Microbiology : The Science of Microbiology is the study of microorganisms and their activities. It is concerned with their form, structure ,reproduction , physiology , metabolism and identification , the distribution in nature their relationship each other and with other living organisms .

♣ Living cells characteristics :

All biological system (all living cells) have the following .

1. The ability to reproduce .
2. The ability to ingest food substances and metabolize the for energy and growth.
3. The ability to excrete waste product .
4. The ability to react to changes in their environment .
5. Susceptibility to mutation .

♣ Distribution of microorganisms in nature

Microorganism occur near every where in nature are carried by air currents from the earths surface to the upper atmosphere they are found in the lacks; they are found on the surface of our bodies, in all our tissues and in our mouth , nose , and other body opening .

Medical Microbiology :

Is the study of micro-organism that play a role in human infection by causing disease and pathogenic effect include (bacteria , viruses, protozoa) .The microbiology is divided into many branches on base of application of micro-organism

The microbiology is divided in to many branches on base of application of microorganism .

1.Air microbiology .
2. Milk and food microbiology
3. Soil microbiology
4. space microbiology.
5. coal and petroleum microbiology
6. microbiology of water and sewage .
7. microbiology of insects .
8. medical microbiology.

♣ The microscopic Observation of microorganisms .

Microscope , the most characteristic instrument of microbiology laboratory, provides the magnification which an able one to see organisms and structure invisible to the naked eye. microscope are available which permit a wide range of magnifications from a few hundred times to hundred thousand of times .

Protista: the kingdom of micro-organism is divided into:-

1. Higher protista : cell are Eukaryotic .example : algae , fungi , protozoa
2. Lower protista-cell are prokaryotic . example : Bacteria , blue- green algae and viruses .

♣ Prokaryotic cell structure .

1. primitive nucleus (no nuclear membrane) .
2. Nuclear division less complex than mitosis .
3. contain peptidoglycan can in cell wall .
4. Gene organization less defined than Eukaryotic .

♣ Group of protista

1. **Bacteria** : unicellular microscope organism .
2. **Viruses** : very small , filter passing obligate intracellular parasite . pathogen for human , Animal , plant . visible and study by electron- microscope.
3. **Algae** : simple plants, most unicellular , contain chlorophyll found in aquatic environment or damp soil .
4. **Fungi** : Plants without chlorophyll , usually multicellular .but not differentiated in to roots, stems , and leaves , reproduce by fission , budding or by means of spores borne on fruiting structure .Some cause disease to human , animal and plant . Fungi divided in to (yeasts and moulds).
5. **protozoa**: A single cell animal , classify on the base of morphological , nutritional and physiological characteristics . cause disease for human and animals .

♣The differences between prokaryotic and Eukaryotic cell .

Character	***pro.cell***	***Eu-cell***
1- cell wall pepdioglycan	yes	No
2- Cell membrane	No usually	Yes
3- mesosome	Yes	No
4- DNA complex with histon	No	Yes
5- Cell division a. mitosis	No but contain Binary division	Yes
b. miosis “sexual”	No	Yes
6- Chromosomes	single	double helix
7- Cytoplasm organelles a. mitochondria	No	Yes
b. chloroplast	No	Yes

Bacteria

Bacteria live every where most of them are saprophytes [Live on dead organic material] . present in soil and water . all bacteria are unicellular , very small in size about 0.5-1.5um and vary in shape (cell wall of bacteria gives it's shape.[

♣Type of bacteria according to shape.

1- Coccus bacteria : OO singular cocci diameter 0.8-1um.

Like: A- Staphylococcus : irregular clusters *Staphylococcus aureus* +ve

-Streptococcus: chain like *Streptococcus viridans* +ve

B. Diplococcus: pairs of cells like *Diplococcus pneumonia*

C. Tetrad : four of cells arranged as square

D. Sarccina : cubical arrangement with 8 cells or more.

E. Neisseria: kidney shape.

2- Bacillus bacteria : short Bacillus

Long bacillus

Like- *Mycobacterium tuberculosis*

1-Vibraio : comma shape like *vibrio choleria*

2-Spirochaete : Spirale Like *Treponema pallidum* cause syphilis.

