وزارة التعليم العالي والبحث العلمي / الجامعة التقنية الشمالية /المعهد التقني الموصل
التخصصات / الطبية (قسم التخدير) السنة الدراسية الأولى
أسم المادة : البكتريا الساعات الأسبوعية (2 نظري و 2 عملي) عدد الوحدات 6
اسم المدرس : زينة يحيى قاسم

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أهداف المادة :-

الأهداف العامة أن يكون الطالب قادراً على معرفة مبادئ الأحياء المجهرية وتطور هذا العلم والعلاقة بين الإصابة والاحياء المسببة للأمراض

الأهداف الخاصة :-

أن يكون الطالب قادراً على:

1- التعرف على الكائنات الحية الدقيقة
2- تعداد الفروع الرئيسية لعلم الاحياء الدقيقة الطبية
3- يميز الطالب بين الخلية حقيقية النواة وبدائية النواة
4- يذكر الأنواع الممرضـــة الرئيســـة للكائنات الدقيقة في غرفة العمليات ووحدة العناية المركزة
5- يعرف الفطريات والفايروسات المسببة للأمراض
6- استخدام المجهر لفحص البكتريا
7- يتعلم زراعة البكتريا

Lecture 1

History for Microbiology

The first named **Protista** by Haeckel 1866 , and divided in to three groups
1. Prokaryotes 2. Eukaryotes 3. Viruses .

- Antony Van Leeuwenhoek gave description of various types of bacteria , and also invented simple microscope in 1683 .
- Louis Pasteur 1857 known (Father of Microbiology) , he established that fermentation was the result of Microbial activity . He introduced techniques of sterilization and developed steam sterilizer , Hot air oven and Autoclave . He developed vaccine for hydrophobia .
- Robert Koch 1876 known (Father of bacteriology) , Isolated Bacillus anthracis , fixed bacteria , staining , isolated bacteria from the solid media , at 1882 discovered the T.b. and discovered the vibrio at 1883 .

Branches of microbiology

Divided in to Four branches:

1. Medical Microbiology
2. Industrial Microbiolog
3. Food Microbiology
4. Soil Microbiology

What is Medical Microbiology?

"The study of micro-organisms (including bacteria, viruses, fungi and parasites) which are of medical importance and are capable of causing diseases in human beings" .it deals with etiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection.

Medical Microbiology include six sciences :-

1. Parasitology : deals with the study of parasites causing diseases in human
.
2. Mycology : deals with the study of fungus causing diseases in human
.
3. Bacteriology : deals with the study of bacteria
.
4. Immunology : is concerned with mechanism involved in the development of

resistance by body to infectious diseases .

5.Genetics : is the study of heredity and variations .

6. Virology: is the study of viruses .

Classification of Microorganisms :-

1. Fungus

2. Protozoa

3. Bacteria

4. Algae

5. Rickettsia

6. Mycoplasma

7. Viruses.

Rickettsia :They are minute organisms having properties in between bacteria and viruses .

Mycoplasma : they are bacteria that lack to cell wall , and so do not possess a stable morphology They are round or oval bodies with interlacing filaments .

Definition of the <u>Bacteria</u>:

Bacteria are unicellular free living organisms , with out chlorophyll , having both RNA and DNA , capable of performing all essential processes of life , growth , metabolism and reproduction . They have rigid cell wall containing muramic acid.

Shape of bacteria

On the basis of shape , bacteria are classified as under :-

1. Cocci are spherical shape or oval cells .

On the basis of arrangement

a. Cocci in cluster ---------- Staphylococci
b. Cocci in chain ----------Streptococci
c. Cocci in pair ---------- Diplococci
d. Cocci in groups of four
e. Cocci in groups of eight

2. Bacilli (rod or cylindrical shape)

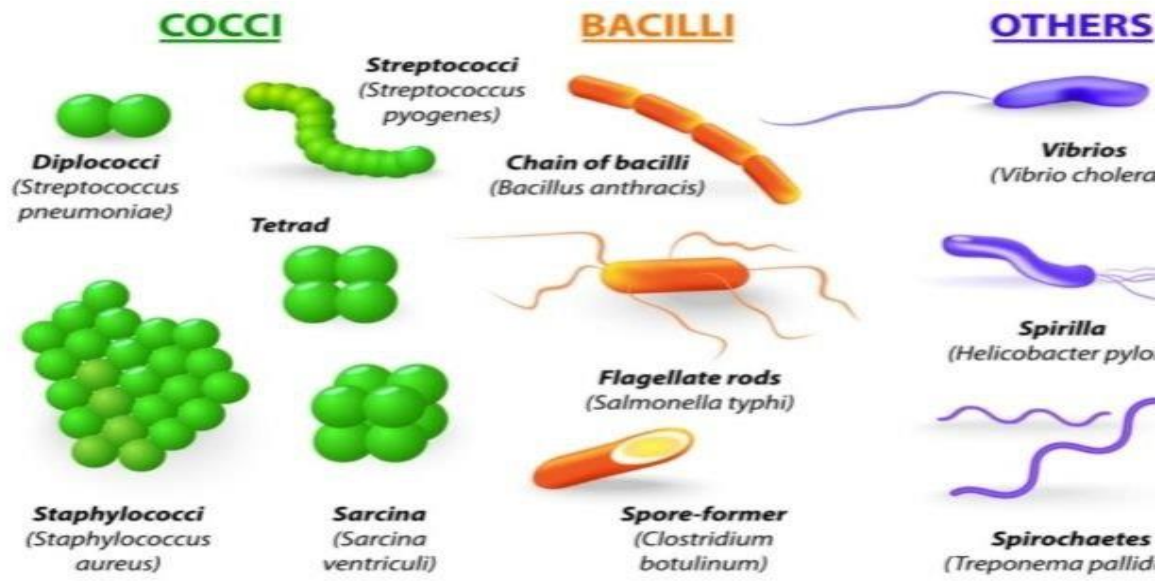
3. Vibrio are comma shaped curved rods

4. Spirochaetes several coils and flexible

5. Chinese letter

6. Actinomycetes (branching filamentous bacteria)

7. Mycoplasma (Do not posses a stable morphology, because lack cell wall) . appears round or oval bodies with interlacing filaments.



Comparison between Eukaryotic and Prokaryotic cells

Differences between	Prokaryotes	Eukaryotes
Examples	Bacteria, green Algae	Fungi, protozoa Slime moulds
<u>Nucleus</u> Nuclear membrane Nucleolus Chromosome Mitotic division	 Absent Absent One Absent	 Present Present More than one Present
<u>Cytoplasm</u> Mitochondria Golgi apparatus Endoplasmic reticulum	 Absent Absent Absent	 Present Present Present
<u>Chemical composition</u> Steroids Muramic acid	 Absent Present	 Present Absent

Lecture 2

General structure of bacteria

The general structure of bacteria include:-

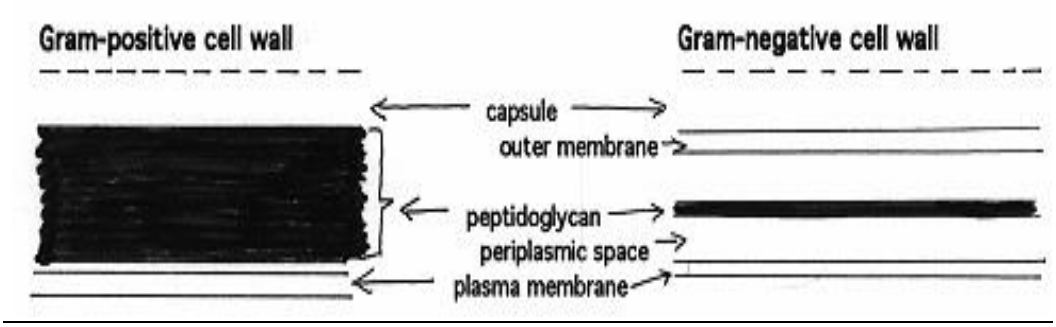
1- Capsule slime layer: It is gelatinous secretion of bacteria which organized as a thick coat a round cell wall.

Function: -

1. Protection a against deleterious agents example lytic enzymes.

2. Contribute to the virulence of pathogenic bacteria by inhibiting phagocytosis.

2-Cell wall: Is the outer most supporting layer which protects the internal structure .Cell wall composed of mucopeptide (muerin), scaffolding formed by N- acetyleglucosamine and N- acetyle muramic acid molecules alternating in chain cross linked by peptide chain .



Lipid	2-4%	15-20 %
Techoic acid	present	absent

Function:

1. Protection of internal structure, (supporting layer) .

2. Gives shape to the cell.

3. Role in division of bacteria.

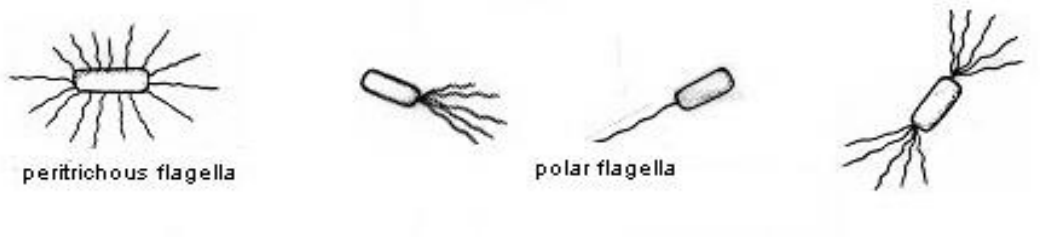
4. Offers resistance to harmful effect of environment.

