STERILIZATION

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure, or filtration.

DISINFECTION

Reducing the number of pathogenic microorganismsto the point where they no longer cause diseases or is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects such as tables,floors,etc.Examples chlorine,hypochlorites, lye, ect.

Bacteriostatic Agent :An agent that inhibits the growth of bacteria, but does not necessarily kill them.

Bactericide: An agent that kills bacteria. Most do not kill Endospores.

Sporicide :An agent that kills spores

Antibiotics

are substances produced by one microbe that inhibits or kills another microbe. Often the term is used more generally to include synthetic and semi-synthetic antimicrobial agents

Antiseptics:microbicidal agent harmless enough to be applied to the skin and mucous membrane should not be taken internaslly such as alcohols, iodine solution and detergents.

PHYSICAL METHODS:

1. Heat (Dry and moist)

Heat is considered to be most reliable method of sterilization of objects that can withstand heat.

A. Dry Heat:. Techniques include:

• **Flaming** *Common uses:* bacterial loops, wires and spatula's.

• **Hot Air oven** (160°C/2hrs or 170°C/1hrs) *common uses:* glassware and metallic instruments.

- **Incineration**

B. Moist Heat:

Moist heat is more efficient in contrast to dry heat; it causes *coagulation* and *denaturation* of proteins.

• **Pasteurization** (At temperature 63°C/30 minutes or 72/15 minutes):is the use oh mild heat to reduce number of microorganism in product or food In the case of pasteurization of milk the time and temperature depend on killing potential pathogen that are transmitted in milk . such as *Staphylococci , streptococci*, *Brucella abortus and Mycobacterium tuberculosis*.

• **Boiling** (At temperature 100°C): 30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores.

• **Intermittent boiling** (At temperature 100°C): three 30 minutes intervals of boiling, followed by periods of cooling kill bacterial endospores.

• **Autoclave** (At temperature 121°C and 15 bar pressure for 15 minutes): kills all forms of life including bacterial endospores. Used to sterilize culture media, surgical instrument and cotton.

Autoclave

2. Radiation

There are 2 types of Radiation: usually destroys nucleic acid

A. Non-ionizing: .

UV Radiation it has a germicidal effect on microorganisms.

• *Common uses:* Surface disinfection, in hospitals, operating theatre and laboratories.

B. Ionizing: 2 types:

(Electron beam): *Common uses:* sterilization of instruments such as syringes, gloves, dressing packs, foods and pharmaceuticals.

(Gamma rays): *Common uses:* sterilization of disposable petri dishes, plastic syringes and fabrics.

CHEMICAL METHODS:

❖ **Ethylene oxide gas:** used to sterilize heat –sensitive objects

- *Common uses:* medical and pharmaceutical products, plastic containers

Formaldehyde: is a water-soluble gas. Formalin is a 35% solution of this gas in water. Formaldehyde is a broad-spectrum germicide for bacteria, fungi, and viruses.The mechanism of action of formaldehyde is based on protein denaturation. Kills endospores

- Common uses*:* Sterilization of instruments.

❖ **Alcohols**. The types of alcohol used in disinfection are ethanol (80%), propanol (60%), and isopropanol (70%). Alcohols are quite effective against bacteria and fungi, less so against viruses. They do not kill bacterial spores.denatures protein

- Common uses: surgical and hygienic disinfection of the skin and hands.

❖ **Halogens**. Chlorine, iodine, and derivatives of these halogens are suitable for use as disinfectants. halogens denatures proteins by binding to free amino groups.

- **Common uses**: Chlorine is used to disinfect drinking water. Iodine used to disinfect skin and small wounds.

❖ **Oxidants**. This group includes ozone, hydrogen peroxide, potassium permanganate, and peracetic acid. Their relevant chemical activity is based on the splitting off of oxygen.

- Common uses: Most are used as mild antiseptics to disinfect mucosa, skin, or wounds.

❖ **Phenols:** Synthetic phenolic compounds are disinfectants with broad-spectrum disinfecting action including a tuberculosis kill.

- Common uses: phenols may be used for surface disinfection.

FILTRATION

Fluids that would be damaged by heat irradiation or chemical sterilization, can be only sterilized by Microfiltration using membrane filters

- commonly uses: for heat labile pharmaceuticals and protein solutions in medicinal drug processing (such as those containing proteins like large molecule drug products, serum, enzymes, sugars, toxins).

Microbiological culture media

(Types of culture media and methods for preparation)

Introduction

The survival and growth of microorganisms depend on available nutrients and a favorable growth environment. In the laboratory, the nutrient preparations that are used for culturing microorganisms are called **media** (singular, **medium**). The media are contained either in test tubes, plates (petri-dishes), flasks or screw capped bottles etc. which must be thoroughly cleaned before use, then the medium and container together are subsequently sterilized by heat. Alternatively, the medium sterilized separately and poured into sterile containers.

Microbiologists use culture media for many purposes and applications:

1- Enrich the numbers of microorganisms.
2- Select for certain microorganism and suppress others.
3- Differentiate among different kinds of microorganisms.

Basic requirements of culture media

1- Nutrients:

- Carbon source.
- Nitrogen source.

2- **Mineral salts**:
(Sulphate, Phosphates, Chlorides and Carbonates of K, Mg and Ca)

3- Dyes and pH indicators

4- **Accessory growth factors**.

