Babylon University/ Nursing College/ Second stage

Practical Microbiology I

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INTRODUCTION

General Laboratory Recommendations:-

For necessary we known that the work in the microbiology laboratories acquired dealing with pathogenic microorganisms and for safety from these microorganisms therefore we must practice all these recommendations:

1-Hand washing with water & soap or with any antiseptic before & after the work.

2-Keep on the general cleaning of laboratory especially the work bench &sterilize it with disinfectant such as alcohol 70%.

3-for keeping of your clothes clean wear tl1e laboratory coat.

4-Avoid the eating smoking.

5-Less the motility inside the laboratory & prevent the air from inter the laboratory by closing the doors & windows and switch off the fans.

6-prevent putting the pipettes in the mouth before the certain from cleaning and sterilizing.

7-Read the steps of any experiment before starting with work.

8-Care from the burning because the fundamentals of microbiology laboratory is Benson or alcohol light.

9-Prevent from put the culture in the lab sink or outside the laboratory but putting it in the autoclave.

10-Culturing the microorganisms inside the hood that specific with the microbiology laboratory.

ll-One of the interesting equipments of microbiology lab is the microscope therefore use it with care and keep it clean.

Lab/1 :- The Microscope:-

Micro: small, Scope: view, It magnifies the image of the object to be visualized through it. The resolving power of the light microscope under ideal conditions is about half the wavelength of the light being used. (Resolving power is the distance that must separate two point sources of light if they are to be seen as two distinct images.).

Types of the Microscope

1-Light Microscope

2-Bright field Microscope

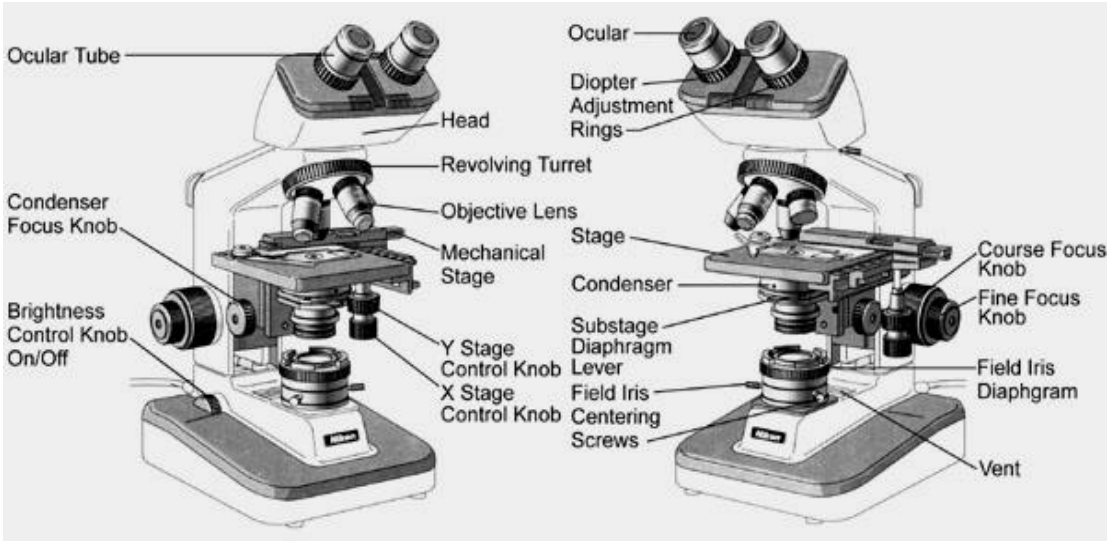
3-Dark field the Microscope

4-Ultraviolet Microscope

5-Fluorescent Microscope

6-Phase contrast Microscope

7-Electron Microscope



Conformation of compound microscope:-

Oculars

The oculars have lenses that magnify images 10 times (10x). Inside the right ocular is a *pointer* which can be moved by rotating the ocular. The right ocular is loose, while the left ocular is secured in place. This is for Köhler illumination. The oculars sit in the *ocular tubes*.

Diopter Adjustment Ring

This ring is used to accommodate the fact that both of your eyes may not be focused the same. Instructions on how to use this part are given below. This ring is found on both ocular tubes

Ocular Tube

The ocular tubes hold the oculars, and can be adjusted for *interpupillary distance*, the distance between your eyes.

Head

This part of the microscope contains a delicate prism system which helps to send an image to the oculars and your eyes.

Body

This part of the microscope houses the *revolving nosepiece or Turret* and *objective lenses*.

Revolving Nosepiece or Turret

This part of the microscope contains four *objectives* at various magnifications.

Objective Lenses

These lenses have different magnification power and divided to two types:-

1. Low power objective lenses (LP): 4X and 10X

2. High power objective lenses (HP):

40X (dry) and 100X (oil immersion).

Arm

This part of the microscope essentially holds all of the other parts, and is used in the transport of the microscope.

Course Focus Knob

This knob located on both sides of the microscope allows you to focus your image in the microscope.

Fine Focus Knob

This knob "fine tunes" the focus of your specimen.

Base

This part of the microscope holds everything in place, and is used in the transport of the microscope.

Mechanical Stage

This is where the specimen is placed for observation. The slide holder has a clamp which can swing out to hold the slide. The lever which opens the clamp is on the left side of the microscope. With a slide in place, it can be moved in the X and Y directions using the *stage control knobs*.

X Stage Control Knob

This knob will move a slide in the X-axis (horizontally) on the mechanical stage.

Y Stage Control Knob

This knob will move a slide in the Y-axis (vertically) on the mechanical stage.

Condenser System

This is a system of lenses which helps to focus light directly on the specimen that is mounted on a slide.

Diaphragm Lever

This lever is used to control the diameter of the *diaphragm*.

Condenser Focus Knob

This knob is used to focus light properly on the mounted specimen.

Field Iris Diaphragm

This system is used to vary the diameter of the field iris diaphragm, limiting the amount of light passing through the condenser system and the specimen.

Brightness Control Knob/Power Switch

This knob controls the brightness of the light, and also acts as the ON/OFF switch.

Illuminator

Housing a 6 V 20 W halogen bulb within the base of the microscope, this system provides light for specimen illumination.

Power Cord

Supplies power to the microscope illumination system.

Magnification:-

Magnification power of microscope = Magnification power of objective lenses x Magnification power of ocular lenses

For example:- the oil immersion have 100x and the ocular =10 , the magnification of microscope =100x10=1000x.

Lab/2:- Sterilization and Disinfection

Sterilization: is the perfect killing of the MO that found on the substances, since become free from this MO (vegetative cell or spores) by using physical methods.

Disinfections: is the removing of MO that hanging with substances by using of disinfectants: chemical agents that have bacteriocidal or bacteriostatic effects.

Bacteriocidal or microcidal: is killing the growth of the MO.

Bacteriostatic or microriostatic: is inhibiting the growth of the MO.

Methods of sterilization

There are three methods:

Physical, Chemical, & Mechanical methods.

Physical methods

A- Heat:

1-Dry heat:

a- Red heat: is sterilizing the tools (loope, needle, and forceps).

b- Flaming is the sterilizing of upper pit of the glasses (test tubes, flasks, and the surface of slides) on the Bunsen light flame with sloping way.

c- Burning: is the burning the clothes and dead infected animals when happened dangerous epidemic microbial diseases such as (anthrax) that caused by *Bacillus anthracis*.

2. Dry hot air: by using apparatus oven (at 160-180°C for 1.5-2 hr) is sterilizing the glass (Petri dish, pipettes, bottles, test tubes) filter papers and metal tools .

3. Moisture heat: is sterilizing the culture media& clothes by autoclave (1.5 bar for 20 mint).

B-radiation (UV, X-ray & Gamma ray):

UV, X-ray & Gamma ray used for this aim since the sterilization with rays depending on if the wave length was short it harmless to microorganisms cell the effect of ultraviolet is equal for G +ve &G-ve bacteria. This type of rays used for sterilization of the culture hood & plastic Petri dish & laboratories but care from it become it have harmless effect of biological tissue.

Chemical methods

There are two terms in these methods: Antiseptic & Disinfectant.

Antiseptic: is the chemical agent that used in sterilization of biological surface (skin).

Disinfectant: is the chemical agent that used in sterilization of non biological surface such as bench.

example of antiseptic are alcohol and iodine. Alcohol is effective in reducing the number of MO on skin, may be used disinfection of contaminated objects.

Alcohol denatures proteins, extracts membrane lipids, and acts as dehydrating agent. All of which contribute to its effectiveness as an antiseptic even viruses are inactivated by alcohol, iodine is another effective antiseptic agent, killing all types of bacteria including spores. It is frequently applied to minor wounds to kill MO that contaminated surfaces for preventing infection. Various dyes used in selective media such as crystal violets, are similarly used as antiseptic agents such as stains are normally effective bactericidal agents at concentrations of less than 1:10000 for examples of disinfectants are phenol 2-5% used for surfaces sterilization of flowers of rooms, surfaces of benches (is killed the bacteria because it act to collecting & coagulation of cell protein of bacteria) while 0.5 concentration used for preservative the serums. formalin (0.04-0.1)% for preservative the microbial suspensions and it used for sterilizing the clothes and polluted surfaces & room flowers . Mechanical methods: such as ultra filtration, these methods using for sterilization the biological fluids; serum, enzyme, antibiotics, these solutions are spoiled in high temperature, that depending on:

1.Size of substances that contaminated the liquid.

2. Nature of liquid.

3. Diameter of filter pits.

4. Electric charge of filter.

5. Electric charge of MO that found in the liquid.

Lab/3:-Culture media

The survival and growth of microorganisms depend on available nutrients and a favorable growth environment. These environments contain energy source, fundamental units & necessary contents for built and conformation the cell compartments, these fundamental units are the sources of carbon (C) & Nitrogen (N) (the materials that all the microorganisms that need them), and these culture media contain high percentage of H2O & other metals such as : Cu , Zn , metallic salts sometimes vitamins and gases such as O2 . The culture media differ depending on microbes needing to nutrition.

The media divided to three types depending on their contents

Natural media: contain natural materials such as meat extract & peptone. used for growth large numbers of microorganisms.

Synthetic media : organic & non organic chemicals inter in the its conformation.

semi synthetic media : contain nature & chemical materials most of culture media due to this type of culture media, while the media can be divided to three types depending on physical state:

Liquid culture media

Semi solid culture media

Solid culture media

Culture media packaging

Liquid culture media (broth) -: (The liquid culture media package before sterilization in test tubes , small flasks, screw capped bottles

solid culture media (agar) :- The solid culture media package in the tubes for getting of deep agar slob before sterilization , while distribution of agar in the petri dishes after sterilization with (pour plate method).

