

# MICROBIOLOGY LAB 6

Gram- positive cocci:

**Streptococci**

lect.

Shaima'a Al-Salihy

# Learning objectives:

After this lab. You must be able to:

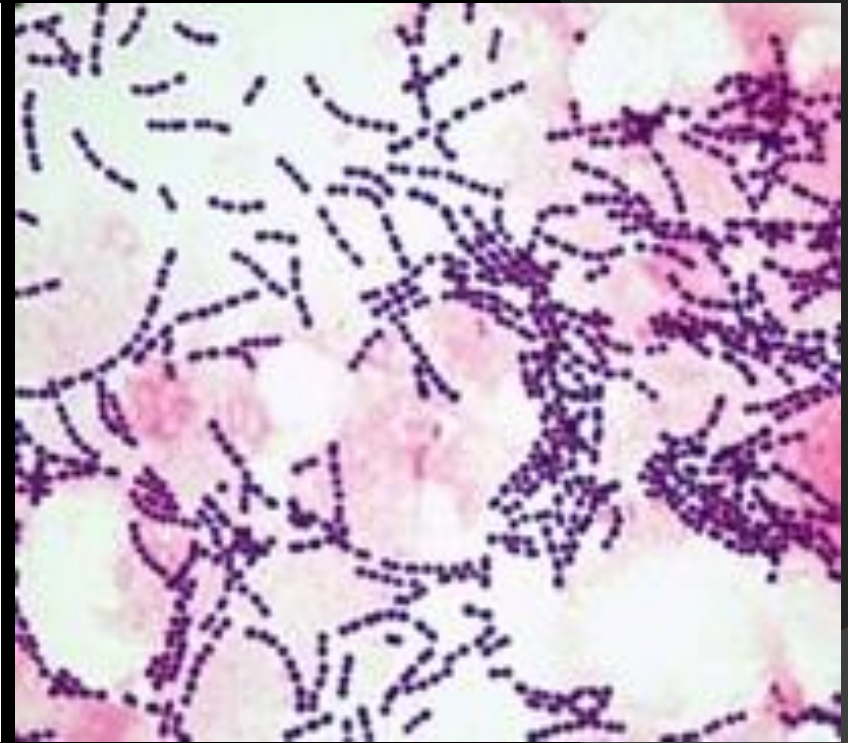
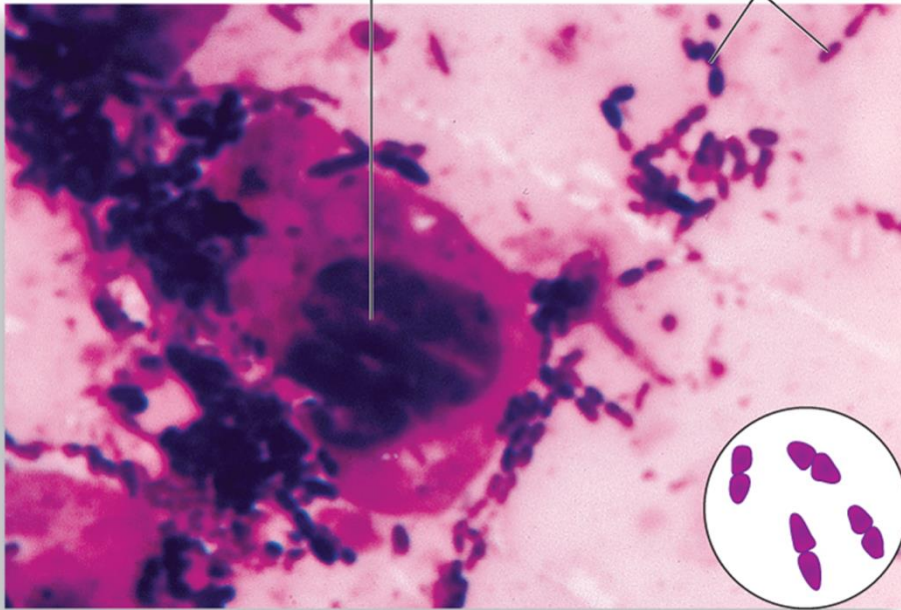
- ⊙ Describe streptococci under microscope.
- ⊙ Classify streptococcus spp. according to hemolysis pattern>
- ⊙ Classify streptococcus spp. according to Lancefield grouping.
- ⊙ List infections caused by each of streptococcal spp.
- ⊙ Differentiate each streptococcus spp. From each other.
- ⊙ Discuss principles of differentiation tests of each streptococcal spp.
- ⊙ Predict streptococcal causative agents causing clinical cases.

# STREPTOCOCCI

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

Phagocyte

Pneumococci



© A.M. Siegelman/Visuals Unlimited

## General characteristics:

1. **Gram-positive cocci, arranged in chains or pairs.**
2. Non motile, non spore forming.
3. **Some** strains are **capsulated**, which are important in pathogenicity.
4. **Catalase-negative.**
5. Majority are facultative anaerobes, few are obligate anaerobes.
6. They are fastidious microorganisms grow on enriched media such as blood agar, have small, pin head, opaque, circular colonies.
7. Sensitive to drying, heat, and disinfectant.

# Classification:

Species of this genus is classified according to the following:

## I. Hemolysis:

- **$\beta$ -hemolysis:** complete destruction of RBCs.  
e.g. *S. pyogenes*
- **$\alpha$ -hemolysis:** partial destruction of RBCs e.g.  
*S. mutans* , *S. pneumoniae*.
- **$\gamma$ -hemolysis:** non-hemolysis.