5- **Solidifying agents** such as agar which is characterized by:

a. Melting point 92-95^{o}C.
b. Solidifying point 42-45^{o}C.
c. Concentration 1.5-2%.

Classification of culture media

Culture media can be classified in at least three ways; based on consistency, based on nutritional component and on its functional use.

1- Classification based on consistency:

A) **Liquid media**: these are available for use in test- tubes, bottles or flasks. It sometimes referred as "broths" (e.g. nutrient broth).

B) **Solid media**: contains agar at a concentration of 1.5-2.0%, is useful for isolating bacteria or for determining the colony characteristics of the isolate **e.g** nutrient agar, MacConkey agar, blood agar, etc…,.

C) **Semi- solid media**: reducing the amount of agar to 0.2- 0.5% renders a medium semi- solid. Such media are useful in demonstrating bacterial motility (e.g. U-tube and Cragie's tube).

2- Classification based on nutritional component

Media can be classified as simple and complex media**.** While most of the nutritional components are constant across various media, some bacteria need extra nutrients. Those bacteria that are able to grow with minimal requirements are said to non-fastidious and those that require extra nutrients are said to be fastidious. Simple media such as peptone water and nutrient agar can support most non-fastidious bacteria, while, complex media such as blood agar used for fastidious bacteria.

3- Classification based on functional use or application:

A- **Basal media**: these are basically simple media that support most non fastidious bacteria (e.g. peptone water, nutrient broth and nutrient agar).

B- **Enriched media**: addition of extra nutrients in the form of blood, serum, egg yolk etc. to the basal medium makes them enriched media. These are used to grow nutritionally exacting (fastidious) bacteria (e.g. blood agar, chocolate agar and Loeffler's serum slope).

C- **Selective media**: are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. (e.g. Mannitol salt agar and Eosin methylene blue)

D- **Differential media**: Differential or indicator media distinguish one microorganism type from another growing on the same medium.This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. These media are used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria. (e.g. MacConkey agar)

E- **Transport media**: clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. (e.g **Stuart transport medium**)

Preparation of culture media

The preparation of media from dehydrated products is simple. Each bottle of dehydrated medium has instructions for preparation on its label. These media are simply reconstituted by weighing the required quantities and by adding distilled water, as per in the manufacturer's instructions. The pH determination can be conveniently done with the use of pH indicator papers.

Sterilization of culture media

Sterilization is the process of rendering a medium or material free of all forms of life. The choice of method to be used to sterilize a medium depends on whether or not the ingredients are decomposed by heat. The most useful approach is autoclaving in which media are sterilized by exposure to steam at 121°C and 15 lbs. of pressure for 15 minutes. Sometimes media made from components that will not withstand heating at 121°C. These components are filtered and may be added separately after the medium is autoclaved.

Storage of culture media

Culture media deteriorate quickly and often contaminated, hence cold storage is necessary.

Antimicrobial susceptibility test

Antimicrobial susceptibility test measure the ability of an antibiotic to inhibit bacterial growth in vitro. This ability may be estimated either by dilution or diffusion method.

Indications for susceptibility testing

1. To guide the clinician in selecting the best antibiotic agent for an individual patient.
2. To control the use of inappropriate antibiotics in clinical practice
3. To accumulate epidemiological information on the resistance of microorganisms of public health importance within the community-.
4. When the susceptibility to the antimicrobial agents of choice is unpredictable and if the isolate is believed to be clinically significant.
5. If the patient is allergic to the antimicrobial agent of choice, testing susceptibility to alternative agents, such as erythromycin for *S. pyogenes*, is reasonable.
6. When an infectious process is likely to be fatal unless treated specifically (e.g., meningitis, septicemia).
7. -Bacteria have the ability to develop resistance following repeated or subclinical (insufficient) doses, so more advanced antibiotics and synthetic antibiotics are continually required to overcome them.

Quantitative susceptibility test

- Broth dilution method

A-Determination of minimal inhibitory concentration (MIC)

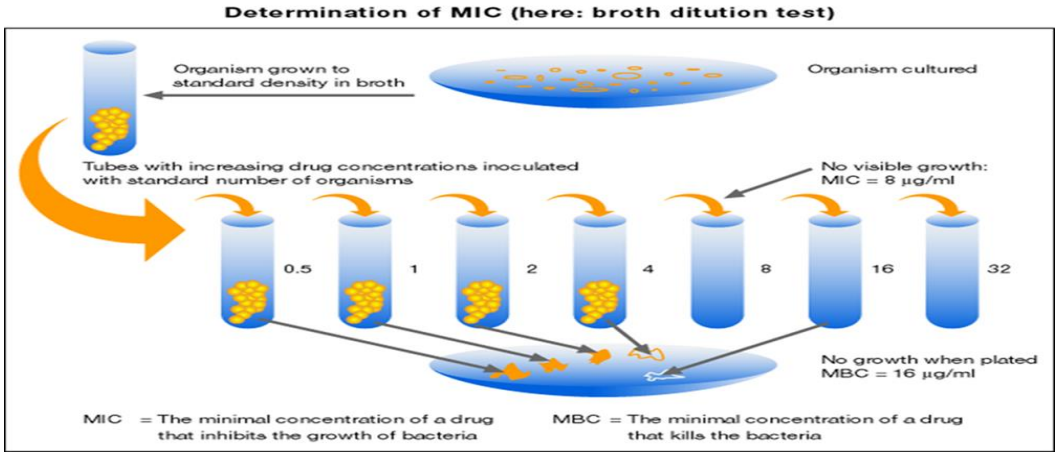
The quantitative determination of susceptibility to antimicrobial agents has proved to be the most reliable means of treating infection. Tube dilution method can be used to determine the MIC, this can be perform by either broth or agar dilution method. The lowest concentration that inhibits growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent.In this method serial dilutions