* Cell wall synthesis may be inhibited by many factors.

Lysozyme: enzyme present in many tissue fluid cause lysis of bacteria.

They act by splitting cell wall mucopeptide linkages.

Flagella: These are long, sinuous, contractile filamentous appendages known as flagella. They are organ of locomotion. They are antigenic and composed of protein.



Salmonella , E. coli — lophotrichous — monotrichous — Amphitrichous

Pseudomonas — Vibrio — Alcaligenes faecalis

Pilli (fimbriae) : They are filamentous , short, thin, straight, hair like .
(0.5 *M* long less than 10nm. Thick).

Function:
1. Organ of adhesion.
2. Conjugation tube through which genetic material is transmitted from doner to recipient cell.
3. They are antigenic.

Differences between Flagella and pilli.

Flagella	Pilli
1.size : larger, thicker .	1. smaller and thinner .
2. Arise from cytoplasm or Cytoplasmic membrane, but not Attached to cell wall .	2. Attached to cell wall .
3. organ of movement .	3. organ of adhesion and conjugation .
4. They are not straight .	4. They are always straight.

Ribosome: These are ribonucleoprotein granules . they are sites of protein

synthesis, measuring (100-200) Angstrom unit .

Mesosome: Sites of respiratory enzymes in bacteria .

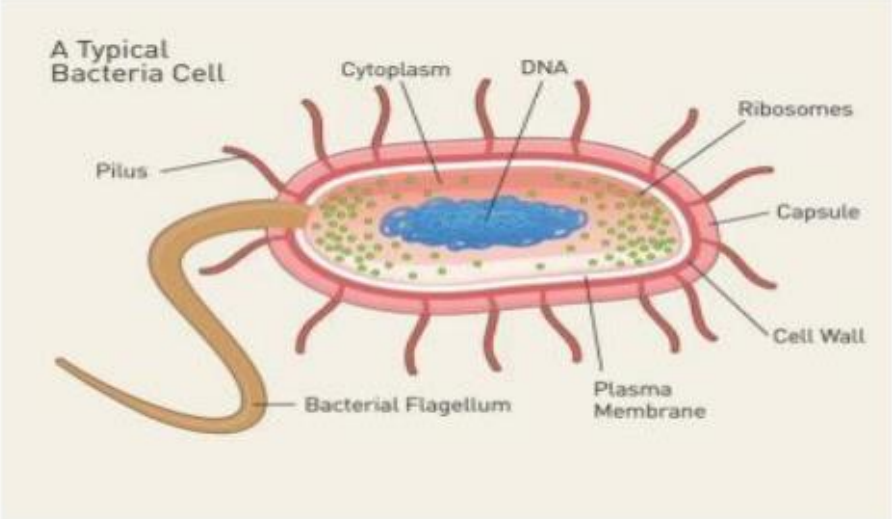
Cytoplasm: Bacterial cytoplasm is suspension of organic and inorganic solutes in viscous watery solution.

Cytoplasmic membrane: It is thin semi permeable membrane which lies just beneath the cell wall.

Function:

1. Controls in flow and out flow of metabolites to and from protoplast.

2. Presence specific enzyme (permease), plays important role in passage through membrane.



The Typical bacterial cell

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Bacterial spores

Spores: They are highly resistant dormant state of bacteria found in certain genera ex. *Bacillus* and *Clostridium* they are not destroyed by ordinary methods of boiling for several hours they are killed when autoclave at 151b pressure at 121 Ċ for 20 mts.

Function:- They make survival of organism possible under unfavourable condition like dry state spores are resistant to heat ,drying , freezing and toxic chemicals for demonstrate the spore used Gram stain, Modified Ziehl- Neelsen method .

Spore may be

1-Central bulging
2-Central not bulging
3-Sub terminal bulging
4-Sub terminal not bulging
5-Terminal spherical
6-Terminal oval

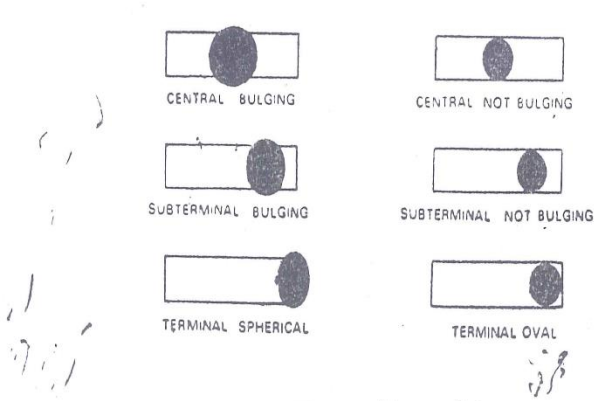
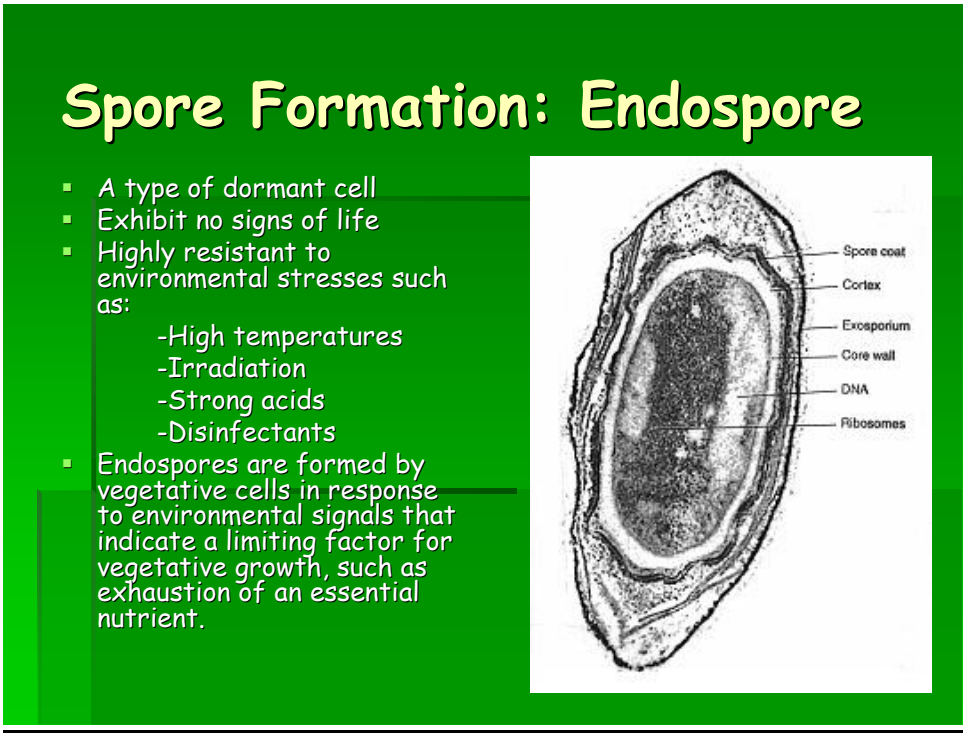


Fig. 9. Types of bacterial spore.



Lecture 3

Nutritional requirement of bacteria

Bacteria may require adequate nutrition of optimum , pH, Temperature and oxygen for multiplication and growth. Bacteria can be classified on the basis of their ability to synthesize essential metabolites in to :-

a. Autotrophic : depends on inorganic sources , use carbon dioxide as the main source of carbon and simple inorganic salts to form new protoplasm of the cell.

b. Heterotrophic : depends on organic compounds , ex. Protein, peptones , amino acid, vitamins and growth factor are supplied from out side .

(Obtain food by eating ex. E. coli)

- Most pathogenic bacteria in human are heterotrophic.

c. Phototrophic gets energy from photochemical reaction, using solar energy e.g. Cyanobacteria .

d. chemotrophic gets energy from chemical reaction , using chemical energy e.g. Archaebacteria .

Nutritional requirement of bacteria: -

1. Minerals.
2. Gas requirement.
3. Moisture.
4. Accessary nutritional requirement.

Physical factors affecting on the growth of bacteria

1. Temperature

2.Oxygen

3. Moisture

4. Osmotic pressure

5. Hydrogen ion concentration (pH)

6. Light

1-**Temperature**: On the basis of temperature degree divided in to 3 groups:-

a. Psychrophilic: grow between (0 – 25° C), mostly soil and water bacteria.

b. Mesophilic: (20- 45° C) includes bacteria producing disease .

c. Thermophilic : (50-60° C) Bacillus , Algae .

