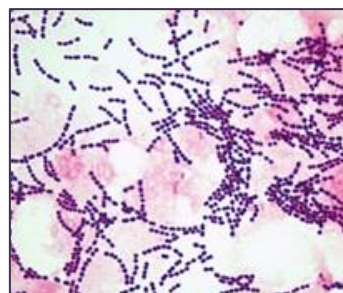


Medical Bacteriology- Lecture 6

Streptococci



Classification of Streptococci based on

(1) - Hemolysis reactions on blood agar) (Brown in 1903)

The type of hemolytic reaction on blood agar has long been used to classify the streptococci.

Beta -hemolysis

complete lysis of red cells surrounding the colony
appearance as Clear zone

Streptococcus pyogenes are beta-hemolytic

alpha-hemolysis

partial hemolysis (Greenish Discoloration) associated with reduction of red cell hemoglobin.

S. pneumoniae are alpha-hemolytic (but can cause β -hemolysis during anaerobic incubation).

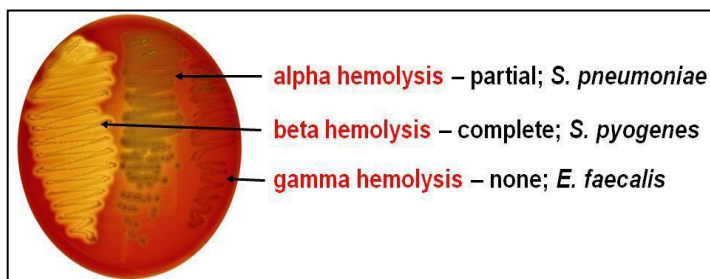
gamma-hemolytic

Non hemolytic colonies.

