The First lab

Sterilization and Disinfection

Sterilization refers to any process that removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents present in a specific surface, object or fluid, for example food or biological culture media.

Sterilization also defined as describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.

Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic.

Disinfection: describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Cleaning: is removal of visible soil (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.

Decontamination: removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

The purpose of Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Because sterilization of all patient-care items is not necessary, health-care policies must identify, primarily on the basis of the items' intended use, whether cleaning, disinfection, or sterilization is indicated.

METHODS OF STERILIZATION

The various methods of sterilization are:

1. Physical Method :

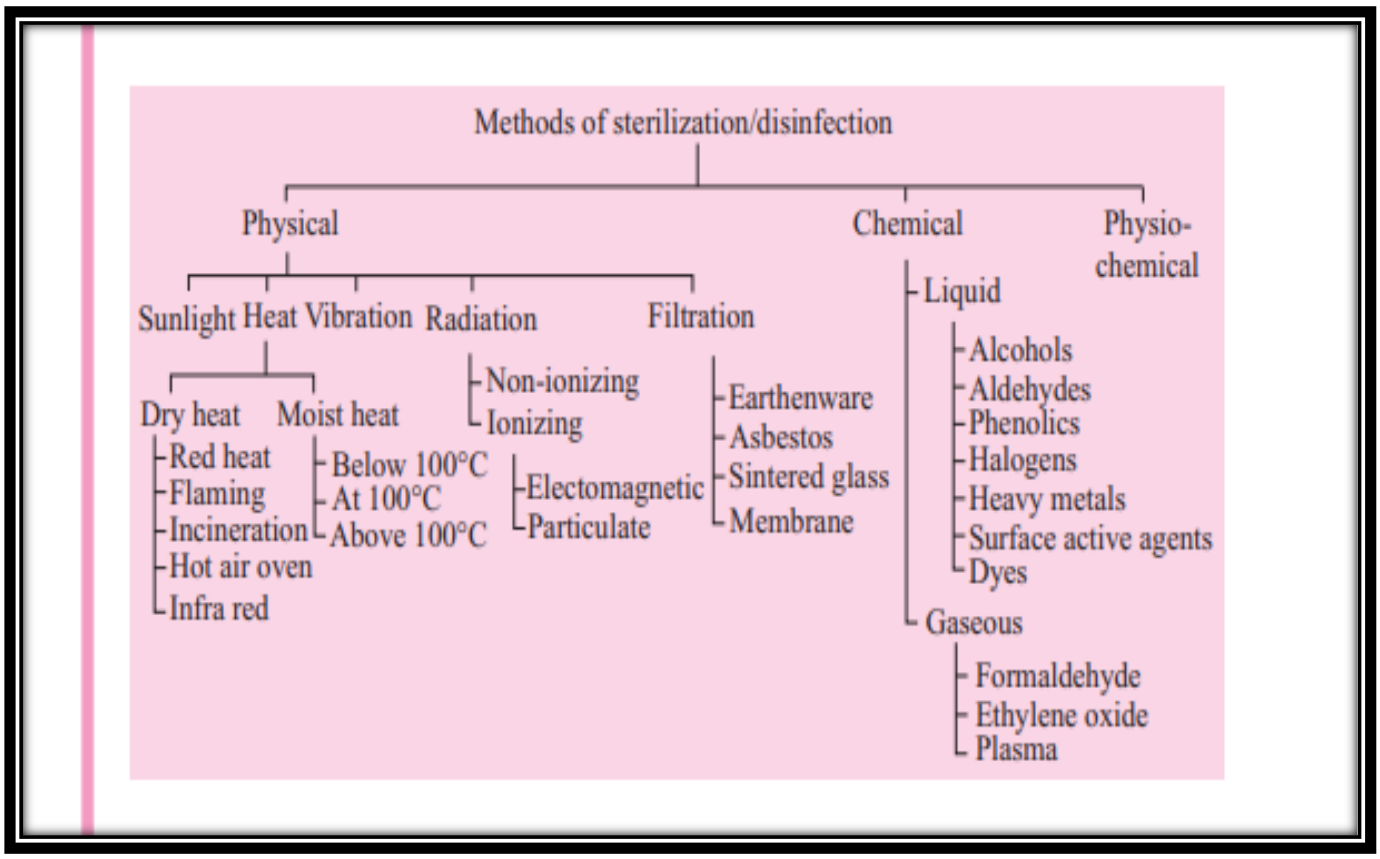
(a) Thermal (Heat) methods

(b) Radiation method

(c) Filtration method

2. Chemical Method :

3. Gaseous Method :



1. Heat sterilization : is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required. This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160- 180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used.

The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water.

Dry Heat Sterilization:

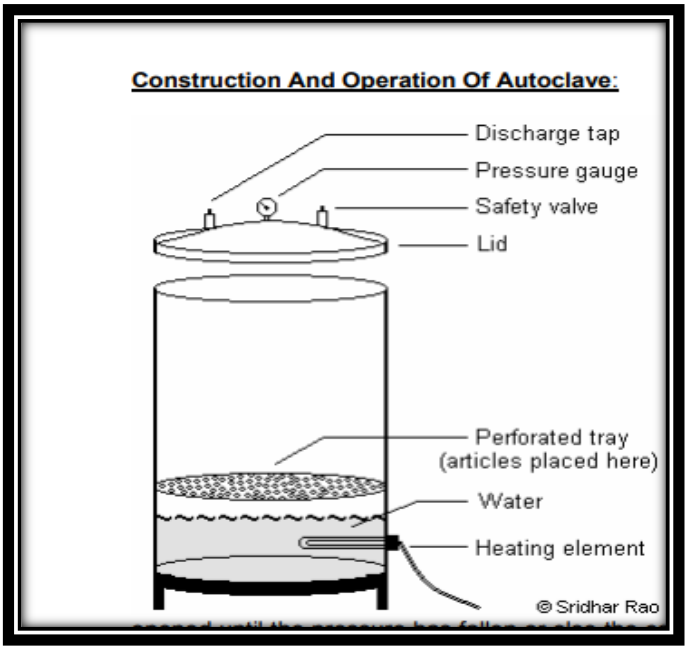
Examples of Dry heat sterilization are:

1. Incineration
2. Red heat
3. Flaming
4. Hot air oven

Moist Heat Sterilization: Moist heat may be used in three forms to achieve microbial inactivation

1. Dry saturated steam – Autoclaving

2. Boiling water/ steam at atmospheric pressure



3. Hot water below boiling point Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

RADIATION STERILIZATION

Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light).The major target for these radiation is microbial DNA. Gamma rays cause ionization and free radical production while UV light causes excitation. Radiation sterilization with high energy gamma rays has proven to be a useful method for the industrial sterilization of heat sensitive products. Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastic syringes .

Filtration Sterilization

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and nonviable particles.

Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas.

Application of filtration for sterilization of gases: HEPA (High efficiency particulate air) filters can remove up to 99.97% of particles >0.3 micrometer in diameter. Air is first passed through prefilters to remove larger particles and then passed through HEPA filters. There are two types of filters used in filtration sterilization (a) Depth filters:. (b) Membrane filters: These are porous membrane about 0.1 mm thick, made of cellulose acetate and some materials, or some other synthetic material.The membranes are supported on a frame and held in special holders. Fluids are made to transverse membranes by positive or negative pressure or by centrifugation.

CHEMICAL METHODS OF DISINFECTION

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used concentration for appropriate duration can be used for sterilization and are called sterilant liquids. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics. An ideal antiseptic or disinfectant should have following properties:

1. Should have wide spectrum of activity

2. Should be able to destroy microbes within practical period of time

3. Should be active in the presence of organic matter

4. Should not be expensive and must be available easily

5. Should be active in any pH, stable .

Classification of disinfectants:

1. Based on consistency :

(a) Liquid (E.g., Alcohols, Phenols)

(b) Gaseous (Formaldehyde vapour)

2. Based on spectrum of activity

(a) High level

(b) Intermediate level

(c) Low level

3. **Based on mechanism of action**

(a) Action on membrane (E.g., Alcohol, detergent).

(b) Denaturation of cellular proteins (E.g., Alcohol, Phenol).

(c) Oxidation of essential sulphydryl groups of enzymes (E.g., H2O2, Halogens) .

(d) Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Formaldehyde) (e) Damage to nucleic acids (Formaldehyde).