2-**Oxygen** : On the basis of oxygen requirement they classified :

a. Aerobes : Grow only in presence of oxygen , e.g. *Bacillus , pseudomonas* .

b. Facultative anaerobes: Can live with or with out oxygen e.g. *E. coli Salmonella , Staphylococcus , Shigella* .

c. Obligate anaerobes : Multiply only in the absence of oxygen . e.g. *Clostridium , Bacteroides* .

d. Carbon dioxide : Some types of bacteria need to CO_2 for metabolic activities , e.g. *Neisseria gonorrhoeae* .

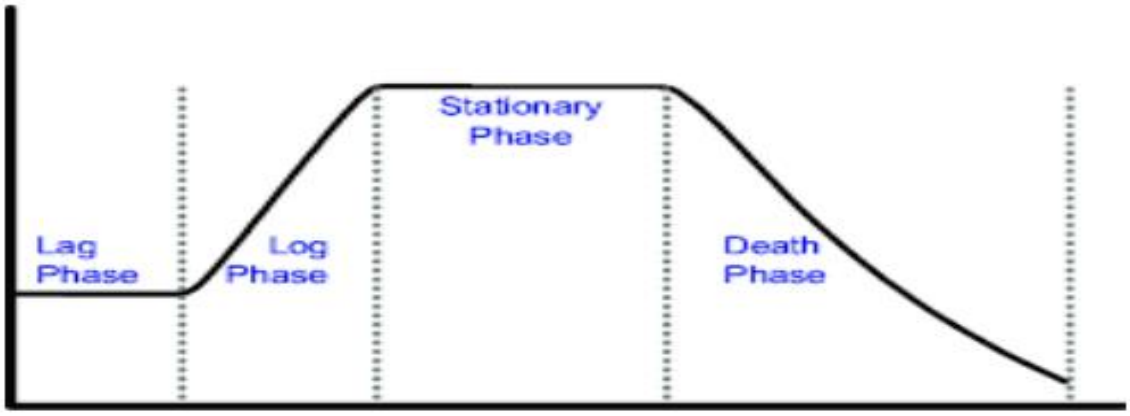
3- Moisture : Bacteria require water for growth , desiccation may kill most of bacteria .

5- pH: Most the bacteria growth best at pH ranged (7.2-7.6) e.g. Vibrio grow at alkaline pH, but Lactobacilli grow at acidic pH.

Stages of bacterial growth curve:

When organisms are cultured in appropriate media there would be increase in size of bacteria only Lag phase .This is followed by multiplication and increase in number of bacteria Log phase .
After some times growth rate becomes Stationary and later on decline or (death phase) .

Growth curve : counting of bacteria at different periods after inoculation and then events of sequences are represented on graph which is called growth curve.



The bacterial growth curve

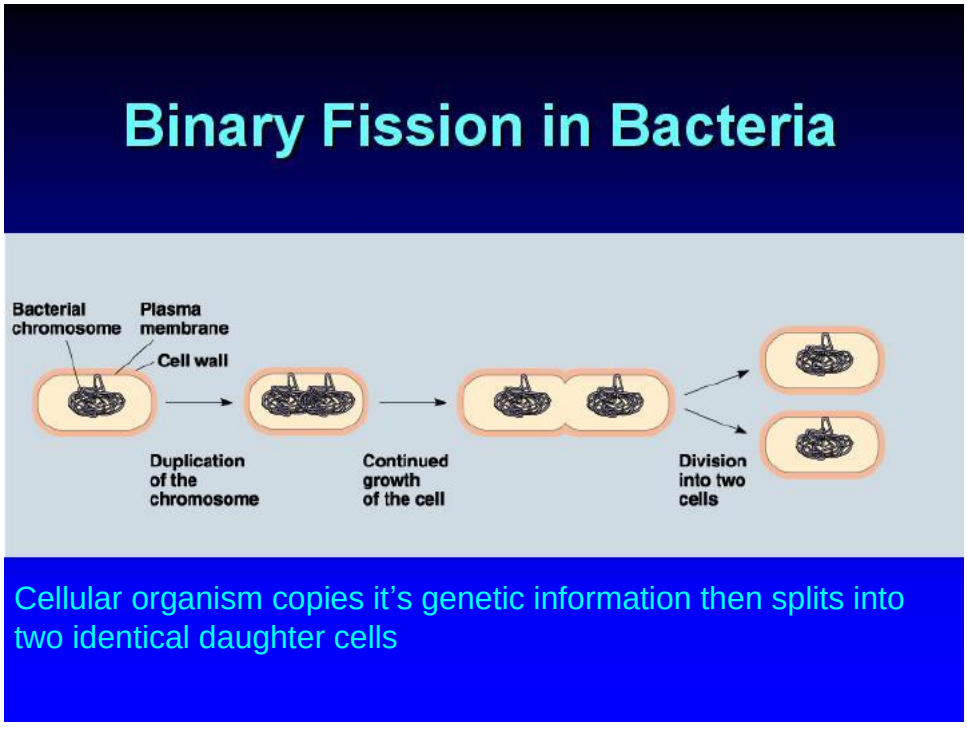
Generation time

The time required for bacterium to produce two daughter cells under optimum condition is called generation time. For example generation time of *E. coli* is 20 minutes.

Reproduction of bacteria :

Bacteria reproduce by: -

- Binary fission
- Conjugation
- Spore formation



Lecture 4

Infection

The lodgement and multiplication of organism in the tissue of host .

Classification of infection :-

1. Primary infection : Intial infection with organism in host constitute primary infection .
2. Reinfection : Subsequent infection by same organism in a host .
3. Secondary infection : When the resistance of host is lowered, preexisting infectious disease a new organism may set up an infection .
4. Local infection : Infection at localized sites , Ex. Appendix , tonsil , etc…
5. Cross infection : When a patient suffering from a disease and new infection is set up .
6. Nosocomial infection : Cross infection occurring in hospital .
7. Subclinical infection : Is one where clinical affects are not apparent .

methods of transmission of infection .

1. **Contact**	Gonorrhoea , Trachoma .
2. **Inhalation**	Influenza , measles.
3**. Infection**	cholera (water), food poisoning (food).
4**. Inoculation**	Tetanus, rabies .
5**. Insect**	dysentery , malaria .

6**. Congenital** syphilis , toxoplasmosis.

7. **Laboratory infection** during procedures like catheterization
,

Source of infection in man :

1-Man (carrier or patient).

2-Animals infection disease transmitted from animals to man are called zoonosis e.g. plague from rats.

3-Insect e.g. typhoid bacilli by house fly or malaria by anopheles mosquite.

4-Some vectors e.g. ticks.

5- Soils e.g. round warms.

6-Water like cholera.

7- Food e.g. food poisoning by staphylococcus

Lecture 5

Sterilization and disinfection

Sterilization :- It is a process by which all forms of life (bacteria , fungi , viruses ….. ect) killed or eliminated .

Disinfection:- It is a process of destruction of pathogenic organisms capable of giving rise to infection.

Antiseptic:- It means prevention of infection by inhibiting growth of bacteria .

Bactericidal agent:- They are those which are able to kill bacteria.

Bacteriostatic agent: - Only prevent multiplication of bacteria and they may remain a live.

Various agents used in sterilization are

A-Physical

1-Sun light.
2-Drying.
3-Dry heat.
4-Moiste heat.
5-Filteration.
6-Radiation.
7-Ultrasonic vibrations.

B- Chemical

1-Acid.
2-Alkalis.
3-Salts.
4-Halogens.
5-Oxidising agent.
6-Reducing agents.
7-Formaldehyde.
8-Phenol.
9-Soap.
10-Dyes.
11-Aerosal

Physical methods

1-Sun light:- this is one of the natural methods of sterilization in case of water in tanks , river and lakes due to ultraviolet rays.

2-Drying:- drying in air has serious effect on many bacteria spores

3-Heat:- The factors influencing sterilization by heat are

1) Nature of heat a- dry b- moist.

2)Temperature and time.
3)Number of organism present.

4)Whether organism has sporing capacity.

Types of heat

1) Dry heat
A . Red heat:- It is used to sterilize metallic object by holding them in flame till they are red hot e.g. inoculating wires, needles, scalpels, forceps.

B .Flaming:- The subject is passed over flame without allowing it to become red hot e.g. cotton wool plugs and glass slides.

C .Incineration:-This is excellent method for rapidly destroying material e.g. soiled dressing, animals carcasses, bedding and pathological material.

D . Hot air oven :- Sterilization by hot air oven requires temperature of 160°C for one hour we can sterilize all glass, syringes, petri dishes, test tube.

2) Moist heat
A) Temperature blow 100 °C

Pasteurization of milk: Temperature employed is either63 °C for 30minutes(Holder method)or 72 °C for 15-20 seconds (Flash method) to kill organisms like *Mycobacterium*, *Salmonella* and *Brucella*.

B) Temperature at 100 °C
Tynddallization:- This is the process by which medium is exposed to steam at 100 °C for 30 minutes each on 3 successive days.

Boiling:- Most of vegetative form of bacteria fungi and viruses are killed at 50-70°C in short time .

