VISION OF THE FACULTY OF DENTISTRY

October 6 University, Faculty of Oral and Dental Medicine educates diverse student body through a challenging learning environment informed by cutting edge scholarships. We fully embrace the social responsibility and public trust of our role as a leader in education and clinical services. In order to accomplish our vision, we promote diversity in thinking and human resources, support creative breakthroughs and commit to ethical and responsible leadership in patient care and scholarships.

MISSION OF THE FACULTY OF DENTISTRY

The mission of the Faculty of Dentistry is to prepare graduates able to provide competent dental care through advanced education and research activities. The Faculty aims at improving community oral health by partnering with students and achieving academic and clinical excellence in the art and science of dentistry.
Preface

This course aims to provide a comprehensive theoretical knowledge of Bacterial cell structure, growth and nutrition. Antibiotics, sterilization and disinfection as well as the fundamental elements of immunology. Also the fundamental types of microorganisms, gram positive & gram negative, diseases (bacterial, viral and fungal). Helps understanding the Infectious diseases, treatment for bacterial infection and how to deal with drug interaction., demonstrate advanced knowledge of nature of viruses, bacteria and fungi, some aspects of dental microbiology such as Microbial flora of the oral cavity, dental plaque, caries and periodontal diseases, know Differential diagnosis of oral ulcerations.
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Part I

General Bacteriology
An Overview of bacteriology

Microbiology is the science that is primarily concerned with the study of microorganisms that are too small to be seen with the unaided human eye.

Medical Microbiology is the study of the causes and management of infectious diseases. These can be caused by viruses, bacteria, microfungi and protozoa.

1. **Viruses**: obligate intracellular parasites, incapable of independent existence. Their sizes vary from 20 to 400 nm & can be visualized by electron microscope.

2. **Bacteria**: Bacteria are generally simple structures. The bacterial cell lacks a membrane-bound nucleus. Because of this, bacteria are described as prokaryotes.

3. **Fungi**: All fungi are eukaryotic, vary in size from 0.5 to 10 \( \mu \text{m} \) e.g. mushrooms & yeast.

4. **Prions**: infectious agents made of protein (no nucleic acid).

Microorganisms are divided into two groups, based on the presence or absence of a nuclear membrane surrounding the nuclear material:

1. **Prokaryotes**: (pro: primitive and karyon: nucleus):
   E.g.: Bacteria and rickettsiae.

2. **Eukaryotes**: (Eu: true and karyon: nucleus)
   E.g.: fungi, protozoa, human and animal cell.
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<th>Eukaryotes</th>
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<tr>
<td>1</td>
<td>Average cell size</td>
<td>0.5 – 10 Mm</td>
</tr>
<tr>
<td>2</td>
<td>Cell Wall</td>
<td>Present except in mycoplasma</td>
</tr>
<tr>
<td>3</td>
<td>Cytoplasmic membrane</td>
<td>Sterols Absent (except in mycoplasma)</td>
</tr>
<tr>
<td>4</td>
<td>Nuclear membrane</td>
<td>Absent</td>
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<td>5</td>
<td>Nucleoli</td>
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<td>Chromosome</td>
<td>Single</td>
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<td>7</td>
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<td>Sedimentation constant 70 S</td>
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<td>8</td>
<td>Multiplication</td>
<td>Binary fission</td>
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<td>9</td>
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<td>Involves cytoplasmic membrane</td>
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**Comparison between prokaryotes & Eukaryotes**
Prokaryotic and Eukaryotic Cells

**Bacterial Identification:**

**SHAPE & ARRANGEMENT:**

- **Cocci:** These are spherical in shape, may be arranged in pairs (diplococci), in chains of variable length e.g. streptococci, in fours (tetrads) or in groups (e.g. staphylococci)

- **Bacilli:** These are rod shaped bacteria which may be arranged in pairs e.g. TB, in angles e.g. diphtheria, or in groups e.g. Leprosy

- **Spirals:** spirella (rigid), and spirochaetes flexible.

- **Pleomorphic:** variable in shape.
Shape and Arrangement of Bacterial Cells

**Staining:** Bacteria can be stained by

a) **simple stain** e.g. methylene blue, crystal violet or fuchsin. Cells and structures stained with them give the same colour, this stain reveals the characteristic shape, size and arrangement.

b) **Differential stain:** requires more than one dye, distinguish between different types of bacteria, giving them different colours and between structures inside or outside a cell e.g. spores or capsules.

**Gram stain** is the most important differential stain in clinical microbiology. It divides bacteria into two main categories Gram positive (purple in colour) and Gram negative (pink in colour).

**Zehil-Neelsen:** It is used to identify the members of the genus mycobacterium.
**Basic Structure Of Bacterial Cell**

All bacteria have **cytoplasm** containing nuclear material enclosed within a **cytoplasmic membrane** which in turn iscoated with a **cell wall**. Beside these basic components, some bacteria have **capsules, flagella and pilli** (fimbriae). The cytoplasmic membrane, cell wall are termed **cell envelope structures**.

![Bacterial Cell Structure Diagram](image)

**Structure of a Bacterial Cell**

**I-Cell Envelope Structures:**

**A-Cytoplasmic membrane:**

It consists of two phospholipid layers within which are embedded proteins of various sizes and composition. It lacks sterol (except in mycoplasma).

**Function of Cytoplasmic Membrane:**

1. Selective transport to different molecules in & out of the cell.
2. Active transport of ions & nutrients (amino acids, sugars…etc) utilizing special enzymes called permeases.
3. Respiratory activity by oxidative phosphorylation & electron transport.
4. Has a role in DNA replication during cell division:
During the replication process, the cytoplasmic membrane is the anchoring site for the ends of the DNA molecule. These membrane attachment sites are the **mesosomes**.

5. Chemotactic system: by attractance & repellance according to their needs.

**Mesosomes**: are invaginated areas of the cytoplasmic membrane which increase the surface area of the membrane and thus increasing its active transport systems & its permeability. The mesosome is the site of chromosomal attachment during cell division.
**B-Cell Wall:**

This is the outer most covering layer of the bacterial cell. It is rigid to protect the bacterial cell. Its strength is due to mucopeptide layer (peptidoglycan) containing mainly N-acetylglucosamine and N-acetylmuramic acid connected by polypeptide cross links. This peptidoglycan layer differentiates Gram positive and Gram negative bacteria.

**In Gram Positive Bacteria:**

The peptidoglycan layer (N-acetyl glucosamine alternating with N-acetyl muramic acid) makes up a major portion of the cell wall up to 90% responsible for rigidity of the wall.

Teichoic acid (TA) is a thin layer present in cell wall of most Gram positive bacteria.

The TA & cell wall associated proteins are the major surface antigens of Gram +ve bacteria.

**In Gram Negative Bacteria:**

The cell wall is thin consists of a very thin rigid inner part of peptidoglycan which constitutes 5-20% of the cell wall. Outside this peptidoglycan layer is an outer membrane that composed of phospholipids bilayer similar to that of cytoplasmic membrane, lipoproteins, lipopolysaccharides and proteins. Some of these proteins called porins, allow small molecules to pass through the phospholipids bilayer.
**Function Of Cell Wall:**

1. Maintains the characteristic shape of bacteria.
2. It protects the cytoplasmic membrane & the bacterial cell contents from environmental hypotonicity, so protects the cell from bursting in hypotonic solution.
3. Plays a role in cell division.
4. Determine the staining reaction.
5. Target for many antimicrobials.
6. It's antigens (polysaccharide & proteins) are used in laboratory identification.
7. Have special receptors (e.g., Bacteriophages).
8. Supports the cytoplasmic membrane against the high internal osmotic pressure.

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**BACTERIA WITHOUT CELL WALL:**
Failure to synthesize cell wall leads to formation of soft, fragile, poleomorphic bodies called **L-forms**.

**L-forms:** When bacteria are treated with *Penicillin* causes impairment of cell wall synthesis, so it also leads to death of bacteria. Also *Lysozyme* which is found in tears and saliva dissolves this rigid cell wall, producing l-form bacteria. **L-form bacteria** survive antibiotic therapy and their reversion to the walled state produce relapse of infection.

**Mycoplasma**

is a unique group of bacteria in that they are naturally lacking the cell wall and yet are free living

**The Capsule:**

It is an organized accumulation of material (protein or carbohydrate) outside the cell wall.

**Function of capsule:**

1. It has a protective role for bacteria against phagocytosis within the host body.
2. Adherence of bacteria to certain sites e.g. dextrans aiding cariogenic bacteria to establish themselves on the teeth.
3. Important in identification & typing of bacteria e.g. in pneumococci.

**II- External Appendages**

1. **Flagella:**

   Motile bacteria have flagella too small to be detected by light microscope. They are demonstrated clearly by electron microscope. They are filamentous structures which arise in the cytoplasm, & pass through the cell wall. They are protein in nature & antigenic

**The arrangement of these flagella on the bacteria may be:**
- Single, at one pole (monotricate).
- Two flagella, one at each pole (amphitricate).
- A group of flagella at one or both poles (lophotrichate).
- Flagella all around the bacterial cell (peritricate).

2- Fimbria (pili).

These are fine surface appendages, protein in nature usually in gram-negative bacilli, and play an essential role in the adhesion of bacterial cells but have no role in motility. There are two types of pili:

Ordinary pili:

They are antigenic, also called colonization antigens which enable the bacteria to adhere to specific receptors on human cell surface.

Sex pili:

Long hollow special tube which is involved in the transport of DNA between two bacteria. This is called conjugation pili.

III-Cytoplasm & Intracytoplasmic Structures:
**Cytoplasm:**

This is a soft gel, containing a large amount of RNA which is collected into granules called ribosomes responsible for the manufacture of bacterial enzymes & proteins. In certain types of bacteria, the cytoplasm also contains inclusion granules formed of lipid, glycogen or polymetaphosphate as the metachromatic granules present in diphtheria bacilli.

**Nucleus:**

It is composed of DNA in the form of a single circular chromosome carrying several genetic characteristics, and there is no nuclear membrane or nucleoli.

**Spores:**

Under unfavourable conditions, lack of nutrition, change in pH, temperature certain types of bacteria such as Clostridium & Bacillus group develop a highly resistant – phase called spore. The spore cytoplasm is dehydrated, there is low metabolic activity and no growth or multiplication of bacteria. **The sporulation:** is composed of a hard outer wall (cortex) containing the chromosome, the cytoplasm, other material needed for germination and the ribosomes all enclosed with spore coat. When adverse conditions become good, it germinates and gives rise to the vegetative form (active form). The spore may be central, subterminal or terminal in position in bacterial cell. It may be the same diameter as that of the cell or may be larger causing a swelling of the cell. Spores are not stained by gram stain. It can be stained by special spore stain.
**Plasmids:**

These are extrachromosomal DNA molecules. They are capable of autonomous replication and carrying genetic information for several characters e.g. resistance to drugs, toxin, production, and enzyme synthesis…etc. They are transmissible to other bacterial cells.

**Bacterial physiology, Metabolism and Nutrition**

The energy required to carry out the many metabolic activities may be supplied to the cell in basically two different forms:

1- **Phototrophs**:
   Take energy in the form of radiation from the sun and converts it to chemical energy. All phototrophic bacteria is of no medical importance.

2- **Chemotrophs**:
   Take energy from chemical compounds (organic & inorganic molecules). All bacteria of medical importance are chemotrophs. Microorganisms can be separated into two types based on their main source of carbon:-
**a- Autotrophs:**

If the carbon source is simple inorganic substances (e.g. CO2). They are free living –non parasitic of no direct medical importance. The energy needed for their metabolism is obtained from light.

**b-Heterotrophs:**

If the carbon source is obtained from more complex organic forms. They live in or on animal body and are called parasitic bacteria (most bacteria of medical importance). They obtain food from plant or animal sources.

**Gaseous Requirements:**

Oxygen & carbon dioxide influence growth of the organism. **As Regard O2, bacteria may be classified into four types:**

a) **a.Obligate aerobes**: These organisms grow only in presence of free O2. e.g. T.B.

b) **b.Obligate anaerobes**: These organisms cannot grow in presence of oxygen e.g. Clostridium group.

c) **c.Facultative anaerobes**: These organisms can grow either in presence or in absence of oxygen e.g. Staphylococci, E.coli,…etc.

d) **d.Microaerophiles**: these organisms can grow in low oxygen concentration killed by higher concentrations e.g. Vibrio.

**AS regards CO2:**

It is necessary in small amounts, i.e. the amount normally present in air is quite sufficient. However in a few cases, more CO2 is required, e.g. Brucella abortus needs 20% CO2 and N.gonorrhoeae needs 5%CO2.
Physical Requirements Of Bacteria:

a-Temperature:

Multiplication of most bacterial pathogens takes place within a given temperature range (18 – 42°C), outside of which no growth occurs. Optimum temperature is that at which growth of the organism is most rapid (maximum activity) & and usually 37°C. Low temperature is less destructive than higher ones.

b-Hydrogen ion concentration (pH):

The optimum pH for most pathogenic bacteria is around 7.4 – 7.6. A few types of bacteria such as Lactobacilli can grow well at acid pH (3 – 4) While others such as Vibrio cholera can grow well at high alkaline pH ( 8.5 – 9 ).

Bacterial Growth Curve

When Bacteria are inoculated (cultured) into a liquid medium, samples of the culture are taken at regular intervals (starting from the time of initial inoculation), and the number of viable bacterial cells in these samples are blotted against time, typical growth curve will be obtained. The curve may be discussed in terms of 4 phases.

1. Lag Phase :
   - It is the stage of preparation for multiplication of the bacterial inoculum, during which the organism adapt itself by synthesis of new enzymes specific for the new medium.
   - There is no increase in number of bacteria but slight decrease due to death of some inoculated bacteria.
- The length of this phase depends upon the nature of the organism, i.e. E.coli has a short lag phase, while T.B. has a long lag phase.
- Clinically corresponds to incubation period of the disease.

2. **Logarithmic Phase:**
- Rapid cell division (the most active phase).
- The numbers of bacterial cells increase steadily by time.
- Clinically corresponds to signs & symptoms of the disease.

3. **Stationary Phase:**
- Total bacterial count (dead & living) rises slowly.
- Any bacterial multiplication is balanced by an increased death rate and the number of living organisms (viable) remains constant.
- Clinically corresponds to signs & symptoms of the disease.

4. **Decline Phase:**
- The number of living organisms decreases until all are dead and the culture becomes sterile.
- Clinically corresponds to recovery & convalescence stage of the disease
Bacterial Growth Curve
BACTERIAL VIRUSES
(BACTERIOPHAGE)

Bacteriophage (or phage) is virus that parasitizes bacteria, i.e. the bacterial cell serves as a host for the virus.

MORPHOLOGY OF BACTERIOPHAGE

In most cases, the bacteriophage consists of:

(1) A head: containing the nucleic acid core (usually DNA, rarely RNA) surrounded by a protein coat (capsid).

(2) A tail: consists of a hollow core surrounded by a contractile sheath which ends in a base plate to which are attached tail fibers.

REPLICATION OF BACTERIOPHAGE:

Two cycles for phage replication are known:

(A) Lytic (vegetative) Cycle

1- Adsorption:

The phage attaches by its tail to specific receptors on the bacterial cell.
2- **Penetration:**
   The tail sheath contracts and the nucleic acid is injected into the cell.

3- **Eclipse phase:**
   In which the viral nucleic acid direct the host cell metabolism to synthesize new enzymes and proteins required to the phage synthesis.

4- **Intracellular synthesis:** of phage nucleic acids, capsids and tails.

5- **Assembly:** The phage components aggregate to form complete phage particle which mature into a typical infectious phage.

6- **Release:** The bacterial cell bursts liberating a large number of phage particles to infect new cells.
Bacteriophage Replication
(B) Temperate (Lysogenic) Cycle:

In this cycle the phage (temperate phage) does not replicate and lyses the bacteria but the phage DNA is integrated with the bacterial chromosome and divide with it to pass into daughter cells. The integrated phage is called prophage and the bacteria carrying it are called lysogenic bacteria.

THE PRESENCE OF PROPHAGE IN LYSOGENIC BACTERIUM MAKES IT:

1- Immune to infection by another phage.

2- Acquire new properties, e.g. Diphtheria bacilli can produce toxin only when lysogenized.

PRACTICAL USES OF BACTERIOPHAGES:

A) Bacteriophages used as cloning vectors in recombinant DNA technology. A fragment of DNA (foreign gene) is carried on the phage DNA (transduction), as the phage infects a bacterial cell e.g. E.coli, its DNA carrying the foreign gene is incorporated into the bacterial chromosome and the gene is replicated in each cell division.

