A light micrograph showing numerous purple-stained Streptococcus bacteria. The bacteria are arranged in various patterns, including long chains, short chains, and small clusters. Some individual bacteria are also visible.

STREPTOCOCCI

STREPTOCOCCI

General characteristics

1- They are Gram positive cocci arranged in pairs or chains of variable length.

2- They are non-motile, non-sporulating, gram- positive facultative anaerobes.

**3- Grow well on ordinary solid media enriched with blood, serum or glucose (fastidious).
Very delicate can die even after 24 hours (unlike Staph.)**

4- They are capnophilic bacteria in which growth is enhanced with 10% carbon dioxide.

5- They are catalase-negative a feature that differentiated from Staphylococci.

**6- They are widely distributed in nature and are found in upper respiratory tract,
gastrointestinal tract and genitourinary tract as normal microbial flora, while others are
potential pathogens to human.**

Classification of Streptococci

There are three methods

- (1) Hemolysis
- (2) Serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens.
- (3) Based on O₂ requirement

Classification based on hemolysis

β-hemolytic Streptococci: Complete hemolysis of RBCs and colonies surrounded by clear zone of hemolysis. Example *Strep.pyogenes*

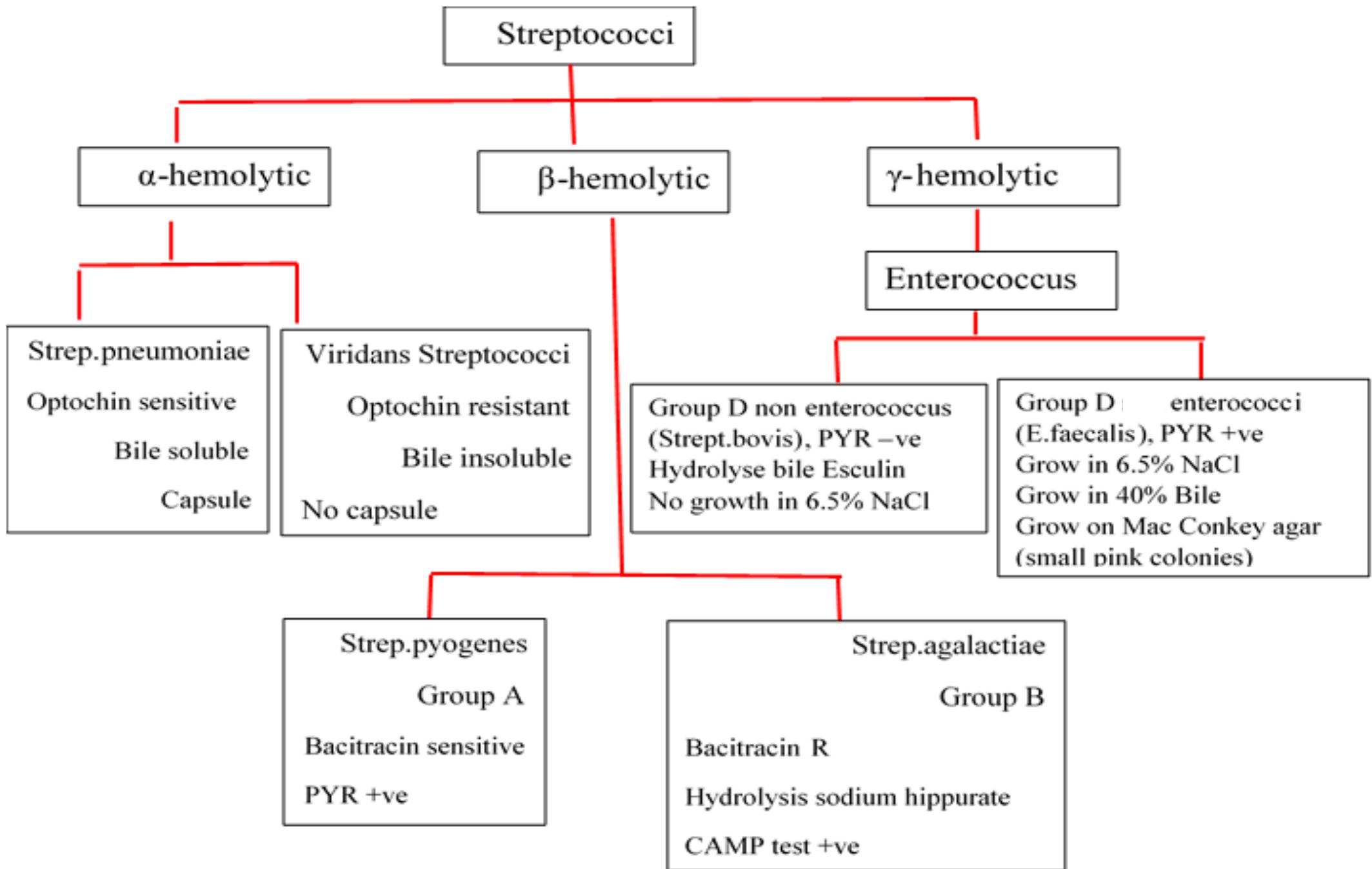
α-hemolytic Streptococci: In complete or partial hemolysis of RBCs and colonies surrounded by a narrow zone of hemolysis or greenish discoloration especially on chocolate agar (reduction of free Hb).