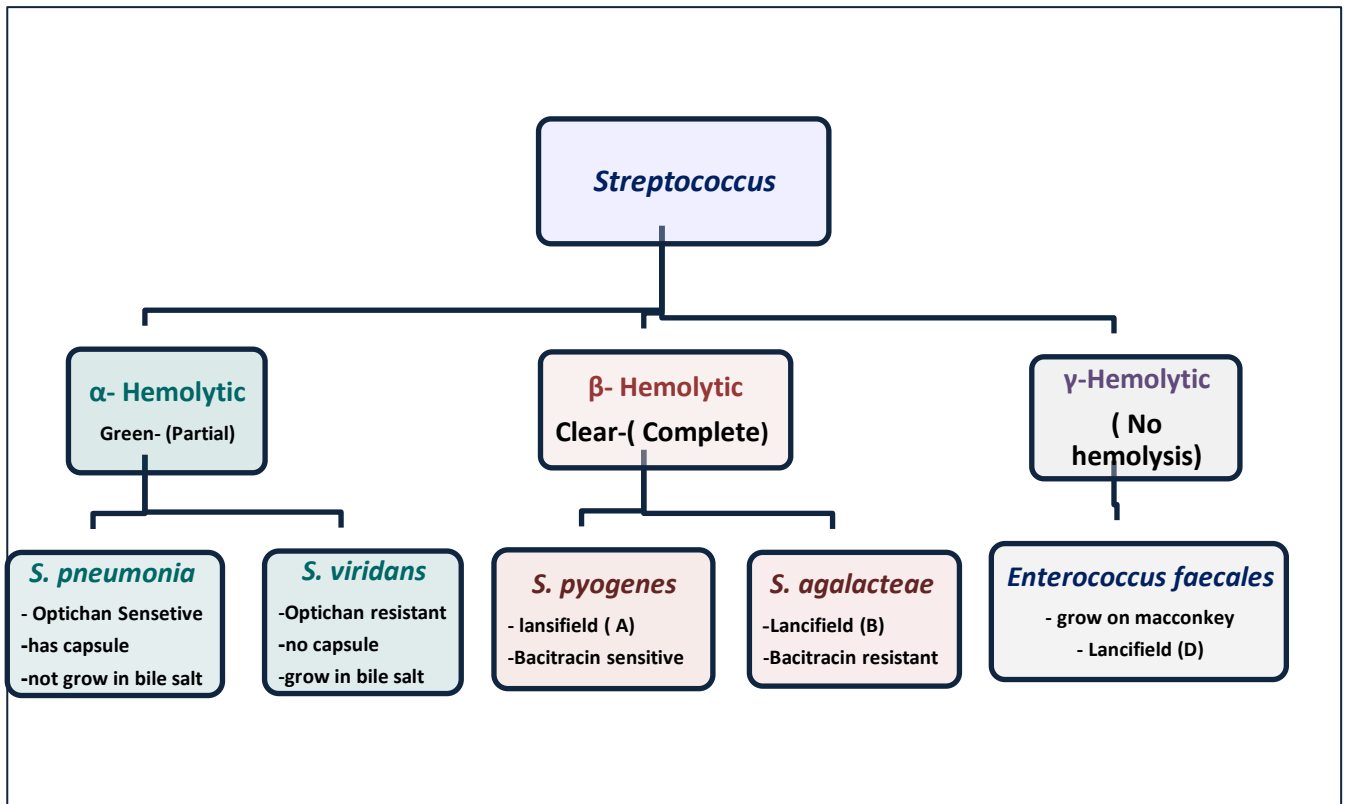
Enterococcus faecalis

Most of oral streptococci and enterococci are non- hemolytic.

The property of hemolysis is not very reliable for the absolute identification of streptococci, but it is widely used in rapid screens for identification of *S. pyogenes* & *S. pneumoniae*.



Groups of Streptococci (1- Hemolysis on blood agar)



(2) - Antigenic types of carbohydrate (Serology)

The cell wall of Streptococci is composed of repeating units of N-acetylglucosamine and N-acetylmuramic acid, (standard peptidoglycan).

The identification of streptococci based on the serologic reactivity of "cell wall" polysaccharide antigens described by **Lancefield classification**.

Lancefield developed serotyping system for classification of **beta-hemolytic streptococci or gamma** based on the antigenic composition of **specific cell wall carbohydrates (C- Carbohydrates) or C- Substrate**

Viridians streptococci & *Strep. pneumonia* are not grouped under Lancefield Classification (have no group-specific antigen).

18 group-specific antigens (Lancefield groups) were known.

The clinically important streptococci are grouped under A, B, C, D, F and G.

The main species and groups of medical importance :

–Group A Streptococci (GAS): *Streptococcus pyogenes*

The cell surface structure *Strep. pyogenes* is the most studied of any streptococci bacteria.

Group A polysaccharide (C substance or group carbohydrate antigen) is a polymer of N-acetylglucosamine and rhamnose.

–Group B Streptococci (GBS): *Strep. agalactiae*

–Group D Streptococci: *Enterococcus faecalis*

(3)- Biochemical /Physiological properties

Bergeys manual of bacteriology, classified Streptococci based on growth at 6.5 % NaCl, 10 and 45 degree centigrade .

Growth in 6.5% NaCL	Temperature		Group
	45 °C	10° C	
-	-	-	<i>Strep. pyogenes</i>
-	+	-	<i>Strep. Viridans</i>
+	+	+	Enterococcus
-	-	+	Lactic acid bacteria

***Streptococcus pyogenes* (Group A streptococcus)**

General characteristics

Gram-positive

Coccus, occurs in chains of varying lengths

Catalase negative

Oxidase negative

Nitrate negative

Facultative anaerobic (the metabolism of *S. pyogenes* is fermentative)

Requires enriched medium containing blood to grow.

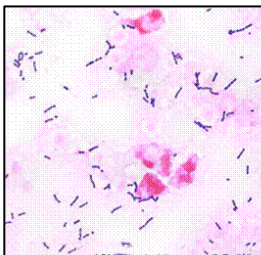
Group A streptococci have a capsule composed of hyaluronic acid

Beta hemolysis on blood agar

Non-motile

Non spore-forming.

