



# GRAM-POSITIVE COCCI

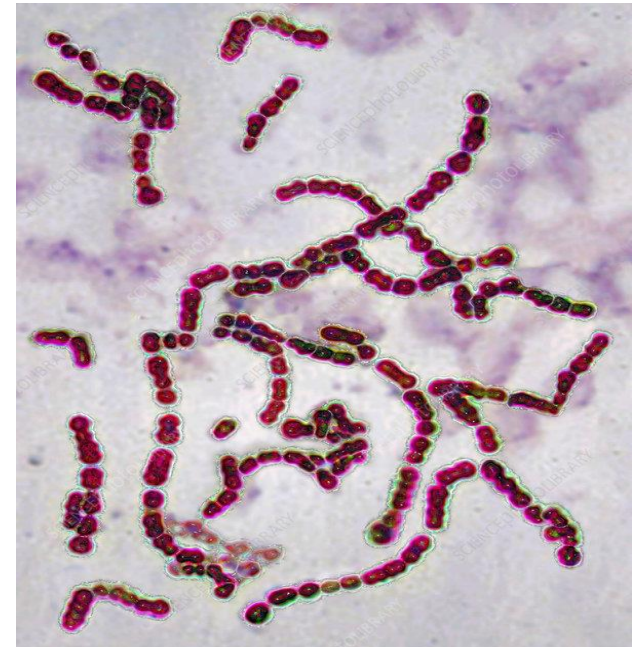
## Streptococci

Lab 5

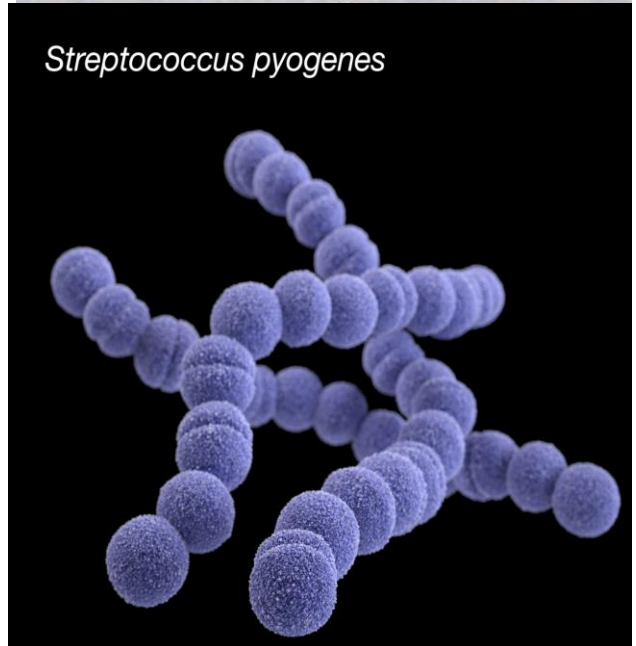
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# Characteristics:

- Streptococci are gram-positive cocci (spherical), usually arranged in chains or pairs.
- Streptococci are nonmotile and do not form spores.
- Some members form capsules composed of polysaccharide complexes or hyaluronic acid.



*Streptococcus pyogenes*



- Streptococci grow best in enriched media (i.e. fastidious) under aerobic or anaerobic conditions (facultative). They are oxidase and catalase negative.
- **Habitat:** Some are members of the normal human flora; Indigenous microbial flora in respiratory tract of animals and humans. Certain species are also found in the gastrointestinal and urogenital tracts of humans.

