PART I HISTORY AND SCOPE OF MEDICAL MICROBIOLOGY

1 History of Medical Microbiology

At a very early stage man developed concept that contagious disease was caused by invisible living things.Invention of the microscope proved it to be a reality. Antony van Leeuwenhoek (1632-1723) designed a single lens microscope and demonstrated the little agents of disease, which he designated as *animalcules*. These animalcules are now well established entities belonging to bacteria, viruses and several other pathogens. The organisms being invisible to naked eye are known as microorganisms.

For many years it was believed that the micro-organisms arose from dead, especially decomposed organic matter. This was known as *theory of spontaneous generation*.

CONTRIBUTIONS OF LOUIS PASTEUR

Louis Pasteur (1822-1895), a French chemist generated strong evidence to show that the microorganisms did not rise *de novo* or spontaneously in the media but were introduced from without. Pasteur showed that these organisms were maximum in the dusty air of towns and minimum in air of hilly areas where human habitation did not exist.

Pasteur also showed that microorganisms were inactivated by

- boiling
- at 120°C under pressure (autoclaved)
- at 170°C (hot air oven)

Pasteur's demonstration of airborne microbes prompted Joseph Lister's (1827-1912) work on wound sepsis. He introduced the practice of protecting the wounds from airborne microbes by applying antiseptic dressing and making medical and paramedical workers wash their hands with antiseptic solution before they touched any exposed part of a patient. He achieved strikingly successful results and brought down tremendously the mortality due to sepsis.

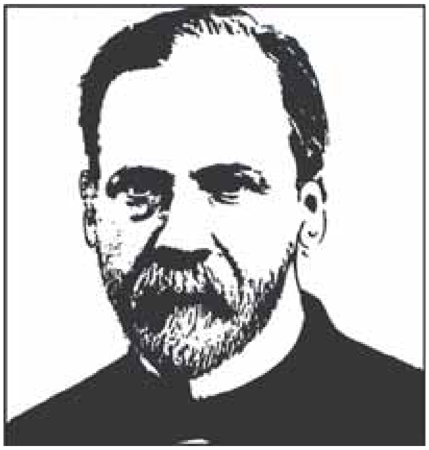

Fig. 1–1. Louis Pasteur—father of microbiology

Pasteur's achievements in the field of sterilization were closely followed by many other workers.Tyndall introduced the method of sterilization by repeated heating with appropriate intervals for germination of spores between them and their subsequent destruction. The method is known as *tyndallization* and practiced even today.

Pasteur developed vaccine against chicken cholera and anthrax by using attenuated suspension of bacteria.

In order to show that the process was akin to Jenner's use of cowpox, he referred to this as vaccination. These attenuated organisms, on injection into animals, protected them from the effects of virulent bacteria. Soon anthrax immunization was widely practised with an enormous reduction in mortality amongst sheep.

However, his epoch making discovery was development of rabies vaccine from the spinal cord of rabbits. It has been responsible for saving innumerable human lives consequent to bites by rabid animals.

The discoveries made by Pasteur can be summarised as follows

CONTRIBUTIONS OF LOUIS PASTEUR

- Microbial theory of fermentation and proving that all forms of life including microorganisms arise from their like and not spontaneously
  - Principles and practice of sterilization
- Development of initial bacteriological techniques
  - Control of diseases of silkworms
  - Development of vaccines against:
    — Anthrax
    — Chicken cholera
    — Rabies

CONTRIBUTIONS OF ROBERT KOCH

Robert Koch's first contribution to science was demonstration of the character and mode of growth of causative bacillus of anthrax.

In 1882, Koch discovered tubercle bacillus and in 1883 the cholera vibrio. For his manifold discoveries in bacteriology, Koch is considered as father of bacteriology.

Koch's Postulates

To confirm the claim that a microorganism isolated from a disease was indeed the cause of this, Koch postulated a set of criteria. According to these postulates, a microorganism can be accepted as a causative agent of an infectious disease only if the followings are satisfied:

A. The isolate should be found in every case of the disease and under conditions which explain the pathological changes and clinical features
B. It should be possible to isolate the causative agent in pure culture from the lesion
C. When such pure culture is inoculated into appropriate laboratory animal, the lesion of the disease should be reproduced

Fig. 1–2. Robert Koch—father of bacteriology

D. It should be possible to reisolate the causative agent in pure culture from the lesion produced in the experimental animal (Fig. 1.3).

