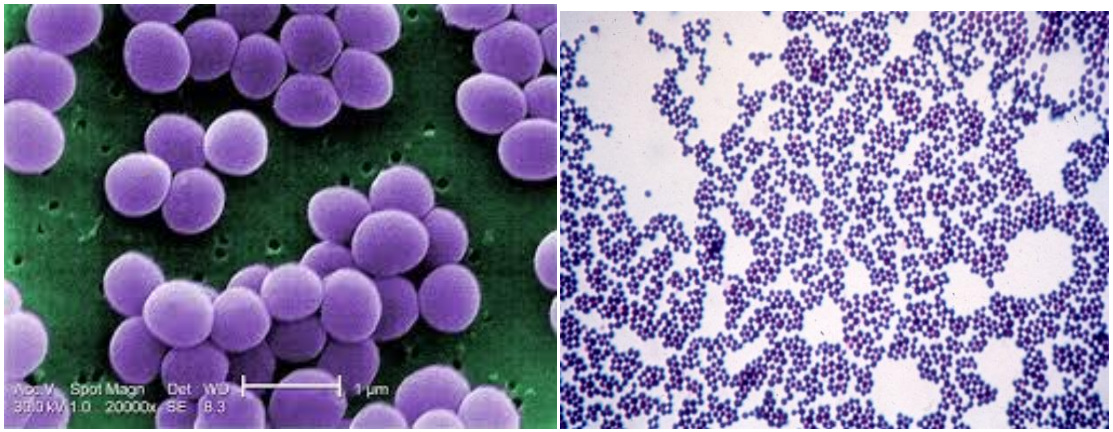
Lec (5-6) **Genus *Staphylococcus*** **Dr .Marwa Kadhim**

- ***Staphylococcus aureus***
- **Morphology: 1**) They are spherical cocci, approximately 1 μm in diameter, 2) arrange in **grapelike clusters**. **3**) Cluster formation is due to cell **division occurring in three planes, with daughter cells tending to remain in close proximity**. **4**) They are **non-sporing**, **non-motile** and usually non- capsulate with the exception of rare strains. They stain readily with aniline dyes and are uniformly **5**) gram-positive but old may be gram- negative.



Electron Microscope Gram Stain (blue color

Cultural Characteristics

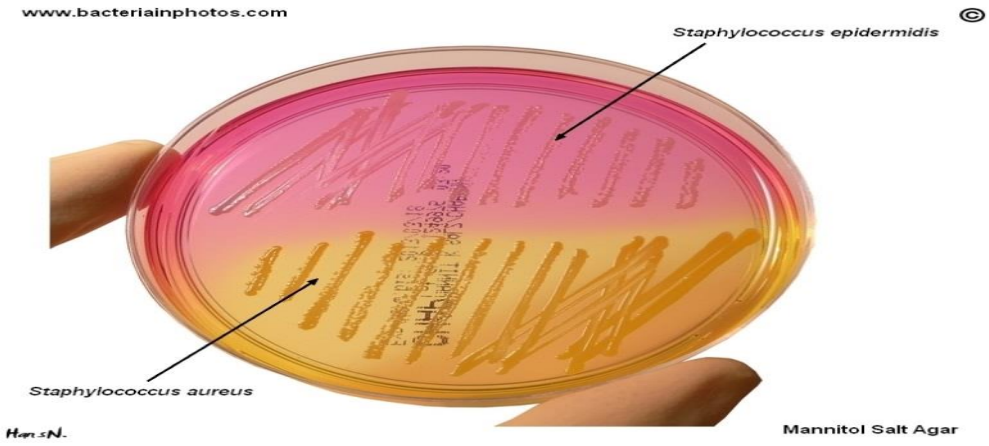
1)They are aerobes and facultative anaerobes. 2) Optimum temperature for growth is 37°C (range being 12-44°C). Optimum pH is 7.5. 3) They can grow readily on ordinary media.

1. Nutrient Agar: After aerobic incubation for 24 h at 37°C, colonies are 1-3 mm in diameter and have a smooth glistening surface, an entire edge, a soft but consistency and an opaque, pigmented appearance. Most strains produce golden- yellow (aureus) pigment, though some strains may form white (non-pigmented) colonies. These white colonized strains of *S. aureus* are fully virulent. Pigmentation is characteristic of this species when grown aerobically .Pigmentation is enhanced on fatty media such as Tween agar, by prolonged incubation, and by leaving plates at room temperature. Non-pigmented strains are not uncommon. Grown anaerobically, colonies are often smaller and grayish in color.

Staph. aureus growing of different culture media:

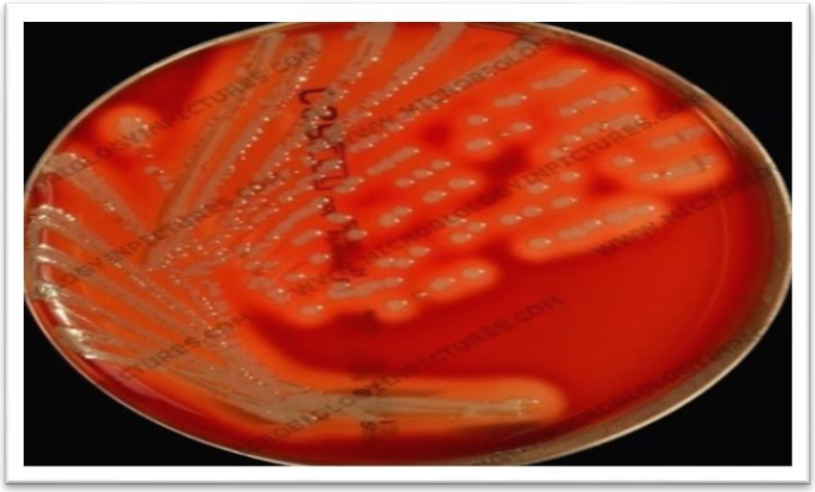
1- On nutrient agar (golden color) On blood agar (beta hemolytic)

2- Mannitol salt agar



3. Blood Agar(see the figure above)

The colonies have the same appearance as on nutrient agar, but may be surrounded by a **zone of β-hemolysis.Hemolysis is more likely to be present if sheep, human or rabbit blood is used** instead of horse blood and if incubation is in air with 20 percent added carbon dioxide. Hemolysis is weak on horse blood agar.



Staphylococcus aureus cultivated on **Columbia agar with 5% defibrinated sheep blood.** Cultivation 24 hours in an aerobic atmosphere, 37°C. Colonies are surrounded by a wide zone of **beta-hemolysis**.

