Chapter 1

Introduction to Microbiology: History of Development

INTRODUCTION

Microbiology is the study of living organisms of microscopic size. This term was introduced by French chemist Louis Pasteur (1822–1895), who demonstrated that fermentation was caused by the growth of bacteria and yeasts. Medical microbiology deals with the causative agents of infectious diseases of man, the response generated by him against them and various methods of diagnosis, treatment and prevention. The term **microbe** was first used by Sedillot in 1878, but it has now been replaced by **microorganism**.

HISTORICAL PERSPECTIVE

Ancient man had thought the infectious diseases to be because of divine wrath and supernatural powers. Later, concepts like bodily constitution, faulty diet and the influence of environment were proposed. From very early times, there have been many suggestions that these diseases result from invasion of body by external agents. In summarizing the history of bacteriology, it is customary, at least, in relation to medicine, to refer to the concept advanced by Fracastorius of Verona in 1546 of a *Contagium vivum* as the cause of infective disease, and to the view suggested by von Plenciz (1762) on the specificity of disease, based on a belief in its microbial origin.

Antonie van Leeuwenhoek (1632–1723)

The construction and use of the compound microscope was an essential prerequisite to study the microbial forms. To Antonie van Leeuwenhoek must be ascribed the credit of placing the science of microbiology on the firm basis of direct observation. This Dutch maker of lenses from Holland devised an apparatus and technique which enabled him to observe and describe various microbial forms with accuracy and care. He observed, drew and measured a large number of minute living organisms including bacteria and protozoa and communicated them to Royal Society of London in 1683.

Antonie van Leeuwenhoek first accurately described the different shapes of bacteria (coccal, bacillary and spiral) and pictured their arrangement in infected material (1683).

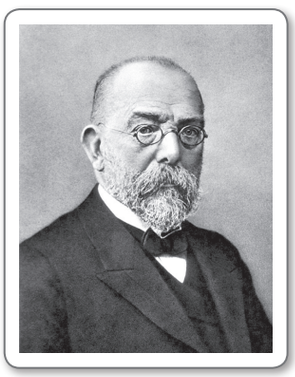
Louis Pastuer (1822–1895)

In 1856, Louis Pasteur was commissioned by an industrialist of Lille to investigate the problem which had arisen in manufacture of alcohol. The beet juice, from which alcohol was derived, was contaminated with a grey material which interfered with alcohol production. During the course of investigation his attention was abruptly focused on the role of microorganisms in alcohol fermentation and spoilage. Undesirable forms of life could be destroyed at temperatures of 50–60°C in a short period of time. Subsequently, this modified process of heating came to be known as pasteurization. Pasteur established that different types of fermentations were due to the activity of different kinds of microbes. In the course of studies, Pasteur introduced the techniques of sterilization and developed steam sterilizer, hot air oven and autoclave.

Joseph Lister (1827–1912)

Joseph Lister (1827–1912), an English surgeon and contemporary of Pasteur, was among the first to appreciate the ramifications of the emerging germ theory of disease. He attributed the frequent disastrous consequences following repair of compound fractures to invasions by airborne microorganisms. Lister advocated the use of carbolic acid as an aerosol during surgery and for impregnation of dressings to reduce the risk of postsurgical infections. He established the guiding principle of antisepsis for good surgical practice upon which the present day specialists depend. For this work he is known as 'father of antiseptic surgery'.

Robert Koch (1843–1910)



Robert Koch (1843–1910), a German physician, perfected the bacteriological techniques, staining procedures and methods of obtaining bacteria in pure culture using solid media during his studies on the culture and characters of anthrax bacillus.

The causative agents of various diseases were reported rapidly by different investigators. Robert Koch discovered the bacillus of anthrax (1876), bacillus of tuberculosis (1882) and cholera vibrio (1883); Hansen described the leprosy bacillus in 1874; Neisser discovered the gonococcus in the pus discharge from urethra in 1879; Alexander Ogston in 1880 described the staphylococci in abscesses and suppurative lesions; Eberth observed the typhoid bacillus in 1880; Klebs (1883) and Loeffler (1884) observed and described the diphtheria bacillus; Rosenbach (1886) demonstrated the tetanus bacillus with round terminal spore; Weichselbaum (1887) described and isolated the meningococcus from the spinal fluid of a patient; Bruce (1887) identified the causative agent of malta fever in 1905 and Schaudinn and Hoffmann described the spirochaete of syphilis.