Subsequently another criterion has been introduced which demands that specific antigens or antibodies to the bacterium should be detectable in the serum of the patient suffering from the disease.

Exceptions to Koch's Postulates

Some of the exceptions of these postulates are:

a. Inability to grow *Treponema pallidum* and *Mycobacterium leprae*-known causative agents of syphilis and leprosy respectively on artificial media
b. Inability to grow many viruses and rickettsial pathogens on artificial media

The lifelong achievements of Robert Koch are summarised as under:

CONTRIBUTIONS OF ROBERT KOCH

Verification of germ theory of diseases

- Introduction of staining techniques for visualization of microorganisms
- Discovery and use of solid medium in bacteriology
- Discovery of causative agents of:
  — Tuberculosis
  — Cholera
  — Anthrax
- Koch's postulates.
- Use of laboratory animals for experimental infections

During the last quarter of the nineteenth century, succession of discoveries were reported which had bearing on the relation of bacteria to human and animal diseases. Table 1.1 shows some important discoveries.

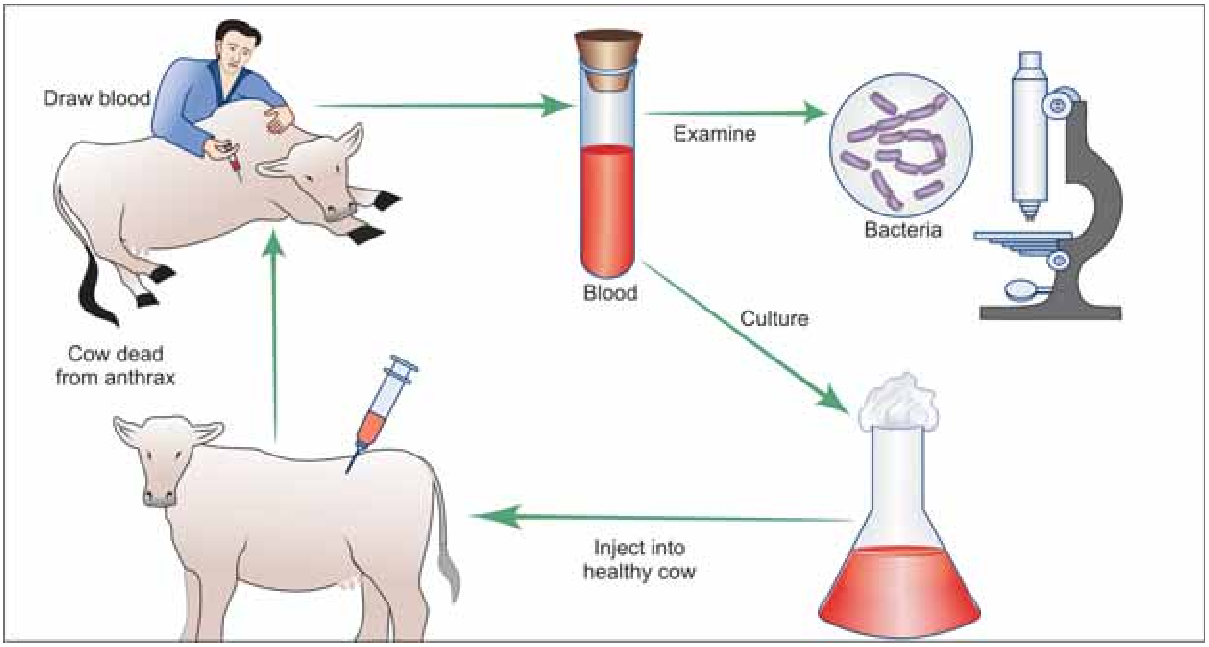

Fig. 1–3. Koch's postulates

Table 1–1. Discoveries of bacteria in 19th Century

Year	Organism	Discovered by
1874	*Mycobacterium leprae*	Hansen
1879	*Neisseria gonorrhoeae*	Neisser
1880	*Salmonella typhi*	Eberth
1881	*Staphylococcus*	Ogston
1881	*Pneumococcus*	Pasteur and Sternberg
1882	*M. tuberculosis*	Robert Koch
1882	*Bacillus glanders*	Loeffler and Shutz
1883	*Vibrio cholerae*	Robert Koch
1883	*Streptococcus*	Fehleisen
1884	*C. diphtheriae*	Loeffler
1885	*Clostridium tetani*	Nicolaier
1887	*Neisseria meningitidis*	Weichselbaum
1887	*Brucella melitensis*	Bruce
1892	*Haemophilus influenzae*	Pfeiffer
1892	*Clostridium welchii*	Welch and Nuttall
1894	*Yersinia pestis*	Yersin and Kitasato
1896	*Clostridium botulinum*	Ermengem
1896	*Shigella*	Shiga

ANTIMICROBIAL AGENTS

With the identification and confirmation of bacteria as causative agents of human diseases, efforts were made to develop effective agents which could kill bacteria in the body of the man without damaging the host tissue. Pioneer work was done by Paul Ehrlich (Fig. 1.4) who is justly described as the father of chemotherapy. In the 1900s he cured one form of trypanosomiasis in rats with the dye trypan red and another form in mice with an organic arsenic compound, atoxyl.

In 1910, Ehrlich successfully treated syphilis using compound '606'' (dioxydiaminoarsenobenzol dihydrochloride) and called it Salvarsan. Fleming discovered penicillin and Waksman streptomycin. Subsequently, several fungi have been used as source of antimicrobial substances.

Fig. 1–4. Paul Ehrlich—father of chemotherapy

CONTRIBUTIONS OF PAUL EHRICH

