Chapter -4
A. Introduction to Microbiology:
B. Epidemiology:

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MICROBIOLOGY :

- The study of unicellular or cell-cluster microorganisms is termed as microbiology.
- The term microbiology was introduced by Louis Pasteur (French chemist) who demonstrated that fermentation was caused by the growth of yeasts.
- Microbiology is the study of microscopic organisms, including bacteria, viruses, fungi, protozoa, and algae.
- These microorganisms are found everywhere, from the human body to extreme environments like hot springs and deep-sea vents.
- Microbiology helps us understand how these tiny life forms interact with humans, animals, plants, and the environment.

Scope of Microbiology:

• **Production of Antibiotics-**

Micro-organism are directly used in pharmaceutical industry for the production of antibiotics by isolating antibiotic producing micro-organism from nature.

• **Diagnosis of disease and treatment E.g.** :- Widal test, Elisa test etc.

• **Sterilization-**

This process involves killing of microbes by different means to make the pharmaceutical product free from microorganism.

• **Testing of Pharmaceuticals**

Endotoxin testing $\frac{35}{17}$ Microbiological assays of antibiotics $\frac{35}{17}$ Antimicrobial preservation efficacy testing.

❑ Common micro-organism Of Microbiology

- Microbiology includes a larger and diverse group of microscopic organisms that exist as a single cell of bacteria, archaea, fungi algae, protozoa and helminths the viruses.

1.   Moulds:

- Mucor, Rhizopus, Botrytis, Aspergillus, Penicillium etc is moulds deteriorate the food.
- The manufacturing of certain food and other related Substance some specific species of mould are used mould ripened cheese, production of oriental foods (Soy, Sauce, etc.

2. Yeast:

- They are mostly non-filamentous & reproduce by fission or budding.
- They maybe both harmful as well useful for food in food industry.
- Example:- Yeast is used in food industry are Saccharomyces, Schizosaccharomyces, Candida etc.

3. Viruses

- Acellular infectious agents that require a host cell to replicate.
- Cause diseases such as the flu (influenza virus), COVID-19 (SARS-CoV-2), and HIV/AIDS.
- Some viruses can be beneficial in gene therapy and biotechnology.

4. Fungi

- Eukaryotic organisms that include yeasts, molds, and mushrooms.
- Some fungi are beneficial, like *Saccharomyces cerevisiae* (used in bread-making), while others cause infections like *Candida* (yeast infections).

5. Bacteria

- Unicellular prokaryotic organisms. Found in soil, water, air, and inside living organisms. Some are beneficial (e.g., Lactobacillus in yogurt), while others cause diseases (e.g., Escherichia coli, Salmonella).

6. Amylases:- These are used in the preparation of starch hydrolysates used in various product formations. Example:- Beer, Vinegar etc.

7. Algae

- Photosynthetic organisms found in water bodies.

- Provide oxygen and serve as food sources for aquatic life. Some algae (e.g., Spirulina) are used as health supplements.

❏ Introduction to Epidemiology:

- **Introduction:** It is the branch of medical sciences that investigates all the factors that determine the presence or absence of diseases and disorders.

- Epidemiology is the study of how diseases spread, their causes, and how they can be controlled in populations. It focuses on identifying patterns, risk factors, and methods to prevent or manage health issues in communities.

- Epidemiology literally meaning the study of what is upon the people is derived from Greek word.

- **Epi-** upon,

- **among demos** - people, district

- **logos** - study

Application of Epidemiology:

- To evaluate health services

- To diagnose the health of the community.

- To identify the syndrome.

- To search for cause of disease.

- Planning and Evaluation

- Elucidate mechanism of disease transmission

- To find causation of the disease.

- Determine the mode of transmission.

Define the term Epidemiology. Enlist and explain different types of Epidemiology? (Definition - 1M; Enlisting Types – 1M; Explanation - 3M (1M each)

- Definition:

- Epidemiology is the study of the distribution and determinants of health-related events and diseases in the population and the application of knowledge to control health problems.