♣The structure of bacteria

A-cell wall:

All bacteria species are surrounded by **a rigid mucopeptid**.

The function of the cell wall are.

A: give the shape of bacteria.

B. support and protection of internal structure.

C. Antigenic determinants.

Bacterial cell are divided in two groups according **to chemical structure of cell wall.**

1-Gram positive bacteria . G +ve.

2-Gram negative bacteria. G-ve.

♣**Gram positive bacteria : G +ve bacteria have cell wall thicker than G-ve bacteria؟**

Because G +ve bacteria cell wall contain **more peplidoglycone**.

1- G +ve bacteria: purple blue in color because it is contain less Lipid (4-11%). (There four it takes the first color stain.
2- Gram Negative bacteria : G-ve bacteria

Some bacteria contain **mycolic acid** such as **mycobacterium tuberculosis.**

G-ve bacteria : Red in color because contain more Lipid in cell wall (11-22%) so it takes the second stain.

♣**All bacteria cell wall have autolysis enzyme that dissolve the peptidoglycan layer is essential for**.

1-cell wall growth

2-cell septation

3-sporulation

4-Trans formation.

Cytoplasmic membrane

Is Located under cell wall, it composed from **phospholipid and protein.**

♣**The function of it are:**

1-Selective permeability.

2-bacterial electron transport.

3-Enzyme biosynthesis activity.

4-mesosome (an in fold of the outer membrane for respiration.)

B: Capsule : (the external structure include capsule , flagella and pili.)

Some bacteria have **a mucous capsule** around the cell wall example: ***klebsiella*** capsule can be Seen by light microscope after special stain of capsule.

♣**The function of capsule:**

1-Protection: prevent drying of bacteria by binding with water molecules.

2-inhibit bacteriophage attachment.

3-Antiphagocytosis /in hibit engulfment of bacteria by white blood cell.

●Virulence , bacteria that contain capsule more virulent than bacteria don't have capsule.

C- Flagella: It's helical appendage originated from cytoplasmic membrane.

all bacteria have flagella are motile except ***spirochetes*** Lack of external flagella but are motile.

D. Some species example ***Nisseria gonorrhoea*** have hair like processes.

Called Pill

1- Sex pilus : serve as part of entry of genetic material during bacterial meeting.

2- Attachment to host cell , play role in infection.

Pili are shorter than **flagella** as well as it is more than flagella in number.

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6-mitochondria	No	Yes
7- chloroplast	No	Yes

♣Cytoplasmic components:

The cytoplasm of bacteria contain

1- DNA " nucleic acid " .In bacterial cell concentrated in the cytoplasm nucloid consist of haploid stranded circular convantly closed DNA molecule in many bacteria a small portion of DNA persist as Plasmids.

2-Ribosomes : for protein synthesis " are a complex globular structure of several RNA molecule as the active centers for protein synthesis.

3- Storage granules : hold excess metabolism.

♣Bacterial spores

Round or oval structure produce by some bacteria in many species of bacteria spore formation occur under unsuitable condition one spore from bacterial cell wall . Example (bacillus , clostridium).

●There are two types of bacterial spores

1- **Endospores** : from inside the bacteria cells . The shape and site of the spore different according to the species of bacteria :**A- Centrally located B – Terminally located C- Sub terminally.**

The endospores has **highly resistance fo**r dry , dyes , radiation , temperature and boiling **because** :

1- Low content of water :

2- High content of Dipicolinic acid (DPA).

The last form complex with CA++ Ions this complex highly resistance .

2-**Exospores :** from extracellular , less resistance than endospores due to low content of (DPA).