4. Selective Salt Media

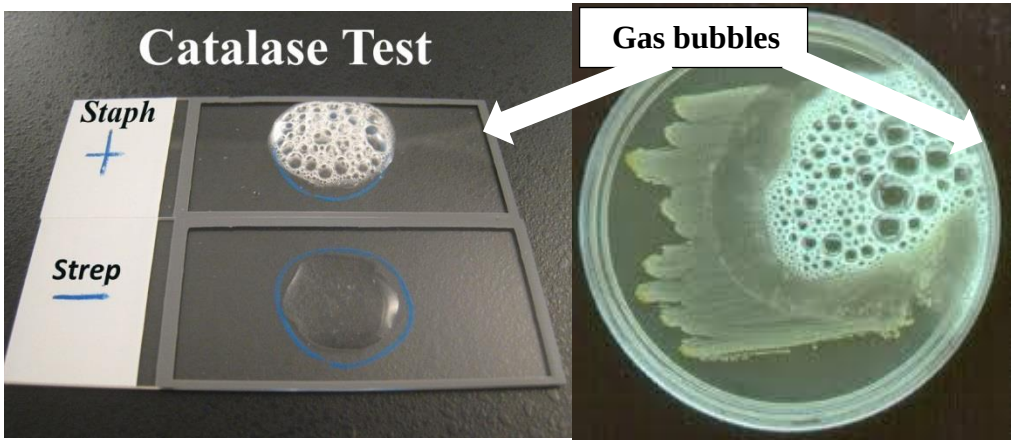
Selective medium may be useful for the isolation and enumeration of staphylococci from materials, such as feces, food and dust, likely to contain a predominance of other kinds of bacteria. Therefore, 7 to 10 percent of sodium chloride may be added to nutrient agar (salt agar) or milk agar (salt milk agar); **mannitol salt agar containing 1 percent mannitol, 7.5 percent NaCl, and phenol red in nutrient agar**; and **Ludlam's** medium containing lithium chloride and tellurite; and salt cooked meat broth (10% NaCl).

5**. Fluid media**: It produces uniform turbidity. No pigment is produced.

<u>Biochemical Reactions:</u>

1. **Sugar fermentation**: *S. aureus* ferments a range of sugars (**glucose**, **maltose**, **lactose**, **sucrose**, including **mannitol**) producing **acid** but no **gas**. Sugar fermentation is of no diagnostic value except for **mannitol**, which is usually fermented anaerobically by *Staph. aureus* but not by other species.

2. **Catalase test**: Catalase positive (unlike streptococci).**By mixing a drop of 3% hydrogen peroxide (H2O2) with a colony of the test bacteria on slide or on plate .Producing air bubbles = positive (+), without air bubbles = negative (+)**



3. **Lipolytic**: When grown on media containing egg yolk, it produces a dense opacity because most strains are lipolytic.

4. **Phosphatase test**: They also produce phosphatase. This is a useful screening procedure for differentiating *Staph. aureus* from *Staph. epidermidis* in mixed cultures, as the former gives prompt phosphatase reaction, while the latter is usually negative or only weakly positive.

5. Penicillinase: This is a secreted form of beta-lactamase producing staphylococci. It disrupts the beta-lactam ring of the penicillin molecule, thereby inactivating the antibiotic.

6. Hyaluronidase ("Spreading Factor"): This protein breaks down proteoglycans in connective tissue**(hyaluronic acid)**.

7. Staphylokinase: This protein lyses formed fibrin clots.

8. Lipase: This enzyme degrades fats and oils, which often accumulate on the surface of our body. This degradation facilitates *Staphylococcus aureus*' colonization of sebaceous glands.

9. Protease: destroys tissue protein.

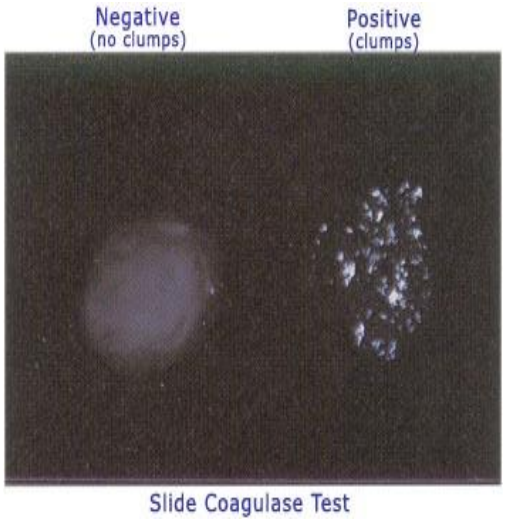
10. Other biochemical tests: Indole **negative**, MR **positive**, VP **positive**, urease **positive**, hydrolyzes gelatin and reduces nitrates to nitrites.

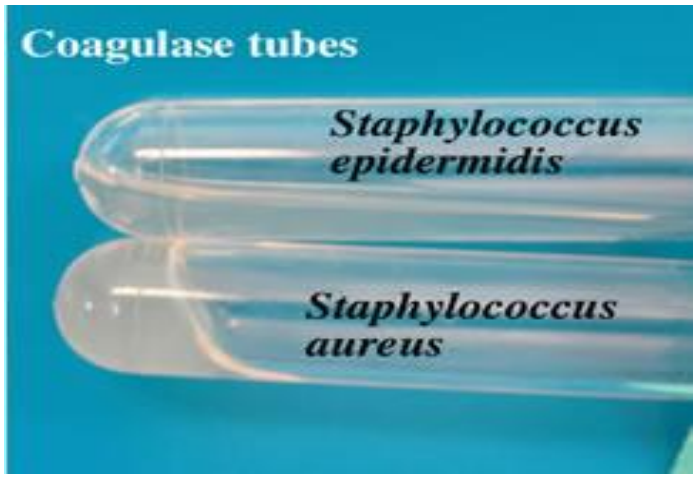
11. Coagulase test:

S. aureus produces an extracellular enzyme called coagulase which brings about clotting of human or rabbit plasma. It acts along with a 'coagulase reacting factor' (CRF) present in plasma, binding to prothrombin and converting fibrinogen tofibrin.**Coagulase does not clotplasma of guinea pigs and some other species because they lack CRF**. CRF is similar to pro- thrombin but is probably not identical with it. ***Staph.aureus* strains usually secrete both coagulase and clumping factor.**

Coagulase test is the standard criterion for the identification of *Staph. aureus* isolates. The role of coagulasein the pathogenesis of disease is speculative, but coagulase may cause the formation of afibrinlayer around a staphylococcal abscess, thus localizing the infection and protecting the organisms from phagocytosis.

