

LAB: 3

Practical Pathogenic bacteria

Genus: *Streptococcus*

General characteristics

1- Streptococci

Bacteria of the genus *Streptococcus* are gram-positive cocci typically arranged in chains or pairs .The genus includes three of the most important pathogens of humans Pyogenic streptococci (Lancefield groups), Pneumococci, Viridans and other streptococci.

2- Streptococci group characteristics

Morphology Streptococci stain readily with common dyes, Demonstrating coccal cells that are generally smaller and more ovoid in shape than staphylococci. Length may vary from a single pair to continuous chains of over 30 cells. Medically important streptococci are not acid-fast, do not form spores, and are non-motile. Some members form capsules. Note: the oval cocci chaining end to end

3- Cultural and Biochemical Characteristics

Streptococci grow best under aerobic or anaerobic conditions (facultative),Some are strict anaerobic .Blood agar is preferred The colonies are small, ranging from pinpoint size to 2 mm in diameter B-hemolysis is clear while α -hemolysis is greening of blood agar .They are catalase negative.

4- Pyogenic Streptococci

Of the many Lancefield groups, the ones most frequently isolated from humans are, groups A (*S. pyogenes*) and B (*S. agalactiae*) which are the most common causes of serious disease.

5- Morphology and growth of Group A Streptococci (GAS). (*Streptococcus pyogenes*) Group A

typically appear in purulent lesions or broth cultures as spherical or ovoid cells in chains of short to medium length (4 to 10 cells). On blood agar plates, colonies are usually compact, small, and surrounded by a 2- to 3-mm zone of β -hemolysis

6- Pneumococci (*Streptococcus pneumoniae*)

It contains a single species, *S pneumoniae*, commonly called the pneumococcus. Its distinctive feature is the presence of a capsule composed of polysaccharide polymers that vary in antigenic specificity. on blood Agar, *S. Pneumoniae* colonies appear small, shiny, flattened, and translucent. They are surrounded by a zone of greenish alpha hemolysis (α -Hemolysis) due to incomplete hemolysis of RBC

Morphology and structure

Pneumoniae (pneumococci) are gram-positive, oval cocci typically arranged end to end in pairs (diplococcus), giving the cells a bullet shape. *Streptococcus pneumoniae* in sputum of patient with pneumonia. Note the marked tendency to form oval diplococci

7- Viridans and other Streptococci

Viridans streptococci are members of the normal oral flora of humans. Their virulence is very low, viridans strains can cause subacute bacterial endocarditis. Viridans streptococci are α -hemolytic or no hemolysis on blood agar and lack both the group carbohydrate antigens of the pyogenic streptococci and the capsular polysaccharides of the pneumococcus. The term encompasses several species, including *S. salivarius* and *S. mitis*.

8- Isolation and identification of group A beta streptococci (Streptococcus pyogenes).

Group A beta streptococci are usually isolated on blood agar. They produce very small, white to grey colonies approximately 1mm in diameter surrounded by a zone of beta hemolysis around 2-3mm in diameter.

