CHAPTER 1

History and Principles of Microbiology

CHAPTER OUTLINE

- How Microbiology Evolved?
- Golden Age of Microbiology
  - Robert Koch and Principles of Microbiology
  - Applications of Microbiology
  - Microbiology in Nursing
  - Antimicrobial Therapy
  - Virology
  - Immunology
  - Scope of Medical Microbiology

INTRODUCTION

Most people are familiar with the concept of microbes, or "germs", and their role in human health. Although a few hundred years ago, before the invention of the microscope, the existence of different types of microbes was impossible to prove. Historical evidence suggests that humans had some notion of microbial life since prehistoric times.

HOW MICROBIOLOGY EVOLVED?

Antony van Leeuwenhoek—Father of Microbiology

History of microbiology began with the invention of the microscope. Microorganisms were first seen in 1675 by a Dutchman *Antony van Leeuwenhoek* (Fig. 1.1). His microscope consisted of a single biconvex lens that magnified about ×200 and resolved microbodies with a diameter of about 1 micron (μm) (Fig. 1.2).

He found microorganisms in materials such as water, mud, saliva and intestinal contents of healthy people and recognized them as living creatures. He called them *animalcules* because they moved about actively. His drawings of the observed forms are now recognized as cocci (spheres), bacilli (rods) and spiral filaments (spirochetes).

Leeuwenhoek observed that large number of bacteria appeared in watery infusions of animal 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 particular organisms, or seeds that were originally present in the materials of the infusion or had entered it from the air. Other scientists suggested that the organisms arose by *spontaneous generation*, i.e., by the spontaneous conversion of dead organic matter into living microbes and with Leeuwenhoek's suggestion a controversy began that lasted for 200 years.

Louis Joblot

In 1718, an experiment by Louis Joblot, who was a French microscopist ultimately settled the matter. He boiled a flask of an infusion of hay for 15 minutes to kill any microbe originally present in it. Covered it with a parchment cap to prevent later entry of other microbes from the air. It was observed that on subsequent standing, it remained sterile, i.e., free from microbial growth.

Fig. 1.1: Antony van Leeuwenhoek

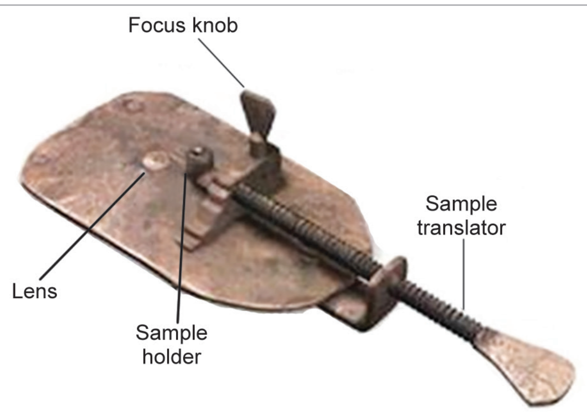


Fig. 1.2: Leeuwenhoek's microscope

John Needham

Needham in 1749, made similar effort, though not identical experiments, in which by contrast, the heated covered infusions showed growth of organisms after a period of time and this supported spontaneous generation.

Francesco Redi

It was later shown by Francesco Redi, an Italian physician in mid-17th century that theory of spontaneous generation does not exist. He performed an experiment by taking pieces of meat and placed them separately in two jars and covered one of these jars. After a few days, maggots appeared in one of the jars which was left open. Redi concluded that because flies visited freely the open jar, the maggots appeared but in other jar that was closed or sealed, flies could not visit the meat piece and no maggots appeared (Fig. 1.3). Although his findings were not accepted. The controversy about the germ theory continued.

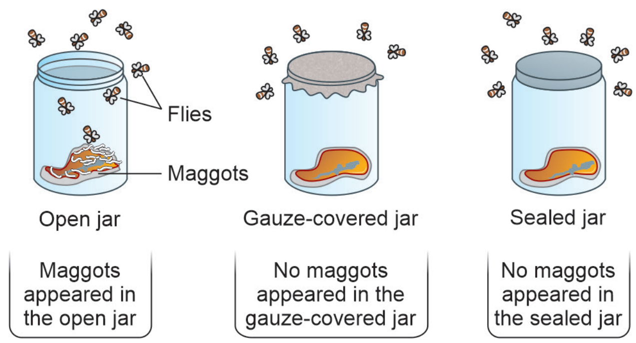


Fig. 1.3: Francesco Redi's experiment

1860–1910 is considered as the period in which maximum discoveries happened and this period is called *Golden Age of Microbiology*.

Louis Pasteur—The Progenitor of Modern Immunology

Pasteur is called *the progenitor of modern immunology* (Fig. 1.4). His discoveries substantiated as foundations for microbiology and immunology. He performed a series of experiments to prove that the solutions could be made free of microorganisms by boiling. If no air is left to come in contact with solutions, there is no emergence of microbial life.

Germ Theory of Disease

In his *swan neck experiment* (Fig. 1.5), he took broth in long necked flask. He softened the neck of flask under the flame and molded it in the shape of a swan neck. Broth was boiled until steaming and then cooled. This solution of broth did not show growth of microorganisms when kept for long periods because the microbes were not able to reach the broth.

Fig. 1.4: Louis Pasteur

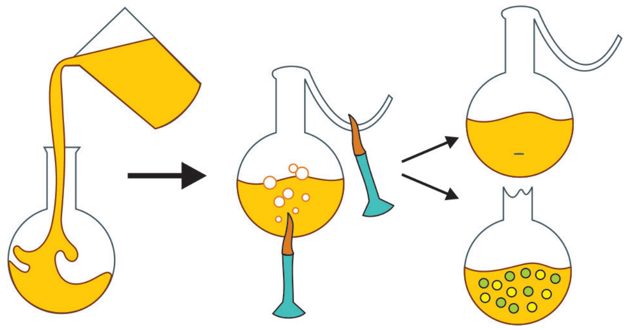


Fig. 1.5: Swan neck experiment

Fig. 1.6: Joseph Lister

This further *rejected the theory of spontaneous generation*. The microbes could not enter the broth due to peculiar shape of the neck of flask. This experiment supported germ theory of disease.