Fibrinogen —Coagulase→ Fibrin (Clotting)

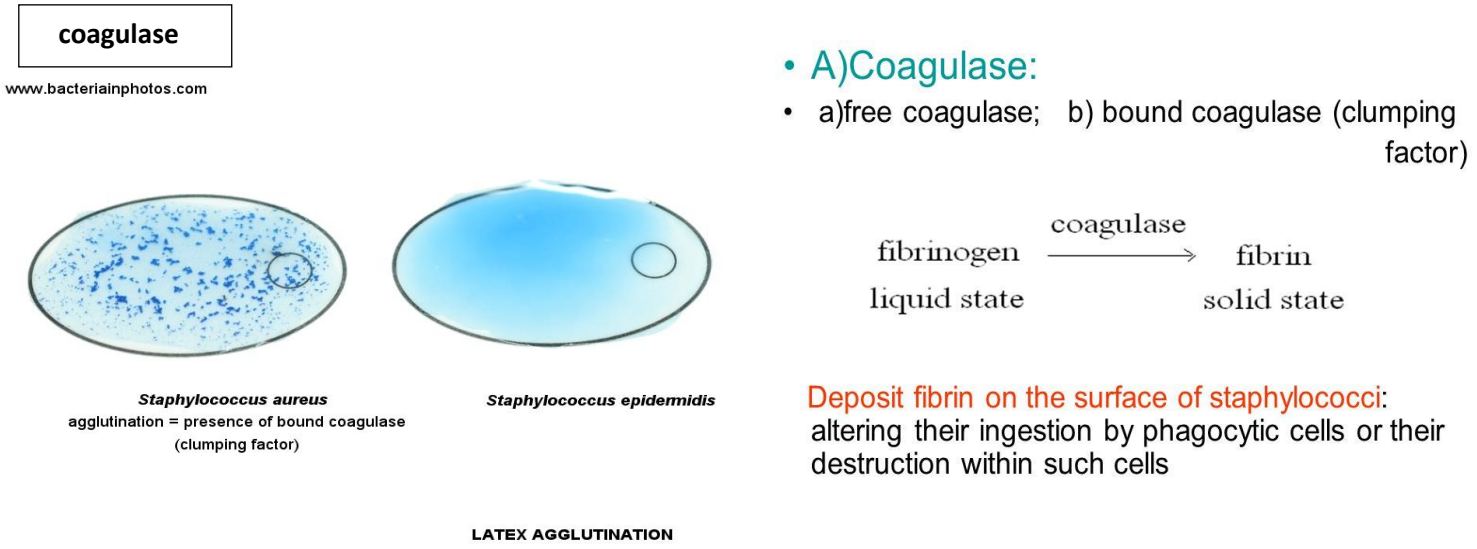




coagulase test/tube method **Coagulase test/slide method**

Clumping Factor (Bound Coagulase): The component on the cell wall of *S. aureus* that results in the clumping of whole staphylococci in the presence of plasma is referred to as the clumping factor (also called bound coagulase). This factor reacts directly with fibrinogen in plasma, converts it to insoluble fibrin ,causing the staphylococci to clump or aggregate.

Slide coagulase test: It can be detected by emulsifying a few colonies of the bacteria in a drop of normal saline on a clean glass slide and mixing it with a drop of rabbit plasma. Prompt clumping of the organisms indicates the presence of clumping factor (bound coagulase). Since this factor is detected by performing the test on a slide ,therefore, the test is known as slide coagulase test.



Comparison between bound coagulase and free coagulase

Bound coagulase (clumping factor)	**Free coagulase**
1. Heat stable.	1. Heat labile.
2. Constituent of cell wall.	2. Secreted **free** into the medium.
3. It **does not require** the cooperation of CRF for its action.	3. **Requires** the cooperation of CRF for its action..
4. Clot plasma of guinea pigs and some other species.	4. Does not clot plasma of guinea pigs and some other species because they lack CRF.
5. Only one type of clumping factor has been identified.	5. Eight antigenic types (A-H) have been described. Most human strains form coagulase type A.
6. Detected by **slide** method test.	6. Detected by **tube** method test.

Laboratory Diagnosis:

1. **Specimens:** The specimens to be collected depend on the type of lesion, for example; Pus from suppurate lesions; sputum from respiratory infections; food remains and vomit from cases of food poisoning; nasal and perineal swabs from suspected carriers. Swabs of the perineum, pieces of hair and umbilical stump may be necessary in special situations.

2. Direct Microscopy: Direct microscopy with Gram stained smears is useful in the case of pus, where cocci in clusters may be seen. This is of no value for specimens like sputum where mixed bacterial flora is normally present.

3. Culture: The specimens are cultured on a blood agar plate. Staphylococcal colonies appear after overnight incubation. Specimens, where staphylococci are expected to be outnumbered by other bacteria (e.g. wound swab and feces), are inoculated on selective media The inoculated media are incubated at 37°C for 18-24 hours. On blood agar plate, look for hemolysis around the colonies.

4. Identification: Relatively simple biochemical tests (e.g. positive reactions for coagulase [clumping factor], heat-stable nuclease, alkaline phosphatase, and mannitol fermentation) can be used to differentiate *S. aureus* and the other staphylococci.

5. Coagulase Test: Coagulase test is done by two methods—slide and tube coagulase test. (Previously mentioned).

6. Antibiotic Sensitivity Tests: As a guide to treatment, antibiotic sensitivity tests should be performed appropriate to the clinical situation. This is important as staphylococci readily develop resistance to drugs.

Characteristics distinguishing three species of the genus *Staphylococcus*			
S. saprophyticus	***S. epidermidis***	***S. aureus***	*Characteristic*
-	+	+	Anaerobic growth and fermentation of glucose
V -	**V** -	+ +	Mannitol fermentation Acid aerobically Acid anaerobically
-	-	+	Coagulase
-	-	+	DNAase
-	**-/weak+**	+	Phosphatase
-	-	+	α-Toxin
-	-	+	**Protein A in cell wall**
Resistant	**Sensitive**	**Sensitive**	**Novobiosin sensitivity**

Treatment

Antibiotics commonly prescribed to treat staph infections include certain cephalosporins, nafcillin or related antibiotics, sulfa drugs, or vancomycin.