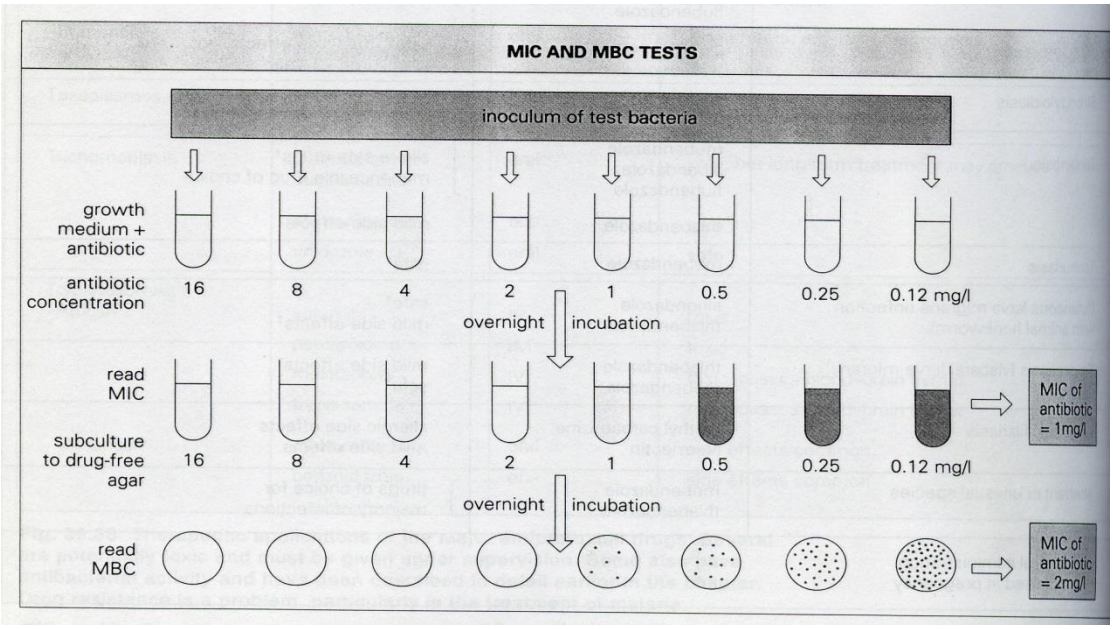
of antimicrobial agent are prepared in several test tubes which contain broth medium ,equal amount of bacterial sample are added to each test tubes, a positive and negative control tests must be prepared to justify the result.The test tubes and control tubes are incubated at 37 ^{o} C for 18-24 hours in reading results turbidity is used as a sign of bacterial growth in the test tubes minimum inhibitory concentration (MIC) is recorded as it is the lowest concentration of antimicrobial agent that can inhibit visible growth of the test organism after overnight incubation

B-Determination of minimal bactericidal concentration (MBC)

The main advantage of the broth dilution method is that it can be readily converted into a bactericidal test. In this case dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37 ^{o}C overnight. The tubes are also incubated overnight at 37 ^{o} C. The MIC is then recorder, subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37 ^{o}C overnight. Compare the amount of growth from the control tube before incubation, which represents the original inoculum. These subcultures may show a similar number of colonies, indicating bacteristasis only, a reduced number of colonies, indicating partial or slow bactericidal activity, or no growth if the whole inoculum has been killed .

MBC is the lowest concentration of the antimicrobial agent which kills 99.9% of the tested bacteria after overnight incubation .

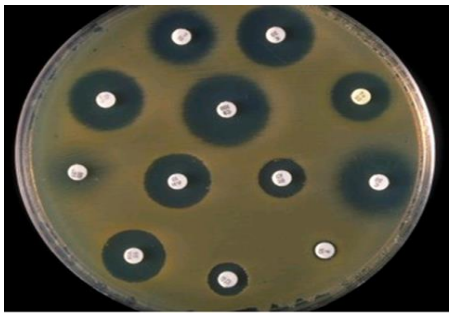




Qualitative susceptibility test

The diffusion test **(kirby-bauer test for antibiotic susceptibility**)

Paper disk, impregnated with the antibiotic are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic forms by diffusion from the disk and the growth of the test organism is inhibited at a distance from the disc . The Mueller-Hinton agar medium being used for the Kirby-Bauer test. If the organism is killed or inhibited by the concentration of the antibiotic, there will be NO growth in the immediate area around the disc: This is called the zone of inhibition. The diameter of the zone of inhibition is measured using a ruler or a pair of calipers. The zone sizes are looked up on a standardized chart to give a result of susceptible, resistant, or an intermediate.



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Systemic Bacteriology

Gram positive cocci

Genus: Staphylococcus spp

The genus Staphylococcus contains about 30 species. Only some of them are important as human pathogens :

1-*Staphylococcus aureus*

Is responsible for most Staphylococcal infections and aureus causes a variety of suppurative (pus-forming) infections. Nasal carriage occurs in 20 – 50% in human.