# Hemolysis on Blood agar

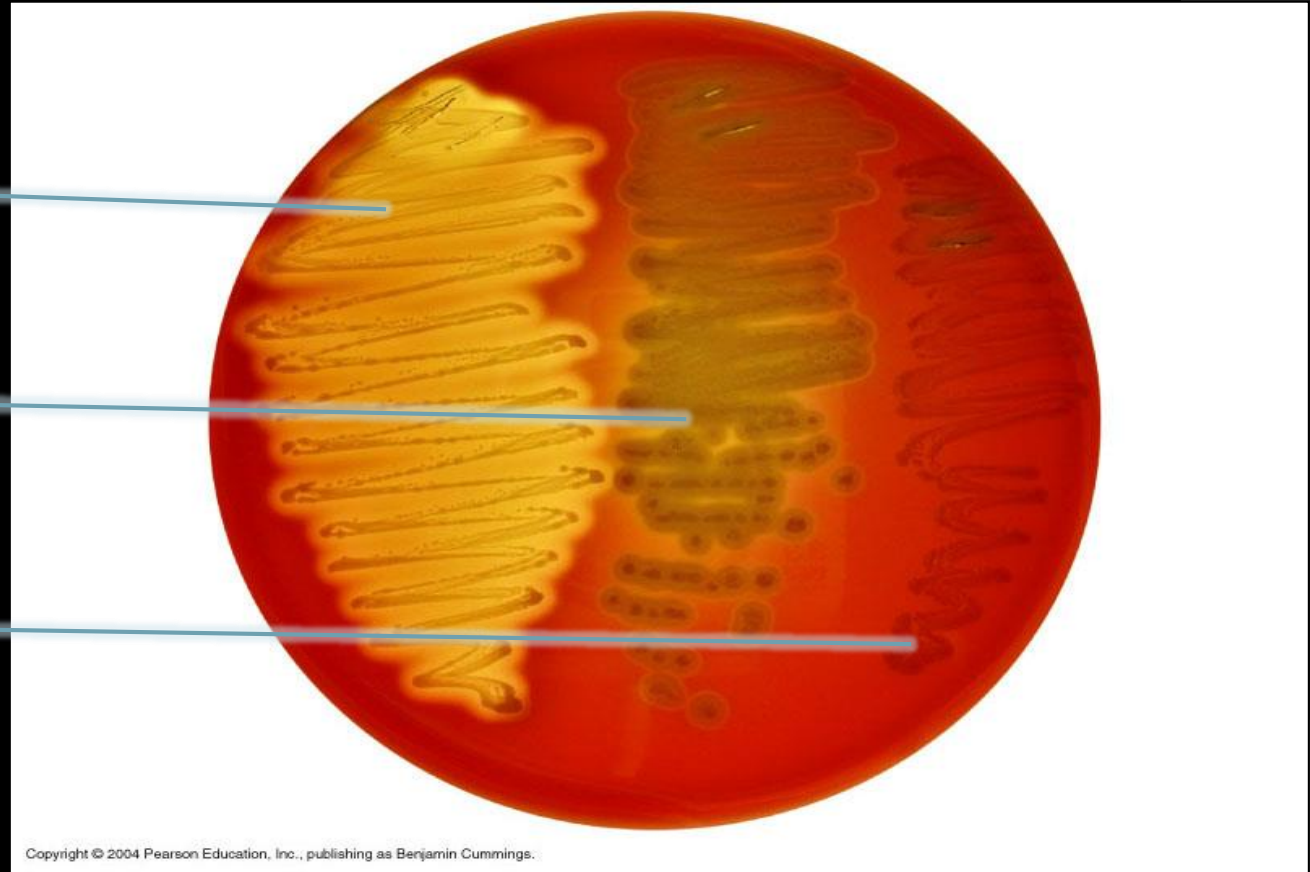
$\beta$ -hemolysis



$\alpha$ -hemolysis



$\gamma$ -hemolysis



Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

# Hemolysis patterns on blood agar

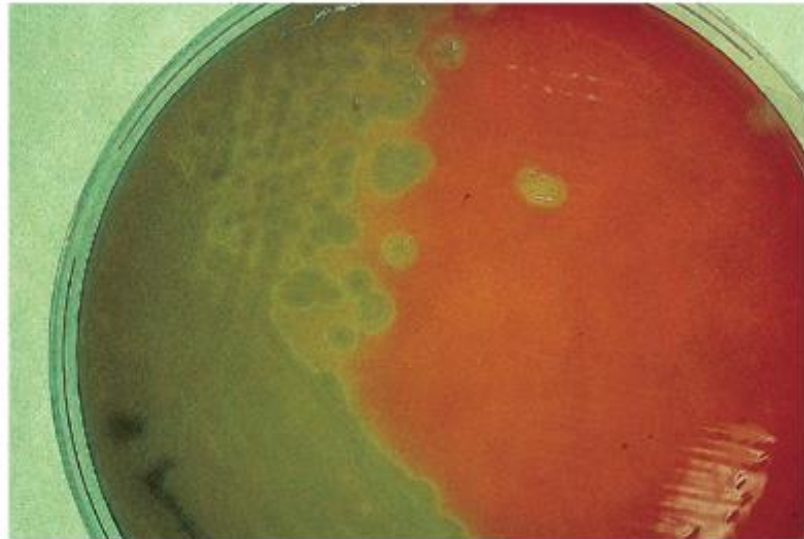
Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

*Streptococcus pyogenes*  
with zones of  $\beta$ -hemolysis



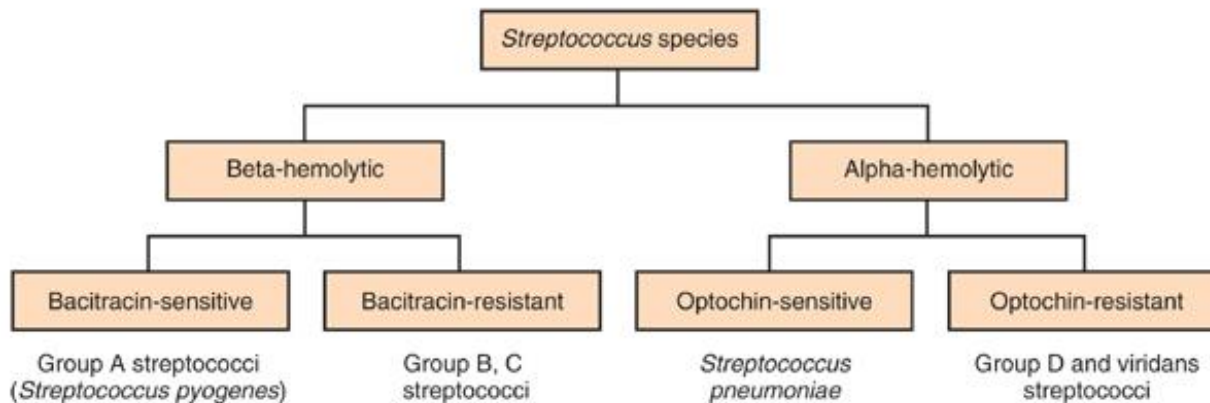
(a)

© Kathy Park Talaro



(b)