Inoculation of Media:-

The word of culture is called of inoculation the microorganism in culture media There are two terms must be know : the(pure culture) : is the culture that have one species of microbes while the (mixed culture) it contain two or more species of microorganisms.

Streak plate technique

Spreading methods

This method using with diluting series of culture and using of L_shaped glass rod.

Pour plate Technique

The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies upon plating this method using for microorganisms count.

Stapping method

Note:-

The liquid media inoculating with loop while slant agar or slob agar in the tube can be inculcated with streaking on the surface of slob agar.

Lab/4 :- Smear preparation and staining (simple and differential stains):-

Simple staining depends on the fact that bacteria differ chemically from their surroundings and thus can be stained to contrast with their environment. The stains are mostly salts (few of them are alkalines and acids),these salts contains of color ions that charging with positive and negative charges, these ions contains of groups carry the stain (chromophere) such as methylene blue.

Methylene blue +CL ⟶ Methylene blue chloride

(Chromophere)

If the chrom-carriers are positive ion that mean the stain is alkaline, while if the chrom-carriers are negative ion that mean the stain is acidic.

The stain that stains the bacterial cells called direct stain. While the stain that stains the glass of slide called negative stain.

Bacterial smear preparation:-

In the case of the **broth culture** :-

Shake the culture tube and with an inoculation loope, transfer 1to2 loopfulls of bacteria to the center of the slide. Spread this out to about a 1/2 inch area disruption of cellular arrangement A satisfactory smear will allow examination of the typical cellular arrangement and isolated cells .

In case of the **solid culture (slant or plate)**:-

Place loopfull of water in center of the slide with the inoculating needle , aseptically pick up a very small amount of culture and mix in to the drop of water. Allow the slide to air dry, or place it on a slide warmer .

Pass the slide through a burner flame 3times to heat-fix and kill the bacteria.

Staining:-

1-Simple staining:-

1. Place the fixed smears on a staining loop or rack over a sink or other suitable place.
2.Stain with any stain for 1 minutes.
3. Wash stain off slide with water for a few seconds.
4. Blot slide dry with bibulous paper.
5. Put the slide on the stage of microscope and begin with low power objective then high power objective lenses lastly with oil immersion objective lens .

2- Differential staining:-

A/ Grams Staining

In 1884, the Danish physician Christian Gram was trying to develop a staining procedure that would differentiate bacterial cells from eukaryotic nuclei in stained tissue sample. What resulted from his work is most important stain in bacteriology, the Gram stain. The Gram stain is an example of a differential stain. These staining reactions take advantage of the fact that cells or structures within cells display dissimilar staining reactions that can be distinguished by the use of different dyes. In the Gram stain, two kinds of cells, gram-positive and gram-negative, can be identified by their respective colors purple and red to pink after performing the staining method.

The Gram stain composed of:

Crystal violet (base or main stain): it's stain the G+ve bacteria with a blue to purple color.

Gram iodine (mordant iodine): that react with crystal violet to make the [Crystal violet-iodine complex] it's colored with purple black.

Ethanol 95% or acetone: it use to remove the first stain.

Safranin (counter stain): it's stain the G-ve bacteria with a pink to red color.

Procedure:

1-Prepare a bacterial smear.

2-Flood the smear with crystal violet and let for l-2 minutes.

3-Wash the slide with water .

4-Cover the slide with gram iodine and let for 1 minute.

5-Decolorize with 95% ethanol for 15 seconds.

6-Wash the slide with water.

Flood the smear with the counter stain (safranin)for 1-2 minutes.-7

8-Wash the slide with water.

9-Dry the slide with bibulous paper.

10-Examine under oil immersion (since the gram-positive bacteria stain with blue to purple while gram-negative stain with pink to red color).

B- Acid fast stain (AFB)(Ziehl-Neelson staining)

Bacteria such as Mycobacterium and Nocardia have cell walls that contain a high lipid content. One of the cell wall lipids is a waxy material called mycolic acid. This material is a complex lipid that is composed of fatty acids and fatty alcohols that have hydrocarbon chains up to 80 carbons in length. It significantly affects the staining properties of these bacteria and prevents them from being stained by many of the stains routinely used in microbiology. The acid-fast stain is an important diagnostic tool in the identification of Mycobacterium tuberculosis the causative agent of tuberculosis, and Mycobacterium leprae the bacterium that causes leprosy in humans. The facilitate staining of these bacteria, it is necessary to use techniques that make the cells more permeable to stain.

The Ziehl-Neelson stain composed of:

Carbol fuchsin solution (red color).

Ethanol 95% acidified with 20% HCL or H2SO4.

Methylene blue solustion.

Procedure:-

1-Prepare microbial smear fiom the sputum specimen & fixed it.

2-Flood the slide with carbol fuchsin stain then heat it on the Benson burner for 5minutes.

3-Wash the slide with water.

4-Decolorize with the acid- alcohol for l0-20seconds.

5-Wash the slide with water.

6-Add methylene blue for 30 seconds .

7-Wash the slide with water & dry it with bibulous paper.

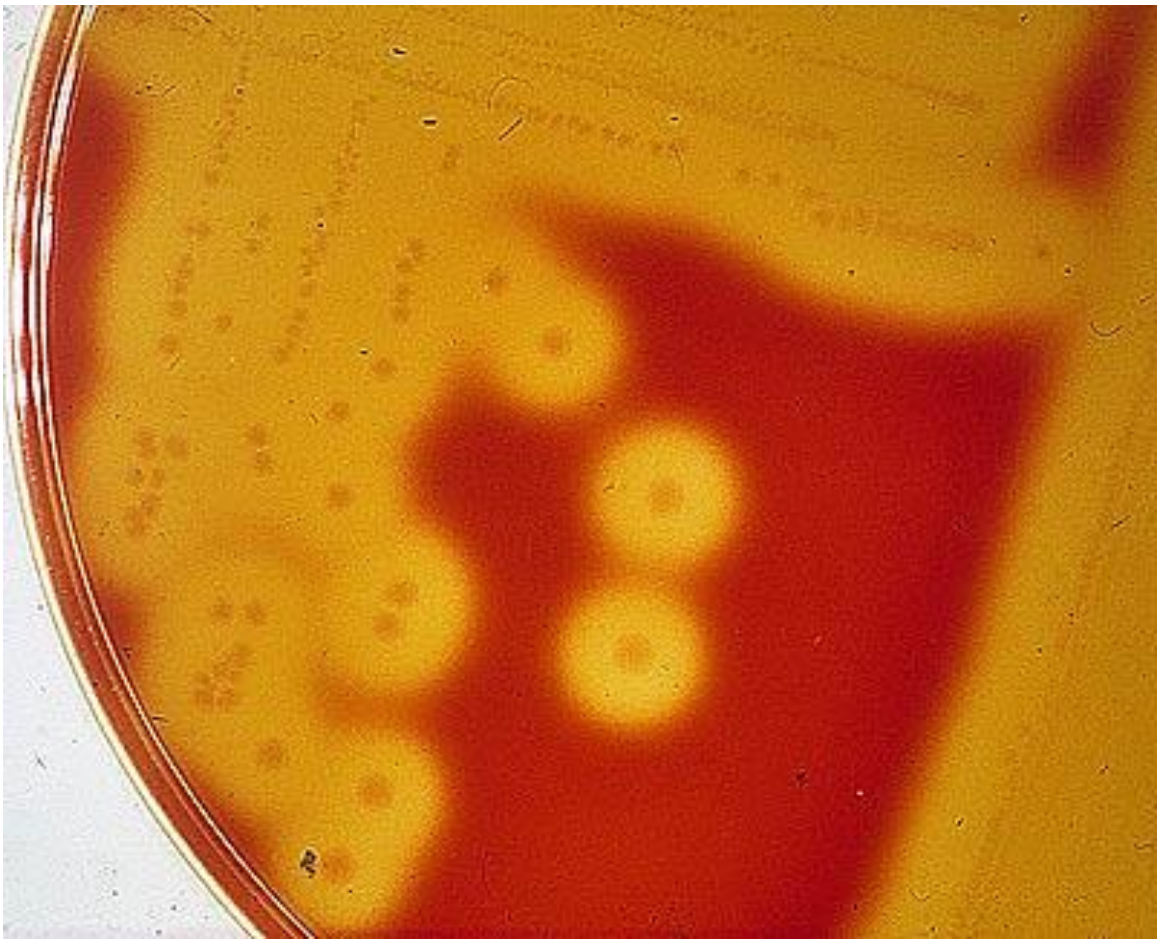
8-Examine the prepared slide under the microscope.

Lab: 5 Biochemical tests used for identification of medical bacteria

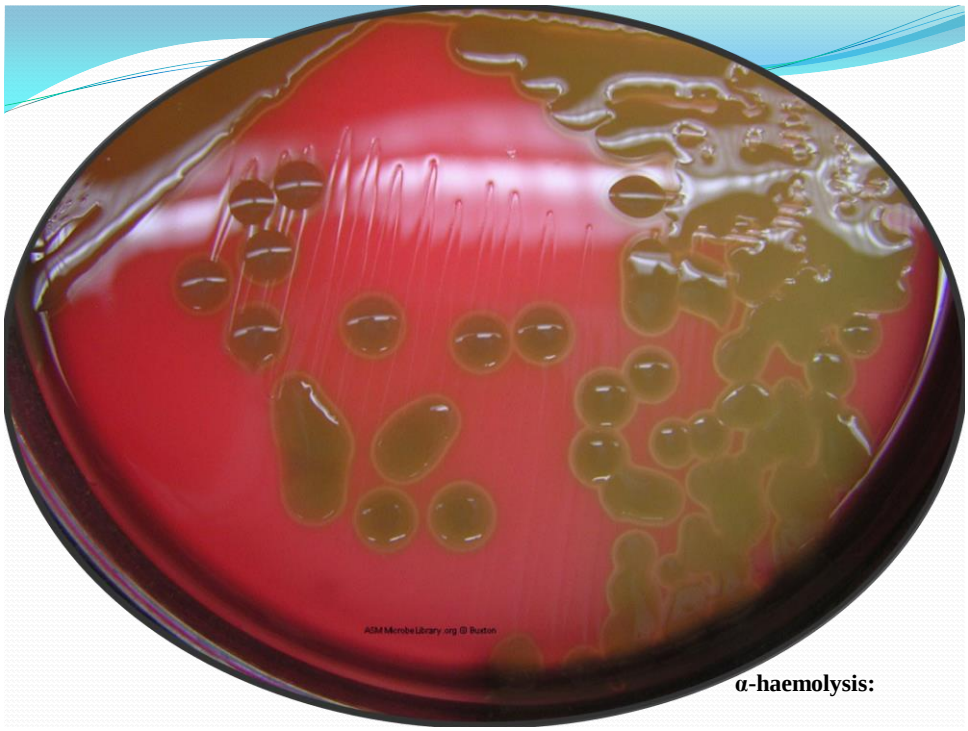
Biochemical tests have an important role in the identification of bacteria to classify bacteria and determine the causative agent of diseases.