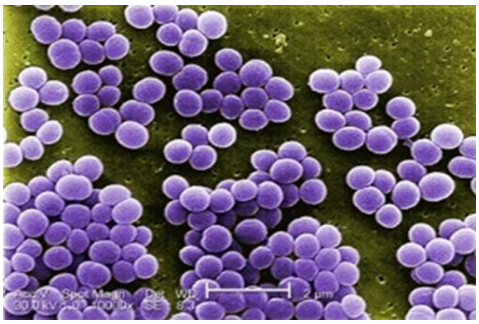
2- *Staphylococcus epidermidis*

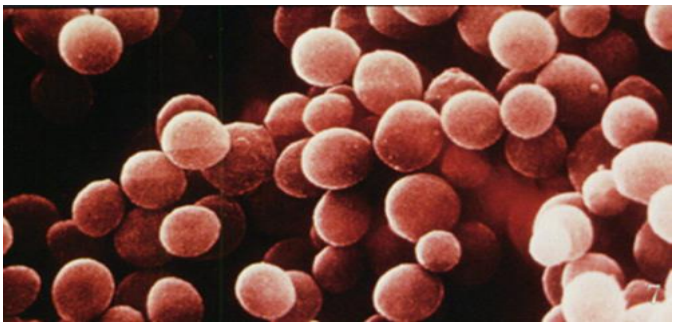
Usually present as normal flora of human skin and mucous membrane (non pathogenic) but may cause infection in immune –compromised patient if accidentally introduced catheterization.

3- *Staphylococcus saprophyticus*. Free living non- pathogens but may produce U.T.I

Microscopically appearance

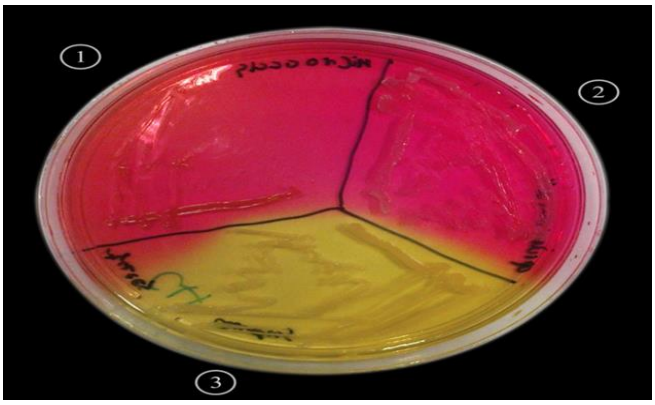
Members of the genus Staphylococcus are gram positive cocci (0.5–1.5 μm in diameter) that occur singly, in pairs, tetrads, short chains (three or four cells), and irregular grape-like clusters. They are non motile, non spore forming, and usually are unencapsulated or have limited capsule formation.





Cultural characteristics

Staphylococcus aureus forms a fairly large yellow colony on rich medium; *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Mannitol salt agar or MSA is selective differential medium for Staphylococcus aureus.It contain :Nacl 7.5%, Mannitol, and phenol Red. The cause of selectivity due to presence of high salt concentration. The cause of differential because contains mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH**.**



Lab diagnosis

The specimen usually sent to the laboratory for isolation Staphylococcus spp. Is pus (from abscess, osteomyelitis, or otitis media),swab are used to collect specimens from throat, nostrils, skin, wounds, urine, C.S.F., or blood in cases of septicemia.

1-Microscopic examination (smear).

2-Culture(Nutrient agar, blood agar and Mannitol salt agar)

3-Coagulase test

Is recognized as the most important test for testing the virulence of staphylococci spp, ***Staphylococcus aureus*** is the only coagulase positive staphylococci, coagulase convert fibrinogen into fibrin. Fibrin can be deposited on the surface of bacteria forming a wall around the bacteria which has an important role in:

A) Protection of bacteria from phagocytosis.

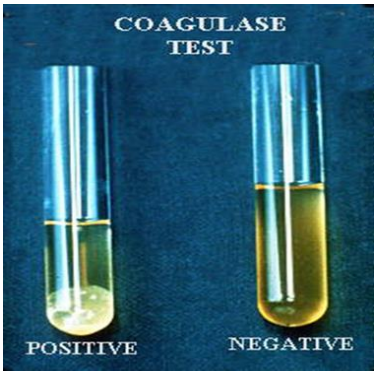
B) Preventing the action of antibiotics.

1-Slide method :

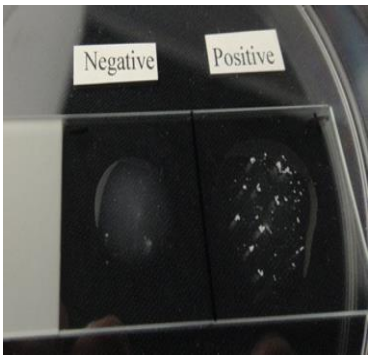
- Used to detect bound coagulase or clumping factor
- Add one drop heavy bacterial suspension and one drop of plasma on clean slide.
- Mixing well and observing for clumping within 10 seconds.

