

INTRODUCTION

The first simple forms of life appeared on earth more than three billion years ago. Their descendants have changed and developed into the several million types of animals, plants and microorganisms recognized today. Of course, thousands more remain to be discovered and officially described.

Microscopic forms of life are present in vast numbers in nearly every environment known, i.e. soil, water, food, air, etc. Since the conditions that favor the survival and growth of many are the same as those under which people normally live.

Many microscopic microorganisms or microbes occur as single cell, others are multicellular, and still others such as viruses, do not have a true cellular appearance. Some organisms called anaerobes are capable of carrying out their vital functions in the absence of free oxygen; whereas other organisms can manufacture the essential compounds for their physiological needs from atmospheric sources of nitrogen and carbon dioxide. Other microorganisms such as viruses and certain bacteria are totally dependent for their existence on the cells of higher forms of life. The branch of science known as microbiology embraces all of these properties of microorganisms and many more.

One of the attractive features of microbiology is a number of investigations and work remaining to be done. Many decisions affecting the future of the world may depend upon and involve the activities of microorganisms in areas like *food production*, *pollution control*, *energy production* and the *control* and *treatment of diseases*. In short, *microbiology* has assumed a position of great importance in modern society.

Medical microbiology (Gr. *mikros*-small, *bios*-life, *logos*-science) is the study of causative agents of infectious disease of human beings and their reactions to such infections. In other words it deals with etiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection.

Obviously, medical microbiology has close link with other disciplines, i.e. pathology, clinical medicine, surgery, pharmacology cum therapeutics and preventive medicine.

Microbiologists are enthusiastic to confirm the diagnosis and cause of macroscopic changes by taking smears and preparing cultures from the lesions to demonstrate microorganisms, e.g. circumscribed boil of staphylococcus, the spreading cellulitis of streptococcus, the red-liver-like appearance of lung in pneumococcal pneumonia, the tubercles followed by syphilis and intestinal ulceration of salmonella organism, etc. The pathology of infection is quite fascinating as it includes affinity of pathogens for certain tissue and initiation of infection and characteristic tissue reaction. Each investigation is regarded as research project in miniature.

Historical Events

Long before the discovery of microorganisms certain processes caused by their life activities, such as fermentation of wine juice, milk, yeast, etc. were known to mankind. In ancient times at the beginning of civilization, man by using these processes learned to prepare kourmurs, sour milk and other products.

◆. **Antoni van Leeuwenhoek (1683)**

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'.

◆. **Robert Hook**, a contemporary of Leeuwenhoek developed compound microscope in **1678** and confirmed Leeuwenhoek's observation.

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

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

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

♦. **Joseph Lister**

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

♦. **Robert Koch (father of bacteriology).** **Robert Koch** provided remarkable contributions to the field of microbiology. He was a German general practitioner (1843- 1910). His contributions are as follows:

- 1- He introduced solid media for culture of bacteria, Eilshemius Hesse, the wife of, one of Koch's assistants had suggested the use of agar as solidifying agents.
- 2- He also introduced methods for isolation of bacteria in pure culture.
- 3- He described hanging drop method for testing motility.

4- He discovered bacteria such as the anthrax bacilli, tubercle bacilli and cholera bacilli.

5- He introduced staining techniques by using aniline dye.

6- Koch's phenomenon: Robert Koch observed that guinea pigs already infected with tubercle bacillus developed a hypersensitivity reaction when injected with tubercle bacilli or its protein. Since then, this observation was called as Koch's phenomenon.

7- He also suggested criteria before blaming the organism responsible for disease. It goes by the name of **Koch's postulate**, according to which:

1-The specific micro-organism should be isolated from all cases of a specific disease, and should not be found in healthy individuals.

2-The specific micro-organism should be isolated from the diseased individual and grown in pure culture on an artificial medium.

3-The isolated micro-organism should reproduce the specific disease when inoculated into a healthy individual.

