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كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: احياء مجهرية طبية I

اسم المادة باللغة الإنكليزية: **Medical Microbiology I**

المرحلة: الثانية

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عنوان المحاضرة باللغة العربية: المكورات الموجبة لصبغة كرام

عنوان المحاضرة باللغة الإنكليزية: **Gram positive cocci**

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.

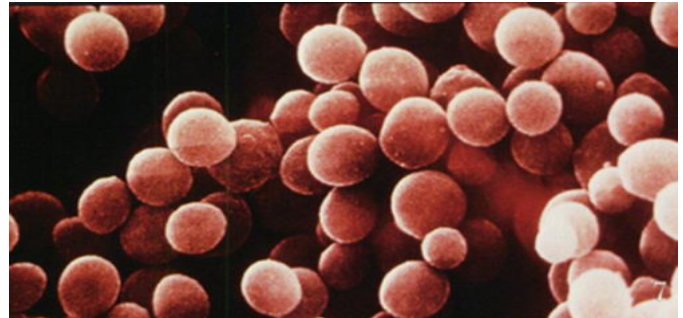
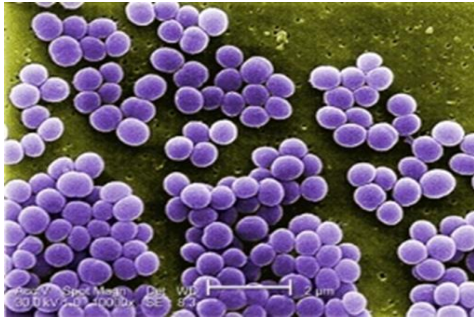
2- *Staphylococcus epidermidis*

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

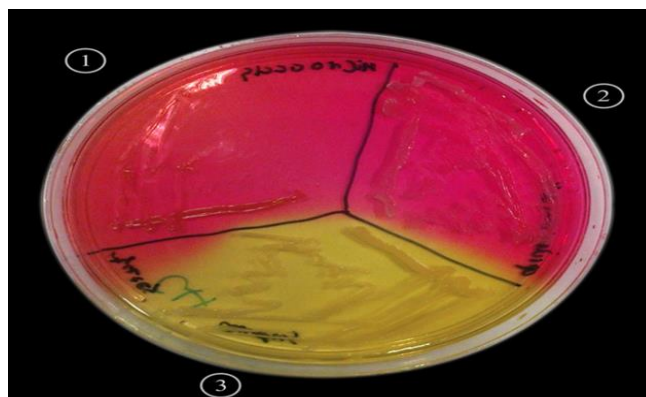
Microscopically appearance

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 contains: 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:

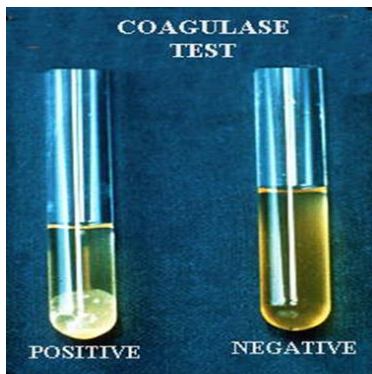
- A) Protection of bacteria from phagocytosis.
- B) Preventing the action of antibiotics.

1-Slide method :

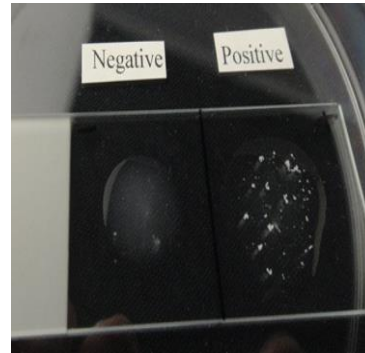
- ❖ Used to detect bound coagulase or clumping factor
- ❖ Add one drop heavy bacterial suspension and one drop of plasma on clean slide.
- ❖ Mixing well and observing for clumping within 10 seconds.