2-Tube method

- Mix 0.1 ml of culture + 0.5 ml of plasma
- Incubate at 37C for 4 h
- Observing the tube for clot formation
- Any degree of clotting constitutes a positive test



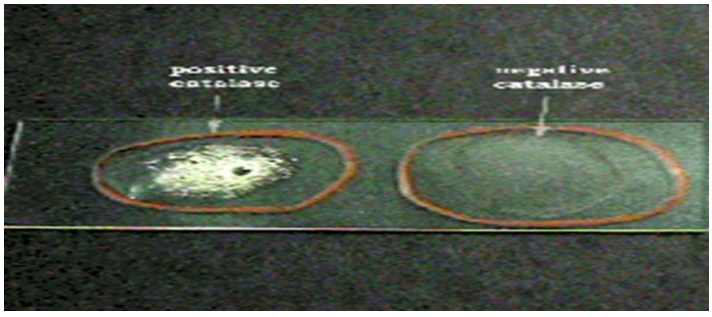
Tube method



Slide method

Catalase test

The catalase test differentiates the staphylococci, which are positive from the streptococci, which are negative. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test. The test should not be done on blood agar because blood contains catalase.



4-Antibiotic Susceptibility / Resistance

Staphylococcul isolates should be tested for antimicrobial to help in the choice of systemic drugs. This done by growing staphylococci on Mueller-Hinton agar, discs impregnated with various antibiotics, in this case Novobiocin are placed onto agar and incubated for 24 hours at 37 °C. The antibiotic in the disc diffuses into the surrounding agar. If the bacterial species is susceptible to the antibiotic there is a circle of "no-growth" around the disc where bacterial growth is inhibited by the antibiotic. If the bacteria is resistant to the antibiotic the cells grow right up the antibiotic disc. The bacterial species or strain is reported as being resistant to the antibiotic (R) or susceptible to the antibiotic (S) depending on the observations made. The diameter of the area of "no-growth" around the disc may determine the susceptibility or resistance of the organism to the antibiotic.

Expected Results

Positive – A zone of inhibition greater than 16mm indicates that the organism is sensitive to the antibiotic.

Negative – A zone of inhibition less than or equal to 16mm is indicative of novobiocin resistance.

Staphylococcus saprophyticus
Resistant (less than 16 mm)

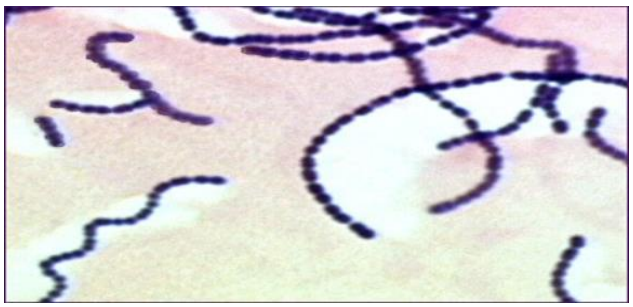
Staphylococcus aureus
Sensitive (greater than 16 mm)

Genus Streptococcus

Members of this genus are widely distributed in environment, some are non pathogenic and could be a part of normal flora present in pharynx, mouth, intestine and female genital tract. Others are pathogenic from simple to complex.

Microscopiclly Appearance

- Gram positive cocci
- 1µm in diameter
- Chains or pairs
- Some strains are capsulated
- Non motile
- Non spore forming



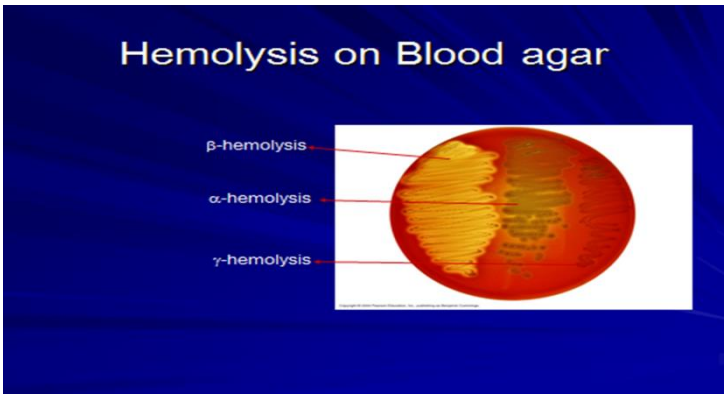
Culture characters:

Growth occur only in enriched media, majority are facultative , can grow under aerobic or anaerobic conditions grow at 37c but is aided by 5-10% CO2. Few are obligatory anaerobic e.g peptostreptococcus. Nutritionally streptococci are fastidious m.o. and only grow on enriched media for example blood agar, chocolate agar and can't grow on simple media like nutrient agar**.**

Species of this genus is classified according to the following:

1- Hemolysis

- **-Beta hemolysis Streptococci (β):** clear zones of hemolysis around the colonies the area appears lightened (yellow) and transparent. e.g. Group A & B (*S. pyogenes* & *S. agalactiae*).
- **Alpha hemolysis Streptococci (α):** partial or incomplete hemolysis around the colony and the agar under colony is dark and greenish ex. *streptococcus viridans* , *Streptococcus pneumoniae*.
- **Gamma hemolysis (γ) :** (Non hemolytic) streptococci: no change on surface of blood agar ex, *Enterococcus faecalis*.



2-Serologic specificity (Lancefield grouping).

It is group specific cell wall antigen which is carbohydrate in nature and it's located in the cell wall of many streptococci and form the basis of serologic grouping. On basic of **this antigen β-hemolytic Streptococci ,named as group from A to U.**

-Group A streptococci (*streptococcus pyogenes*)

Habitat:- Throat, skin

Common and Important Diseases

Pharyngitis, tonsillitis, impetigo, glomerulonephritis & rheumatic fever.