1-Haemolysis: Some types of pathogenic bacteria are able of producing haemolysin enzyme that lyses Erythrocytes (RBCS). This can be detected *in vitro* on blood agar plates. There are three types of haemolysis:

A- **β**-haemolysis: Complete clear circular zone around the bacterial colonies due to complete lysis of red cells. e.g. *Streptococcus pyogenes and Staphylococcus aureus*



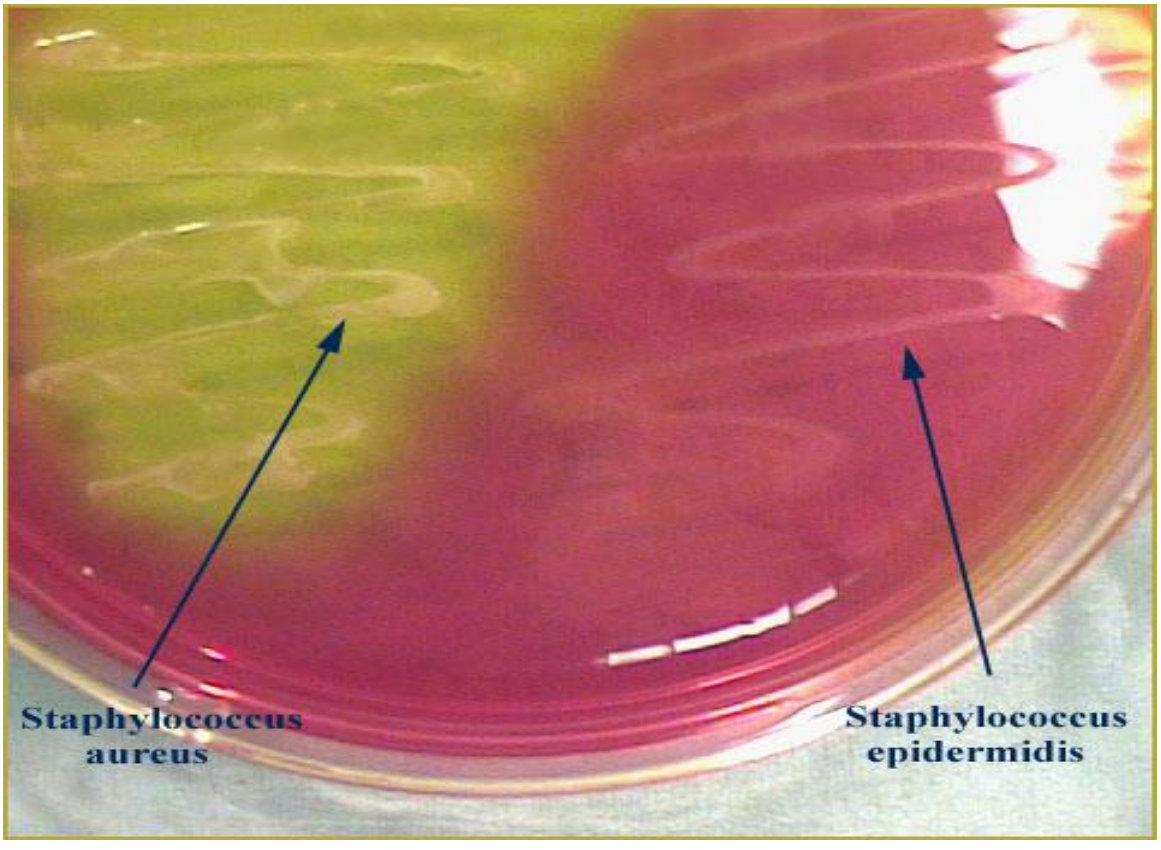
B- **α**-haemolysis: appear as greenish zone around the colonies· due to partial haemolysis of RBCs. e.g. *Streptococcus viridians*



α-haemolysis:

C- γ-haemolysis: (no haemolysis) no any obvious changes around the colonies e.g. *Enterococcus faecalis*

2- Mannitol fermentation: This can be detected *in vitro* on mannitol salt agar plates. *Staphylococcus aureus* can be ferment the sugar (mannitol) in this media &become yellow, while *S. epidermidis* cannot ferment the sugar &become white.



3-Pigment production: Some type of bacteria able to produce a characteristic pigments. There are two types of pigments:

Endopigment: Remain bound to the body of the M.O. and doesn't diffuse to the surrounding media e.g. *Serratia and Staphylococcus*

Exopigment: Soluble which readily diffuse into the surrounding media e.g.*Pseudomonas aerogenosa* produce four types of pigments Pyocyanin (blue-gree) Pyoveridin (green), Pyorubin (red) and Pyomelanin (black)

4- Motility test: Motility of bacteria can be detected by several methods; used to determine whether an organism is equipped with flagella or not e.g:-

1- Hanging drop technique

2-Stabbing of semisolid medium- .

3-Flagellar stain

Motile bacteria such as *Salmonella, Proteus and E coli*

5-Catalase production test: Some aerobic bacteria able to produce catalase enzyme that catalyses H_2O; (Hydrogen peroxide) and releases O_2 and H_2O

$$2O_2 + 2H \xrightarrow{\text{catalase}} O2 + 2H2O$$

Procedure: A small amount of bacterial culture to be tested is picked from nutrient agar by stick or glass rod and put it on the surface of a clean slide, where a drop of (3 %H2O) was added. Formation of gas bubbles indicates a positive result. A false positive reaction may obtain if the

culture medium contain catalase (Blood agar) or if iron loop is used.

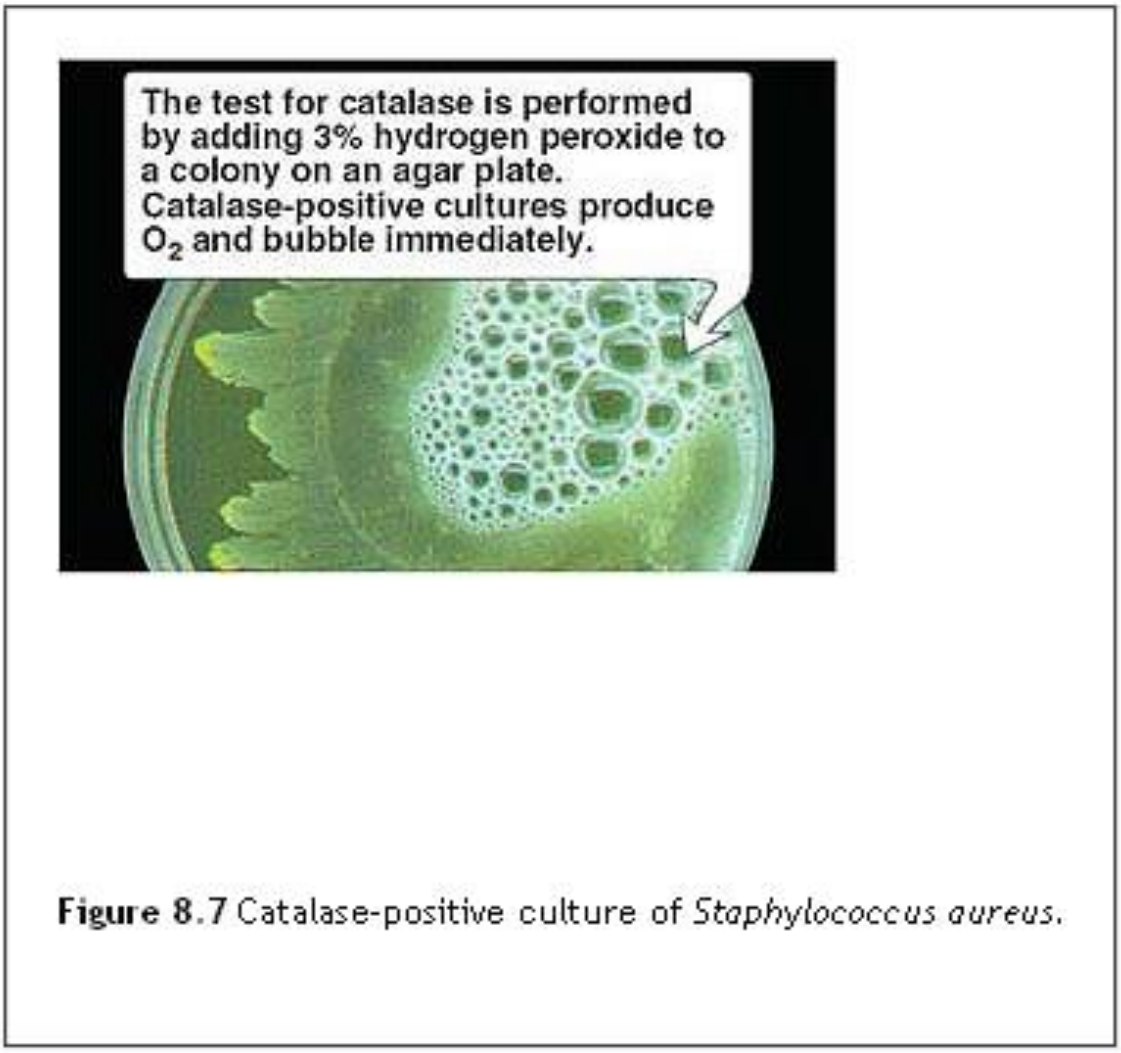


Figure 8.7 Catalase-positive culture of *Staphylococcus aureus*.

6-Coagulase production: Some bacteria produce coagulase enzyme that converts soluble fibrinogen protein to insoluble fibrin protein (coagulation of plasma).Coagulase is a virulence factor of *Staphylococcus aureus*. The formation of clot around an infection caused by this bacterium will protects it from phagocytosis

A-Bound coagulase (**Slide method)**

B- Free coagulase (**Tube method)**

7-Oxidase test: Use to detect the production of cytochrome oxidase which related to respiratory electron transport chain and it produced by strictly aerobic bacteria e.g. *Pseudomonas and Neisseriae*.

Procedure: A small area of filter paper is soaked with a freshly prepared 1% oxidase reagent (Tetramethyl-p-pheuylene Diamine Dihydrochloride) bacterial colony to be tested is picked from agar by stick or glass rod and put it on the soaked area. A positive result is indicated by formation of deep purple color due to reduction of this dye by oxidase enzyme.

