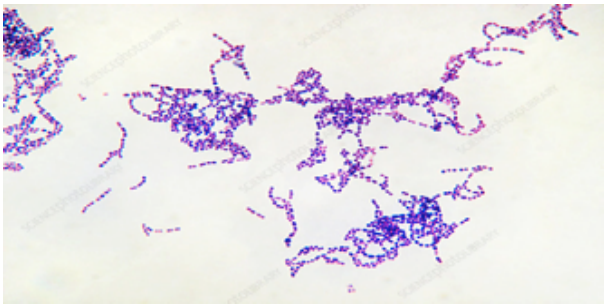


(Streptococcus)

Greek-streptus =flexible: coccus = sphere spherical or ovoid cells, occurring in short or long chins, pairs, not in groups. Capsules are not regularly formid but develop in some species. **Gram positive, better growth on enriched media, non motile, non sporing catalase negative,** responsible for a variety of diseases of man and animals. Some are commensals in the upper respiratory and intestinal tracts. Many are aerobes or facultative anaerobes.

Classification of Streptococcus

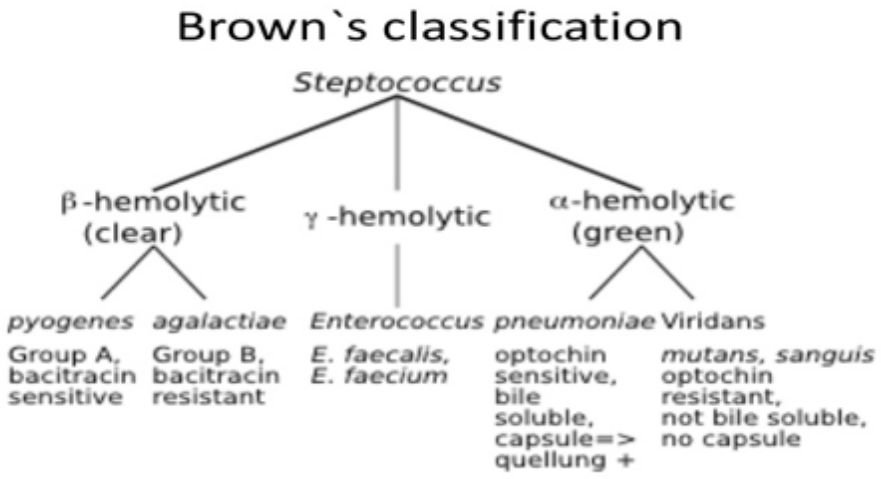
Streptococci are placed in the family streptococcaceae . Strptococcus pyogenes (Streptococcu shaemolvticus)The streptococci are gram positive spherical cells, measuring 0.8 -1 p.m in diameter, they divide in only one plane and the tendency of the cells to remain united results in the development of the characteristic chains of sphere giving the organisms their name. Chain formation results form the fact that a connecting link between the cocci probably composed of material link that forming the cell wall is retained following the cell division. These inter cellular bridges are not easily broken.