Germ theory is also known as *theory of pathogenesis*. Leeuwenhoek's findings started building a foundation for germ theory. His contemporary scientific workers had this view that disease originates due to some curse when a person displeases the God—theory of spontaneous generation. But with the invention of microscope and other laboratory techniques, many scientists discovered pathogenic organisms and it slowly disapproved theory of spontaneous generation.

In the golden age of microbiology, which covered a time period of 1857 to 1914, many microorganisms were discovered by scientists from time to time.

Neisser discovered gonococcus in 1879, **Ogston** discovered the staphylococci, **Loeffler** isolated the diphtheria bacillus, **Frankel** described the pneumococcus, **Bruce** identified the causative agent of Malta fever, **Schaudinn and Hoffmann** discovered the spirochetes of syphilis in 1905 and many more microbes were discovered.

Contributions to Germ Theory of Disease

Fracastoro in 1546 told that "the disease is caused by minute 'seed' and it spreads from person to person". In 1796, Jenner used cowpox virus to immunize against smallpox. He tried to protect against smallpox by inoculating a less virulent virus and Bassi in 1835 demonstrated that silkworm disease was due to microbial infections. Oliver Wendell Holmes (1809–1894) suggested that sepsis during child birth was due to some germs being transmitted from mother to child. In 1840, Ignaz Semmelweis observed that by hand washing, transmission of puerperal sepsis was prevented.

In 1845, Barkley proved that potato blight was caused by fungus *Phytophthora infestans*.

Davaine observed that anthrax bacillus was transmitted by inoculation of infected blood.

Pasteur in 1861 proved that microorganisms are present in the environment and later he associated germs with diseases. He found the cause of wine souring and related this to growth of bacteria in the wine barrels. He discovered vaccines to protect against the diseases like cholera, rabies and anthrax.

In 1860, Joseph Lister (Fig. 1.6) applied a phenolic compound for dressing the surgical wounds and found that it was effective in preventing surgical wound infections. He is also known as *"Father of Antiseptic Surgery"*. On getting good results by the application of phenol on wounds, he also introduced spray of carbolic acid in operation theaters (OTs) so that the growth of microorganisms could be prevented.

Koch contributed by giving four postulates which proved as foundation in microbiology. He discovered causative organisms for tuberculosis, anthrax and cholera. He gave many microbiological techniques like staining technique, use of solid culture media and smear fixation by heat, etc.

Applied Microbiology

Knowledge of germ theory touched the working life of nurses and other health care workers and gave the understanding as follows:

- A specific disease is caused by a *specific microorganism*.
- *Transmission of a disease* is due to the presence of existing microorganism in the environment.
- *Hygienic practices* were introduced in medical technology based on the germ theory.
- *Sterilization and disinfection methods* were introduced after understanding germ theory.
- *Antibiotics* were used for the first time in medical treatment.
- Germ theory is the foundation for *diagnostic microbiology*.
- The epidemic spread of disease can be prevented.

Alexander Fleming discovered first antibiotic penicillin in 1928 that proved as wonder drug in Second World War and saved many precious lives.

Contributions of Louis Pasteur

In 1856, Pasteur was called by wine makers of Lille to investigate the cause of souring of alcohol being produced. He investigated and found that there was a gray material growth in the substrate used for manufacturing alcohol. This microorganism growing in alcoholic product was the cause of souring of wine. He found that these undesirable microorganisms could be killed by heating the wine at 50°–60°C for a short period of time. This method is now popularly known as *pasteurization* and involves moderate heat treatments of the product to kill microorganisms. This process is now backbone of wine industry. Nowadays, pasteurization is widely used in milk industry, food and beverage industries.

We can summarize his contributions as follows:

- Pasteur discovered that *fermentation of a substrate is caused by microorganisms*.
- Pasteur performed *swan neck experiment* to disapprove the theory of spontaneous generation.
- He was the first one to use terms like *aerobics* for those organisms that can live in the presence of oxygen and *anaerobic* for those organisms that prefer to live in the absence of oxygen.
- He discovered the process of *pasteurization* that is used nowadays to keep the food products for a long time, i.e., to increase shelf life of the product.
- He *developed vaccines* against cholera, anthrax and rabies, which were very deadly diseases at that time.
- He *discovered silkworm disease* known as pébrine and isolated the causative microorganisms of this disease.
- He *introduced sterilization techniques* and developed steam sterilization method, hot air oven and autoclave. These methods are backbone of the sterilization techniques and are widely used in health care industry, and food and beverage industry.
- He *isolated microorganisms responsible for chicken cholera and rabies*. He demonstrated that the causative agents of these diseases were too small to be seen through microscope and these could pass through filters. Later on this filterable agent of disease was known as virus.
- He *suggested methods to control cross infections in hospitals*.
- He *introduced attenuated live vaccines* for prophylactic use.
- He *discovered microorganisms such as Staphylococcus, Streptococcus and Pneumococcus*.

GOLDEN AGE OF MICROBIOLOGY

ROBERT KOCH AND PRINCIPLES OF MICROBIOLOGY

Robert Koch (Fig. 1.7) was a German physician and is well known for his contributions in the field of medical techniques, and is called *Father of Microbial Techniques* and *Father of Medical Microbiology*. He brought perfection in bacteriological techniques, *staining methods*, *pure culture techniques*, use of *solid media to culture* microorganisms. He isolated anthrax bacillus (1876), *Mycobacterium tuberculosis* (1882) and *Vibrio cholerae* (1883).

As the reports were just pouring about the causative organisms of different diseases by different investigators, it became necessary to introduce tests to prove that the microorganisms isolated were indeed the causative agents of that particular disease. These *tests serve now as a guideline* to prove a relationship between the disease and the causative organism and these should be fulfilled before the organism can be confirmed as a real cause of disease. These criteria are known as Koch's postulates and these are considered as principles of microbiology.