More than 20 extracelluar products that are antigenic are elaborated by group A streptococci including: Hemolysins: β- hemolytic streptococci elaborate 2 hemolysins:

1-Streptolysin O *sensitive to oxygen,

*antigenic ASO

Streptolysin O is a protein that is hemolytically active in the reduced state but rapidly inactivated in the presence of oxygen, its antigenic and combines quantitively with antistreptolysin O (ASO) this antibody blocks hemolysis by streptolysin O.

2. streptolysin S * Not sensitive to oxygen

*Not an antigenic

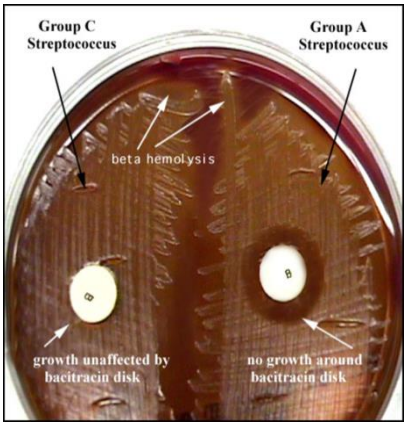
It's responsible for hemolysis around streptococcal colonies on blood agar surface.

Lab diagnosis – *Strep. Pyogenes*

- Specimens: throat swab, pus, blood
- Microscopy :Gram stain - Gram positive cocci in chains
- Culture: on blood agar under 5-10% Co2 pinpoint colonies which are circular with entire margins and exhibits beta (b) hemolysis; the complete breakdown of the red blood cells surrounding the colonies.

- **Bacitracin susceptibility Test**

S.pyogens is the only streptococci sensitive for this antibiotic which causes a zone of growth inhibition.



- Serology: (Lancefield grouping). The ASO titer can be applied in the diagnosis of streptococcal infections. Serum titer >200 I.U. is considered abnormal.

–Group B streptococci Beta hemolytic (*Streptococcus agalaectiae)*

Habitat:-Normal flora in lower GIT, female genital tract

Common and Important Diseases

Neonatal sepsis and meningitis.

The following tests can be used to differentiate between Beta-hemolytic streptococci:

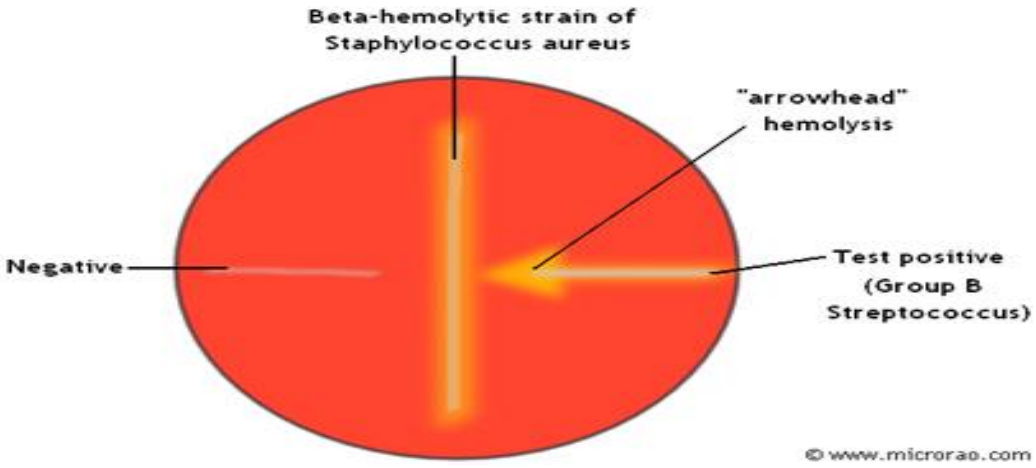
1-**Lanciefield Classification**

2-**Bacitracin susceptibility Test**

Specific for *S. pyogenes* (Group A)

3-CAMP test:

Specific for *S. agalactiae* (Group B)



Enterococcus e.g *Enterococcus faecalis*,
Normal flora in GIT, lower genital tract
Cause UTI, wound infection, endocarditis.
Non hemolytic

α – hemolytic Streptococci

It includes:

1-*Viridans streptococci* (Group D)(e.g., *S. mutans, S.mitis,* S. *sangius, S.salivaris*)

This microorganism has low virulence and often colonize in the URT. They considered as commensal m.o of the mouth and they can act as opportunistic pathogen and attack tissue. It is associated with dental caries and they are the leading cause of subacute bacterial endocarditis (SBE) following dental extraction. *S. mutans* causes tooth carries

2. Streptococcus pneumonia

Also called Diplococcus pneumoniae or pneumococcus

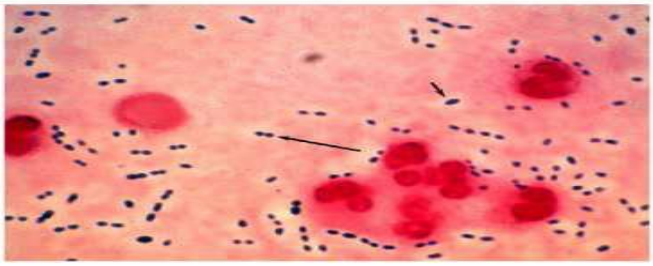
-Capsulated possessing a polysaccharide capsule

-Normal inhabitant of the upper respiratory tract (URT) of human

-some times may cause important human diseases such as **pneumonia**, **bronchitis**, **sinusitis, otitis media** and less frequently it invades blood stream producing bacteremia, and the most important complication of bacteremia includes: meningitis and septic arthritis.