Vancomycin increasingly is required to treat serious staph infections because so many strains of staph bacteria have become resistant to other traditional medicines. But vancomycin and some other antibiotics have to be given intravenously.

Antibiotic resistance

Staph bacteria are very adaptable, and many varieties have become resistant to one or more antibiotics. For example, only about 10 percent of today's staph infections can be cured with penicillin.

The emergence of antibiotic-resistant strains of staph bacteria — often described as methicillin-resistant Staphylococcus aureus (MRSA) strains — has led to the use of IV antibiotics, such as vancomycin, with the potential for more side effects, such as vancomycin.

Streptococcus

General characters: They are Gram **positive cocci** arranged in **chains**, **non-motile** and **non-sporing**. They require media enriched with blood serum for growth. They are human pathogens causing pyogenic infection with a characteristic tendency to spread. They are also responsible for non-supportive lesions like (**acute rheumatic fever and glomerulonephritis**). **Group A streptococci have a hyaluronic acid capsule.**

Pathogenesis:

Virulence factors of group A streptococci include :

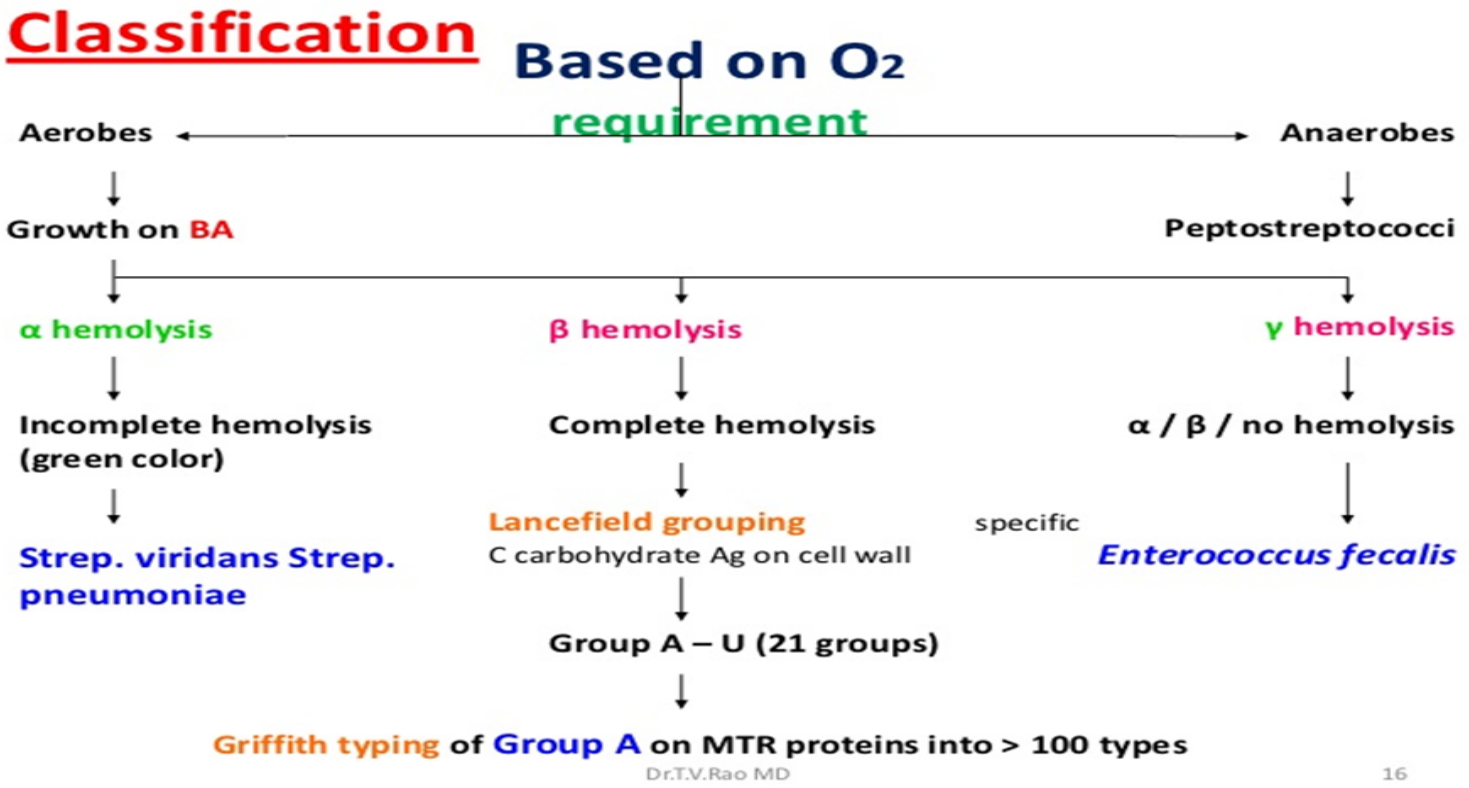
(**1**) **M** protein and **lipoteichoic acid for attachment.**