Koch's Postulates

Three postulates were formulated by Robert Koch and Friedrich Loeffler in 1884. In 1905, EF Smith added fourth postulate. These postulates were applied by Koch to find out the course of diseases like anthrax and tuberculosis (Fig. 1.8). These postulates are applicable for other diseases too.

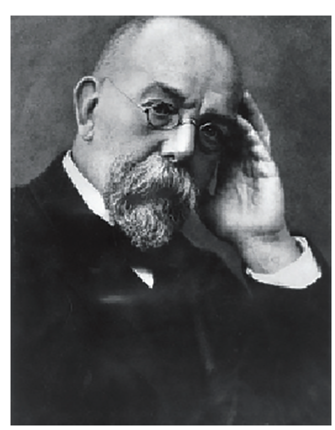


Fig. 1.7: Robert Koch

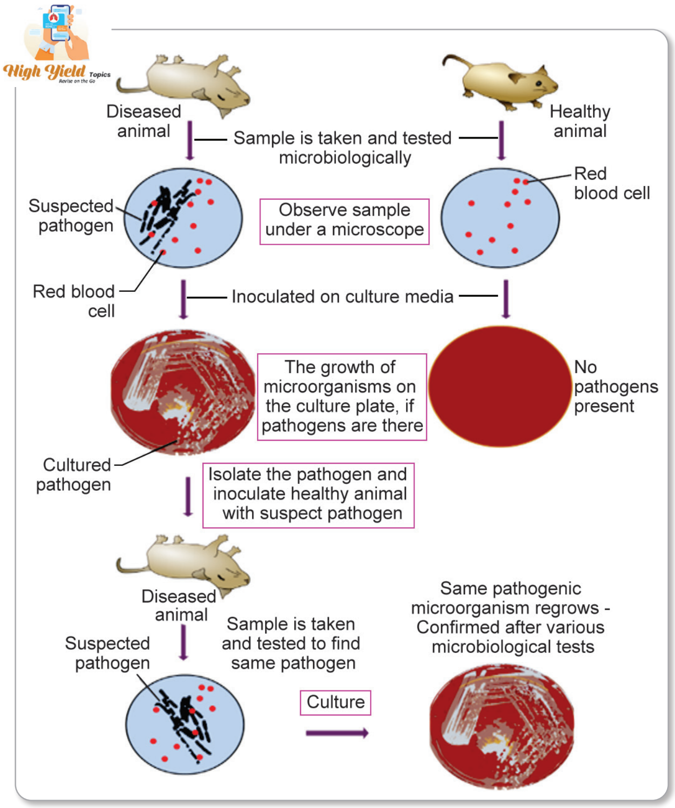


Fig. 1.8: Koch's postulates—A summary

Postulate I—Association: The microorganism must be present in the body of suffering animal and should not be found in healthy animal. A particular *microorganism should be associated with a particular disease*.

Postulate II—Isolation: The causative organism must be isolated from the body of diseased animal. This isolated organism must be cultured in laboratory to get a *pure culture*. Its growth characteristics and biochemical characteristics must be noted down.

Postulate III—Inoculation: The isolated organism from pure culture *must produce similar disease* in a healthy laboratory animal.

Postulate IV—Reisolation: The microorganism must be reisolated from the inoculated laboratory animal and identified as causative microorganism. The *reisolated microorganism must show exactly the same growth and biochemical characteristics* as recorded in second step.

Later, a *fifth criterion* was also introduced, which clarifies that *specific antibodies to the microorganism should be demonstrated* in the patient's serum suffering from the disease.

Limitations of Koch's Postulates

Koch's postulates have severe limitations too, which even Koch realized as:

- There are microorganisms which do not follow Koch's postulates, e.g., the pathogens like *Mycobacterium leprae* and spirochetes.
- He believed that cholera and leprosy were caused by microorganisms but all criteria set according to Koch's postulates are not fulfilled by these pathogens. Further, he proved that *Vibrio cholerae* could be isolated from both sick and healthy people, which *invalidated postulate 2*.
- The limitations of Koch's postulates are even more intense in case of viral diseases where it was found that many viruses do not cause illness in every infected individual—it was the *violation of postulate 1*.
- *Postulates 2 and 3 cannot be fulfilled for viruses* that do not replicate in cell culture, or for infections for which a suitable animal species has not been identified like spirochetes or *Mycobacterium* species.

Facteria

Modern Concepts of Koch's Postulates

Here are Koch's postulates for the recent times as suggested by Fredericks and Relman:

- The antigen in the form of nucleic acid (from a causative organism) *must be present in majority of suffering people* having same type of disease. The nucleic acid must be present in diseased organ when anatomically observed.
- The nucleic acid of the pathogenic organism *must not be present in a healthy person*.
- Nucleic acid of pathogen must *disappear* or reduce when the disease resolves. The nucleic acid sequence of a pathogen should:
  - Establish *association with disease*.
  - Be *biologically consistent* with characteristics of that particular group of organisms.
  - The results obtained from nucleic acid sequences must be reproducible in other labs.

Contributions of Robert Koch

Robert Koch has contributed in various ways in the field of microbiology. Some of these are mentioned here:

- Before Koch, it was emphasized that the disease begins spontaneously. With his experiments, he *proved the germ theory of disease* and explained that disease is not due to curse but it originates from germs.
- His four postulates are a guideline when an association between disease and causative microorganism is to be established.
- In order to study morphology of an organism, he introduced *staining techniques*.
- Nowadays, we all are familiar with *heat fixing of the smear* before staining. This was also introduced by Koch.