B) Phage typing: as the phages are used to identify and type bacteria according to the pattern of lysis. In epidemiologic tracing of outbreaks of infection e.g. wound infection or food poisoning.
"Antimicrobial agents"

The substances that can kill or inhibit the growth and multiplication of microorganisms and suitable for systemic use.

Chemotherapeutic agents:

These substances, chemically synthesized and they are used in treatment of infectious diseases.

Antibiotics are antimicrobial substances produced by a living microorganism and are effective against other microorganisms, used in treatment of infectious diseases.

Characteristics of antibiotics:-

- **Bactericidal**: It is a chemotherapeutic that affects bacteria by killing them.

- **Bacteriostatic**: It is a chemotherapeutic that affects bacteria by inhibiting their growth and multiplication.

Spectrum of action:-

**Broad spectrum**: It is the chemotherapeutic that affect wide range of bacteria (both Gram- positive and negative organisms).

**Narrow spectrum**: Effective mainly against only Gram positive or Gram negative organisms.

**Limited spectrum**: Effective only against single organism or disease.
For an antimicrobial drug to be suitable for systemic use, it should have selective toxicity i.e. harmful to the microorganism at the therapeutic doses but harmless to the host.

MECHANISM OF ACTION OF ANTIMICROBIAL AGENTS

1- Inhibition of cell wall synthesis:
   E.g. penicillin, cephalosporins, cycloserine and vancomycin. These drugs inhibit the synthesis of peptidoglycan and internal osmotic pressure will be high so the bacteria burst.

2- Inhibition of cell membrane function:
   E.g. amphotericin B, colistin and polymyxins, these drugs disrupt the functional integrity of the membrane as a result ions escape from the bacteria and it will damage.

3- Inhibition of protein synthesis:
   E.g. chloramphenicol, erythromycin, tetracyclins and aminoglycosides (streptomycin, gentamycin...etc) leads to inhibition of cell growth.

4- Inhibition of nucleic acid synthesis:
   E.g. Quinolones inhibit DNA synthesis.
   Rifampicin inhibits RNA synthesis.

5- Competitive Antagonism:
   E.g. Sulphonamides compete with P-aminobenzoic acid (the essential metabolite of folic acid) which is the precursor to the synthesis of nucleic acids. Sulphonamides are structural analogues to PABA. They enter into the reaction in place of PABA and compete for the active center of the enzyme.
   Nonfunctional analogue of folic acid is formed so nucleic acid synthesis is inhibited.
RESISTANCE TO ANTIMICROBIAL DRUGS

*Its mechanisms are due to:*

1- Microorganisms produce enzymes that destroy the active drug, e.g. penicillin; cephalosporins are destroyed by β-lactamase produced by Staphylococci.

2- Microorganisms develop an alternative pathway to bypass the reaction inhibited by the drug, e.g. bacteria resistant to sulphonamides capable of using preformed folic acid and do not require PABA.

CROSS RESISTANCE

Microorganisms resistant to a certain drug may develop resistance to another drug that is closely related chemically.

COMPLICATION OF CHEMOTHERAPY

(1) **Toxicity:**

Tetracycline, if given to pregnant women or to an infant, cause permanent staining of the teeth. Streptomycin is toxic to eighth cranial nerve causing deafness.

(2) **Allergy:**

Penicillin can cause allergic reaction which varies from simple urticaria to anaphylactic shock.

(3) **Drug resistance:**

Due to abuse of antibiotics, it is recommended to do in vitro sensitivity tests before giving antimicrobials.
(4) **Superinfection:**

Due to elimination of normal flora and flourishing of drug resistant organisms e.g. Staphylococci, leading to staph enterocolitis. Candida leading to oral thrush or vulvovaginitis.

**ANTIBIOTIC COMBINATION**

*Indications:*

- Severely ill patients suspected to have serious microbial disease e.g. staphylococcal and gram negative sepsis in immunocompromized patients.
- To delay the emergence of drug resistance mutants e.g. T.B.
- Mixed infections.

**ANTIMICROBIAL CHEMOPROPHYLAXIS**

*CHEMOPROPHYLAXIS:* Is the administration of antimicrobial drugs to *prevent occurrence of infection by particular organism, e.g.*:

1- Use of penicillin or erythromycin before dental procedures to prevent bacterial endocarditis.

2- Use of long-acting penicillin to prevent recurrent throat infections with Strept.pyogens in rheumatic patients.

3- Use of Rifampicin to contacts of a case of epidemic cerebrospinal meningitis.

4- In surgery: In clean operations there is no need for chemoprophylaxis but in large bowel surgery, war wounds and major cardiac surgery, the use of pre- or post-operative antibiotics is recommended.
Good practice with antibiotics:

- Be sure that the patient actually requires an antibiotic.
- Avoid treating colonized not actually infected patients.
- Don’t change the antibiotic if the condition improving.
- If no response within 72 hours, so, the diagnosis, antibiotic therapy and secondary infection should be reconsidered.
- Give the antibiotic for the minimal length of time being effective. Review the duration therapy after 5 days.
- For surgical prophylaxis start with induction of anesthesia & continue for maximum 24 hours.
Host- Parasite Relationship

This study to know the relationship between bacteria and the host

There are three levels of that relationship:-

- **Commensalism:** The organism (parasite) colonize the host at different sites of the body, where a balance is achieved between them with only minor changes of the host. Neither the host nor the parasite is harmed.

- **Infection:** The organism establishes and colonizes the host can elicit an immune response but very minimal tissue damage and no clinical signs and symptoms of the disease.

- **Disease:** The establishment and colonization of the organisms into the host, development of an immune response and marked tissue damage and/or disturbance of the physiologic functions of the host enough to elicit the signs and symptoms of the disease.

**Factors affecting the host-parasite relationship:**

These factors can be recognized into:-


A- Microbial factors:

1- **Saprophytic bacteria:** are those which live freely in nature, on decaying organic matter, in soil or water.

2- **Opportunistic pathogens:** bacteria that do not causing a disease under normal conditions but in immunocompromized or when enter the body from another site than their normal habitat.

3- **Pathogenic bacteria:** bacteria which capable of causing disease.

**Pathogenicity:** it’s the capability of the organism to cause disease.

**Virulence:** it’s the degree of pathogenicity. The Virulence factors of bacteria are:
1- **Adherence factors:** certain bacteria have specialized structure e.g. fimbria or glycocalyx allows them to adhere to human cell causing the disease.  

2- **Invasion factors:** Invasion of tissue followed by inflammation, **so bacteria can cause a disease. This invasion is helped by:** Enzymes secreted by bacteria e.g. collagenase&lecithinase.  

**Antiphagocytic factors:** e.g.capsule of pneumococci.

3- **Toxin production.**

**Toxigenicity:** it’s the ability of the organism to produce toxins whether exotoxin or endotoxin. The major difference between the two types of toxins are outlined in the following table:-

**N.B. Toxoid:** Is the treatment of exotoxin by formalin to remove toxicity and keep antigenicity.

An example of toxigenic bacteria is Corynebacterium diphtheria & Clostridium tetani.

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<tr>
<td>Secreted by the living organisms mainly gram positive</td>
<td>It’s the integral part of the cell wall of gram negative organism liberated upon cell disintegration</td>
</tr>
<tr>
<td>Protein</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>Very highly toxic</td>
<td>Low toxigenicity</td>
</tr>
<tr>
<td>Every individual toxin has specific action.</td>
<td>All give fever &amp; shock.</td>
</tr>
<tr>
<td>Can be converted to toxoid.</td>
<td>Cannot.</td>
</tr>
<tr>
<td>Highly antigenic.</td>
<td>Poorly antigenic.</td>
</tr>
<tr>
<td>Unstable to temperature above 60oC</td>
<td>Stable to temp.above 60</td>
</tr>
</tbody>
</table>
The essential steps for the pathogen to enter into a relationship with the host are:

1. **Entry** into the host by inhalation, ingestion or by inoculation through skin or mucus membrane.

2. **Establishment and reproduction** within the host, the organism from portal of entry may spread locally through tissues or spread via blood &lymphatic to reach target organs. **An effective mean for transmission to** new host must present then the organism leave the body through **portal of exit**

B. **Host factors:**

The most host factor that determine the outcome of the host parasite interaction is his **resistance or immunity whether natural or acquired.**

**Course of infectious diseases**

- The incubation period: Course of infectious diseases: it’s the interval between introduction of an organism to susceptible host and the onset of illness.

- The phase of illness: with the characteristic symptoms and signs follows the incubation period.

- The period of convalescence: After the illness subsides.

**Types of infectious diseases:**

- Acute infections; rapid onset short duration of illness.
- Chronic infections; Develop more slowly &long duration.
- Latent infections: never completely eliminated, become reactivated when immune response is decreased.
- **Carriers:** individuals harboring infectious agents for long time (months or years) spread pathogen continually even though they show no signs or symptoms of the disease.
Bacterial Genetics

Bacterial cell has a single circular chromosome composed of two polynucleotide chains of DNA twisted together in a double helix then replicated in each cell division. In each chain, purin and pyrimidin bases are arranged as G-C or A-T and the two chains are held together by hydrogen bonds between alternative bases. Each gene lying on the chromosome has specific character. And the set of genetic determinants carried by a cell is called its genotype. The observable properties of the cell are called phenotype.

MODEL OF DNA MOLECULE SHOWING THE INTERACTION AND HELICAL NATURE OF THE TWO STRANDS

A:Adrenine & T:Thymine & G:Guanine & C:Cytosine

Bacterial variation:

Phenotypic variation: are the changes in bacterial characters under the influence of the environment with no genetic changes. Its reversible when the environmental cause is removed. Its not heritable e.g. S-R variation in colony appearance which leads to loss of antigenicity, loss of virulence and the organism easily phagocytosed.

Genotypic variation: is a heritable irreversible variation due to changes in genetic constitution. Genetic variation occurs through:
a- Mutation **without gene transfere**.

b-Transformation, conjugation and transduction **with gene transfere**.

**a-Mutation:**
It’s a change in base sequence of gene nucleotide leads to appearance of altered phenotype, mutation can occur by base substitution, deletion or insertion. Mutation can occur **spontaneously** or can be **induced** by mutagenic agents such as X-ray, U-V light).

**b-Gene transfere:**
its an inter-strain transfere of DNA from donor to recipient bacterium .There are three mechanisms of gene transfere that alter the DNA gene content of bacteria:

1- Transformation.
2- Transduction.
3- Conjugation.

1- Transformation: it’s the uptake of naked fragments of DNA released from destructed bacterial cell by the recipient cell. then recombination with the bacterial chromosome takes place and transformation will occur which then express new genes.

2- Transduction: its transfere of chromosomal fragments from donor to recipient bacterium .There are two major types of transduction: a-

**Generalized Transduction:** During the lytic cycle of (bacteriophage).Piece of (donor) bacteria by accident is enclosed within the phage particle instead of normal phage DNA. When this phage infect another bacterium (recipient) ,the DNA from (donor) recombine with DNA of (recipient) ,so any length of chromosome can be transferred in this type of Transduction.

**Specialized or Restricted Transduction** This occurs during temperate (lysogenic) cycle of phage replication. Few genes of bacterial chromosome that adjacent to insertion site of the bacteriophage are transferred i.e. transfere of specific DNA segments.
Conjugation: It involves two types of bacterial cells donors that have fertility factor(F), recipient lacking fertility factor( F- ) Sex pilus facilitates the transport of DNA between donor and recipient cell.

**PLASMIDS**

- Small extrachromosomal double stranded circular DNA.
- Replicate autonomously independent of cell chromosome.
- Dispensable ( not necessary for cell life ).
- Transmissible to other bacterial cells( by conjugation, transformation and transduction)

**TRANSPOSONS.**

- Short DNA sequences.
- Can be transferred from one genetic compartment to other one within the same bacterial cell.
- It is possible for a gene to undergo transposition between plasmids, from plasmid to chromosome and vice versa.

**RECOMBINANT DNA TECHNOLOGY**

- In the course of the process of conjugation, transformation and transduction, DNA is transferred from a donor to recipient bacterium then followed by DNA recombination.
- Cloning vectors; are used to carry and introduce foreign DNA fragments into a host cell e.g. plasmid & bacteriophage.

**Construction of DNA molecule**

Foreign DNA and a vector with sticky ends. hydrogen bonds are formed between complementary strands( hybrid DNA) then the recombinant plasmid is introduced into proper host by process of transformation.
Application of DNA technology:-

1- Production of important biological products in large amount and great purity such as: human insulin, monoclonal antibodies and interferons.& highly effective vaccines.

2- Diagnosis of infectious diseases by DNA probe.

3- Gene therapy. to treat immunodeficiency diseases by using gene delivery viruses.
Sterilization And Disinfection

**Sterilization:** Is complete destruction of all living microorganisms contaminating an article (including spores), whether pathogenic or nonpathogenic.

**Disinfection:** Elimination of pathogenic microorganisms on surfaces (not including spores) but too toxic & irritant to be applied directly to tissue.

**Sepsis:** Is the presence of pathogenic microbes in living tissues.

**Antiseptic:** A chemical substance less toxic or irritant than disinfectant & can be safely applied locally against pathogenic microbes on living tissues.

**Cleaning:** is the removal of organic materials, dust that interfere with sterilization & disinfection it is done by using soap and water.

**Methods of Sterilization:**

*Five main methods are used:*

1- **HEAT**
   At temperature above 100 under carefully controlled conditions, is the most reliable & widely applicable method.
   - Dry heat
   - Moist heat

2- **Filtration.** : to remove bacteria. From liquids that are reliable to be spoiled by heat.

3- **Ionizing Irradiation.**: Both Beta & Gamma rays are employed.

4- **Sterilant gases:** As formaldehyde gas.

5- **Sterilant Liquids:** As glutaraldehyde (applied when no other method is available).
1- Heat:

a- Dry heat

- **Incineration**: It is a burning to destroy contaminated materials.
- **Direct flame**: For tips of forceps, scalpels, bacteriological loops and mouths of glass ware.
- **Hot air oven**: Metal chamber operated at temperature of 160°C–180°C for 1–2 hours. Used for materials not denaturated by high temperature such as glasswares-metallic instruments, oil and powders. (E.g. talcum powder).

b- Moist heat:

1-Moist heat at 100°C (Boiling &Steaming):

**Boiling:**

Articles may be disinfected by boiling in water for 20 min, all non sporing and some spores will be killed.

**Steaming:**

- Koch Steamer is used.
- Water is allowed to boil at normal atmospheric pressure. The steam temperature is 100°C will replace the air
- Used to sterilize culture media e.g. media containing gelatin or carbohydrates that might be destroyed by exposure to higher temperature.
- These are steamed for 20–30 min, in each of 3 successive days.
- A method is known as intermittent steaming or **Tyndallization**
- On the first occasion vegetative bacteria are killed, any spores that survive, will germinate at 37°C for 24 hours. Then vegetative forms will be killed by the second or third steaming.
**2- Moist heat above 100C (steam under pressure):**

*The autoclave:*

It is a metal chamber with a closely fitting lid which is connected to (1) a steam discharge tap, (2) a safety valve and (3) a manometer.

To sterilize articles in the autoclave these direction will be as followed:

1- Water is placed in the bottom and the articles to be sterilized are placed on the top of the perforated tray above the level of water.

2- The lid is tightly closed, the water is heated electrically, the steam discharge tap, is opened and the safety valve is adjusted to double atmospheric pressure.

3- When steam is released in a continuous stream, which is indicating that no air is left inside the autoclave, the steam tap is closed.

4- When the steam pressure reaches (2 atmospheric), the safety valve will permit excess steam to escape.

5- The sterilization time is calculated (20 – 30 min).

6- The heater is turned off and the autoclave is allowed to cool down before opening the lid.
The autoclave is used to sterilize surgical instruments, bed linen, surgical dressing, gowns, cotton, gauze and any culture media not destroyed by heat.

**PRECAUTIONS**

1- Do not overload the autoclave for better penetration of steam and contact with articles.

2- Make sure that, there is no air left inside before closing the steam tap.

3- Start timing from the moment the manometer read 2 atmospheric pressure.

4- Do not open the lid before the pressure goes down to one atmospheric pressure, otherwise the articles will be blown up and accident an happen.
How to test the efficiency of the autoclave:

- **Chemical method:** A glass tube contains a red indicator solution that turns green when an adequate temperature had been maintained for a sufficient period.

- **Biological method:** A preparation of dried bacterial spores is placed within the load and at the end of sterilization they are tested for viability. *Bacillus stearothermophilus* is usually used since its spores are killed at 120°C in about 10-20 minutes.