S. pyogenes is usually an exogenous secondary invader, following viral disease or disturbances the normal flora.



Gram stain of *Strep. pyogenes* in a clinical specimen and from pure culture.

Pathogenesis of *Strep. pyogenes*

It is a major success pathogen to its ability to colonize, rapidly multiply and spread in host while evading phagocytosis and confusing the immune system.

It is found usually in the respiratory tract, without signs of disease. *Strep. pyogenes* can infect when defenses are compromised or when its able to penetrate the constitutive defenses.

Streptococcal diseases are associated in **respiratory tract (pharyngitis or tonsillitis)**, **bloodstream**, or the **skin (pyoderma)**.

Summary of diseases caused by *Strep. pyogenes*

Suppurative infections (associated with pus occur in the throat, skin & systemically).

Acute diseases:

- **Respiratory tract infections**

Throat: *S. pyogenes* is the leading cause of **pharyngitis (strep throat)**. It is acquired by inhaling aerosols by infected individuals. The symptoms reflect the inflammatory events at the site of infection.

sinusitis, otitis, and pneumonia.

- **Skin**

Impetigo (superficial) infection of epidermal layers of skin.

Cellulitis (deep) occurs when the infection spreads subcutaneous tissues.

Necrotizing fasciitis (destructive wound infections) infection of the fascia & may proceed rapidly to underlying muscle. It is **severe invasive infections**, prompting descriptions of "flesh eating bacteria".

- **Systemic**

Scarlet fever (rash); a severe complication of streptococcal infection, but now, because of antibiotic therapy, it is little more than streptococcal **pharyngitis** accompanied by rash. It caused by **erythrogenic toxin**.

Toxic shock; caused by a few strains that produce a toxic shock toxin (Superantigens). Scarlet fever & streptococcal toxic shock syndrome are systemic responses to circulating bacterial toxins.

Invasive, toxigenic streptococci cause **joint or bone infections**, and **myositis, meningitis** and **endocarditis, Bacteremia**.

Non-suppurative Sequelae:

Two post streptococcal sequelae rheumatic fever & glomerulonephritis, may follow streptococcal disease, and occur in 1-3% of untreated infections.

Due to immunological reactions to *Strep. pyogenes* antigens. Some of the antibodies produced during the above infections cross-react with certain host tissues. These can indirectly damage host tissues after the organisms cause non suppurative complications.

Rheumatic fever. M protein cross reacts with sarcolemma.- Antibodies cross-react with heart tissue, fix complement, and cause damage.

Glomerulonephritis. Antigen-antibody complexes may be deposited in kidney, fix complement, and damage glomeruli- Only a few M-types are nephritogenic.

Virulence Factors of *S. pyogenes*

Strep. pyogenes produces a wide array of virulence factors, include:

(1) **Protein M** (major virulence factor) , fibronectin-binding protein (**Protein F**) and **lipoteichoic acid** for adherence (inhibit phagocytosis).

(2) **hyaluronic acid capsule** (non antigenic) as an immunological disguise and to inhibit or prevent opsonized phagocytosis by neutrophils or macrophages.

(3) **invasins** such as Haemolysins, **streptokinase**, **hyaluronidase** and **streptolysin** (streptolysin O, streptolysin S) (ASO test)

Hemolysin	Streptolysin O	Streptolysin S
Stability of Oxygen	No	yes
Antigenic	Yes	No (small)

(4) **Exotoxins**, such as **pyrogenic (erythrogenic) toxin**; causes the rash in **scarlet fever** and systemic **toxic shock syndrome**.

5-DNAase (streptodornase- 4 types)

6-Protease

7- Amylase

8- C5a peptidase (evasion of phagocytosis)- C5a enhances chemotaxis of phagocytes

Host defenses against *Strep. pyogenes* infections

In the normal human the **skin** is an effective barrier against invasive streptococci, and **nonspecific defense** mechanisms prevent the bacteria from penetrating the superficial epithelium of the **upper respiratory tract** (cilia movement, coughing, sneezing and epiglottal reflexes).