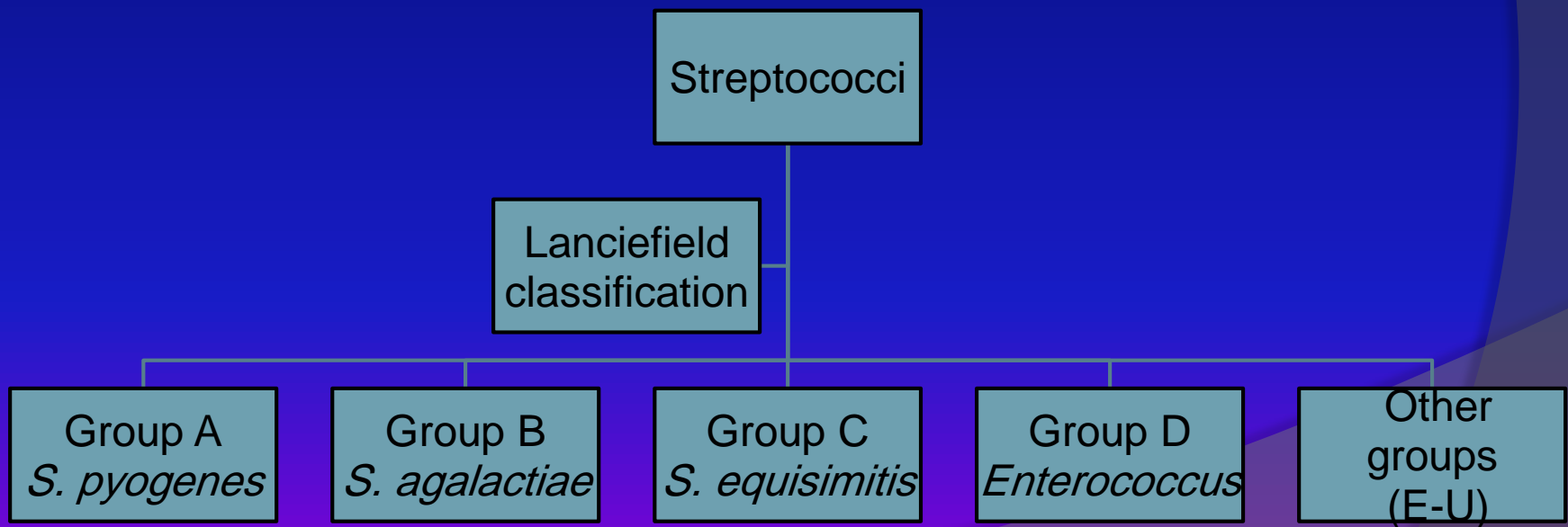
© L.M. Pope and D.R. Grote/Biological Photo Service



(c)

## II. Serology (Lancefield grouping):

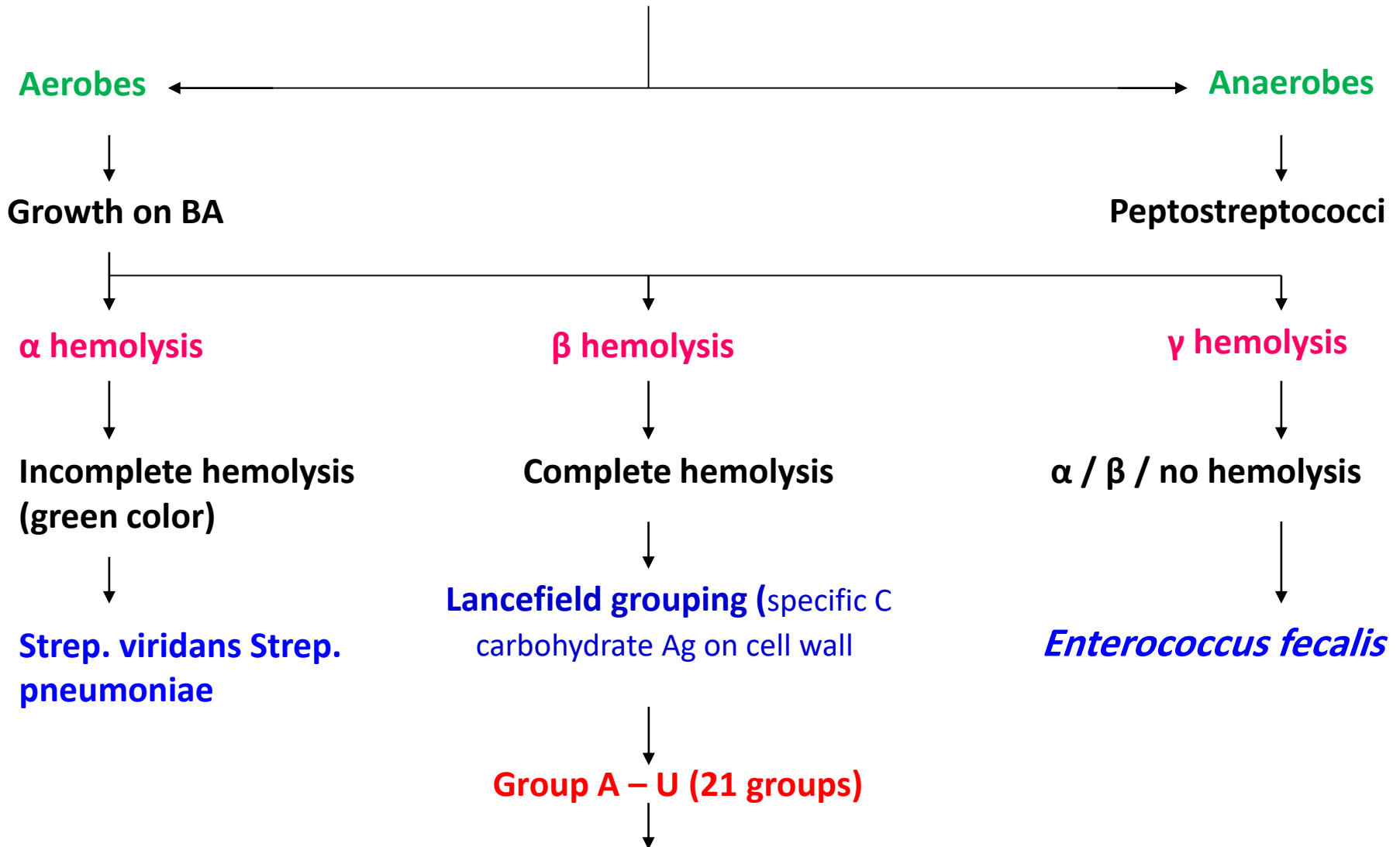
There are differences in the polysaccharide antigens (group-specific carbohydrate) of the cell wall. Depending on these specific polysaccharide antigens, streptococci are named as groups from A-H & K-U.





# Classification

Based on O<sub>2</sub>



Griffith typing of Group A on MTR proteins into > 100 types

# HUMAN STREPTOCOCCAL PATHOGENS

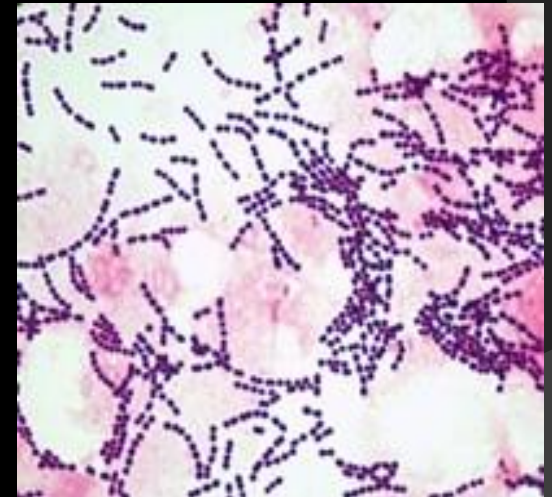
- *S. pyogenes*
- *S. agalactiae*
- Viridans streptococci
- *S. pneumoniae*
- *Enterococcus faecalis*

# Human streptococcal pathogens:

➤  $\beta$ -hemolytic

I. Group A streptococci - *S. pyogenes*:

- Most serious streptococcal pathogen
- Inhabits throat, nasopharynx and occasionally skin



# Pathogenicity of *S. pyogenes*:

- a) **Pyogenic infections:** sore throat, streptococcal pyoderma (impetigo), erysipelas, cellulitis, necrotizing fasciitis (flesh eating), septicemia,
- b) **Toxin-mediated:** puerperal fever, scarlet fever, and streptococcal toxic shock syndrome.
- c) **Post streptococcal (immunogenic) infection:** a hypersensitivity response - rheumatic fever (after sore throat), acute glomerulonephritis (after skin infection).