2- **STERILIZATION by IRRADIATION:**

Two types of ionizing radiation are commonly used for sterilization:

1) **Gamma rays:** which are emitted by radioactive elements such as cobalt-60.

2) **High energy electrons (B-rays):** which are produced by electron accelerators. Ionizing radiation has a high penetrating power and is, therefore, a practical means for sterilization of prepacked disposable items such as plastic syringes, gloves, catheters, and I.V. infusion sets.

3- **STERILIZATION BY FILTRATION:**

Fluids can be rendered free of bacteria by passage through filters with a pore size of less than 0.45 μm. It is used for preparation of fluids which would not withstand heat e.g. **antibiotics, hormones, vitamins,** etc. The most reliable filters used are the **membrane filters**. They filter rapidly, do not affect the filtrate in any way, and adsorb very little of the substance being filtered.

Filters can be used to remove microorganisms from air supplied to critical sites such as operating theaters, drug factories, and laminar flow ventilation systems. High efficiency particulate air (HEPA) filter can
achieve 99.997%, arrestance to particles of 0.5 um, and can produce sterile air at the filter face.

4- STERILIZATION BY GASEOUS PROCESSES:

(1) ETHYLENE OXIDE: It is a highly penetrative, non corrosive, and microbicidal gas which is used for sterilization of heat-sensitive medical devices such as plastic syringes and catheters.

(2) FORMALDEHYDE GAS AND LOW TEMPERATURE STEAM:

The effect of the gas combines with that of the steam thermal effect which generated at sub atmospheric pressure. Although effective and kill spores, it requires safety measures which make this process inappropriate for routine hospital use.

5- Sterilant liquids: e.g. glutaraldehyde.

METHODS OF DISINFECTION: used for inanimate objects (tables, floor & utensils):

(1) Cleaning

Thorough cleaning is a successful disinfection method. A valuable method for disinfection is the detergents used in cleaning and the dilution effect of through rinsing further reduces the microbial load.

(2) Disinfection by moist heat:

- Washing or rinsing laundry or eating utensils in water at 70-80°C for few minutes will kill most non – sporing micro-organisms.
- Exposure to boiling water (100°C) for 20 minutes achieves high effective disinfection. It can be useful in emergencies if no sterilizer is available.
• **Pasteurization of milk** by heating at 63 C for 30 min. or at 72C for 20 sec. destroys pathogenic organisms transmitted from diseased animals e.g. M.bovis, brucella and Salmonella.

(3) **Disinfection by Ultraviolet radiation:**

Is a low-energy, non-ionizing radiation with poor penetration power. It is produced by mercury lamps. It is used in operation rooms and laboratory safety cabinets.

(4) **Disinfection by chemicals:**

a) **Alcohols**: ethyl and isopropyl alcohol at 70 % concentration are used as skin disinfectants.

b) **Aldehydes**: formaldehyde and glutaraldehyde are used to disinfect equipments that cannot be sterilized by heat such as endoscopes. Glutataldehyde is irritant to eyes, skin and respiratory mucosa.

c) **Biguanides**: Chlorhexidine is commonly used for disinfection of skin and mucous membrane (as a mouth wash). It is often combined with compatible detergents for hand washing or with alcohol as a hand rub.

d) **Halogens**:

1. **Hypochlorite** are used as laboratory disinfectants on bench surfaces and in discard pots.
2. **Chlorine** is used to disinfect water supply and to treat swimming pools.
3. **Iodophores** are better than iodine as they are less irritant and cause less staining. They are widely used in preoperative preparation of the skin.
e) (e) **Phenolics**: are preferable for tuberculous material, for non-enveloped viruses, on metals and where much organic matter has to disinfect e.g. floors.

f) **Oxidizing agents**: as potassium permanganate and hydrogen peroxide which has limited application for the treatment of wounds
Part II

Immunology
IMMUNITY

There are two defense mechanisms that protect an individual from microorganisms and potentially harmful agents:

(1) Innate or natural immunity.

(2) Acquired or adaptive immunity.

(1) Innate Immunity

In this type of immunity the individual is protected from microorganisms by a number of very effective mechanisms. These mechanisms do not depend upon prior exposure to any particular organism. They are non specific. These mechanisms are:

I) Mechanical barriers:

- Intact skin and mucus membranes.
- Sticky mucus covers mucus membranes and trap any foreign material.
- Cilia of respiratory tract epithelium sweep foreign materials out.
- Blinking, sneezing and coughing.

II) Chemical barrier (surface secretions):

- Sweat and sebum contain (lactic acid and ammonia) that inhibit microorganisms.
- Saliva, tears, mucus secretions of respiratory, alimentary and genitourinary tract contain lysozyme.
- Gastric acidity, acid pH of the vagina inhibit growth of microorganisms.
III) Normal bacterial flora:

- Produce bacteriocins and acids (inhibitory substance) that destroy microorganisms.
- Compete with pathogenic bacteria for essential nutrients.
- Suppression of normal flora by antibiotics may lead to superinfection with potential pathogens.

IV) Humoral defence mechanism (Bactericidal substances in serum and body fluids)

- **Lysozyme**: it is a basic protein found in most body fluids, split sugar off peptidoglycan of cell wall of bacteria causing its lysis.
- **Properdin**: a complex system of serum proteins includes two enzymes that activate complement in alternative pathway.
- **Acute phase proteins**: e.g. C-reactive protein that binds to microorganisms and activates the complement.
- **Complement**: a group of proteins present in all body fluids except urine and CSF. It can bind to bacterial surface causing their lysis. Also, facilitate phagocytosis and promote inflammation.
- **Interferons**: important in the non specific immunity against viral infection.

V) Cellular defence mechanisms

- **Phagocytosis**: organisms that enter the tissue fluids or blood are rapidly engulfed by phagocytic cells which are:
  a- Polymorphonuclear leucocytes (microphages)
  b- Mononuclear cells (macrophages): e.g. monocytes in blood and macrophages in tissues.
These phagocytes are the link between innate and acquired immunity, they are activity phagocytic and contain digestive enzymes that degrade ingested materials.

**Stages of Phagocytosis**

(1) **Chemotaxis and attachment:**
   - Microorganisms and injured tissues elaborate chemotactic factors that attract macrophages.
   - Microorganisms attach to phagocyte surface receptors.

(2) **Ingestion:** cytoplasm and cytoplasmic membrane of phagocyte form pseudopod that extended around the microorganism to engulf it forming a vacuole termed as phagosome. Lysosomal granules then fuse with the phagosome forming phagolysosome.

(3) **Intracellular killing:** once ingested, most microorganisms are digested and solibilized by lysosomal enzymes.

The process of phagocytosis
• **Natural killer cells**: large lymphocytes causing non specific lysis to virus infected cells, tumour cells and graft cells.

**IV) Inflammation**: the events that occur during inflammation, vasodilatation, increased vascular permeability and cellular infiltration will lead to increase in chemical mediators and cells that can combat the invading agent.

(2) **Acquired Immunity**: -

Microorganisms that escape the innate mechanisms come up against the host's second line of defence, the acquired immunity. It is a specific immune response, discriminates between self and non-self and develops immunologic memory (first exposure to Ag the immunologic memory will develop and at second exposure to the same Ag, a more rapid, longer response will develop)

**Antigen (Immunogen)**

An antigen (Ag) or immunogen is any foreign molecule that when introduced into an animal stimulate the immune system to produce an immune response (humoral and/or cell mediated) and this Ag react specifically with:

The Abs produced or, Sensitized T-lymphocytes induced.

**☞ Hapten**

This is a low molecular weight substance which is incapable of inducing immune response alone but when coupled with a carrier molecule (protein) it can act as an antigen e.g. cosmetics and polysaccharide capsules of pneumococci.
**Epitopes or antigenic determinants:**

Specific structurally distinct areas on the surface or within the antigen molecule. The immune system does not recognize the Ag molecule as a whole but it reacts with these specific areas (epitopes). They are very small, composed of just four to five amino acids or monosaccharide residues. They determine the specificity of the Ags.

**Types of antigens**

1- **Bacterial Ags**: somatic (O), flageller (H), surface (Vi and K), fimbrial, capsular antigens, bacterial toxins, enzymes and other bacterial products.

2- **Viral antigens**: protein coat Ags and soluble Ags that diffuse in the surrounding fluids, e.g. nucleoprotein Ag of influenza and mumps viruses, and HBs antigen.

3- **Tissue antigens**:
   - **Blood group Ags**: A, B, Rh antigens on the surface of RBCs.
   - **Major histocompatibility complex antigens (MHC)**:
     These are glycoprotein Ags of two classes. Class I present on all nucleated cells and class II present on the surface of immuno competent cells, e.g. T-cells, B-cells and macrophages. Individuals vary in their MHC cells except the identical twins (this is very important in graft rejection).

4- **Superantigens**: These antigens nonspecifically activate a large fraction of T cells (up to 25%). There is hyperactivation of immune system with subsequent release of huge amount of biologically active cytokines by activated T cellse.g. Staphylococcal toxic shock toxin & exfoliating toxins.
5-Other antigens: e.g. drugs and some food proteins which are important allergens in hypersensitivity reactions.

Mechanism of acquired (specific) immune response:

1- The humoral immune response:
   - Abs (immunoglobulins) produced by B-lymphocytes
   - Able to recognize and bind specifically to Ag that induced their formation and not other Ags.

2- The cell mediated immune response:
   - Responsible for generation of certain T-lymphocytes. These cells are able to recognize foreign Ags (by surface receptors). These Ags as microorganisms or infected cells.
   - They attack and destroy them:

   Directly by release of soluble mediators (lymphokines)
Acquired immune response
(1) Phagocytes

a) **Macrophages**: originated from bone marrow released in blood as monocytes. Some of these blood monocytes migrate outside the blood vessels and differentiate to become tissue macrophages.

b) **Microphages**: these are the blood neutrophils.

(2) Lymphocytes

In peripheral blood, lymphocytes form about 25-35% of total leucocytes. These lymphocytes are composed of 3 types namely: B-lymphocytes, T-lymphocytes and natural killer cells.

**B-lymphocytes**: This type of lymphocytes matures in Bursa of fabricus in birds which equivalent in man to foetal liver or bone marrow "hence the name B". They are found in peripheral blood forming about 15-30% of total peripheral blood lymphocytes. They are also found in germinal centre of LNs, white pulp of the spleen and in gut associated lymphoid tissue.

**Stimulation**: Under the influence of Ags stimulation and in presence of growth factors secreted by T cells, these mature B-lymphocytes multiply. Some of these B cells differentiate to plasma cells that secrete immunoglobulins. The remaining B cells will be memory B-cells.

- Plasma cells: they are the antibody producing cells. These cells result from differentiation of B-lymphocytes under the influence of Ags stimulation.
**T-lymphocytes:**

T- Lymphocytes form about 65-75% of circulating lymphocytes. There is no difference in morphology between B- or T-lymphocytes except in surface markers. T-cells maturate in the thymus gland; then they leave thymus and settle in thymus dependent areas (spleen – lymph nodes also peripheral blood).

**Sub populations of T-cells:**

a) T4 (CD4) or T-helper (T_H):

These cells are stimulated by the processed Ag together with MHCII. They constitute 65% of total lymphocytes. There are 2 subsets of T_H cells:

- T_H1 or T_DTH (T-delayed type hypersensitivity): they produce IL2, INFγ and INFβ responsible for granuloma formation of DTH, macrophage activation and T-cell growth.
- T_H2: they produce IL 4, 5, 6 and 10: they concerned with cell activation and differentiation.

b) T₈ (CD₈):

These cells are stimulated by the processed Ag together with MHC-I. They constitute about 35% of total T-lymphocytes. There are 2 subsets:

- T-cytotoxic cells (Tₗ): against abnormal cells.
- T-suppressor (Tₜ): regulate immune response.
Natural Killer Cells (NK cells):

Large granular lymphocytes. Lack most surface markers of B and T cells (previously known as null lymphocytes). They comprise 5-10% of the total lymphocytes in peripheral blood.

The main function of NK cells is immune surveillance against abnormal cells (tumour cells, virally infected cells, old cells or transplanted cells). They exert direct cytotoxic activity against abnormal cells.
Humoral Immune Response

Humoral immunity is mediated by immunoglobulins or antibodies. These are produced by B-lymphocytes that change into plasma cells in response to Ag stimulation. The antibodies bind specifically to the Ag that induced their formation. Most immunoglobulins are present within the gamma globulin fraction of serum proteins when separated by electrophoresis. There are five classes of antibodies or immunoglobulins, IgG, IgM, IgA, IgE, and IgD.

Antibody structure:

I) IgG: The major immunoglobulin of serum. It represents about 75% of the total serum immunoglobulins composed of single basic unit (monomeric), and having a MW of 150,000 in human biological activities:

- The major Ab in secondary immune response.
- Found in both serum and tissue fluids
- Can pass the placental barrier to the foetus.

II) IgA:

This class of immunoglobulin exists in two forms:

- Monomeric form (serum IgA), which is found in serum and similar in structure to IgG molecule.
- Dimeric form (secretory IgA), which is produced by submucosal plasma cells and is found in the mucosal secretions (saliva, tears, colostrums, respiratory, GIT and genitourinary secretions). It acts as a first line of defence against invading bacteria and viruses at the mucous surfaces.
**III) IgM:** This immunoglobulin represents about 10% of the total serum immunoglobulins. IgM is a pentamer, composed of five basic units held together by disulphide bonds and a single J chain. It is mainly confined to the intravascular pool because of its large size (MW 1000,000).

**Biological activities:**

- It is the predominant antibody in the primary immune response to most antigens.
- It can not cross the placenta hence its presence in newborn blood indicates intrauterine infection.

**Basic structure of an immunoglobulin molecule (IgG)**
IV) **IgD**: It is a monomeric antibody, represents less than 1% of circulating antibodies. It has been found on the surface of circulating B-cells and it can act as an antigen receptor on them.

V) **IgE**: it is a monomeric antibody present in trace amounts (0.005%) in normal serum. Elevated level is found in type I hypersensitivity it has a protective role in parasitic infestation.
**Primary and Secondary Antibody Response**

When an antigen is introduced into the body for the first time, there is an interval before antibody can be found in the blood. This interval is called the "latent period" or "induction period". During this time, the antigen is processed by macrophages and recognized by B- and T-cells. The B-cell differentiates into plasma cells to produce antibodies. The isotope produced by plasma cells is IgM. This takes 7-10 days. Antibody concentration rises to a peak within few weeks, decline rapidly to undetectable levels. In re-exposure to the same Ag, there is more rapid Ab response (induction period several hours) that reaches high concentrations, the Ab formed is IgG and persists for long periods. The very short and rapid induction period in 2ry response is due to presence of "memory cells" that immediately stimulated to form Abs. This immunologic memory is the cause of long lasting immunity after some diseases e.g. diphtheria, mumps and measles.
Monoclonal Antibodies:

Highly specific Abs against a single epitope produced by a single clone of cells (hybridoma cells). These cells result from fusion of two types of cells.

1- Neoplastic plasma cells that grow indefinitely in culture (myloma cells).

2- B-cells from spleen of injected mouse with certain antigen (epitope).

These cells will have the ability to grow indefinitely (from myloma) and the ability to produce the specific antibody (from the B-cells).

The hybridoma cells producing the required antibody can be frozen and kept for a long time for use.

Uses of Monoclonal Abs:

1- Diagnostic uses:
   - Identification of Ags on cells or microorganisms
   - Detection of CEA and α fetoproteins (Tumour markers)
   - Hormonal assay

2- Therapeutic uses:
   - Antitumour therapy
   - Immunosuppressive therapy
   - Drug toxicity
   - Anti-RhD to prevent Rh –incompatibility
   - Passive immunotherapy: for protection against viral infection.
II- Cell–Mediated Immune Response

In contrast to the B-cell response, T-cells serve a large number of different functions that collectively result in cell-mediated immunity. The cell-mediated immunity (CMI) reactions are responsible for a wide variety of protective mechanisms, e.g.:

- Resistance to many infectious agents, e.g. viruses, M. tuberculosis.
- Resistance to fungal & protozoal infections.
- Resistance to tumours.

CMI also play a role in delayed type hypersensitivity reactions, graft rejection and autoimmune diseases.

**Stages of CMI:**

1) Ag processing and presentation by macrophage or B-cells in association with MCHII molecules to be recognize by T\(_H\). Activation of T\(_H\): this activation by 2 signals, 1st, the recognition of Ag with the MHCII on cell surface and 2nd, IL\(_1\) secreted by macrophage activate T\(_H\) which secrete IL\(_2\) that also stimulate T\(_H\).

2) Activation of T\(_C\): by 2 signals, 1st recognition of Ag in association with MHCI, 2nd IL\(_2\) which activate T\(_C\) to proliferate and kill target cells.