- Discovery of salvarsan as a chemotheraputic agent against syphilis. This opened the new field of antimicrobial agents which has saved the lives of millions of people till date
- Identified that mycobateria have acid fastness nature
- Proposed a theory for the production of antibody called as "side chain theory"
- Refined the science of staining the organisms
- Advocated standardization of biologicals including toxins and antitoxins to ensure uniformity

DISCOVERY OF VIRUSES

By the end of nineteenth century many infectious diseases had been proven to have a bacterial aetiology. The trend continued in twentieth century. But yet there remained many diseases of common occurrence for which no bacterium could be demonstrated. These included smallpox, chickenpox, measles and common cold. Advent of electron microscopy in 1934 by Ruska made morphological examination of viruses possible. The first human disease proven to have a virus aetiology was yellow fever.

Table 1–2. Nobel laureates in microbiology and immunology

Year	*Nobel Laureate*	*Contribution in/discovery of*
1901	Von Behring	Diphtheria antitoxin
1902	Sir Ronald Ross	Malaria
1905	Robert Koch	Tuberculosis
1908	Paul Ehrlich	Theories on immunity
1908	Metchnikoff	Phagocytosis
1913	Richet	Anaphylaxis
1919	Bordet	Immunity
1928	Nicolle	Typhus
1930	Landsteiner	Blood group
1939	Domagk	Prontosil as antibacterial
1945	Fleming, Chain & Florey	Penicillin
1951	Marx Theiler	Yellow fever
1952	Waksman	Streptomycin
1954	Enders, Weller and Robbins	Poliomyelitis
1958	Beadle, Tatum and Lederberg	Bacterial genetics
1960	Burnet and Medawar	Immunological tolerance
1962	Watson, Crick and Wilkins	Structure of DNA
1965	Jacob, Monod and Lwoff	Protein synthesis in bacteria
1968	Holley, Khorana and Nirenberg	Genetic code
1969	Delbruck, Hershey and Luria	Mechanism of viral infection of bacteria
1972	Edelman and Porter	Nature and structure of antibody
1975	Dulbecco, Temin and Baltimore	Reverse transcriptase and causation of cancer
1976	Blumberg and Gajdusek	Chronic viral infections and cancers
1977	Yalow	Radioimmunoassays
1978	Nathans, Smith and Arber	Restriction enzymes
1980	Benacerraf,Snell and Dausset	MHC genes and transplantation
1983	Barbara McClintok	Transposons
1984	Milstein,Kohler and Jerne	Monoclonal antibody
1987	Susumu Tonegawa	Genetics of antibody production
1989	Bishop and Varmus	Oncogenes
1990	Murray and Thomas	Use of immunosuppressive drugs in transplantation
1993	Sharp and Roberts	Gene splicing
1996	Doherty and Zinkernagel	Recognition of viruses by immune system
1997	Prusiner	Prions
1999	Gunter Blobel	Intrinsic signals in proteins
2001	Hartwell, Hunt and Nurse	Key regulators of cell cycle
2002	Brenner, Horvitz and Sulston	Genetic regulation of organ development and cell death