- The different epidemiological methods are as follows:

1. Descriptive epidemiology

2. Analytical epidemiology

3. Experimental epidemiology.

1. Descriptive epidemiology:

- Descriptive studies are concerned with the distribution of disease or health related characteristics in human populations and identifying the characteristics with which the disease in question appears to be associated i.e., Time of the disease occurrence, place where the disease is occurring and who is affected by the disease (population).

- The time distribution means the study of, "when does the disease occur? For example, water borne gastrointestinal infections occur more frequently during summer season. In place distribution we can study the occurrence of diseases in a particular geographical area.

2. The analytical studies:

- These comprise of two distinct types of observational studies.

A. Case control study

B. Cohort study

A. Case Control Study

- Retrospective study : Already available data are scrutinized..

- From effect to the cause

- Studying the disease and see if you can associate risk factors to it.

B. Cohort Study

- Prospective study : Record Developed from the study of population.

- Example: Tracking a group of smokers and non-smokers for 20 years to compare lung cancer rates.

- From cause to the effect

- Studying the risk factor to see if disease is associated with it.

- From both these study designs one can determine whether a statistically significant association exists between, a disease and a suspected factor and if one exists, the strength of association.

3. Experimental epidemiology

- The aims of experimental studies are as follows:

- To provide scientific proof of etiological or risk factors which may permit the modification or control of those diseases.

- To provide a method of measuring the effectiveness and efficiency of health services for the prevention, control and treatment of disease and improve the health of the community.

- The experimental studies can be conducted in animals or human beings.

❑ **Understanding of following terms:**

- **Epidemic: (Rapid mass spread):**

- Epidemic can be defined as sudden increase of a case of disease which is higher than normal in a specific population.

- It is the quick and rapid spread of the number of cases of a disease above what is normally expected in that population in that area.

- Epidemics happen when an agent and susceptible hosts are present in adequate numbers, and the agent can be effectively transmitted from a source to the susceptible host.

- E.g.: Cholera outbreak, diarrhoea outbreak, chicken pox in school going children etc.

- **Pandemic:-** (world widespread):

- It Is defined as the growth rate skyrockets and each day grow more than the day prior.

- The virus has nothing to do with virology, population immunity or disease severity.

- It means a virus covers a wide area, affecting several countries and populations.

- Example: COVID-19 (declared a pandemic in 2020).

* **Endemic:-** (particular area):

 A disease outbreak is endemic when it is consistently present but limited a particular region. The disease spread and rates predictable.

Eg: malaria ,Ebola

Modes of transmission :

An infectious agent may be transmitted from its natural reservoir to a susceptible host in different ways. There are different classifications for modes of transmission. Here is one classification:

▪ **Direct:**   a) Direct contact   b) Droplet spread

▪ **Indirect :** a) Airborne b) Vehicle borne c) Vector borne (mechanical or biologic)

- **Isolation** : separates sick people with a contagious disease from people who are not sick. Separating **infected individuals** from healthy ones to prevent disease spread.

☐ Example: Hospital isolation wards for COVID-19 patients.

- **Quarantine separates** and restricts the movement of people who were exposed to a contagious disease to see if they become sick.

☐ Example: Travelers being quarantined after arriving from high-risk countries during COVID-19.

- **Incubation period:**

- The time elapsed between exposure to a pathogen's organism a chemical or radiation and when symptoms and signs are first apparent.

- The period of time between harmful bacteria or viruses entering a person's and animal body or entering a plant and the effects of a disease.

- **Contact tracing:**

- People in close contact with someone who is infected with a virus, such as the Ebola virus, are at higher risk of becoming infected themselves, and of potentially further infecting others.

- Closely watching these contacts after exposure to an infected person will help the contacts to get care and treatment, and will prevent further transmission of the virus. This monitoring process is called contact tracing.