3) Activation of T\(_S\): signals are not known T\(_S\)-cells regulaboth humoral and CMI response.

**Cytokines:**

- They include lymphokines (secr. By lymphocytes), monokines (secr. By monocytes and macrophages), interleukins and interferons. They are low MW soluble proteins that regulate the amplitude and duration
of the immune-inflammatory responses. Include IL-1, IL-2, colony stimulating factor, TNF and IFNs.

**Interferon IFNs:**

IFNs are groups of low molecular weight glycoproteins produced by the host cells in response to viral infection causing inhibition of viral replication. Three different interferons (IFNs) are now identified:

- IFNα produced by leucocytes.
- IFNβ produced by fibroblasts. Both are induced by viral infections or by double standard RNA.
- IFNγ which called immune interferon and is produced by T-cells when stimulated by specific antigen.

**Activities of interferon:**

1. **Anti-viral:** INFs stimulate cells to produce certain protein that prevent synthesis of viral proteins and stop viral replication.

2. **Anti-proliferative:** IFNs stimulate certain enzyme in the cells called "protein kinase" which inhibit protein synthesis and slow down the rate of cell multiplication.

3. **Immunomodulatory:** it enhances activities of macrophages' B- and T-lymphocytes, Tc and NK cells.

**Therapeutic uses of IFNs:**

1- Treatment of viral hepatitis.

2- Treatment of some cases of malignancies

3- IFNγ is given in small doses to enhance the activities of both B- and T-lymphocytes and also macrophages.
Superantigens: -

A class of bacterial toxins including:

- Staphylococcal enterotoxins.
- Toxic shock syndrome toxin.
- Group A streptococcal pyogenic toxin A.
- Superantigens are not processed and they are active at very low concentrations, irrespective of their antigenic specificity, to be stimulated causing the release of large amounts of cytokines including IL-1, IL-2 and TNF.
- This method of stimulation is not specific for the pathogen and does not lead to acquired immunity.
Transplantation Immunology

Transplantation is surgical techniques that are used to replace damaged and diseased tissues or organs with normal ones from a donor. A high proportion of organ transplants fail because of immune-rejection by the recipient.

**Types of grafts:**

1) *Autograft*: is a graft removed from a site and placed in another site of the same individual, e.g. skin graft.

2) *Isograft*: transfer of tissues between genetically identical individuals, i.e. identical twins.

3) *Allograft*: transfer of tissues between individuals of the same species, e.g. man to man.

4) *Heterograft*: transfer of tissues between two different species.

**Types of antigens responsible for immune-rejection:**

1- **Blood group antigens**: ABO and Rh present on RBCs surfaces.

2- **Major histo-compatibility complex (MHC) antigens**: they are glycoprotein antigens coded by a gene complex. These Ags are also known as (HLA). MHC gene complex is located on chromosome 6. They are polymorphs (i.e. multiple alleles). Thus, it is uncommon that two individuals will have exactly the same MHC antigens.

**Graft survival rates:**

When the transplant comes from HLA identical twin, transplanted organ have a survival rate more than 90%. This drops to 70% if the HLA antigens match (Never identical).
**Mechanisms of graft rejection:**

Both cell mediated and antibody mediated mechanisms have their roles:

(1) Ags on the graft sensitize T-cells and cause their activation

- $T_c$ destroys the foreign graft by direct contact.
- $T_h$ secretes lymphokines that activate macrophages, NK cells and polymorphs leading to destruction of the graft.

(2) Abs against the graft activates the complement and cause cell lysis. Abs also enhances phagocytosis and mediate attack of the graft by macrophages. NK cells and polymorphs through (Type II hyper sensitivity).

**Rejection reactions:**

1- **Hyperacute rejection:** it occurs within hours mainly due to preformed Abs to some donor Ags (humoral) or when donor and recipient do not match for ABO blood group.

2- **Acute rejection:** it occurs 10 to 30 days after transplantation. T-lymphocytes start to develop and attack the graft. It’s a CMI response.

3- **Chronic rejection:** a slow loss of tissue function occurs over a period of months or years through weak Ags of HLA. The rejection CMI, humoral or both
Part III

Medical Bacteriology
Staphylococci

Staphylococci are:

- Gram-positive cocci
- Arranged in clusters (grape-like)
- The genus staphylococcus include 3 important species:
  1) Staph aureus (the most important pathogenic member)
  2) Staph epidermidis (it is a normal flora of skin and oral cavity).
  3) Staph saprophyticus It is a very rare (it causes urinary tract infection)

Virulence factors are toxins, enzymes like coagulase which changes fibrinogen to fibrin, which forms a barrier and cause localization of lesions.

Diseases caused by S. aureus:

Abscess, boils, osteomyelitis, post-operative wound infection and food poisoning.

Diagnosis:

- Gram stain
- Culture on nutrient and blood agar. On nutrient agar it gives golden yellow colonies. On blood agar it causes β-haemolysis.

Treatment:

It is recommended to do in vitro culture and sensitivity.
Streptococci

Gram-positive cocci arranged in chains. Streptococci are classified according to:

(A) $O_2$ requirements into:

1- Aerobic: Which classified into 3 groups:
   - Alpha-haemoloytic: e.g. viridans streptococci and pneumococci.
   - β-haemolytic: e.g. strept. pyogenes.
   - Non-haemolytic: e.g., strept. foecalis.

2- Anaerobic: it is called peptostreptococcus which is normally present in vagina, intestine and upper respiratory tract. It causes puerperal sepsis, urinary tract infection and abscesses.

(B) Carbohydrate (C) presents in the cell wall, lancefield classified β-hemolytic streptococci into groups from A to O.

The most important pathogenic group is: Group A Beta – Haemoloytic Streptococci (streptococcus pyogenes)

   It produces many toxins and enzymes e.g., streptokinase enzyme which dissolve fibrin in clots and thrombi.

Diseases caused by strept. Pyogenes:

a) Diseases due to the local infection or invasion by the organism:

   Acute follicular tonsillitis, scarlet fever, impetigo and streptococcal stomatitis.

These diseases to be diagnosed we must take specimen from the site of infection and examine by microscope (Gram stain) and culture on
blood agar. This group is differentiated from other groups by its sensitivity to bacitracin.

b) Post streptococcal diseases:

Rheumatic fever & Acute glomerulonephritis.

These diseases occur 1-3 weeks after inadequately treated streptococcal infection. Antibodies in blood of the patient not differentiate between streptococcal Ags and Ag of endocardium or glumeruli and react with them causing rheumatic fever or acute glomerulonephritis. These diseases are considered allergic complication to streptococcal infections.

**Diagnosis of post streptococcal diseases:**

1. Antistreptolysin – O (ASO) titre: If more than 1/200 it is significant.
2. Detection of C-Reactive Protein (CRP)
3. High erythrocyte sedimentation rate (ESR)

*N.B.:* streptolysin-O is a toxin secreted by group A –Beta hemolytic streptococci which is antigenic, antibodies to it "antistreptolysin–O" increase in post-streptococcal disease.

Alpha haemolytic streptococci (Viridans Streptococci):

These are the predominant group of organisms in the oral cavity (oral streptococci) that includes the following species:

1- **Strept. Mutans:** it is found on enamel surface, pits and fissures. It forms glycocalyx from sucrose with which the organism stick to the tooth enamel. Other bacteria become entrapped in the glycocalyx forming a layer known as plaque (first step of dental caries).
2- **Strept. Sanguis**: it is common in dental plaque. It has no role in dental caries.

3- **Strept. Salivarius**: it predominates on the dorsum of the tongue because it has high affinity to keratinized surface. It has no role in dental caries.

4- **Strept. Mitis**: it is a common component of dental plaque. It has no role in dental caries.

5- **Strept. Milleri**: it is present in all sites in the mouth. It has no role in dental caries.

**Diseases caused by viridans streptococci:**

1- **Dental caries**

2- **Subacute bacterial endocarditis**: it is an endogenous infection usually following tooth extraction or tonsillectomy in individuals with congenital heart, rheumatic heart or prosthetic heart valves. The organisms pass from the mouth via blood stream and settles on the deformed valve leading to subacute bacterial endocarditis.

   - Individuals with deformed heart valves should be given penicillin or erythromycin few hours before and 48 hours after oral surgical procedures.

   - Subacute bacterial endocarditis is diagnosed by blood culture then subculture on blood agar to detect the organism streaming in patient blood.
**Streptococcus Pneumoniae (Pneumococci)**

Gram positive cocci arranged in pairs, surrounded by capsule (virulence factor) which appears unstained halo around the organism. It causes lobar pneumonia, sinusitis, otitis media, conjunctivitis and meningitis. Pneumococci are differentiated from viridans strept by being pathogenic to mice causing its death on I.P injection.

**Neisseriae**

Many of the members of genus Neisseria are commensals of the upper respiratory tract and mouth and collectively named N. Pharyngis. There are in addition two highly pathogenic members called: N. gonorrheae (gonococcus) and N. meningitidis (meningococcus).

*Morphology:*

Neisseriae are gram-negative cocci arranged in pairs with the adjacent sides flattened (kidney shaped). The pathogenic members are characteristically found inside the pus cells of the inflammatory exudates such as CSF or urethral discharge. Extracellular cocci also occur.

*Culture:*

They grow on chocolate agar and Thayer-Martin medium. Cultures are incubated at 37°C in presence of 5-10% CO₂.

1- Neisseria Gonococcus

*Pathogenicity:*

1) Venereal: gonorrhoea

2) Non-venereal: ophthalmia neonatorum and vulvo-vaginitis in small girls & oral infection.
**Way of infection:** Gonorrhoea is transmitted by sexual intercourse (characterized by urethritis with yellow creamy pus and painful urination). Chronic cases are asymptomatic.

- Ophthalmia neonatorum by contamination of the infant's eye during labour through the birth canal of a gonorrhoeal mother.
- Vulvo-vaginitis through contaminated toilet seats, and contaminated towels.
- Oral infection: It varies from pharyngitis to severe painful erythema and ulceration of soft palate, gingival and buccal mucosa.

**Treatment of gonorrhoea:**

Penicillin. Anti-biogram is recommended because resistance to penicillin has gradually increased. Ciprofloxacin or streptomycin is recommended.

2- Neisseria Meningitidis

**Pathogenicity:**

**Epidemic cerebrospinal meningitis.** But it can cause pharyngitis and rarely myocarditis.

**Way of infection:**

By droplet infection of a case or a carrier, because the organism is present in the nasopharynx of the carrier (about 5-10% of population are carriers of meningococci). The patient has sudden onset of fever, vomiting, stiff neck, haemorrhage, skin rash and coma in few hours.

The CSF (cerebrospinal fluid) is obtained through lumbar puncture under complete aseptic precautions then the diagnosis of the case in the laboratory.
**Diagnosis:-**

It depends on isolation and identification of the organism from CSF and blood, by blood culture. Diagnosis of suspected cases, by nasopharyngeal swab which the only way for diagnosis of carriers. Meningococci isolated from nasopharynx should be differentiated from commensal neisseriae.

**Treatment:**

Penicillin G is the drug of choice. Chloramphenicol or a third – generation Cephalosporin is used in persons allergic to penicillin.

**Prevention and control:**

1-Rifampicin for 2 days to contacts.

2-Vaccination.
Aerobic gram positive spore forming bacilli. This genus includes:

**Bacillus anthracis**: one of the warfare germs. It causes anthrax (a disease of animals that can infect man).

**Anthracoids**: they are saprophytes present in the environment, e.g., air, water and soil.

**B. cereus** is one of its members having medical importance. It is the cause of food poisoning.

Some members produce antibiotic, e.g. polymyxin. And others test the efficiency of sterilization procedures, e.g.:

- Spores of B. stearothermophilus for the autoclave.
- Spores of B. subtilis for ethylene oxide sterilizer.
- Spores of B. pumilis for ionizing radiation.
**Corynebacteria**

These are group of aerobic, non-spore forming gram-positive bacilli. *Corynebacterium diphtheriae*. This is the cause of diphtheria in man

**Morphology:**

Gram positive, non sporing, non capsulated, non motile slender bacilli with one swollen end, arranged parallel or at acute angles, giving rise to Chinese letter appearance. They contain phosphate (volutin) granules that give the organism beaded appearance when stained by methylene blue.

**Diphtheria:**

This is a serious infectious disease, affecting mainly children. **I.P:** 2-5 days.

**Mode of transmission:**

Droplet infection from a case or carrier. It affects man only.

Tonsillar or pharyngeal diphtheria is the commonest type. Less commonly, (diphtheria affect skin through opened wound or the conjunctiva through contact).

The organism multiplies locally on the mucosal surface producing exotoxin. Local inflammation and tissue necrosis will occur, Producing an adherent grey-black pharyngeal membrane). This pseudomembrane contains fibrin, blood, inflammatory cells, and epithelial cells, any attempt to remove this membrane resulting in bleeding. The membrane and oedema may compress the airway leading to asphyxia. The absorbed
toxin through the membrane is carried by blood to distant sites with
tropism for cardiac, neural and renal cells.

**Diagnosis:**

Diphtheria must be suspected on clinical grounds, treatment should
not await laboratory confirmation. But laboratory diagnosis aims at
isolation of the organism from throat swab as follows:

1) Direct smear with gram stain and methylene blue stain.
2) Culture: on Loffler’s serum and blood tellurite
3) Toxigenicity or virulence tests: isolated organism from culture should
   be tested for the production of the exotoxin especially when isolated
   from carrier.

**Treatment:**

a) **Antitoxin:** started as soon as the diagnosis is suspected clinically to
   neutralize free toxin. 40,000-12,000 units of horse antiserum IM or
   IV. To avoid sensitivity to horse serum, patient should be tested for
   hypersensitivity. Cortisone and antihistamines for treatment of
   anaphylaxis must be available.

b) **Antibiotics:** erythromycin or ampicillin for at least 7 days to eradicate
   the organism and stop further toxin production.

**Prevention and control:**

**A- Active immunization**

Diphtheria toxoid combined with that of tetanus and pertussis
vaccine called DPT is given to infants at 2, 4 and 6 months of age.

Booster doses are also given at 1½ years and before going to
school.
**B- Passive immunization**

By antitoxic serum in a dose of (5.000-10.000 units) should be given to contacts.

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**Anaerobic, gram positive spore forming bacilli:**

Most of the members of this genus are saprophytes in soil and water. Some are commensals in the intestine of man and animals. They produce diseases through their toxins and enzymes. The pathogenic species are:

1- Cl. Perfringens causing gas gangrene

2- Cl. tetani causing tetanus

3- Cl. botulinum casing botulism (type of food poisoning)

4- Cl. difficile causing enterocolitis (superinfection due to oral antibiotic treatment complication).

**Gas Gangrene**

The commonest cause is *Cl. Perfringens*.

**Virulence factors:**

1- α- Toxin (lecithinase) that cause membrane lysis.

2- Collagenase and hyaluronidase help in spreading of the disease.

**Pathogenesis:**

- In presence of foreign bodies, dead bodies, blood clots, local ischaemia in atherosclerosis and diabetes and presence of pyogenic infection
• Contamination of deep lacerated wounds with soil containing Cl. Perfringens spores

• Under all the previous anaerobic condition is established and the spores germinate, the vegetative bacilli will produce toxins and enzymes that spread to adjacent tissues and muscles where the sugars are fermented with production of large amount of gases that press on blood supply giving rise of more ischaemia and necrosis. Affected area becomes tense, oedematous, brick red and odourless.

• Proteolytic clostridia start the process of putrifaction of dead tissues, so becomes black with foul odour due to H₂S.

• Tissue necrosis extends providing an opportunity for increased bacterial growth, sever toxaemia and death.

**Laboratory diagnosis:**

*The specimen is taken from wound discharge then the following will be done:*

1- Gram stain shows large gram-positive bacilli

2- Culture on blood agar anaerobically

**Treatment:**

- Surgical debridement
- Penicillin and metronidazole
- Polyvalent anti gas gangrene serum
- Hyperbaric oxygen
Clostridium Tetani

Ways of infection:

After burns or wounds contaminated with soil or dust in which spores are present.

Cl. tetani occurs in the intestine of domestic animals and from their stools it reaches the soil Another way of infection is after surgical sutures due to badly sterilized cat-gut. The spores vegetate and the organism multiply locally at the site of infection producing exotoxin which spread via blood to the spinal cord and affects the motor nerves to produce stiffness in the jaw muscles leading to trismus (lock jaw), dysphagia, and finally spasm of most of the body's muscles.

