolving nose piece that bears three to four objectives with lenses of differing magnifying power (4x, 10x, 40x and 100x).

3. **Illuminating parts:**

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

Fig. 1.2. Bright-field microscope.



Source: Nikon Alphaphot *(with permission).*

Working Principle

The rays emitted from the light source pass through the iris diaphragm and fall on the specimen. The light rays passing through the specimen are gathered by the objective and a magnified image is formed. This image is further magnified by the ocular lens to produce the final magnified virtual image **(Fig. 1.3)**.

Dark-field Microscope

In dark-field (or dark ground) microscope, the object appears bright against a dark background.

- This is made possible by use of a special dark-field condenser
- *Applications:* It is used to identify the living, unstained cells and thin bacteria like spirochetes which cannot be visualized by light microscopy **(Fig. 1.4)**.

Phase Contrast Microscope

In this type of microscope, the contrast is enhanced.

Fig. 1.3. Light pathways of bright-field microscope.

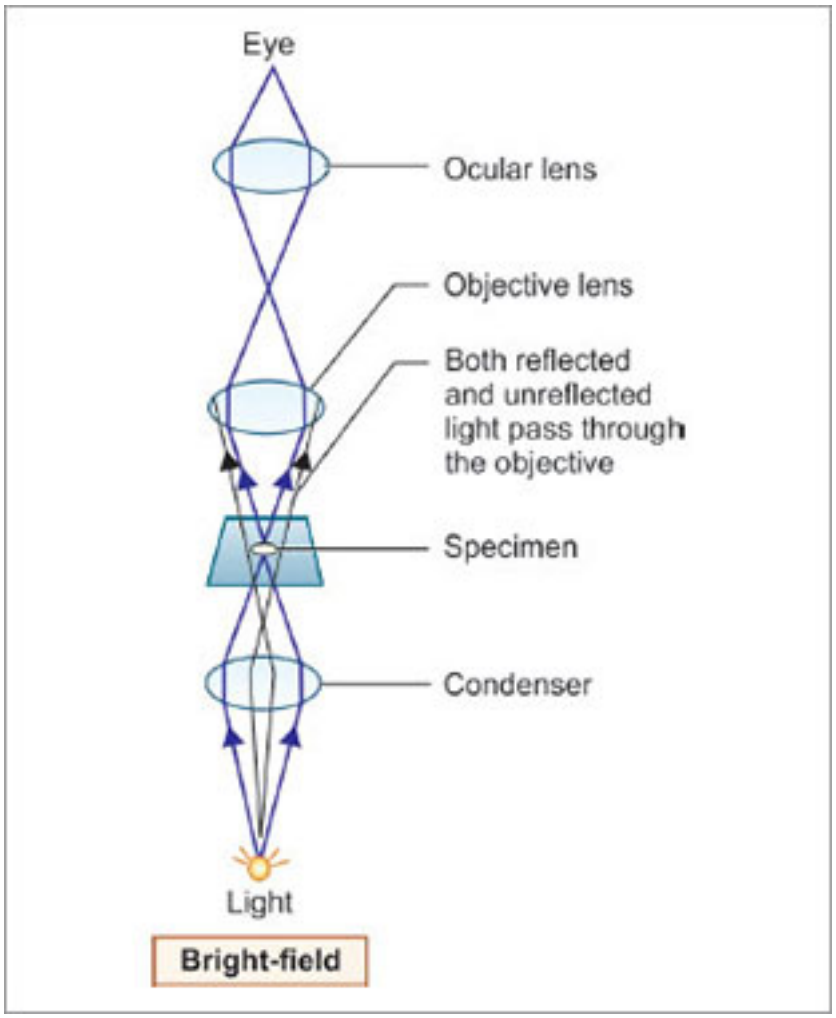
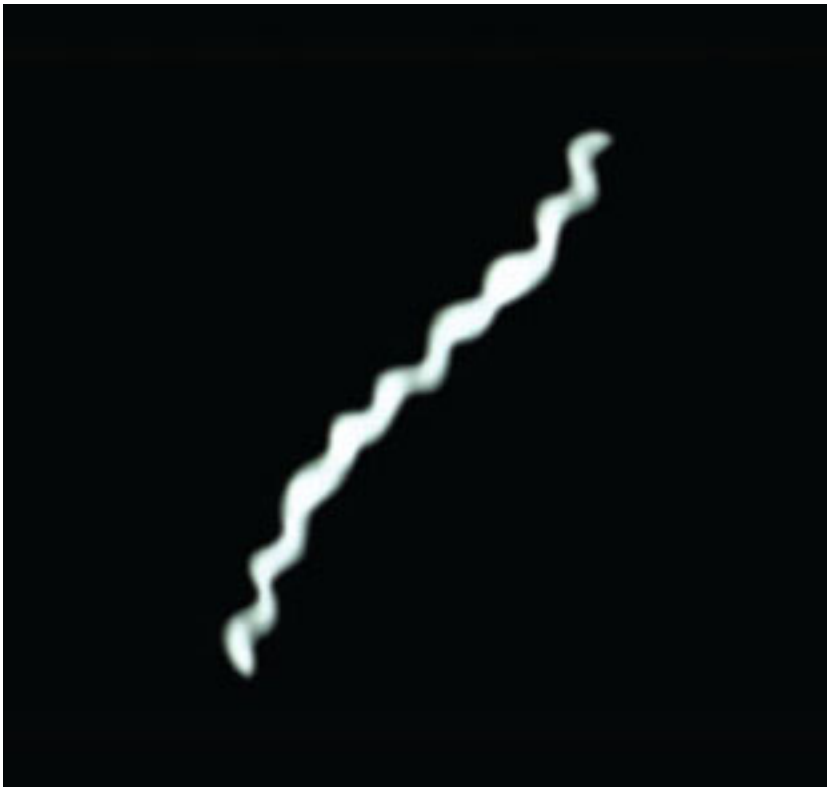
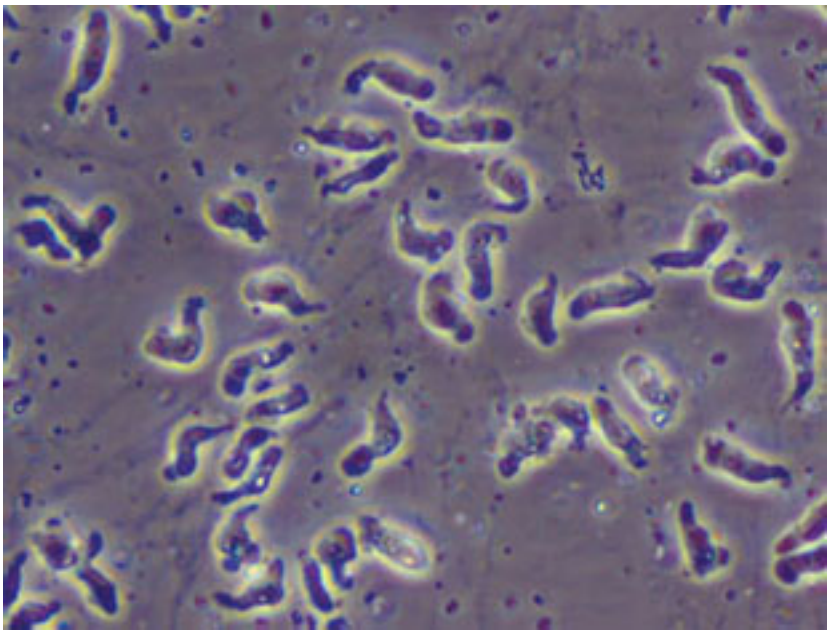


Fig. 1.4. Dark ground microscopic picture demonstrating spirally coiled bacteria (spirochete).



Source: Public Health Image Library, ID# 2043; Centers for Disease Control and Prevention (CDC), Atlanta *(with permission).*

Fig. 1.5. Phase-contrast microscopic picture demonstrating *Naegleria fowleri* trophozoites (free-living amoeba)



Source: Centers for Disease Control and Prevention (CDC), Atlanta *(with permission)*.

This microscope visualizes the unstained living cells by creating a difference in contrast between the cells and water.

