Practical Pathogenic bacteria **LAB-5**

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.

3- Morphology Streptococcus gram stain.
Note: the oval cocci chaining end to end

4- Cultural and Biochemical Characteristics

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

5- 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.

6- Group A Streptococci (Streptococcus pyogenes)
Morphology and growth Group A streptococci (GAS).

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

7- Pneumococci

This category 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. S pneumoniae is α-hemolytic

8- Streptococcus pneumoniae

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

9- Viridans and other Streptococci

Viridans streptococci are α-hemolytic 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. Viridans streptococci are members of the normal oral flora of humans. Their virulence is very low, viridans strains can cause subacute bacterial endocarditis

10- Isolation and identification of group a beta streptococci (Streptococcus pyogenes).

Beta hemolysis on blood agar 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.

11-streptococci (Streptococcus pyogenes).

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

12- Isolation and identification of group a beta streptococci (Streptococcus pyogenes).

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.

13- Isolation and identification of pneumococci and viridans group

Isolation on blood, *S. Pneumoniae* colonies appear small, shiny, flattened, and translucent. They are surrounded by a zone of alpha hemolysisα-Hemolysis: This is a streak plate of *Streptococcus pneumoniae* demonstrating α-hemolysis. The greenish zone around the colonies results from incomplete lysis of red blood cells.

14- Isolation and identification of pneumococci and viridans group

Isolation on blood agar: The viridans streptococci show alpha hemolysis or no hemolysis on blood agar

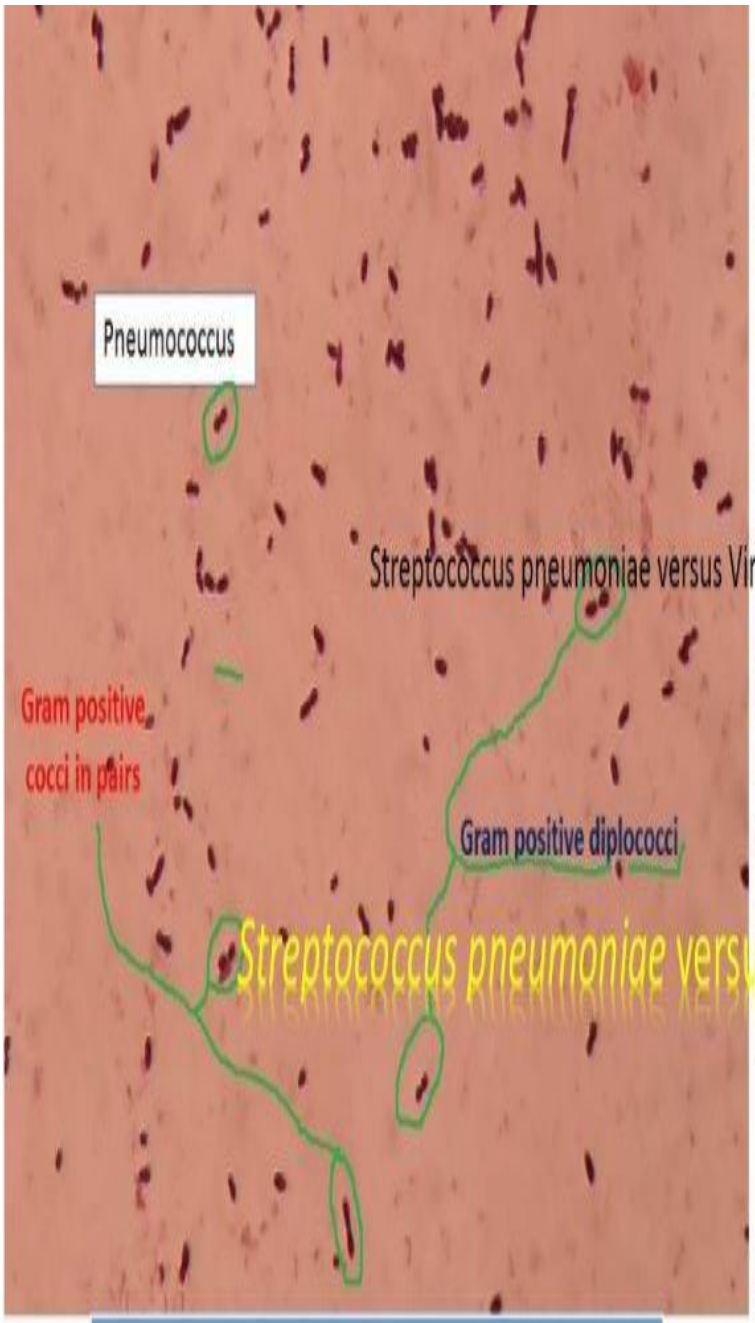
15-Isolation and identification of pneumococci and viridans group