Diagnosis:

1) On clinical evidences i.e., lock jaw, wound and convulsions. Antitoxic treatment should be started without waiting for laboratory diagnosis.

2) Laboratory diagnosis is done for confirmation

Treatment:

1) Sedation and mechanical ventilation if needed

2) Antitoxic serum: intravenous drip 10,000 units human serum or 100,000 units of horse serum to neutralize the toxin in the blood before it reaches the CNS.

3) Penicillin and metronidazole to kill the organism so stop further production of toxin.

4) Cleaning the wound and surgical debridement: the wound is left open and washed with H₂O₂.
**Immunization**: passive and active

1- **Passive immunization**: antitetanic serum (1.500-3.000 I.U.) is given S.C. or I.M. to patients with wounds contaminated with soil and foreign bodies or deep puncture wounds.

2- **Active immunization**:

   a) Formal-toxoid: prepared as formal toxoid of diphtheria
   
   Dose: ½ ml  Dose: ½ ml
   
   6 Weeks $\frac{1}{2}$ ml  6 Months $\frac{1}{2}$ ml  6 Years $\frac{1}{2}$ ml as a booster dose

   b) D.P.T. vaccine (Diphtheria, pertussis and tetanus)

   **Non-Spore Forming Anaerobes**

   **Bacteroides**:

   Gram-negative bacilli, non spore forming strict anaerobic, highly pleomorphic, and are capsulated.

   Normal flora of upper respiratory tract, intestine, oral cavity and female genital tract.

   **Prevotella**:

   They are normally present in the mouth and produce infections, e.g., periodontitis.

   Previously a member of the bacteroids.

   **Porphyromonas**:

   - Previously belong to bacteroides
   - Normal inhabitants of oral cavity
   - Produce advanced periodontitis and dental root canal infections
**Fusobacterium:**
- Large gram negative bacilli, fusiform or cigar-shaped.
- F. nucleatum is frequently isolated from mixed infections of the head and neck, including dental abscess.
- In association with spirochaetes, they produce fusospirochaetal disease in the oral cavity.

**Lactobacilli:**
- Gram-positive bacilli, non spore forming, and anaerobic.
- Powerful acidogenic (produces acid) and aciduric (grow in acidic media).
- Normal commensal of oral cavity, L-odontolyticus is present in the mouth and is involved in dental caries.
- For culture: tomato juice agar or whey agar containing 0.5% acetic acid is selective media.

**Propionibacterium:**
It was previously placed in genus Corynebacterium, typically diphteroids in morphology. P.acneis present in human skin & one of the causes of acne.

**Mycobacteria**

1- Pathogenic members of mycobacterium which cause important human infection include:

- Mycobacterium tuberculosis complex which cause tuberculosis
- M. leprae, which cause leprosy
- Non tuberculous mycobacteria (NTM)

2- Opportunistic and saprophytic species
**Mycobacterium Tuberculosis**

It causes tuberculosis in man and animals by a group of very closely related species (M. tuberculosis complex). This complex comprises M. tuberculosis, M. Bovis and M. Africanum.

*Morphology:*

The organism is slender, straight, non motile, non sporing, non capsulated occurs singly or in small groups. By Zeihl-Neelsen (Z.N.) stain, the bacilli appear as thin red beaded rods against a blue background.

*Culture:*

The tubercle bacilli are obligate aerobic; grow on Lowenstein Jensen media (L.J) containing egg and malachite green. Optimal temp. is 35-37\(^0\)C appears in about 2-8 weeks. The tubercle bacilli are sensitive to heat, destroyed by pasteurization, killed when exposed to sunlight and survive in dark-damp areas.

**Tuberculosis**

It is a chronic granulomatous disease affecting any organ in the body but mainly, it affects the lungs resulting in pulmonary tuberculosis.

*Mode of transmission:*

1. **Inhalation:** the organism is present in droplets expelled in cough, talking and sneezing of infected person.

2. **Ingestion:** of contaminated milk.

3. **Direct inoculation:** through skin abrasion.
Pathogenesis:

I. Primary tuberculosis

After inhalation of the tubercle bacilli, they are engulfed by alveolar macrophages and multiply forming Gohn's focus at the apex of the lung which together with enlarged lymph node forming primary complex. The primary complex may also the primary focus in tonsils with enlarged cervical L.N. or the focus is in the intestine with enlarged mesentery L.N. This complex will become latent or dormant till reactivation occurs.

II. Post Primary Tuberculosis

It is a reactivation of the primary latent complex, it may be endogenous reactivation or exogenous through reinfection. Because it is acquired cell-mediated immunity, the lesion is localized.

Oral manifestation of tuberculosis: There are four forms of tuberculosis in the mouth:

I) Primary complex

This primary complex involves the oropharyngeal region in the form of oral ulcer at the site of entry; it is a painless and progressive ulcer. The submaxillary and cervical L.Ns are enlarged may caseate and discharge through sinus tracts.

II) Oral ulceration secondary to pulmonary tuberculosis:

Present in oral mucosa, the ulcers are very painful. Present on lateral sides of the tongue or palate. The regional L.Ns are not enlarged.
III) Lupus vulgaris:

It is a form of skin tuberculosis affecting the face, cheek and nose due to exogenous infection or secondary spread from blood or lymphatic. However, the oral mucosa may be affected in the form of small nodules.

IV) Periapical infection and osteomyelitis:

Secondary to pulmonary tuberculosis, the M. tuberculosis may colonize the periapical granuloma associated with teeth and roots leading to cold dento-alveolar abscess with lack of pain and inflammation. Tuberculous osteomyelitis of the jaw is a rare form occurs when tubercle bacilli transmitted via the blood stream in systemic tuberculosis and reach the bones via a carious tooth or via an extraction socket.

Laboratory diagnosis:

1- The diagnosis of T.B. is based on detection of acid fast Microscopy: specimens are stained with Zeihl-Neelsen stain.

2- Culture: On Lowenstein-Jensen after decontamination and concentration of the specimen.

3- PCR: it is a rapid (1-2 days) method but cannot replace culture then drug sensitivity tests.

   Tuberculin test: intradermal injection of 0.1 ml of purified bacilli in clinical specimens (sputum, CSF, urine, pus, tissue biopsy… etc) by:

   protein derivative (PPD) of the tubercle bacilli containing 5 I.U.

   This is called Mantoux Test. An indurated area of 10mm or more, 48-72hrs after injection, is regarded as a positive test. It is a cell-mediated hypersensitivity test.
**Treatment:** by antituberculous drugs that are given in combination of not less than two drugs to reduce the chance of emergence of resistant mutants.

**Prevention and control:**

1) General measures:
   - Good housing, prevent smoking, good nutrition
   - The Dentist must wear a mask during examination of the patient to prevent direct contact.

2) Public health measures:
   - Early diagnosis and treatment
   - Eradication of T.B. from animals
   - Pasteurization of milk
     4) Vaccination: a living attenuated vaccine called Bacillus-Calmette-Guerin (BCG) is the currently used vaccine. It is given to infants in the first month of life and to tuberculin negative adults especially the high risk group as doctors, nurses… etc.

**Important Gram-Negative Bacilli**

**Enterbacteriaceae:**

*Gram negative bacilli that may cause pneumonia and soft tissue infections.* Cultured on simple media and Macconkey, smedia on which they are differentiated into:-
(1) Lactose fermenters e.g.:

* Ecoli:
  - Gram-negative motile bacilli
  - They are normal intestinal flora
  - Transient flora of oral cavity
  - Commonest cause of urinary tract infection
  - It causes postoperative wound infections and some strains cause diarrhea.

* Klebsiella:
  - Gram-negative capsulated bacilli
  - K. pneumoniae: causes pneumonia in man. It may also cause urinary tract infection.
  - K. rhinoscleromatis: causes rhinoscleroma. (granulomatous disease of the nose)

(2) Non Lactose fermenters: e.g.

* Salmonella:
  - Gram-negative motile bacilli
  - It causes enteric fever by S.typhi, S.paratyphi A, B and C. It is transmitted by contaminated food and drinks.
  - Some groups cause food poisoning.

* Shigella
  - Gram-negative, non-motile bacilli
  - They cause bacillary dysentery
- They are transmitted by contaminated food and drinks.

*Proteus*

- Gram-negative, highly motile bacilli.
- They cause swarming growth on nutrient agar.
- Normal intestinal flora – it causes wound infection, UTI and otitis media.

*Pseudomonas*:

- Gram-negative motile bacilli
- They cause greenish discolouration on nutrient agar due to pyocyanin (exopigments).
- They cause UTI, wound infection and otitis media. They are normally present in soil, sewage, water and intestine.

(3) Slow Lactose fermenters *e.g.*

- *Serratia marcescens* it causes nosocomial infections, wound infections and septicaemia, resistant to antibiotics, produces red-pigmented colonies on nutrient agar.

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**Spirochetes**

Spirochaetes are large heterogeneous group of long, spiral, motile bacteria.

**There are three genera:**

- Treponema
  - Borrelia
  - Liptospora
**Treponema**

They are motile, slender, helical rods with regular spaced spiral coils, cannot stained with gram stain and are stained by **Fontana stain**. They can be demonstrated in fresh smears by dark ground illumination.

**Syphilis**

Treponema. Pallidum is the causative agent of **syphilis**. It is a sexually transmitted disease. It is a progressive disease with primary, secondary, latent and tertiary stages.

**Mode of transmission of infection:**
- Sexual intercourse
- Vertical from mother to foetus
- Close direct contact (medical personnel can attain infection)

**Clinical finding:**

**Incubation period:** 2-10 weeks

**Primary stage:**

Chancre results from penetration of T. Pallidum to intact mucosa or through abraded skin and its multiplication through the I.P. The chancre is painless ulcer on external genitalia. It usually heals spontaneously.

**Secondary stage:**

2-10 weeks later: T. Pallidum invades blood and disseminate throughout the body giving rise to fever, skin rash on palms and soles
and mucous patches in mouth and condylomata at the oral mucosa, vulva and anus.

- **Latent stage:**
  
  Secondary lesions subside and period of latent infection starts, in which no clinical manifestations are evident but serological evident of infection persists.

- **Tertiary stage:**
  
  Slowly progressive, destructive inflammatory affect any organ. There are three common forms which are neurosyphilis, cardiovascular and gummatous syphilis. No T. Pallidum is detected in tertiary lesions.

**Oral manifestations of sexually acquired syphilis:**

1. **Primary stage:**
   - This occurs in the form of chancre (ulcer).
   - Commonly develops on the tongue and lips
   - Slightly painful with enlarged lymph nodes.

2. **Secondary stage:**
   - Recurrent oral mucosal patches, maculopapular eruptions and condyloma.
   - Snail track ulcers develop on the tongue and palate.

3. **Tertiary stage**
   - Gummata and painful ulcers develop in the mouth
   - Neurosyphilis leads to parathesia of the lip and tongue

**Oral Manifestation of congenital syphilis:**
It is a transplacental infection from syphilitic mother to the foetus that leads to:

The oral manifestations of delayed dentition and enamel hypoplasia that also leads to abnormal development of 2 groups of permanent teeth:

Upper central incisors, which appear widely separated and notched (Hutchinson's teeth)

a) First molars, which show defective cusps and pinched appearance.

**Hutchinson's Triad:**

1) The previous dental abnormalities (a and b)
2) Recurrent Keratitis (leads to blindness)
3) Affection of 8\(^{th}\) cranial nerve (leads to deafness)

**Laboratory diagnosis**

a) **Microscopy**

1- Dark ground microscopy of unstained fresh preparation
2- Direct immunofluorescence: by fluorescein – labeled anti-treponemal Abs.
3- Fontana stained smear

b) **Serological tests**

* **Non treponemal tests**

In these tests using cardiolipin antigen(phospholipids from beef heart muscle, cholesterol&lecithin) for detection of non-specific antibodies called regain: **VDRL test & Wasserman test**
* **Treponemal tests**

Highly specific tests using T.pallidum as antigen for detection of antitreponemal antibodies

- FTA = fluorescent treponemal antibody test
- TPHA = T. pallidum haemaglutination test
- TPI = T. pallidum immobilization
- ELISA = to detect specific IgM or IgG

**Treatment:**

Penicillin is the drug of choice.

**Fusospirochaetal Diseases**

Treponema and Borrelia are normal flora of the mouth; mostly isolated from the deeper parts of the gingival crevice. Examples include: T. denticola, T. oralis, T. macrodentium, T. microdentium, B. buccalis and B. vincentii.

In debilitated children (nutritional deficiency or concomitant infection), the normal spirochaetes of the mouth, together with anaerobic fusiform bacilli find suitable conditions for multiplication (endogenous infection). They increase in number causing:

1) Acute necrotizing ulcerative gingivostomatitis (ANUG).

2) Vincent Angina: clinically simulating pharyngeal diphtheria and follicular tonsillitis. The ulcers extend to the pharynx and tonsils. There is pseudomembrane formation.

**Treatment:** Penicillin & correction of the underlying condition.
Actinomycetes

- Large filamentous gram positive bacilli.
- They are saprophytic live in soil.
- They cause Actinomycosis, Nocardia and Stretptomycyes.

Actinomycosis

A chronic suppurative and granulomatous disease. The organism is present as a normal flora in mouth, tonsils, intestine and vagina. Infection spreads by continuity forming sinuses discharging sulfur granules, which represent collection of the branching actinomycetes.

Clinical types of actinomycosis:

1) Cervicofacial: involving the face, neck and mandible.
2) Abdominal: involving the lungs and thoracic wall.
3) Thoracic: involving the lungs and thoracic wall.
4) Pelvic: in females using intrauterine device (IUD).

Diagnosis:

The sulphur granules are subjected to:

- Microscopical examination
- Culture under anaerobic condition, for 2 weeks.

Treatment:

Penicillin is the drug of choice

E.g. Cervicofacial actinomycosis:
This disease is manifested by painless swollen erythematous lesion in the Jaw, progressing to fluctuant mass draining fistulae and extends to adjacent tissue, bone and lymph nodes of head and neck. Multiple sinuses develop that discharge pus containing yellowish sulphur granules. Infection is extended to the adjacent tissues, e.g. gingival, palate and oral mucosa. Periostitis develops and rarely osteomyelitis. **Tooth extraction preceded by pyogenic infection favours the growth of actinomycosis.**
The genus Candida presents as normal commensals in the oral cavity, vagina and intestine. C. albicans (monilia) is an oval budding yeast which produce pseudo hyphae. It may predominate in these sites and cause superinfection (candidiasis).

**Predisposing factors to candidiasis:**

- After broad spectrum antibiotic
- Diabetes
- Steroid therapy
- Immunosuppression

Candidiasis is clinically manifested as oral & vaginal thrush, paronychia (nail infection) and may cause diarrhea in debilitated children on antibiotics. Progressive systemic candidiasis may occur in immunosuppressed patients affecting the lungs/or kidney.

**Laboratory diagnosis** :-

It depends on detection of Candida in large numbers in specimens from mouth, vagina, sputum… etc. Specimens are examined by:

1- Direct microscopic examination of smears stained with gram. This shows large gram-positive budding yeast cells with pseudohyphae.

2- Cultures are done on blood agar and Sabouraud's dextrose agar. Large cream coloured colonies develop after 2-3 days at room temperature.
**Oral Candidiasis**

Candida albicans is present in about 30% of mouths of adults as part of their normal oral flora. C. albicans is the most common cause of oral candidiasis. Oral Candidiasis may occur in the following forms:

1. **Acute Candidiasis:**
   
a) **Pseudomembranous candidiasis (thrush).**

   It occurs mainly in newborn infants who acquire the infection from the vagina during birth or through contaminated food bottles. Oral thrush may occur in adults suffering from debilitated diseases (cancer, diabetes …etc). The oral mucosa is covered by loosely adherent whitish patches or pseudomembrane that can easily be rubbed off leaving red raw bleeding painful surface.

b) **Acute atrophic candidiasis:**

   It occurs as a result of administration of broad spectrum antibiotics particularly tetracyclines that used topically in the mouth. There are red patches of atrophic red raw painful mucosa (resemble thrush after removal of the pseudomembrane).

2. **Chronic Candidiasis**

a) **Denture stomatitis (chronic atrophic)**

   The infection occurs in the palatal surface in contact with an ill fitting upper denture, which cut the mucous membrane from salivary antibodies (normal defense mechanism). C. albicans also colonizes the denture base forming a reservoir for repeated infection.

   The denture-bearing area of the palate appears oedematous red with sporadic white patches of thrush.
b) **Chronic hyperplastic candidiasis (Candida leucoplakia):**

It occurs most commonly among heavy smokers, the lesions appear as white adherent, raised patches that affect any part of oral mucosa, most commonly the tongue. After treatment of Candida infection by antifungal drugs, the hyperplastic epithelium persists, may ulcerate and is regarded as **potentially premalignant**.