# Classification:

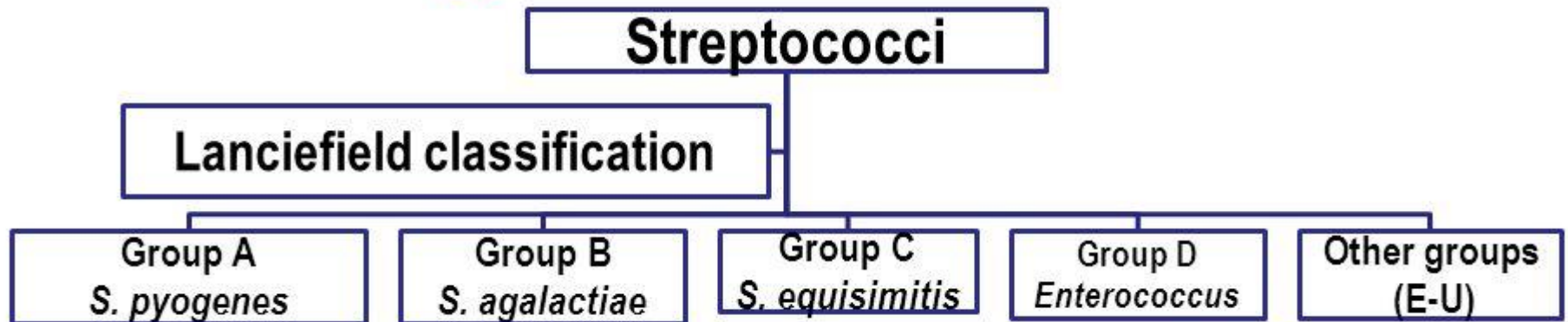
## Streptococci can be classified according to:

- 1. Serology (Lancefield Classification):** Streptococci are classified into many groups based on the C- carbohydrate antigen of the cell wall. They are called groupable, one or more species per group, the ones most frequently isolated from humans are A, B, C, D, F, and G. Of these, groups A, B, and D are the most frequent causes of serious disease.
  - **Groupable streptococci:**
  - Group A: *S. pyogenes*
  - Group B: *S. agalactiae*
  - Group C: *S. equisimitis*
  - Group D: *Enterococcus* spp

**2. Biochemical reactions:** used for species that cannot be classified into the Lancefield classification, they are called non groupable streptococci.

- **Non-groupable streptococci:**
- *S. pneumoniae* (pneumonia)
- Viridans streptococci e.g. *S. mutans*

# Serology: Lancefield Classification



- Streptococci classified into many groups from A-K & H-U
- One or more species per group
- Classification based on C- carbohydrate antigen of cell wall
  - **Groupable streptococci**
    - A, B and D (more frequent)
    - C, G and F (Less frequent)
  - **Non-groupable streptococci**
    - *S. pneumoniae* (pneumonia)
    - viridans streptococci
      - e.g. *S. mutans*
      - Causing dental carries

### 3. Hemolysis on Blood Agar

- **Alpha  $\alpha$ -hemolysis:** Partial hemolysis to RBCs, with green discoloration around the colonies. e.g. non-groupable streptococci (*S. pneumoniae* & Viridans streptococci)
- **Beta  $\beta$  –hemolysis:** Complete hemolysis to RBCs, with a clear zone of hemolysis around the colonies. e.g. Group A & B (*S. pyogenes* & *S. agalactiae*)
- **Gamma–hemolysis:** It does not affect RBCs (No lysis), e.g. Group D (*Enterococcus* spp)



# Disease

- **Beta-hemolytic group A streptococci** cause 4 types of disease by local invasion and/or exotoxin release. These include Streptococcal pharyngitis, Streptococcal skin infections, Scarlet fever, and Streptococcal toxic shock syndrome. Group A can also cause 2 delayed antibody-mediated diseases: Rheumatic fever and Glomerulonephritis.
- **Beta-hemolytic group B** these organisms cause neonatal (< 3 months of age) meningitis, pneumonia, and sepsis.
- **Group D:** causes Urinary tract infections, septicemia, and endocarditis.
- **The viridans streptococci** *S. mutans* cause dental caries.
- ***Streptococcus pneumoniae*** causes Pneumonia, meningitis, and otitis media.



- It is extremely important to know how to differentiate staphylococci from streptococci. You can do 3 things to differentiate them stain, catalase test, and culture.
1. **Gram stain:** *Staphylococci* lie in grape-like clusters as seen on Gram stain. While *streptococci* lie in the chain.
  2. **Catalase test:** All staphylococci have the enzyme catalase (streptococci do not!).
  3. **Culture:** *Staphylococcus aureus* and certain streptococci are beta-hemolytic (completely hemolyze red blood cells on an agar plate), but *Staphylococcus aureus* can be differentiated from the other beta-hemolytic cocci by their elaboration of a golden pigment on blood agar.