1. Alcohols
2. Aldehydes
3. Phenol
4. Halogens
5. Heavy Metals
6. Hydrogen Peroxide

Gaseous Sterilization

The chemically reactive gases such as formaldehyde, and ethylene oxide (CH2)2O possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas. The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids.

1. **Ethylene oxide sterilizer**
2. **Low temperature steam formaldehyde (LTSF) sterilizer**

The Second Lab

Practical Medical Microbiology

Lec.Amera A. / Lec Ibrahim T. / Ass.Lec Rasha W.

Culture Media

What is a culture media for Bacteria ?

A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents (for solid media). Also can we defined culture media as **A population of bacteria grown in the laboratory** . A pure culture contains only one single type; a mixed culture contains two or more different bacteria

There are Six types of Culture media

(1) Basal media

(2) Enriched media

(3) Selective media

(4) Indicator media

(5) Transport media

(6) Storage media.

What is the use of culture media

Culture media are used for :

- Quality control tests of nonsterile materials and finished products as well as for microbial contamination (sterility) tests in applications such as , sterilization process validation and determination of the effectiveness of preservatives and antimicrobial agents.
- It is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological tests.

What is the principle of culture media ?

In preparing a culture media for any microorganism, the primary goal is to provide a balanced mixture of the required nutrients, at concentrations that will permit good growth. No ingredients should be given in excess because many nutrients become growth inhibitory or toxic as the concentration is raised.

What are the components of Bacterial culture media?

Some of the ingredients of culture media include :

- Water– Source of hydrogen and oxygen.
- Electrolytes– NaCl & other electrolytes.
- Peptone– Mixture of partially digested proteins (animal or vegetable). ...
- Meat extract, yeast extract – Protein degradation products/carbohydrates/Inorganic salts/Growth factors.
- Blood– It enriches media.
- Solid media commonly contain **1.5% agar** per weight to solidify the liquid media.

Classification: Bacterial culture media can be classified in at least three ways; Based on consistency (Solid , Liquid & Semi liquid), based on nutritional component and based on its functional .

How is culture media prepared in microbiology?

Suspend 15 g of nutrient agar in 100 cm^3 distilled water. Bring to the boil to dissolve completely. Heat 40 g of soluble starch in 100 cm^3 of distilled water to form a suspension. Allow to cool and then mix with the nutrient agar solution.

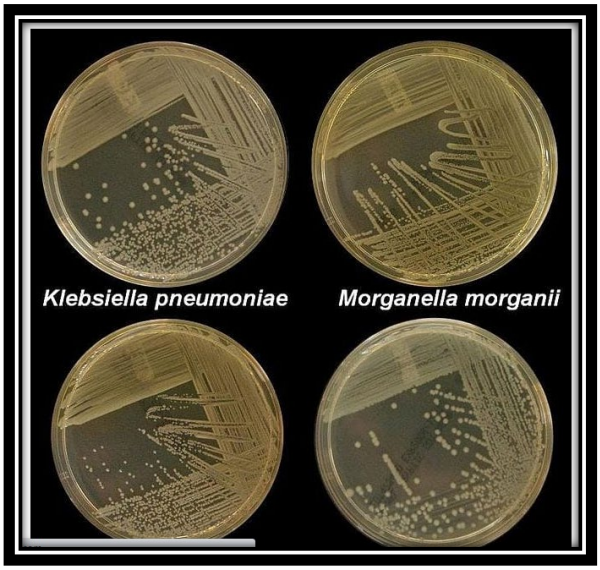
What are agar plates?

An agar plate is a thin layer of nutrient gel in a Petri dish, used to grow bacteria and fungi in the microbiology laboratory. polysaccharide derived from the cell walls of red sea weed. A variety of nutrients can be added to the agar to preferentially grow different bacteria .

There are Four types of agar :

- Nutrients agar
- Blood agar
- MacConkey agar
- Chocolate agar

What is general media in microbiology?



Examples of standard general purpose media that will support the growth of a wide variety of bacteria include **nutrient agar**, **tryptic soy agar**, and **brain heart infusion agar**. A medium may be enriched, by the addition of blood or serum

Why can some bacteria not grow on any known culture media at all?

Some of the possible reasons are that a required nutrient is not present in the culture medium, that the culture medium itself is toxic, or that other bacteria in the sample produce substances inhibitory to the target organism. ... Of course, a bacterium dependent on another will not be able to grow independently in vitro.

What is inoculation of culture media?

The Inoculation meaning in microbiology is that transfer from culture for their growth. It is the direct transfer from the culture of microorganisms to inoculation needle. Also refers to the act of introducing micro-organisms or suspensions of microorganisms (e.g. bacteria into a culture medium).A sterile inoculation loop is placed in bacterial culture and then either spread on an agar.

What is the name of bacteria that are difficult to culture?

The more restrictive term fastidious microorganism is used in microbiology to describe microorganisms that will grow only if special nutrients called growth factors such as vitamins, and other essential nutrients to promote the growth of fastidious organisms, that cannot make certain nutrients and require them to be added to the medium. Thus fastidiousness is often practically defined as being difficult to culture, by any method yet tried contains

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of

microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth .

What is blood agar plate used for?

Blood agar is a general purpose, enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties.

Do all bacteria grow on blood agar?

Do all bacteria that grow on blood agar ? ... No, the only thing that grows on blood agar is the bacteria because it breaks down RBCs. Blood agar is a differential medium because all the bacteria will grow on it due to the nutrients and iron

What type of media is blood agar?

Blood agar is differential media because 3 different types of hemolysis, or lysing of red blood cells, which is occur under enzymatic effect produced by microorganisms that can be seen on this plate . These hemolysis are : Alpha Beta and Gamma haemolysis?

- alpha hemolysis is the partial destruction of red blood cells in the blood .
- Beta-hemolysis (β-hemolysis), sometimes called complete hemolysis, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells.
- Gamma (γ) hemolytic: formerly Group D Streptococci, re-classified as Enterococcus faecalis and Enterococcus faecium.

What is a MacConkey agar plate used for?

MacConkey Agar (MAC) is a selective and differential medium designed to isolate and differentiate enterics based on their ability to ferment lactose. Bile salts and crystal violet inhibit the growth of Gram positive organisms.

What bacteria grows on MacConkey agar?

MacConkey agar only grows gram-negative bacteria, and those bacteria will appear differently based on their lactose fermenting ability as well as the rate of fermentation and the presence of a capsule or not .MacConkey's is a selective medium that inhibits the growth of Gram-positive bacteria due to the presence of crystal violet and bile salts. Gram-negative bacteria grow well on MAC.

What is chocolate agar used for?

Chocolate agar is used for growing fastidious respiratory bacteria, such as Haemophilus influenzae and Neisseria meningitides. Which consider as a differential medium for gram-positive cocci.

How do you make a chocolate agar?

Chocolate agar is prepared by heating blood agar, which in turn ruptures the red blood cell (RBC) and releases nutrients that aid in the growth of fastidious bacteria, most notably Haemophilus and Neisseria species. The name is derived from the fact that the lysis of RBC gives the medium a chocolate-brown color.

All labeling is done on the bottom of the agar plate.

Place all inoculated material in **incubator** .Culture tubes should be stored upright in plastic beakers, while Petri plates should be incubated upside‑down. These plates will be incubated at 37° C for 24 hours present in O2 or Co2 gas . if we need it then stored at refrigerator (4 C) until used it.