❑ Differentiate between prokaryotic and eukaryotic microorganisms.

Parameter	Prokaryotic Cells	Eukaryotic Cell
Membrane bound organelles	Absent	present
Nucleus	There is the absence of a well-defined nucleus. Furthermore, there is the presence of nucleoid here which is an open region that contains DNA	There is certainly the presence of a well-defined nucleus here. Furthermore, a nuclear membrane encloses it
Cell size	The cell size is smaller (0.1- 5 μm)	The cell size is larger (10-100 μm) in contrast
Cell structure	Unicellular structure	Most eukaryotic cells are multicellular. However, some are unicellular
DNA Form	They have a circular DNA form	They have a linear DNA form

Complexity	Simpler	More complex in comparison to prokaryotic cells
Cell wall	The cell wall is present here. Furthermore, it comprises of mucopeptide or peptidoglycan	Usually, there is an absence of cell wall here. However, in case it is present, it comprises cellulose
Mitochondria	Absent	Present
Ribosome	70S	80S
Shape of DNA	Double-stranded circular DNA	Double-stranded Linear DNA
Mode of Reproduction	Asexual	Sexual reproduction is certainly the most common here
Endoplasmic Reticulum	Absent	Present
Cell Division	Binary fission, transduction, conjugation, and transformation	Mitosis
Number of chromosomes	Only one	More than one
Replication	These cells have a single origin of replication	These cells have multiple origins of replication
Examples	Archea and bacteria	Fungi, protists, animals

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• Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents.

• The Gram stain procedure distinguishes between Gram positive and Gram-negative groups by colouring these cells red or violet.

• **Procedure:**

1. Place slide with heat fixed smear on staining tray.

2. Gently flood smear with crystal violet and let stand for 1 minute.

3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.

4. Gently flood the smear with Gram's iodine and let stand for 1 minute.

5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.

6. The smear will appear as a purple circle on the slide.

7. Decolorize using 95% ethyl alcohol or acetone.

8. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.

9. Immediately rinse with water.

10. Gently flood with safranin to counterstain and let stand for 45 seconds.

11. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.

12. View the smear using a light-microscope under oil-immersion.

Observation:

• **Gram-Positive:** Bacteria which retain the crystal violet dye and appear deep violet color.

• **Gram-Negative:** Bacteria which loose violet when washed with alcohol and are stained with red dye safranine and appear red.

❏ Acid Fast Staining Method /Ziehl-Neelsen Method:

- **Acid Fast Microorganisms :**
- *Mycobacterium tuberculosis.*
- *Mycobacterium leprae*
- **Ziehi-Neelsen's Staining Procedure :**

  Scientist Ziehl and Neelsen discovered this method. It is Important for separation of 'Mycobacteria Group'. The method is commonly used for a diagnostic aid and identification species.

Procedure of Staining:

- A smear is prepared from the given tubercule sputum and fixed on slide by heat.
- On the smear a Ziehl-Neelsen carbolfuchsin stain is added and allowed to react for 10 min.

- *Precautions* were taken to avoid boiling of stain.
- Wash the smear with water.
- Add 20% H_2SO_4 for one minute and wash it with water.

- Add methylene blue or malachite green for 30 sec.

- Wash the slide and dry and observe under oil immersion lens.

➤**Observation:**

- Cell those appear pinkish red are acid fast cell/bacteria .

- Cell those appear blue/green are non-acid-fast bacteria.