♣Environmental factors that influence microbial growth

1- temperature : All chemical reaction in the cell influence by temperature the range of temperature for microbial growth can be express as three types :

A- The minimum temperature :is the lowest temperature its activities are inhibited

B- : **The maximum temperature** : is the highest temperature at which growth and metabolism can proceed if the temperature rise the enzyme and nucleic will eventually become in activated and the cell die .

C- The optimum temperature : converse a small range intermediate between the minimum and maximum which promotes the faster rate of growth and metabolism the terms used for these ecological group are :

1- Psychrophilic : microorganisms grow best below 15c

2- Mesophilic : microorganisms grow best between 20- 40 c

3- Thermophilic : microorganisms grow best greater than 45c

Most human pathogens have optimum temperature (37c).

2- Gas requirements (oxygen and carbon dioxide).

The atmospheric gases that most influence microbial growth are oxygen and co2.Oxygen has the great test effect on microbial growth play role not only as important respiratory gas but it is also a powerful oxidizing agent that exists in many toxic forms with respect to oxygen requirements several general categories are recognized .

A- aerobic. Grow well in the presence of normal atmospheric oxygen and posses the enzyme needed to process toxic products .Example Shigella spp .
mycobacterium .
B- Obligate aerobic . microorganisms that cannot grow without oxygen .
C- Facultative anaerobic can grow in presence and absence of oxygen .
D- Microaerophilic . microorganism cant grow at normal atmospheric tensions of oxygen but requires a small amount of it in metabolism .
E- An aerobic microorganism doesn't grow in normal atmosphere .Example: Clostridium tetani , Treponema palladium .
F- Canophilic . grow best at a higher co2 tension than normally in the atmosphere.

3- Light

Darkness is favorable condition for growth of bacteria and direct sun light cause injury and killing of bacteria and another factor sun light such as ultra violet ray (U.V. Rays).

4- moisture . Bacteria require water for their growth dehydration may kill most of bacteria because the water is very important for bacterial metabolism .

5- Osmotic pressure : bacteria are usually resistant to change of osmotic pressure but it cant resist to highly concentrated media such as ,Jam, salted meat .

6- Suitable PH: bacteria vary widely I n their tolerance of acidic or alkaline condition ranging from ph 4-9 human pathogens generally prefer ph with in the rang 7.2- 7.6 . but there are exception Vibrio cholera , Lactobacilli . grow best at ph 4.

●Hydrogen Ion concentration (PH).

1- The bacteria growth well at optimum ph ,so some bacteria grow in ph (8- 10.5) this called **Alkalophilic bacteria** .
2- Neutrophilic bacteria .most of bacteria grow better at a neutral to slightly alkaline ph (7.2- 7.6).
3- Acidophilic bacteria : Grow in acidic ph Lactobacillus about (3- 4.5)ph.

♣Preparation for light microscope examination

Two techniques are employed to provide material suitable for microscopic examination :

1- The wet amount preparation :

Permit examination in a normal living condition suspended in fluid .wet preparation are made by placing a drop of the fluid containing the organisms on a glass side and covering the drop with a cover slip ,the method permits examination of organisms in normal living condition suspended in fluid (hanging drop method preparation).

Are made by placing a drop of the bacteria suspension on a cover slip and inverting it over the concave are of a hallow ground slid .used for detection of the motility of bacteria .

2- Fixed stained smears (preparation)

Fixed stain preparation are most frequently used for the observation of the morphological characteristics of bacteria . The advantages of this procedure are that :

1- the cells are made more clearly visible after they are colored .

2- Differences between cells of different species and within the same species .

The essential step in the preparation of affixed stained smear are :

a- preparation of the film or smear .

b- fixation the film on the glass slid by heat .

c- Application of one or more staining solution .

♣Microbial stains :

A large number of colored organic (dyes) are available for staining microorganism the dyes are acidic , basic or neutral dyes .the process of staining may involve ion exchange reaction between the stain and active site at the surface or within the cell .

The exchange can be represented by the following equation.