Example *Strep.viridans* (viridans=green)

Non-hemolytic or γ-hemolytic Streptococci: Neither beta nor alpha hemolysis for example *Strep.faecalis* as shown in figure 1.



Fig.1. Types of hemolysis



Serological (Lancefield) grouping

Beta hemolytic Streptococci are divisible into 20 serogroups or Lancefield groups: A, B, C, D, E, F, G, H, K, L, M, N, O, P, Q, R, S, T, U, V, on the basis of group specific carbohydrate (C-substance) in their cell wall. Most hemolytic Streptococci that cause infection in human are belong to the Group A Streptococci (*Strep.pyogenes*).

Not all serogroups infect human. Not all serogroups are beta hemolytic. Not all Streptococci are groupable. Among 20 serogroups, only serogrouping is available for A,B,C,F and G.

Classification based on oxygen requirement

A- Aerobic and facultative anaerobic Streptococci

B- Anaerobic Streptococci called *Peptostreptococcus* found as normal flora in GIT and vagina. Participate in mixed anaerobic infections like abscess in the abdomen, brain, pelvis, lung and uterus. They sensitive to penicillin and metronidazole.

Group A Streptococci (*Streptococcus pyogenes*)

Morphology

Gram positive, non-motile, non-spore forming, catalase negative cocci arranged in chains of variable length.

Most of them are encapsulated and their capsule is helpful for pathogenesis by preventing phagocytosis but non immunogenic, because it composed of hyaluronic acid similar to that of our tissue.

Beside of the presence of group specific antigen (C-substance), there are another proteins like M, T, R which act as type specific (Griffiths typing) antigen. There are more than 80 serotypes of Strept. Pyogenes Based on M protein.

These bacteria have pili protruding from their surfaces and consist of both M protein and teichoic acid. M proteins act as antiphagocytic factor while teichoic acid mediates attachment to the epithelial cells (figure 2).

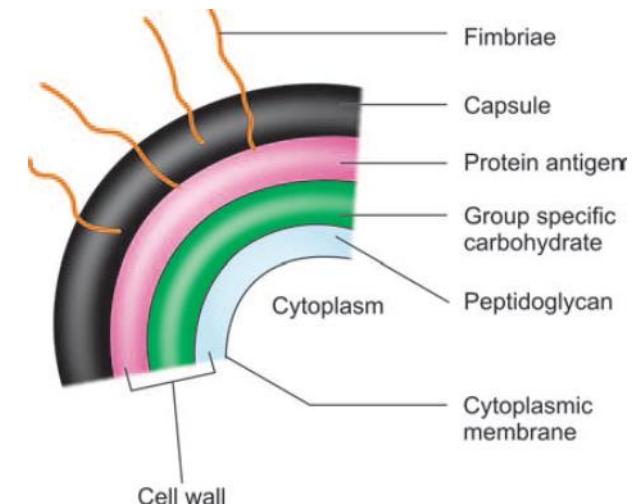


Fig.2. Schematic diagram of the cell wall of *Strep. pyogenes*

Cultural characteristics

Strep.pyogenes is a fastidious bacteria requires enriched media like blood agar producing pin point colonies surrounded by wide zone of beta hemolysis.