The **host phagocytic system** is a **second line** of defense against streptococcal invasion. Organisms can be opsonized by activation of the **complement** pathway and by **anti-streptococcal antibodies** in the serum.

Strep. pyogenes is rapidly killed following phagocytosis enhanced by specific antibody.

The bacteria do not produce **catalase** or significant amounts of **superoxide dismutase** to inactivate the oxygen metabolites (hydrogen peroxide, superoxide) produced by the oxygen-dependent mechanisms of the phagocyte. Therefore, they are quickly killed after engulfment by phagocytes. The streptococcal defense must be one to stay out of phagocytes.

In **immune individuals**, **IgG antibodies** reactive with **M protein** promote phagocytosis which results in killing of the organism. This is the major mechanism to terminate Group A streptococcal infections.

Antibody against M protein antigen is the only effective protective antibody, but there are more than 50 different M types (antigenic variation), and subsequent infections may occur with a different M serotype

Treatment and prevention

Penicillin is still effective in treatment of GAS disease and to prevent sequelae.

No effective vaccine has been produced, but specific M-protein vaccines are being tested.

M protein vaccines are a major candidate for use against rheumatic fever, but certain M protein types cross-react antigenically with the heart and themselves may be responsible for rheumatic carditis. This risk of autoimmunity has prevented the use of Group A streptococcal vaccines.

Streptococcus agalactiae

Beat hemolytic

Lancefield group B

Regularly resides in human **vagina, pharynx and large intestine**

Can be transferred to infant during delivery and cause severe infection.

Diseases;

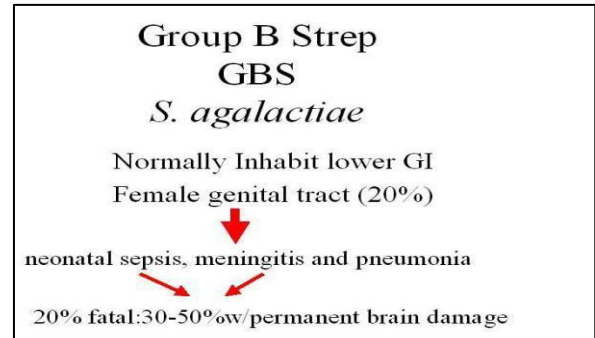
Puerperal sepsis

Neonatal sepsis

pneumonia

meningitis

Septic abortion



Groups A & B Streptococci are treated with penicillin/ Erythromycin.

No vaccines available

Enterococcus faecalis

Lancefield Group D

normal colonists of human large intestine

Causes; **Nosocomial Infections, opportunistic urinary**, wound and skin infections

Grow in the presence of 6.5% NaCl.

Grow on MacConkey agar

Usually non-hemolytic or α hemolytic

Naturally high levels of antibiotic resistance

Sensitivity testing needed for enterococci

Treatment (Penicillin+ Gentamycin)

No vaccines available

Viridians streptococci

They possess no Lancefield antigens.

Non haemolytic- alpha haemolytic.

Optochin resistant.

Not soluble in bile salts

Heterogeneous group of organisms

- Human commensals
- Pathogens

Causes; endocarditis, bacteremia

eg. *Streptococcus mutans* (dental caries)

Streptococcus salivarius

Streptococcus pneumoniae (*Diplococcus pneumoniae*)



Gram-positive, cocci. Usually, pairs of cocci (diplococci)

alpha hemolytic, cultured in media that contain blood (**fastidious**) - growing best in 5% Co₂.

Possess a capsule of polysaccharide that permits typing with specific antisera

Young colonies resemble dew-drops due to capsule- spontaneous autolysis of older bacteria.

Fermentative aerotolerant anaerobe

Special tests such as inulin fermentation, bile solubility, Quelling reaction, optochin antibiotic.

Like other streptococci, they lack catalase and ferment glucose to lactic acid.

Do not display C- substrate of cell wall composition.

normal inhabitant of the human upper respiratory tract

can cause **pneumonia**, sinusitis, otitis media, meningitis- It also causes osteomyelitis, septic arthritis, endocarditis, cellulitis and brain abscesses.

usually secondary to one of the former infections.