1-Classification on the basis of Oxygen requirement

i. Aerobic or facultative anaerobes: eg. *Streptococcus* spp

ii. Obligate anaerobes: eg. *Peptostreptococcus*

2-Brown classification; on the basis of haemolytic pattern on sheep blood agar

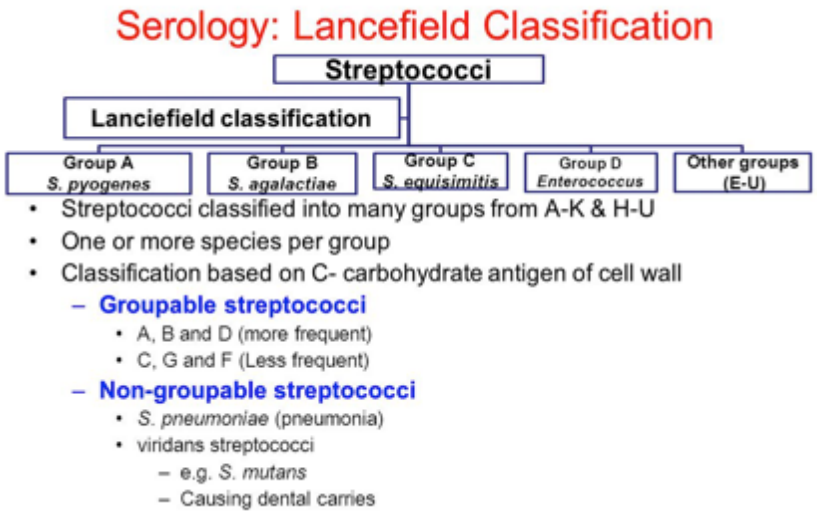


Dr. Ban Oday Abdulsattar

i. Alpha-haemolysis group:
Form incomplete haemolysis on blood agar. Shows greenish discoloration around colony and persistence of some unhaemolyszed RBCs.
Streptococcus pneumoniae, Viridians streptococci
ii. Beta-haemolysis group:
Form complete haemolysis on blood agar. Give 2-3 mm diameter zone of haemolysis
Streptococcus pyogens
iii. Non-haemolysis group
Does not cause haemolysis at all. These are non-haemolytic group
Streptococcus faecalis

3-Shermann's classification; on the basis of physiological characteristics
i. Pyogenic streptococci: Streptococcus pyogens
ii. Lactococci
Found in dairy products
They are non-haemolytic group
eg. Lactoccus
iii. Enterococci:
They are normal flora of human intestine
Enterococcus
iv. Viridans streptococci

4-Lancifield classification; serological classification



This classification is based on the difference in the structure of cell wall carbohydrate ie. group specific polysaccharide antigen. Most strain of β-haemolytic group and some strain of α- hemolytic and non-haemolytic group are classified on the basis of cell wall polysaccharide.
Streptococci are classified into 20 lancifield group from A to V except I and J.
Group A; *Streptococcus* pyogens
Group B; Streptococcus *agalactiae*
Group C; *Streptoccus equi*

Group D; *Enterococcus*
other (group E to V)
Group A streptococci ie. *S. pyogens* is further sub divided into approximately 80 serotypes by Griffith according to their specific surface protein (M, T and R). M-protein is the most important one.
The pathogenicity of Streptococci depends on the presence of hyaluronic acid capsule and surface M-protein.

5. Biochemical classification:
i. On the basis of sugar fermentation test;
Streptococci ferments most sugar with production of lactic acid but no gas.
Accumulation of lactic acid in media terminates bacterial growth.
ii. On the basis of production of certain enzymes
iii. On the basis of antibiotic susceptibility test
iv. On the basis of colony characteristics on different media
v. On the basis of haemolysis pattern

6. Classification on the basis of analysis of 16s rRNA sequence

Cultural characters:

The majority are aerobes and facultative anaerobes, the optimum temperature for growth is 37°C. Fastidious bacteria grow on medium containing blood or serum on serum broth or glucose broth: after 24hs granular growth is obtained and the medium remains clear with granules or powdery deposit.
On blood agar : This is the medium of choice, and after 24hr the colonies are circular, translucent, low convex, showing beta haemolysis (complete haemolysis) on fresh blood agar medium. The bacteria require about 15 amino acids and almost all the known members of the vitamin B complex are needed. Human blood is inferior to both horse and sheep blood in media for the identification of streptococci. The beta-haemolysis produced by pyogenic streptococcus on blood agar plates incubated aerobically is usually due to the action of an oxygen stable S-haemolysin improvement in haemolysis by anaerobic incubation is due to additional action of the oxygen labile haemolysis-O. streptococci may produce a haemolysin but may not cause beta-haemolysis. *Streptococcus pneumoniae* which causes greening broth aerobically and anaerobically, forms an oxygen sensitive haemolysin. The mucoid character of some strains is associated with the production of hyaluronic acid capsules. ((group-A streptococci are significantly more sensitive to bacitracin than strains of all other groups)) 0.1 unit of bacitracin per disc is employed on blood agar as a convenient method of differentiating *Str. Pyogenes* from other haemolytic streptococci.
Biochemical reactions: These reactions are replaced by serological procedure to identify and classify these organisms . Streptococci are catalase negative with the Possible exception of Peptostreptococcus and a few strains of group -D**.**