As the agents were being reported in such profusion it became necessary to introduce criteria for proving the claims that a microorganism isolated from a disease was indeed causally related to it. Henle indicated such criteria but were enunciated by Koch which consisted of guidelines for the association of particular microorganisms with specific infectious diseases. A microorganism can be accepted as the causative agent of an infectious disease only if following postulates, known as Koch's postulates, are satisfied:

- The organism must be present in the lesions in every case of the infectious disease.
- It should be possible to isolate the organism in pure culture from the lesions.
- Inoculation of the pure culture into suitable laboratory animals should produce a similar disease.
- It should again be possible to reisolate the organism in pure culture from the lesions produced in the experimental animals (Fig. 1.1).

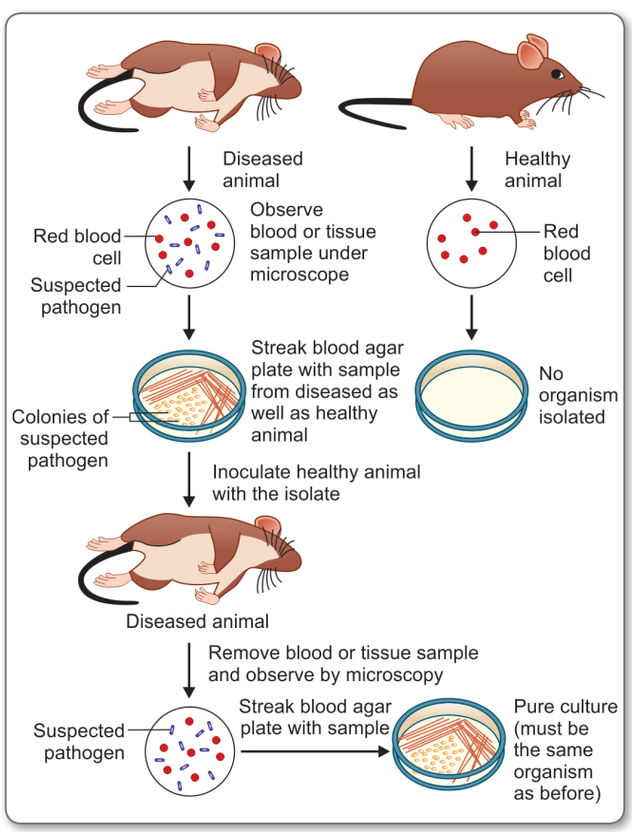
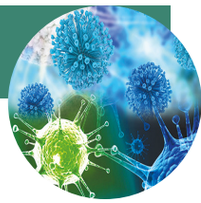


Fig. 1.1: Koch's postulates.



A fifth criterion introduced subsequently states that specific antibodies to the organism should be demonstrable in the serum of the patient suffering from the disease. These postulates have proved extremely useful in confirming the authenticity of doubtful claims made regarding the causative agents of infectious diseases.

Exceptions to Koch's Postulates

Koch's postulates have remained a mainstay of microbiology; however, many microorganisms that do not meet the criteria of Koch's postulates have been shown to cause disease. For example:

- *Treponema pallidum* and *Mycobacterium leprae*, causative agents of syphilis and leprosy respectively, cannot be grown *in vitro*; however, there are animal models of infection with these agents.
- In another example, *Neisseria gonorrhoeae*, which causes gonorrhoea, there is no animal model even though the bacteria can readily be cultured *in vitro*.

The Beginning of Virology

For many years the term virus was used to describe any poison or microbial agent capable of causing an infection. In a large number of diseases such as smallpox, chickenpox, measles, influenza, poliomyelitis and the common cold, no bacterial cause could be established. Pasteur had suspected that rabies in dogs could be caused by a microbe too small to be seen under the microscope.

The first man to describe a filtered extract capable of causing disease in plants was Dmitri Iwanowski (1864–1920), a Russian scientist, who started his studies on diseases of tobacco while he was still a student. He reproduced mosaic disease in tobacco plant by applying juice from diseased plants to healthy leaves from which all bacteria had been removed by passage through fine filters (1892). In 1898, Martinus Beijerinck, unaware of Iwanowski's work, attributed the cause of tobacco-mosaic disease to *Contagium vivum fluidum*, a living liquid virus.