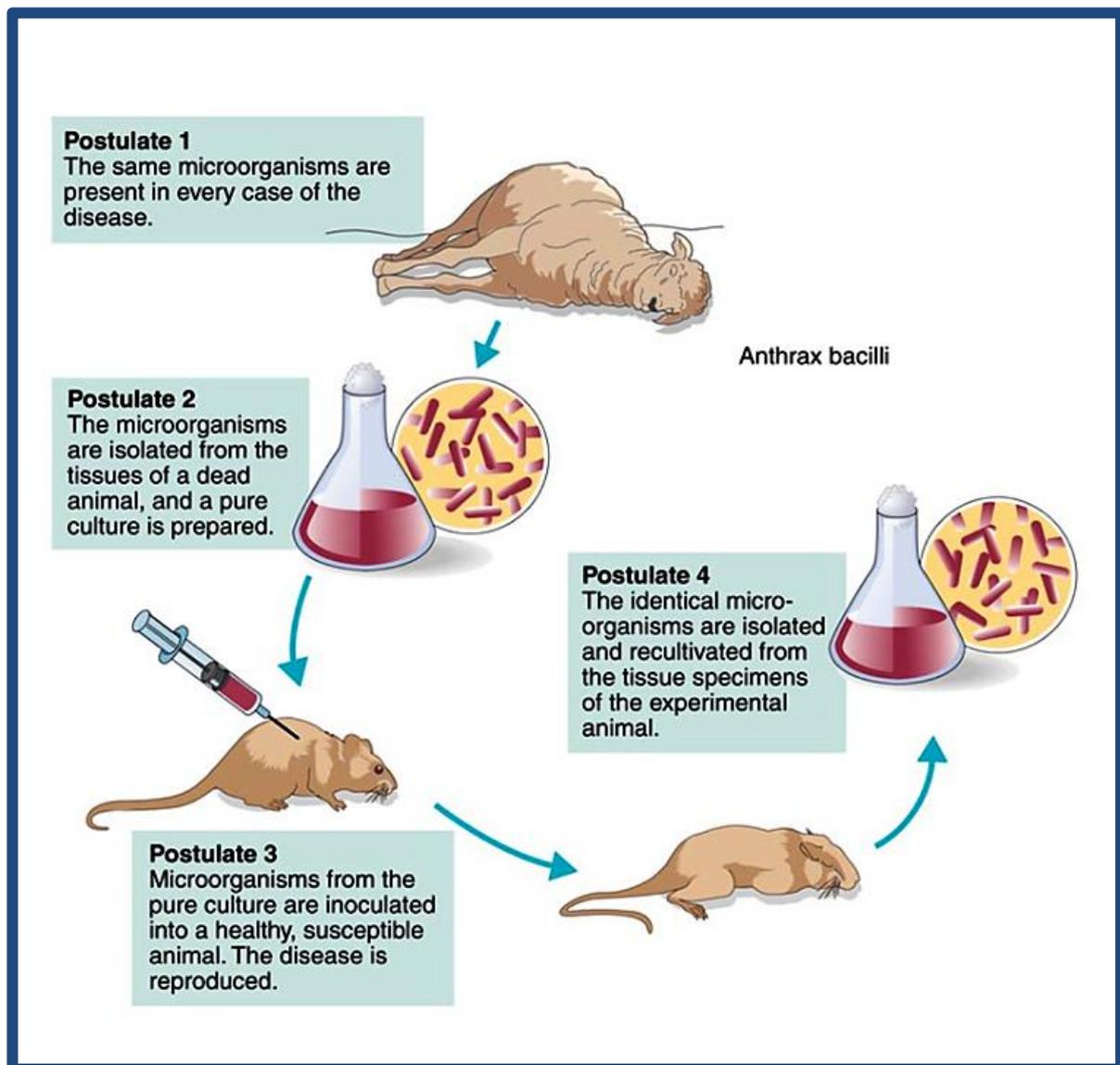
4-The specific micro-organism should be re-isolated in pure culture from the experimental infection.

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

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

(i)- *Mycobacterium leprae* and *Treponema pallidum*: They cannot be grown *in vitro*; however, they can be maintained in experimental animals.

(ii)- *Neisseria gonorrhoeae*: There is no animal model; however, it can be grown *in vitro*.



- ◆. *Neisser* (1879) described gonococcus
- ◆. *Ogston* (1881) discovered staphylococcus
- ◆. *Loeffler* (1884) isolated diphtheria bacillus.
- ◆. *Loeffler and Frosch* (1898) observed that foot and mouth disease of cattle was caused by a microbe, i.e. filter passing virus.
- ◆. *Walter Reed* (1902) observed that yellow fever was caused by filterable virus and that it was transmitted through the bite of mosquitoes.

◆. *Landsteiner* and *Popper* (1909) showed poliomyelitis was caused by filterable virus.

◆. *Towert* (1951) and *Herelle* (1917) discovered lytic phenomenon in bacterial culture. The agent responsible was termed as bacteriophage (viruses that attack bacteria).

◆. *Alexander Fleming* discovered penicillin in 1928 (He made the accidental discovery that the fungus penicillium produces a substance which destroys staphylococci).