**Diagnosis of oral candidiasis:**

The specimen is a swab from pseudomembrane or white patches and in case of denture stomatitis; samples from both the affected palatal mucosa and the opposing denture base should be collected and examined as previously mentioned.

**Treatment of oral candidiasis:**

- Antifungal drugs, e.g. nystatin mouth wash or lozenges.
- Correction of the predisposing factors, e.g. denture replacement, control diabetes…etc.
Part IV

Medical Virology
General properties of viruses

- Viruses are the smallest infectious agents known.
- They contain one type of nucleic acid (RNA or DNA) as a genome.
- They do not have protein synthesizing apparatus (ribosomes). So, they are metabolically inert and can only replicate inside the living cells.

Structure of viruses:

Each virus particle is composed of protein coat (capsid) which encloses nucleic acid and is called nucleocapsid.

Viral capsid:

It is composed of protein subunits called capsomers that arranged in a way to determine virus symmetry.

Viral capsid:

- Protect the viral genome (RNA or DNA)
- Participate in attachment of the virus to susceptible cells.
- Determine antigenicity and the antibodies that formed against the protein coat antigens neutralize virus infectivity. Protein coat antigens neutralize virus infectivity.

Viral nucleic acid (Genome):

- Viruses contain DNA or RNA but not both.
- It is the infectious part of the virus
- It carries the genetic information

Viral envelope:
- Some viruses are surrounded by a lipid or lipoprotein envelope that make these viruses sensitive to ether. The loss of lipids by ether results in disruption of virus and loss of its infectivity.

**Diagnosis of virus infections:**

1- **Direct detection of viruses or Ags in clinical specimens by:**

   a) **Microscopy**
      - Light microscopy to visualize large viruses as poxviruses.
      - Electron microscopy
      - Immuno-electron microscopy.
      - Immunofluorescent microscopy

   b) **Solid phase immunoassays (RIA)**
      - Radioimmunoassay
      - Enzyme linked immunosorbent assay (ELISA)

   c) Nucleic acid hybridization using DNA probes

   d) Polymerase chain reaction (PCR)

2- **Serological diagnosis through detection of antibodies in patient's serum**

   - Detection of IgM antibodies to the virus indicates recent infection
   - Detection of arising Abs titre to the virus, the first sample is collected early after infection and the second sample is collected 1-2 weeks later.
**Pathogenesis of virus infections:**

It refers to interaction of viral and host factors that leads to disease. The virus reaches the target cell (tropism), replicates and produces its effects on the cells.

Some viruses find their target cells at portal of entry and spread locally, e.g. Influenza virus in respiratory epithelium. The virus produce localized infection after short incubation period. Other viruses spread and reach a distant target cells, e.g. Polioviruses enter through GIT, pass to lymphatics then blood stream to reach CNS and spread along the nerve fibers, this occurs after long incubation period.

**Treatment of virus infection:**

- Antiviral drugs, e.g., Amantadine for influenza A infection

- Interferons, e.g., in treatment of HBV and also, interferons are used as anticancer agents.

![Diagram Illustrating the components of the complete virus particle (virion)](image-url)
Herpes viruses

DNA viruses, they establish life long latent infection which may be activated.

Types of herpes viruses:

1) Herpes simplex virus (HSV)
2) Varicella-Zoster virus (VZV)
3) Epstein–Barr virus (EBV)
4) Cytomegalovirus (CMV)
5) Human Herpes Virus 6 (HHV6)
6) Human Herpes Virus 7 (HHV7)
7) Human Herpes Virus 8 (HHV8)

Herpes Simplex Virus (HSV):

There are two types of HSV:

- Herpes Simplex Virus type 1 (HSV-1)
- Herpes Simplex Virus type 1 (HSV-2)

Herpes Simplex Virus type 1 (HSV-1):

It affects mainly the face causing:

a) Acute herpetic gingivostomatitis. (oral manifestations):

It is the most common type, affect children 1-5 years old. The lesion appears in gingiva, tongue occasionally the lips and skin around the mouth. Gingival ulceration precedes oral ulceration. Gingival ulceration is not accompanied by loss of marginal gingival nor interdental papillae. The submandibular and upper deep cervical L.Ns are enlarged.
b) **Herpes Labialis (Cold Sores)**

Vesicles and ulcers appear at the mucocutaneous junctions of the lips and nose. Recurrence usually appears in the same site.

c) **Keratoconjunctivitis:** corneal ulcers and conjunctival lesions. Recurrence causes blindness.

d) **Encephalitis and meningitis:** usually accompanied with fever and altered mental status.

e) **Herpetic whitlow:** infect fingers, commonly seen in dentists and nurses and thumb-sucking children.

**Pathogenesis of HSV-1:**

- HSV-1 is probably transferred by direct contact. At the site of primary infection in a broken skin, the virus replicates in the epithelial cells causing vesicular lesions that contains (serous fluid with the virus particles). When the vesicle ruptures, an ulcer is formed. This heals without scarring.

- After recovery of the primary lesion, the virus passes through the neuron to reach the sensory (dorsal root) ganglia "Trigeminal ganglion cells" where it remains latent.

- Exposure to stress or disease, e.g. influenza reactivates the virus from the latent state, the virus migrates down the neuron to reach again the skin, replicates and causes recurrent lesion.

**Herpes Simplex virus type 2 (HSV2)**

*It affects mainly the genitalia causing:*

a) **Herpes genitalis:** it is a venereal disease that causes vesicular lesions and ulcers in male and female genitals. It may be asymptomatic.
b) **Neonatal herpes** which presented by generalized disease or encephalitis in newborn.

c) **Cervical carcinoma**

**Pathogenesis of HSV-2:**

The same as HSV-1 but it differs in location. The virus is transmitted by: sexual contact and neonates get infection through birth canal during labour. The virus replicates in mucous membranes and skin of male and female genitals forming the **primary lesions**. After recovery the virus remain latent at the sacral ganglion cell.

**Laboratory diagnosis of HSV infections:**

1) Isolation of the virus: the vesicular fluid from the site of lesion is cultured on human or monkey cells.

2) Detection of HSV in vesicle fluid by electron microscopy

3) Detection of viral antigen by direct immunofluorescence.

4) Detection of viral DNA by PCR.

5) Serological diagnosis to demonstrate a rising Abs titre and shorten the duration of lesion.

**Treatment:**

- Acyclovir and vidarabine, which inhibit viral DNA synthesis and shorten the duration of lesion. It is available for topical, oral and IV use.

- For eye infection, topical trifluridine is effective.

**Prevention and control:**

- Avoid contact with vesicular lesions and ulcers.
• Vaccination: purified HSV- glycoproteins must be given before the primary infection.

**Varicella – Zoster Virus (VZV)**

This virus causes 2 different diseases:

Vericella = chicken pox in primary infection in children.

Zoster = shingles in reactivated infection in old age.

**Mode of infection:**

- Inhalation
- Direct contact

**Pathogenesis:**

VZV infects upper respiratory tract mucosa, then spreads via blood stream to reach the skin forming vesicular rash.

The lesion heals without scarring. The virus becomes latent in dorsal root ganglia, reactivated later when cell-mediated immunity is reduced. The virus causes painful vesicular skin lesions in different sites than that of primary infection.

**Clinical pictures:**

**Varicella (chicken pox):**

IP is 2 weeks. Common in children. Starts by fever, malaise then rash in the trunk spreads to head and extremities.

**Zoster (Shingles):**

It is common in adults (old age). It results from reactivation of latent varicella infection in the neurons. Painful vesicles appear unilateral along the course of sensory nerve of the head or the trunk. The patient can infect his susceptible contacts causing varicella.
**Epstein – Barr Virus (EBV)**

- EBV is the causative organism of **infectious mononucleosis** (glandular fever), characterized by sore throat, fever, skin rash, lymphadenopathy and marked leucocytosis.

- EBV – associated with some diseases:
  - Nasopharyngeal carcinoma
  - Burkitt's lymphoma

**Cytomegalovirus Virus (CMV)**

Infection occurs by close contact, sexual intercourse, breast feeding, transplacental (congenital), blood transfusion, and organ transplantation. The congenital infection may cause abortion or congenital abnormalities.

**Hepatitis Viruses**

Viral hepatitis is a viral infection in which the liver is the main target. The hepatitis viruses are: **hepatitis A (HAV)**, **hepatitis B (HBV)**, **hepatitis C (HCV)**, **hepatitis D (HDV)**, hepatitis E (HEV) and **hepatitis G (HGV)**. Hepatitis A and E are transmitted by feco-oral route, but B, C, D and G are transmitted parenterally.

**Other viruses causing hepatitis:**

These viruses cause inflammation of the liver as a complication in the course of their infection, e.g. yellow fever virus, CMV, EBV, rubella virus, HSV and entero viruses.

**Hepatitis 'A' Virus (HAV)**

It is a **single stranded RNA, non enveloped** virus.
Reaction to physical and chemical agents:

- It is resistant to:
  
  * ether and acid (as there is no envelope).
  
  * Heat at 60°C for 1 hour.

- It is destroyed by:
  
  * Autoclaving, boiling for 5 min or dry heat (160°C-1hr)
  
  * Ultraviolet irradiation
  
  * Formalin (1/4000 for 3 days at 37°C
  
  * Sodium hypochlorite 1%

Pathogenesis & Mode of transmission:

The virus is transmitted by the foeco-oral rout. HAV affects mainly children and young adults (5-15 y) in autumn. It enters liver. It damages the hepatic cells then the cells are repaired. No chronic infection occurs.

I.P: 2-4 weeks (shorter than HBV).

Clinical Picture:

Asymptomatic or acute hepatitis with fever, nausea, anorexia, vomiting followed by jaundice with dark urine and pale stool.

Recovery occurs spontaneously 2-4 weeks. No hepatocellular carcinoma.

→ HAV – IgM appears at the time of jaundice. After 1-3 weeks.

→ HAV – IgG appears and provides life long immunity.
Laboratory diagnosis:

1- Abnormal liver functions (liver enzymes are elevated).

2- PCR to detect viral RNA

3- Detection of anti-HAV IgM antibodies by ELISA or RIA which is diagnostic of acute illness.

4- Detection of anti-HAV IgG antibodies during convalescence and persists for years indicating immunity.

Prevention and Control:

1- Proper hygiene

2- Vaccine: inactivated HAV vaccine (Havrix) in 2 doses with interval of 6-12 months between the two doses.

3- Immune gamma globulin decreases the severity of the disease if given 1-2 weeks after exposure to HAV.

Hepatitis 'B' Virus (HBV)

It is a double stranded DNA with viral envelope causes serum hepatitis. Composed of outer shell of lipoprotein containing hepatitis B surface protein antigen (HBs Ag), which is important for diagnosis of infection and for vaccination and inner core antigen (HBc Ag) and a core related Ag (HBeAg).

Reaction to physical and chemical agents:

1- HBV is destroyed by:

   - 1% Na hypochlorite
   - 2% glutraldehyde
   - 70% ethanol
   - Boiling at 100°C for 1 hr
- Autoclaving at 121\(^{0}\)C for 30 min.

2- On the other hand, **HBV survives in** dried blood for long period. Heat stable pH – resistant.

**Mode of transmission:**

1- **Parenteral:** by blood transfusion and shared contaminated needles, tattooing, haemodialysis and shared razors and scalpels.

2- **Sexual intercourse**

3- **Perinatal:** from mother to newborn either transplacental or during birth.

**Pathogenesis:**

HBV pass through the blood reaching the liver cells where it replicates. Persistence of infection of HBV in hepatocytes leads to chronic carriers (in 10% of cases). Also, hepatocellular carcinoma may occur.

**Clinical Pictures:**

- Acute hepatitis: as that HAV but more severe.

- Chronic hepatitis: persistent infection may lead to liver cirrhosis and death.

- Extra hepatic manifestations, e.g., skin rashes and arthritis

- Hepatocellular carcinoma

- 90% of children that born from infected mothers are chronic carriers and 25% of these carriers will have hepato-cellular carcinoma.

**Laboratory diagnosis:**

a) Liver enzymes are elevated

b) Hepatitis B virus marker (HBV Ags and Abs) are used in diagnosis
• **HBsAg:**
  - Appears during the incubation period and acute disease.
  - Declines within a period of 3 months.
  - It's persistence for more than 6 months, Indicates carrier state, chronic hepatitis and hepatic carcinoma.

• **HBsAb:** it appears late after the disappearance of HBsAg. It indicates resolution of infection.

• **HBcAg:** it is not detected in serum. It is detected in the infected liver cells.

• **HBcAb:** in recent infection IgM antibodies are detected, but IgG persists indefinitely as a marker of past infection.

• **HBeAg:** starts to appear during the incubation period and present during the acute illness.

• **Anti-HBeAb:** its detection is a strong evidence of recovery.

The window phase: it is the period from about 6-8 months when neither free HBeAg nor its antibody can be detected. The best tool for diagnosis of an acute HBV infection during the window is the presence of anti-HBC IgM.

**Prevention:** Either by vaccine or hyperimmune globulin or health care:

1) **Recombivax vaccine:** its HBsAg produced in yeasts by recombinant DNA technology. It is recommended for:
   - All newborns as part of immunization schedule
   - Health care personnel
   - Patients receiving multiple drug transfusion or hemodialysis.
2) **Hepatitis B immune globulin (HBIG).**

It contains high titer of HBsAb. It provides passive protection to individuals exposed to infection, e.g., accidental needle prick, or infants born from infected mother. It should be given immediately post exposure together with HBV vaccine (passive – active immunization).

3) **Health care**

- Screening of blood donors
- Disinfecting instruments by autoclaving
- Use of disposable syringes

**Hepatitis 'C' Virus (HCV)**

The virus causes chronic hepatitis and hepatocellular carcinoma.

*Mode of transmission:*

The virus is transmitted mainly parenterally and also perinatal.

*Clinical picture:*

- Asymptomatic = Carriers
- Acute hepatitis: milder than other viral hepatitis cases.
- Chronic hepatitis: is more common and leads to cirrhosis and hepatocellular carcinoma

*Laboratory diagnosis:*

1) Liver enzymes are elevated
2) Detection of HCV antibodies in serum
3) Detection HCV – RNA by PCR
**Treatment:** Alfa interferon and ribavirin according to the clinical condition.

**Hepatitis Delta Virus (HDV)**

HDV can only replicate in patients with HBV infection. Infection is either a coinfection, the patient acquires both viruses at the same time, or a superinfection on top of a chronic HBV infection. The virus can be controlled through control of hepatitis B infection.

**Diagnosis:**

By detection of anti-HDV antibodies.

**Hepatitis 'E' Virus (HEV)**

An RNA virus characterized by foeco-oral transmission and a short incubation period (6 weeks). There is a little risk of development of chronic hepatitis, but there is a high mortality rate due to disseminated intravascular coagulation especially in pregnant females.

**Hepatitis 'G' Virus (HGV)**

It is widely distributed in humans and can be transmitted parentally or sexually. Most patients show evidence of co-infection with other viruses such as HBV or HCV.
RETROVIRUSES

Retroviruses are RNA viruses. They are termed retroviruses due to their possession of the reverse transcriptase enzyme which enable them to carry out reverse from RNA to DNA. They include:

1. Oncovirinae: which includes human lymphotropic viruses (type I & II)

2. Lentivirinae: the most important member is the human immunodeficiency virus HIV (type 1 and type 2).

Human Immunodeficiency Virus (HIV – 1,2)

- It is the primary etiologic agent of acquired immunodeficiency syndrome (AIDs)

- HIV-1 is world wide, HIV-2 is found primarily in West Africa.

Mode of transmission:

1- Sexual contact: in homosexuals and heterosexuals.

   The virus is present in semen, saliva, as well as all body secretion.

2- Percutaneous transmission by:

   a) Blood transfusion with infected blood or blood products

   b) Contaminated syringes, especially used by drug addicts, tooth-brushes, razors, tattooing …etc.

3- Transplacental: from mother to fetus.

Pathogenesis:

HIV has a selective tropism to CD₄ cells (T-helper cells) resulting in their destruction and depletion leading to reversal of CD4: CD8 ratio
which is normally (1.5) and changed to about (0.5) causing marked lowering of CMI.

**Clinical Picture:**

The entire course of the disease varies from 2-15 years including 3 stages:

1) **Stage of primary infection:**
   - It lasts 8-12 weeks.
   - Significant decrease of CD$_4$ cells.
   - Widespread of viruses all over the body.
   - Fever, sore throat and enlarged lymph nodes

2) **Stage of clinical latency:**
   - It lasts for 10 years.
   - The patients are infectious
   - No symptoms
   - Anti – HIV antibodies are increased associated with decrease of CD$_4$ cells.