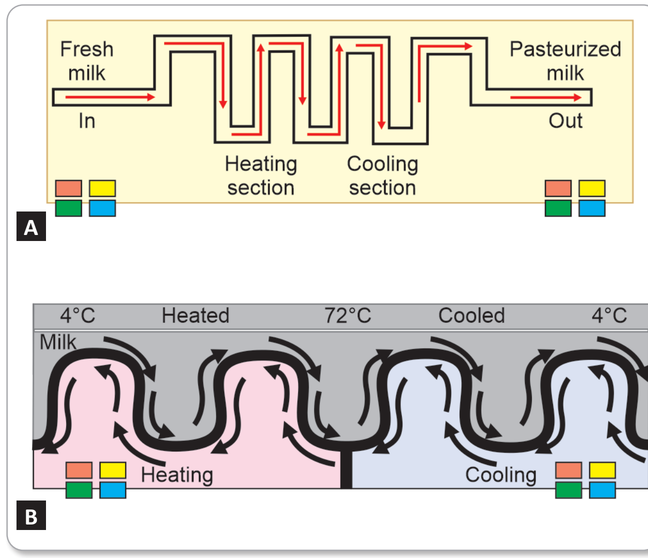
- He introduced the dyes for staining microorganisms like *aniline dyes* which are easy to work with, fast to act and give good results.
- Technique to obtain *pure cultures* of microorganism was developed by him.
- Agar is used in many European dishes as a solidifying agent from a long time. Koch was first to use agar as solidifier in culture media. This facilitated a scientist to observe colonial morphology of an organism when grown in labs.
- He introduced a skin test for diagnosing tuberculosis which is a hypersensitivity reaction.

APPLICATIONS OF MICROBIOLOGY

Pasteurization

Pasteurization is a *process of heating and then rapidly cooling* liquids like milk, honey, juices or food items like eggs, butter and cheese in order to kill microorganisms that may spoil them or cause diseases (Figs 1.9A and B). Although every single microorganism in the product is not destroyed, for example bacterial spores. Today, the method is used widely in the dairy industry and other food processing industries to achieve *food preservation and food safety*. The pasteurization process is based on the use of one of the following time and temperature relationships.

- **High temperature short treatment (HTST):** This process uses higher heat for less time to kill pathogenic bacteria, e.g., milk is pasteurized at 161°F (72°C) for 15 seconds. HTST causes less damage to the nutrient composition and sensory characteristics of food as compared to low temperature long treatment (LTLT).
- **Low temperature long treatment:** This process uses lower temperature for a longer time to kill pathogenic bacteria. For example, *milk* is pasteurized by this method by exposing it to 145°F (63°C) for 30 minutes.



Figs 1.9A and B: **A.** Pasteurization–mechanism showing passage; **B.** Use of high and low temperature in process

- **Flash pasteurization:** In order to pasteurize food, "Flash pasteurization" method is used. It involves HTST in which *pourable products* like juices are heated for 3–15 seconds at a temperature of 74°C which destroys harmful microorganisms. After heating, the product is cooled and packed. Most drink boxes and pouches are pasteurized in this way because it allows *extended unrefrigerated storage*.
- **Steam pasteurization:** This technology uses heat to control or reduce harmful microorganisms in *beef/meat*. Beef is exposed to pressurized steam for approximately 6–8 seconds (Fig. 1.10). The steam raises the surface temperature of beef to 191°–200°F (88°–93°C). The carcasses are then cooled with a cold water spray. This process kills microorganisms like *Escherichia coli, Salmonella* and *Listeria*.

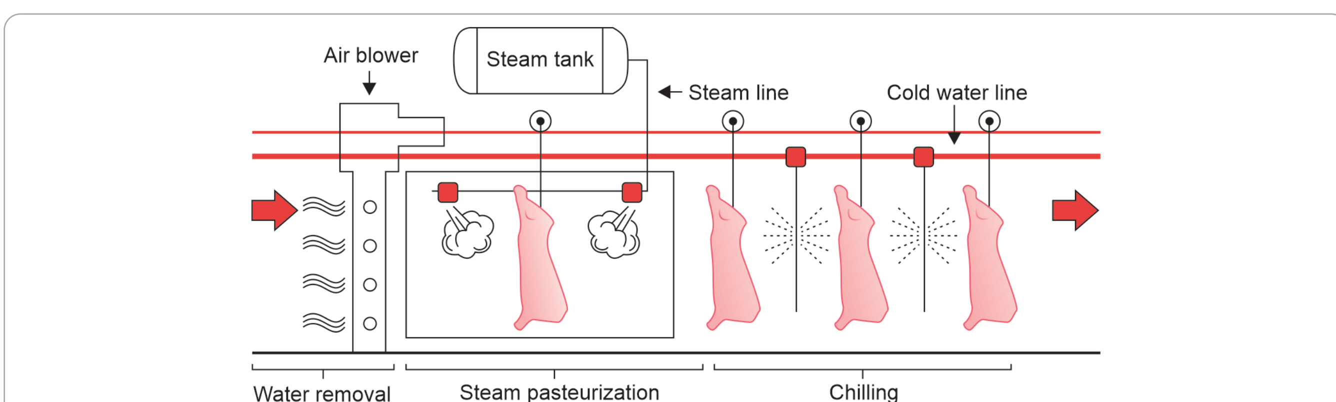


Fig. 1.10: Steam pasteurization used in meat processing factory

- **Irradiation pasteurization:** *Poultry products, red meat, spices, fruits and vegetables* are all subjected to small amounts of gamma rays. This process effectively kills vegetative bacteria and parasitic food-borne pathogens. Shelf life of food items is also increased.

Tyndallization

Tyndallization was discovered by John Tyndall (Fig. 1.11), who was a student of Pasteur. It is a process used for sterilizing substances, usually food and is also called *fractional sterilization*. He concluded that bacteria occur in two forms known as *heat labile*, which are easily killed when exposed to heat. Another form of bacteria is *heat stable*, which could not be killed by exposing to high temperature and are heat resistant. They could not be killed by continuous boiling of the broth and grow in the broth after some period of time. He further demonstrated that if such broths are subjected *to intermittent (discontinuous) boiling-steaming at 100°C on three consecutive days for 30 minutes of each* exposure, these heat resistant forms can be killed in broth and no microbial growth will occur thereafter in such broths as broth becomes germ free.

At first exposure, the vegetative cells are killed, but spores may remain. At intervals, spores germinate and grow into vegetative cells which are killed during second exposure. On third exposure, the broth becomes completely free of germs and no growth occurs for prolonged period of time.