Optochin test: The optochin test is used to differentiate *streptococcus pneumoniae* from other α-hemolytic streptococci isolation and identification of pneumococci and viridans group

16- Isolation and identification of pneumococci and viridans group

Optochin is an antibiotic that interferes with ATPase activity and ATP production in susceptible bacteria. A 6 mm disc (containing 5 µg 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).

Note: . Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes.



Streptococcus pneumoniae in Gram stain

Streptococci in Gram stain stained smear

Streptococcus pneumoniae

Characteristics

- **Gram** - Positive
- **Shape** – Diplococcus, "lancet shaped"
- **Motility** – None
- **Oxygen Requirements** - Facultative anaerobe
- **Oxidase** – Negative
- **Catalase** – Negative
- **Hemolysis** – Alpha
- **Optochin** - Sensitive
- **Capsule** - Polysaccharide
- **Bile** – Soluble
- **Virulence Factors**
  - Pneumolysin
    - Toxic to the bacteria, once the bacteria are dead, it is released into the environment
    - Kills endothelial cells, paralyzes respiratory cilia, reduces phagocytic ability, increases anti-inflammatory cytokines, causes platelet activation
  - IgA protease
  - Choline Binding Protein
    - Attachment to epithelial cells

Epidemiology and Risk Groups

- Worldwide
- Part of normal flora of skin and nose
- Patients with Sickle Cell Disease
- Patients without a spleen

Symptoms

- Pneumococcal meningitis
  - Fever, headache, stiff neck, nausea
  - Most common bacterial cause
- Otitis Media
- Pneumoniae
  - Causes a unilobular pneumonia with rust-colored sputum
  - Most common cause of bacterial pneumonia in the world
  - Shaking chills, high fever, hemoptysis, chest pain
- Acute Rhinosinusitis

Transmission

- Contaminated air droplets
- Part of the normal flora

Treatment

- ✓ Children get the protein-conjugated vaccine to activate T-cells
- ✓ Adults over 65 can get the polysaccharide vaccine to activate B-cells
- ✓ Treat with Azithromycin or cephtriaxone

Diagnosis

- Culture and biochemical tests

Viridans Streptococci

Characteristics

- **Gram** - Positive
- **Shape** – Cocci
- **Motility** – None
- **Oxygen Requirements** - Facultative anaerobe
- **Oxidase** – Negative
- **Catalase** – Negative
- **Hemolysis** – Alpha
- **Optochin** - Resistant
- **Bile** – Insoluble

Epidemiology and Risk Groups

- Worldwide
- Part of normal flora of mouth

Transmission

- Contaminated air droplets
- Part of the normal flora

Symptoms

- Dental cavities
  - *Streptococcus mutans* associated with this
- Subacute infective endocarditis
  - Grabs onto previously damaged heart valves and creates a fibrin-platelet aggregates to form a vegetation
  - *Streptococcus sanguinis* is associated with this

Diagnosis

- Culture and biochemical tests

Treatment

- ✓ Good dental hygiene
- ✓ Amoxicillin for dental procedures

Differentiation between

Streptococcus species

Tests	*S.pyogenes*	*S.pneumoniae*
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

Hemolysis

- Beta (β) hemolysis
  - Complete lysis of RBC's surrounding colony causing a clear zone or clearing of blood from the agar
- Alpha (α) hemolysis
  - Partial lysis of RBC's surrounding colony causing a greenish discoloration in media
- Non-hemolytic
  - Also known as Gamma hemolysis (δ)
  - No lysing of RBC's and no color change of the medium surrounding colony

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