Growth is better under microaerophilic conditions (5-10% CO₂ by candle jar).

It is a delicate pathogen and can dye rapidly when exposed to dryness.

Antigenic structure

A- Group specific antigen (Lancefield antigen) is a CHO (C-substance) found in the cell wall of most Streptococci and divided into 20 different serogroups.

B- Another antigens are M,T and R proteins. M protein is the most important one because

a- Act as type specific antigen (Griffiths typing) by which *Strep.pyogenes* divided into more 80 (no cross immunization) serotypes. These serotypes further divided into nephritogenic types and rheumatogenic types (Responsible for post streptococcal non-suppurative immunological diseases).

b- Act as a major virulence factors (without it, it becomes avirulant) by impeding phagocytosis.

c- M-protein is associated with teichoic acid as hair-like protrusion from surface of bacteria (mediate attachment).

d-Immunity to *Strep.pyogenes* infection is related to the presence of type specific antibodies to M-protein.

e-M-proteins and other proteins have an important role in the pathogenesis of rheumatic fever in which Antibodies formed against certain M types can cross reaction with the heart muscle leading to rheumatic fever.

Toxins and enzymes

A-Streptokinase (Fibrinolysin)

Convert plasminogen into plasmin which dissolve blood clot. Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thrombosis.

B- Deoxyribonucleases (Streptodornase) liquefies pus and help bacteria to spread with tissue. The viscosity of pus is due to the presence of deoxyribonucleoprotein that released from lysed PMNs during infections.

C- Hyaluronidase (Spreading factors) important in skin infection

D- Pyrogenic Exotoxins (Erythrogenic Toxin)

- Lysogenic strain of Strep.pyogenes produce this toxin
- Associated with streptococcal toxic shock syndrome and scarlet fever.
- Act as superantigen

D- Hemolysins (Streptolysin)

A- Streptolysin O is oxygen labile and responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates. It antigenic producing Anti-Streptolysin O antibodies . ASOT is a serological test used for diagnosis of rheumatic fever.

Streptolysin S is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is heat stable and non- immunogenic.

Diseases caused by Group A Streptococci (*Strep.pyogenes*)

1- Streptococcal sore throat (pharyngitis) with tonsillitis (most common infections)characterized by fever, malaise, enlarged cervical lymph nodes.

2- Streptococcal pyoderma or impetigo (superficial skin infection especially of the lower extremities characterized by little pus but with diffuse cellulitis

3- Streptococcal Toxic Shock Syndrome

4- Scarlet fever is due to production of erythrogenic toxins by localized streptococcal infection like pharyngitis or skin or soft tissue infection (myositis –flash eating bacteria, cellulitis). Clinically characterized by skin rashes, strawberry tongue and fever.

5- Puerperal fever: it is infection of the uterus occurring after child birth and the source of infection is the nasopharynx of doctors, nurses and attendants.



Tonsillitis



Sore throat or pharyngitis



Pyoderma or impetigo



Cellulitis

6- Non-suppurative complications: They are:

A- Acute rheumatic fever

It is a systemic nonsuppurative inflammatory condition characterize by fever, pancarditis, migratory polyarthritis sometimes chorea (uncontrolled involuntary movements) and subcutaneous nodules. Usually develop after 1-3 weeks from tonsillitis caused by rheumatogenic strains of S.pyogenes. It is due to the cross reaction between certain streptococcal antigens (M protein) and that of heart. Can be reactivated after recurrent streptococcal infection, therefore require prophylaxis doses of Benzyl penicillin.

B- Acute glomerulonephritis

It develops 1- 4weeks after S pyogenes skin infection (pyoderma ,impetigo) or pharyngitis. Caused by nephritogenic types. The main mechanism for this is due to the precipitation of the immune complexes in the kidney that leads to glomerulonephritis. No reactivation and no prophylaxis treatment.