Tyndallization process is usually effective in practice. But it is not very reliable because there are chances that some of the spores may survive which later germinate.

Tyndallization method is mainly *used for sterilizing plant seeds* these days.

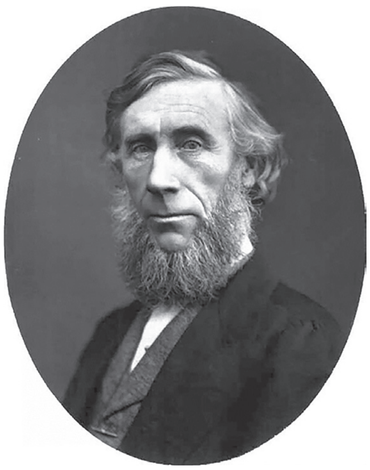


Fig. 1.11: John Tyndall

Fermentation

Fermentation is a process where *carbohydrates are broken down into alcohols and organic acids during anaerobic respiration*. Here, carbon and energy (in the form of ATP) provide an electron donor and an electron acceptor in a series of coupled oxidoreduction reactions. This process leads to the formation of a variety of waste products like ethanol in cultures of yeasts, organic acids and alcohols in cultures of bacteria and a mixture of lactic, acetic, formic and succinic acids by the enterobacteria.

Fermentation is accompanied by the production of both acids and gases like carbon dioxide and hydrogen. The fruit juices on incubation, in presence of yeast, give rise to wine and this is the main reaction taking place in wine production, but when this substratum gets contaminated by bacteria the wine gets sour. Pasteur solved this problem and he found that on heating this mixture, bacteria were destroyed and a good quality wine was obtained.

Note Other applications include Sterilization and Disinfection; Aseptic Techniques; Culture methods; Isolation Techniques; Staining Methods; Use of PPE, etc. All these are discussed at relevant places in this book.

MICROBIOLOGY IN NURSING

The nurses have to take care of patients—both indoor and outdoor. They are involved in various other aspects in a hospital such as collection of sample from appropriate sites, report reading, patient care, sterility checks in OTs, sterilization of medical equipment, disinfection of the wards and biomedical waste management. To perform these duties sincerely and accurately, a nurse must be well aware of the microbiological concepts. Some of the duties performed by a nurse in correlation with microbiology are mentioned here:

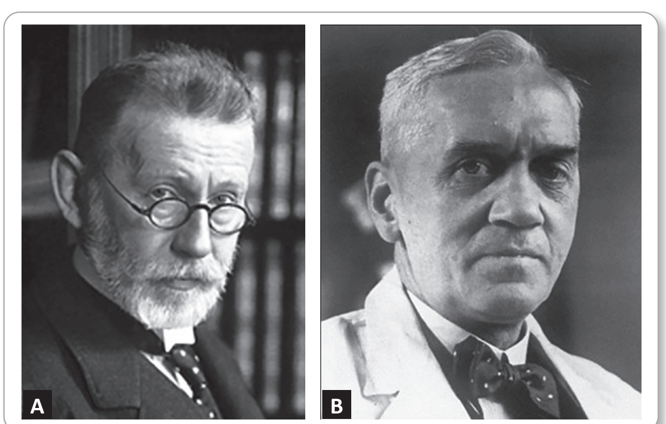
- Knowledge of microbiological aspects imparts a nurse with knowledge about *handling a patient* and his/her *clinical samples*, without infecting oneself.
- Water is sufficient to kill microorganisms when boiled to high temperature. This knowledge is applied *to sterilize instruments* used in hospitals like metal instruments, surgical knives, blades, scissors and needles.
- The *use of antiseptics* to minimize septic conditions caused by growth of microorganisms in the wounds.
- Universal hand washing technique followed by all nurses is based on the fundamental principle of microbiology as hand washing reduces/removes the load of microorganisms and prevents transmission of hospital acquired infections too.

- Different *blood groups are identified* in labs by a nurse based on the knowledge of immunology, which is a necessary task before blood transfusion.
- *Hypersensitivity reaction like Mantoux test* is used to check tuberculosis. This test is again based on immunological knowledge.
- The knowledge about *reactions between specific antigens and antibodies* is fundamental for the tests like radioimmunoassay (RIA), complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), immunodiffusion and agglutination reactions. These tests are very useful in *diagnostic labs*.
- The *aseptic procedures* followed in OTs and minor OTs are based on the principles of sterilization— both gaseous and liquid states of sterilizing agents are used in this process.
- In a situation when a patient's body does not respond to antibiotic treatment, antibiotic sensitivity test is done on the isolated organism from the infected site. It helps in selection of targeted treatment.
- The *mass immunization programs* are based on the knowledge of microbiology and immunology. These immunization derives have helped in eradicating dreadful diseases like smallpox and providing protection for diseases like mumps, hepatitis and diphtheria, etc.
- *Biomedical waste management* is necessary part of the hospital. Collection, segregation, transportation and disposal of biomedical waste are based on the principles of microbiology.

ANTIMICROBIAL THERAPY

Paul Ehrlich

Paul Ehrlich (Fig. 1.12A) was one of the earliest pioneers in the field of antimicrobial therapy. He along with Sahachiro Hata discovered *Salvarsan to treat syphilis* and *Trypan red dye* for treating African sleeping sickness. This was the foundation of chemotherapy. He also developed a method to stain the tubercle bacilli—the causative agent of tuberculosis.