Antigenic structure:

A. M Protein

This substance is a major virulence factor of *S pyogenes.* M protein appears as hairlike projections of the streptococcal cell wall. When M protein is present, the streptococci are vir- ulent, and in the absence of M type-specific antibodies, they are able to resist phagocytosis by polymorphonuclear leuko- cytes by inhibiting activation of the alternate complement pathway. *S pyogenes* that lack M protein are not virulent.

B. T Substance

This antigen has no relationship to virulence of streptococci. Unlike M protein, T substance is acid labile and heat labile. It is obtained from streptococci by proteolytic digestion, which rapidly destroys M proteins. T substance permits differen- tiation of certain types of streptococci by agglutination with specific antisera, but other types share the same T substance. Yet another surface antigen has been called **R protein.**

C. Nucleoproteins

Extraction of streptococci with weak alkali yields mixtures of proteins and other substances of little serologic specificity, called **P substances,** which probably make up most of the streptococcal cell body.

Toxins and enzymes:

More than 20 extra cellular products which are antigenic are elaborated by group A-streptococci, the important toxins and enzymes formed by Streptococcus pyogenes **are:**

1-Streptolysin-S
2-Streptolysin-O
3-Erythrogenic toxin
4-Streptokinase
5-Diphoshopyridine nucleotidase (DPN-ase)
6-Hyaluronidase
7-Deoxyribonuclease
8-Streptococcal proteinase
9-Amylase
10-Streptococcal opacity factor

1-Streptolyein-S: Is responsible for the zones of haemolysis, which surrounded colonies of Streptococcus pyogenes on the surface of blood agar plates. The designation "S" refers to serum soluble due to the fact their haemolysin appears to be extracted from living streptococci, which they are shaken with serum. It is oxygen stable and sensitive to heat and acid. Streptolysin-S is cell bound and its release from the cell depends on its association with some carrier molecules such as serum albumin or ribonucleic acid. It is not antigenic

and no antibodies are formed.

2-Streptolysin-O:

Oxygen labile and produced in serum free broth. Under aerobic conditions it fails to produce haemolytic zone around colonies of streptococcus, but under reducing conditions such as deep colonies in pour plate technique or anaerobic culture haemolysin is produced. All strains of group-A streptococci produce streptolysin-O. Streptolysin-O is protein in nature and is strongly antigenic. Antibody to it is found in the sera of many patients following streptococcal infection. Antibody is known antistreptolysin. Antistreptolysin-O (ASO) titer of 200 or more is generally considered to be indicative of recent infection. Measurement of serum antistreptolysin-O has become widely used as a test for establishing the occurrence of recent streptococcal infection. Streptolysin-O is toxin for leucocytes and has cardiotoxicity.

3-Erythrogcnic toxin:

It is a protein , antigenic, relatively heat stable but destroyed by boiling for one hour. This toxin is responsible for characteristic skin rash with pharyngitis and tonsillitis in scarlet fever and its mode of action is not known. Tt is also known as **Dick toxin** after its discoverer.

Dick test:

The skin test depending on the intradermal injection of erythrogenic toxin in humans, is called **Dick test** a positive Dick test is an erythrematous and often oedematous area of more than 10mm diameter on intradermal injection of 0.2m1 of 1/1000 dilution of a filtrate of broth culture containing erythrogenic toxin. It appears with in 6-24 hours. The effect of the toxin is neutralized by antibody. A susceptible child shows positive Dick test. The same toxin, previously heated to destroy the Dick toxin is used as a control on the opposite arm. Inhibition of the effects of the toxin by antibody is further demonstrated by the fact that the intradermal injection of specific potent antitoxin into an area of scarlet fever rash, will result in **blanching** of the rash in 6-18hours during early stage of scalet fever and this is known as **Schultz and Charlton reaction.**

4-Streptokinase:

It is known as streptococcal fibrinolysin. It transforming the plasminogen of human plasma into plasmin, an active proteolytic enzyme which digests fibrin and other proteins allowing the bacteria to escape from blood clots.