Microscopic appearance of *Streptococcus pneumonia*

Gram positive, diplococci



As both *S. viridins* and *S.pneumonia* are α hemolysis on blood agar, we expect misdiagnosis between them. So we depend on the following differential points:

character	Pneumococci	Viridans streptococci
Morphology	Capsulated, lanceolate, diplococci	Oval or rounded in chains
Quellung test	+	-
Bile solubility	+	-
Capsule swelling (Quellung reaction)	+	-
Optochin sensitivity	+	-
Animal pathogenicity	Virulent for white mouse	Avirulent

Lab diagnosis of *S .pneumonia*

-Specimen: CSF, blood, sputum, pus, swabs

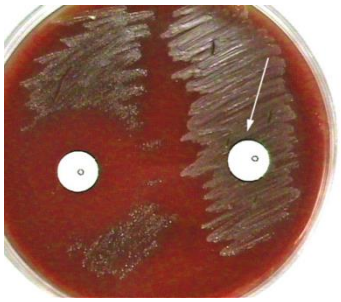
-**Microscopy**: Gram stain – G+ve C in pairs, capsulated, lanceolate shaped diplococci.

-Culture on blood agar, chocolate agar alpha hemolytic colonies

-Identification tests

A- Optochin Susceptibility Test

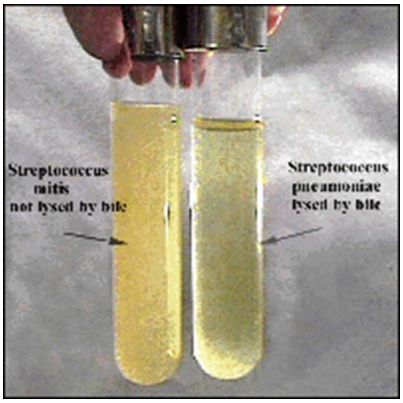
Chocolate agar streaked with *S.pneumonia* then apply Optochin disc. Demonstrate a zone of inhibition around disk. This test used to differentiate *S.pneumonia* from *viridins streptococci* because both of them are α hemolysis on Chocolate agar.



B- Bile solubility test

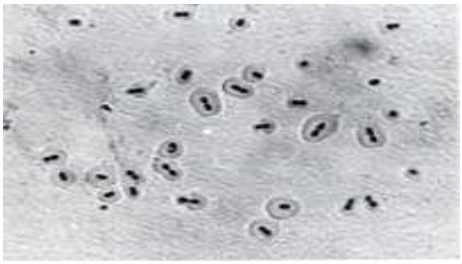
S. pneumoniae produce a self-lysing enzyme to inhibit the growth .The presence of bile salt accelerate this process

- Add ten parts (10 ml) of the broth culture of the organism to be tested to one part (1 ml) of 2% Na deoxycholate (bile) into the test tube.
- Incubate at 37oC for 15 min
- Positive test appears as clearing in the presence of bile while negative test appears as turbid
- *S. pneumoniae* soluble in bile whereas *S. viridans* insoluble



c-Quelling reaction test

Pneumococcus of certain type when mixed with anti- capsular antibodies on microscopic slide, the capsule swells markedly and can be visualized by examination of the slide under low power.



Proteus spp.

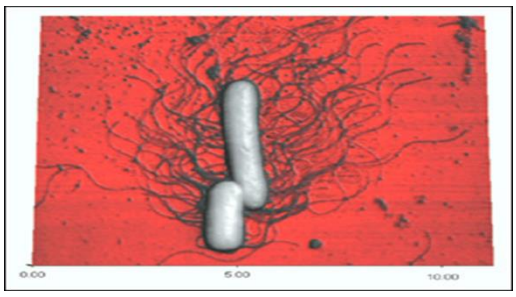
Bacteria, which belong to enterobacteriaceae, are commensal in human intestine .However this group also becomes pathogenic under opportunistic conditions causing infections such as(UTI following catheter & surgery, wound infection, otitis media and septicemia

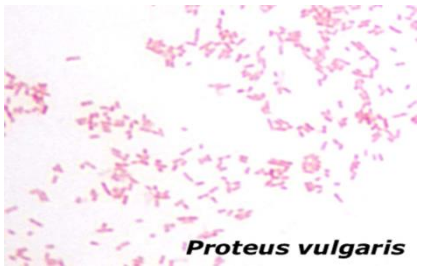
The species of proteus are differentiated in to:

1. *proteus mirabilis.*
2. *proteus vulgaris.*
3. *proteus morganii*
4. *proteus rettgeri*

Morphology:

Gram-negative, facultatively anaerobic rod-shaped, pleomorphic , noncapsulated, non spore forming and motile show Swarming motility.





Swarming motility of *proteus* spp.

Cultural Characteristics:-

They grow readily on all ordinary media,producing fishy smell. Swarming type of growth is observed on soild media such as nutrient agar and blood agar. Swarming is inhibited on macConkey ́ s agar and produce pale yellow colonies which moderate in size, moist and low convex, Non-fastidious bacteria. Urease producing organisms which decompose urea.

How to prevent swarming????

- ❖ Increase the agar concentration (from 1.2-1.5% to 6%).
- ❖ CLED agar (electrolytes deficiency).
- ❖ To add chemicals: chloral hydrate, sodium azide, sulphonamide & paranitroglycerol.
- ❖ MacConkey agar (Bile salt).