◆. *Ruska* (1934) introduced electron microscope and hence detailed study of morphology of virus was possible.

New agents of infectious diseases continue to emerge, e.g. HIV (identified in 1980). The outbreaks of plague in 1994, cholera in 1995, and dengue hemorrhagic fever in 1996. As such many workers in medicine have been awarded **Nobel Prizes** for their outstanding contributions in microbiology (Table -1).

The methods of many infectious diseases and vaccine production have been revolutionized, e.g. **recombine DNA technology, PCR, nuclear anaprobcs, radioimmunoassay, ELISA**, etc.

One of the best ways of learning microbial nomenclature is to look up the characteristics of each genus and species of organism that is encountered in the classroom, in the laboratory, or in practice. With this practice, a lot of information will quickly become a part of working knowledge, on which one can build the sound practice of the profession.

TABLE 1: Noble prize winners in microbiology

1901	Behring	Antitoxins
1902	Ross	Malaria
1905	Koch	Bacteriology
1907	Laveran	Malaria
1908	Ehrlich and Metchnikoff	Theories of immunology
1913	Richet	Anaphylaxis
1919	Bordet	Immunology
1928	Nicolle	Typhus fever
1930	Landsteiner	Blood groups
1939	Domagk	Sulfonamide
1945	Fleming, Florey and Chain	Penicillin
1951	Theiler	Yellow fever vaccine
1952	Waksman	Streptomycin
1954	Ender	Cellular culture of polio virus
1958	Lederberg, Tatum and Beadle	Genetics
1959	Ochoa Kornberg	Genetics RNA
1960	Burnet and Medawar	Theories of immunity
1961	Watson and Crick	Genetic code, structure of DNA
1965	Jacob, Mond and Lwoff	Genetic episome and prephage
1966	Rous	Viral etiology of cancer
1968	Nirenberg, Holley and Khuranna	Synthesis of DNA
1969	Dulbecco, Luria and Delbruck	Genetics, mutations
1972	Porter and Edelman	Structure of immunoglobulin
1974	Christian	Lysosomes
1975	Dulbecco, Baltimore and Teonin	Genetics and mutations
1976	Gajdusck and Blumberg	Slow virus and Australia antigen
1977	Rosalyn Yalow	Radioimmunoassay
1978	Arber, Nathans and Smith	Restriction enzyme
1980	Snell, Dausset and Benacerrof	Major histocompatibility complex (MHC) and genetic control of immune response
1983	Barbara McClintoch	Mobile genetic element
1984	Georges Koehler and Danish Niele Jerne	Monoclonal antibodies
1987	Tonegawa Susuma	Generation of immunoglobulin diversity
1988	Gertrude Elion, George Hitchings and James Black	Discoveries of important principles that have resulted in the development of a series of new drugs including Acyclovir for herpes and AZT for treating AIDS
1989	Michael Bishop and Harold Varmus	Discovery of cellular origin of viral oncogenes
1993	Richard J Robert	Split genes
1997	Stanley B Prusiner	Prion
2005	Barry J Marshal and Roben Warren	<i>Helicobacter pylori</i> as causative agent of gastritis and peptic ulcer
2006	Andrew Fire and Craig Mello	RNA interference—gene silencing by DS RNA

Branches of Microbiology

Microbiology is one of the largest and most complex of the biological sciences because it integrates subject matter from many diverse disciplines. Microbiologists study every aspect of microbes their genetics, their physiology, characteristics that may be harmful or beneficial, the ways they interact with the environment, the ways they interact with hosts, and their uses in industry and agriculture.

Each major discipline in microbiology contains numerous subdivisions or specialties that deal with a specific subject area or field (Branches of Microbiology).

★. *Branches of Microbiology*

1-*Medical microbiology*

2-*Industrial microbiology*

3-*Food microbiology*

4- *Microbial Genetics & Molecular Biology* deals with the function of genetic material and biochemical reactions of cells involved in metabolism and growth.

5- *Microbial Ecology* deals with Interrelationships between microbes and the environment; the roles of microorganisms in the nutrient cycles of soil, water, and other natural ecosystems.

Here we are concerned with *medical microbiology*. It is studied under following headings:

A- *Parasitology* deals with the study of parasites causing diseases in human being.

B- *Mycology* deals with the study of fungi causing diseases in human beings.

C- *Immunology* is concerned with mechanism involved in the development of resistance by body to infectious diseases.

D- *Bacteriology* deals with the study of bacteria.

E- *Genetics* is the study of heredity and variations.

F- *Virology* is the study of viruses.