8-Triple sugar iron (TSI) and Kligler's iron agar (KIA)

TSI medium contain (glucose, lactose and sucrose)

KIA contain only (glucose and lactose)

*pH indicator: phenol red (red in alkaline pH and yellow in acidic pH).

*Ferrous sulfate as an indicator of H_2S production

These media are used to detect ability of bacteria to ferment these sugars and this aid in the identification and classification of enteric G-ve bacilli (enterobacteriaceae).

Three criteria can be detected:

l- Bacterial ability to produce gas from sugar fermentation. This makes the media to push up or break up.

2-H_2S gas production can be detected by the production of black precipitate in the bottom of the media. As H2S react with iron in the media to form black ferrous sulfide in the butt.

3-Ability to ferment sugars that can be detected by color changes from red to yellow. Position of the color change distinguishes the acid production associated with glucose fermentation from the acidic products of lactose or sucrose fermentation. Bacteria that ferment glucose produce acid that turn the color of the pH indicator to yellow in the butt but not in the slant (result——> K/A). While lactose or sucrose fomenters produce more acid that turn both butt and slant to yellow (result—> A/A).

9-Urease test: This test is used to identify bacteria able of hydrolyzing urea using the enzyme urease to make ammonia and carbon dioxide. The hydrolysis of urea raises the pH to above 7.0 and the pH indicator (phenol red) turns the medium from yellow to red pink.

$NH_2\text{-}CO\text{-}NH_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$

Urea ammonia

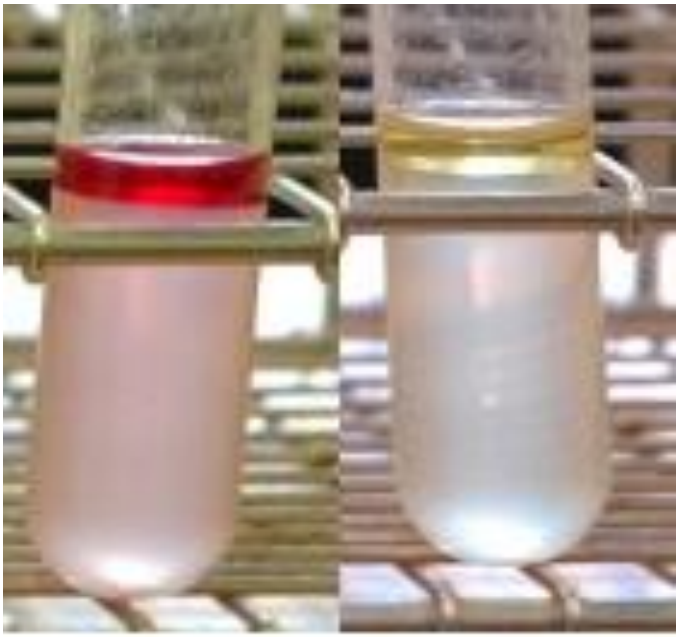
Ex: of urease producer are *Helicobacter pylori and V. cholera , Klebsiella & Proteus*

10-IMVC: These are a group of biochemical test that help in the identification and differentiation between enteric G-ve bacilli (enterobacteriaceae).

A-Indole production test: It tests for the bacterial ability to produce indole. Bacteria use an enzyme, tryptophanase to break down the amino acid (tryptophan) to give indole, ammonia and pyruvic acid.

Tryptophan — Tryptophanase —> Indole + ammonia + pyruvic acid

Peptone liquid medium containing tryptophan is inoculated the- tested bacteria and incubated at 37 °C for 24 hrs. Few drops of kovac's reagent are added to the bacterial growth. The presence of red rig in the superficial layer of the medium indicate +ve result of indole production e.g. *E.coli.* Yellow ring indicate —ve result e.g. *Klebsiella.*



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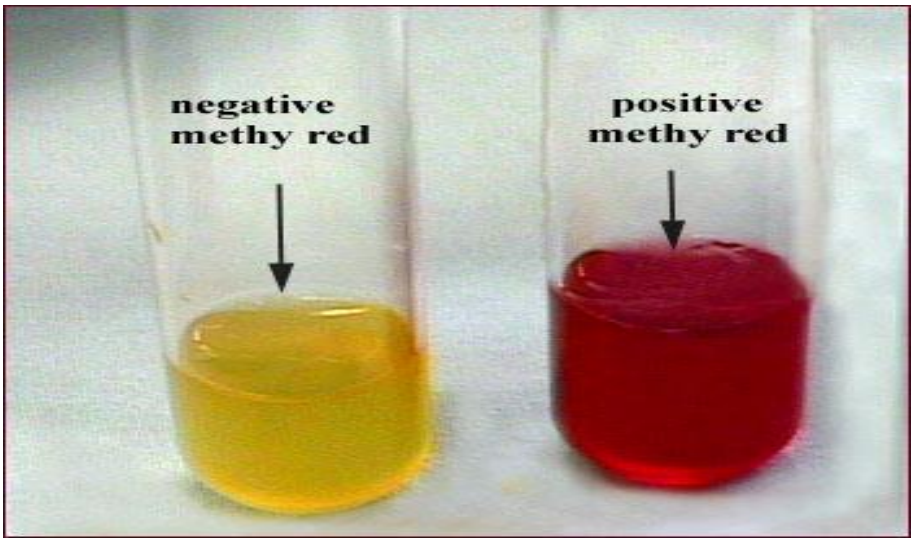
B- Methyl red/ Voges-Proskauer tests: Both MR and VP tests are used to determine what end products result when the tested organism degrades glucose (for energy production) and this depend on the type of enzyme that the bacteria have.



MR- used to detect acid as an end product from complete glucose fermentation.

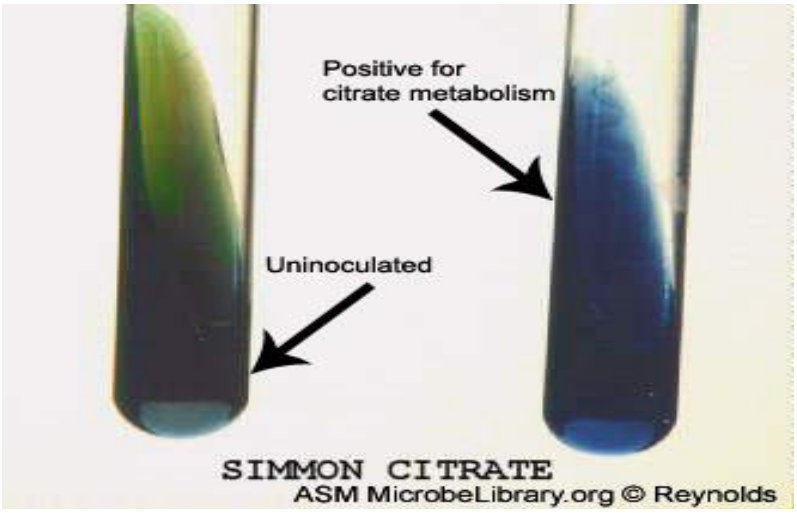
VP- used to detect acetoin (acetyl methyl carbinol) production from partial glucose fermentation.

Glucose phosphate peptone water medium is used for both tests; it's inoculated with the test bacteria, alter incubation at 37 °C for 24hrs.

In MR; 5 drops of methyl red indicator are added. Color changes of the medium to red indicate positive result e.g. E. coli and yellow in negative result e.g. *Kebsiella.*

ln VP; Voges proskauer reagent (Barritt reagent) is added to the medium. This reagent is consists of reagent A (5% or-naphtbol) and reagent B (40% KOH). Positive reaction can be detected by developing a pink-burgundy color within 20-30 min. e.g. of +ve result is *Enterabacrer aerogener and Klebsiella* while -ve result as *E. coli*

C- Citrate utilization: It used to test the ability of bacteria to consume citrate as a sole source of carbon. Simmon's citrate agar can be used with bromthymol blue as pH indicator.The tubes will be incubated after inoculation by stabbing, +ve result is blue (meaning the bacteria metabolised citrate) e.g. *Enterobacter and Klebsiella* and –ve result remains green e.g. *E coli.*



Lab/6:- Gram positive bacteria :-

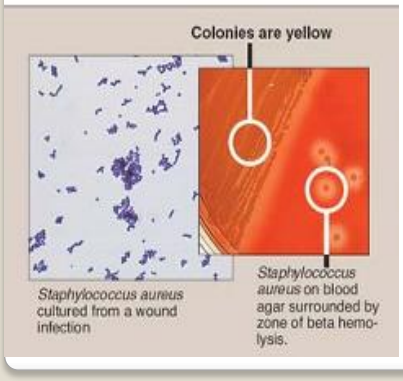
A/Gram + cocci (**Staphylococci and Streptococci**).

1. Staphylococci:

I- General features:

- Staphylococci are G+ve cocci (spherical or grapes shape).
- Are non motile, non capsulated, non spore forming.
- Staphylococci are oxidase negative & catalase positive which one feature that distinguishes from Streptococci.
- Staphylococci are part of normal flora of human skin, nose, respiratory and gastrointestinal tracts. Are also found in air, dust and other in human environments.
- *Staphylococcus* has at least 30 spp., three spp of clinical importance are *Staphylococcus aureus (S. pyogenes), S. epidermidis (S. albus), S. saprophyticus (S. citrus).*

Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation
S. aureus	Common	+	Golden yellow	+
S. epidermidis	Common	–	White	–
S. saprophyticus	Occasional	–	Variable	–



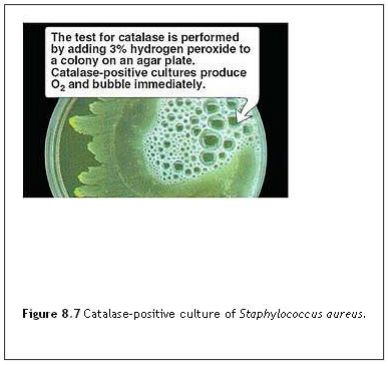


Figure 8.7 Catalase-positive culture of *Staphylococcus aureus*.

II- Transmission:

S. aureus is major pathogenic spp for human.

Transmission of bacteria from human to human by inhalation of respiratory secretion or consumption of contaminated food.