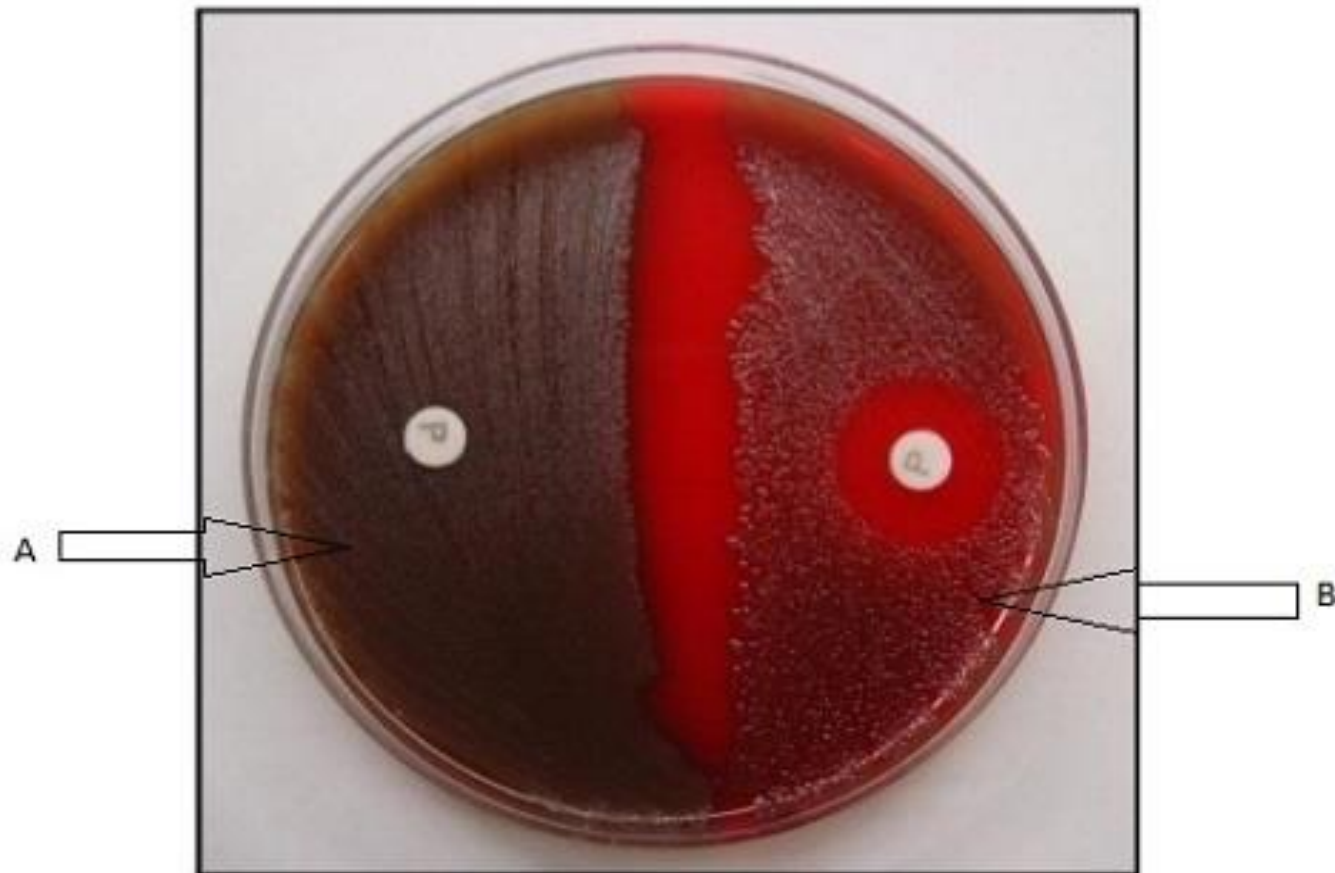
# Diagnosis of Streptococci

- **Gram's Stain:** Gram's +ve cocci arranged in: chains or pairs.
- **Catalase Test:** it is a differential test to separate *Staphylococci* which are catalase +ve from *Streptococci* which are catalase –ve.
- **Culture (Colony morphology on blood agar)**
  - Small to medium, smooth or mucoid, grayish-white colony on blood agar.
  - *Streptococcus* produces three types of hemolysis on blood agar:
    - **Alpha  $\alpha$ -hemolysis:**
    - **Beta  $\beta$  –hemolysis:**
    - **Gamma  $\gamma$  –hemolysis:**