Isolation of bacteria

- The third and fourth lab
- Practical medical microbiology
- Lec. Amera A./Lec.Ibrahim T. /Ass.Lec.Rasha W

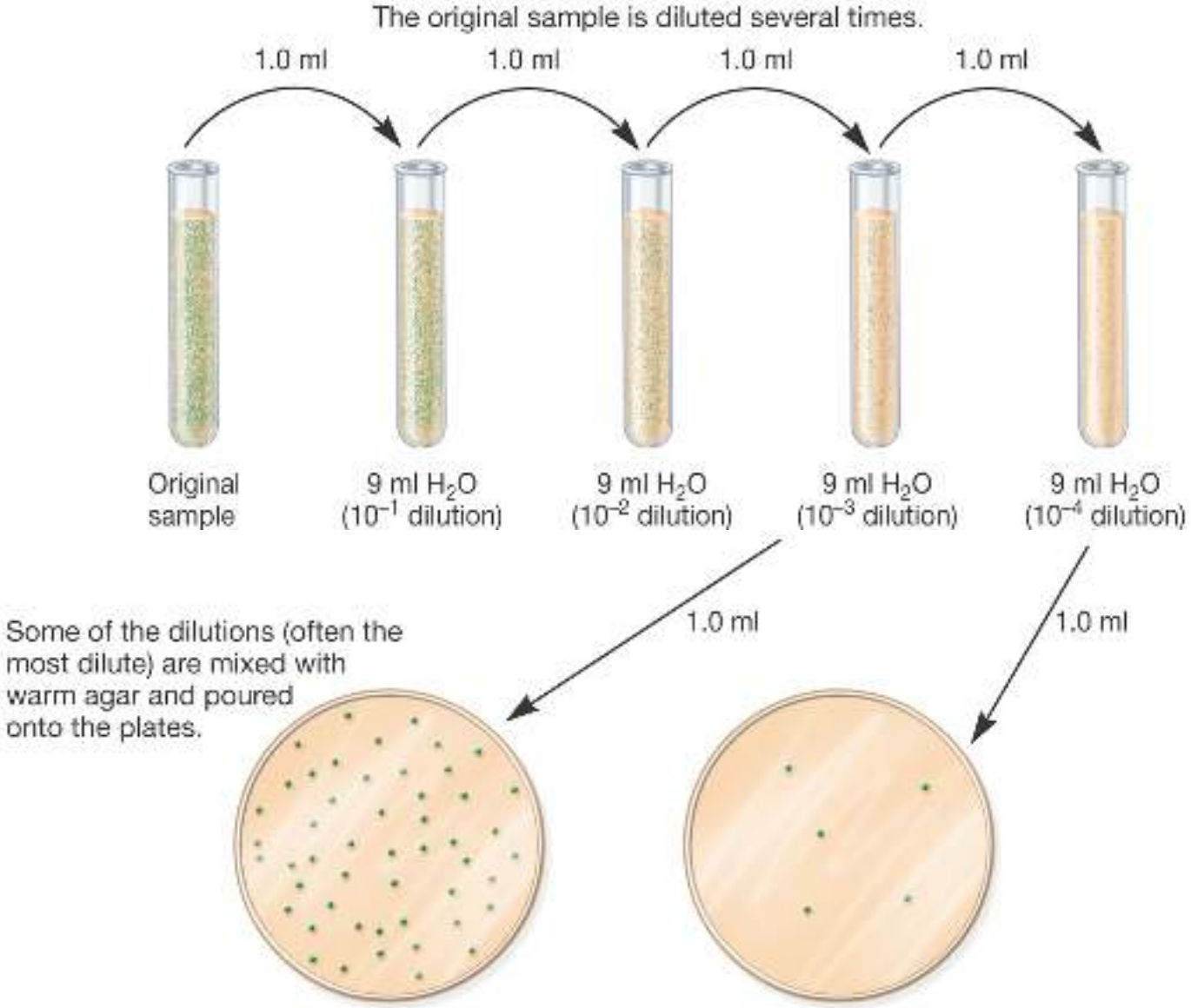
Isolation of microorganisms

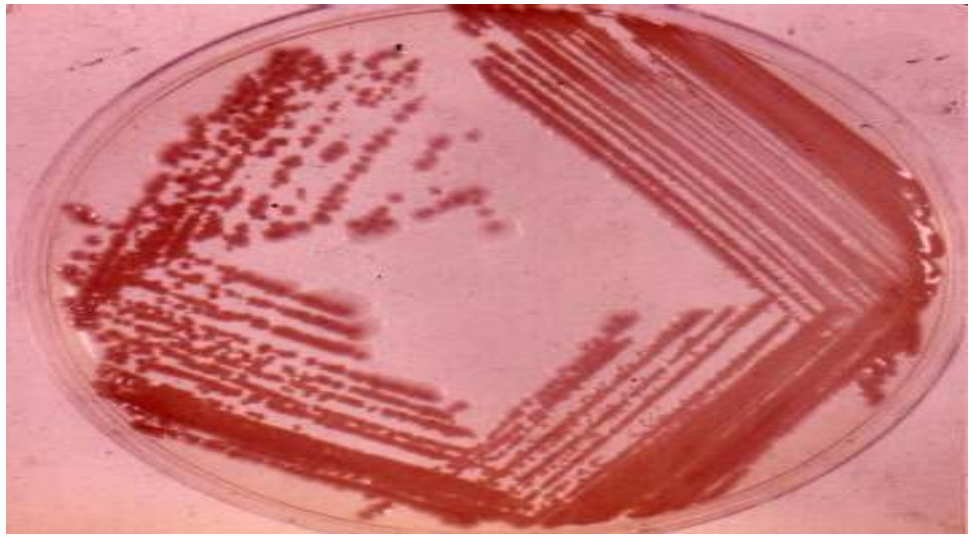
- Natural habitat, microorganism grow in complex, mixed populations with many spp.
- Need a **pure culture** to study and characterize an individual species
- Pure culture =contain one type of microorganisms
- Techniques to prepare pure culture:

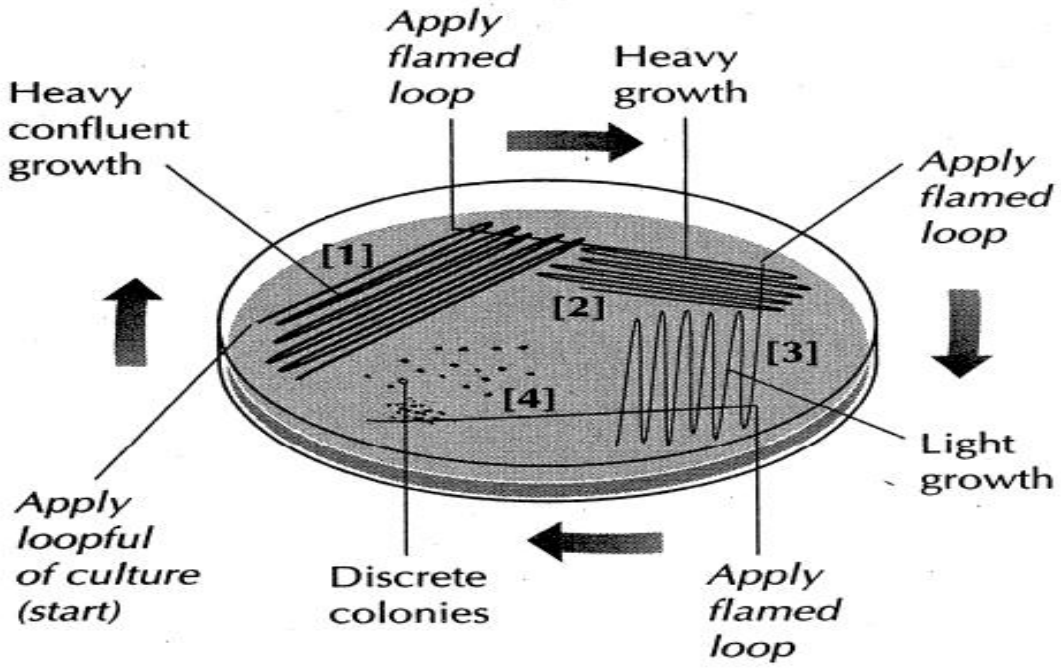
-spread plate

-streak plate

-Pour plate






Apply flamed loop
Heavy growth
Heavy confluent growth
Apply flamed loop
[1]
[2]
[3]
[4]
Light growth
Apply loopful of culture (start)
Discrete colonies
Apply flamed loop

Preparation of bacterial smear

- Bacteria are microscopic organisms. They are also colorless for the
- most part. In order to visualize them to study their structure, shape
- and other structural characteristics, it becomes necessary to make
- them more easily visible.

- This means that the structures have to be contrasted from their
- environment so that they can be seen easily.

- Staining is one such contrasting process where in chemicals (stains)
- are used to impart their color to the cells structures with which they
- come in contact.

- Staining procedure can also be classified in different ways.

- **Simple staining** involves the use of only 1 dye and is used primarily
- as a means to study the morphology and structure of organisms.

- **Differential staining** uses more than 2 dyes and is also used to
- differentiate the organisms into one of two groups.

- Simple staining – there are two methods:
- positive staining - where the actual cells are themselves colored
- and appear in a clear background;
- negative staining – where the cells remain clear (uncolored) and the
- background is colored to create a contrast to aid in the better
- visualization of the image.

Morphology of bacteria:

- The basis for staining is to study the morphology and structure of
- Bacteria primarily have distinct shapes;
- spherical (coccus/cooci) and
- rod shaped (bacillus/bacilli)
- filamentous.