Steam at atmosphere pressure (100) °C :- Here free steam is used to sterilize culture media which may decompose if subjected to higher temperature.

Steam under pressure :- For bacteriological and surgical work boiling is not sufficient because spore survive boiling hence high pressure sterilizer or autoclave is used.

4-Filteration
This is a method of sterilization useful for antibiotic solutions and serum

5-Radiation
Ultraviolet radiation it is chief bactericidal factor present in sun light it causes following changes in cell

1- Denaturation of protein .

2- Damage to DNA

3-Inhibition of DNA replication

Ultraviolet lamps are used in
a-Killing of organism.

b-Making bacterial and viral vaccines.

6- Ultrasonic and sonic vibrations
They are bactericidal causing mechanical agitation and rupture of bacteria

Chemical methods

1-Acids and alkalis :- They are inhibitory to the growth of bacteria Mycobacteria are more resistant to acid than alkalis .

2-Distilled water:- causes loss of viability this action may be due to traces of metal in distilled water.

3- Metallic ions:-$HgCl_2$ and $AgNO_3$ prevents the growth of many bacteria in concentration less than (1ppm) part per million.

4-Oxidising agents: - They are weak antiseptic e.g. H_2O_2 potassium permanganate.

5-Halogens:-Iodine is used chiefly for skin chlorine combines with water to form hypochloric acid which is bactericidal

6-Formaldehyde:- 5-10% solution in water kills many bacteria it is bactericidal sporicidal and lethal to viruses also .

7- Phenol:- It is used for sterilizing surgical instruments and for killing culture accidentally spread in the laboratory .

8-Soap and detergents: -They are bactericidal and bacteriostatic for gram positive bacteria.

9-Alcohol:-Ethyl alcohol is most effective in 70% solution than 100% alcohol it does not killing spores.

10-Dyes:- Gentian violet and malachite green etc. are active against gram positive bacteria .

11- Aerosols and gaseous disinfectant such as SO_2 , chlorine and formalin vapour have been used as gaseous disinfectant .

Lecture 6

Medical bacteria

Cocci

Gram negative	↓	Gram positive
Neisseria		Staphylococcus Streptococcus Pneumococcus.

Staphylococcus spp.

- Morphology : Spherical cells , arranged in irregular cluster ,gram positive , non motile , non sporing .

- Cultivation : grows on most bacteriological media under aerobic conditions optimum growth temperature is 37Ċ .

- On the basis of pigments production three types of Staphylococci are identified :

1. *Staphylococcus*. *aureus* produce golden yellow colonies and are pathogenic .

2. *Staphylococcus*. *albus* produce white colonies and are non-pathogenic .

3. *Staphylococcus*. *citreus* produce lemon yellow colonies and are non-pathogenic.

Staphylococcus.aureus

General characters: They are oval or spherical (0.8to 0.9m) non-motile non-capsulated non-sporing, Gram positive they are arranged in cluster (grape like). Cluster formation is due to active aggregation of multiple cells in to one location .

Characteristic of pathogenic strain: -

1. Coagulase positive.

2. Mannitol fermentation.

3. ß- hemolysis.

4. Golden yellow pigments.

5. liquify gelatin.

Pathogenesis : *Staphylococcus* are one of the most important causative agents of hospital acquired infection, especially post operative wound infection. It cause the majority of acute pyogenic lesions in human. Staphylococcal disease may be classified as :-

a. Cutaneous lesions: styes, boils, abscess, impetigo, eye infection in new born, (pemphigus neonatorum) .
b. Deep infection: acute osteomyelitis, tonsillitis, pharyngitis, abscess breast (mastitis), Staphylococcal septicemia.
c. Staphylococcal Food poisoning, this occurs when food (Meat. fish, milk products) contaminated with enterotoxin B Produced by staphylococci after 6 hours of taking contaminated food leading to diarrhea and vomiting.

Toxins

1-Haemolysin: *Staph. aureus* produces at least 3 types of haemolysine known as alfa, beta, and gamma.

2- Leucocidin (leukotoxins) lyse white blood cells .

3-Staphylococcal enterotoxins cause nausea , vomiting and diarrhea and the toxins cause food – borne disease.

4- Fibrinolysin:- *Staph. aureus* produces staphylo kinase during the later stage of growth which causes lysis of fibrin

Other toxins
(a) Nucleases

(b) Lipases

(c) Proteases

(d) Scarlatina toxin.

Treatment : The antibiotic therapy for S. aureus infections include cephalexin , dicloxacillin , and nafcillin.

Culture media :Selective media are widely used to detect small number of S. aureus such as broth of mannitol salts agar

Staphylococcus albus

They are coagulase negative staphylococci which are part of skin flora .

Cultural character

Nutrient agar: Cololnies are pin head size and white in colour.

Blood agar : Colonies are white and there is no haemolysis .

P**athogenecity**: They are non pathogenic they may act as opportunist pathogens causing acne pustules , stitch abscess .

Staphylococcus citreus

It is found as saprophyte and is never pathogenic . On nutrient agar and blood agar they form lemon yellow pigment colonies . On blood agar they are never haemolytic . They don't ferment sugar and they don't produce toxin or coagulase.

Lecture 7

Streptococcus

General characters : spherical or oval cells about 1*M* in diameter, arranged in chains, gram positive , non-motile, non-sporing and some times capsulated , required media enriched with blood ,serum or ascitic fluid for their growth . *Streptococcus* can be classified in to the following groups according to their haemolytic activity :-

1.Beta- hemolytic :Break down the red blood cells and hemoglobin completely .leaves clear zone around the bacterial growth or colony , (colorless zone) e.g. *Streptococcus pyogenes* .

2-Alfa- hemolytic : Partially break down the red blood cells and leaves agreenish color on blood agar plates. e.g. *Streptococcus viridans* .

3.Gamma-hemolytic : (non-haemolytic) many streptococci do not produce any kind of haemolysis , generally commensal e.g. *Streptococcus faecalis* .

Pathogenecity:

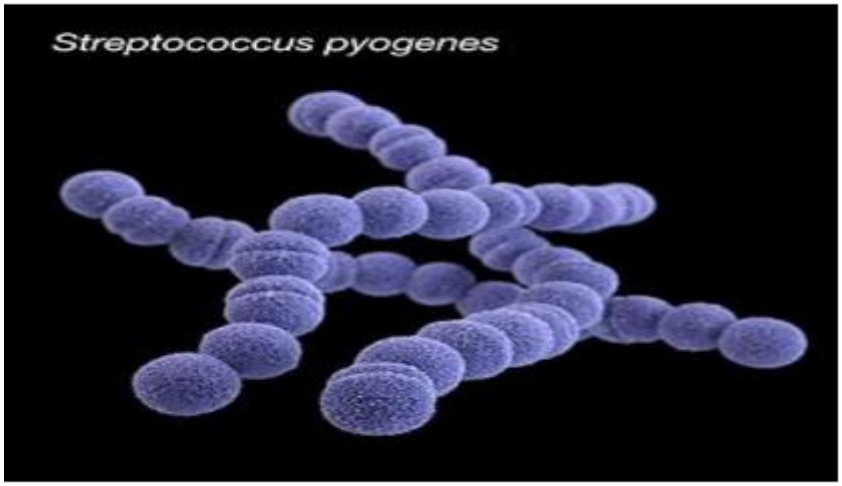
Streptococcus pyogenes

1. Respiratory infection like throat infection, tonsillitis , pharyngitis .
2. Skin infection like suppurative infection of skin e.g. wound, burn.
3. Scarlet fever is caused by astrain producing erythrogenic toxin . it is characterized by a bright red rash on the body , accompanied by ahigh fever and sore throat .
4. Genital tract infection causing puerperal sepsis.

   Toxin : Erythrogenic toxin refer to this bacteria induces inflammation .

Treatment : Penicillin or amoxillin is the antibiotic that treat *Streptococcus pyogenes* infections

Culture media: usually grown on agar media supplemented with blood which allow the detection of β-hemolysis for identification of *Streptococcus*



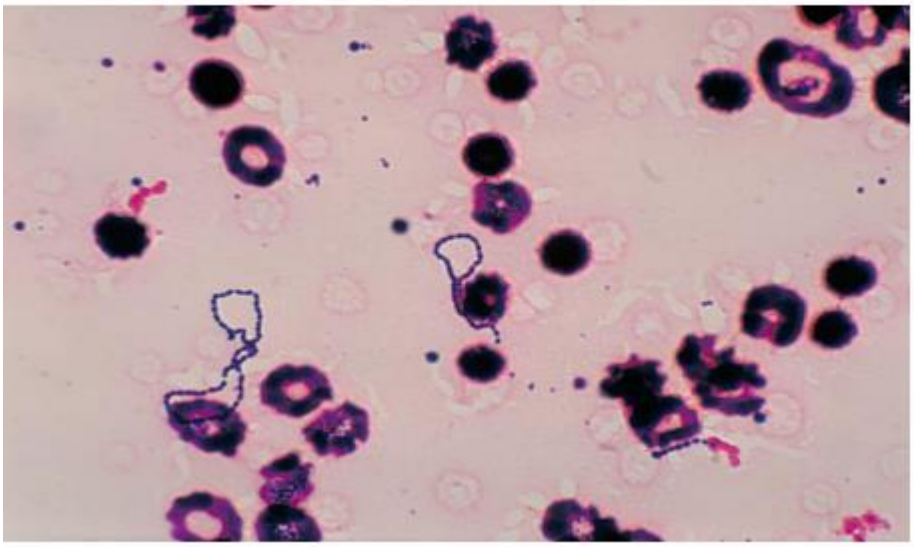
Streptococcus viridans : this organism may be cause sub acute bacterial endocarditis which is an infection of heart involving damaged valves or endothelium .