5-Diphosphopyridine nucleotidase:

It is antigenic and associated with leucotoxicity and thus produces destruction of leucocytes.

6-Hyaluronidase: (spreading factor)

It is an enzyme, which splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase acid in spreading microorganisms (spreading factor).

7-Deoxyribonuclease:

Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus.

8-Streptococcal proteinase:

It is not known whether streptococcal proteinase causes. Tissue damage in the course of streptococcal infection. Some believe that it is capable of breaking down the M-protein on which the virulence of the organisms depends and thus might contribute to the patients recovery.

9-Amylase:

This enzyme hydrolyses glycogen, amyloprotein and starch.

10-Streptococcal opacity factor: (OF)

It is an alpha-lipoproteinase produced by certain serotypes which rise to opacity in serum broth.

Pathogenesis:

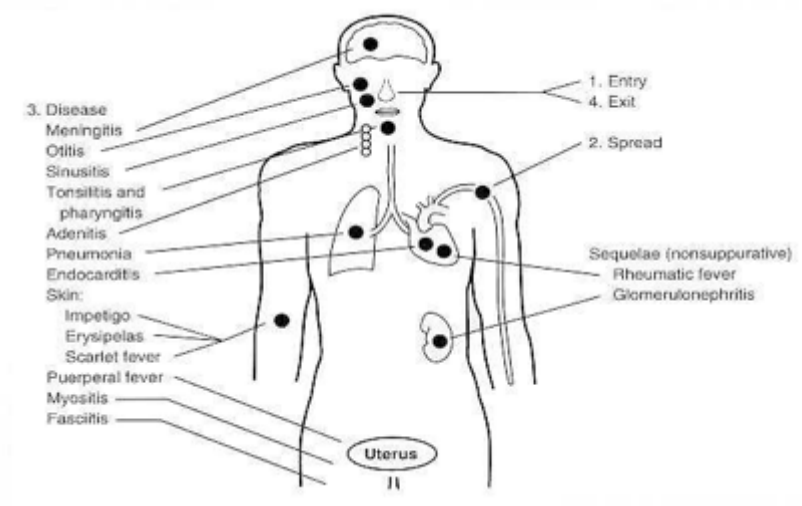
A) Acute infections: it may be 1)Tonsillitis and quinsy 2)Pharyngitis 3)otitis media 4)Paranasal sinusitis 5) Meningitis 6)Scarlet fever 7)Pneumonia 8) Erysipelas 9) Cellulitis 10) Impetigo 11) Puerperal sepsis 12) Septicemia.

B)Non suppurative complications:

1-Acute glomerulonephritis: This sometimes develops 1–4 weeks after *S pyogenes* skin infection (pyoderma, impetigo) or pharyngitis. Glomerulonephritis may be initiated by antigen– antibody complexes on the glomerular basement membrane.

2. Rheumatic fever—This is the most serious sequela of *S pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens, patients with more severe streptococcal sore throats have a greater chance of developing rheumatic fever.

ASOT (Rheumatic fever) In rheumatic fever and acute glomerulonephritis, the diagnosis of streptococcal infection may be established by demonstrating high levels of antibody to streptococcal toxins . The most frequent and widely employed test **is "antistreptolysin-O-titer(A.S.O.T.) higher levels are generally found in rheumatic fever than in acute glomerulonephritis"**



Laboratory diagnosis:

The specimens to be obtained for the diagnosis depend on the nature of streptococcal infection.

A) Acute pyogenic infection by demonstrating the causative organism. The morbid material depends on the site of the lesion. It may be throat swab, pus swab, CSF, blood. The morbid material may be subjected to the following examination.