SCOPE OF MICROBIOLOGY

- 1-** Diagnostic, e.g. isolation and identification of causative organism from the pathological lesions. We can also diagnose typhoid fever by doing Widal's test.
- 2-** Prognosis of disease, e.g. in Widal's test rising titer signifies active disease and ineffective treatment. Falling titer means effective treatment and curing of disease.
- 3-** Guidance in treatment, e.g. by culturing the organism in pure form and then performing drug sensitivity test we can suggest the effective drug for the treatment of that particular infection.
- 4-** Source of infection, e.g. in sudden outbreak of infectious disease we can find out the source of infection.
- 5-** Detection of new pathogens and then development of vaccines.

BACTERIAL TAXONOMY

Bacterial taxonomy comprises of three separate but interrelated important areas.

- 1-Classification:** It is the arrangement of bacteria into taxonomic groups or taxa (in singular, taxon) on the basis of similarities or differences in their biochemical, physiological, genetic, and morphological properties.
- 2-Nomenclature:** It refers to the naming of taxa according to their characteristics, by following the international rules.
- 3-Identification:** It is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon.

CHAPTER CHECKPOINTS



Taxonomy is the formal filing system scientists use to classify living organisms. It puts every organism in its place and makes a place for every living organism.

The taxonomic system has three primary functions: classification, nomenclature, and identification of species.

The eight major taxa, or groups, in the taxonomic system are (in descending order): domain, kingdom, phylum or division, class, order, family, genus, and species.

The binomial system of nomenclature describes each living organism by two names: genus and species.

Taxonomy groups organisms by phylogenetic similarity, which in turn is based on evolutionary similarities in morphology, physiology, and genetics.

Evolutionary patterns show a treelike branching from simple, primitive life forms to complex, advanced life forms.

The Woese-Fox classification system places all eucaryotes in the Domain (Superkingdom) Eukarya and subdivides the procaryotes into the two Domains Archaea and Bacteria.

The Whittaker five-kingdom classification system places all bacteria in the Kingdom Procaryotae and subdivides the eucaryotes into Kingdoms Protista, Myceteae, Animalia, and Plantae.

BIOLOGICAL WEAPONS

Now it is quite clear and understandable that biological warfare is not new at all. Biological warfare was reported by early Romans who polluted water sources of their enemies by dumping animal carcasses. Then British distributed blankets to Indians in 1763. These blankets had been used by smallpox patients. Hence Indian users contracted smallpox. British had detonated an experimental anthrax bomb in Gruinard Island in Second World War.

In 1984, 750 people fell ill to food poisoning in Oregon because of spread of salmonella that had been cultured in the laboratory.

Biological warfare may be defined as intentional use of doses to harm or kill an adversary military forces, population, food or livestock and includes any living, or nonliving organisms or its bioactive substance (toxin). Hence, germ warfare can be spearheaded by bacteria, viruses, fungi, toxins, etc.

Organisms which can be used for biological war are *Bacillus anthracis* (causing pneumonia with a mortality rate 95%, if untreated), **smallpox** (contagious with high mortality rate), *Yersinia pestis* (plague causing bacteria), *Francisella tularensis* (tularemia), Ebola and Marburg viruses (hemorrhage fever), *Clostridium botulinum* (botulinism), etc.

Molecular Epidemiology

Molecular epidemiology is defined as a science that focuses on the contribution of potential genetic and environmental risk factors, identified at molecular level, to the etiology, distribution and prevention of diseases within families, countries and continents. Genetic variability within and between infectious agents/disease pathogens forms the foundation of molecular microbiology.

Table-2 Epidemic Prone Diseases

Ancient	Middle Era (killer diseases)	Middle Era (emerging/ reemerging)	Current Era	Threat of Rare Diseases
Smallpox	Cholera	O ₁₃₉ V. cholerae	Viral hepatitis	Ebola virus
Plague	Malaria	Dengue	HIV.	Yellow fever
Anthrax	Typhoid	Leptospira		Rift valley
	Tuberculosis			

-Application of Molecular Epidemiology

- 1- Tracing the source and origin of infection.
- 2- Tracking the routes of pathogen transmission.
- 3- Identifying reservoirs sustaining transmission.
- 4- Identifying new, emerging and reemerging pathogens.
- 5- DNA finger printing in actual diagnosis of pathogens.
- 6- Characterizing drug resistant strains.
- 7- Monitoring the progress of disease and central activities.
- 8- Identifying links between cases and infections.
- 9- Linking pathogen variants to endemicity and epidemicity.
- 10- Monitoring impact of immunization program, e.g. polio eradication.

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