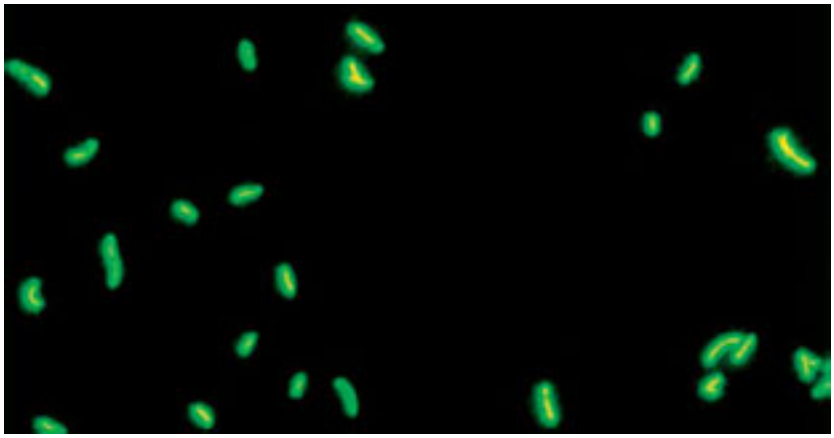
- This is made possible by the use of a special condenser, similar to the dark-field condenser, and a special optical disc located in the objective called a phase plate
- **Applications:** A phase-contrast microscope is useful for studying **(Fig. 1.5):**
  - Microbial motility
  - Determining the shape of living cells
  - Detecting microbial internal cellular components, such as the cell membrane, nuclei, mitochondria, chromosomes, Golgi apparatus, inclusion bodies, etc.

Fluorescence Microscope

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

- The source of light may be a mercury lamp that emits UV light rays
- The specimen should be stained with fluorescent dyes for visualization
- **Applications:** Certain microbes fluoresce when they are stained nonspecifically by fluorochrome dyes
  - Acridine orange dye is used for the detection of parasites such as *Plasmodium* and filarial nematodes
  - Auramine phenol is used for the detection of tubercle bacilli **(Fig. 1.6).**

Fig. 1.6. Tubercle bacilli seen under fluorescence microscope.



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

Electron Microscope

An electron microscope (EM) uses accelerated electrons as a source of illumination. EM has a much better resolving power than a light microscope; hence, it can reveal the details of flagella, fimbriae and intracellular structures of a cell. It was invented by German physicist **Ernst Ruska** in 1931. Electron microscopes are of two types:

1. Transmission electron microscope (TEM, the most common type)

2. Scanning electron microscope (SEM).

EXPECTED QUESTIONS

1. I. Write short notes on:

1. Contributions of Louis Pasteur to Microbiology.
2. Koch's postulates.
3. Principle and uses of bright-field microscope.

2. II. Multiple Choice Questions (MCQs):

1. **Who has described the germ theory of life?**
   a. Antonie van Leeuwenhoek
   b. Louis Pasteur
   c. Robert Koch
   d. Paul Ehrlich
2. **Who has introduced the sterilization techniques?**
   a. Louis Pasteur
   b. Edward Jenner
   c. Robert Koch
   d. Paul Ehrlich
3. **Who discovered tubercle bacilli?**
   a. Edward Jenner
   b. Alexander Fleming
   c. Robert Koch
   d. Joseph Lister
4. **Which of the following microscope, the object appears bright against a dark background?**
   a. Light microscope
   b. Phase-contrast microscope
   c. Dark ground microscope
   d. Electron microscope
5. **Which of the following organism follows Koch's postulates?**
   a. *Mycobacterium leprae*
   b. *Treponema pallidum*

c. *Neisseria gonorrhoeae*

d. *Bacillus anthracis*

6. **Electrons are used as a source of illumination in:**

a. Light microscope

b. Dark field microscope

c. Phase contrast microscope

d. Electron microscope

7. **Who developed smallpox vaccine?**

a. Louis Pasteur

b. Edward Jenner

c. Robert Koch

d. Paul Ehrlich

Answers

1. b	**2.** a	**3.** c	**4.** c	**5.** d	**6.** d	**7.** b