B: Clinical significance, Staphylococcal infections are classified as:

- **Skin infections**; such as abscess, pyoderma (impetigo), furuncles, carbuncles, styes, boils, folliculitis, cellulites, toxic shock syndrome, and scalded skin syndrom.
- **Respiratory tract infections;** such as tonsillitis, pharyngitis, sinusitis, pneumonia, and Otitis media.
- **Other infections**; endocarditis, osteomyelitis, meningitis, and nosocomial infections.
- **Food poisoning (Staphylococcal gasteroenteritis).**

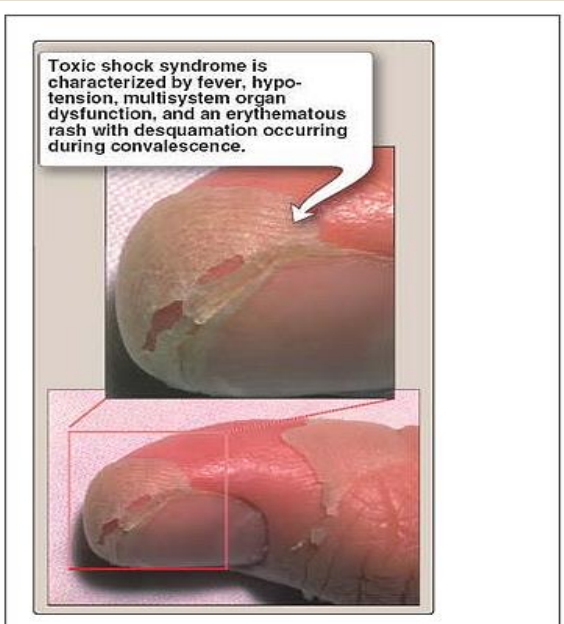
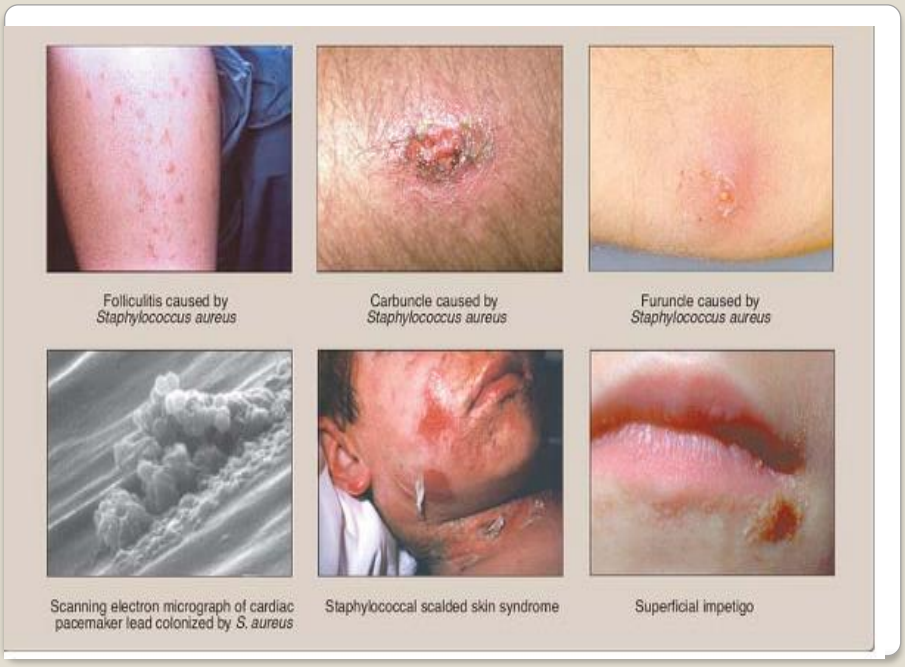


Figure 8.6 Desquamation of skin in toxic shock syndrome.



IV- Laboratory diagnosis:

A: smear examination, stained smear shows G+ve cocci arranged in cluster.

B. culture of *S. aureus,* the sample is plated on blood agar, showing yellow colonies with Beta hemolytic. Identifications of bacteria is confirmed by catalase positive, coagulase test positive, mannitol fermentation, and grow in high concentration (7.5%) of NaCl.

V- Control

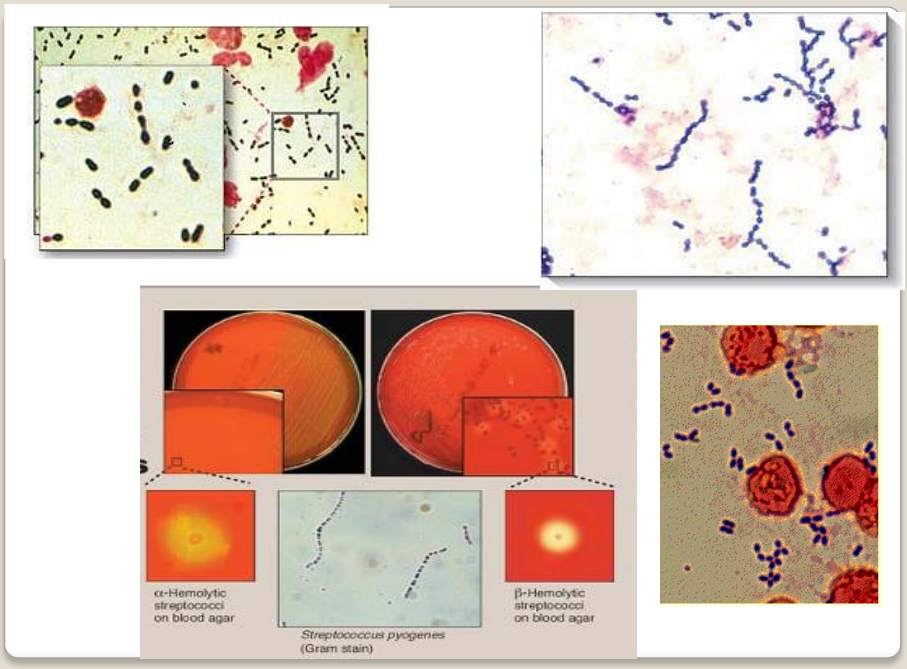
2. Streptococci

I- General features:

1. 1. Streptococci are G+ve cocci (spherical, chain or pairs shape).
2. 2. Are non motile, non spore forming and non capsulated (some strain have capsule).
3. Streptococci are oxidase & catalase negative which one feature that distinguishes the Streptococci from Staphylococci.
4. Streptococci are member of normal flora skin, respiratory tract and some are normal flora of enteric and genital tracts of human.

A streptococcus has at least 20 spp. *S. pyogenes,* and *S. pneumoniae* are clinical

Importance for human.



II- Transmission:

Respiratory tract infections (*S. pyogenes*) are transmitted by inhalation of respiratory droplets. Skin infection occurs after direct contact with infected individuals or contaminated fomites.

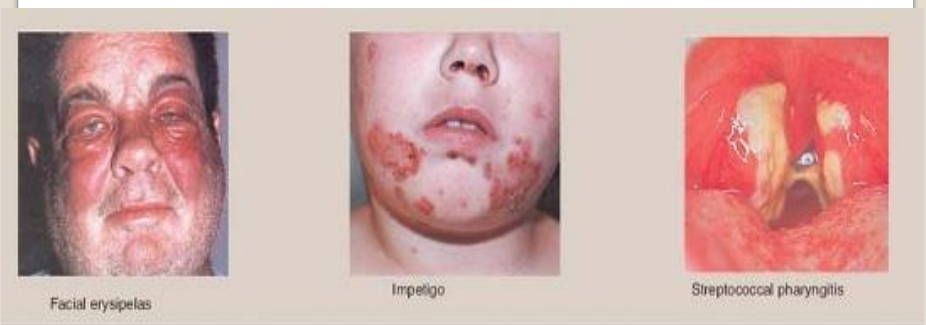
Clinical features Streptococcal infections are classified as:

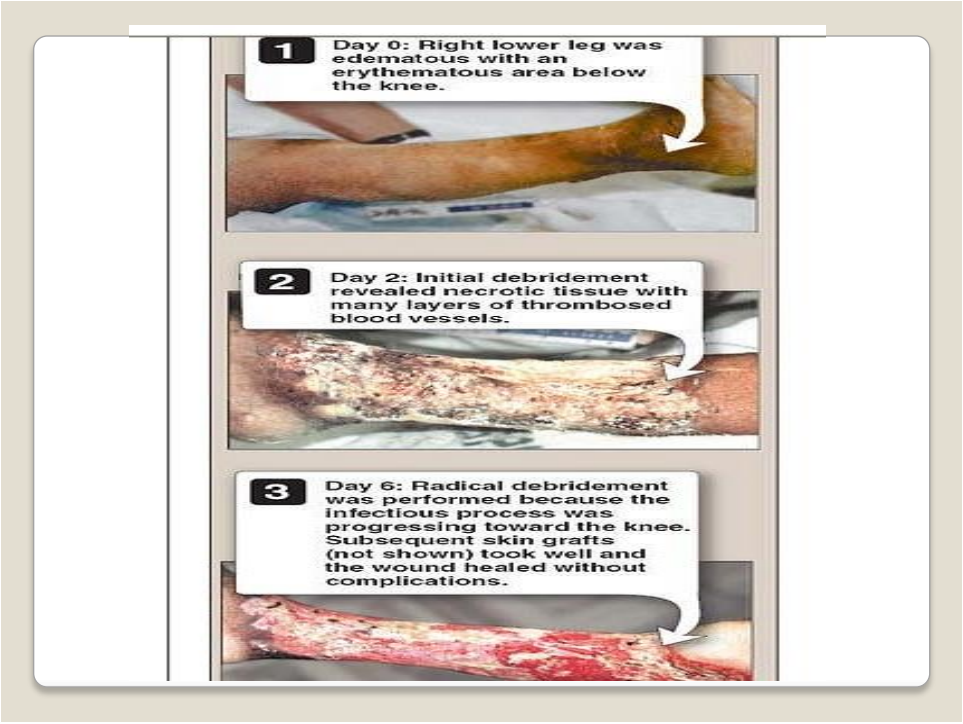
1- pyogenic infections (skin & respiratory tract infection)

2- Respiratory tract infection;

3- Sore throat (tonsillitis) after incubation periods (1-3 days), or its may be invade pharynx and causes pharyngitis.

4- It may be causes severe pneumonia with fever and cough.