Toxin : Erythrogenic toxin also referred to *Streptococcus pyogenic* toxins which induced inflammation .

Treatment : Penicillin and amoxicillin is the antibiotic that treat the infection .

Culture media : grow in agar media supplemented with blood .

***Streptococcus viridans*:**



Streptococcus faecalis :this organism is often referred to as enterococcus ,because of their intestinal habitat .Most strain are non-haemolytic .

Pathogenesis : It may cause disease in man when introduced in to the blood stream or urinary tract accidentally.

Treatment: Penicillin and amoxicillin

Culture media : this organism can grow in media containing bile salt. On blood agar colonies are little bigger than *Streptococcus pyogenes* on MacConkey agar they are tiny and dark pink in colours.

***Streptococcus faecalis*:**

Lecture 8

Neisseria

General characters: Gram negative, cocci, aerobic, arranged in pairs , mostly non-capsulated.
Two important pathogens :-

1. Neisseria meningitidis or known (meningococcus)

2. Neisseria gonorrhoeae or known (gonococcus)

3. Neisseria catarrhalis (commensal of respiratory tract) Normal flora

Morphology: Approximately 1 *M* (micron) in diameter , slightly elongated arranged in pair with long axis parallel and flattened in their opposite surfaces .

Culture media: Grow best in atmosphere containing (5-10)% of CO2 on heated blood agar (chocolate agar) .

Diagnostic test : Oxidase test , all the genus of Neisseria give oxidase reaction positive .(Tetramethyl-p-phenylene diamine dihydrochloride)1%.

Pathogenesis :

Neisseria gonorrhoeae_ is responsible for gonorrhea , venereal disease ,it is transmission is almost always by sexual intercourse In male there is usually urithritis with yellow creamy pus ,but in female the infection extends from urethra and vagina .

Neisseria meningitidis: They produce cerebrospinal meningitis and meningococcal septicemia .

Treatment : cephalosporine , and ceftriaxone

Toxin :endotoxin is released by the organism

Lecture 9

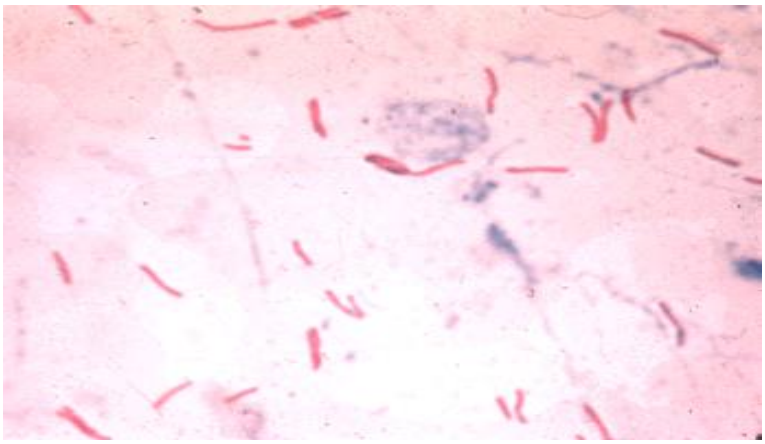
Mycobacterium

Consist of many saprophytic and pathogenic species , pathogenic species are :

Mycobacterium tuberculosis causes tuberculosis in human (T.b.).

General characters: M. tuberculosis is an aerobic, Gram negative, non-spore forming, non-motile bacillus with a high cell wall content of high molecular weight lipids, which comprise approximately 60% of the cell wall structure. According to cell wall composition, mycobacteria stain poorly with Gram stain but are described as acid-fast, as once stained with hot carbol-fuchsin it resists decolorization with acidified organic solvents (Ziehl–Neelsen stain). The high lipid concentration in the cell wall accounts for its resistance to antimicrobial agents, and resistance to killing by acidic and alkaline compounds in both the intra and extracellular environment

Mycobacterium tuberculosis



Pathogenesis : causes T.B

Culture media : used Lowen stein-Jensen media ,incubation time for(4-8) weeks at 37 Ċ .

Toxins: Mycobacterium tuberculosis (Mtb) induces necrosis of infected cells to evade immune responses. Mtb utilizes the protein CpnT to kill human macrophages by secreting tuberculosis necrotizing toxin (TNT) that induces necrosis.

Treatment: For initial empiric treatment of TB, the administration of 4-drug regimen: isoniazid, rifampin, pyrazinamide, and either streptomycin.

BCG: (Bacillus Calmette Guerin)

This is bovine strain of tubercle bacillus lose completely avirulent by culturing repeatedly on glycerin potato medium

The vaccine is given over deltoid region.

Complication of BCG

Local
1-abcess
2-ulcer
3-keloid

General
1-fever
2-mediastinal adenitis

Mycobacterium leprae

General characters: They are long, slender, slightly curved or straight occurring in bundles of parallel packets. They are weakly acid fast (5% H_2SO_4 is used for decolorization) . In smear living bacilli are uniformly stained while dead bacilli are fragmented and irregular. Generation time of Lepra bacilli is 20 days.

Pathogenesis : Lepra bacilli are obligate parasite of man portal of entry is most probably skin and nasal mucosa . It needs close and prolonged contact with infective patient for contract. Incubation period is 5-8 year.

The discase occur in two forms
1- Tuberculoid and
2-Lepromatous.

Laboratory diagnosis
Smear from leprous nodules , skin scrapping or nasal mucosa are stained with (Ziehl–Neelsen) method using 4% H_2SO_4 for decolorization . Smear shows acid fast bacilli arranged in packed bundles is lepra cells.

Lecture 10

Clostridium

General characters : The genus Clostridium is Gram positive, anaerobic,(4-6) micron in length, spore forming , pleomorphic bacilli, spindle shape
.

Pathogenic species

1-. ***Clostridium tetani***
Spore are terminal, oval and projecting out side the bacilli, this appearance is called drum stick. On blood agar medium produce alfa hemolysis .

Toxins: It is an obligate anaerobic bacterium whose spores produce two distinct toxins, i.e., tetanolysin, which causes local tissue destruction and tetanospasmin that leads to causes clinical tetanus.

Pathogenesis: cause tetanus

Spores implanted in wound multiply only if conditions are favorable. Toxin so produced is absorbed by motor nerve ending. Toxin travels along the axis cylinders of peripheral nerve and reach central nervous system .It exacts mode of action is not known but it may act at synaptic junctions between anterior horn cell and related internuncial neurons leading to abolition of spinal inhibition.

Treatment: Acute treatment of tetanus is based on wound cleaning and the administration of antibiotic, such as intravenous metronidazole or penicillin.

Culture media: When grown on blood agar medium, it produces alfa haemolysis.

2. **Clostridium perfringens:**

General characters: Gram-positive, anaerobic bacterium that is widely distributed in the environment; it is found in soil, however, commonly inhabits are the gastrointestinal tract of humans and animals. This bacterium is a major cause of histotoxic and enteric diseases.

Toxins: Toxins are the main cause of lesions and symptoms associated with diseases caused by its infection. There are two main groups of toxins; major and minor. The major toxins are alpha, beta, epsilon and iota toxins. Theses toxins are lethal and necrotizing agents. Minor toxins are eta, theta, kappa and enterotoxin

Pathogenesis: Toxins produced by the bacterium results in a broad range of diseases including gas gangrene, various enterotoxaemias, food poisoning and necrotic enteritis.

Treatment: Oral rehydration or, in severe cases, intravenous fluids and electrolyte replacement can be used to prevent or treat dehydration. Antibiotics are not recommended.

Culture media: Lactose-gelatin medium and Sporulation broth.

Clostridium botulinum:

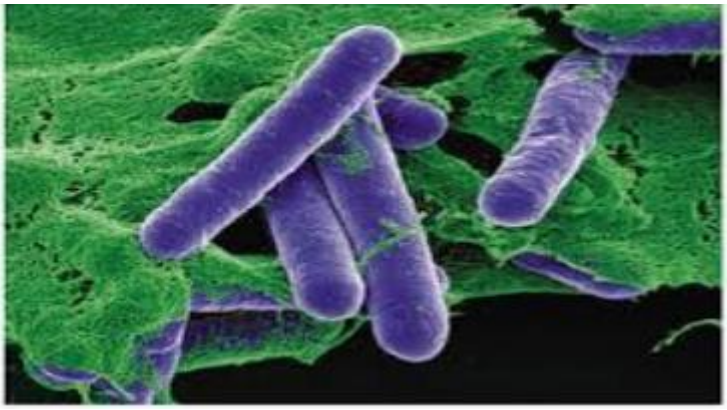
General characters: Gram positive, obligate anaerobic, spore-forming, rod-shaped bacterium. C. botulinum organisms are commonly found in soils and marine sediments throughout the world. It also colonizes the gastro-intestinal tract of fishes, birds and mammals.