In 1898, Loeffler and Paul Frosch from Germany reported that the causative agent of foot-and-mouth disease in cattle would pass through a bacterial filter. Walter Reed (1902) in Cuba proved that the causative agent of yellow fever was not only a filterable virus but also transmitted through the bite of infected mosquitoes. The term 'filterable' was dropped in time and the tiny infectious agents were merely called viruses. Larger viruses could be seen under light microscope after appropriate staining but their detailed morphology could only be studied by electron microscope introduced by Ruska (1934). The technique of growing them on chick embryos developed by Goodpasture in 1930s and the application of tissue culture in virology expanded the scope of virological techniques considerably.

Ellerman and Bang (1908) suggested the possibility that virus infection could lead to malignancy. Peyton Rous (1911) isolated a virus causing sarcoma in fowls. Several viruses have been blamed to cause natural and experimental tumours in birds and animals. Experimentally, viruses can cause malignant transformation of infected cells in tissue cultures. The discovery of viral and cellular oncogenes have put forth the possible mechanisms of viral oncogenesis.

IMPORTANCE OF MICROBIOLOGY TO NURSING

Laboratory diagnosis of microbial infections can be divided into pre-examination processes, examination processes and post-examination processes. Nurses play an important role in the pre-examination processes. This includes collection of primary samples, labelling of primary samples, safe disposal of materials used in collection and transportation of the samples to the laboratory such that they are transported:

- Within a time frame appropriate to the nature of requested examinations;
- Maintaining cold chain; and
- In a manner that ensures safety of the carrier.

Quality of examinations is improved if proper precautions are taken during pre-examination processes.

Hospital-associated infections (HAI) or nosocomial infections are increasingly becoming a major concern all over the world. The source of infecting organisms may be exogenous from another patient or a member of the staff including nursing staff or from inanimate objects in the hospital like medical equipment (endoscopes, cystoscopes, catheters, needles, etc.), bed pans, food, water, hospital air and surfaces contaminated by the patients' secretions, excretions, blood and body fluids. It may be endogenous from patient's own flora which at the time of infection may invade the patient's tissues spontaneously or be introduced into them by surgical operation, instrumental manipulation or nursing procedures. Nurses can help in prevention of HAI by:

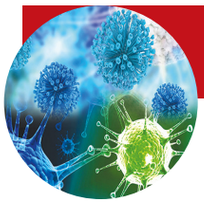
- The provision of sterile instruments, dressings, surgical gloves, face masks, theatre clothing and fluids; and
- Thorough hand washing after any procedure involving nursing care or close contact with the patient.
- Infection control nurses are important members of infection control committee which meets regularly, at least once in a month and as often as required to discuss measures to control infection in a hospital. Nurses play an important role in biomedical waste management.

CONTRIBUTIONS OF VARIOUS SCIENTISTS IN THE FIELD OF MICROBIOLOGY

A large number of scientists has contributed in the field of microbiology. Principal contributions of some of them are given below.

Louis Pasteur

Louis Pasteur (1822–1895) was born in the village of Dole, France on December 27, 1822, the son of humble parents. His father was



a tanner. He was originally trained as a chemist, but his studies on fermentation led him to take interest in microorganisms. His discoveries revolutionized medical practice, although he never studied medicine.

1. The term **microbiology**, as the study of living organisms of microscopic size, was coined by Pasteur.
2. He also coined the term **vaccine**.
3. He used various forms of nutrient fluid to grow micro-organisms.
4. He showed that some organisms were not destroyed by boiling. For the sterilization of fluids he advocated heating to 120°C under pressure and for glassware the use of dry heat at 170°C. He showed that cotton plugs (a primitive air-filtration device) could prevent microbes from reaching otherwise air-exposed sterile broths.
5. In 1860–61, he disapproved the **theory of spontaneous generation**. In a series of classic experiments, Pasteur proved conclusively that all forms of life, even microbes, arose only from their alike and not *de novo*.
6. In 1860–64, he gave experimental evidence that fermentation and putrefaction are effects of microbial growth.
7. In 1863–65, he devised the process of destroying bacteria, known as **pasteurization**.
8. In 1877, Koch and Pasteur demonstrated that anthrax is caused by bacteria. Pasteur grew the organisms in sterilized yeast water and kept them in the laboratory for several months, transferring them frequently to new culture fluid, in which they multiplied readily, and showed that these cultures would always cause anthrax when inoculated into healthy animals.
9. In 1880, he prevented chicken cholera by injection of live attenuated culture. He found that pure cultures of the germ of this disease which had been kept in the laboratory for some time would not kill his animals as fresh cultures did, but would merely cause a passing illness from which the chickens recovered. Then he discovered that the animals that had recovered from a previous inoculation of weakened germs were immune, and did not succumb to the disease. Pasteur immediately perceived that it might be possible to make individuals resistant by inoculating them with the weakened (and therefore harmless) germs of a particular disease.
10. In 1880, he first cultured staphylococci in liquid medium and produced abscesses by inoculating them into rabbits.
11. In 1881, he developed **live attenuated anthrax vaccine**.
12. In 1881, pneumococci were first noticed by Pasteur and Sternberg independently.
13. The crowning achievement of Pasteur was the successful application of the principle of vaccination to the prevention of rabies, or hydrophobia, in human beings and developed **Pasteur rabies vaccine** in 1885. He obtained fixed rabies virus by serial intracerebral passage in rabbits. The rabies vaccine was prepared by drying pieces of spinal cord from rabbits infected with fixed virus. Rabies vaccine prevented the development of this fatal disease if the inoculations are given soon after the bite of the rabid animal. He gave the first treatment for rabies in 1885 to a young boy bitten by a rabid dog.
14. In 1887, Pasteur and Joubert first described *Clostridium septicum* and called it *Vibrion septique*.