# Pharyngitis and tonsillitis

- *S.pyogenes* is leading cause of uncomplicated bacterial pharyngitis and tonsillitis
- Common in winter and early spring in children over age 3
- Typical symptoms:
  - Pus in throat
  - Reddened and inflamed tonsils and uvula
  - Tiny, reddish-brown spots at back of throat
  - Swollen lymph nodes and tongue



# Streptococcal skin infections

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



(a)



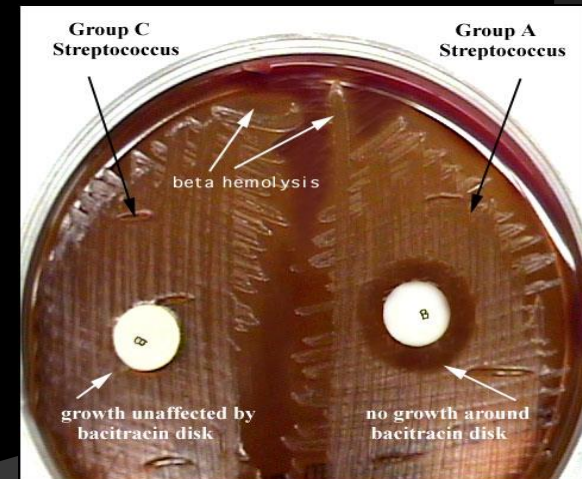
(b)

## Erysipelas



# Lab diagnosis – Strep. pyogenes

- ◎ **Specimens:** throat swab, pus, blood
- ◎ **Microscopy :** Gram stain - **GPC in chains**
- ◎ **Culture:** BA - **beta hemolytic colonies**
- ◎ **Identification tests -**
  - Catalase Negative
  - **Bacitracin sensitive**
  - ASO titer / Ab. titer: normal < 200 < positive result.





# Anti- Streptolysin O Test (ASOT):

## ◎ Principle:

- ASOT this test is serological test used in post streptococcal infection complications depending on the presence of antistreptolysin-O antibody in the blood of patient's previously infected with *S. pyogenes*.
- Ab. titer: normal  $< 200$  < positive result.

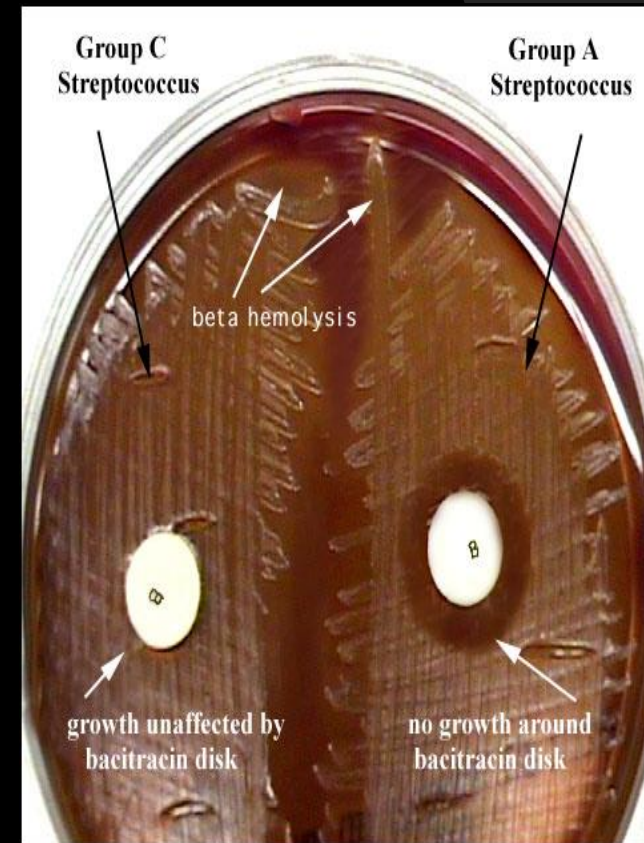
# Bacitracin sensitivity

## 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 group A *Strep. pyogenes* giving zone of inhibition around the disk

## Procedure:

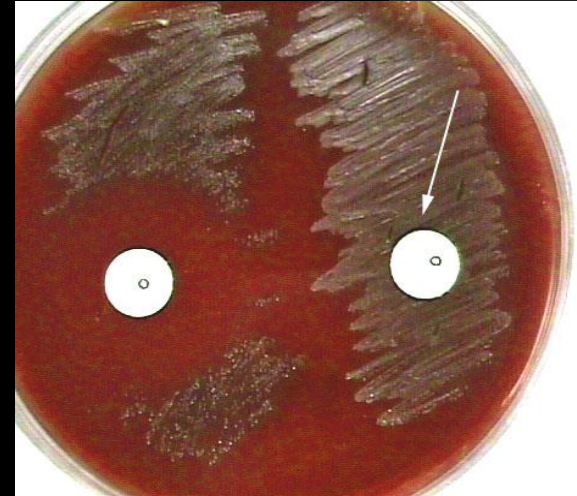
- Inoculate BAP with heavy suspension of tested organism
- Bacitracin disk (0.04 U) is applied to inoculated BAP
- After incubation, any zone of inhibition around the disk is considered as susceptible



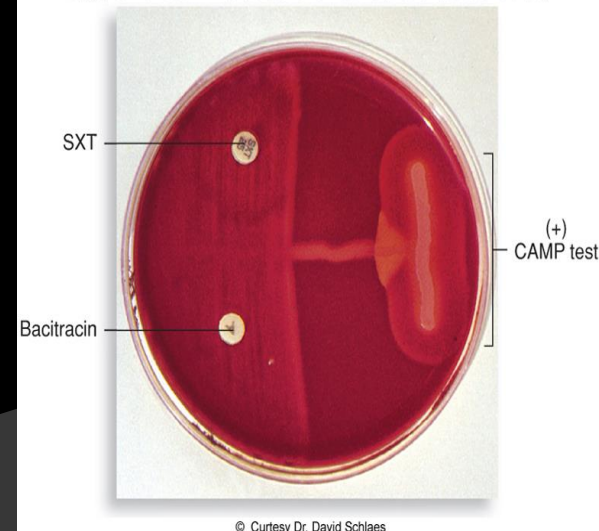
## ➤ $\beta$ -hemolytic :