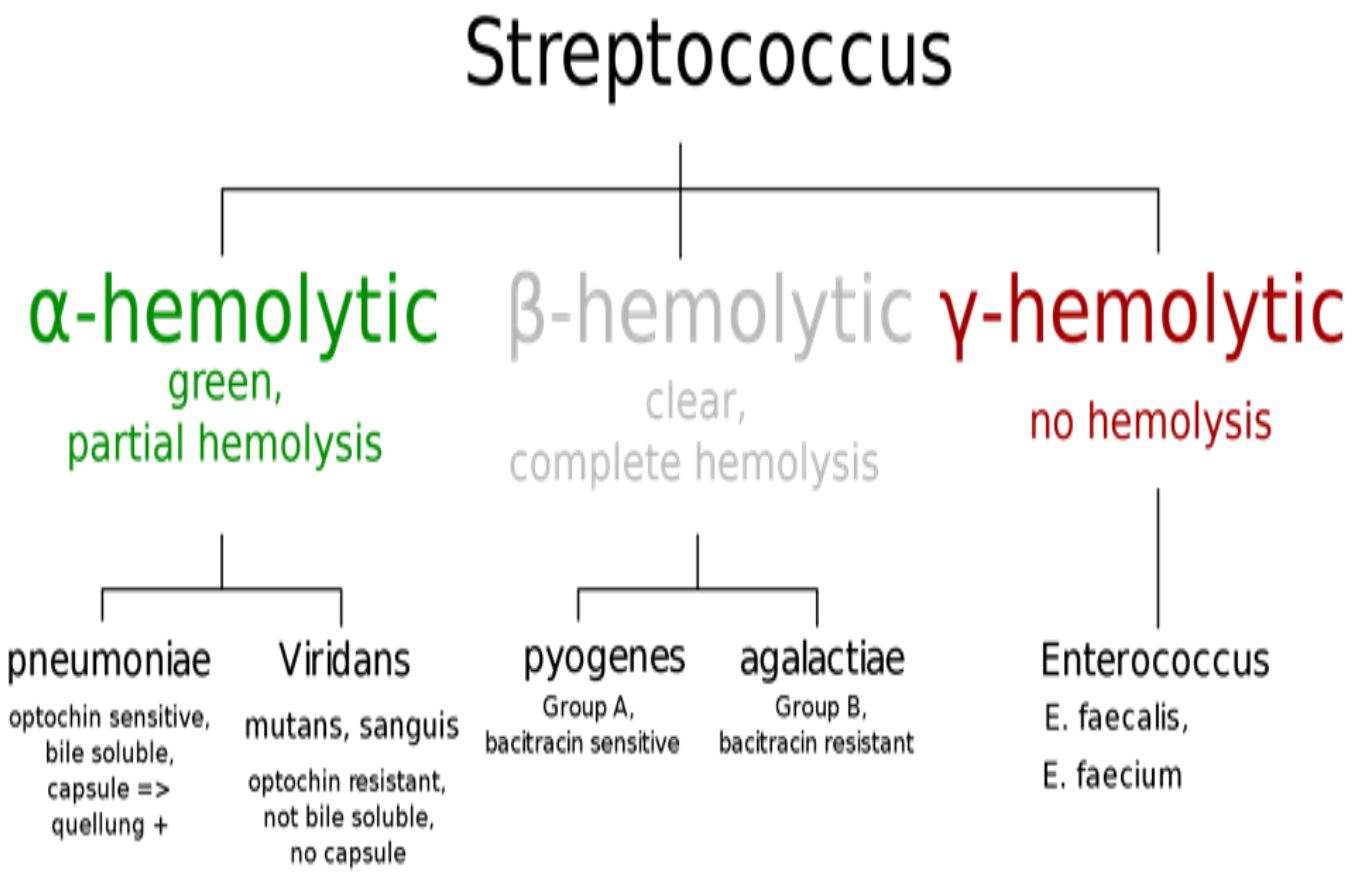
(**2**) a **hyaluronic acid capsule that inhibits phagocytosis.**

(**3**) extracellular products, such as **pyrogenic** (**erythrogenic**) **toxin**, which **causes the rash of scarlet feve**r.

(**4**) **streptokinase**, **streptodornase** (DNase B), and **streptolysins**. It causes **immune-mediated sequelae** (**acute rheumatic fever and glomerulonephritis**) **.**

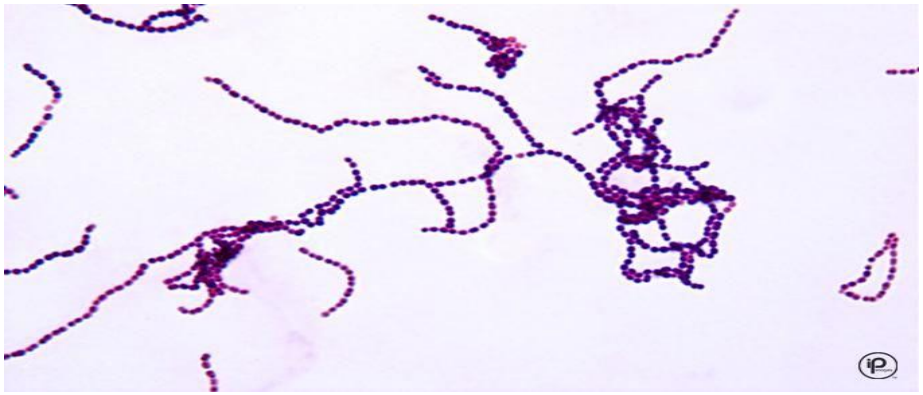
Classification of Streptococci according to different factors:




Streptococcus
α-hemolytic
green,
partial hemolysis
β-hemolytic
clear,
complete hemolysis
γ-hemolytic
no hemolysis
pneumoniae
optochin sensitive,
bile soluble,
capsule =>
quellung +
Viridans
mutans, sanguis
optochin resistant,
not bile soluble,
no capsule
pyogenes
Group A,
bacitracin sensitive
agalactiae
Group B,
bacitracin resistant
Enterococcus
E. faecalis,
E. faecium

Streptococcus pyogenes

Morphology: It is 0.5 to 1 μm in diameter and arranged in chain. Chain formation is due to cocci **dividing in one plane only and failure of daughter cell to separate completely**. The length of a chain depends upon medium in which organism is grown. It is usually encapsulated, non-spore forming and non-motile. When capsule is present it is composed of hyaluronic acid.

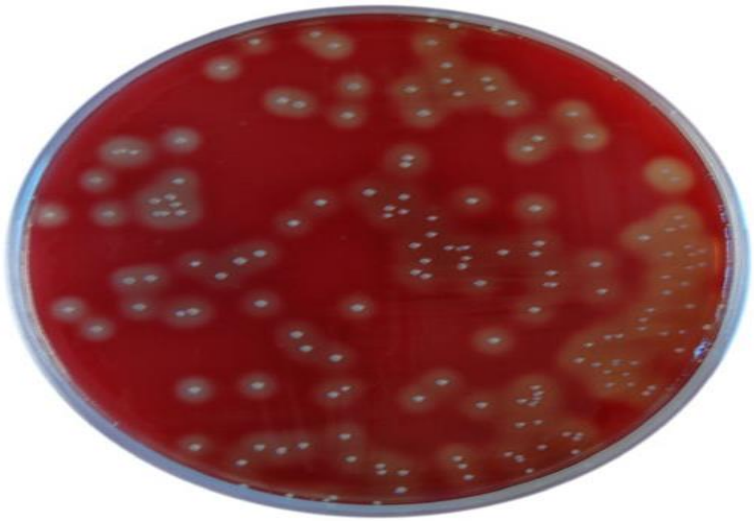


Cultural character: *Streptococcus pyogenes* is aerobic and facultative anaerobes with optimum temperature of growth being 37°C. Enriched media with whole blood, serum, or glucose favors rapid growth.

a. **Fluid media***:* Serum broth, 24 hours after culture shows granular growth with powdery deposits. There is no pellicle formation.

b. **Blood agar***:* After 24 hours' incubation colony is small, 0.5 to 1 mm (pin point colonies), circular, transparent, low convex with area of hemolysis.Strains with capsules produce mucoid colonies. Virulent strains produce matted colonies (granular). A virulent strain produces glossy colonies.