## Differentiation between $\alpha$ -hemolytic streptococci

The following definitive tests used to differentiate between *S. pneumoniae* & *Viridans streptococci*

<i>Streptococci</i>	Hemolysis	Optochin sensitivity	Bile solubility	Inulin Fermentation
<i>S. pneumoniae</i>	$\alpha$	Sensitive ( $\geq 14$ mm)	Soluble	Not ferment
<i>Viridans strep</i>	$\alpha$	Resistant ( $\leq 13$ mm)	Insoluble	Ferment



**Fig. Optochin Test using optochin disk**  
**A. *S. viridians*, Resistance to optochin**  
**B. *S. pneumoniae*, Susceptible to optochin**

# Bile Solubility Test:

## Principle:

*S.pneumoniae* produces a self-lysing enzyme to depress the growth of old colonies. The presence of bile salt accelerate this process.

## Procedure:

- 1) Add 1 ml 10% bile salt solution to 10 ml broth culture of the test organism
- 2) Incubate at 37°C for 15 min
- 3) Observe for the visible clearing of the turbid culture

## Results

Visible clearance



*S.Pneumoniae*



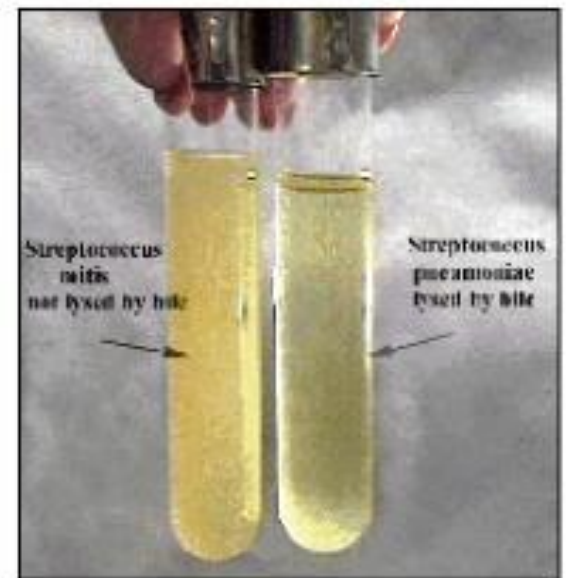
Remain turbid



*Viridans Streptococci*

# Bile Solubility test

- Results:
  - 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



# Differentiation between $\beta$ -hemolytic streptococci

- The following tests can be used to differentiate between  $\beta$ -hemolytic streptococci
- Bacitracin susceptibility Test: Specific for *S. pyogenes* (Group A)
- CAMP test: Specific for *S. agalactiae* (Group B)

<i>Streptococci</i>	Hemolysis	Bacitracin sensitivity	CAMP test
<i>S. pyogenes</i>	$\beta$	Susceptible	Negative
<i>S. agalactiae</i>	$\beta$	Resistant	Positive