Figs 1.12A and B: A. Paul Ehrlich; **B.** Alexander Fleming

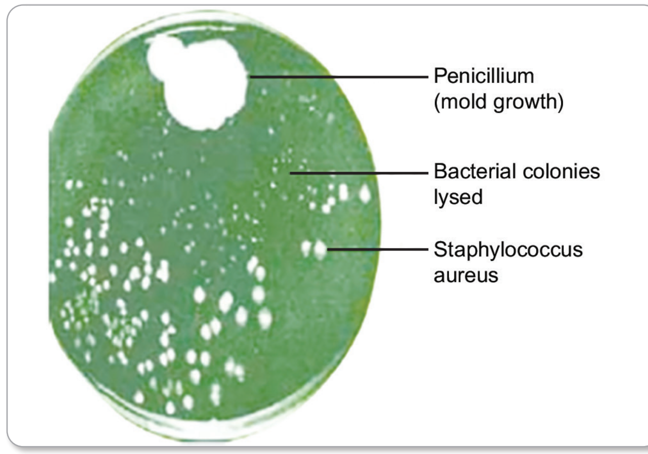


Fig. 1.13: Action of penicillin—Original culture plate of Alexander Fleming

Alexander Fleming

Sir Alexander Fleming (Fig. 1.12B) discovered the wonder drug Penicillin from mold *Penicillium notatum* in 1929. The discovery was accidental. He found that the fungus *Penicillium* produced a substance that destroyed bacterial growth of *Staphylococcus*. He named it as penicillin.

He also showed that the culture filtrate of mold inhibited the growth of *Staphylococcus aureus*. He called this substance penicillin (Fig. 1.13), which acted on Gram-positive bacteria.

For the discovery of this antibiotic, Fleming, Florey and Chain got Nobel Prize in 1945. Penicillin eventually came into use during World War II as a result of the work of a team of scientists led by Howard Florey of the University of Oxford.

Selman Abraham Waksman

Waksman and his coworkers isolated Actinomycin in 1940, Streptothricin in 1942, Streptomycin in 1943, and Neomycin in 1949.

Streptomycin is produced by *Streptomyces griseus*. It is a secondary metabolite produced by *Streptomyces griseus* which is not required for its growth but helps it to compete with other bacteria for food and space in the environment. Streptomycin is used in the treatment of tuberculosis.

For commercial purpose *Penicillium chrysogenum* is used in place of *P. notatum* as it gives more yield. This drug was later used in Second World War. The *antibiotic era* began from here. Later *Streptomyces griseus* was obtained from Streptomyces by SA Waksman in 1944 and he received Nobel

Prize for this discovery in 1952. The Streptomycin was used in treatment of tuberculosis.

The *role of opportunistic pathogens* among immunocompromised patients in crowded hospitals was also analyzed and understood in 20th century. Efforts were made to eradicate diseases globally by various eradication programs and one of the dreadful diseases like smallpox was finally eradicated in 1977 worldwide.

Later many new pathogens emerged like: AIDS (1981), Zika virus (2016), Ebola virus (2014), SARS coronavirus and avian influenza virus (1997). Middle East respiratory syndrome coronavirus (MERS-CoV) reported from Oman and Saudi Arabia on 19th December, 2016 was responsible for outbreak. The most newly emerged viruses are COVID-19 and Omicron. Many new pathogens will keep on introducing themselves in future.

VIROLOGY

By the end of nineteenth century, bacterial etiology of a large number of diseases had been confirmed. But there were many diseases, like measles, smallpox, influenza, etc., for which bacterial etiology could not be assigned. The field of virology was introduced with *Pasteur's discovery of rabies*.

Later Ivanovsky (Fig. 1.14A) in 1892 showed that tobacco mosaic disease was caused by applying filtered juice of diseased plants to healthy plants. These filterable but virulent agents that were alive only in a host's body were termed virions (poison).

Beijerinck in 1898 confirmed previous findings and gave the name "virus" for such filterable infectious agents.

The first human disease of viral origin was yellow fever discovered by Sir Walter Reed in 1902.

Goodpasture (Fig. 1.14B) in 1930 developed a technique of virus cultivation in chicken eggs. Much development took place in field of virology with the *discovery of electron microscope by* Ruska (Fig. 1.14C) in 1934. With introduction of tissue culture, cultivation of viruses became easy.

The possibility that a viral infection could lead to cancer was first put forth by Ellerman and Bang in 1908. Later in 1980, when human T cell leukemia was isolated, this theory was proved.

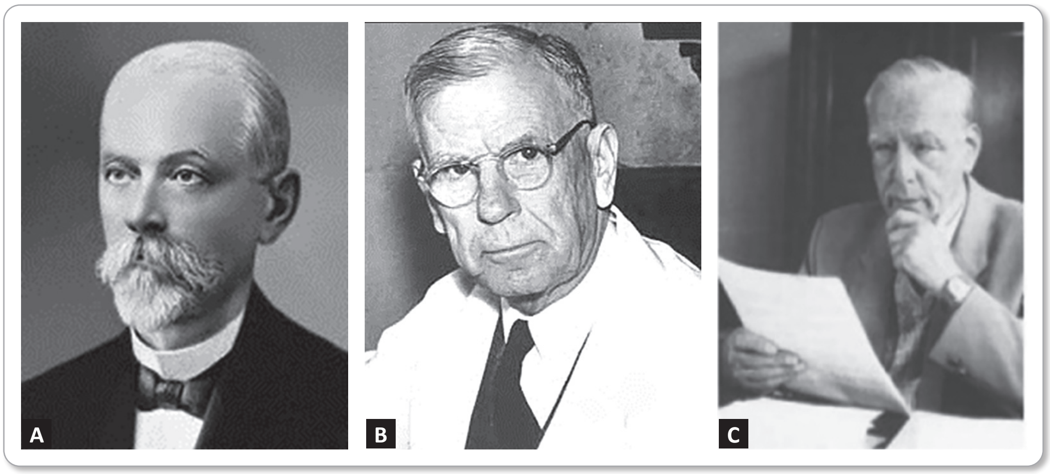
IMMUNOLOGY

The study of host response to infection was also studied in the field of microbiology. In 1888, Nuttall observed that defibrinated blood or serum could kill bacteria and in 1889, Buchner showed that this effect can be removed by heating the sera for 1 hour at 55°C. This factor was given the name of Alexine, which was renamed as complement by Bordet in 1895. Antibody was discovered by von Behring and Kitasato in 1890 (Figs 1.15A and B).