Streptococcus pyogenes growth of blood agar medium



Columbia Agar Base with 5% Defibrinated Horse Blood Streptococcal Selective Supplement. It is **selective medium for the isolation of *Streptococcus* spp.** from clinical samples. It is made selective by the addition of **Colistin** and **Oxolinic Acid**.

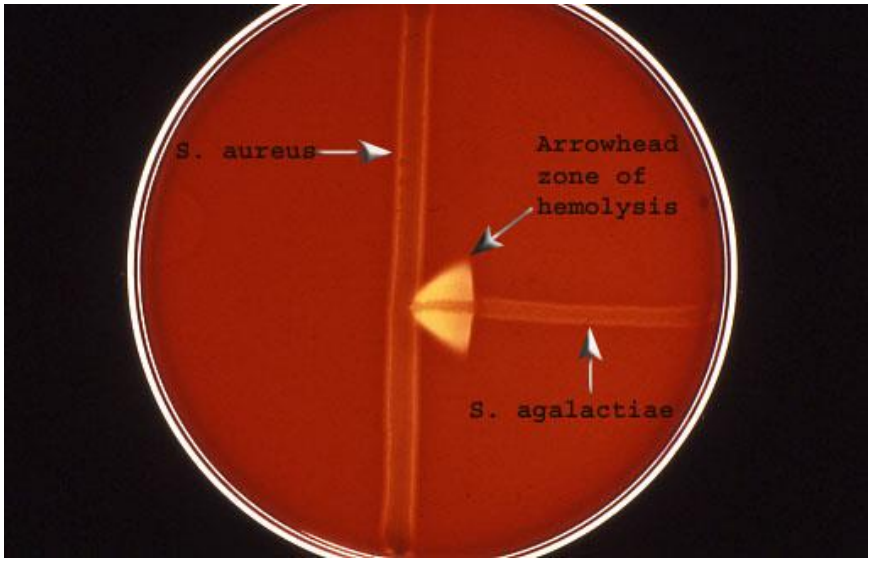
Biochemical reactions: It ferments lactose, glucose, salicin, sorbitol, maltose, dextrin, etc. producing acid but no gas. It is catalase negative. It does not liquefy gelatin and is not soluble in 10 percent bile. It hydrolyze pyrrolidonyl naphthyl amide (PYR test), producing red colors. It does not ferment ribose.

Streptococcus agalactiae

Streptococcus agalactiae belongs to Lancefield group B and is the only species that carries the group B antigen. Human pathogenic group B strains possess a polysaccharide capsule which appears to confer virulence. Nine capsular serotypes have been identified, antibodies to which confer type specific protection.

Identification of *S. agalactiae*:

Presumptive identification method is based on their ability to 1- **rapid hydrolyse hippurate.** They may be identified by the **CAMP** reaction (Christie, Atkins and Munch-Peterson), which can be demonstrated as an **2- accentuated zone of hemolysis (arrowhead-shaped area of enhanced hemolysis)** when *Str. agalactiae* is inoculated perpendicular to a streak of *Staph. aureus* grown on blood agar **(see the fig., below, please)**. *S. agalactiae* produces a CAMP factor that enhances the lysis of sheep red cells by staphylococcal β-lysin. Group B streptococci are occasional strains are bacitracin sensitive.



CAMP reaction

Enterococcus

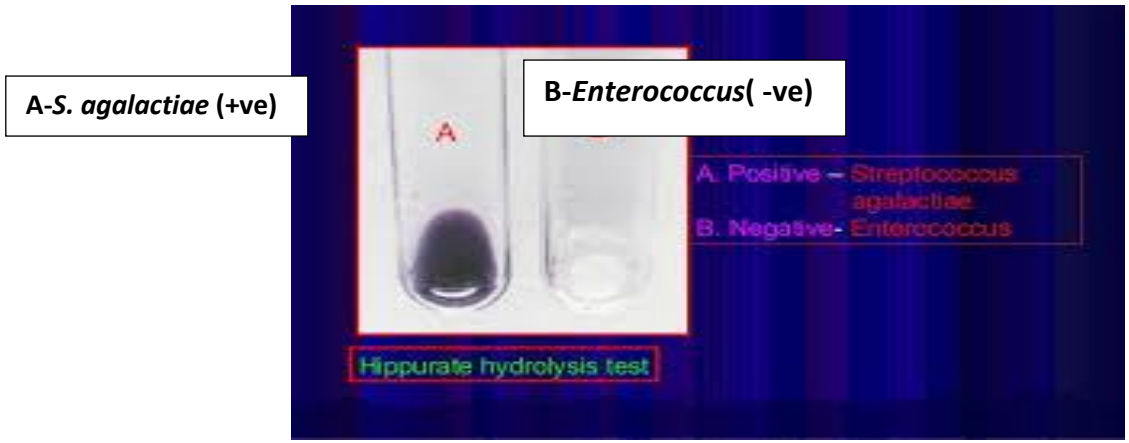
The enterococci ("enteric cocci") were previously classified as group D streptococci. In 1984, the enterococci were reclassified into the new genus *Enterococcus*. Enterococcus can be differentiated from group D streptococci by a number of tests. There are currently 16 species in this genus. This group consists of gram-positive cocci that are natural inhabitants of the intestinal tracts of humans and animals.

Characteristics of Enterococci

The enterococci are gram-positive cocci typically arranged in pairs and short chains, and is non-motile and non-capsulate. The cocci are facultative anaerobic and grow optimally at 35°C, although most isolates can grow in the temperature range l0ºC to 45°C. They grow readily on blood agar media, with large, white colonies appearing after 24 hours of incubation; the colonies are typically non-hemolytic but can be α-hemolytic or β-hemolytic. It grows readily on ordinary nutrient media .

Distinctive Features of Enterococci

The Enterococci possess several distinctive features separating them from streptocooci: The enterococci grow in the presence of 6.5 percent NaCl, 40 percent bile, at pH 9.6, at 45°C and in 0.1 percent methylene blue. It survives heating at 60°C for 30 min, a feature distinguishing it from streptococci, and also grows within a wider range of temperatures (10-45°C). They do not hydrolyze hippurate**.**



Viridance streptococci

The viridans streptococci are commensals of mouth and upper respiratory tract infection. The viridans group of streptococci are a heterogeneous collection of α-hemolytic and non-hemolytic streptococci.