In 1888, in recognition of his incomparable achievements, the Pasteur Institute of Paris was built by public contribution during his lifetime for investigations of infectious diseases and preparation of vaccines. Acclaimed the world over for his epoch making discoveries, Pasteur died in Paris on September 28, 1895. His body lies in Pasteur Institute of Paris. Today the Pasteur Institute is a thriving research centre—an appropriate memorial to its founder.

Robert Koch

Contributions of Robert Koch, to microbiology, are variegated and enormous.

1. In 1876, Robert Koch reported the isolation of anthrax bacillus in pure culture, formation and germination of its spores and the proof of its infectiousness. This agent as the sole cause of anthrax was confirmed by Pasteur.
2. In 1877, he introduced the method of making smears of bacteria on glass slides, and of staining them with the aniline dyes.
3. In 1881, he described means of cultivating bacteria on solid media, thus making it possible to obtain pure cultures by transferring material from a single colony.
4. The **hanging-drop method** of studying bacteria as used today is a product of his genius.
5. In 1882, Koch startled the world by announcing his discovery of **tubercle bacillus** (*Mycobacterium tuberculosis*), the causative agent of tuberculosis. He described a special staining method for detection of this organism and grew it in pure cultures in the laboratory.
6. In 1883, he discovered the causative agents of cholera (*Vibrio cholerae*), Egyptian ophthalmia (pink eye) and Koch Weeks bacillus.
7. In 1884, Koch expounded the postulates or laws by which an organism may be proved to be the cause of a particular disease. These are known as **Koch's postulates**.
8. Koch continued his work on tuberculosis and in 1890–91 he showed how a normal guinea pig and an already infected guinea pig behaved differently to an infection with tubercle bacillus. This is known as **Koch's phenomenon** (*see* Chapter 17).

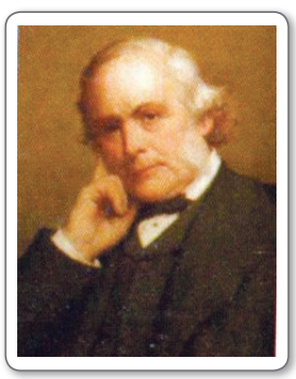
In 1905, he was awarded the **Nobel Prize in Medicine**' for his work on tuberculosis. Together with Louis Pasteur, he laid the foundations of modern bacteriology.

Antonie van Leeuwenhoek

Antonie van Leeuwenhoek was expert in the grinding of simple magnifying lenses. He made these lenses of same bits of glass, polished them very carefully, and mounted each separately between two brass, copper, silver, or gold plates, to which he fastened an adjustable holder for the object to be examined. He constructed many of these 'microscopes' each containing a single lens ground by himself. The best of lenses magnified about 200 times. He observed, drew and measured a large number of living organisms including bacteria and protozoa in materials such as rain water, pond and well water, and saliva and the intestinal contents of healthy subjects and communicated them to the Royal Society of London in 1683. He was the first to accurately describe different shapes of bacteria (coccal, bacillary and spiral) and picture their arrangement in infected material.