Laboratory diagnosis of sore throat

There are different causes of sore throat

- 1- *Strept.pyogenes*
- 2- *Corynebacterium diphtheriae*
- 3- *Staph.aureus*
- 4- *Haemophilus influenza*
- 5- *Candida albicans* (Yeast)
- 6- Infectious mononucleosis
- 7- Adenovirus infection

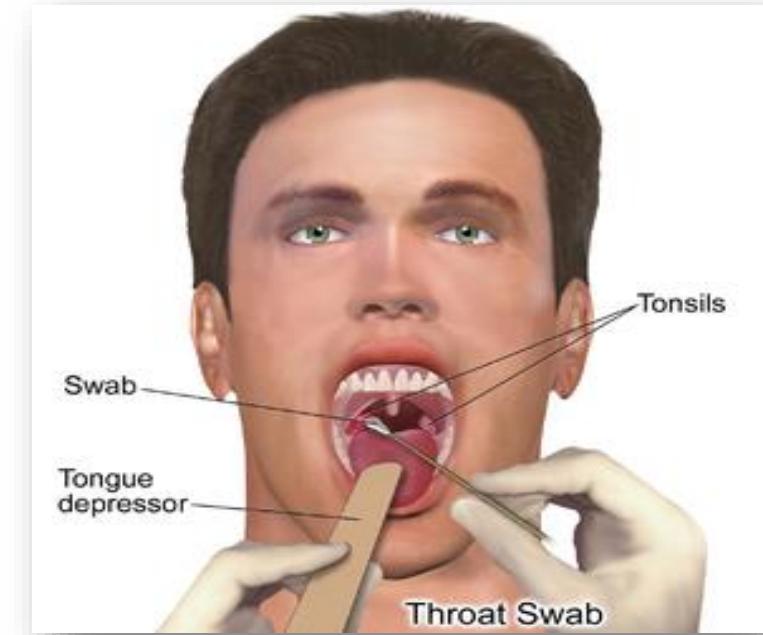


Fig.4. Taking throat swab

Throat swab

2 swabs are collected from inflamed area of the tonsils or pharynx or from gently rubbed pseudomembrane under a bright light with depressed tongue by tongue depressor as shown in figure 4.

One swab is used for smear preparation and staining procedures like Gram stain and Alberts stain, while other submitted for culture.

Smear is useful for the following cases

- Detection of *Corynebacterium diphtheriae* which appear as Gram positive club shaped bacilli with Chinese letter arrangement. Alberts stain detect volutine granules.
- In case of oral candidiasis (thrush) will reveal Gram positive yeast.
- In Vincents angina caused by Spirochetes and Fusiform bacilli.

Culture

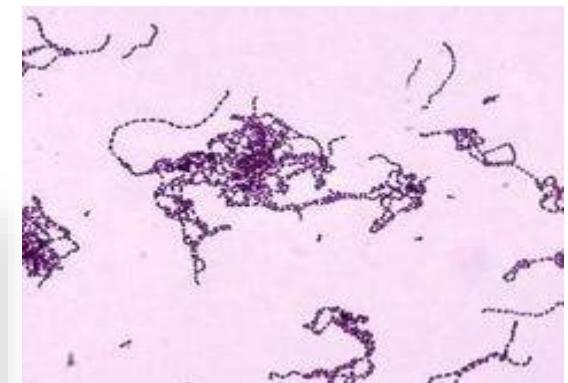
The second swab streaked on the surface of blood agar and incubated under microaerophilic conditions (5-10% CO₂) for 24-48 hrs)

Look for pin point colonies surround with large zone of beta hemolysis as shown in figure 5.



Fig.5. Beta hemolytic colonies of S.pyogenes on blood agar

Gram stained smear from colonies reveal Gram positive coccis in chains as shown in figure 6.



Bacitracin disc test= sensitive (Presumptive diagnosis) as shown in figure 7

PYR test = +ve as shown in figure 8

Lancefield grouping using group A specific antigen for colonies or directly from swab before culture as shown in figure 9.