### II. Group B streptococci- *S. agalactiae*:

- Normal flora of female vaginal tract and cause **neonatal meningitis**.
- Bacitracin resistant
- CAMP test +ve** (Christie, Atkins, Munch-Peterson)
- hipurate hydrolysis test +ve** (split sodium hippurate and give deep purple color indicate +ve response in this test)



Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



© Curtesy Dr. David Schlaes

# CAMP test

## ◎ Principle:

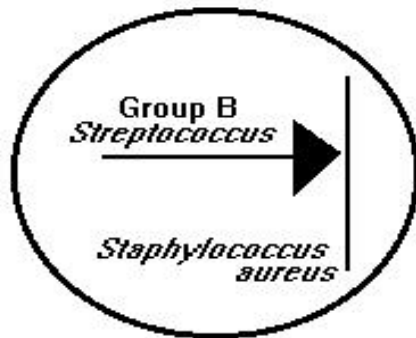
- Group B streptococci produce diffusible extracellular protein (CAMP factor)
- CAMP act synergistically with staph.  $\beta$ -lysin to cause lysis of RBCs

## ◎ Procedure:

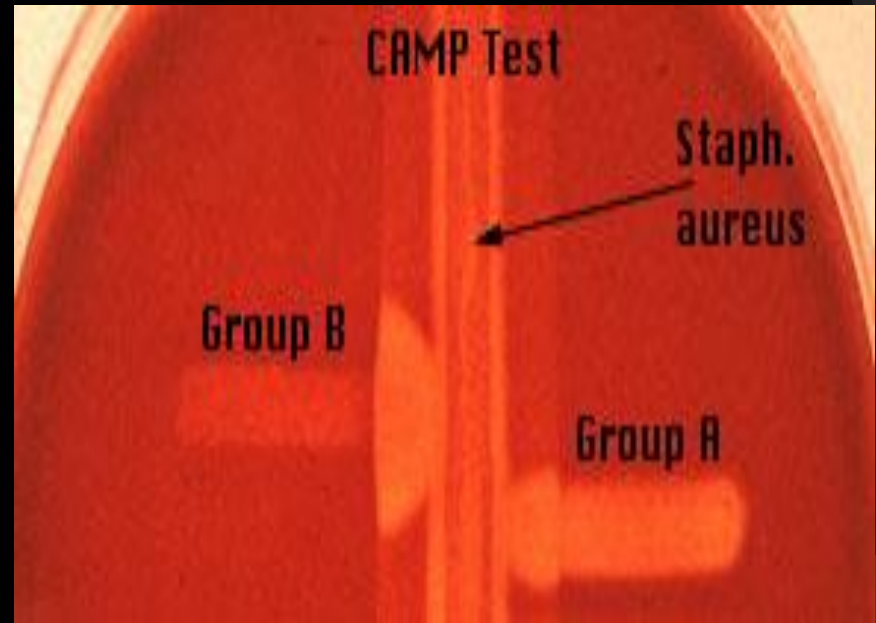
- Single streak of unknown *Streptococcus* to be tested and a *Staph. aureus* are made perpendicular to each other
- 3-5 mm distance was left between two streaks
- After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis
- *S. agalactiae* is CAMP test positive while non group B streptococci are negative

# CAMP test

Positive Control:  
*S. agalactiae*



Negative Control:  
Group A or Group D Strep.



## • Group D streptococci:

### *Enterococcus faecalis* and *Enterococcus faecium*:

- ⊙ Streptococcal-like with group D antigens were at first classified in the genus *Streptococcus* but studies has revealed that they differ in many biological aspects.
- ⊙ They are **normal flora of GIT**.
- ⊙ **Bile- esculin +ve**
- ⊙ Causes UTI, wound infection, bed sore, endocarditis.
- ⊙ One of the most frequent cause of nosocomial infections particularly in ICU.
- ⊙ Very resistant to antibiotics ( many isolates are resistant to cephalosporines even vancomycin).