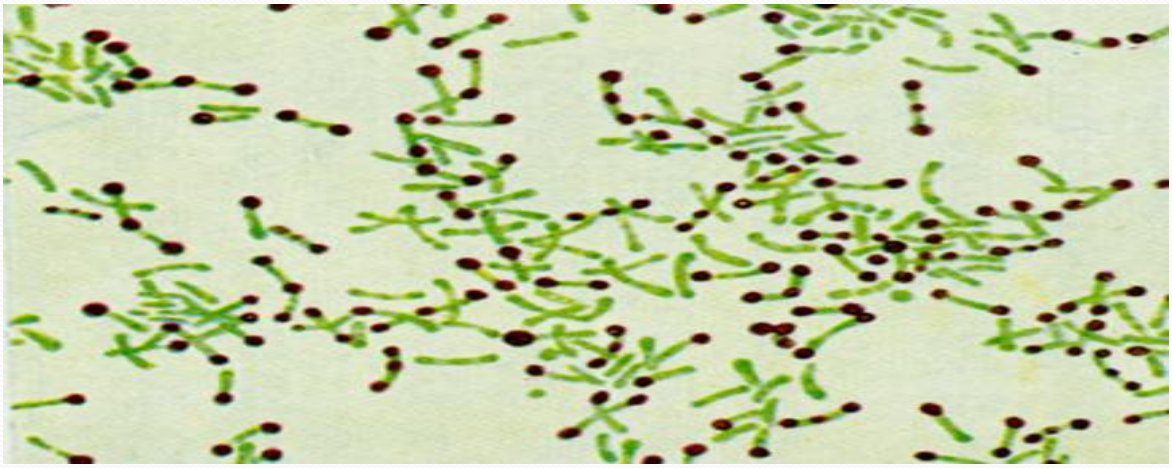

1
Day 0: Right lower leg was edematous with an erythematous area below the knee.
2
Day 2: Initial debridement revealed necrotic tissue with many layers of thrombosed blood vessels.
3
Day 6: Radical debridement was performed because the infectious process was progressing toward the knee. Subsequent skin grafts (not shown) took well and the wound healed without complications.

Lab/7

B/Gram + bacilli (**Corynebacterium diphtherae and mycobacterium tuberculosis)**

Corynebacterium diphtheriae

Corynebacteria are small, slender, pleomorphic, gram-positive bacilli & Chinese letters. They are non motile, un encapsulated, and do not spore formation, catalase positive , oxidase nagative containing chromatin granules called volutin granules or Babes- Ernest granules present in cytoplasm (staining by Albert stain).



The genus *corynebacterium* have many species represent normal flora of human skin and mucous membranes such as *C. hoffmanii, xerosis and diphtheriae*

corynebacterium diphtheriae is the principle human pathgen with its exotoxin which filtaration to circulation system and infected the heart muscles and produce diphtheria in human.

C/Gram + bacilli spore forming

Bacillus anthracis

It is gram-positive rods, nonmotile, encapsulated, **spores forming,** and facultative aerobes.

Pathogenesis:

B. anthracis possesses a capsule that is antiphagocytic and is essential for full virulence. The organism also produces three exotoxins;

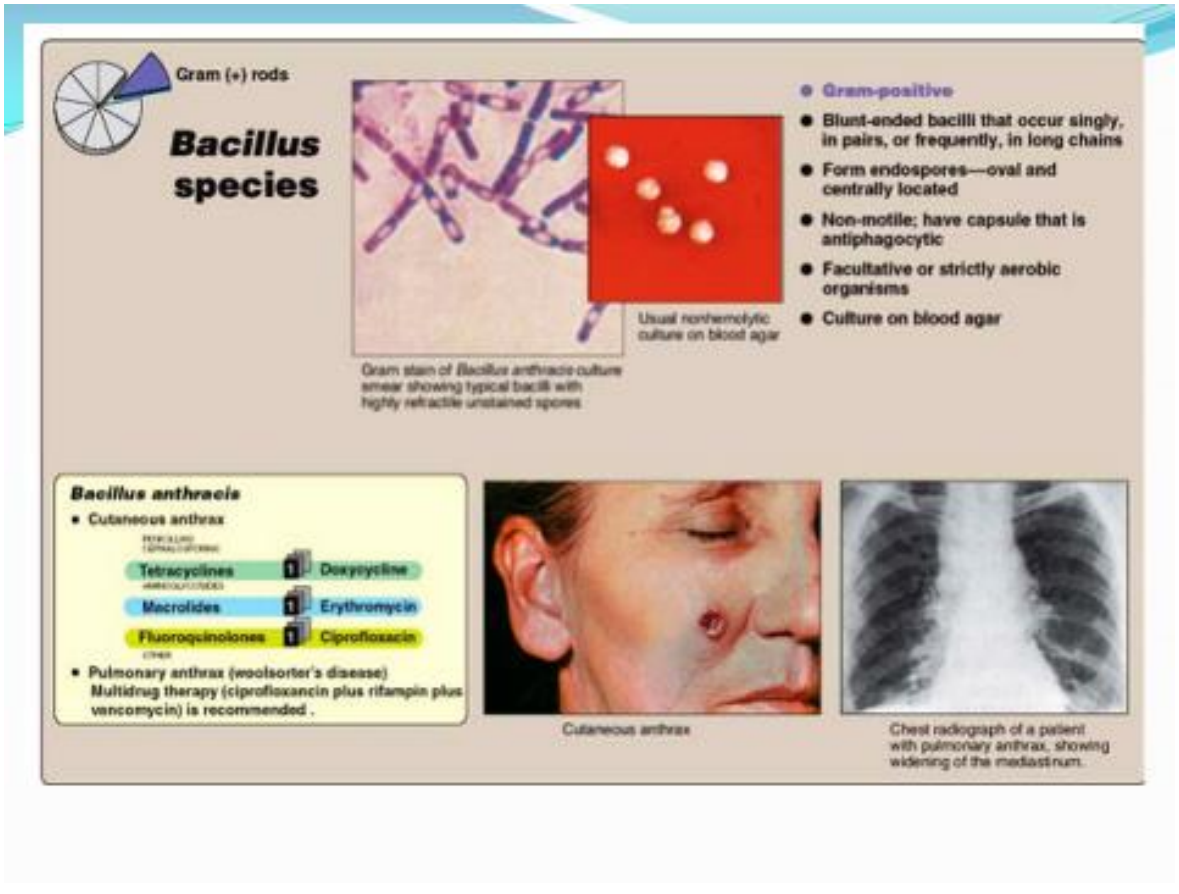
edema factor is responsible for the severe edema usually seen in B. anthracis infections;

lethal toxin is responsible for tissue necrosis;

protective antigen mediates cell entry of edema factor and lethal toxin

Laboratory identification:

B. anthracis is easily recovered from clinical materials. Microscopically, the organisms appear as blunt-ended bacilli that occur singly, in pairs, or frequently in long chains. They do not sporulate often in clinical samples, but do so in culture. The spores are oval and centrally located. On blood agar, the colonies are large, grayish, and nonhemolytic, with an irregular border.



Clinical significance

1. **Cutaneous anthrax**: About 95% of human cases of anthrax are cutaneous. Upon introduction of organisms or spores that germinate, a papule develops. It rapidly evolves into a painless, black, severely swollen malignant pustule, which eventually crusts over. The organisms may invade regional lymph nodes and then the general circulation, leading to fatal septicemia. Although some cases remain localized and heal, the overall mortality in untreated cutaneous anthrax is 20%.
2. **Pulmonary anthrax** (wool sorter's disease) is caused by inhalation of spores. It is characterized by progressive hemorrhagic lymphadenitis (inflammation of the lymph nodes), and has a mortality rate approaching 100 percent if left untreated.

***Mycobacterium tuberculosis* or tubercle bacillus (TB)**

TB is long, slender rods, aerobic that are non motile and do not spore formation. TB have thick cell walls, they are high lipid, (mycolic acids)

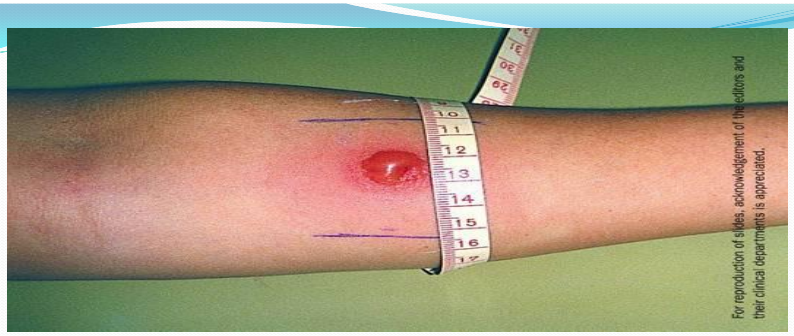
Mycobacterium tuberculosis causes tuberculosis

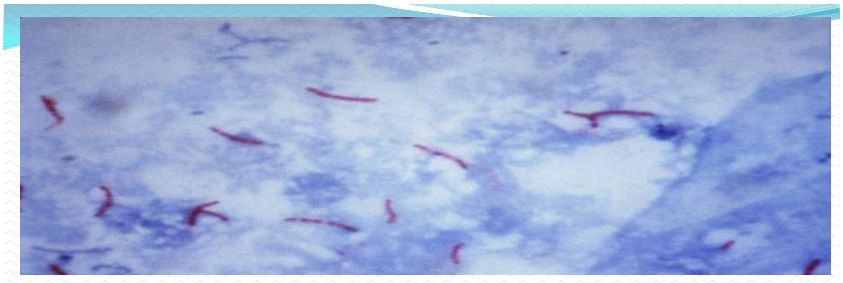
Mycobacterium leprae causes Leprosy

A microscopic search for acid-fast bacilli using the **Ziehl-Neelsen stain** is the most rapid test for mycobacteria.

Culture: Lowenstein-Jensen medium , appeare white color with mucoid

Tuberculin test : In the routine procedure, a measured amount of PPD (purified protein derivative) is injected intr-dermally in the forearm. It is read 48 to 72 hours later for the presence and size of an area of induration (hardening) at the site of injection, which must be observed for the test to be positive.





Lab/8:- Gram negative bacteria :-

A/Gram – cocci (Neisseria)

Neisseria

Gram – , (diplococcus), catlase and oxidase positive, non motile, non hemolytic. Two important spp are pathogenic for human;

N. gonorrhoeae : Diplococcus in kidney shape

N. meningitidis : Diplococcus in spherical shape.

It is fastidious grow on enriched media (chocolate agar) and selective media (Thayer-Martin medium) which contain 3 antibiotic (VCN) , Vancomycin , Colistin & Nystatin.

incubated under 5-10% CO2.