PCR for direct detection from swab

API 20 STREP as shown in figure 10



Fig.9. Lancefield grouping



Fig.10. API 20 STREP

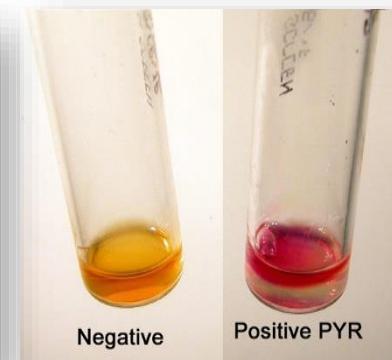


Fig.8. PYR test

Fig.6. Gram positive Strep.pyogenes



Fig.7. Bacitracin sensitivity

Immunity

Immunity is M type specific without cross immunization among types

ASOT doesn't indicate immunity (not all antibodies are protective) just inhibit hemolysis.

Treatment

1- S. pyogenes has not acquired resistance to penicillin G (procaine penicillin), which remains the antibiotic of choice for acute streptococcal disease such as tonsillitis.

2- In a penicillin allergic patient, a macrolide such erythromycin is the preferred drug.

Prevention

Rheumatic fever is prevented by rapid eradication of the infecting organism. Prolonged prophylactic antibiotic therapy (Benzathine penicillin/monthly for years) is indicated after an episode of rheumatic fever

Group B beta hemolytic Streptococci (*Strep.agalactiae*) (GBS)

β-hemolytic and produce zones of hemolysis that are only slightly larger than the colonies (1–2 mm in diameter).

Hydrolyze sodium hippurate and give a positive response in the so-called CAMP (Christie, Atkins ,Munch-Peterson) test.

Part of the normal vaginal flora and lower gastrointestinal tract in 5–30% of women

It is mainly infect newborn during delivery from colonized mother birth canal and can cause Pneumonia, meningitis and sepsis.

There is early onset of the disease (during 7 days of deliver) or late onset after 7 to 3 months

Lab.Diagnosis

Specimens: CSF, Blood , vaginal swab

Culture on blood agar : beta hemolytic Streptococci

CAMP test positive : *Strep.agalactiae* produce CAMP factor (a diffuse heat stable protein) that enhances the hemolytic activity of beta hemolysin of *Staph.aureus* and appear as arrow head at the transection of two bacterial line as shown in figure 11.

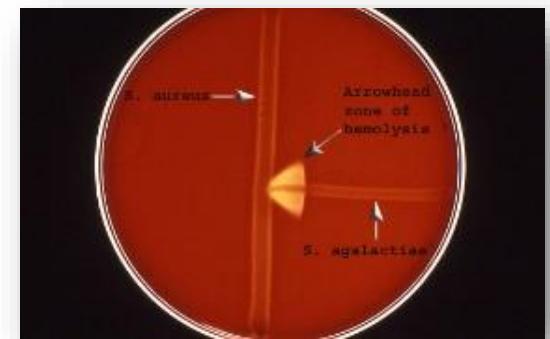


Fig.11. CAMP test

API 20 STREP

Serological test using group specific antigen

PCR

C - Christie; A - Atkins; MP - Munch-Peterson (3 researchers)

Group D Streptococci

It can be divided into two groups: Group D Streptococci or Enterococci and group D non enterococci Streptococci (Strep.bovis). *Enterococcus faecalis*, *E.faecium*, others.

E.faecalis is the most important Enterococci

Normal flora of lower intestinal tract and vagina.

Can grow in 6.5 percent sodium chloride, 40 percent bile and at 45°C.

On sheep blood agar may produce alpha, beta hemolysis or may be non-hemolytic.

Tiny, deep pink colonies appear on MacConkey agar medium.

Survive heat upto 60°C for 30 minutes.

PYR test positive.

Most strains are resistant to penicillin

This bacteria important in hospital acquired infection can cause UTI, wound infection, endocarditis, biliary tract infection, peritonitis, suppurative abdominal lesions and septicemia.

Strep.bovis can grow in bile esculin like enterococci but cant grow in 6.5% NaCl may cause urinary tract infection, endocarditis, septicemia. They may be non- hemolytic and susceptible to penicillin.

α -hemolytic Streptococci (non-groupable Streptococci)

2 types Streptococcus pneumonia and viridans Streptococci

Streptococcus pneumonia (Pneumococcus) or diplococcus pneumoniae

General characteristics

The pneumococci (*S. pneumoniae*) are gram-positive diplococci, often lancet shaped or arranged in chains.