1- Smear examination

2-Cultural examination:

Blood agar is the medium of choice for the growth of haemolytic streptococcus, special medium for the isolation of streptococci is **crystal violet blood agar, contains 1/10^{6}concentiation of crystal violet.** Aerobic and anaerobic incubation may be done, depending on the site of the lesion.

3-Fluorescent antibody technique:

Smear from serum broth culture of throat swab 2-3 hours old, may be stained with group A specific antibody conjugated with fluorescent dye, for the most rapid identification of group A streptococci in clinical disease.

4-Bacitracin sensitivity:

A disc impregnated with 0.1 unit of bacitracin is placed on the surface of a heavily inoculated blood agar culture plate before incubation to ensure rapid recognition of group A strains which are sensitive to this antibiotic.

5-Serotyping of streptococci:

Is of academic interest and a research tool, which can be only carried out in laboratories well, equipped with different antisera.

Chemotherapy: Penicillin is the treatment of choice in most cases of severe streptococcal infections.

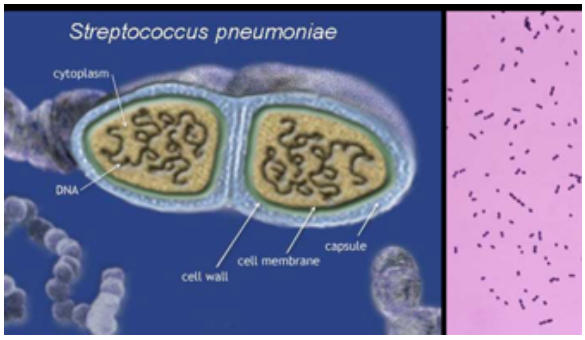
Streptococcus viridans

Many streptococci isolated from human source do not possess Lancefield polysaccharide, and they do not belong to any serological group. They **produce alpha haemolysis on blood agar.**

Biochemical reactions: Based on biochemical reactions. Streptococcus viridans group have been classified into five species (Streptococcus salivarus ; Strep. mutans ;Strep. sangius ;Strep. mitior and Strep. milleri). The biochemical reactions are very helpful in differentiating Strepococcus viridans from pneumococcus.

Streptococcus pneumoniae

Gram positive coccus, lancet shaped, in pairs, capsulated, non motile and spore former, lysed by bile salts, inhabited of upper respiratory tract of man and some animals. Causes infection primarily of the respiratory tract, conjunctivitis, otitis media, peritonitis and meningitis.



Cultural characters:

It may be cultured on the following media:

1-Glucose broth and serum broth.

2-Blood agar

3-Chocolate agar

The growth on ordinary culture media is poor, but it is greatly improved by the addition of glucose, blood, and serum. It is aerobic and facultative anaerobe. Optimum temp for growth is 37C cultivation Man atmosphere of 5-10% CO_2 by using candle jar and the **pH** of the medium should be between 7.2-7.4 after sterilization.

In liquid culture media: After 24h of incubation there is inform turbidity of the medium and prolonged incubation may produce clearing of the culture medium due to autolysis of the organisms.

Blood agar: After 24h the colonies are small about 1mm in diameter semitransparent and surrounded by **a zone of alpha-hemolysis**. The colonies first are dome shaped and later on become draughtsman colonies.

Chocolate agar: The zone of alpha -haemolysis is better seen on this medium. The pneumococci are structurally very delicate organisms and autoly much more readily than most of the other bacteria and this is due to intracellular ferments. Autolytic enzymes action the muramic acid of the cell wall only when the choline containing teichoic acid is present.

Morphology: Smear direct form the morbid material shows gram positive diplococci lanceolate and showing clear zone of halo around the diplococci. **Capsule:**

May be demonstrated either by special capsular staining or by the presence of a halo around the diplococci.

Bile salt solubility test: The bile solubility of pneumococci is a constant characteristic although different strains vary in their sensitivity to bile. The test contains of adding one part of sterilized 10% solution of sodium taurocholate in normal saline, to 10 parts of a broth culture, PH of which should not be lower than 6.8 alternatively, 0.2m1 of 10% solution of sodium deoxycholate may be added to 10m1 broth culture. The PH of the broth should be in neutral range.The lysis occurs within fifteen minutes 37C.