B/Gram – bacilli (Enterobacteriaceae)

G-bacilli / Enterobacteriaceae

	E.coli	*Klebsiella*	*Salmonella*	*Shigella*
Cause	GIT,UTI	GIT,RTI	Food poisoning, Typhoid	GIT
Motility	Motile	Non	Motile	Non
Capsule	Non	Thick Capsule	Non	Non
Lactose ferment.	+	+	-	-

Non spore formation , glucose fermentation , Oxidase - , Catalase + , Reduce $No_3 \longrightarrow No_2$

Differential Media

1- MacConky agar

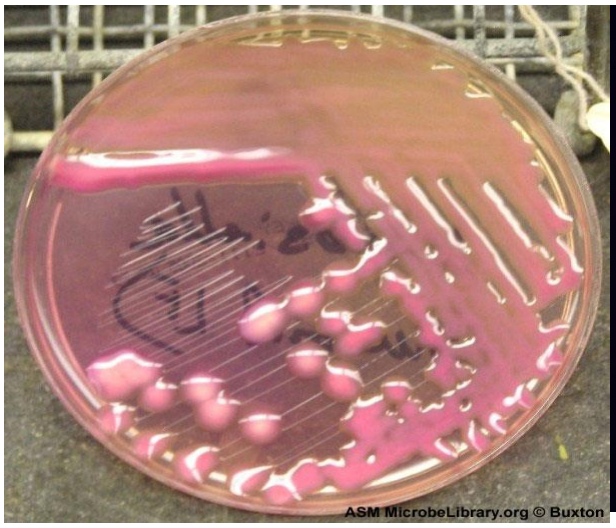
1- MacConky agar

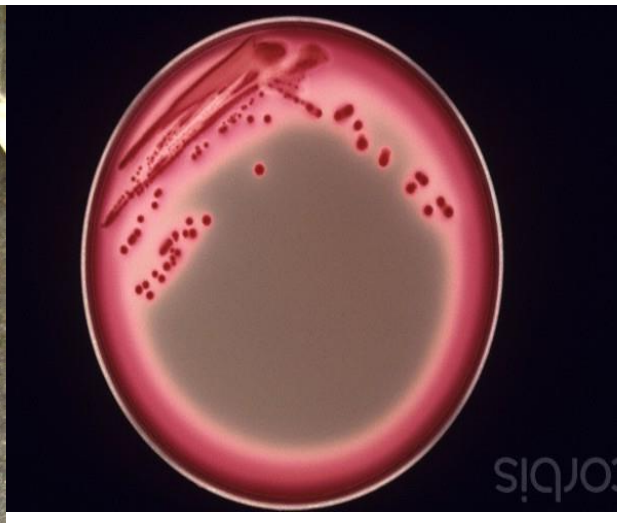
- ***E.coli pink***
- ***Klebsiella pink + Mucoid***

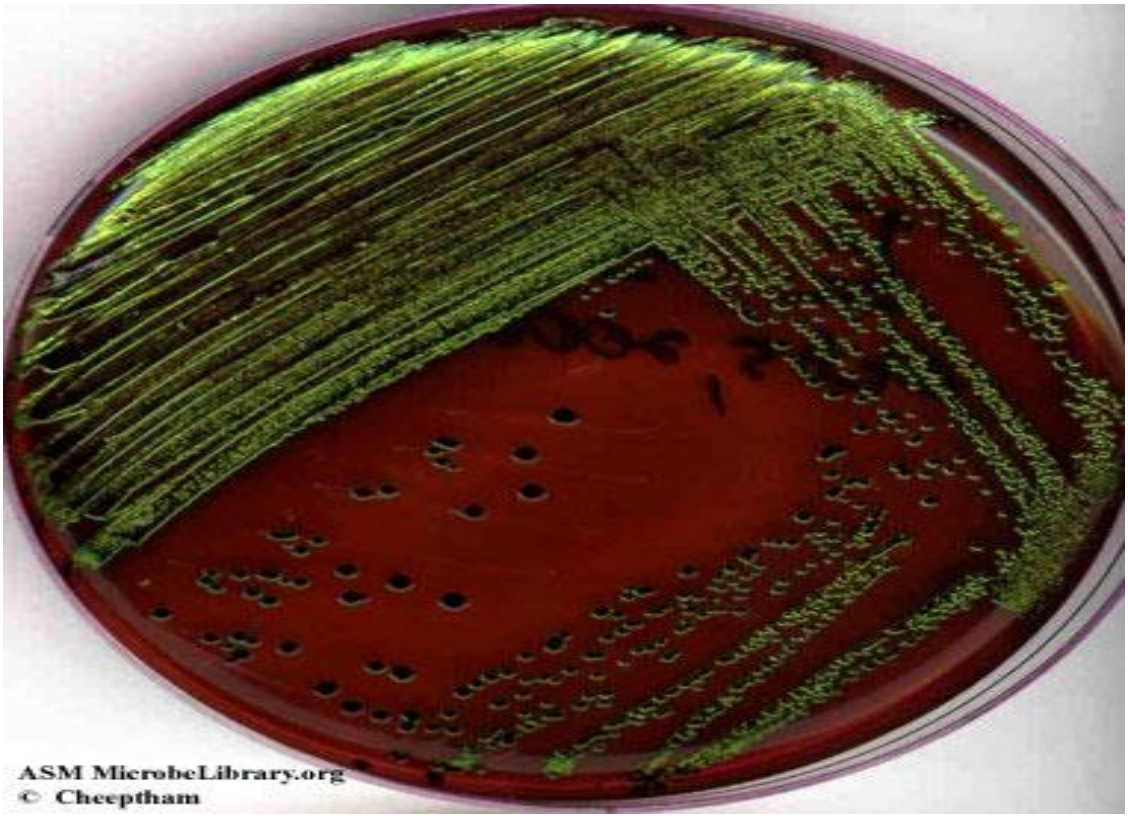
2- Eosine Methylene Blue (EMB)

2- Eosine Methylene Blue (EMB)

- ***E.coli dark and large with metalic green sheen***
- ***Klebsiella pink + Mucoid***







3- *Salmonella* , *Shigella* agar (S.S agar)

Colorless with black center (H_2S)

Colorless

MB-S1394 Salmonella Shigella (SS) Agar
Cultivated Shigella flexneri (L)
/ Salmonella typhimurium (R)

Vibrio cholerae

Members of the genus Vibrio are short, curved, rod-shaped organisms. They are rapidly motile by means of a single polar flagellum. O and H antigens are both present, but only O antigens are useful in distinguishing strains of vibrios that cause epidemics, which cause gastroenteritis and extraintestinal infections.

Laboratory identification:

V. cholerae grows on s blood and MacConkey agars. Thiosulfate citrate bile salts sucrose (TCBS) medium can enhance isolation. The organism is alkaloids and oxidase-positive

Treatment and prevention:

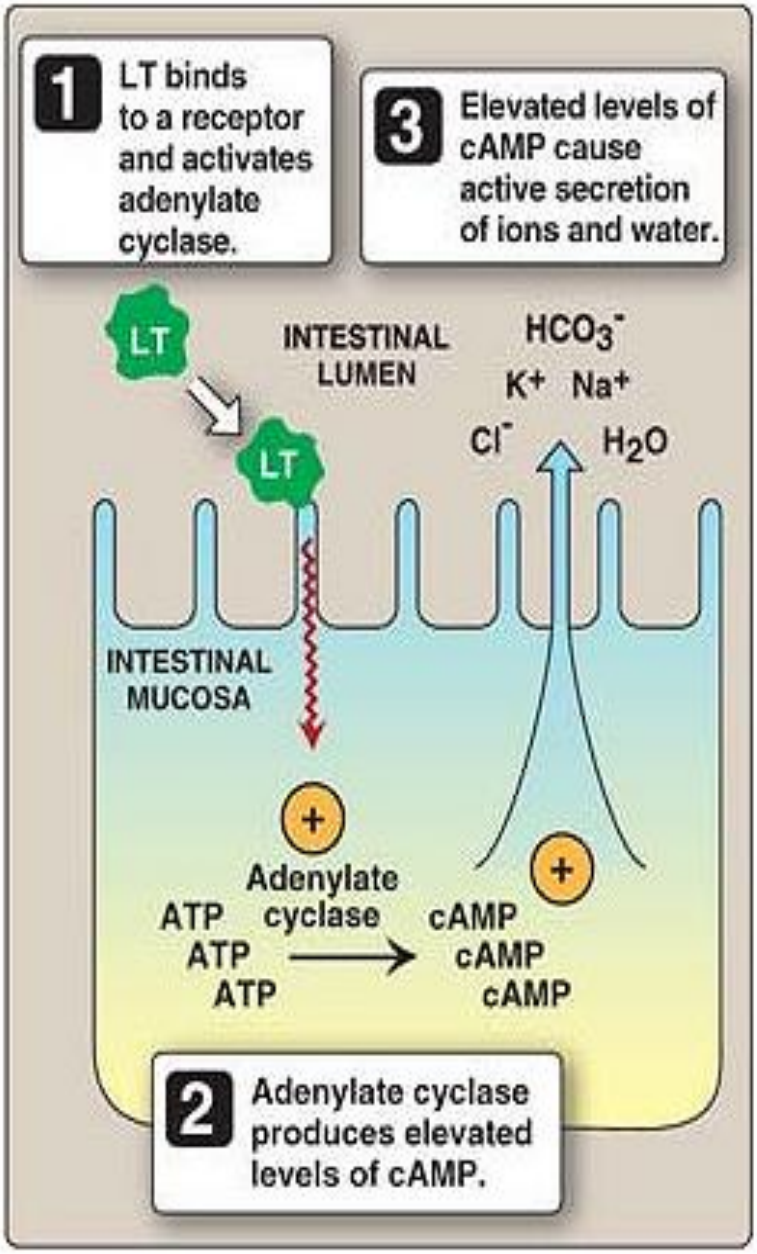
Replacement of fluids and electrolytes is crucial in preventing shock. doxycycline can shorten the duration of diarrhea and excretion of the organism

Clinical significance

cholera is characterized by massive loss of fluid and electrolytes from the body. After an incubation period ranging from hours to a few days, profuse watery diarrhea (rice-water stools) begins. Untreated, death from severe dehydration causing hypovolemic shock may occur in hours to days. Patients with suspected cholera need to be treated prior to laboratory confirmation, because death by dehydration can occur within hours.

Pathogenesis

Following ingestion, V. cholerae infects the small intestine. Adhesion factors are important for colonization and virulence. The organism is noninvasive, and causes disease through the action of an enterotoxin that initiates an outpouring of fluid. Cholera toxin is protein bound Gs protein. Gs protein activates adenylate cyclase, which produces elevated levels of intracellular cAMP. This, in turn, causes an outflowing of ions and water to the lumen of the intestine. `


1 LT binds to a receptor and activates adenylate cyclase.
3 Elevated levels of cAMP cause active secretion of ions and water.
LT
LT
INTESTINAL LUMEN
HCO_3^-
K^+ Na^+
Cl^-
H_2O
INTESTINAL MUCOSA
+
Adenylate cyclase
ATP ATP ATP
cAMP cAMP cAMP
+
2 Adenylate cyclase produces elevated levels of cAMP.

Lab/9:- Antimicrobial susceptibility testing

Antimicrobials: is agent killing the disease-causing bacteria.