# Lab diagnosis - Enterococcus



**Specimens: urine, pus, blood**

**Microscopy: Gram stain - GPC in pairs or short chains**

**Culture: BA - alpha / beta / no hemolysis**

**Identification tests - Catalase Negative**

**- Bile esculin positive**

**- Growth in 6.5% NaCl**

**- Penicillin resistance**

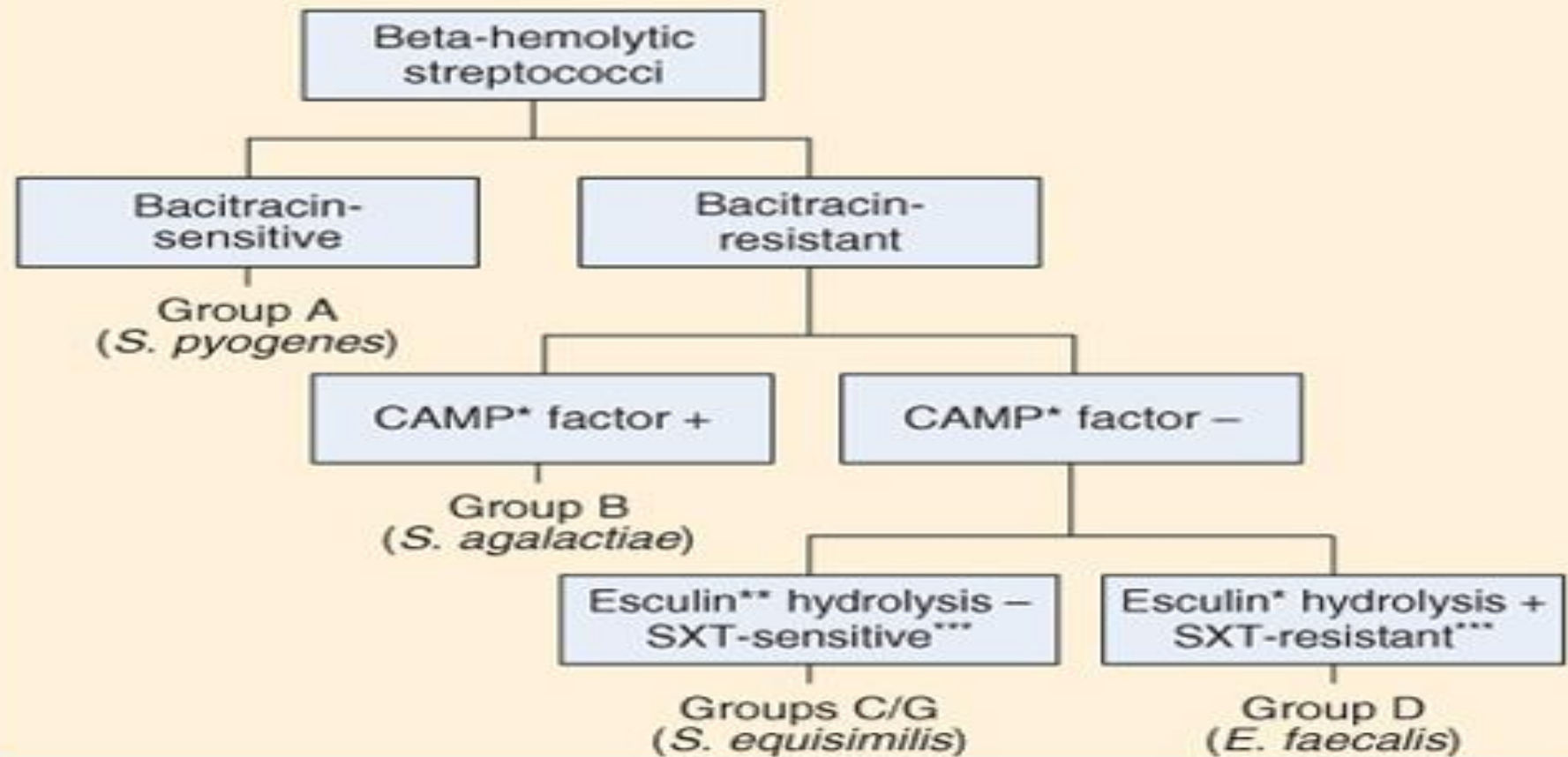


# Differentiation between $\beta$ -hemolytic streptococci

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



**TABLE 18.4** Scheme for Differentiating Beta-Hemolytic Streptococci



\*Name is derived from the first letters of the names of its discoverers. CAMP is a diffusible substance of group B, which lyses sheep red blood cells in the presence of staphylococcal hemolysin.

\*\*A sugar that can be split into glucose and esculetin. Group D streptococci can accomplish this in the presence of 40% bile.

\*\*\*Sulfa and trimethoprim. The test is performed (like bacitracin) with discs containing this combination drug.

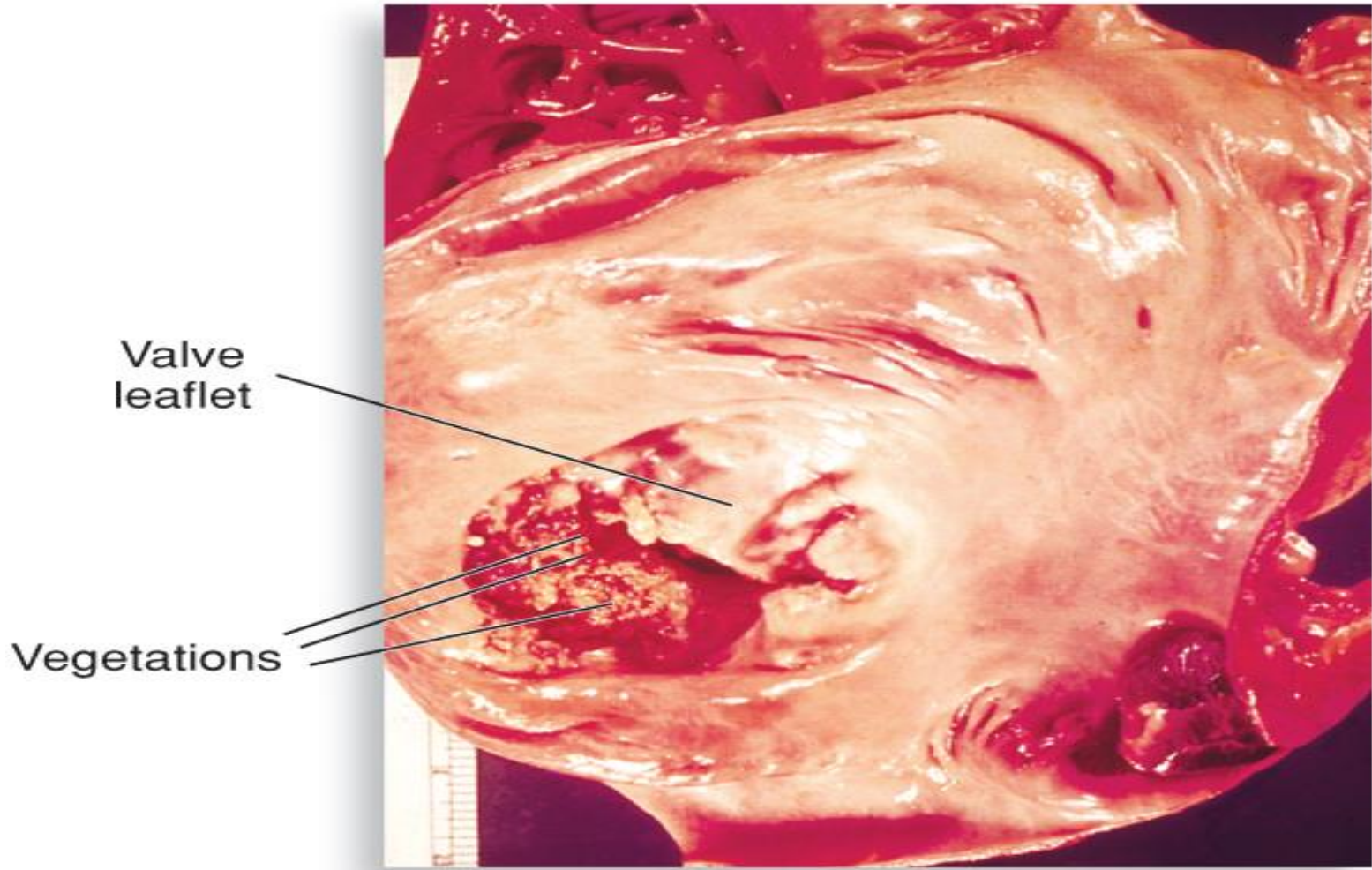
# Alpha hemolytic streptococci

## Streptococcus viridans

- ⦿ They contain many species.
- ⦿ Normally present on teeth, throat, colon & female genital tract
- ⦿ They adapted to non-invasive mode of life and enforced to enter human body.
- ❖ **Pathogenicity –**
  - ⦿ The most serious infection - **Subacute bacterial endocarditis**- Blood-borne bacteria settle and grow on heart lining or valves
  - ⦿ Colonization of heart by forming biofilms.
  - ⦿ Dental caries