Structure of bacterial Cell
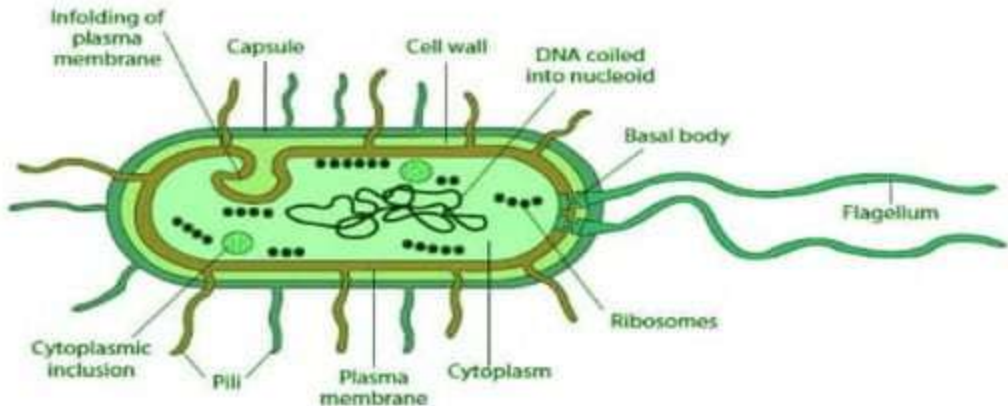
Generalized Structure of a bacterium

II. Cytoplasmic membrane- The most important function of cytoplasmic membrane of bacteria is to form a permeable barrier, regulating the passage of solutes between the cell and the outer environment.

ii. Flagella- It helps an organism in movement. They act as sensory organs to detect temperature and pH changes.

iii. Capsule- It protects bacteria from toxic compounds and desiccation and allowing them to adhere to surfaces and to escape the immune system of the host.

iv. Cell wall- It gives the cell its shape and surrounds the plasma/cytoplasmic membrane, protecting it from the environment.

v. Plasmid: Plasmids are small, circular fragments of DNA that are found in the cytoplasm of bacterial cells. They act as delivery vehicles, or vectors, to introduce foreign DNA into bacteria.

❑ Classify bacteria depending upon their shape.

• Bacteria depending upon their shape are classified as-

   (i) Cocci- Bacteria spherical or round in shape

   (ii) Bacilli- Rod shaped bacteria

   (iii) Spirilla – Rigid spiral or spring shaped bacteria

   (iv) Vibrios- Comma shaped bacteria

   (v) Actinomycetes – Branching filamentous bacteria

   (vi) Mycoplasmas – Round or oval bodies as they lack cell wall. So, shape is not
   fixed.

□ **Nosocomial infections. Write prevention and control of Nosocomial Infections.**

- **Nosocomial Infections or Hospital Acquired infections:**

- Hospital acquired or nosocomial infections are the infections acquired by the patients after they have been admitted to the hospital and prior to the hospital admission, the patient do not have the said infection.

- Common nosocomial infections include infections of urinary tract, respiratory tract, alimentary tracts, wound infections, skin infection, septicaemia etc.

❑ Types of Nosocomial Infections

1. **Urinary Tract Infections (UTIs)** – Related to catheter use (CAUTI).

2. **Surgical Site Infections (SSIs)** – Common after surgery

   (*Staphylococcus aureus*).

3. **Pneumonia** – Especially **ventilator-associated pneumonia (VAP)**.

4. **Bloodstream Infections (BSIs)** – Often linked to central lines (CLABSI).

5. **Gastrointestinal Infections** – *Clostridioides difficile* (C. diff) infections.

- **Sources / Causes of Nosocomial Infection :**

- Staff attending the patients such as sweepers, nurses, doctors, suffering from infection.

- Due to objects like surgical instruments, dressing etc.

- Due to infected syringe and needle.

- Examples : Hepatitis B, HIV infections, UT infections , Wound Infections , Tuberculosis, Pneumonia.

➤ Control and Prevention:

• To achieve this, a committee needs to be appointed in the hospital and they need to monitor following aspects on regular basis

   i) Cleanliness in the hospital

   ii) Proper sterilization of instruments and maintaining aseptic conditions

      wherever required

   iii) Controlling overuse of antibiotics

   iv) Maintaining Health and hygiene of hospital staff

   v) Avoiding water, food contamination

   vi) Proper isolation of infectious patients