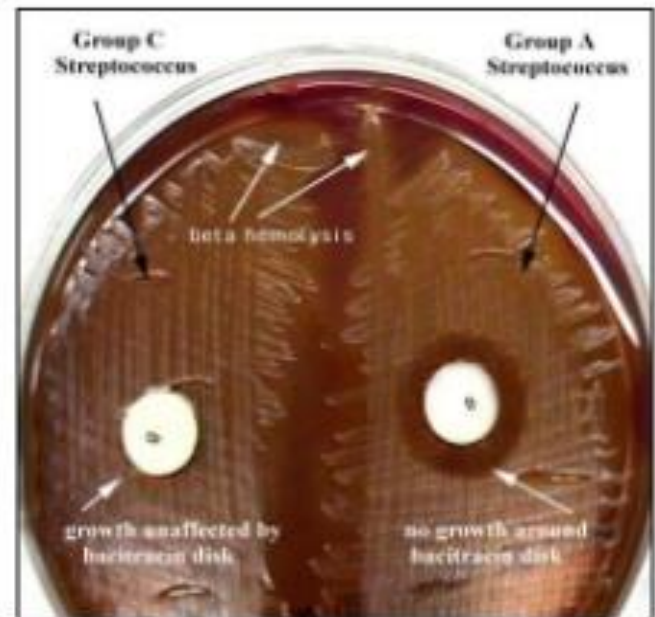
# Bacitracin sensitivity

- **Principle:**

- Bacitracin test is used for presumptive identification of group A
- To distinguish between *S. pyogenes* (susceptible to B) & non group A such as *S. agalactiae* (Resistant to B)
- Bacitracin will inhibit the growth of gp A *Strep. pyogenes* giving zone of inhibition around the disk

- **Procedure:**

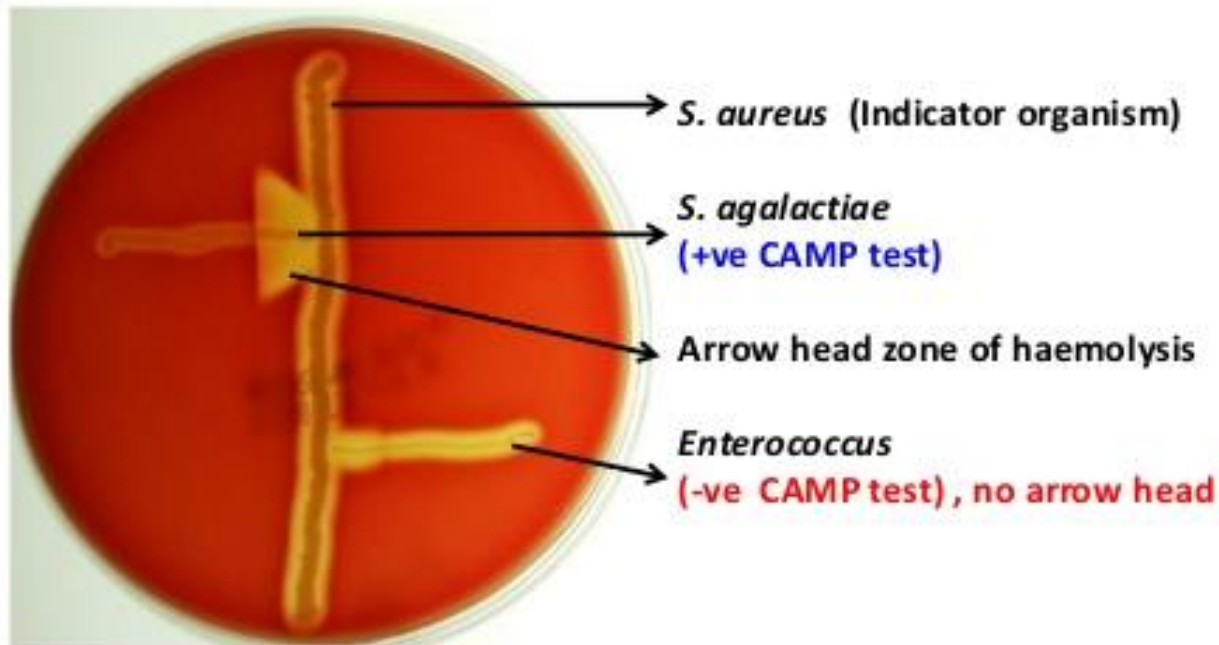
- Inoculate BAP with heavy suspension of tested organism
- Bacitracin disk (0.04 U) is applied to inoculated BAP
- After incubation, any zone of inhibition around the disk is considered as susceptible





# BIOCHEMICAL TESTS

4. **Christie Atkins Munch-Petersen (CAMP) test:** is used for the presumptive identification of Group B beta-hemolytic streptococci, (*Streptococcus agalactiae*).



# Gram Positive Cocci Flow Chart