Toxins: The toxin types are classified as A, B, C, D, E, F and G. Human botulism has been described with the strains of Clostridium botulinum that produce toxin types A, B and E

Pathogenesis: It cause botulism (Food poising),

It growing in media like

1- Cooked meat media
2- Nutrient agar
3-Blood agar.



Lecture 11

Bacillus anthracis

General characters: It is a Gram-positive, rod-shaped bacterium, with a width of 1.0–1.2 μm and a length of 3–5 μm. When grown in culture, they tend to form long chains of bacteria. On agar plates, they form large colonies that are generally white or cream colored. Most B. anthracis strains produce a capsule that gives colonies a slimy mucus-like appearance.

Toxin :production of a toxic complex consisting of three proteins known as protective antigen (PA), lethal factor (LF), and edema factor (EF)

Pathogenesis: B. anthracis bacterium (responsible of anthrax disease).

Treatment: Many antibiotics target B. anthracis, including penicillin, amoxicillin, levofloxacin, and ciprofloxacin.

Culture media: It can be grown in an ordinary nutrient medium under aerobic or anaerobic conditions.

Corynebacterium diphtheria

General characters: Is a Gram-positive, non-motile, aerobic, rod-shaped bacterium. Strains grow in tissues or old cultures, causing diphtheria, can be characterized as toxigenic or non-toxigenic, or those causing diphtheria and those that don’t, respectively

Toxin: The main virulence factor of C. diphtheriae is diphtheria toxin (DT), an exotoxin, released by the bacteria after entering the human body. The

major function of the toxin is to enter the cytoplasm and inhibit protein synthesis in host cells.

Pathogenesis: Diphtheria can cause a thick gray coating to build up in throat or nose making it difficult to breathe and swallow.

Treatment: The common form of treatment is the administration of erythromycin or penicillin.

Culture media: Loeffler Medium is used for growing Corynebacterium diphtheria, which contains horse serum, beef extract, dextrose and proteose peptones.

Corynebacterium diphtheriae

Lecture 12

Enterobacteriaceae

Enterobacteria : The bacteria present in the intestine of human and animals called enterobacteria .

Characters :

1. They are all gram negative bacilli.

2. Mostly are motile with peritrichous flagella .

3. They grow on simple medium like nutrient agar , and MacConkey agar .

4.They are all ferment various carbohydrate like glucose , lactose, sucrose, mannitol, etc…

5. They reduce nitrates in to nitrites . $NO_3 \rightarrow NO_2$

6. They are all oxidase negative .

Lactose fermenting

Escherichia coli

General characters: Gram negative bacilli, motile, non-capsulated, non-spore forming ,lactose fermenting (L.F.) considered opportunistic bacteria , Pink colonies on MacConkey agar.

Pathogenesis : E. coli causes

1. Gastroenteritis.
2. Summery diarrhea .
3. Urinary Tract Infection (U.T.I) .
4.Pyogenic infection ex. Wound infection .

* When E. coli presence in water indicates ,water pollution by human faecal matter .

Treatment: Currently, the antibiotics of choice are fluoroquinolones or azithromycin, with an emerging role for rifaximin.

Culture media: E. coli grows in a variety of defined laboratory media, such as lysogeny broth, or any medium that contains glucose, ammonium phosphate monobasic, sodium chloride, magnesium sulfate, potassium phosphate dibasic, and water.

Non- Lactose Fermenting

Salmonella spp.

General characters: Salmonella spp. are a group of bacteria, which reside in the intestinal tract of human beings and warm blooded animals and are capable of causing disease. They are facultative anaerobic, motile, Gram-negative rod-shaped bacteria. Salmonella spp. are members of the Enterobacteriaceae group.

* Salmonella typhi ----------causes---------- typhoid fever.
* Salmonella Para typhi A}
  Salmonella Para typhi B} ---------causes ----- paratyphoid fever
  Salmonella Para typhi C}

Toxins: Salmonella enterica (S. Typhi) is the cause of typhoid fever in humans, which results from secreting Typhoid toxin. Typhoid fever is accompanied by various symptoms including fever and abdominal pain.

Treatment require treatment with antibiotic drugs, including ciprofloxacin, ceftriaxone and ampicillin.

Culture media : Non-Lactose Fermenting (N.L.F) ,when inoculated on MacConkey agar .
Enrichment media used selenite broth or tetrathionate broth .
Serological test : To diagnose Salmonella infection used ,
Widal Agglutination Test (W.A.T).

Shigella

General characters : Gram negative bacilli, non-motile , non-capsulate
Culture media : Non-lactose fermenting , on MacConkey agar , colonies are colorless due to the absence of lactose fermenting.
Culture media used selective media for Shigella is (D.C.A.)
Deoxy cholate citrate agar .

Serological test : no H antigen.

Divided in to 4 species on the basis of O- antigen .
* *Shigella dysenteriae*
**Shigella* . *flexneri*
* *Shigella boydii*
* *Shigella sonnei* , which ferments lactose late and form pink colonies .

Toxins : toxins are cytotoxins that cause severe gastrointestinal disease caused by Shigella dysenteriae serotype 1 damage caused by Shiga toxins in the colon, kidneys, and central nervous system

Treatment: ciprofloxacin, Pivmecillinam, ceftriaxone and Azithromycin.

Pathogenesis: Causes Bacillary dysentery. Ingested bacilli may infect villi of large intestine and multiply inside them.

Lecture 13

Proteus

Cultural characters : Gram negative bacilli , motile , non-lactose fermenting

Culture media: grow on simple media like nutrient agar and MacConkey agar

Species :

Proteus vulgaris
Proteus mirabilus
Proteus morgani
Proteus rettigeri.

Pathogenesis : causes urinary tract infection , wound infection

(ear or respiratory tract),
Proteus morgani causes infantile diarrhea .

* when the Proteus inoculated on nutrient agar can be seen the spreading of the colony through the Petri dish ,this phenomena known **swarming** .
Q. What is swarming?
Swarming may be due to progressive surface growth spreading from the edge of parent colony of proteus , or spreading occurs in successive wave and concentric rings are formed .

Brucella

General character : Gram negative ,Coccobacilli, which is zoonosis (transmitted from animals to man) .
Culture media : strict anaerobic , optimum temperature at 37Ċ ,under 10% CO2, main species Brucella abortus is responsible for abortion in cows .

Pathogenesis : Causes brucellosis ,acute brucellosis it is also called undulant fever .Incubation period of this disease is (4-30) days.

* Bacteriological investigation : used Blood culture ,(sample of blood patient inject in vial containing Brain heart infusion or Trypticase soy broth and incubated at 37Ċ for 21days) .Can be shown the appearance of vial,

Clear ---------- no infection

Turbid ---------- infection (growth of bacteria)

Serological method: used Rose Bengal Card Test, depending on antibody antigen reaction.

Lecture 14

Vibrio cholerae

General characters: Gram negative, comma shaped, motile by polar flagellum (monotrichous) , non-lactose fermenting , give Indole and Oxidase test positive (+ve) .

Toxin : V. cholerae secretes cholera toxin, a protein that causes profuse, watery diarrhea (known as "rice-water stool").

Pathogenesis: in the intestinal lumen, V. cholerae bacterium uses fimbriae (short pilli) to attach to the intestinal mucosa. After that it secretes cholerae toxin that leads to secreting of water into the intestinal lumen, causing watery stool or rice watery stool..

Treatment : Doxycycline is recommended as first-line treatment for adults, while azithromycin is recommended as first-line treatment for children and pregnant women.

- Zinc treatment has also been shown to help improve cholera symptoms in children.

Culture media: Selective thiosulfate–citrate–bile salts agar (TCBS).

Bordetella

General characters :they are Gram negative , non- motile ,coccobacilli, The genus Bordetella contains three species *Bordetella pertussis* *Bordetella parapertussis* and *Bordetella bronchiseptica*.

Bordetella pertussis

Gram negative cocco-bacillus, non motile, non sporing and Adherence to capsulated, ciliated cells

Cough,Violent coughing followed by whooping sound

Strict aerobic, causes Whooping cough .

Culture : used Charcoal Cephalexin Blood Agar (C.C.B.A.) ,or Bordet-Gengou penicillin, or Glycerine Potato blood agar medium .

Medical Mycology

Mycology : is the study of fungi, the diseases they cause are called **mycoses** .

Fungi : are saprophytic or parasitic eukaryotic microorganisms , distinct from plants and animals. Fungi are usually classified as part of the plant kingdom, unlike most the plants they lack completely the chlorophyll , pigment , therefore they are forced to live as saprophytes or parasites in order to find the food necessary to their development .

