

# Introduction to Medical Microbiology

## Microbiology generally

- the study of microbes or micro-organisms
  - small living things
  - majority require magnification to enable visualization
    - diameter may be less than 0.1 mm

## General classification of micro-organisms

- broadly based on cellular structure
  1. **eukaryotic organisms**
    - consist of well developed cellular structures with a nucleus nucleolus and nuclear membrane
    - include protozoa and fungi
  2. **prokaryotic organisms**
    - relatively undeveloped or primitive cells
    - unicellular approximately  $1\ \mu\text{m}$  diameter
    - no nuclear membrane or proper nucleus
    - includes bacteria

## Medical microbiology

- study of micro-organisms or microbes which infect humans
  - the micro-organisms are grouped as
    - a. bacteria
    - b. fungi
    - c. viruses
    - d. parasites
  - each group constitutes a specific discipline of study

## Medical bacteriology

- developed over centuries as a result of research by several scientists from different parts of the world
- important steps in early development include
  1. biblical information on some diseases which could spread from person to person
  2. ideas of infection and epidemics recorded by Hippocrates in the 4<sup>th</sup> century B.C.
  3. evidence of micro-organisms as causative agents of disease and modes of transmission described in a book titled De Contagion published in 1546

4. invention of the microscope by Leeuwenhoek in 1675

- enabled

- a. observation of objects too small to be seen without magnification

- b. recognition of the small objects as living creatures some of which were motile

## **Establishment of the discipline of bacteriology formally in the 18<sup>th</sup> and 19<sup>th</sup> centuries**

- was based on recognition of contributions from different workers including
  1. demonstration of sexual transmission of micro-organisms and associated disease
  2. concept of vaccination in prevention of smallpox
  3. cholera outbreak linked to lack of clean water
  4. establishment of attenuated organisms by artificial culture of some microbes which cause disease and development of vaccine against anthrax for animals using the principle
  5. prevention of wound sepsis by applying antiseptic techniques and use of carbolic acid

6. establishment of techniques required to isolate and propagate bacteria by Dr. R. Koch
  - medical doctor referred to as **Great founding father of Medical Microbiology**
    - a. established the bacterial causative agents of **anthrax tuberculosis and cholera**
    - b. used agar to grow bacteria in pure cultures
    - c. formulated proposals associating specific micro-organisms with causation of specific diseases
  - proposals led to principles referred to as **Koch's postulates**
    - used to substantiate claims that a particular organism caused a specific illness

## **Koch's postulates**

- for a particular organism to be a causative agent of a disease
  1. the organism must be demonstrated from the lesions in every case of the disease
  2. the organism must be isolated and propagated in pure culture in vitro
  3. inoculation of a pure culture of the organism by a suitable route into a suitable animal should reproduce the disease
  4. the organism must be re-isolated from the animal

- organisms which fulfilled Koch's postulates were referred to as pathogens to distinguish them from the non-pathogens
- however
  - other several human bacterial pathogens have been identified many years after the postulates
  - many diseases caused by clearly identified organisms do not fulfill one or two or three or all of the above postulates

7. discovery and introduction of Gram's stain

## Development of some antimicrobial agents in the 20<sup>th</sup> century

1. synthetic arsenical compound was developed and used for treatment of syphilis
2. discovery and development of penicillin
3. discovery and development of sulphonamides

## Further advances in technology in relation to micro-organisms

- allowed more and better understanding of the nature and functions of micro-organisms
- enabled revelation of the structure of DNA which led to further development in molecular biology and its analytical applications

- discoveries based on molecular biology include
  1. cellular forms of life can be classified or identified according to the DNA and other genetic components
  2. molecular basis of pathogenesis of infection
  3. development of molecular techniques in the diagnosis of infections
    - techniques include polymerase chain reaction (PCR)
      - a sensitive method of detection and amplification of specific DNA or RNA sequences
  4. development of molecular techniques in detection of bacterial resistance to antibiotics

## Methods of study in medical bacteriology

### 1. Microscopy

- involves the use of various types of microscopes for visualization of micro-organisms
- a. commonly used type of light microscope for
  - general observation of shape size and arrangement of stained bacteria
  - examination of unstained organisms for various characteristics such as motility
  - examination of tissues and other specimens for micro-organisms and associated abnormalities

**b. special microscopes**

- for visualization of finer structures or very slender bacteria include

**1. phase contrast microscope**

**2. dark field microscope**

- 1) and 2) are fitted with special condensers to enable visualization of refractive and slender unstained organisms

**3. fluorescence microscope**

- utilizes fluorescent light and fluorescence staining methods of micro-organisms

**4. electron microscopes**

- for higher resolutions of fine structures of micro-organisms

## 2. **Staining of bacteria and other materials**

- **unstained bacteria are refractive or translucent**
- **staining**
  - a. **imparts colour and makes bacteria more visible**
  - b. **enables differentiation of special features of each bacterial cell**

### **staining techniques**

#### **a. simple staining**

- **utilizes one type of stain**

#### **b. negative staining**

- **stains the background and enables certain features to be visualized clearly against stained background**

c. **differential staining**                      **various methods**

- utilize more than one reagent
- depends on permeability of the cell wall and entry of the stain into the cell
- involves more complex procedures and reactions of the reagents and cell contents
- several methods based on the type of organism under study include
  - Gram's stain
  - Ziehl-Neelsen staining
  - spore stain
  - various staining methods for specific bacterial cell structures such as  
   flagella                      DNA                      capsules

## a. Fluorescence staining or Immunofluorescence staining

- used for examination of specimens or identification of organisms by
  - a. staining with fluorescent materials or stains bound chemically with specific proteins including antibodies against antigens on a particular organism
  - b. examination by fluorescence microscope and ultra-violet light

### 3. **Culture or growth or isolation of bacteria**

- involves growing the **desired bacteria in artificial media**
- **bacteria differ in their physiological and nutritional requirements for growth**
  - **suitable nutritional and other growth requirements must be provided for successful isolation of each organism in**
    - a. **artificial media**
    - b. **atmospheric growth environment**
    - c. **duration and temperature of incubation**

## artificial culture media

- semi-solid or liquid substances prepared and dispensed into petri-dish or bottle
  1. basic contents are water nutrients and agar
    - agar is a substance extracted from sea-weed
  2. other substances depending on intended use
- bacteria divide numerous times and form visible growth as
  - a. turbidity in a liquid medium
  - b. multiple heaps of cells on the surface of semi-solid media referred to as colonies
    - each colony
      1. arises from a single bacterial cell
      2. has specific features for the type of bacterium on each medium