Pneumococci (diplococcus pneumoniae, *Streptococcus pneumoniae*)

Morphology

Pneumococci are gram-positive cocci in pairs (diplococci). The cocci are about 1 μm, slightly elongated cocci, with one end broad or rounded and the other pointed, presenting a flame shaped or lanceolate appearance. They may occur singly, in pairs, or in short chains but most often are seen as pairs (diplococci), with the broad ends in apposition. They are non-motile and non-sporing.

All freshly isolated strains are capsulate. The capsule encloses each pair. The capsule may be demonstrated as a clear halo in Indian ink preparations or may be stained directly by special techniques or by use of homologous type-specific antibody in the **Quellung** reaction.

Cultural Characteristics

They are aerobes and facultative anaerobes. It grows best in air or hydrogen with 5-10 percent CO_2, for which some strains have a strict requirement. Optimum temperature being 37°C (range 25-40°C) and pH 7.8 (range 6.5-8.3). The pneumococcus has complex nutritional requirements and grow only in enriched media. It can grow on ordinary media, but better on media with serum, blood or heated blood, which supplies nutrient, pH buffers and catalase.

On blood agar, after incubation for 18 hours, the colonies are small (0.5-1 mm), dome shaped and glistening, with an area of green discoloration (alpha=hemolysis) around them similar to the greenish discoloration observed with the viridans streptococci. On further incubation, the colonies become flat with raised edges and depressed centrally, so that concentric rings are seen on the surface when viewed from above which is due to **autolysis by amidase enzyme of bacteria within the flat pneumococcal colonies.**

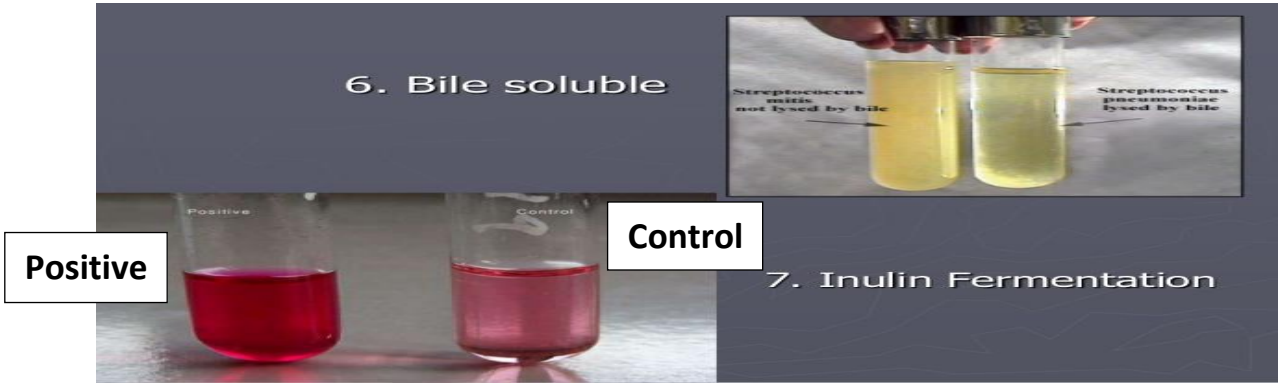
Some strains, e.g. of type 3, which form very large capsules, tend to form larger, mucoid colonies.

Under anaerobic conditions, however, a zone of beta hemolysis is produced around the colony by an oxygen labile pneumolysin-O. In liquid media such as glucose broth, growth occurs as uniform turbidity. The cocci readily undergo autolysis in cultures due to the activity of intracellular enzymes (amidase enzyme). Autolysis is enhanced by bile salts, sodium lauryl sulphate and other surface active agents. Heat killed cultures do not undergo autolysis

Biochemical Reactions

1. Inulin Fermentation : Pneumococci ferment several sugars—glucose, lactose, sucrose and inulin with the production of acid without gas. Fermentation is tested in Hiss's serum water or serum agar slopes. Fermentation of inulin by pneumococci is a useful test for differentiating them from streptococci as the latter do not ferment it.

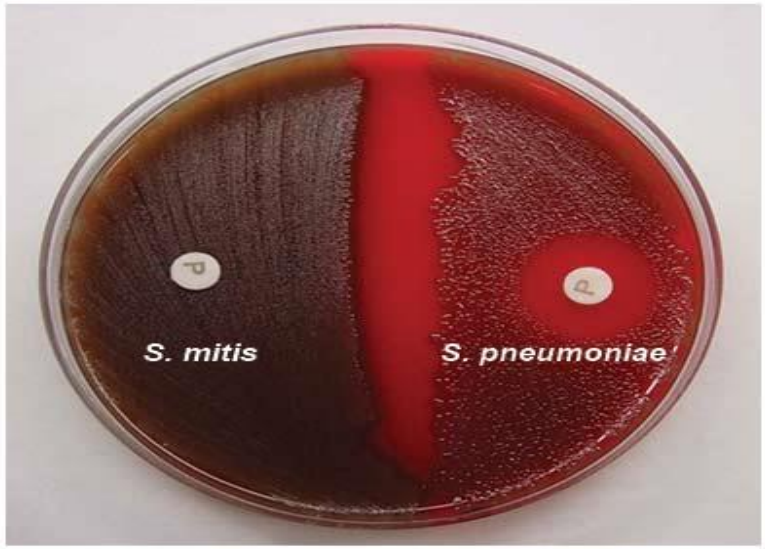
2. Bile Solubility Test : For bile solubility test, grow the isolate to be tested for 18 hours at 37°C in 5 ml serum, digest broth or infusion broth. While still warm, add 0.5 ml of 10 percent sodium deoxycholate solution and re-incubate at 37°C. Pneumococci are lysed within 15 minutes and the initially turbid culture becomes clear and transparent. Pneumococci are soluble in bile; viridans and other streptococci are not.



3. Pneumococci are Catalase and Oxidase negative

Optochin Sensitivity

Pneumococci are highly sensitive to killing by **optochin (ethyl hydrocuprein hydrochloride),** in a concentration of 1/500,00 and is useful in distinguishing them from viridans streptococci. For testing, place a paper disk containing 5 μg of optochin on an area of a blood agar plate inoculated with pneumococcus-like colonies from the primary diagnostic plate. A growth of pneumococcus will be inhibited in a zone extending radially for at least 5 mm from the margin of the disk on incubation. Viridans streptococci will grow right up to the disk.