Possessing a capsule of polysaccharide that permits typing with specific antisera into more than 85 serotypes.

Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause different infections when predisposing factors are present.

Producing characteristic bottom like colonies called draughtsman colonies.

Cultural characters:

- ❖ It requires serum or whole blood for growth.
- ❖ It grows best at 37°C and at pH 7.6.
- ❖ It is aerobic and facultative anaerobic.
- ❖ Growth is improved by providing them 5 to 10 percent CO₂.
- ❖ On blood agar colonies are small (0.5 to 1 mm) ,dome shaped with area of greenish discoloration (alpha hemolysis) around them.On further incubation the colonies become flat with raised edges and central umbonation (draughtsman appearance).
- ❖ Sensitive to optochin
- ❖ Soluble in bile (bile solubility test is positive) (few drops of 10 percent sodium desoxycholate solution are added to 1 ml of overnight broth culture)
- ❖ It is catalase and oxidase negative.

Predisposing factors for pneumococcal infections

- 1-Viral and other respiratory tract infections that damage surface cells
- 2-Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material
- 3 .Abnormal circulatory dynamics (eg, pulmonary congestion ,heart failure)
- 4- Other mechanisms, such as malnutrition, general debility ,sickle cell anemia, hyposplenism, nephrosis, or complement deficiency

Clinical infections

Pneumococcal pneumonia or lobar pneumonia

Otitis media

Sinusitis

Bacteremia with meningitis, endocarditis and septic arthritis

Laboratory diagnosis

Specimen: sputum, blood, CSF, wound exudates

Smear examination: Gram staining shows flame-shaped cocci arranged in pairs and they are Gram-positive capsulated.

Culture: Material is inoculated on blood agar plates and incubated at 37°C under 5 to 10 percent carbon dioxide. Growth occurs after overnight incubation.

Blood culture: It shows flat, umbonated colonies showing alpha hemolysis

Biochemical test

- 1- Catalase negative
- 2- Optochin sensitive
- 3- Bile solubility

Immunological method like Quellung test or capsular swelling reaction



Optochin susceptibility test

Principle: Optochin (Ethyl hydrocuprein HCl) is a detergent that dissolve (lyse) choline in the cell wall of Strep.pneumonia, while viridans Streptococci are resistant as shown in figure 13.

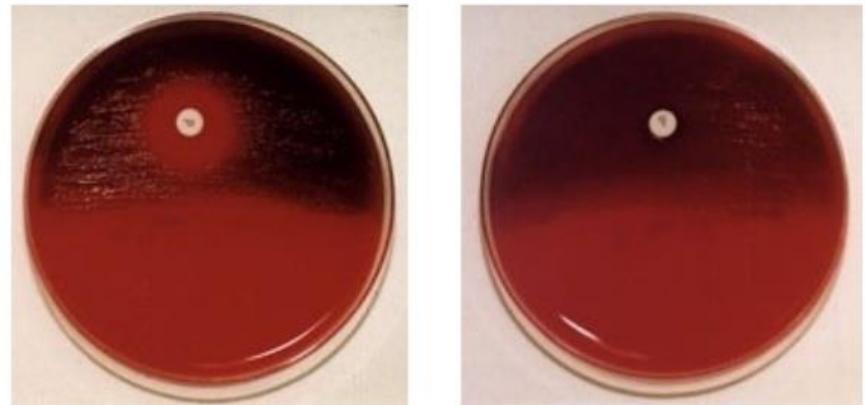
Bile Susceptibility test

Procedure of bile solubility test:

Preparation of 2% sodium deoxycholate (bile salt) solution

Dissolve 2 g of sodium deoxycholate into 100 ml sterile distilled water.