- Based on the planes of divisions seen in the organism bacteria may
- also have specific arrangements of the cells.
- Diplococci are formed when the plane of division is vertical
- and the resultant two coccal cells do not completely separate
- from each other.
- If the cells divide in the vertical plane continually and the
- cells do not separate it results in a chain of coccal cells called a
- streptococci/streptobacilli.
- Other arrangements include: tetrad (4), sarcina (8), staphylococcus
- (irregular clusters).

- Bacterial Smear preparation:

- Smear is a distribution of bacterial cells on a slide for the purpose
- of viewing them under the microscope.

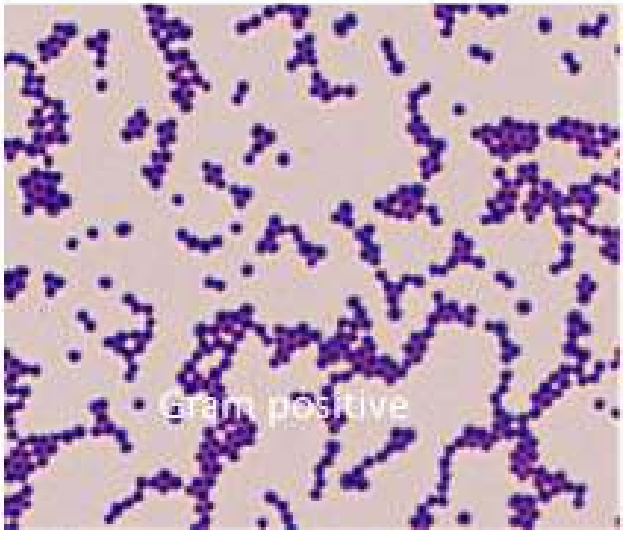
- Method:
- -Aseptically a small sample of the culture is spread over a
- slide surface.
- -This is then allowed to air dry.
- -The next step is heat fixation to help the cells adhere to the
- slide surface.
- -The smear is now ready for staining

PRACTICAL
GRAM STAINING

- REAGENTS USED IN GRAM STAIN
- Gram Crystal Violet .
- Gram Iodine.
- Gram Decolorizer
- Methanol
- Acetone
- Gram Safranine

REAGENTS USED IN GRAM STAIN

1. Gram Crystal Violet 0.5%
2. Gram Iodine
   a. Potassium Iodide 2%
   b. Resublimed Iodine 1%
3. Gram Decolorizer
   a. Methanol 80%
   b. Acetone 20%
4. Gram Safranine 1%

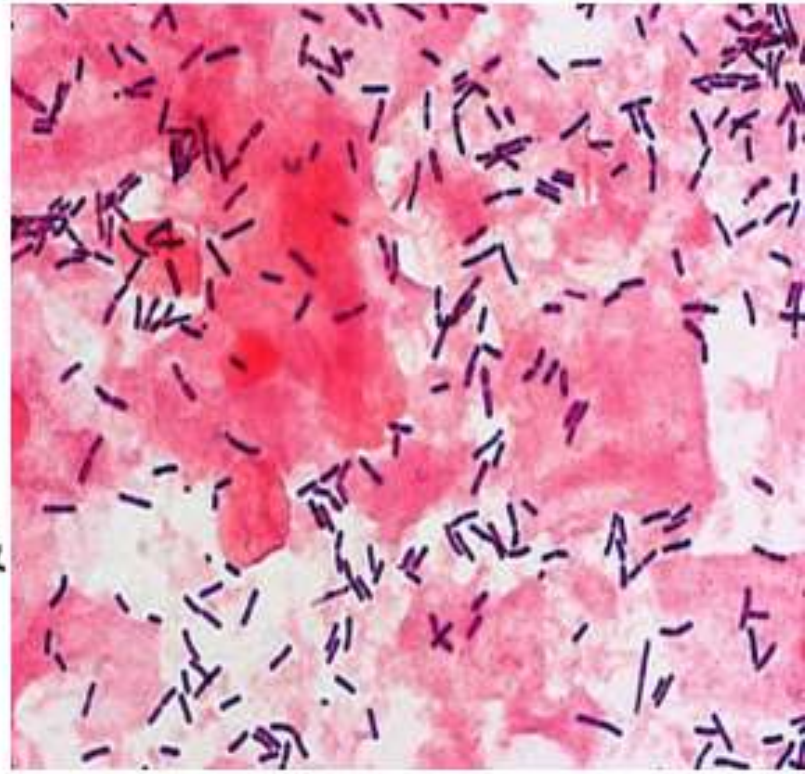


REAGENTS USED IN GRAM STAIN

1. CRYSTAL VIOLET
   - Primary stain
   - Violet colored, stains all micro-org
2. GRAM IODINE
   - Mordant
   - Forms Crystal violet Iodine complexes
3. DECOLORIZER
   - Acetone + Methanol
   - Removes Crystal violet Iodine complex from thin peptidoglycan layers
   - Dissolves outer layer of Gram negative org

REAGENTS USED IN GRAM STAIN

4. GRAM SAFRANINE
   - Counter stain
   - Red colored
   - Stains thin walled Gram neg org
   - Pus cells cytoplasm & lobes of nuclie also stain red



The Fifth Lab

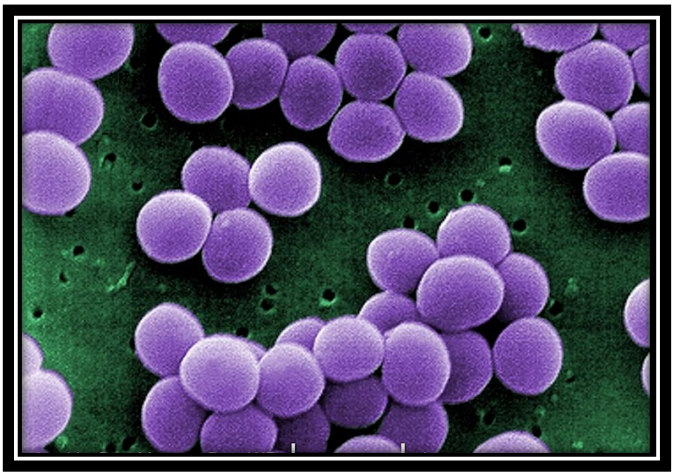
Practical Medical Microbiology

Lec.Amera A. / Lec Ibrahim T. / Ass.Lec Rasha W.

Staphylococcus

What is Staphylococcus

- Staphylococcus (staph) is a group of bacteria
- Gram-positive round-shaped bacteria
- It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.
- it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies.



- The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved.
- There are more than 30 types. A type called *Staphylococcus aureus* causes most infections. Staph bacteria can cause many different types of infections, including. Skin infections, which are the most common types of staph infections and others such as *Staphylococcus saprophytics , Staphylococcus epidermiditis.*

Staphylococcus aureus

- It is a facultative aerobic, Gram-positive coccal bacteria also known as "golden staph" . It is nonmotile and does not form spores . It appears as staphylococci (grape-like clusters) when viewed through a microscope, It has large, round, golden-yellow colonies, often with hemolysis when grown on blood agar plates. It reproduces asexually by binary fission, the daughter cells remain attached to one another and appear as clusters. It is catalase-positive (meaning it can produce the enzyme catalase). Catalase converts hydrogen peroxide (H_2O_2)

to water and oxygen. It can cause skin infection , Food poising , bone & joint infections and Bacteremia .

Virulence factor

1. **Enzymes**

S. aureus produces various enzymes such as :

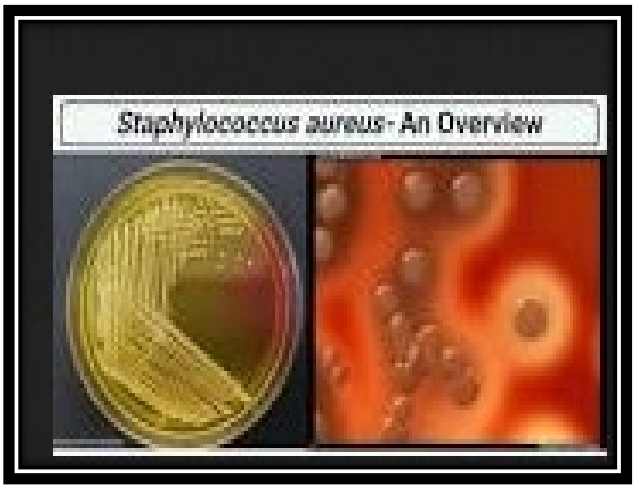
- coagulase (bound and free coagulases) which clots plasma and coats the bacterial cell, probably to prevent phagocytosis.
- Hyaluronidase (also known as spreading factor) breaks down hyaluronic acid and helps in spreading it.
- Deoxyribonuclease, which breaks down the DNA, protects *S. aureus* from neutrophil extracellular trap-mediated killing.
- *S. aureus* also produces lipase to digest lipids,
- staphylokinase to dissolve fibrin and aid in spread, and beta-lactamase for drug resistance.