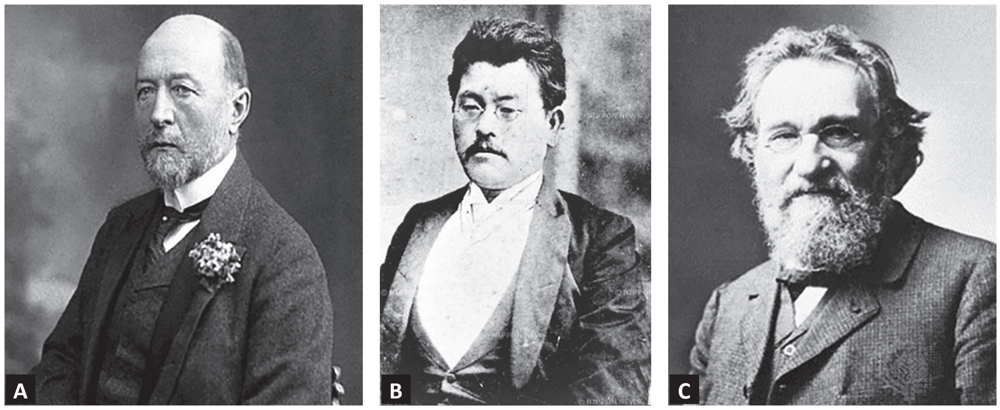
Metchnikoff (Fig. 1.15C) discovered phenomenon of *phagocytosis* and suggested that it was important in the body's defense mechanism against the microbial invasions of tissues. Later on this led to the idea that cells were important in immunity and *cellular concept of immunity* came into existence.

Immunization

An accidental discovery was made by Pasteur when culture of chicken cholera bacillus was left on the bench for several weeks. He found that this culture lost its ability to cause disease but could still protect against subsequent infection by this particular organism. Later on this process was given the name of attenuation and was used to prepare attenuated vaccines in 1881.



Figs 1.14A to C: **A.** Ivanovsky; **B.** Goodpasture; **C.** Ruska



Figs 1.15A to C: **A.** Von Behring; **B.** Kitasato Shibasaburō; **C.** Élie Metchnikoff

Fig. 1.16: Lady Mary Montagu

Lady Mary Montagu (Fig. 1.16) used the artificial induction of immunity by immunizing agents. She used the immunization practice for smallpox in Asia. Material from pustule of an infected person was scratched into the skin of a healthy person to be immunized. It resulted in mild form of smallpox and gave lifelong immunity.

Edward Jenner in 1796 observed that his milkmaid who suffered from cowpox infection was protected against smallpox when they came in contact with smallpox virus. He introduced the term and technique of vaccination (vacca—cow), and used a similar but attenuated organism in healthy individuals. Later on, Pasteur developed attenuated vaccines against chicken cholera and anthrax in 1880 and against rabies in 1885.

SCOPE OF MEDICAL MICROBIOLOGY

Medical microbiology is one of the recent and youngest sciences helpful in the diagnosis of the diseases. The other branches of microbiology like immunology, bacteriology, virology and mycology have already emerged as separate independent sciences. Many microorganisms have been studied in detail and still studies are going on.

Medical microbiology is mainly concerned with etiology, pathogenesis, laboratory diagnosis and treatment of infection along with the epidemiology and control of infection in the community. In order to manage a bacterial infection in a patient, first step is to establish a *clinical diagnosis*. Then the causative agent of the disease is to be isolated from the clinical sample. The antibiotic susceptibility test is determined in laboratory to give specific treatment before giving appropriate antibiotic. The whole therapeutic strategy is monitored. If antibiotic therapy is not successful then antimicrobial agents are observed in body fluids to give target treatment. Confirmation of bacterial cure is indicated by disappearing of signs and symptoms of the disease—known as follow-up.

Mostly the physicians give treatment based on the signs and symptoms of a disease. But there are many diseases which may have same clinical conditions caused by a large number of organisms. In such situations, laboratory is needed so as to diagnose the organism and target the causative microorganism of the disease. Many times even when the clinical symptoms are evident like in urinary tract infections, it becomes difficult to prescribe an appropriate chemotherapeutic agent because a variety of organisms exist which can cause similar infection. In such cases, the laboratory investigation can provide confirmation about the causative organism. On the basis of investigation, it could be decided which drug is to be given. A clinical cure does not always indicate an absolute eradication of infection therefore a lab's help is always needed.

Major discoveries of bacteria and names of the Nobel Prize winners in microbiology are given in Table 1.1 and Table 1.2, respectively.

TABLE 1.1: **Major discoveries of bacteria**

Organism	Discovered by	Year
Mycobacterium leprae	GH Hansen	1861
Bacillus anthracis	Robert Koch	1876
Micrococcus amorphous	Thomas Burrill	1878
Neisseria gonorrhoeae	Albert Neisser	1879
Malarial parasite	CL Laveran	1880
Staphylococcus	Ogston	1881
Pneumococcus	Pasteur and Sternberg	1881
Mycobacterium tuberculosis	Robert Koch	1882
Bacillus of glanders	Loeffler and Schutz	1882
Corynebacterium diphtheriae	Klebs and Loeffler	1883
Vibrio cholerae	Robert Koch	1883
Streptococcus	Fehleisen	1883
Clostridium tetani	Nicolaier	1884
Escherichia coli	Escherich	1885
Neisseria meningitidis	Weichselbaum	1887
Brucella melitensis	Bruce	1887
Tobacco mosaic virus	Ivanovsky	1892
Clostridium perfringens	Welch and Nuttall	1892
Babesia microti	Smith and Kilbourne	1893
Yersinia pestis	Yersin	1894
Spirillum desulfuricans	Beijerinck	1894
Clostridium botulinum	Ermengem	1896
Shigella	Shiga	1896
Malarial parasite	Ronald Ross	1899
Viral origin of yellow fever	Walter Reed	1900
Leishmania donovani	Leishman	1903
Treponema pallidum	Schaudinn and Hoffmann	1905
Causative organism of Rocky Mountain spotted fever	Ricketts	1909
Trypanosoma cruzi	Carlos Chagas	1909