Optochin test



Sensitive
(Pneumococci)

Resistant
(Viridans streptococci)

Fig.13. Optochin test

Performing the bile solubility test

1. Grow the isolate(s) to be tested for 18-24 hours on a blood agar plate at 35-37°C with ~5% CO₂ (or in a candle-jar).
2. Add bacterial growth from the overnight blood agar plate to 1.0 ml of 0.85% saline
3. Divide the cell suspension equally into 2 tubes (0.5 ml per tube).
4. Add 0.5 ml of 2% sodium deoxycholate (bile salts) to one tube. Add 0.5 ml of 0.85% saline to the other tube. Mix each tube well.
5. Incubate the tubes at 35-37°C in CO₂.
6. Vortex the tubes.
7. Observe the tubes for any clearing of turbidity after 10 minutes. Continue to incubate the tubes for up to 2 hours at 35-37°C in CO₂ if negative after 10 minutes. Observe again for clearing as shown in figure 14. Strep.pneumonia soluble in bile (positive) while viridans strep insoluble (negative)

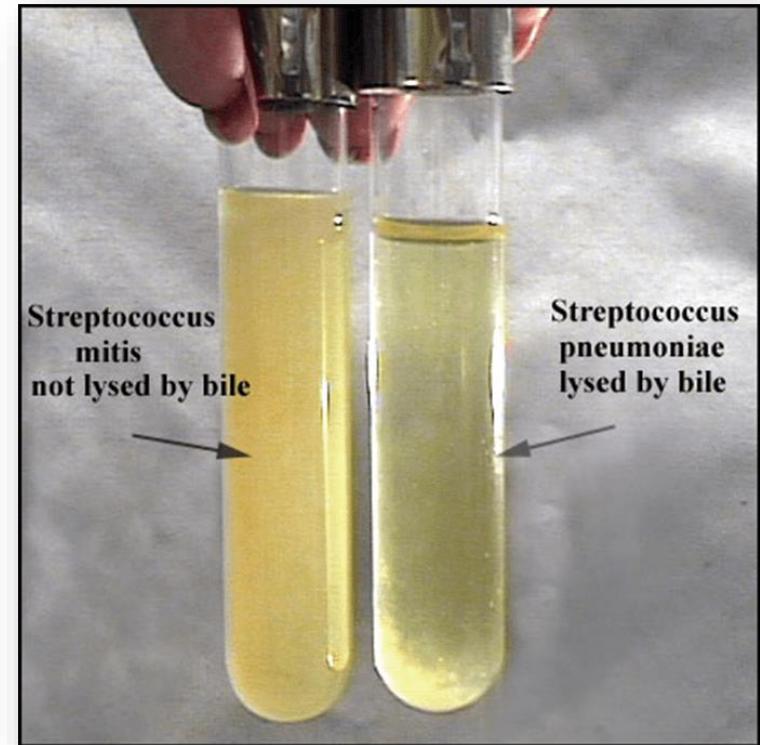


Fig.14. Bile solubility test

Quellung reaction (swelling reaction)

Mix specific anti-capsular antibodies of Strep.pneumonia with a suspension of suspected Strep.pneumonia and examine under oil immersion lance. Strep.pneumonia appear as swollen as shown in figure 15

Viridans Streptococci

Streptococcus viridans: It is present as a commensal on mucosa of mouth, nasopharynx and saliva of man. On the basis of biochemical reactions it is classified into 5 species (*Streptococcus salivarium, mutans, sanguis, mitior, milleri*). On blood agar it produces alpha Hemolysis. Non pathogenic but can cause subacute bacteria endocarditis and susceptible to penicillin.