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

Laboratory Diagnosis

1. Specimens

Sputum, **pleural fluid**, **cerebrospinal fluid** (**CSF)** or **blood** are collected **according to the site of lesion**. Sputum specimens must be mucus expectorated from the lungs rather than samples of saliva. Blood culture is useful in pnemococcal septicemia.

2. Collection and Transport

All the specimens should be collected in sterile containers under all aseptic conditions. They should be processed immediately. CSF specimen should never be refrigerated in case of delay and should be kept at 37°C (*H. influenzae*, another causative agent of pyogenic meningitis may die at cold temperature).

3. **Microscopy and Antigen Detection**

Gram stain of sputum specimens is a rapid way to diagnose pneumococcal disease. If the smears are positive for gram-positive lancet-shaped diplococci, a presumptive diagnosis of pneumococcal pneumonia may be made. A centrifuged deposit of the CSF should be examined immediately in a

Gram film in case of meningitis and presumptive diagnosis may be made by finding gram-positive diplococci both inside the polymorphs and extra-cellularly.

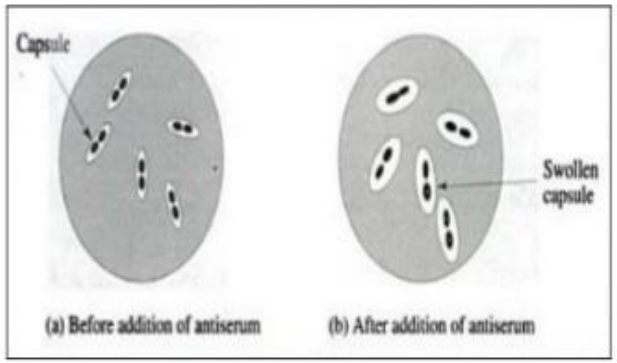
Pneumococcal antigen is often detectable by co-agglutination (COA), latex agglutination (LA) or counter immune-electrophoresis (CIE) and ELISA. COA test for antigen gives positive result in larger proportion of specimens than either Gram film or culture. Moreover, by COA test, result is available within a short time.In addition to CSF, capsular polysaccharide can be demonstrated in the blood and urine by counter immune-electrophoresis

4. Capsule Swelling Tests (Quellung reaction)

If typing sera are available, the most simple, rapid, and accurate method for the identification of pneumococci by direct examination is the **quellung reaction.** In this test, **polyvalent anti-capsular antibodies are mixed with the bacteria**, and then the mixture is examined microscopically. A greater refractiveness around the bacteria is a positive reaction for *S. pneumoniae.*

DIAGNOSIS:

- Positive Quellung test: swelling when tested against antiserum containing anti-capsular antibodies
  - Quellung reaction: technique used to detect encapsulated bacteria (such as S. pneumonia and H. influenza)



Quellung Antibody reaction

57

5. Culture

Specimen is inoculated on plates of blood agar and heated blood agar incubated in air with 5-10% CO_2 for 18-24 hours. Typical colonies develop with **α-hemolysis.** The colonies are small (0.5-1 mm), **dome** shaped and **glistening**, with an area of green discoloration (**α-hemolysis**) around them.

6. Identification

Procedures commonly used to distinguish *S. pneumoniae* from the viridans streptococci are 1) optochin susceptibility, 2) bile solubility, and 3) the quellung reaction. *S pneumoniae* is susceptible to optochin, whereas other α-hemolytic species are resistant (see the table below, please). Additional biochemical, serologic, or molecular diagnostic tests can be performed for a definitive identification.

Differences between Viridans Gp & Pneumococci

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

Species	Optochin disk	Bacitracin disk	Growth in 6.5% NaCl	Hydrolyze Bile-Esculin	Hippurate hydrolysis
S. pneumoniae	S	R	–	–	–
S. pyogenes	R	S	–	–	–
E. faecalis, faecium	R	R	+	+	–
S. agalactiae	R	R	–	–	+
Viridans streptococcus	R	R	–	–	–

7. **Blood Sample Culture** : In the acute stage of pneumonia, the organism may be obtained from blood culture in glucose broth. The finding of pneumococci in the blood is much better evidence of their pathogenic role in the lung than is their finding in sputum. Isolation of pneumococci from blood indicates bad prognosis.

Treatment of streptococcus : If you're diagnosed with strep throat, your doctor will prescribe an antibiotic to treat the infection. These medications inhibit the spread of bacteria and infections. Several types of antibiotics are available. However, penicillin and amoxicillin are the most common medications given for a strep infection.

Characteristics and clinical significance of medically important streptococci and enterococ…..See Lancefield groups……

Table 25.1: Characteristics and clinical significance of important streptococci and enterococci

Species	*Lancefield group*	*Hemolysis*	*Natural habitat*	*Associated diseases*	*Laboratory tests*
Str. pyogenes	A	beta	Throat, skin	Pharyngitis, scarlet fever, pyoderma, erysipelas, cellulitis, necrotizing fasciitis, streptococcal toxic shock syndrome, bacteremia, rheumatic fever, glomerulonephritis	Bacitracin sensitive; PYR test positive; Ribose not fermented
Str. agalactiae	B	beta	Female genital tract, rectum	Neonatal sepsis, meningitis, puerperal fever, pyogenic infections	Hippurate hydrolysis, CAMP test
S. equisimilis	C	beta	Throat	Pharyngitis, endocarditis	Ribose and trehalose fermentation
Enterococcus sp. (*Enterococcus faecalis* and other enterococci)	Group D	variable hemolysis	Gastrointestinal tract, oral cavity, gallbladder, urethra, and vagina	Urinary tract infections, endocarditis, bacteremia, abdominal infections	Growth in 6.5% NaCl; PYR positive
Nonenterococcal group D species (*Streptococcus bovis*)	Group D	alpha-hemolytic or nonhemolytic	Gastrointestinal tract	Neonatal meningitis	No growth in 6.5% NaCl
Str. anginosus group	A, C, F, G, untypable	Beta (alpha, gamma)	Throat, colon, female genital tract	Pyogenic infections	Group A strains, bacitracin resistant, PYR negative colony variants of other groups
Viridans streptococci (*Str. mitis*, *Str. mutans*, *Str. salivarius*, and many other species)	Not typed	Alpha (gamma)	Mouth, throat, colon, female genital tract	Dental caries; endocarditis	Optochin resistant, species classification on biochemical properties