Leeuwenhoek observed that very large numbers of bacteria appeared in watery infusions of animals or vegetable matter which were left to stand for a week or two at room temperature. He believed that these huge populations were the progeny of a few parental organisms, or seeds, that were originally present in the materials of the infusion or had entered it from the air. The significance of these observations was not realized then and to Leeuwenhoek the world of '*little animalcules*' represented only a curiosity of nature. Their importance in medicine and other areas of biology came to be recognized two centuries later.

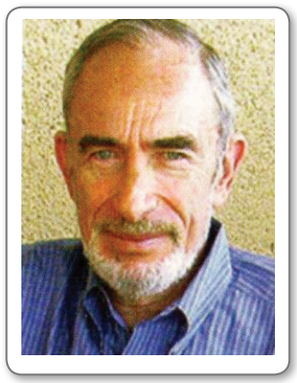
Edward Jenner (1749–1823)



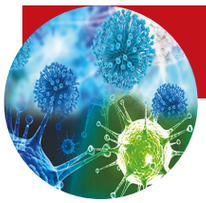
Edward Jenner *introduced the modern method of vaccination to prevent smallpox*. He observed that milkmaids who contracted cowpox or vaccinia while milking were subsequently immune to smallpox. On May 14, 1796, he devised a brave experiment. He performed a vaccination against smallpox by transferring material from a cowpox pustule on the hand of a milkmaid, Sarah Nelmes, to the arm of a small boy named James Phipps, his gardener's son. 6 weeks later the boy was inoculated with smallpox. He failed to develop the disease. *The terms vaccine and vaccination were first used by Pasteur out of deference to Jenner*.

In 1967, the World Health Organization masterminded a final global plan to eradicate smallpox. Success was announced in 1980 with the declaration: Smallpox is dead. Thanks to Jenner. Edward Jenner's discovery has now been developed into one of the most important parts of modern medicine—**immunology**.

Paul Ehrlich (1854–1915)



1. In 1882, he reported the *acid-fastness* of tubercle bacillus.
2. From 1890 to 1900, he did important research in immunology. He soon found that the specific effect of immune serum could be demonstrated *in vivo* and *in vitro* and introduced methods of standardizing toxin and antitoxin. To him goes the credit of *minimum lethal dose*.
3. In 1898, he proposed *side chain theory of antibody production*.
4. In 1909, he introduced salvarsan, an arsenical compound, sometimes called the '*magic bullet*'. It was capable of destroying the spirochaete of syphilis with only moderate toxic effects. He continued his experimentation until 1912 when he announced the discovery of neosalvarsan. Thus he created a new branch of medicine known as *chemotherapy*.



Key Points

- Microbiology is the biology of ***microscopic organisms***, its subjects being microorganisms.
- Microorganism is an organism that ***cannot be seen without the use of a microscope.***
- ***Medical microbiology*** deals with those ***organisms which are responsible for infectious diseases of humans***.
- A microorganism is generally accepted as the causative agent of an infectious disease if it satisfies ***Koch's postulates***.
- *Treponema pallidum*, *Mycobacterium leprae* and *Neisseria gonorrhoeae* do not fulfil all the criteria of Koch's postulates; the first two ***cannot be grown in vitro*** and for the third there is ***no animal model***.

Assess Yourself

Short Answer Question

1. Write short notes on:
- (a) Koch's postulates
- (b) Contributions of Robert Koch in microbiology
- (c) Contributions of Antonie van Leeuwenhoek in microbiology
- (d) Contributions of Louis Paster in microbiology

Multiple Choice Questions

1. Which of the following organisms **does not** meet all the criteria of Koch's Postulates?
- (a) *Streptococcus pneumoniae*
- (b) *Treponema pallidum*
- (c) *Leptospira interrogans*
- (d) *Mycobacterium scrofulaceum*

2. The techniques of sterilization were introduced by:
- (a) Louis Pasteur
- (b) Robert Koch
- (c) Ferdinand Cohn
- (d) John Needham

3. Bacillus of tuberculosis was discovered by:
- (a) Hansen
- (b) Loeffler
- (c) Robert Koch
- (d) Bruce

4. The term microbiology, as the study of living organisms of microscopic size, was coined by:
- (a) Antonie van Leeuwenhoek
- (b) Robert Koch
- (c) Louis Pasteur
- (d) Edward Jenner

5. Which of the following is **not** a condition of Koch's postulates?
- (a) Isolate the causative agent of a disease
- (b) Cultivate the microbe in the laboratory
- (c) Inoculate a test animal to observe the disease
- (d) Produce a vaccine

Answers to MCQs

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