2. **Toxins**

Depending on the strain, *S. aureus* is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases. **hemolysin, leukotoxin, exfoliative toxin, enterotoxin, and toxic-shock syndrome toxin-1 (TSST-1).**

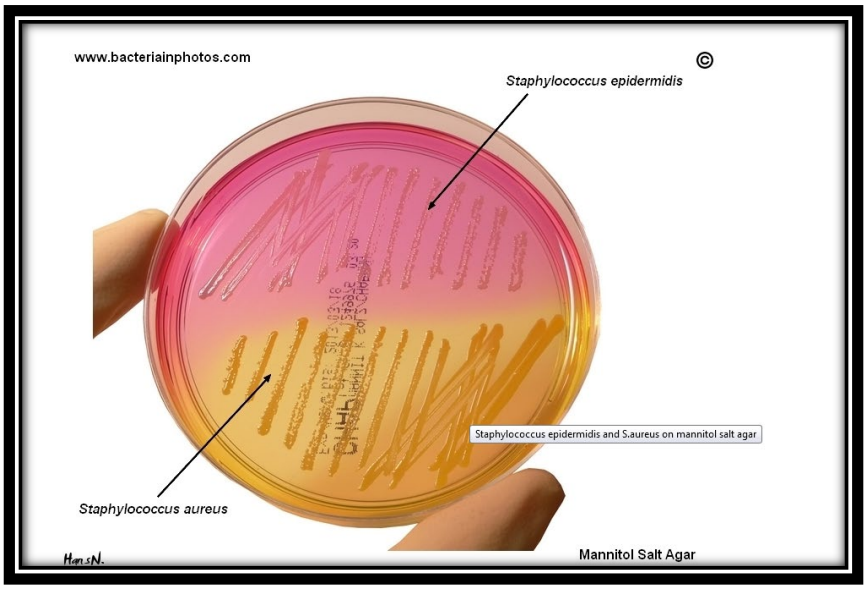
Does Staphylococcus aureus grow on Blood agar ?

On blood agar plates, colonies of Staphylococcus aureus are frequently surrounded by zones of clear beta-hemolysis. The golden appearance of colonies of some strains is the etymological root of the bacteria's



What is the Mannitol salts agar ?

- Mannitol salt agar or MSA is a commonly used selective and differential medium in microbiology.
- It encourages the growth of a group of certain bacteria while inhibiting the growth of others. This medium is important in medical laboratories as one method of distinguishing pathogenic microbes in a short period of time.
- It contains a high concentration (about 7.5–10%) of salt (NaCl) which is inhibitory to most bacteria - making MSA selective against most Gram-negative and selective for some Gram-positive bacteria (*Staphylococcus*, *Enterococcus* and Micrococcaceae) that tolerate high salt concentrations.
- It is also a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol and the indicator phenol red, a pH indicator for detecting acid produced by mannitol-fermenting staphylococci. *Staphylococcus aureus* produces yellow colonies with yellow zones, whereas other coagulase-negative staphylococci produce small pink or red colonies with no colour change to the medium.
- If an organism can ferment mannitol, an acidic byproduct is formed that causes the phenol red in the agar to turn yellow. It is used for the selective isolation of presumptive pathogenic (pp) *Staphylococcus* species.



Does Staphylococcus aureus grow on MacConkey?

MacConkey agar selects for organisms like Escherichia coli (Gram negative bacilli) while inhibiting the growth of organisms like Staphylococcus aureus (Gram positive cocci)

Stapylococcus epidermidis

- *S. epidermidis* is a Gram-positive bacterium.
- It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora and also found in marine sponges.
- It is a facultative anaerobic bacteria .
- *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection. These infections are generally hospital-acquired.
- *S. epidermidis* is a particular concern for people with catheters or other surgical implants because it is known to form biofilms that grow on these devices. Being part of the normal skin flora,
- *S. epidermidis* is a frequent contaminant of specimens sent to the diagnostic laboratory.

Staphylococcus saprophyticus

Staphylococcus saprophyticus is a Gram-positive bacterium that is a common cause of uncomplicated urinary tract infections, especially in young sexually active females. It is also responsible for complications including acute pyelonephritis, epididymitis, prostatitis, and urethritis.

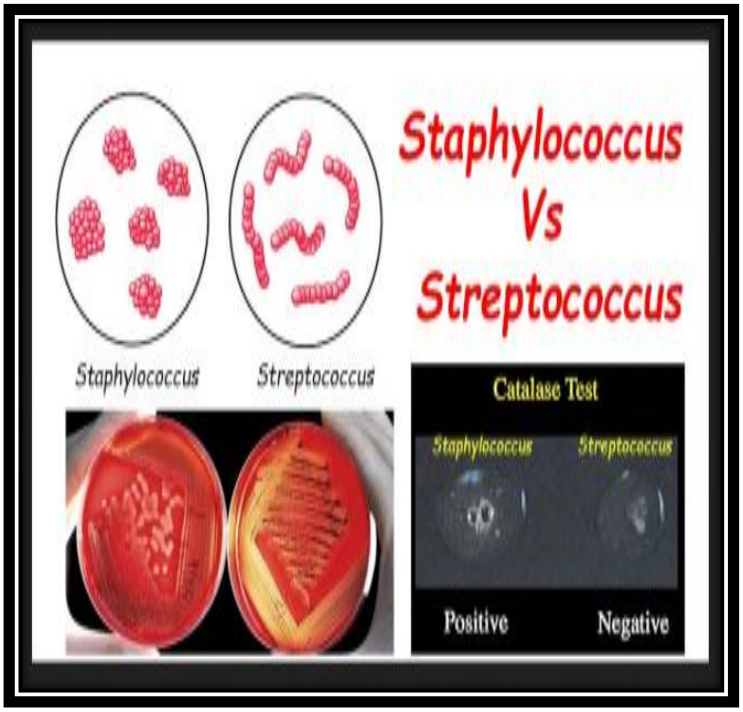
The Sixth Lab

Practical Medical Microbiology

Lec.Amera A. / Lec Ibrahim T. / Ass.Lec Rasha W.

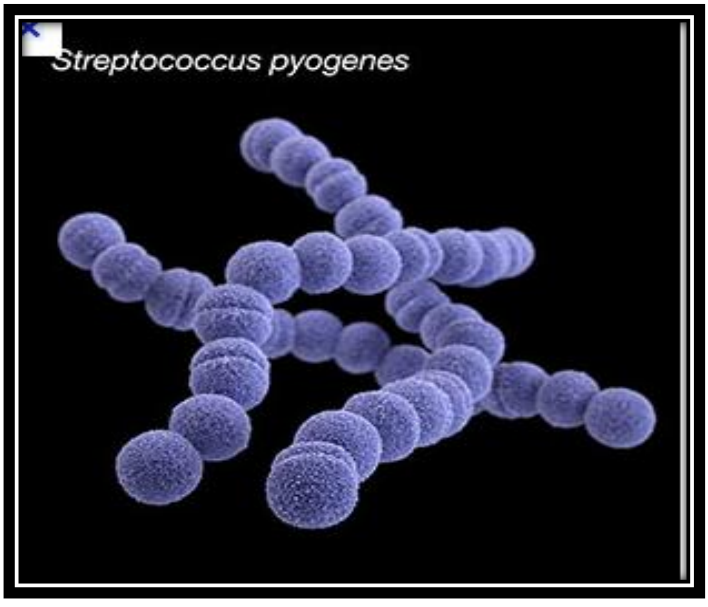
Genus : Streptococcus

- ***Streptococcus*** is a genus of gram-positive *coccus* (plural *cocci*) or spherical bacteria , non motile & non spore forming.
- Cell division in streptococci occurs along a single axis, so as they grow, they tend to form pairs or chains that may appear bent or twisted. This differs from staphylococci, which divide along multiple axes, thereby generating irregular, grape-like clusters of cells.
- Most streptococci are oxidase-negative and catalase-negative, and many are facultative anaerobes (capable of growth both aerobically and anaerobically).