(bacterial cell)$^-$ + Na $^+$ + MBCL___ Bacterial cell-MB$^+$ +Nacl.

(MB mean) methylene blue dye.

●Staining method include

1- Simple staining method

2- Gram staining method and fast staining method

Simple staining method the coloration of bacteria by application of a single solution of stain of affixed smear is termed simple staining .

Procedure of the method include the following steps

a- the fixed film is flooded with dye solution for 1 minute (example methylen blue solution dye).

b- The slid washed by water and then blatted to dry

c- Examine under 100x power . Result :the microbial cell stain uniformly blue.

3- Differential staining technique.

Staining procedure that elicit difference between bacterial cells or parts of bacteria cell are termed differential staining techniques .The cell may be exposed to more than one dye solution staining regard.

A- Gram staining method

One of the most important and widely used differential staining techniques in

Microbiology is gram staining in the process the fixed bacterial smear is subjected to the following solution crystal violet , Iodine solution , alcohol(decolorizing agent) and safranin or the suitable counter stain the steps of the gram stain include

1- fix smear by heat
2- cover with crystal violet for one minute
3- wash by water don't blot
4- cover with iodine for one minute
5- wash by water
6- Decolorized for 10 -30 sec with alcohol
7- Wash by water
8- Cover for 10- 30 sec with safranin
9- Examine under 100 power .

B- Acid – fast stain (Ziehl- nielsen stain).

1- fix smear by heat
2- cover with carbol fuchsin , steam gently for 5 minutes over direct flame ,don't dry or boiling.
3- Wash with water
4- Decolorize in H2so4(20%).until only a fain pink color remain
5- Repeat 3 and 4 steps 3 times wash with water
6- Counter stain for 10 -30 sec with methylen blue
7- Wash with water and then let to dry and examination under 100 x

●-**Sterilization** :It's killing or remove all living microorganism either in vegetative or spore state .

●- **Disinfection**: It is a process of destruction of pathogenic organism but not spores.

●-**Bacteristatic agent**: Only prevent multiplication of bacteria and they may remain alive inhibit like tetracycline

●-**Bactericidal agent**: Any agent that able to kill the bacteria like penicillin.

♣**There are two method of sterilization** .

A/ physical B/ Chemical

A/ physical sterilization.

Methods in which no chemical substance is used include:

1. Thermal method B-not thermal method.

Thermal method : the metabolic activity of an organisms are the result of chemical reactions and since chemical reaction are influenced by temperature it follow that the life process of organisms are also influenced by temperature. Heat is one of the most effective reliable and economic sterilization agent widely used. temperature above a maximum will exert a killing effects where as temperature below minimum are regarded as producing a static effect .

Vegetative cells can be killed at temperature 50-60c but a much higher temperature is required to kill spores .

Example :Yeast cells , vegetative cell kill by 50-60 at 10 minutes, spore killed by 70-80c for 10 minutes moist heat more active than dry heat because it is denaturation of the cellar protein .

Example: spores of clostridium botulinum are killed in 120c by moist heat for 5 minutes where as it needed to two hours at 120c in dry heat.

A-Dry heat.

1. **Incineration**: the actual burning of material destroying microorganisms and the method used for .

A- Infected Laboratories animals .

B- Contaminated clothes and materials .

2. **Flaming** : Sterilization the platinum wire of the loop and mouth of tubes .

3. **hot air oven 160-220c sterilization**. Destroyed by dry heat only when exposed to extremely high temperature for long period of time effect of dry heat that destroy microorganisms through oxidation , of their intracellular constituent use in sterilization of Laboratory glass (Petri dishes) .

B - **moist heat** this is much efficient than dry heat method because the moist heat .penetrate easier destruction and coagulation of protein .

1. **Boiling:** to kill all vegetative bacteria, fungi ,virus that contaminated material will destroyed with in minutes by exposure to boiling water spore not killing by this method.