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



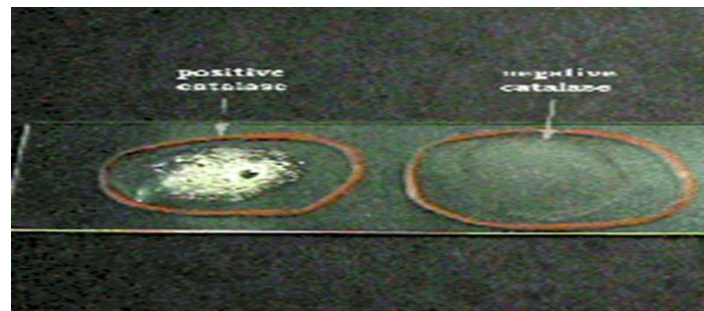
Tube method



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

Staphylococcal 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

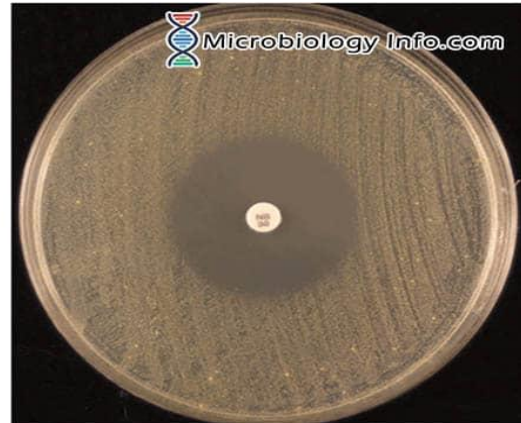
Positive – A zone of inhibition greater than 16mm indicates that the organism is sensitive to the antibiotic.

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



Staphylococcus saprophyticus
Resistant (less than 16 mm)

NOVOBIOICIN



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.

Microscopically Appearance

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



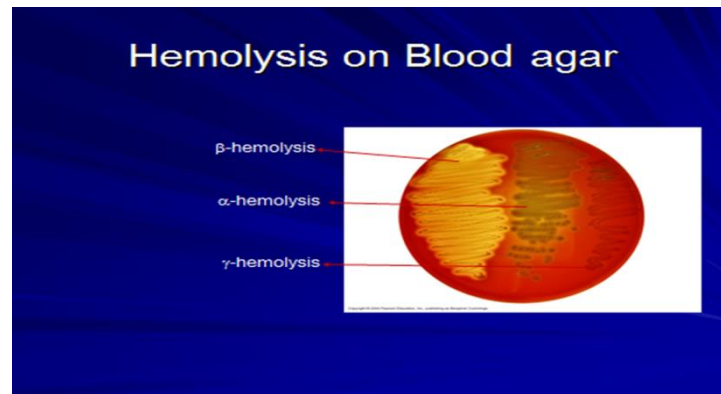
Culture characters:

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 basis 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 extracellular 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, it's antigenic and combines quantitatively with antistreptolysin O (ASO) this antibody blocks hemolysis by streptolysin O.

2. streptolysin S * Not sensitive to oxygen

*Not an antigenic

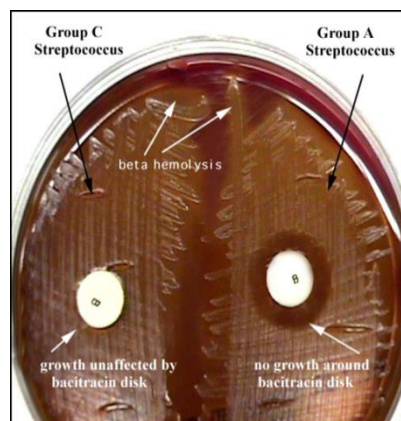
It's responsible for hemolysis around streptococcal colonies on blood agar surface.

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 (β) hemolysis; the complete breakdown of the red blood cells surrounding the colonies.

❖ Bacitracin susceptibility Test

S. pyogenes 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 agalactiae*)

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:

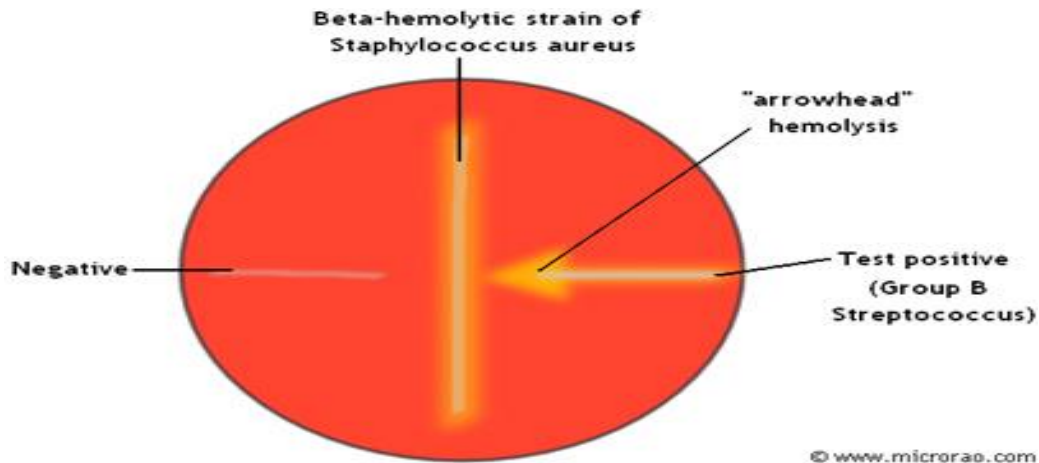
1-Lanciefield Classification

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* (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

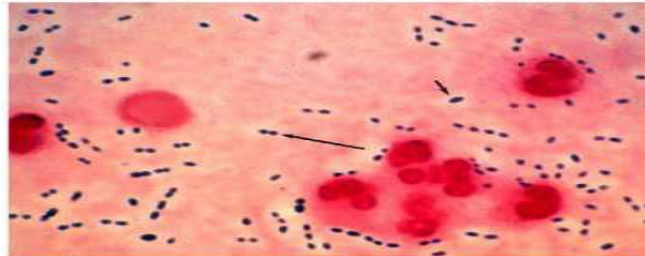
-Normal inhabitant of the upper respiratory tract (URT) of human

-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 pneumoniae*

Gram positive, diplococci



As both *S. viridans* and *S.pneumoniae* 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)	+	-
Inuline fermentation	+	-
Optochin sensitivity	+	-
Animal pathogenicity	Virulent for white mouse	Avirulent

Lab diagnosis of *S .pneumoniae*

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

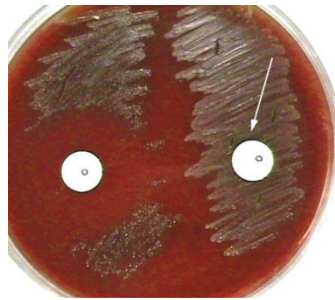
-**Microscopy:** Gram stain – G+ve C in pairs, capsulated, lanceolate shaped diplococci.

-**Culture** on blood agar, chocolate agar alpha hemolytic colonies

-**Identification tests**

A- Optochin Susceptibility Test

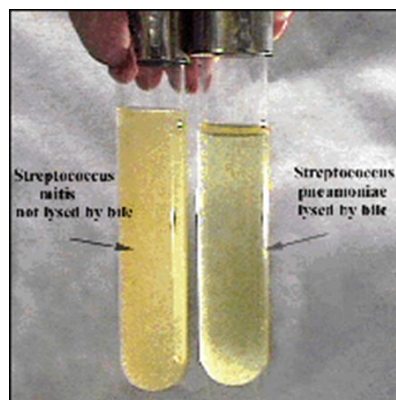
Chocolate agar streaked with *S. pneumonia* then apply optochin disc. Demonstrate a zone of inhibition around disk. This test used to differentiate *S.pneumonia* from *viridins streptococci* because both of them are α hemolysis on blood agar.



B- Bile solubility test

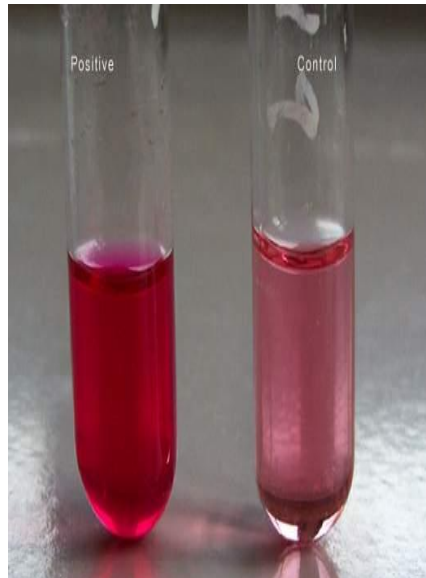
S. pneumoniae produce a self-lysing enzyme to inhibit the growth .The presence of bile salt accelerate this process

- ❖ 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- Inulin fermentation test.

This test is useful to differentiate Pneumococci from Streptcocci as the latter do not ferment Inulin sugar while Pneumococci do ferment it.Positive test produce red color while negative result media remain unchanged, pink color.



Inulin Test

d-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.