Pathogenesis and classification

- In addition to streptococcal pharyngitis (strep throat), certain *Streptococcus* species are responsible for many cases of pink eye, meningitis, bacterial pneumonia, endocarditis,. However, many streptococcal species are not pathogenic, and form part of the commensal human microbiota of the mouth, skin, intestine, and upper respiratory tract.
- Species of *Streptococcus* are classified based on their hemolytic properties. Alpha-hemolytic species cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar such



as *S. pneumoniae* and *Streptococcus viridans* group. Beta-hemolytic species cause complete rupture of red blood cells. On blood agar, this appears as wide areas clear of blood cells surrounding bacterial colonies such as group A strep" and "group B strep. Gamma-hemolytic species cause no hemolysis such as Enterococcus faecalis

Species	Host	Disease
S. pyogenes	human	pharyngitis, cellulitis, erysipelas
S. agalactiae	human, cattle	neonatal meningitis and sepsis
S. dysgalactiae	human, animals	endocarditis, bacteremia, pneumonia, meningitis, respiratory infections
S. gallolyticus	human, animals	biliary or urinary tract infections, endocarditis
S. anginosus	human, animals	subcutaneous/organ abscesses, meningitis, respiratory infections
S. sanguinis	human	endocarditis, dental caries
S. suis	swine	meningitis
S. mitis	human	endocarditis
S. mutans	human	dental caries
S. pneumoniae	human	pneumonia

Group A streptoccoci causes :

- Strep throat a sore, red throat, sometimes with white spots on the tonsils
- Scarlet fever an illness that follows strep throat. It causes a red rash on the body
- **I** mpetigo a skin infection
- Toxic shock syndrome
- Cellulitis and necrotizing fasciitis (flesheating disease)

Group B Streptococci causes :

- Blood infections, pneumonia and meningitis in newborns.
- Adults can also get group B strep infections, especially if they have health problems.
- Strep B can cause urinary tract infection & skin infections

Group D Streptococci (Enterococcus)causes :

- The most commonly isolated are

E. (S.) faecalis
E. (S.) faecium.
E. (S.) faecalis

- Can cause nosocomial infections, urinary tract infections, bacteremia, endocarditis, meningitis
- Can be found in wound infections along with many other bacteria

How do you get streptococcus?

These bacteria are spread by direct contact with discharges from the nose and throat of infected people or by contact with infected wounds or sores on the skin. The risk of spreading the infection is highest when a person is ill, such as when people have "strep throat" or an infected wound.

STREPTOCOCCI SEROLOGIC PROPERTIES TESTING:

1. **Bacitracin sensitivity** test : is a polypeptide antibiotic interfering with the synthesis of peptidoglycan, a unique chemical fabric bacteria include in their cell walls

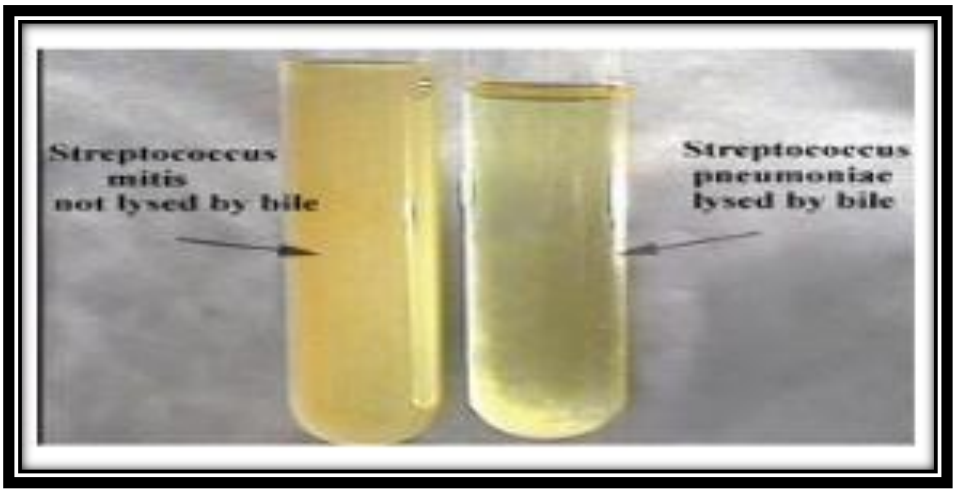
Principle:

Bacitracin test is used to determine the effect of a small amount of bacitracin on an organism. Streptococcus pyogenes is inhibited by the small amount of bacitracin in the disk (visible zone of inhibition of growth).

2. **Optochin test:** is a chemical material like disc used in cell culture techniques for the Streptococcus pneumoniae which is optochin sensitive (positive result), from other alpha hemolytic streptococci such as Streptococcus viridans which are resistant.

3. **Bile solubility test :**

The bile (sodium deoxycholate) solubility test distinguishes Streptococcus pneumonia from all other alpha hemolytic (viridans) streptococci. Streptococcus pneumonia is bile soluble whereas all other alpha hemolytic streptococci are bile resistant. Sodium deoxycholate (2% in water) will lyse the pneumococcal cell wall. A clearing of the turbidity in the bile tube indicates a positive test**.**



Laboratory tests for *Streptococcus* spp.

1. **Anti streptolysin O(ASO)** is the antibody made against streptolysin O, an immunogenic, oxygen labile hemolytic toxin produced by most strains of group A and many strains of group G streptococci. The O in the name stands for oxygen labile the other related toxin being oxygen stable streptolysin.
2.
3. **C-reactive protein (CRP)** is found in the blood and is a response to inflammation in the body. It can also be an indicator of the presence of infection, trauma or serious illness. Chronic inflammation can keep CRP levels elevated, which can increase the risk of cardiovascular conditions such as heart attacks or stroke. Creactive protein is produced by the liver . the level of CRP rises when there is inflammation throughout the body. It is not a specific test.

The Seventh Lab

Practical Medical Microbiology

Lec.Amera A. / Lec Ibrahim T. / Ass.Lec Rasha W.

Family :Enterobacteriaceae

- Are a large family of Gram-negative bacteria (Gr-) that includes a number of pathogens such as Escherichia coli , Klebsiella, Enterobacter, Citrobacter, Salmonella, Shigella, Proteus, Serratia and other species.
- Members of the Enterobacteriaceae are bacilli (rod-shaped), facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. They are typically 1-5 μm in length and they have Gram-negative stains. Most have many flagella used to move about, but a few genera are non-motile. They do not form spores. Most members of Enterobacteriaceae have fimbriae involved in the adhesion of the bacterial cells to their hosts.
- Enterobacteriaceae are found in nature; their presence in the intestinal tracts of animals results in their wide distribution in soil, water, and sewage. They are also found in plants.
- Enterobacteriaceae can cause a wide range of illnesses, which include wound infections, urinary tract infections, gastroenteritis, meningitis, pneumonia and septicemia. Not all are regarded as truly pathogenic – some are regarded as opportunistic.
- These pathogens can spread to the bloodstream resulting in life-threatening complications.
- Are all kinds of Enterobacteriaceae coliforms?

Coliforms are a group of grams negative bacteria which are lactose fermenting. Coliforms belong to Enterobacteriaceae. Hence, all **coliforms are members of Enterobacteriaceae**. But not all Enterobacteriaceae are coliforms.

How do you identify Enterobacteriaceae?

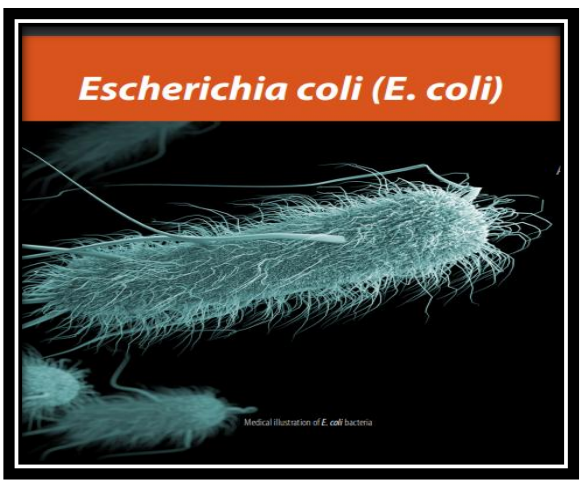
Members of the Enterobacteriaceae family are identified based on **their biochemical properties**.Tests for identification of members of Enterobacteriaceae family

- Citrate utilization Test.
- Indole Test.
- Motility Test.
- Methyl Red (MR) Test.