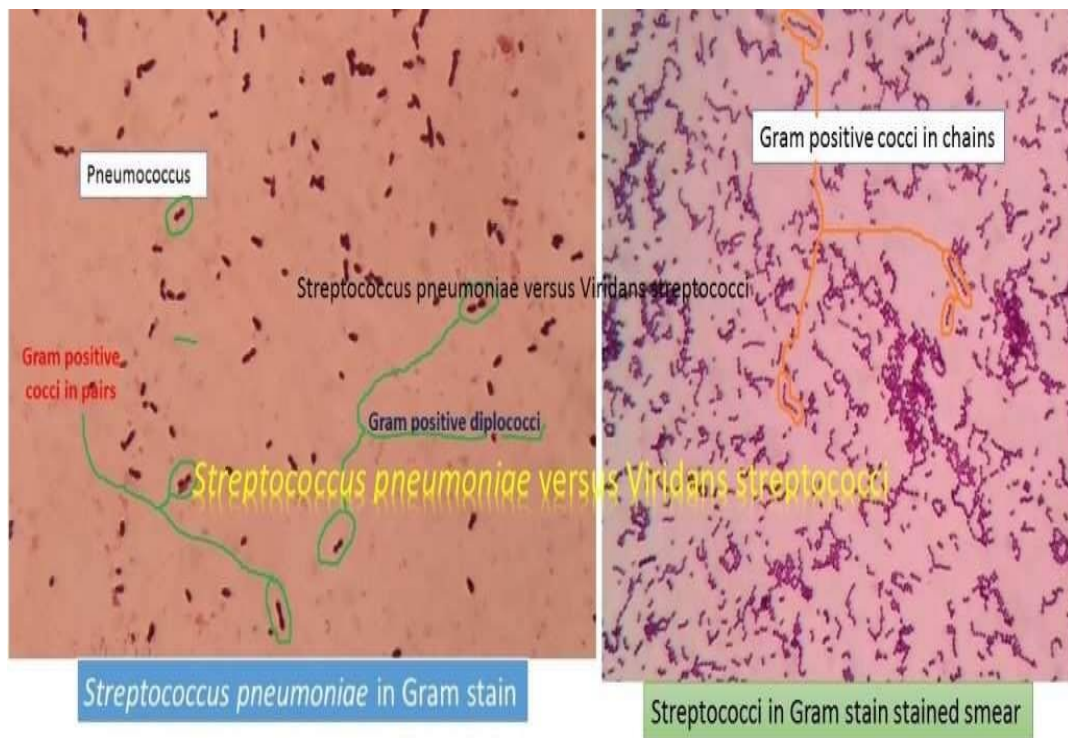
Bacitracin test:

The bacitracin test is used to differentiate and identify β -hemolytic group A streptococci (*Streptococcus pyogenes*) from other β -hemolytic streptococci.

Bacitracin, produced by *Bacillus licheniformis*, is a powerful peptide antibiotic that inhibits bacterial cell wall synthesis. Any zone of clearing 10 mm or greater around the disk is interpreted as bacitracin susceptibility.

Optochin test: The optochin test is used to differentiate *Streptococcus pneumoniae* from other α -hemolytic streptococci .

Optochin is an antibiotic that interferes with ATPase activity and ATP production in susceptible bacteria. A 6 mm disc (containing 5 μ g of optochin) should produce a zone of inhibition 14 mm or more to be considered optochin susceptible (*S. Pneumoniae*), while viridans group considered optochin resistance (-ve results).



Differentiation between *Streptococcus* species

| Tests | <i>S.pyogenes</i> | <i>S.pneumoniae</i> |
|------------------------|---------------------|-------------------------|
| Gram stain | Gram+ve & cocci | Gram+ve & cocci |
| Arrangement | chain | pair |
| Catalase | Negative | Negative |
| Oxidase | Negative | Negative |
| Streptokinase | Positive | Negative |
| Capsule | Negative | Positive |
| Inulin | None ferment | Ferment |
| Bile solubility | Negative | Positive soluble |
| CAMP reaction | Negative | Negative |
| Blood hemolysis | Beta | Alpha |
| Bile esculin | Negative | Negative |
| Nacl 6.5% | Negative | Negative |
| Motility | Negative | Negative |
| Spore forming | Negative | Negative |
| Optochin | R | S |
| Bacitracin | S | R |

Differences between
Str. pneumoniae & Viridans Streptococci

| Property | Pneumococci | Viridans Streptococci |
|---|------------------------|--|
| Morphology | Lanceolate, diplococci | Spherical / oval cocci In long chains |
| Capsule | Present | Absent / Slime |
| Colony | Draughtsman | Dome |
| Bile solubility | Soluble | Insoluble |
| Optochin sensitivity | Sensitive | Resistant |
| Animal pathogenicity (mouse) | Virulent | Avirulent |

Laboratory Diagnostic

1-Gram stain

2-Catalase test

3-Oxidase test

4-Sterptokinase test

5-Bacitracin & Optochin test

6-Bile solubility test

7-Bile esculin test

8-Growth on 6.5% NaCl

9-Hippurate hydrolysis test

10-Blood agar: for hemolysis

11- CAMP test