Antimicrobial susceptibility testing: An *in vitro* test; done to check the effectiveness of a drug against a bacterium and to select the best drug that acts against the bacterium.

Antimicrobial agents can be divided into two categories:

1- Natural antimicrobial agents: are the substances produced as secondary metabolites by living organisms and which are active against other organisms, and called antibiotics.

2- Synthetic antimicrobial agents: are simple compounds, obtained by synthesis of the agents in the laboratory, such as sulfonamide and trimethoprime.

-The antimicrobial agents have two types of effect, either bacteriostatic or bacteriocidal.

-Some antimicrobial agents are active against several types of M.O. called (Broad-spectrum),whereas other are active against few types of M.O. called (Narrow-spectrum).

Mode of action:

Antibiotics have selective inhibition of growth of M.O. without damage to the host cell. This selectivity due to the differences between the metabolism and structure of M.O. and human. Mode of action according to the site of effect:

1. Inhibition of cell wall synthesis, ex, penicillins and cephalosporines.

2. Inhibition of cell membrane synthesis, ex, polymixine and garamicine.

3. Inhibition of nucleic acid synthesis, ex, rifampicine and quinolones.

4. Inhibition of protein synthesis, ex, erythromycin and tetracycline.

Resistance to antibacterial agents:

The major mechanisms that mediate bacterial resistance to drugs:

1. Certain bacteria produce enzymes that destroy the drug, ex, Beta-lactamase enzymes can inactivate penicillines and cephalosporines by cleaving the beta-lactam ring of the drug.

2. Certain bacteria synthesize modified target site of drug action.

3. Certain bacteria change their permeability to the drugs.

4. Certain bacteria increase the export of drug to the outside of the M.O.

Notes:

A.Bacteria have the ability to develop resistance following repeated or insufficient doses, so more advanced and synthetic antibiotics are continually required to overcome them.

B.Certain bacteria are not only resistant to drug but require it for growth, called drug-dependent bacteria.

C.Most drug resistance is due to a genetic change in bacteria(1)due to mutation in bacterial chromosome, inherited (2) acquired resistance due to acquisition of genetic materials.

Methods of antimicrobial susceptibility testing:

Because the bacteria rapidly develop resistance, therefore; they should be tested for antimicrobial susceptibility by one of the following methods:

Conventional testing methods, such as disc diffusion and dilution tests.

Commercial testing methods, such as E-test.

Factors affecting antimicrobial susceptibility in vitro:

Type and components of the medium.

Type of inoculating method.

Number and activity of inoculated bacteria.

Stability and concentration of the used drug.

Temperature and time of incubation.

Disc diffusion method (Kirby and Bauer, 1966)

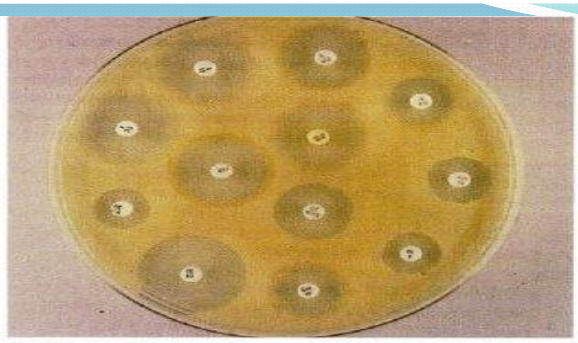
Preparation of bacterial suspension by taking 5 colonies of young culture and adding it to tube contains 5 ml of broth medium then this tube will be incubated at 37°C for 5 hours.

The turbidity of bacterial suspension will be compared with the standard McFarland tube for determination of bacterial cell number that used in the inoculation.

About 0.1 ml of the bacterial suspension is spread over a solid medium (Muller-Hinton agar medium) by swabbing (vertical and horizontal directions). The inoculated plate is left to dry at room temperature for 15 minutes.

Antibiotic-impregnated filter paper discs are placed, 4-6 discs on each plate by a sterile forceps. The plate will be incubated at 37°C for 18-24 hours.

The result will be recorded by measuring the inhibition zones (in mm). The interpretation of these results as sensitive or resistant according to a standard document of CLSI (clinical and laboratory standard institutes). The efficacy of drug combination can be estimated as synergistic (enhancement of efficacy of drug in the presence of other drug), or antagonistic (impairment of efficacy) or indifferent (no change in the presence of other drug).



5-12 Antimicrobial susceptibility testing of *S. aureus*. Most community-acquired strains of *S. aureus* are resistant to penicillin.

L-10/:-Immunity

Immunity is defined as body's resistance to invasion by microorganisms and damage by foreign substances vertebrate possess a sophisticated immune response that protects them from invading pathogens when a vertebrate is exposed to foreign macromolecules called antigen , the immunologic system produces proteins called antibodies , which react specifically with the antigen responsible for their synthesis the immune response may involve blood . Blood consist of a fluid portion called plasma. die cells in blood that are of immunologic importance are the leukocytes (white blood cells), serum is the fluid portion of blood that remains after blood has clotted. Blood serum that contains the specific antibody is referred to as antiserum. Not only antibodies play a major role in the resistance to invading microorganisms but they are also very important in the identification of both pathogenic microorganisms and a variety of proteins and other antigens.

Serology is the branch of immunology that studies the antigen — antibody reaction in vitro.

There are many types of immunological test -

1-Agglutinationtest.
2- precipitation test

3-complement test.
4-Immunoflorescence test.
5- Enzyme — linked Immunosorbant Assay(ELISA) test.
6- Neutralization test.
7- haemagglutination test & haemgglutination inhibition test.
8- Gel diffusion test.

1-Agglutination test:-

In *this* test, the Ag is particulate (eg, bacteria & red blood cells) and when red cells are used , the reaction is called hearnagglutination or the Ag is an inert particle (Latex beads) coated

with an Ag.

Ab , because it is divalent or multivalent, cross—links the antigenically multivalent particles and forms a lattice work, and clumping (agglutination) can be seen. the application of agglutination test in clinical medicine

1- In determine a person's ABO blood group for transfusion.

2- To identify bacterial cultures .

3- To detect the presence relative amount of specific Ab in patients serum.

4- widely used for rapid diagnosis of several disease such as: -

a- widal test — typhoid fever (salmonellosis)

b- Rose bengal — Malta fever (Brucellosis)

c- VDRL (veniral Disease References Lab) for syphilis (*Treponema pallidum)*

c- ASOT (Anti — streptolysin 0 test).

2- prccipitation : - (p.p.t.)

In this test, the Ag is insoluble , the Ab *cross* — links , Ag molecules in variable proportion, and aggregates (precipitates) forms.

Application of precipitation Reaction : -

1- Ring test : - ex : typing of streptococci and pneumococci

c- reactive protein test.

2- slide test:- ex. : VDRL (diagnosis syphilis) the reaction appears in the form of floccules,

3- tube flocculation test . (ex. : Kahn test for syphilis and also used for standardization- of toxins & toxoids)

3- Complement Fixation Test: -(CFT)

For many decades , CFT remained a main step for syphilis diagnosis , Modern Technology this technique used for detection of Ab against a Varity of viruses , fungi & bacteria.

It used mainly for diagnosis of encephalitis , meningococcal meningitis & histoplasmosis.

4- Immuno fluorescence test: -

Fluorescencent dyes, ex Fluorescein which emits an apple — green and

rhodamine, which emits orange — red higher fluorescent dye can be covalently to antibody molecules and made visible by ultra — violet (UV) light in fluorescence microscope. such labeled antibody can be used to identify antigens , ex. on the surface of bacteria (such as streptococci and treponemes) , in cells in histologic section, or in other immunofluorescent reaction is direct when known, labeled antibody interacts directly with unknown antigen and indirect when a two — stage process is used.

5- Enzyme — linked Immunosorbent Assay (ELISA):-

This method can be used *for* quantitation of either antigens or antibodies in patient specimens . It is based on covalently linking an enzyme to a known antigen or antibody. 'reacting the enzyme -linked material with patients specimen, and then assaying for enzyme activity by adding the substrate of the enzyme . the method is nearly as sensitive
as RIA (radioimmuno assay) yet requires no special equipment.

6- Neutralization test: -

These use the ability of antibodies to block the effect of toxins or the infectivity of viruses . they can used in cell culture (eg, inhibition of cytopatic effect and plaque reduction assays) or in host animal (eg , mouse protection test).

7- Hemoagglutination test : -

a- active hemagglutination - many viruses clump red blood ccii from one species or another,
b- hemagg1utinjion inhibition - hemoagglutination can by inhibited by antibody specifically directed against the viruses and can be used to measure the titer of such antibody
c- passive hemagglutination :- Red blood cells also can absorb many antigens and, when mixed with matching antibodies will clump.

<u>widal test</u>

the widal test is a reaction involving the agglutination of typhoid bacilli when they are mixed with serum (containing typhoid antibodies, the agglutinin) from an individual having typhoid fever.

procedure :-

first period

1- using the wax pencil , label nine clean serology test tubes with proper dilution as indicated in the figure2, and place the tubes in a test tube rack
2- pipette 0 9 ml of 0 85 % saline in to tube 1 and 0 5 ml in to the other eight tubes
3- Added 0.1 ml of *Salmonella* antiserum to tube I with 0.1 ml serological pipette. Mixed by drawing the solution in to the serological pipette and slowly blowing it out with a pipette.
4- Transfer 0.5 ml with the same pipette to the second tube Mix as before and continue transferring 0.5 ml aliquots through tube 8. Discard 0.5 ml from tube 8 after mixing. Tubes 1-8 constitute a dilution series from 1/15 through 1 / 1280 tube 9 is the antigen control tube and contains 0.5 ml antigen + 0,5 ml saline.
5- Add 0.5 ml of *Salmonella* o antigen or heated *S.typhi* culture to each dilution tube (2-9) . Mix by gently shaking the test tube rack to agitate all tubes.
6- Incubate the tubes in 37C water bath for 1 hour.
7- After the 1 hour period, check tube 9 first for non specific agglutination (macroscopic floccules), then tubes 1-8 for specific agglutination with a good light source against a black background.
8- Refrigerate until the next laboratory period

Second period

1- observe the bottom of each tube for agglutination . examine all tubes without shaking and rank them for agglutination tube 9 should show no agglutination and be ranked o. *A* tube would be given a 4 + ranking if all cells had clumped and settled to the bottom of the tube . the saline control is seen as a compact button, with cloudy supernatant If tube show agglutination one observe a compact mass on the bottom with a clear supernatant. In serology , controls very important, because some bacterial suspensions agglutinate spontaneously. If this occurs the test must be repeated with different cell suspension. 2-the titer of antiserum is an indication of its antibody level .