LAB- Diagnosis:-

- Specimen: according to the side of infection (Pus, swabs, blood, urine, sputum……etc).
- Colonial morphology. Culture & incubation**.**
- Gram stain
- Biochemical reaction.
    - It forms acids and gas from glucose fermentation (except *P. rettgeri*).
- It characteristically deaminates phenylalanine to phenyl pyruvic acid(PPA). Phenylalaine deaminase test is used to differentiate

members of the genera *Proteus,* (+ve) from other members of **Enterobacteriaceae** which give negative results.

Is used to test the ability of microorganism to produce enzyme deaminase this enzyme remove amine group from phenylalaine and produce phenyl pyruvic acid. Phenylalanine agar, also known as phenylalanine deaminase medium is used as test medium. positive result give green color while negative result no change in media yellow color.

- Hydrolsis of urea is another characteristic property of proteus spp.
- It is MR positive and VP negative.
- It is non lactose fermenter.
- *proteus mirabilis* and proteus vulgaris can produce H2S while *proteus morganii* and *proteus rettgeri* are H2S negative.
- Indole is not produced by *proteus mirabilis*
- Citrate utilization test is positive in *proteus rettgeri* and negative in *proteus morganii*

Pseudomonas

They are saprophytic and innately resistant bacteria causing opportunist infections in man, found in nature water, soil and other moist environments, causing suppurative and inflammatory lesions.

Pseudomonas spp:-

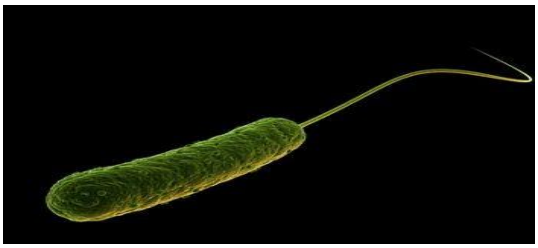
1. *Pseudomonas aeruginosa*
2. *Pseudomonas fluorescens.*
3. *Pseudomonas putida*
4. *Pseudomonas stutzeri*
5. *Pseudomonas alcaligenes*
6. *Pseudomonas pseudomallei*

The species most common associated with diseases is *Pseudomonas aeruginosa* .It is commonly encountered in secondary infections of wounds, burns and chronic ulcer of skin as well as urinary tract infections.

Pseudomonas aeruginosa

Morphology

It is slender gram negative bacilli, 1.5 – 3 x 0.5 microns in size, actively motile by a by single or multiple polar flagella, non capsulated and its non–spore forming.



Cultural Characters

It is aerobic, growing on simple media with optimum temperature of 37 ^{o}C.

In broth forms dense turbidity with surface pellicle. Bluish green pigment due to water soluble Pyocyanin is seen.

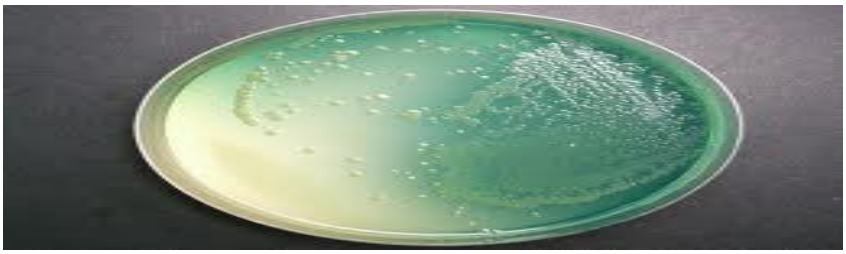
Nutrient agar: producing large opaque irregular colonies with distinctive musty or earthy smell.

Pseudomonas spp. are Produce water soluble pigments which diffuses in medium.There are several types of pigments:

a) Pyocyanin (bluish green).
b) Pyoverdin (yellowish green).
c) Pyorubin(Reddish- Brown)

d) Pyomelanin (Brown – Black).

Some strain may be non –pigmented.



Blood agar: It shows beta type of hemolysis.

MacConkey agar: It produce non lactose fermenting colonies.

Laboratory diagnosis:

1- **Specimens:** According to the side of infection (swabs,blood ,urine,sputum).
2- **Smears:***pseudomonas aeroginosa* is gram negative bacillus often sees.
3- **Culture :**on nutrient agar media characteristic bluish green colonies appear.
4- **Biochemical tests**
   - Glucose is utilized oxidatively forming acid only.
   - Indole, MR and VP and H2 S tests are negative.
   - Utilizes Citrate as a sole source of Carbon.
   - Catalase and Oxidase are positive.

OXIDASE TEST

The oxidase test is a key test to differentiate between the families of Pseudomonadaceae and Enterobacteriaceae. Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Presence of cytochrome oxidase can be detected through the use of Oxidase test reagent (**1% tetra methyl-para –phenylenediamine**) .This reagent acts as an electron donor to cytochrome oxidase. If the bacteria oxidize the Oxidase test reagent (remove electrons) the reagent will turn purple indicating a positive test. No color change in one minute indicates a negative test.

THE PROCEDURE:

1. Pick a good-sized amount of inoculum (already incubated and grown) from a plate culture or slant culture and place it on a piece of filter paper.

2- Add one drop of the reagent. A positive reaction will usually occur within 10-15 seconds be a bluish-purple color that progressively becomes more purple.