2. **Pasteurization** : to kill most of the pathogenic bacteria 62c for 30 minutes used to sterilization of milk/ cream / alcohol .

3. **fractional sterilization (tyndalization)**: use for sterilization of some substances, bacteriological media and chemical that effected by high temperature 100c.

Expose the material to a temperature below boiling temperature (60-70c) for ½ hour for 3 days in this days the temperature kill the vegetative cells only (not the spore) when the material cool to the room temperature the spore convert to vegetative cell which will die during heating in the second day the heating in third day is to be sure to get astral material .

4. **steam under pressure heat**: in the form of saturated stem under pressure is the most practical and dependable agent for sterilization steam under pressure provides temperature above those obtained by boiling the advantages are :

a. rapid heating b- penetration.

c. moisture in a abundance which facilitate the coagulation of proteins the Laboratory apparatus designed to use steam under pressure is called auto clave use for sterilization of media. The time of sterilization depend on the type of nature of container nature and material being sterilized Example: media in test tubes require 10 minute , but media in bottle 10 liter quantity require one hours.

2. not thermal method

A. Radiation.

1. **ultra violet radiation** : it has lethal effect on bacteria because it is absorbed in the nucleic acid of the bacteria also forms ozone gas (0_3) in the air and hydrogen peroxide H_2O_2 un the water which kill bacteria it is used to sterilized laboratories and operation rooms , it can not penetrate the glasses.

2. Ionizing radiation.

Ex: Gamma- rays , it penetrate deeper than ultra violet it is used to sterilized. Syringe and threads which is used in the operation .

B. **Filtration** : by using filters which has a very small pores which prevent the passage of bacteria through it , It used for sterilization media and biological solution which effected by thermal method .

●Chemical sterilization

1. salts : used in high concentration as bacteria static .
Ex: NACL is used for preservation of meat and fishes .

2. Acid and Base : bacteria grow in a natural media and it can not grow in acidic or basic medium , so the acid and base used to stop the growth of bacteria .
Ex: acetic acid , benzoic acid are used in preservation of food .

3. Halogen:

a. **chlorine** : it is used 1-2 ppm to kill the bacteria in water .

b. Iodine: it is used in a concentration of 2-5% in water or 1-2% in alcohol to disinfect the wounds.

4. Alcohols Ex: Ethyl alcohol in concentration 70% kill a high numbers of pathogenic bacteria.

5. Gaseous disinfectant.
Formaldehyde (37%) a quos solution called formalin) is disinfectant , but it has an irritating audar .

6. days : a number of days are toxic to microbe and able to inhibit the growth of bacteria (gram +ve) bacteria more sensitive to day than gram -ve

7. surface – active substance : soap and fatty acids change the bacteria cells negative
charge and acquire positive charge with impairs the normal formation of cytoplasm
membrane the substance not penetrate into the cells.

الاسبوع الثامن

♣Medical microbiology .

●Medical bacteria :

* Pathogens **:** Is the micro-organisms that can cause disease to the host (human or animal).
• Non pathogen : micro-organisms not capable causing disease .
• Carrier **:** a person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.
Infection : multiplication of an infection agent within the body of the host invasion the process where by bacteria, animal parasites, fungi and viruses, enter host cells or tissues and spread in the body .
Virulence : the quantitative ability of an agent to cause disease .

●*The degree of virulence depend on the following :*

1. **Surface components :**

A. Capsule , help to increase the resistance of bacteria to phagocytosis and resist the action of bactericidal substance in the body fluid Example : pneumonia disease cause by *pneumococci SPP*.

B. M-protein , some species of bacteria have a layer of "polysaccharide" outside their cell wall increase the pathogencity of bacteria. Example **:** *Enterobacteriace* .

2. Adherence to the surface : (adhesion , attachment) the process by which bacteria stick to the surface of host cell once bacteria have entered the body adherence is a major initial step in the inflammation process .Example : pili .