Optochin sensitivity: The pneumococcus is sensitive to optochin (ethyl-hydro-cuppreine hydrochloride). Sterile filter paper discs 8mm in diameter, impregnated with 51.1g of optochin, are placed over the radially streaked cultures on blood agar plate. The pneumococci are inhibited in a zone of at least 5mm from the edge of the disc strains of *Streptococcus viridans* grow up to the disc edge.

Inulin fermentation: The fermentation of inulin has been used as a differential test to distinguish pneumococcus from *Streptococcus viridans* while it is true that inulin fermentation is a property of pneumococcus, it is not reliable test when used it self, since certain strains of streptococcus especially those of the salivarus group share this property.

Antigenic structure: Penumococci have the following important antigenic components:

1-Capsular polysaccharide: (specific soluble substance(SSS)): this substance present on the surface of pneumococci diffuses into the culture medium or infective exudates and tissues. It is called specific soluble substance, at least 85 specific serotypes of pneumococci have been recognized on the basic of differences in the capsules which surrounded the cells. The capsule composed of species specific polysaccharide antigen which is immunologically distinct for each type. The type identification can be established by means of agglutination or by capsular swelling or Quellung reaction it is carried out by mixed sputum or a saline suspension of fresh growth from blood agar or loopful and mixed is cover with a. cover glass and examined under oil immersion objective. The capsule becomes apparently swollen and enlarged with in 1-2 minutes.

2-Somatic M-protein: As the indicates, this is somatic protein of pneumococci which contains an M-protein antigen. This is deep in the cell and is characteristic for each type.

Antigen structure of pneumococci

3-Somatic C-arbohydrate:

Antigen is common to all pneumococci.

The C-reaction protein, a substance found in the serum of certain patients suffering from inflammatory or destructive lesions . Antibodies to these antigens are not protective.

C-Reactive proteins (CRP)

These are abnormal proteins **(beta globulin)** that precipitate with somatic C-antigen of pneumococcus. This appears in the acute phase sera of cases of pneumonia and other acute infections. This is known as the C-reactive protein(CRP) . it is not an antibody, but is an acute phase substance whose production is stimulated by bacterial infections, inflammatory reaction and tissue destruction. The test is performed by passive agglutination using latex particles coated with anti CRP-antibody.

Pathogenicity: It may cause lobar pneumonia, otitis media,meningitis, eritonitis, sinusitis, conjunctivitis and septicemia.

Laboratory diagnosis: Common material referred to the laboratory for isolation and identification of pneumococci are:

1-sputum 2-laryngeal swab 3-C.S.F. 4-pus 5-pleural fluid 6-blood

The examination of the material may be considered under the following heading:

1-Direct smear examination.
2-Capsular swelling reaction (Quellung reaction)
3-Culture
4-Animal pathogenicity

Streptococcus pneumoniae **can be differentiated from Viridans streptococci by various features:**

	Properties	**Pneumococcus**	**Viridans streptococci**
1	Morphology	Lanceolate or flame-shaped	Round/oval
2	Arrangement	Gram-positive cocci in pairs	am-positive cocci in long chains
3	Capsule	Present	Absent
4	On blood agar	Draughtsman or carom coin colony	Convex shaped colony
5	Liquid medium	Uniform turbidity	Granular turbidity
6	Bile solubility	Soluble in bile	Insoluble in bile
7	ulin fermentation	Fermenter	Non-fermenter
8	Optochin	Sensitive	Resistant
9	lice Pathogenicity	Pathogenic	Non-pathogenic
10	Quellung Test	Positive	Negative
11	Hemolysis	Alpha-hemolytic (under aerobic conditions) or Beta-hemolytic (under anaerobic conditions)	Alpha hemolytic or non-hemolytic
12	Pathogenesis	Causes pneumonia	Causes mainly oral infections