# Two effects of streptococcal colonization

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



© Iclw/ Carroll H. Weiss/Camera MD Studio

# Alpha hemolytic streptococci

## *Streptococcus pneumoniae* (Pneumococcus)

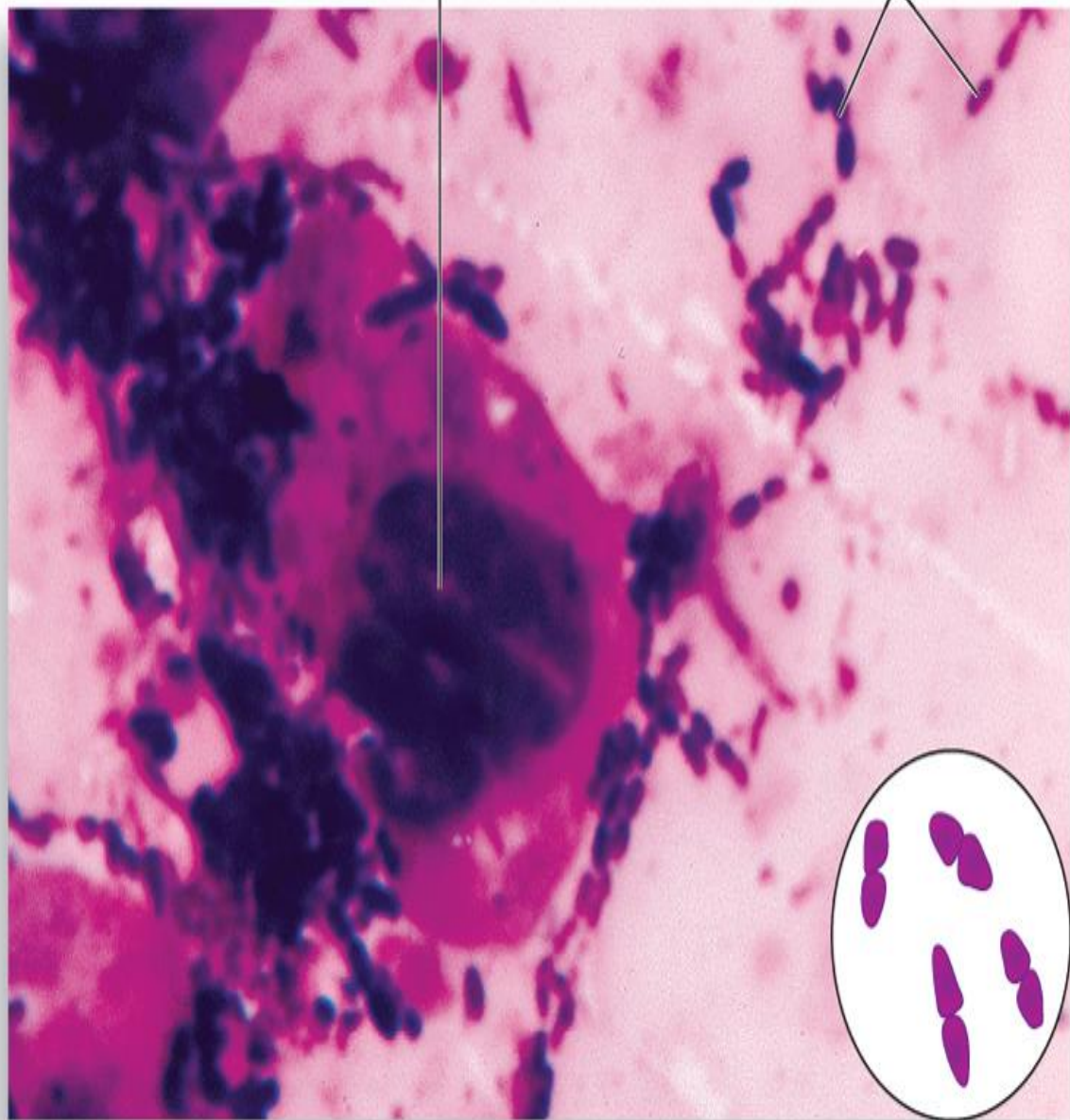
---

### ❖ General features:

- Causes 60-70% of all bacterial pneumonias
- Small, lancet-shaped cells arranged in pairs and short chains
- Culture requires blood or chocolate agar, Growth improved by 5-10% CO<sub>2</sub>
- 5-50% of all people carry it as normal flora in the nasopharynx; infections are usually endogenous
- Virulence factor – capsule

Phagocyte

Pneumococci



## ❖ Pathogenicity

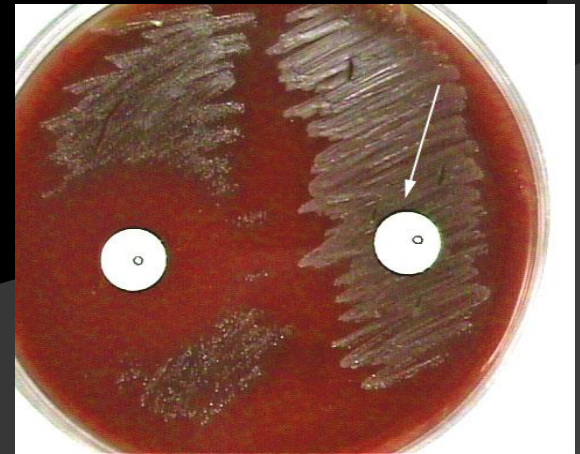
- Otitis media, sinusitis – commonest
- Pneumonia (Pneumonia occurs when cells are aspirated into the lungs of susceptible individuals, Pneumococci multiply and induce an overwhelming inflammatory response)

Gains access to middle ear by way of eustachian tube.

- Meningitis
- Other suppurative lesions - Pericarditis, conjunctivitis, arthritis, peritonitis

## Lab. diagnosis:

- ◎ Gram stain: GPC arranged in pairs. (lancet-shaped diplococci) presumptive identification to differentiate from pneumonia caused by viruses.
- ◎ Rapid diagnostic test: **Quellung test or capsular swelling reaction** for *S. pneumoniae* :is a **Rapid diagnostic test** on sputum or culture. **By mixing *S. pneumoniae* with specific antipolysaccharide (capsule component) on microscopic slide. The capsule swells due Ag-Ab reaction.**
- ◎ Culture: BA-  $\alpha$ -hemolytic
- ◎ Biochemical test:
  - optochin sensitivity: sensitive
  - bile solubility: soluble (+)
  - inulin fermentation (+)



# Bile Solubility test

## ◎ Principle:

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

## ◎ Procedure:

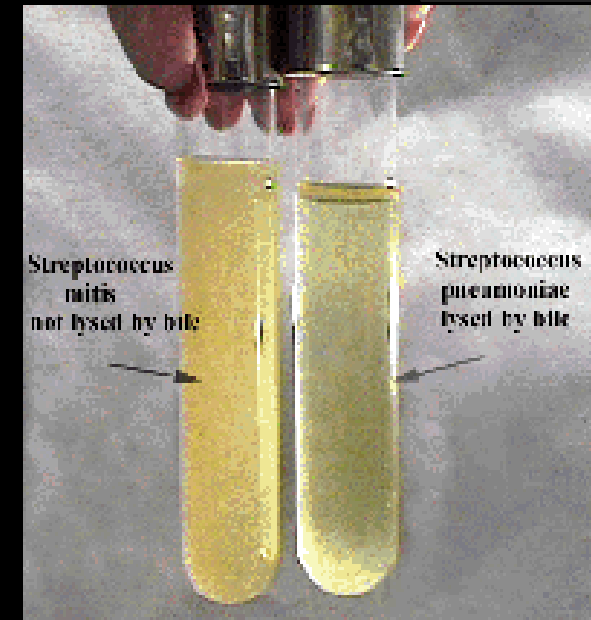
- Add ten parts (10 ml) of the broth culture of the organism to be tested to one part (1 ml) of 2% Na deoxycholate (bile) into the test tube
- Negative control is made by adding saline instead of bile to the culture
- Incubate at 37°C for 15 min
- Record the result after 15 min