3) **Stage of late symptoms:**
   - Continuous decrease of CD4 leads to depression of the immune status.
   - Invasion of the opportunistic microorganisms

*The opportunistic microorganisms include the following:*

a) **Fungi:** e.g., pneumocystis carinii, Candida albicans.

b) **Bacteria:** e.g., M. avium and Listeria monocytogenes
c) **Viruses:** CMV, HSV, EBV and adenovirus

d) **Protozoa:** e.g., toxoplasma gondii.

Other manifestations like malignant diseases or AIDS – related complex (ARC) in the form of lymphadenopathy, fever, night sweat, loss of weight and diarrhea.

**Laboratory diagnosis:**

1) Virus isolation and identification.

2) Detection of virus-RNA in serum by PCR.

3) Detection of HIV – antibodies by ELISA then confirmed by Western blot technique in positive cases.

**Treatment:**

1) Antiviral drugs

2) Treatment of opportunistic infection and tumours.

**Prevention & Control:-**

1- **Elimination of virus transmission by:**

   a) Screening of all blood-donors for HIV antibodies.

   b) Public health measures as:

   - Dental instruments should be well sterilized.

   - Avoid sharing of toothbrushes and razors.

   - Disposable syringes and equipments must be used.

   - Sex education programs

   - Programs to combat drug addiction.
2- **Vaccination**: various approaches to develop a proper vaccine are being investigated.

**N.B.**: The disinfection and inactivation of HIV are by the following methods:

- HIV is inactivated at room temperature for 10 min with:
  
  - 10% household bleach
  - 50% ethanol
  - 0.3% hydrogen peroxide
  - 0.5% Lysol

- Also HIV is inactivated by:
  
  - Extremes in pH (1.0 and 13.0)
  - Heating at 560°C for 10 minutes

**POLIOVIRUS**

- It causes disease named as poliomyelitis in man.
- Infection occurs by ingestion of contaminated food and drink.
- It causes paralysis in less than 1% of infected persons.
- Prophylaxis: 2 vaccines are available:
  
  - Salk vaccine: it is given subcutaneously (Formalin inactivated).
  - Sabin vaccine: it is given orally and provides better protection (living attenuated).

**COXACKIE VIRUSES**

*Cause many disease conditions, e.g.*:
• Herpangina: fever and vesicles on soft palate, uvula, pharynx and rarely the tongue. The vesicles rapidly rupture leaving ulcers covered with grayish white membranes. The disease is self-limited.
• Hand-foot and mouth disease: painful stomatitis with vesicular rash on the hand and feet.

MEASLES
• Acute highly infectious disease of childhood and non-immune adults.
• Transmitted by droplet infection.
• Incubation period: is 9-11 days followed by fever, sneezing, coughing, conjunctivitis and Kopliks' spots (small bluish yellow spots on the buccal mucosa opposite the lower molars).
• After 4-5 days, the characteristic skin rash appears.

Prevention:

By living attenuated vaccine which is given subcutaneously to children at 15 months of age, usually in combination with rubella (German measles) and mumps vaccines (MMR).

MUMPS
• An acute infectious disease results in a non-suppurative inflammation of parotid glands.
• Transmitted by droplet infection.
• Infection is followed by long-lasting immunity.

Prevention:

By living attenuated vaccine usually combined with measles and rubella vaccines (MMR).
RABIES

- It is a fatal disease that affects man.
- The virus is present in saliva of infected animal and is transmitted to man through the bite of such an animal.
- The virus replicate at the site of the bite propagated through the nerve to the CNS leading to fatal encephalitis.
- The incubation period varies from 2-16 weeks according to the site of the bite.

*Treatment:*

The bitten person must be managed by the following:

1) The wound is cleaned and anti-rabies immunoglobulin is given (local and I.M.)

2) Rabies vaccine is given in case of:
   a- The animal is not available
   b- The animal proved to be rabid
   c- Severe bites in the head and neck

   Human diploid cell vaccine is preferred than nerve tissue and duck embryo vaccines because it is devoid of allergic complications.
Part V

Dental Bacteriology
MICROBIAL FLORA OF THE ORAL CAVITY

Development of the Oral Flora

1) At birth:

The oral cavity is sterile during intrauterine life and at birth even though subjected to normal vaginal flora during delivery. Within 8 hours after birth, the mouth acquires organisms as well as the attendants. These organisms are aerobic and the most important members are Strept. salivarius, Staph. epidermidis and Neisseria spp.

2) After 6 months:

With eruption of deciduous teeth, the environment in the oral cavity changes inviting some anaerobic bacteria to establish themselves as Lactobacilli, Bacteroides, Spirochetes, Actinomyces and anaerobic streptococci.

The tooth surface itself allows the growth of 2 important members of the genus streptococci, these are Strept. Mutans and Strept. sanguis. With increase in the number of teeth and changes in the diet, the oral flora become more complex and more anaerobes become established.

3. Adult:

Permanent teeth are usually accompanied by various degrees of dental plaque and chronic periodontal diseases that influence the number and types of bacteria present. Aerobic organisms include Strept. mutans, salivarius, sanguis, and mitior, together with Staph. epidermidis. Neisseria spp., diphtheroids and sometimes Coliforms are present as E. coli and Kiebsiella spp., but usually as transient flora.

Mycoplasma can also be detected in the adult oral cavity and different species of anaerobic bacteria as Lactobacilli, Bacteroides,
Actinomyces, Veillonella and Spirochetes. Candida, as a member of the oral flora is present in 20-80% of adults.

Also, some protozoa may be detected in the oral cavity as Entamoeba gingivalis and Trichomonas temax.

4. Old Age: (Edentulous patients)

With loss of teeth, the number of microorganisms decreases specially Lactobacilli, Strept. mutans and yeasts. But with complete dentures usually yeasts increase again and so do Strept. mutans.

HOST DEFENSE MECHANISMS

Despite the presence of large number of organisms and many traumatic incidents, acute infections of the oral tissues are rare. This is a reflection of the host defense mechanisms that operate in the mouth as:

* **Mechanical Factors**

The flow of saliva, desquamation of the oral mucosa and the motion of the lips, cheeks and tongue, remove enormous number of microorganisms and help cleanse the surface of the teeth.

* **Chemical Factors:**

The saliva contains lysozyme, lactoferin and glycoproteins that aggregate certain microorganisms and prevent their attachment to teeth and oral mucosa. Also, the saliva contains secretory IgA interferes with adherence of organisms to oral tissues. This is the basis of vaccines against dental caries.

* **Gingival Sulcus:**

The fluid that seeps into the sulcus from gingival tissues contains IgG, IgA, IgM, complement, as well as phagocytic cells and neutrophils.
All contribute to normal host defense mechanisms within the gingival tissues.

*Saliva:

This is a mixture of secretions of the 3 salivary glands; parotid, sublingual and mandibular. The viscosity of saliva is due to its content of mucin, which is a glycoprotein.

Saliva is composed of 99.5% water and 0.5% solids of organic and inorganic substances. Salivary pH is between 5.7-7 (mean 6.7). This is kept constant by the action of more than one buffer system the most important of which is the bicarbonate system. Slight change in salivary pH can be detected after chewing gum, increase breath rate and fatigue.

Saliva is saturated with Ca phosphate. This is important because it prevents dissociation of calcified tissues of the teeth. If the pH of saliva is increased e.g. due to action of acidogenic bacteria, Ca phosphate may be precipitated and thus calculase is formed.

**Saliva has an antibacterial effect through:**

1. Lysozyme and peroxide, which cause hydrolysis of cell wall mureins.
2. Antibodies: secretory IgA is the predominant. It causes bacteria to agglutinate thus loosing its ability to adhere to tissues. Some bacteria can produce protease which breakdown IgA.
DENTAL PLAQUE

After cleaning of the teeth, a thin amorphous film starts to collect on the surface of the teeth called the acquired pellicle. It is formed of salivary glycoproteins adsorbed to the enamel.

Certain oral microorganisms including streptococci and actinomycetes adsorb to this pellicle. Other bacteria starts to bind to those already present and, within few hours, microcolonies and aggregates of bacteria can be observed. As organisms multiply, some produce extracellular polysaccharide polymers as glucans.

Microcolonies eventually coalesce to form a continuous mass of bacteria and polysaccharide polymers together with some salivary constituents, all firmly adherent to the surface of the tooth. This is the dental plaque. Thus dental plaque is a heterogeneous material of microbial flora and matrix that differ considerably with aging of the plaque as well as its site of formation.

The first organisms to attach are the aerobes including streptococci as Strept.sangius and mutans, as well as Neisseria. With maturation of the plaque, anaerobic bacteria start to appear as veillonella and actinomycetes. Later, fusobacteria, and Bacteroids colonize the plaque.

Bacteria make up about two thirds of the bulk of dental plaque and the number of organisms may be as high as $10^{10}$-$10^{11}$ bacteria per gram wet weight of plaque. Intracellular materials, of bacterial or salivary origin, make up to the plaque matrix.

Dental plaque which accumulates on the tooth above the level of the gingival margin is called supragingival plaque, while that formed below this level is called subgingival plaque. Their bacterial populations are different, so that supragingival can produce plaque, while subgingival
plaque induces periodontal disease. **Plaque, if not removed by tooth brushing or flossing, predisposes to calculus formation, periodontal diseases or dental caries.**

Saliva contains a supersaturated solution of calcium and phosphate. Bacteria (veillonella, neisseria, Strept. sanguis, and actinomycetes), present in saliva and plaque promote calculus formation.

*Calculus:*

The calculus is produced by calcification of supra and subgingival dental plaque. As the calcium phosphate deposits, the hardness of the calculus varies. Dental plaque may cover the established layer of calculus. Calculus contains a large variety of organisms. Strept.sangius and Actinomycetes predominates.

**Factors can affect plaque formation:**

1. Oral hygiene
2. Orthodontic or prosthetic appliances as they may interfere with oral hygiene.
3. Structure and position of the tooth. Roughness of enamel whether developmental or acquired encourages plaque accumulation.
4. Diet greatly affects plaque formation. Rough fibrous diet and the movement of mastication reduce plaque, while a sucrose rich diet encourages plaque deposition through sticky polymers.
Control of Dental Plaque:

1) Oral hygiene.

2) Diet: Plaque increase considerably in the presence of carbohydrates, especially sucrose. Limiting dietary carbohydrates has a dramatic effect on plaque volume.

3) Physical removal of plaque: e.g. tooth brushing, use of dental floss & interdental wooden wedges.

4) Ultrasonic scaling devices:
   - These remove plaque and calculus mechanically.
   - Ultrasonic vibration disrupts bacteria in subgingival plaque.

5) Antiseptics: Chlormexidine & providine-iodine reduce bacteria. Its action may extend for sometime.

DENTAL CARIES

Dental caries is the progressive and irreversible destruction of the hardest and most calcified tissues of the body, the enamel and dentin of the teeth. It is characterized by an initial demineralization of the inorganic material of these structures, followed the destruction of their organic components. Demineralization is due to the acids produced by the fermentation of dietary carbohydrates by bacteria found in the oral cavity. Once initiated, caries progresses into the enamel layer of the tooth and, eventually, to the dent enamel junction, where the infection spreads laterally to involve large numbers of dentinal tubules. If not treated, the lesion often progresses to the dental pulp.

Predisposing factors leading to Dental caries are:

1) Susceptible sites on the tooth.

2) Diet, especially fermentable carbohydrates.
3) Microorganisms.

1. **Susceptible Tooth:**

   Caries does not attack the teeth in a uniform manner; rather, certain areas of the teeth are more vulnerable than others.

   1. Pit and fissure caries affects and is initiated by the trapping of food debris and microorganisms in the pits and fissures that characterize these surfaces.

   2. Smooth surface caries affects certain sites on the smooth surfaces of the teeth. It is seen primarily on the interproximal surfaces teeth near the point at which they contact the adjacent teeth.

   3. Smooth surface caries is related to the formation of dental plaque, which holds microorganisms and their products in close contact with the tooth enamel.

      Some teeth are more susceptible than others as, upper teeth, posterior teeth, malposed and crowded teeth.

2. **Diet:**

   Carbohydrates were found to be the only food component that can cause caries. Carbohydrates between meals, is related to its cariogenic effect. Refined, carbohydrates are more cariogenic. Sticky carbohydrate is more cariogenic as they adhere to the tooth surface for a longer period.

   Monosaccharide, sucrose in particular, has been described as the main element for dental caries. This is probably due to:

   a. Its low molecular weight allows it to diffuse into the plaque.

   b. It is broken down rapidly by plaque bacteria to produce acids.
3. Microorganisms:

Bacteria that are related to development of caries are those which are:

a. Acidogenic and aciduric.

b. Isolated from all stages of caries development.

c. Able to produce caries when introduced in germ-free animals.

d. Can produce caries whether introduced into oral cavity or directly on the tooth.

Most studies point to Strept. Mutans as the initiator of caries and Lactobacilli contribute to the progression of the lesion.

Many theories have been but forward to explain dental caries as acidogenic theory, proteolytic theory and proteolysis chelating theory. The last two lack support. These theories emphasis only the role of proteolytic bacteria, which proved unable to produce caries alone in germ-free animals.

The acidogenic theory, on the other hand, stresses the role of acidogenic bacteria in caries production through its action on fermentable carbohydrates producing acids especially lactic acid. Acids cause demineralization of the enamel and the dentine. Proteolytic bacteria then can cause digestion of the organic dentine matrix. Dental caries is preceded by the formation of dental plaque.

Microbiology of Dental Caries:

The two most important genera of bacteria involved in dental caries are **Streptococci and Lactobacilli.**
Role of Streptococci:

Streptococcus mutans is the species of streptococci most incriminated in dental caries as:

- There is a strong positive correlation between the presence and numbers of Strept. mutans in plaque and the development of caries.

- Strept. mutans produces glucosyltransferase which synthesize glucan polymers from sucrose. These water insoluble glucans contribute to plaque development and microbial colonization of the smooth surface of the teeth.

- Strept. mutans produces higher levels of acids than other organisms in plaque and remain metabolically active at low pH (aciduric).

- Strept. mutans stores large amounts of intracellular polysaccharide that may be used when dietary carbohydrates are not available.

- They only appear after tooth eruption and disappear after teeth loss.

- Strept. mutans are unable to colonize mucosal surfaces of the oral cavity, but colonies only certain sites on the teeth which readily become diseased. There is limited movement of Strept. mutans in the oral cavity.

- The number of Strept. mutans in samples taken from carious areas are higher than those from adjacent normal surface areas.
Role of Lactobacilli:

Lactobacilli are important in dental caries. They are present in large numbers in carious plaque and their count in saliva is taken as an index of caries activities. Lactobacilli are also acidogenic. Although lactobacilli produce less acids than Strept. mutans, they are highly aciduric (pH less than 4). Since lactobacilli are present in saliva more than in plaque and are unable to produce sticky polymers and have low affinity to teeth surfaces, it is said that lactobacilli do not initiate caries but are secondary invaders and contribute to the progression of caries. Lactobacilli can produce pit and fissure caries.

Role of other Bacteria:

- Actinomycetes are involved in plaque in gingival margin, thus can initiate root caries.
- They are also more resistant to fluorides than other oral bacteria.
- Veillonella can metabolize acids including lactic acid thus reducing caries.

Prophylaxis against caries:

1. Good oral hygiene with frequent brushing and flossing.
2. Topical use of antiseptics, by oral gurgle and mouth rinse to reduce the bacterial load in the oral cavity.
3. Sometimes antibodies are used to destroy cariogenic bacteria.
5. Modification of diet to avoid or reduce fermentable sugars.
6. The use of fluoride in dental paste:

Fluoride has more than one mechanism of action:
a. At low concentration, fluoride inhibits bacterial enzymes (Enolase) that ferment sugars to produce acids.

b. At higher concentration it inhibits bacterial growth.

c. At higher concentration, it unites with the organic matter of the enamel (hydroxyl apatite) to produce fluoro-apatite which is more resistant to acid.

d. Chronic high concentration produce blackening of the teeth (Fluorosis)

7. Sealants can be used to seal pits and fissures thus decreasing the number of sites capable of harboring bacteria.

8. Immunization against dental caries:

   Strept. mutans is the important organism causing dental caries, so trials have been made to produce vaccines containing this organism or its produces to enhance the immune response of the body against this organism.

   **The vaccines available are:**

   1. Formaline-killed suspension of Strept. mutans in a capsule taken orally.

   2. Mutants of Strept. mutan has been isolated which lack the enzyme lactate dehydrogenase. These do not produce acids but can colonize the oral cavity. They can be used in replacement therapy. Replacement therapy by this mutated strain must be supplied either.