- Voges–Proskauer (VP) Test.
- Triple Sugar Iron (TSI) Agar Test.
- Urease Test

Genus : Escherichia coli or E. coli?

- ***Escherichia coli*** also known as ***E. coli*** is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes (EPEC, ETEC etc.) can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonisation of the intestine with pathogenic bacteria, *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.
- Although most strains of E. coli are harmless, others can make people sick. Some kinds of E. coli cause disease by making a toxin called Shiga toxin.
- The bacteria that make these toxins are called “Shiga toxin-producing E. coli”, or STEC for short. STEC bacteria live in the intestines of many animals and are usually transmitted to people when they eat foods contaminated with the bacteria.
- The most commonly reported type of STEC in the United States is O157. Other STEC are called non-O157. Some types of STEC frequently cause severe disease, including bloody diarrhea and hemolytic uremic syndrome, which is a type of kidney failure. E.coli grow on MacConkey agar with pink colony because it can ferment lactose.



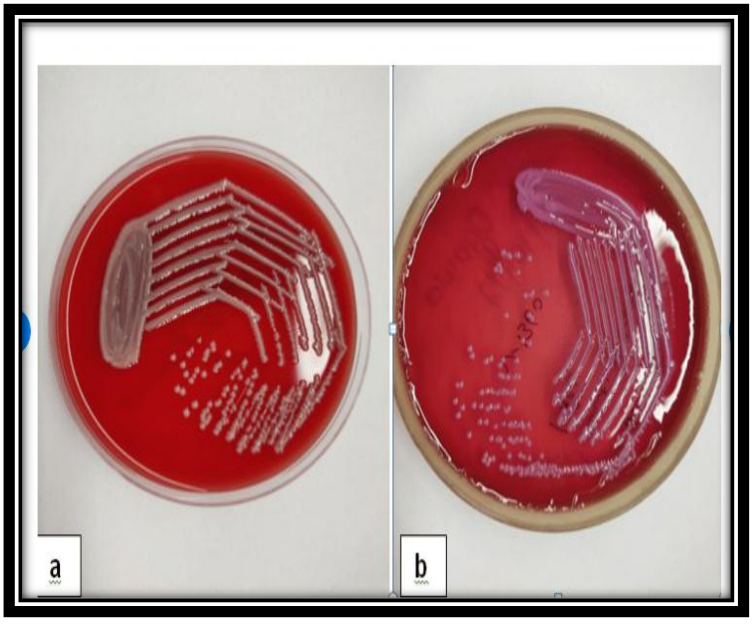
Genus : Klebsiella spp

- It is a genus of Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule.

- *Klebsiella* species are found everywhere in nature. This is thought to be due to distinct developing specific adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects and other animals including humans.

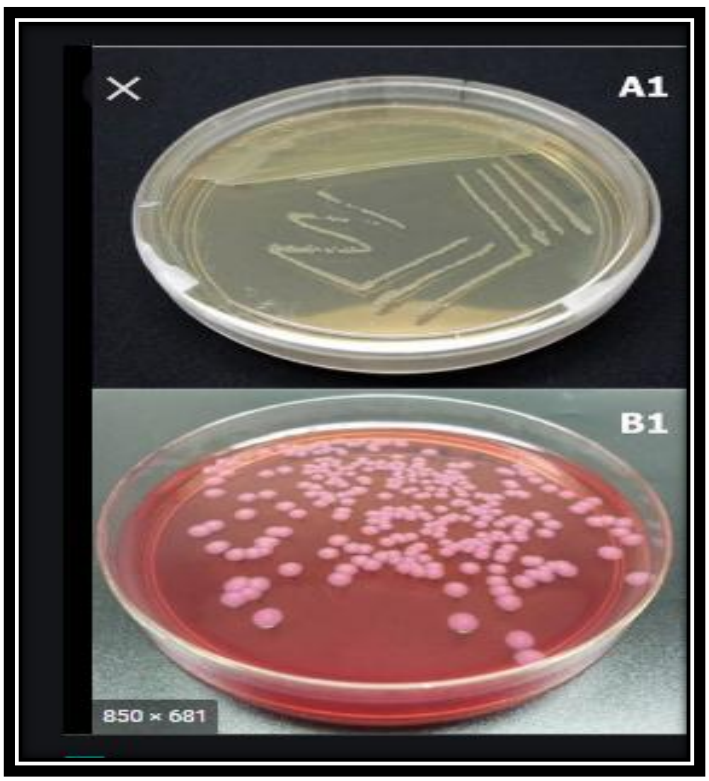
- The species of *Klebsiella* are all gram-negative and usually non-motile. They tend to be shorter and thicker when compared to others in the family Enterobacteriaceae. The cells are rods in shape and generally measures 0.3 to 1.5 µm wide by 0.5 to 5.0 µm long. They can be found singly, in pairs, in chains or linked end to end like *K. pneumonia & K. oxytoca*.

- *Klebsiella* can grow on ordinary lab medium and do not have special growth requirements, like the other members of Enterobacteriaceae. The species are aerobic but facultatively anaerobic. Their ideal growth temperature is 35° to 37 °C, while their ideal pH level is about 7.2.



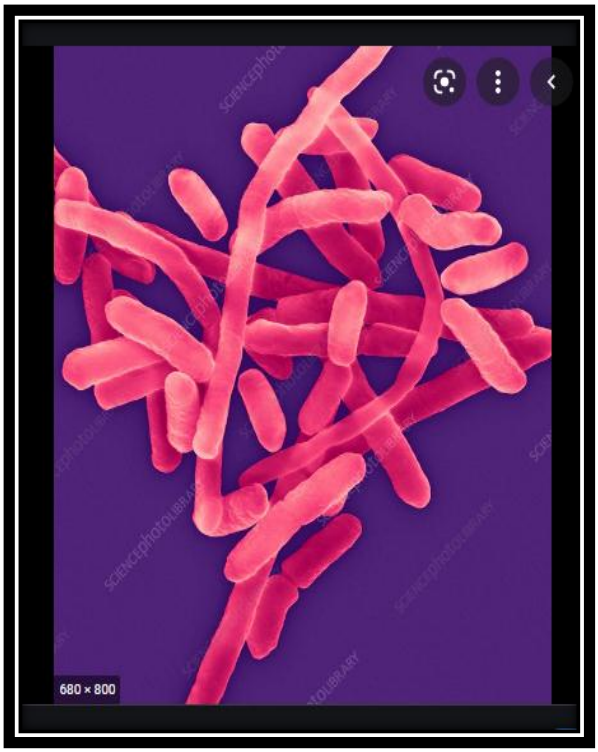
Genus : Enterobacter spp

- Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae.
- Enterobacter species are responsible for causing many nosocomial infections, and less commonly community-acquired infections, including urinary tract infections (UTI), respiratory infections, soft tissue infections, osteomyelitis, and endocarditis, among many others.
- The source of infection may be endogenous (via colonization of the skin, gastrointestinal tract, or urinary tract) or exogenous, resulting from the ubiquitous nature of Enterobacter species.
- Enterobacter can be found on human skin, plants, soil, water, sewage, intestinal tracts of animals, including humans, dairy products; and clinical specimens such as feces, urine, blood, sputum, and wound exudates.
- The best example for Enterobacter spp is Enterobacter cloacae