# 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



# Optochin Susceptibility Test

## ◎ Principle:

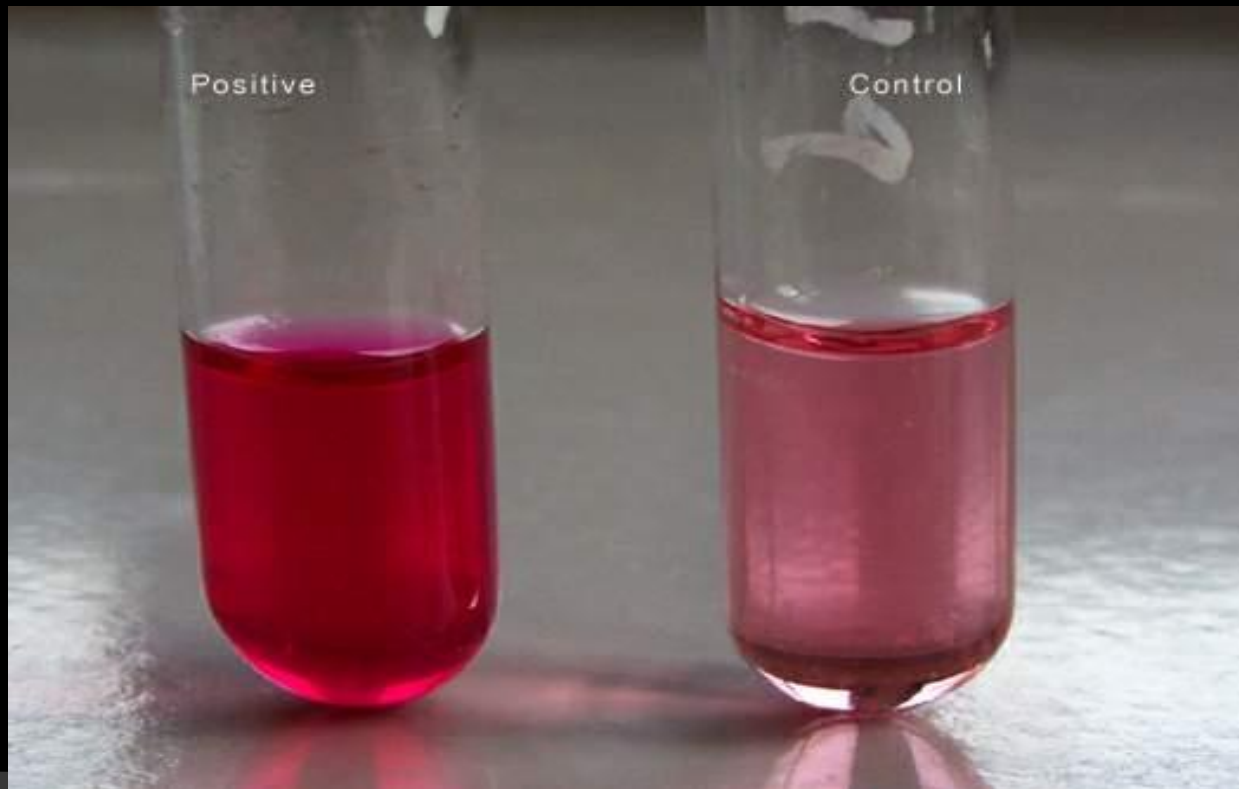
- Optochin (OP) test is **presumptive test** that is used to identify *S. pneumoniae*
- *S. pneumoniae* is inhibited by **Optochin reagent** (<5 µg/ml) giving a inhibition zone ≥14 mm in diameter.

## ◎ Procedure:

- BAP inoculated with organism to be tested
  - OP disk is placed on the center of inoculated BAP
  - After incubation at 37°C for 18 hrs, accurately measure the diameter of the inhibition zone by the ruler
  - ≥14 mm zone of inhibition around the disk is considered as positive and ≤13 mm is considered negative
- ◎ *S. pneumoniae* is positive (S) while *S. viridans* is negative (R)

# Inulin fermentation Test

This test is useful to differentiate **Pneumococci** from other **streptococci** as the latter do not ferment Inulin sugar while Pneumococci do ferment it.



# Differentiation between $\alpha$ -hemolytic streptococci

- ◎ The following definitive tests used to differentiate *S. pneumoniae* from viridans streptococci
  - Optochin Test (sensitive)
  - Bile Solubility Test (+)
  - Inulin Fermentation (+)

## Differences between Viridans Gp & Pneumococci

<b>Point</b>	<b>Pneumococci</b>	<b>Viridans Gp</b>
<i>Morphology</i>	<b>Capsulated, lanceolate, diplococci</b>	<b>Oval or rounded in chains</b>
<i>Quellung test</i>	<b>+</b>	<b>-</b>
<i>Colonies</i>	<b>Dome shaped→ Draughtsman</b>	<b>Dome shaped</b>
<i>Growth in liquid</i>	<b>Uniform turbidity</b>	<b>Granular turbidity with powdery deposits</b>
<i>Bile solubility</i>	<b>+</b>	<b>-</b>
<i>Inulin fermentation</i>	<b>+</b>	<b>-</b>
<i>Optochin sensitivity</i>	<b>+</b>	<b>-</b>
<i>Intraperitoneal inoculation in mice</i>	<b>Fatal Infection</b>	<b>Non-pathogenic</b>

**GRAM POSITIVE COCCI**

Note: *Strep. viridans* are alpha hemolytic and negative for all the tests below

**Catalase**



***Staphylococcus* (Clusters)**

**Coagulase**



***S. aureus***  
β hemolytic  
mannitol  
yellow










***S. epidermidis***  
nonhemolytic (usually)  
mannitol  
white



***Streptococcus* (pairs & chains)**

**Hemolysis**

- (1) BETA: Bacitracin  → *S. pyogenes* (group A)  
CAMP/Hippurate  → *S. agalactiae* (group B)
- (2) ALPHA: Optochin/Bile Solubility  → *S. pneumoniae*
- (3) GAMMA: Bile Esculin  6.5% NaCl  → Group D<sup>+</sup>  
*Enterococcus*  
Bile Esculin  6.5% NaCl  → Group D<sup>+</sup>  
*Non-Enterococcus*

(\*can also be Beta or Alpha hemolytic)



**THANK YOU  
FOR  
LISTENING**