Eumycetes classify on the basis of morphology in to four groups :-

1. Molds .

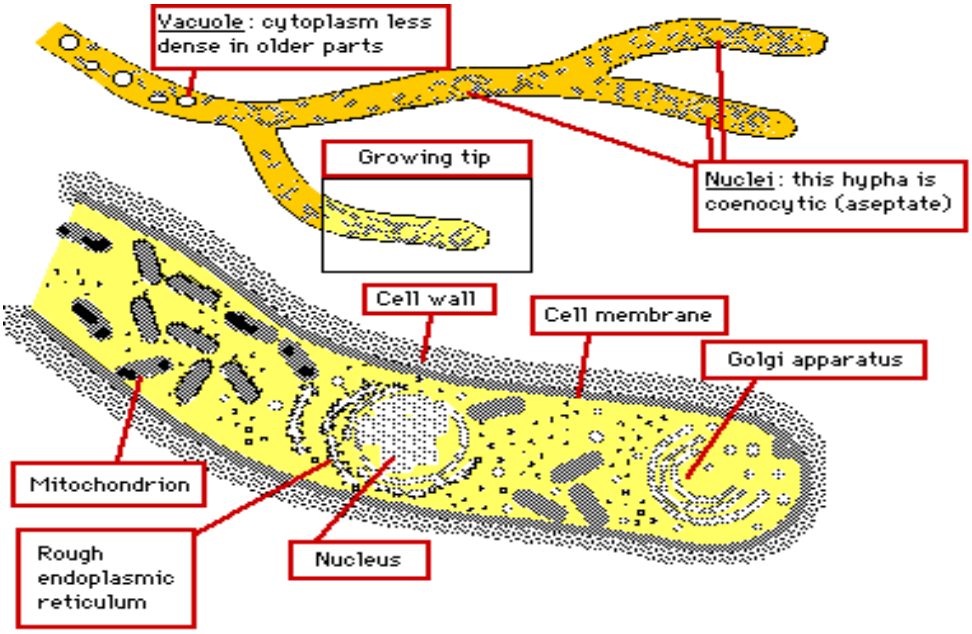
2. Yeast .

3. Yeast like fungi .

4. Dimorphic fungi .

1.**Molds** :

Most fungi consist of microscopic branching filaments called **hyphae** , these are normally divided in to cells by cross-walls referred to as septa .
A visible mass of interwoven hyphae is called a **mycelium** .
Reproduced by spore forming .



2.**Yeast** :

When the fungi appear unicellular, spherical or oval shaped, and reproduce by budding e.g. Cryptococcus neoformans .

3. **Yeast like fungi** :

When the hyphae represented only by pseudohyphae, which are elongated budding cells often linked in branching chains which superficially resemble hyphae .e.g. Candida albicans

.

Candida albicans.

4. **Dimorphic fungi** :
The term dimorphism is used to describe a fungus which occurs in two different forms according to the environment culture . Appear filaments at 22 Ċ , but appear yeast on the culture media at 37Ċ or in the human body . For example some pathogenic fungi are filamentous (mycelial) in Culture and yeast- like in infected tissues . e.g. Histoplasma capsulatum .

Characters of Fungi :

1. All species of fungi are aerobic.

2. Nutritional requirement to growth of fungi is simple sometimes need enriched media e.g. saburate dextrose agar .

3. Optimum temperature to growth of fungi at 28Ċ for 7 days incubation.

4. Fungi affected by physical and chemical agents.

5. Resist spores destroyed by sterilization method used for antibacterial or used heavy metals and chemical antiseptic, considered antifungal.

6. Growth in pH 6 (acidic).

7. Treatment material used for antifungal Nystatin , but used clotrimazole for anti -yeast .

8. Fungi may reproduce sexually or asexually or by both.

9. Cell wall may contain chitin and glucose.

Spore formation

Most fungi reproduce by forming spores. The spores may be produced in many different ways and their size, shape, color and manner of production are of value in identifying individual species. The principal type of spores and the ways in which they develop are as follow :-

1. **Chlamydospore**
   A resting stage formed when a cell swells up and develops a thick resistant wall .

2. **Arthrospore**
   Spore formed septation followed fragmentation of hyphae.

3. **Blastospore**
   A single vegetative cell of yeast which is produced by budding .

4. **Conidium**

   A spore produced externally on a specialized hypha called a conidiophore. e.g., Aspergillus conidia, penicillium conidia.

5. **Sporangiospore**
   A spore produced with in a swollen cell (sporangium) at the end of specialized hypha called a sporangiophore. e.g., Mucor.

Asexual reproduction

1. Fragmentation
2. Fission
3. Budding
4. spores (differ in shape and color) .

- When the fungus in human called anthrophilic .
- When the fungus in animals called zoophilic .
- When the fungus in soil called geophilic.

Classification of fungi according to their sites of infection

Mycoses can be divided in to **Four** groups differencing in the level of the infected tissue .

1.**Superficial mycoses**

These affect the (skin, hair, nails) Do not directly involve living tissues . e.g., Ring worm (dermatophytosis), Tinea corporis (body surface), Tinea capitis (scalp), Tinea unguium (nails) , Tinea pedis (foot)
.

2.Cutaneous mycoses skin hair, nails ,eye, ear . (dermatomycoses).

3. **Sub cutaneous mycoses** Nasal mucosa ,smooth skin ,e.g. Mycetoma.

4. **Systemic mycoses** primary infection in lung ,bone , kidney . e.g.

Aspergillosis ---------- Asp. Flavus
Histoplasmosis ---------- Histoplasma capsulatum

Viruses :

Viruses are unicellular ultramicroscopic particles containing either RNA or DNA. Reproducing inside living cell .

Characters :

1-donot posses cellular organization .

2-contain one type of nucleic acid either RNA or DNA never both.

3-lack enzymes necessary for protein and nucleic acid synthesis .

4-they multiply by complex process inside living cell called replication not by binary fission .

5-they are not affected by antimicrobial or antibiotic .

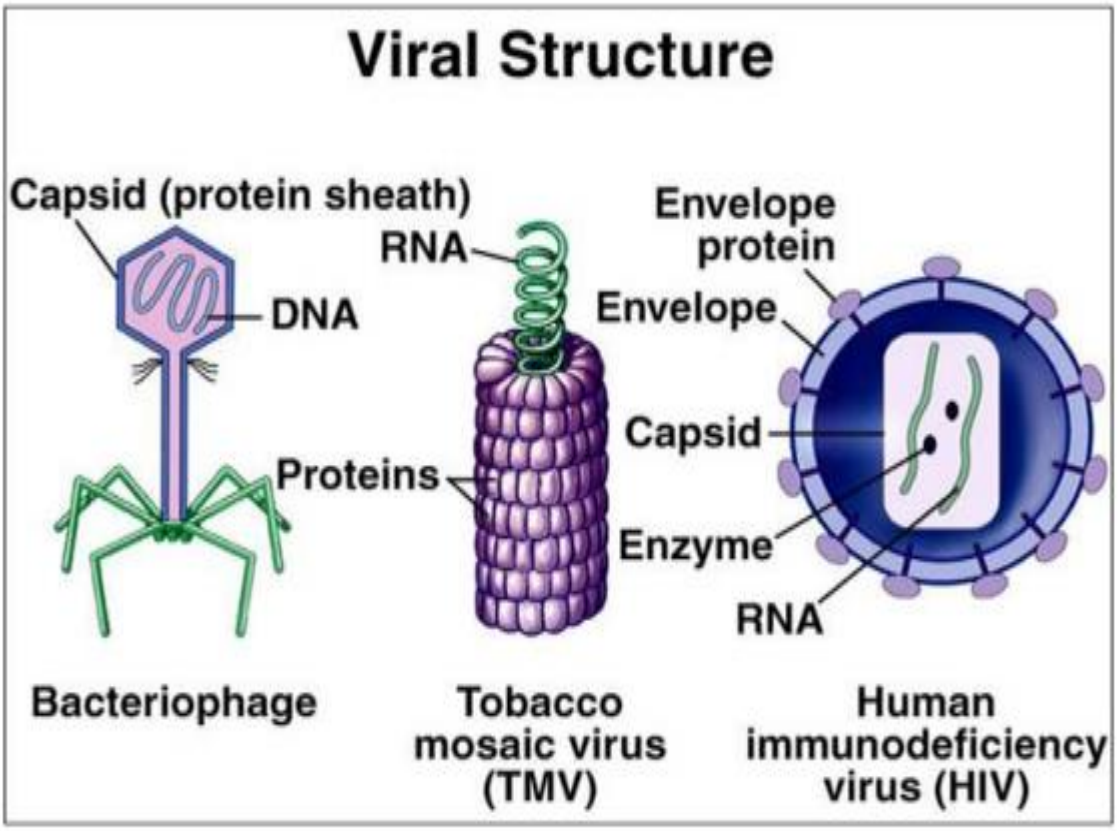
6-they are sensitive for interferon , which is chemical substances give resistance to the immunity system.