TABLE 1.2: **Nobel Prize winners in microbiology**

Year	Name of the scientist	Contributed to the...
1901	Behring	Diphtheria antitoxins
1902	Ross	Malarial transmission
1905	Koch	Cultured tubercle bacilli
1907	Laveran	Malarial parasite
1908	Ehrlich and Metchnikoff	Theories of immunity and phagocytosis
1945	Fleming, Chain and Florey	Penicillin
1952	Waksman	Streptomycin
1953	Krebs	Krebs cycle
1954	Enders, Weller and Robbins	Cultured polio virus in cell culture
1958	Lederberg, Beadle and Tatum	Genetic control of biochemical reactions
1962	Watson, Crick and Wilkins	Physical structure of DNA
1965	Jacob, Monod and Lwoff	Regulation of protein synthesis
1966	Rous	Cancer-causing viruses
1969	Delbruck, Hershey and Luria	Mechanism of viral infection in bacteria
1972	Edelman and Porter	Nature and structure of antibodies
1980	Paul Berg	DNA recombinant technology
1982	Aaron Klug	Structure of TMV
1987	Susumu Tonegawa	Genetics of antibody production
1989	Michael Bishop and Varmus	Cancer-causing genes
1997	Stanley Prusiner	Prions and relation to neurological diseases
2005	Marshall and Warren	*Helicobacter pylori* causes peptic ulcers
2008	Francoise Sinoussi and Montagnier	Discovery of HIV
2011	Beutler, Jules A Hoffmann and Steinman	Role of dendritic cells in adaptive immunity
2015	William C Campbell and Satoshi Ōmura	A novel therapy against infections caused by roundworm parasites

Contd...

Recent Updates

- Minal Dakhave Bhosale from Pune created India's first coronavirus testing kit in 2020.
- Gita Ramjee, world-renowned virologist from India died after contracting COVID-19, on her return from London in April 2020.
- Dr Krishna Ella from Bharat Biotech is behind the manufacture of COVID-19 vaccine.

Timeline of microbiology developments has been shown in Table 1.3.

TABLE 1.3: Timeline of microbiology developments

Timeline	In Year
Observed "little animals" (Antony Leeuwenhoek)	1677
First scientific Smallpox vaccination (Edward Jenner)	1796
Advocated washing hands to stop the spread of disease (Ignaz Semmelweis)	1850
Disproved spontaneous generation (Louis Pasteur)	1861
Supported Germ Theory of disease (Louis Pasteur)	1862
Practiced antiseptic surgery (Joseph Lister)	1867
First proof of Germ Theory of disease with Bacillus anthracis discovery (Robert Koch)	1876
Growth of bacteria on solid media (Robert Koch)	1881
Outlined Koch's postulates (Robert Koch)	1882
Developed acid-fast stain (Paul Ehrlich)	1882
Developed Gram stain (Christian Gram)	1884
First Rabies vaccination (Louis Pasteur)	1885
Invented Petri dish (JR Petri)	1887
Discovered viruses (Dmitri Iosifovich Ivanovsky)	1892
Recognized viral dependence on cells for reproduction (Martinus Beijerinck)	1899
Proved mosquitoes carried the yellow fever agent (Walter Reed)	1900
Discovered cure for syphilis (Paul Ehrlich)	1910
Discovered Penicillin (Alexander Fleming)	1928
Developed a method to sequence DNA (W Gilbert and F Sanger)	1977
Polymerase Chain Reaction invented (Kary Mullis)	1983
First microbial genomic sequence published (H Haemophilus) (TIGR)	1995

ASSESS YOURSELF

Long Answer Questions

1. Write down the contributions of Koch in microbiology? How has it affected the medical microbiology?
2. Write about the contributions of Louis Pasteur in microbiology.
3. What are the principles of microbiology in nursing?

Short Notes

Write notes on:

1. Pasteurization
2. Tyndallization
3. Fermentation
4. Edward Jenner
5. Robert Koch and his postulates

Multiple Choice Questions

1. What is the name of process used in dairy industry?
   a. Fermentation
   b. Tyndallization
   c. Pasteurization
   d. None of these
2. The process by which a bacterial virulence power is reduced is known as:
   a. Disinfection
   b. Sterilization
   c. Attenuation
   d. None of the above
3. Agar-agar was used in culture media as a solidifying agent by:
   a. Pasteur
   b. Joblot
   c. Neisser
   d. Koch
4. Discoveries about microorganisms blossomed after the introduction of:
   a. Staining techniques
   b. Microscope
   c. Culture media
   d. All of these
5. Pasteur performed swan neck experiment to disapprove:
   a. Theory of spontaneous generation
   b. Germ theory of disease
   c. Both (a) and (b)
   d. None of the above

6. Who gave the terms—aerobic and anaerobic for organisms growing in presence and absence of oxygen, respectively?
   a. Koch b. Leeuwenhoek
   c. Hansen d. Pasteur
7. The vaccine as a term was used by:
   a. Pasteur b. Jenner
   c. Landsteiner d. None of these
8. Discoverer of penicillin was:
   a. Pasteur b. Leeuwenhoek
   c. Fleming d. Jenner
9. Who is called as father of antiseptic surgery:
   a. Pasteur b. Loeffler
   c. Lister d. Fraenkel
10. Who is known as the Father of Microbiology?
    a. Paul Ehrlich b. Joseph Lister
    c. Louis Pasteur d. None of these
11. Who introduced vaccination method for prevention of smallpox?
    a. Edward Jenner
    b. Joseph Lister
    c. Robert Koch
    d. None of the above
12. Who introduced methods of obtaining bacteria in pure cultures using solid media?
    a. Robert Koch b. Louis Pasteur
    c. Joseph Lister d. Paul Ehrlich

Answer Key

Multiple Choice Questions

1. c	**2.** c	**3.** d	**4.** b	**5.** a	**6.** d	**7.** b
8. c	**9.** c	**10.** c	**11.** a	**12.** a		