      - Prior to colonization with Strept. mutans.
      - After reduction of Strept. mutans by iodine application.
PERIODONTAL DISEASES

Diseases of the periodontium affect those tissues that support and anchor the teeth. These include the gingiva, the periodontal ligament, the cementum and the alveolar bone.

Periodontal diseases:

1- Acute gingivitis may be seen primarily in young adults as acute necrotizing ulcerative gingivitis (ANUG), which is characterized by pain and bleeding commonly with grayish membrane covering the margins of the affected gingiva.

2- Adult periodontitis with formation of periodontal pocket leading to accumulation of debris and plaque formation. The periodontal ligament is eventually destroyed and resorption of alveolar bone is seen resulting in increased mobility and loss of affected teeth.

3- Localized juvenile periodontitis is an uncommon form of periodontal disease, which affects permanent molars and incisors with reduced number of bacteria compared to the adult one.

Microbial pattern of periodontal diseases

Oral microorganism play a role in the initiation of gingivitis and destructive periodontal disease. Also, the gingival inflammation can be reduced or eliminated by antimicrobial agents. Organisms normally present in gingival sulcus are gram-positive species primarily Streptococci and gram negative cocci as Veillonella.

Acute narcotizing ulcerative gingivitis is characterized by increased number of spirochetes and anaerobic gram-negative bacilli. On the other hand, about 75% of bacterial population found in the gingival sulcus in adult periodontitis consists of gram negative organisms mainly...
obligate anaerobes as Porphyromonas, Prevotella, Bacteroides, Fusobacterium and Actinobacillus. Porphyromonas produces several proteases that may contribute to the tissue damage seen in periodontitis. Actinobacillus and Actinomycetes are present in high number in localized juvenile periodontitis. They produce several substances including leucocidin and endotoxins that contribute to the pathogenic processes.

**Pathology of Periodontal Disease:**

The disease usually begins by dental plaque, which consists of a mass of bacteria that are strongly attached to the tooth surface. These organisms start the inflammatory process by production of enzymes and endotoxins that are chemotactic for cells like polymorphs and lymphocytes and results in oedema fluid that accumulates in dental cervices. The lysozomal enzymes released from polymorphs result in tissue destruction, which may affect the periodontal ligaments and lead to loose tooth.

**Infection of the dental pulp and peripheral tissues (Pulpitis)**

**Infection can reach the pulp through the following routes:**

1. Extension of caries through the enamel and dentin into the pulp chamber.

2. Infection can reach the pulp from a nearby periodontal pocket.

3. Exposure of the pulp by surgical procedure.

4. Infection can reach the pulp through blood stream.

The end result of this pulpitis depends on virulence of the organism. Highly virulent organism will produce large volume of inflammatory
exudates that may lead to interference with blood supply to the tooth causing necrosis and death of the tooth.

The infection may spread to the periapical tissues resulting in periapical abscess and a sinus may be produced through which pus can come out.

Another possibility is that infection may be not so acute, resulting in formation of a chronic granuloma in the periapical tissues. The organism involved in this pulp infection is usually one of the oral flora or one of organisms in dental plaque. In majority of cases anaerobic organisms are usually the causative organisms. The strict anaerobes include Porphyromonas endodontalis, P. gingivalis and Prevotella intermedia (all of which produce black colonies on blood agar) and Peptostreptococci.
Viruses Secreted In Saliva

1. Herpes simplex 1 and rarely 2
2. Mumps
3. Cytomegalovirus (CMV)
4. Measles
5. Rabies
6. Hepatitis B virus (HBV)
7. HIV which causes AIDS

Viruses Transmitted by Contact With Blood

HBV & HCV & HDV & CMV & EBV & HIV.

Surgical infections & infections related to hard tissues:

Ludwig’s angina:

- A rapidly developing cellulitis. Spreads above and below the mylohyoid.
- Resulting from infection (usually a dental abscess) spreading posteriorly into the tissue spaces.
- There is marked fever & oedema of pharynx and larynx.
- May result in death from asphyxia.
- Anaerobes especially P. melaninogenica and B. gingivalis are isolated from infected tissues.
**Pericoronitis:-**

- Inflammation of tissues surrounding a partially erupted mandibular third molar.

- Bacteria involved are usually the result of multiplication of organisms in this area of stagnation resulting from the plentiful supply of nutrients.

- P.melaninogenica and B.gingivalis are dominant.

- Mechanical cleaning is necessary prior to surgical removal of implanted tooth.

**Infected surgical Wounds:-**

- Not common after intraoral surgery despite the large number of bacteria that surround the wound edges.

- Facial incision are more commonly infected than wounds in the mouth. Staphylococci are the commonest cause of infections.

- Oral streptococci, staphylococci and anaerobes are also cause wound infection in the mouth.

- Drainage and antibiotics are recommended.
Causes of Ulcers in the Oral Cavity

Oral ulceration may be due to:


b. Microbial infections:
   - Bacterial: acute necrotizing ulcerative gingivitis, tuberculous, syphilitic and gonococcal ulcers.
   - Fungal: candidiasis
   - Viral: herpes simplex, herpes zoster, herpangina, hand, foot and mouth disease, infectious mononucleosis and AIDs.

c. Malignant neoplasm: squamous cell carcinoma.

d. Immunologic reaction: aphthous ulcers, Behcet's syndrome, pemphigus vulgaris, mucous membrane pemphigoid, bullous pemphigoid, Lichen planus, epidermolysis bullosa, Lupus erythematosus and drug eruption.


F. Gastrointestinal disease: celiac disease, ulcerative colitis and Crohn's disease

g. Drugs: cytotoxic drugs as anticancer drugs.
PRACTICAL MICROBIOLOGY
Microscopy

Types of Microscopes

- Student microscope.
- Dark ground microscope.
- Electron microscope.
- Fluorescent microscope.

The student microscope:

- To examine unstained specimens use the low and high power lens (magnifying power is 10 and 40 respectively), with the condenser is screwed down.

- To examine stained specimens use the oil immersion lens (magnifying power is 100), with the condenser is screwed up.

Types of Stains:

- Simple stain (e.g. methylene blue & fucchsin).

- Differential Stain.
  - Gram stain.
  - Zeihl –Neelsen stain.

Gram Stain:

Prepare a smear for staining then:
- Cover the smear with methyl violet for 30 seconds.
- Pour off the methyl violet.
- Wash with iodine solution.
- Add iodine & leave for 1 min then pour it.
- Add 95% alcohol & reapply it till no violet colour comes off.
- Wash with water.
- Counter stain with basic fuchsin for 30 seconds.
- Wash with water then leave to dry.
- Place small drop of immersion oil on the smear.
- Examine with oil immersion lens.
- Rack condenser up.

**Zeihl–Neelsen Stain:**

Prepare a smear for staining then:

- Flood the smear with strong carbol–fuchsin for 5-10 min. intermittently don’t allow the stain to boil or dry.
- Wash with water.
- Flood the smear with 3% hydrochloric acid in 95% alcohol or 20% H₂SO₄ allow to act for 1 min., then wash with Water.
- Repeat the process several times till it becomes pale.
- Wash thoroughly with water
➢ Flood the smear with 95% alcohol for 2 min. (This step is only to be used when using H$_2$SO$_4$).

➢ Wash with water

➢ Counterstain with methylene blue for 2 minutes

➢ Wash with water

➢ Dry and examine by oil immersion lens.

**Types of Culture media:**

There are several types of media which vary in their chemical composition and nutritive value:

I. *simple media*

a) **Peptone water**

- It is composed of 1% peptone and 0.5 NaCl.
- It’s the base for sugar media and for indole production tests.

b) **Nutrient broth**

- It is composed of meat extract to which is added peptone water; it is a clear yellowish fluid media which becomes turbid when bacteria grow in it.

c) **Nutrient agar**

i. It is composed of nutrient broth to which we add a solidifying agent (Known as Agar Agar which is certain Seaweed).

The agar melts at 100 °C and remains liquid till 45 °C when it solidifies.

II. *Enriched media*
Some bacteria are fastidious and their growth requires the presence of highly nutritive substance e.g. blood, serum or egg.

a) Blood agar

- It’s composed of nutrient agar to which is added 10% sheep, horse or human sterile blood at 55 °C then it is poured in plates or tubes to be solid at 45 °C.
- It’s opaque and red in colour.
- Some bacteria cause zones of clear haemolysis around their colonies e.g. Staph. aureus and Strept. pyogenes. Other bacteria cause greenish discoloration of blood e.g. Viridans strept and Pneumococci.

b) Chocolate Agar

- It is prepared like Blood agar then the temperature of the medium is raised to 100 °C before pouring.
- It is suitable for the growth of Neisseria and Haemophilus groups.
- It is opaque brown in colour.

c) Loffler’s serum

- It’s composed of three parts sterile sheep or horse serum and one part glucose broth inspissated at 80 °C for two hours.
- Its opaque white suitable for the growth of diphtheria bacilli.

III. Selective media

- These media contain substances that inhibit all unneeded bacteria and leave the needed one.
- These substances may be chemicals, dyes or antibiotics.

a) Lowenstein Jensen medium (L.J.):
- It is composed of beaten egg; mineral salts and malachite green. The latter inhibit the growth of bacteria other than T.B.

- The medium is rendered solid by heating in the inspissator at 80 °C for one hour.

- The medium is opaque green suitable for growth of Tubercle bacilli.

b) **Blood tellurite**

- It is composed of blood agar to which we add potassium tellurite.

- It is suitable for growth of diphtheria bacilli and can be helpful in differentiation of the types of diphtheria.

(c) **Thayer-Martin medium**

- It is composed of chocolate agar which contains many special growth factors required by N.gonorrhoeae.

(d) **T.C.B.S**

- It is composed of thiosulphate, citrate, and bile as selective substance and sucrose as test sugar.

- Bromothymol blue as indicator give yellow colour in acidic pH.

- T.C.B.S is a green semitransparent medium suitable for growth of V.cholerae.

IV. **Enrichment media**

a) **Selenite broth**
• It is a liquid medium that inhibits coliform bacilli but permits bacilli of the typhoid group to grow freely.

b) Tetrathyonate

• It’s a liquid medium which inhibits coliform bacilli but permits bacilli of the typhoid and schigella.

V. Differential (indicator) media

This media contain a substance that is changed visibly as a result of metabolic activities of particular organisms e.g. MacConkey’s medium.

• MacConkey’s medium is composed of lactose as a test sugar and Neutral red as an indicator that changes pink in the presence of acid

That produced as a result of the lactose fermentation. It also contains bile salts that inhibit non intestinal bacteria. It is a reddish transparent medium. This medium can differentiate between:

1) The lactose fermenters which give pink colonies (coliforms group).

2) The non lactose fermenters which give pale colonies (salmonella and schigella).

VI. Sugar media

Bacteria vary in their fermentative action on different sugars. This property is used in the identification of bacteria.

N.B. Some media are both selective and enriched e.g. L-J, blood tellurite. Others are both selective and indicator e.g. MacConkey and T.C.B.S.

ANAEROBIC CULTIVATION

The anaerobic bacteria include clostridia, bacteroides and actinomyces.
Anaerobioses can be achieved either by:

1. Complete removal of oxygen e.g. anaerobic Gas Pack Jar.

2. The addition of reducing substance to culture media Example:

   a) Robertson’s cooked meat medium (the reducing substances are haematin and glutathione are present in meat).

   b) Thioglycollate broth which contains sodium thioglycollate as a reducing substance.

The two media (a) and (b) are suitable for growth of clostridium group and other anaerobes.

Identification of Bacteria

The main lines of identification are:

1) **Microscopic examination:**

Examination of unstained preparations will help in demonstrating motility. While examination of gram stained preparation will determine the staining reaction of the organisms (G+ve or G-ve), their morphology, size and arrangement.

2) **Cultural appearance:**

This includes the colony morphology, its size and shape, whether it’s opaque or translucent, mucoid or dry. Pigment producing organisms will have colored colonies, if it is an endopigment producer organism as in Staph. Aureus (Golden yellow). If it is an exopigment producer, the colour will diffuse in the surrounding medium as in Ps.pyocyanea colonies. Proteus has a characteristic swarming broth in solid media.

On blood agar, some organisms cause complete haemolysis of red blood cells (Clear zones of haemolysis) e.g. Staph. aureus and Strept.pyogenes. Others may cause partial haemolysis or greenish discoloration of blood agar.
e.g. Viridans, strept and pneumococci. On MacConkey’s medium we can differentiate lactose fermenters (pink colonies) from non lactose fermenters (Pale colonies).

3) **Biochemical reactions**

   **a. Sugar fermentation**

This method depends on the varying ability of bacteria to ferment sugar with acid production + or – evolution of gases.

It is tested for by growing the organisms on peptone water to which 1% sugar is added, which usually include glucose, lactose, maltose, mannite and sucrose Andrade’s indicator is added which changes to pink if acid is produced. An inverted (Durham tube) is immersed and the collection of bubbles at its apex reveals gas formation.

   **b. Indole production**

This test demonstrate the ability of certain bacteria to decompose the amino acid tryptophane- present in peptone- to indole, tested for by adding Kovac’s reagent which gives pink ring in presence of indole (indole +Ve) at 37°C for 24 hours.

If a yellow ring is produced, it is considered indole negative.

   **c. Voges Proskuer’s reaction (V.P.).**

Some bacteria ferment glucose with the production of a substance. The substance when tested gives an eosin- pink colour.

   **d. Methyl red (MR)**

Some bacteria have the ability to produce large amount of acid on fermentation of glucose thus lowering the pH of the medium below 4. After incubation of the organism on glucose at 37 °C for 24 Hours few drops of the methyl red indicator are added. A positive test gives bright red colour & negative test gives a yellow colour.

M.R. is +Ve & V.P. is –Ve in case of foecal pollution of water.
e. Urease test

Proteus organism grow on a medium containing urea and phenol red indicator will turn the medium deep pink after 4 to 24 hours due to production of Urease enzyme.

f. Catalase test

Staphylococci colony immersed in a drop of hydrogen peroxide on a slide will produce rapid effervescence indicating oxygen production due to catalase enzyme produced by Staphylococci.

g. Oxidase test

Some bacteria e.g. vibrio and pseudomonas produce oxidase enzyme that reduce the oxidase reagent to deep purple colour.

h. Commercial kit system

1- The API system for the identification of the organisms.

2- Automated bacterial identification system

The Vitek system is one of the main systems used to determine the presence of growth as well as identifying the organism and its antibiotic sensitivity.

4) Serological identification by antigen antibody reactions

5) Animal inoculation
The laboratory animals are used to identify certain pathogenic bacteria e.g. tubercle bacilli which produce characteristic lesions when injected in laboratory animals.

6) Bacteriophage typing

7) Molecular identification and typing methods This involves the detection of microbial nucleic acid.
Staph. in culture

Staph. in pus

Staph. endopigment on nutrient agar

Catalase test (positive)
Slide coagulase test

Tube coagulase test

Bacteriophage typing
Strept. in culture

Strept. in pus

B-haemolytic organism on blood agar (C1)
α-haemolytic organism on blood agar (C2)
Non-haemolytic organism on blood agar (C3)
Bacitracin sensitivity test

Antistreptolysin-0 test (titre: 1/400)

Pneumococci in tissue

Optochin sensitivity
Neisseria in culture
Pathogenic neisseria in pus

West's nasopharyngeal swab
Chocolate agar
Oxidase test

Diphtheria bacilli (Gram stain)

Diphtheria bacilli (M.B. Stain)

Ordinary swab
Löffler’s serum

Elek’s test

Diphtheroides

B. anthracis in culture (Gram stain)
B. anthracis (Spore stain)

Clostridium tetani

Cooked meat medium

Gaspak system (anaerobic jar)
M. tuberculosis (Z.N. stain)  
L-J medium  
M. leprae (modified Z.N. stain)
Gram negative bacilli

Klebsiella in tissue

Lactose fermenter on MacConkey’s medium (Left) and Lactose non-fermenter (Right)
Indole test

Sugar fermentation test (different effects)

Effects of different bacteria on TSI agar
API – 20E

Proteus on nutrient agar (swarming growth)

Urease test
Vibrios on T.C.B.S.

Vibrios Gram stain

Pseudomonas exopigment on nutrient agar
Vincent anigna

Spirochetes
(Dark field microscope)

Spirochetes
(Fontana stain)

Borrelia in blood film
(Leishman stain)
Candida (Gram stain)

A mixture of staph. & Gram negative bacilli

Single radial immunodiffusion
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