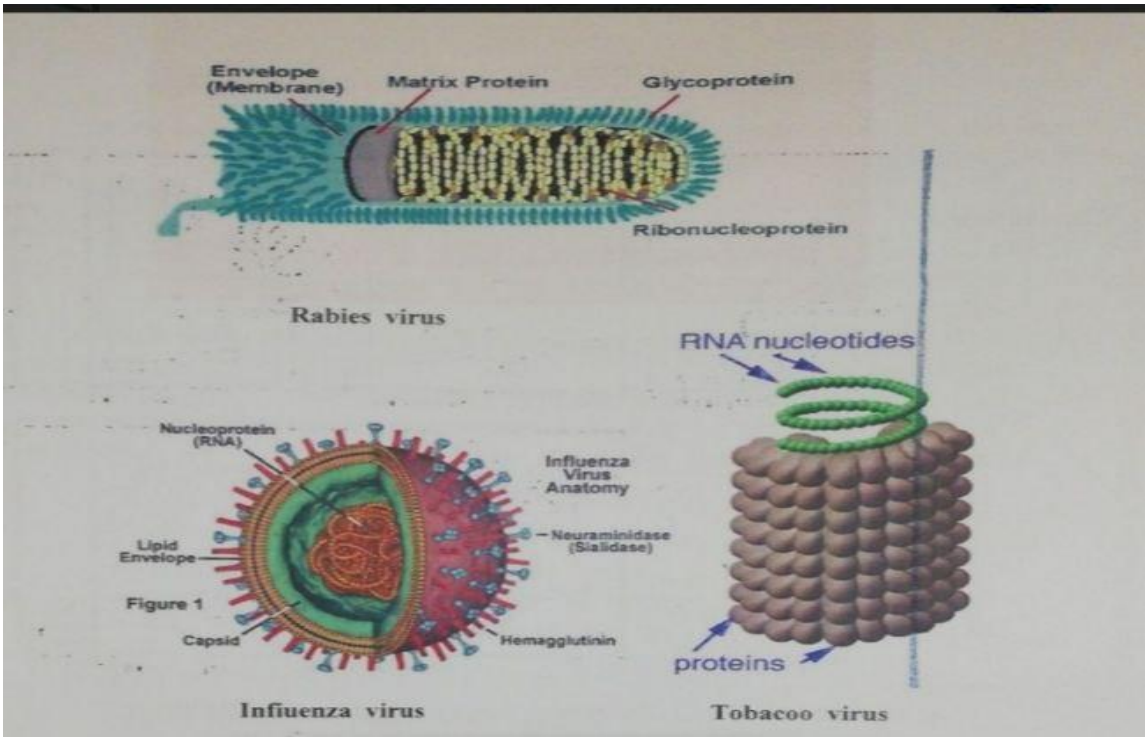
Morphology:

1-size: viruses are widely in size, the largest virus among them is pox viruses measuring about 300 nano micron, while the smallest viruses are food and mouth virus measuring about 20 nano micron.

2- shape : viruses are also widely in shape , some have characteristic shape like Rabies virus have bullet shape , pox virus have brick shape . Tobacco mosaic virus have rod shape, Influenza virus have spherical shape, while Bacteriophage have head, neck and tail.

3- structure: viruses contain the following components: 1-a nucleic acid genome and 2- a protein capsid that covers the genome. Together this is called the nucleocapsid. In addition, many animal viruses contain a 3- lipid envelope . The entire intact virus is called the virion. The structure and composition of these components can vary widely.


Viral Structure
Capsid (protein sheath)
DNA
Proteins
RNA
Envelope protein
Envelope
Capsid
Enzyme
RNA
Bacteriophage
Tobacco mosaic virus (TMV)
Human immunodeficiency virus (HIV)


Envelope (Membrane)
Matrix Protein
Glycoprotein
Ribonucleoprotein
Rabies virus
RNA nucleotides
Nucleoprotein (RNA)
Influenza Virus Anatomy
Neuraminidase (Sialidase)
Lipid Envelope
Figure 1
Capsid
Hemagglutinin
proteins
Infiuenza virus
Tobacoo virus

General step in viral replication cycles:

1-Atachment

2- penetration

3-viral genome replication

4-mutration

5-Release

Pathogenesis:
is the process by which an infection leads to disease Pathogenic mechanisms of viral disease include :

1- implantation of virus at the portal of entry.

2- local replication.

3- spread to target organs (disease sites).

4- spread to sites of shedding of virus into the environment.

Factors that affect pathogenic mechanisms are:

1- accessibility of virus to tissue.

2- cell susceptibility to virus multiplication.

3- virus susceptibility to host defenses.

Some viral disease :

1-Influenza virus

2-Mumpus virus

3-Small pox virus

4- Measles virus

5- Poliomyelitis virus

6- Aids virus (immunodeficiency syndrome)

Human Immunodeficiency Virus (HIV virus)

Pathogenesis : Human Immunodeficiency Virus (HIV) cannot survive outside the bloodstream or lymphatic tissue. Furthermore, virus is easily inactivated by the exposure to common detergents and disinfectants. Thus, virus transmission requires the directed exposition to infected blood or secretions in the presence of skin damage by needles or sharp tools, or abrasions in mucosal tissues within sexual intercourses. Transmission of HIV is highly dependent on the

1- Biologic properties of the virus

2- Its concentration in the infected body fluid

3- Host susceptibility

The HIV replication cycle can be summarized in six steps;

1) Binding and entry

2) Uncoating

3) Reverse transcription

4) Provirus integration

5) Virus protein synthesis and assembly

6) Budding

Differences between virus and bacteria

	Bacteria	Virus
1	Unicellular	Do not have cells
2	Considered as a living organism	Not considered
3	Larger and visible under light microscope	Smaller and visible under electron microscope
4	Contain a cell wall with peptidoglycan	Contain protein coat rather than a cell wall
5	Have a single, circular chromosome	Has DNA/RNA strand
6	Do not need a host organism for reproduction	Replicates only inside the host
7	Caused localized infections	Cause systemic infections
8	Can be either beneficial or harmful	Usually harmful
9	Infections can be prevented by Antibiotics.	Spread of viruses can be prevented by vaccines

Antimicrobial drugs include :-

1. Antibiotics .
2. Chemical antimicrobials (chemotherapeutic agents) .

1. Antibiotics : Substances produced by some living microorganisms, which inhibit some other microorganisms .They include the culture extracts and filtrates of fungi such as , penicillium, cephalosporium , and bacteria such as Bacillus and streptomyces .Important antibacterial antibiotics include the :- Penicillin , cephalosporin , tetracycline , rifampicin, chloramphenicol erythromycin , clindamycin ,amino glycoside .

2. Chemical antimicrobials : They are synthetically produced antimicrobials compounds. They include sulphonamide , trimethoprim, cotrimoxazole , Nitrofurantion.

Q. Various ways anti microbials act on bacteria, How ?
Various ways anti microbials act on bacteria as follows :-

1. Inhibiting **cell wall** forming ,leading to cell lysis. Exp. Penicillin , cephalosporin.

2. By damaging the bacterial **cell membrane** , leading to loss of cell contents so to cell death .Exp. polymyxin.

3. By inhibiting **protein production** and therefore arresting bacterial growth. Exp. Tetracycline, erythromycin, chloramphenicol .

4. By inhibiting the production of **nucleic acid** and therefore, preventing bacteria from reproducing . Exp. Naldixic acid prevents DNA synthesis, Rifampicin inhibit RNA synthesis .

Q. How can you choose the antimicrobial drugs ?

1. Greater resistance to beta-Lactamase and other antimicrobial inactivating or destroying enzymes .

2. Increased activity and stability .

3. Simpler methods of administration .

4. Better diffusibility in to remote areas of the body and slower rates of excretion .

5. Greater selective toxicity .

Q. How can you antibiotic to be tested invitro ?

By using **sensitivity test** .

Immunology

Immunity : The resistance offered by the host to the harmful effect of pathogenic microbial infection is called immunity , or immunity against infectious diseases of different types .

↓ ↓

Non specific immunity
Or Innate immunity

Specific immunity
or Acquired immunity

Types of innate immunity :-

1. Mechanical defenses .
2. Chemical defenses .
3. Phagocytosis .
4. Inflammation .
5.Temperature .

Factors affect on the innate immunity :-

1. Age 2. Species and strain 3. Sex 4. Hormones 5. Foods .

Acquired immunity

↓

↓

Passive

active

↓

↓

Artificial	Natural	Artificial	Natural
1. Adminstration of serum.	1.Transplacentation .	Vaccination	Infection
2.Adminstration of lymphocyte . (T and B cell lymphocyte)	2.Via colostrum.		a-clinical inf. b-sub clinical infection

.

Antigen : Any substance can promote the immune response (humoral or cellular immunity) .

Antibody : Immunoglobulin as humoral substance (gamma globulin), produced in response to an antigenic stimulus .Five groups of immunoglobulin have been distinguished IgG 80% , IgA 15% , IgM 10% , IgD 0.2-1% ,IgE 0.04% .