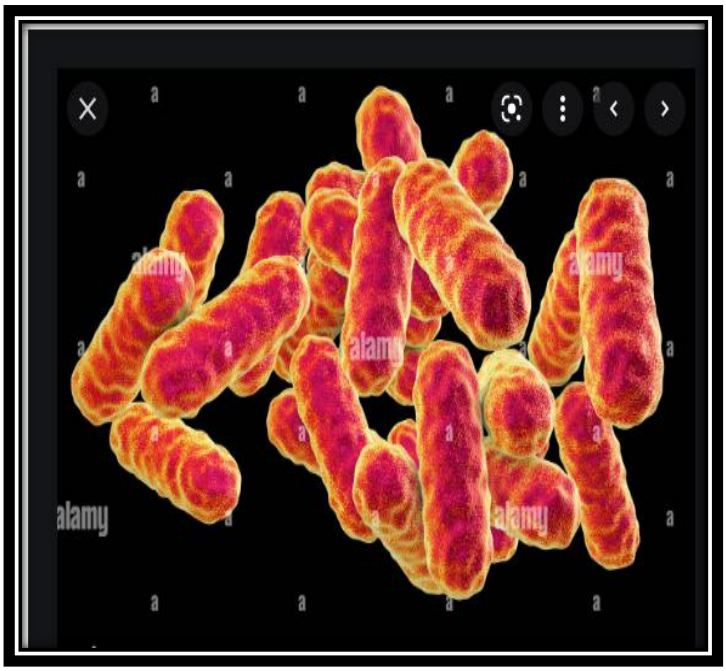
Genus : Citrobacter spp

- *Citrobacter* is a genus of Gram-negative coliform bacteria in the family Enterobacteriaceae. The species C. koseri, and C. freundii can use citrate as a sole carbon source. Citrobacter species are differentiated by their ability to convert tryptophan to indole, ferment lactose, and use malonate··
- *Citrobacter* may be spread by direct contact with hospital staff members, mother to child transmission or through ingestion of environmental sources (fecal-oral route) but person-to-person transmission is more prevalent ,In the pediatric population, infections due to *Citrobacter* spp. occur most commonly in neonates. Organisms can be transmitted by vertical transmission from mothers or by nosocomial spread.
- For patients with C. koseri brain abscesses, surgical drainage or aspiration of the abscesses should be strongly considered, but it is not always feasible because the abscesses are often multiple or inaccessible. Brain abscesses caused by C. koseri have been reported to be cured by medical therapy alone ·



Genus : Serritia spp

- ***Serratia*** is a genus of Gram-negative, facultatively anaerobic, rod-shaped bacteria of the family Yersiniaceae. According to the List of Prokaryotic names with Standing Nomenclature (LPSN), there are currently 19 species of *Serratia* that are credibly published with accurate names as of 2020.
- *S. aquatilis*, *S. entomophila*,& *S. marcescens* They are typically 1–5 μm in length, do not produce spores, and can be found in water, soil, plants, and animals.
- Some members of this genus produce a characteristic red pigment, prodigiosin, and can be distinguished from other members of the order Enterobacterales by their unique production of three enzymes: DNase ,lipase, and gelatinase.
- *Serratia* was thought to be a harmless environmental bacteria until it was discovered that the most common species in the genus, *S. marcescens*, is an opportunistic pathogen of many animals, including humans.
- In humans, *S. marcescens* is mostly associated with nosocomial, or hospital-acquired, infections, but can also cause urinary tract infections, pneumonia, and endocarditis. *S. marcescens* is frequently found in showers, toilet bowls, and around wetted tiles as a pinkish to red biofilm but only causes disease in immunocompromised individuals. Aside from *S marcescens*, some rare strains of the *Serratia* species *such as S. plymuthica, S. liquefaciens* have been shown to cause infection such as osteomyelitis and endocarditis.



Genus : Proteus spp

- ***Proteus*** is a genus of Gram-negative bacteria . *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial.

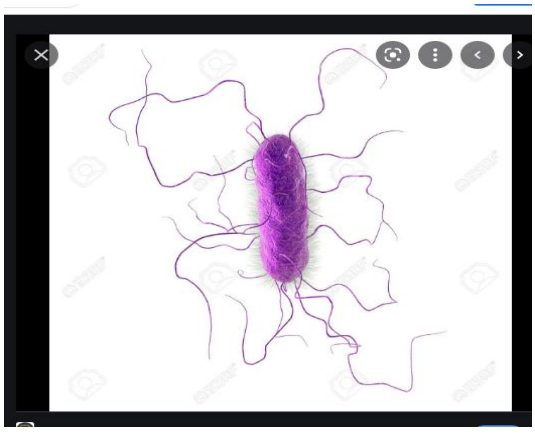
- The term Proteus signifies changeability of form . The first use of the term "Proteus" in bacteriological nomenclature was made by Hauser (1885), who described under this term three types of organisms which he isolated from putrefied meat.

- Three species *P. vulgaris*, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens. *Proteus* includes pathogens responsible for many human urinary tract infections. grow on media with swarming phenomena.

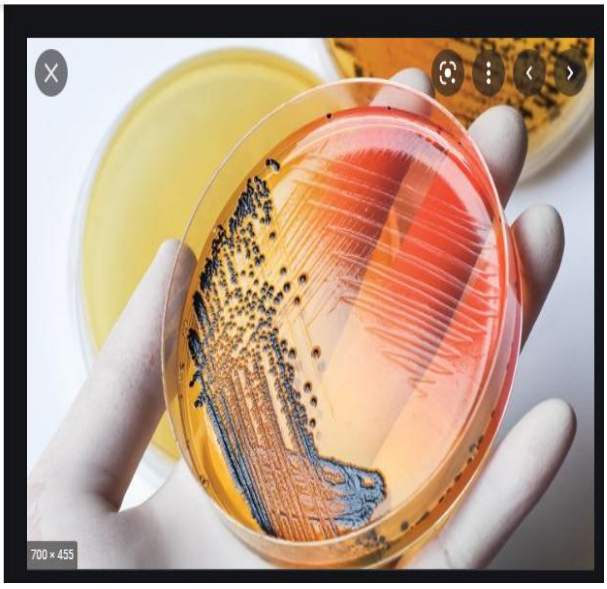
- *P. mirabilis* causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins. *P. vulgaris* is not sensitive to these antibiotics . However, this organism is isolated less often in the laboratory and usually only targets immunosuppressed individuals.

- *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of animals, soil, and polluted waters. *P. mirabilis*, once attached to the urinary tract, infects the kidney more commonly than *E. coli*. *P. mirabilis* is often found as a free-living organism in soil and water.

- About 10–15% of kidney stones are struvite stones, caused by alkalinization of the urine by the action of the urease enzyme (which splits urea into ammonia and carbon dioxide) of *Proteus* (and other) bacterial species.



Genus : Salmonella spp

- Salmonella is a genus of rod-shaped Gram-negative bacteria of the family Enterobacteriaceae. Facultative anaerobic , motile .The two species of Salmonella are Salmonella enterica and Salmonella bongori. S. enterica is the type species and is further divided into six subspecies that include over 2,600 serotypes.
- Salmonella infection is caused by a group of salmonella bacteria called Salmonella. The bacteria are passed from feces of people or animals to other people or animals. Contaminated foods are often animal in origin. They include beef, poultry, seafood, milk, or eggs.
- Salmonella spp. grow on MacConkey , SSA and other media with H_2S producing
- They generally cause diarrhea. Salmonella can also cause typhoid fever. It can spread to other parts of the body. Symptoms of a salmonella infection usually include diarrhea, fever (Typhi fever), abdominal cramps, chills, headache, nausea, or vomiting.
- **Salmonella Causes**
  1. Raw and undercooked meat, including chicken, turkey, duck, beef, veal, and pork.
  2. Raw fruits or vegetables.
  3. Unpasteurized milk and other dairy products, including soft cheese, ice cream, and yogurt.
  4. Raw or undercooked eggs.
  5. Processed foods like chicken nuggets and nut butters
-  

Genus : Shigella spp

- Shigella is a genus of bacteria that is Gram-negative, facultative anaerobic, non-spore-forming, nonmotile, rod-shaped and genetically closely related to E. coli. The genus is named after Kiyoshi Shiga, who first discovered it in 1897.

- **People become infected with Shigella by:**

1. Eating food or drinking liquids contaminated by an infected person.
2. Touching contaminated surfaces or objects and then touching their mouth or putting a contaminated object into their mouth.

- Foods that have been identified in Shigella outbreaks include salads (potato, tuna, chicken, turkey, macaroni, fruit), chopped turkey, rice balls, beans, pudding, strawberries, spinach, raw oysters, luncheon meat, and milk. Contamination of these or other foods is through the fecal–oral route.

- **Risk factors**

1. Being a child. Children under age 5 are most likely to get shigella infection.
2. Living in group housing or participating in group activities. Close contact with other people spreads the bacteria from person to person.
3. Living or traveling in areas that lack sanitation.

- Shigella cause dysentery (Diarrhea) Shigellosis (Shigellosis is common in the United States with about a half-million cases every year. It's far more deadly in poorer countries (about 165 million cases and about 1 million deaths worldwide every year).

- by Shigella dysentery , Untreated, the colon may rupture and cause peritonitis, a life-threatening condition requiring emergency surgery. The other relatively rare complication that can occur with a Shigella infection is the development of hemolytic uremic syndrome (HUS).