Table 1-3. Recently recognized pathogenic microbes and infectious diseases

Year	*Microbe*	*Type*	*Disease*
1973	Rotavirus	Virus	Major cause of infantile diarrhoea worldwide
1975	Parvovirus B-19	Virus	Aplastic crisis in chronic heamolytic anaemia
1976	*Cryptosporidium parvum*	Parasite	Acute and chronic diarrhoea
1977	Ebola virus	Virus	Ebola haemorrhagic fever
1977	*Legionella pneumophila*	Bacteria	Legionnaires disease
1977	Hantaan Virus	Virus	Haemorrhagic fever with renal syndrome (HRFS)
1977	*Campylobacter jejuni*	Bacteria	Enteric pathogen distributed globally
1980	Human T- lymphotropic virus I (HTLV-1)	Virus	T-cell lymphoma -leukaemia
1981	Toxin producing strains of *Staphylococcus aureus*	Bacteria	Toxic Shock Syndrome
1982	*Escherichia coli* O157:H7	Bacteria	Haemorrhagic colitis; heamolytic Ureamic syndrome
1982	HTLV-II	Virus	Hairy cell leukaemia
1982	*Borrelia burgdorferi*	Bacteria	Lyme disease
1983	HIV	Virus	AIDS
1983	*Helicobacter pylori*	Bacteria	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhoea
1986	*Cyclospora cayatanensis*	Parasite	Persistent diarrhoea
1988	Human herpes virus-6 (HHV-6)	Virus	Roseola subitum
1988	Hepatitis E Virus	Virus	Enterically transmitted hepatitis
1989	*Ehrilichia chafeensis*	Bacteria	Human ehlichiosis
1989	Hepatitis C Virus	Virus	Parenterally transmitted liver infection
1991	Guanarito virus	Virus	Venezuelan haemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjuctivitis ;disseminated disease
1991	New sps. of *Babesia*	Parasite	Atypical babesiosis
1992	*Vibrio cholerae* O139	Bacteria	New strain associated with epidemic cholera
1992	*Bartonella henselae*	Bacteria	Cat –scratch disease;bacillary angiomatosis
1993	Sin nombre virus	Virus	Adult respiratory distress syndrome
1993	*Encephalitozoon cunculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian haemorrhagic fever
1995	HHV-8	Virus	Associated with Kaposi sarcoma in AIDS patients
1999	Nipah	Virus	Encephalitis
2002	SARS CoVirus	Virus	SARS
2004	Influenza virus H5N1	Virus	Avian influenza

In 1930s viruses could be grown in bacteria free, living chick embryo—a technique perfected by Goodpasteur. By 1940, growth in tissue culture of susceptible mammalian cells was established. The availability of well defined cell lines have now replaced tedious methods of growing viruses in the living animals.

NOBEL LAUREATES

A number of Nobel laureates in Medicine and Physiology were awarded this prize for their work in Microbiology and Immunology (Table 1.2).

Discovery of New Organisms

The discovery of new microorganisms is a continuous phenomenon. A large number of new organisms have been discovered in recent past (Table 1.3). Some of these have acquired considerable importance because of the mortality and morbidity caused by them (HIV, hepatitis B, hepatitis C) and others have the capability to cause international scare (SARS CoVirus)