***Strep. pneumoniae* is currently the leading cause of invasive bacterial disease in children and the elderly.**

do not form spores.

non-motile.

sensitivity must be routinely employed to differentiate the pneumococcus from *Strep. viridans*.

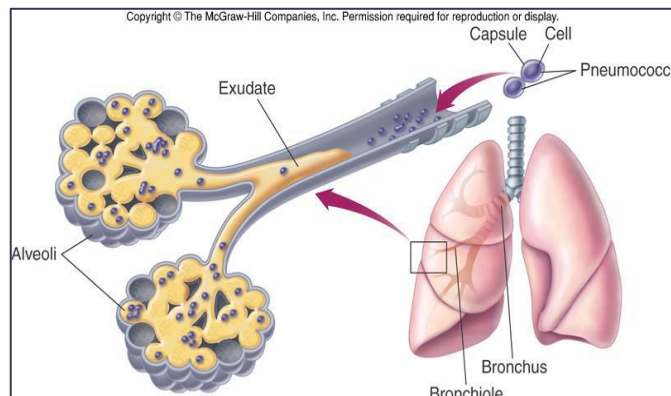
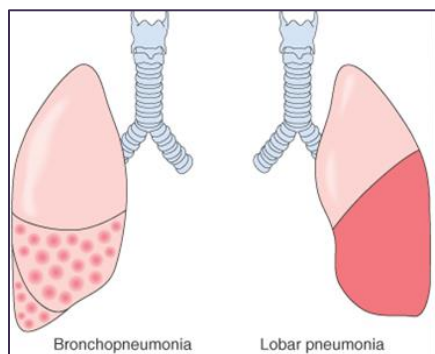
Pneumonia is a disease of the lung that is caused by a variety of bacteria including *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Haemophilus*, *Chlamydia* & *Mycoplasma*, several viruses, and certain fungi and protozoans.

The disease may be divided into two forms,

bronchial pneumonia and lobar pneumonia.

Bronchial pneumonia: most prevalent in **infants, young children and aged adults**. It is caused by **various bacteria, including *Strep. pneumoniae***. produces Patchy Pneumonic Consolidation

Lobar pneumonia: occur in **younger adults**. A majority (**more than 80%**) of the cases of lobar pneumonia are caused by ***Strep. pneumoniae***. causes consolidation of whole lobe.



Virulence factors of *Strep. pneumoniae*

1- Capsule (polysaccharide)

Completely envelops the pneumococcal cells.

The capsule is an essential determinant of virulence.

The capsule interferes with phagocytosis by preventing complement C3b opsonization of the bacterial cells.

90 different capsule types of pneumococci have been identified and form the basis of antigenic serotyping of the organism.

2- IgA protease

Treatment and vaccination

- Traditionally treated with

Amoxicillin

Chloramphenicol

Third generation Cephalosporins

- **Anti-pneumococcal vaccines** are based on formulations of various capsular (polysaccharide) antigens derived from the highly-prevalent strains

Review of Questions

- 1- What are major characteristics of *Streptococcus pyogenes*?
- 2- Give four examples of acute disease that causes by GAS, two examples of non suppurative sequels?
- 3- What are virulence factors of *S. pyogenes*?
- 4- What is the effective antibiotic to treat CAS infections? And what is the ineffective antibiotic against *S. aureus* infections?
- 5- You studied three categories to classify Streptococcal species, what they are, discuss haemolytic basis and serology classification with give examples?
- 6- Why the alpha haemolytic Streptococci not included with Lancifield classification?
- 7- Compare between *Strep. pneumonia* & *Strep. viridians* - *Strep. pyogenes* & *Strep. agalactiae*?
- 8- Compare between two types of pneumonia?
- 9- What is the major virulence factors of *Strep. pneumoniae*?
- 10- Give two examples of *Strep. agalactiae* infections?
- 11- What are major characteristics of *Enterococcus faceless*, *Strep. pneumoniae*?