3. Toxin production : (Toxogenicity): toxins produced by bacteria are generally classified into two groups :

A: **Exotoxins :** Secreted by living growth positive bacteria Example : tetanus disease caused by toxics of *clostridium titan* and it divided to :

1- Cytotoxins 2- Neurotoxins 3- Enterotoxin .

B. **Endotoxins** : Excreted by gram Negative bacteria (**G+**) after death of bacteria: Example bacillary dysentery cause by *shigella spp*. Enteric fever cause by *Salmonella spp.*

Toxemia : production of toxins by bacteria to the blood and to all parts of the body of host .

♣Pathogenicity and virulence of micro-organism depend on the following factors :

1.Toxins and enzymes .
2. Number of bacteria .
3. Types of infected tissues .
4. Age and resistance (immunity)

♣The Rout of infections.

1-Skin and mucous membrane
2- Lungs (Respiratory tract).
3-Gastro-intestinal tract.
4- Uro-genital system .

Gram positive (G+ve)bacteria

1-Staphylococcus : SPP

Morphology : it's a G+ve bacteria , cocci , non motile , non capsulated , non spore, growth at 37C° on most bacteriological forming catalase +ve and arranged in cluster .

Species: Staphylococcus divided in to 3. groups according to the pigment production :

1. *Staphylococcus aureus* (coagulase +ve) : Produce golden yellow colonies on nutrient agar and it is pathogenic.
2. *Staphylococcus albus* (coagulase -ve) : Produce white colonies on nutrient agar and it is non pathogenic. It found normaly on skin.
3. *Staphylococcus citreus* :Produce Lemon yellow colonies on nutrient agar, it is non pathogenic.

Habitat and transmission :

It is often found in upper respiratory tract (nose) intestine and some times on the skin especially in hospital stuff and patients transmission by the hand , food and it can live in high concentration of salt. out side the body they could be also found in air Soil and water .

Characteristics : *This bacteria have many characteristics :*

1. They are aerobic and facultative anaerobic , grow easily in nutrient broth and agar after 24 hrs of incubation in 37.
2. Coagulaes positive Bio-chemical test.
3. blood hemolysis ---- haemolysin.
4- manitol fermentation.
5- Oxidase (- ve).

By these characters (2/3/4 /5) we can distinguish the Staph . aureus from other Staph . SPP.

Toxins

1. Hemolysin
2. Enterotoxin (Food poisoning)
3. fibrinolysin cause Lysis of fibrin .

Pathogenicity :

The infecton by staphylococcal disease may be classified in to :

1. Cutanous infection : Cutaneous lesions, abscess, Boils, eye infection.
2. Deep infection : Tonsillitis , pharyngitis , acute osteomyelitis
3. Staphylococcal food poisoning : Food which contaminated by entero toxin of bacteria staphylococci, for example : Milk, fish and meat .

2-Streptococcus SPP.

General Characters :

1. Gram +ve , cocci bacteria , arranged in chains
2. Non motile .
3. Non spore forming , catalase -ve (Bio-chemical test).

4. Aerobic or facultative anaerobic.
5. Some time capsulated.

The classification on the base of hemolytic.

Streptococci divided according to their haemolytic activity in to :

1. ά (Alpha) hemolytic . produce a green zone around the colony as a result of incomplete lysis of red blood cells . Example: *Streptococcus viridance*

2. β (Beta) hemolytic from a clear zone around their colonies as a result of complete lysis of red Blood cells . Example : *Streptococcus pyogenes*

3. γ (Gamma) hemolytic : non hemolytic. Example : *Streptococcus faecalis* .

Pathogenicity :

1. Strepococcus pyogen :

- Respiretory system infection : sorethroot and tonsillitis .
- Scarlet fever : Erythrogenic rash.
- Skin infection : wound and burns .
- Rheumatic fever .
- Genital tract.

2. **Streptococcus viridans :** cause subacute of endocarditis.

3. **Streptococcus faecalis** : cause urinary tract infection in the case of contamination.