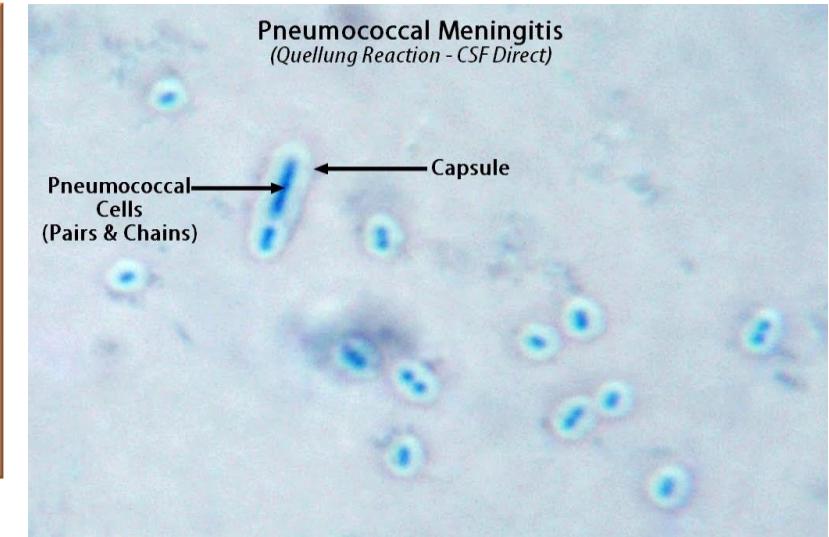


Fig.15. Quellung reaction

TABLE 24.1: Differentiation between *Pneumococcus* and *Streptococcus viridans*

<i>Pneumococcus</i>	<i>Streptococcus viridans</i>
1. Morphology	
a. Capsulated	Non-capsulated
b. Flame shaped diplococci	Oval or round arranged in chain
2. Quellung test positive:	Negative
3. Colonies Initially dome shaped and later on draughtsman colonies	Dome shaped
4. Growth in liquid media shows uniform turbidity	Granular turbidity and powdery deposits
5. Bile solubility is positive	Negative
6. Inulin fermentation is positive	Negative
7. Optochin sensitivity is positive	Negative
8. Intraperitoneal inoculation in mice brings fatal infection	Non-pathogenic

Treatment

Choose the drug according to the result of sensitivity test

Prevention

There is a vaccine composed of many capsular serotypes licensed in USA and used for elderly and immunosuppressed individuals.

Case #4A

A premature baby delivered by birth canal and developed high fever after one week. Procalcitonin test (A serological test used for detection of bacterial septicemia) was positive. Blood culture gave small hemolytic colonies. Gram stained smear from colonies revealed Gram positive club shaped bacilli arranged in Chinese letter like.

Questions

- 1- What is ur tentative diagnosis?
- 2- What make you to think for ur first diagnosis?
- 3- What is the source of this infection?
- 4- Which bacterium makes confusion with this pathogen?
- 5- What can cause in normal individuals?
- 6- Name two unique cultural characteristics of this pathogen?
- 7- Is this pathogen zoonotic?
- 8- Is this pathogen extracellular or intracellular?

Case #4B

Beta hemolytic colonies were isolated from sore throat of 2-years-old boy with fever and enlargement of both tonsils. Gram stained smear revealed Gram positive cocci arranged in chains (25 mark).

- A. What is the suspected pathogen?
- B. Describe of this pathogen on blood agar.
- C. Name the test that used for presumptive diagnosis of this pathogen.
- D. Name the confirmatory test used for definitive diagnosis of this pathogen.
- E. Name one complication of this infection that require monthly injection with benzathine penicillin.

Case #4C

Ear swab cultured on blood agar revealed growth of alpha hemolytic Gram positive oval shaped diplococci.

- A. Name the suspected isolate.
- B. How to confirm your diagnosis?
- C. What is the characteristic of colonies on blood agar?
- D. What is the most common clinical infection of this isolate?
- E. What is the major virulence factor of this isolate?

Case #4D

An 8-year-old girl develops Sydenham's chorea ("St. Vitus dance") with rapid uncoordinated facial tics and involuntary purposeless movements of her extremities, strongly suggestive of acute rheumatic fever. She has no other major manifestations of rheumatic fever (carditis, arthritis, subcutaneous nodules, skin rash). The patient's throat culture is negative for *Streptococcus pyogenes* (group A streptococci). However, she, her brother, and her mother all had sore throats 2 months ago. A test that if positive would indicate recent *Streptococcus pyogenes* infections is

- (A) Antistreptolysin S antibody titer
- (B) Polymerase chain reaction for antibodies against M protein
- (C) ASO antibody titer
- (D) Esculin hydrolysis
- (E) Antihyaluronic acid antibody titer

1- Describe *Streptococcus pyogenes* in Gram stained smear under microscope.

2- How diagnose this pathogen using cultural methods?

3- What are the structures of this bacteria that responsible for: attachment to the host tissues, antiphagocytic, type specific and group specific?

4- Mention clinical infections both suppurative and non suppurative.