True bacteria – Cocci- Gram positive cocci

Streptococci

Most of *Strep*. species are commensal resident of mouth, throat, so several may act as opportunistic pathogens, also few spp. like *Strep*. *pyogenes* and *Strep. agalactiae* act as primary pathogen.

Classification of Streptoccoci

Strep. are classified by 2 major methods:

1- Classification by hemolytic activity:

The initial classification of the *Strep*. depends on the type of hemolysin produced on blood agar (under anaerobic conditions about (5-10 % Co_2): (Figure 1).

a- Strains that produce soluble hemolysin(Streptolysin O or S) colonies that produce streptolysin causing complete clearness around the colony in blood agar are called β-hemolytic *Streptococci e.g Strep. pyogenes, Strep. agalactiae.*

b- Strains that produce insoluble hemolysin which causes partial lysis called α-hemolytic Streptococci e.g *Strep. pneumonia*, *Strep.viridans.*

c- Starins that are non-hemolytic, give no change around the colonies called γ-hemolytic streptococci e.g *Strep. faecalis*.

2- Serological classification (Lancefield classification).: (Figure 2)

Made by Lancefield, based on the presence of polysaccharide antigen called "C-substance" or "C-carbohydrate" present in the cell wall which differ from group to another .A,B,C,D which are implicated in human infections, and E,F,H,G and K-U which are implicated in animal infections.

➔ Group A causes tonsillitis in human.

➔ Group B causes mastitis which is transmitted from cows to human through contaminated milk.

➔ Group C causes strangles disease which transmitted to human from horses.

➔ Group D which are enterococci present as normal flora in intestine of human.

All above are β- hemolytic *Strep*. and the clinical important groups are types A and B.

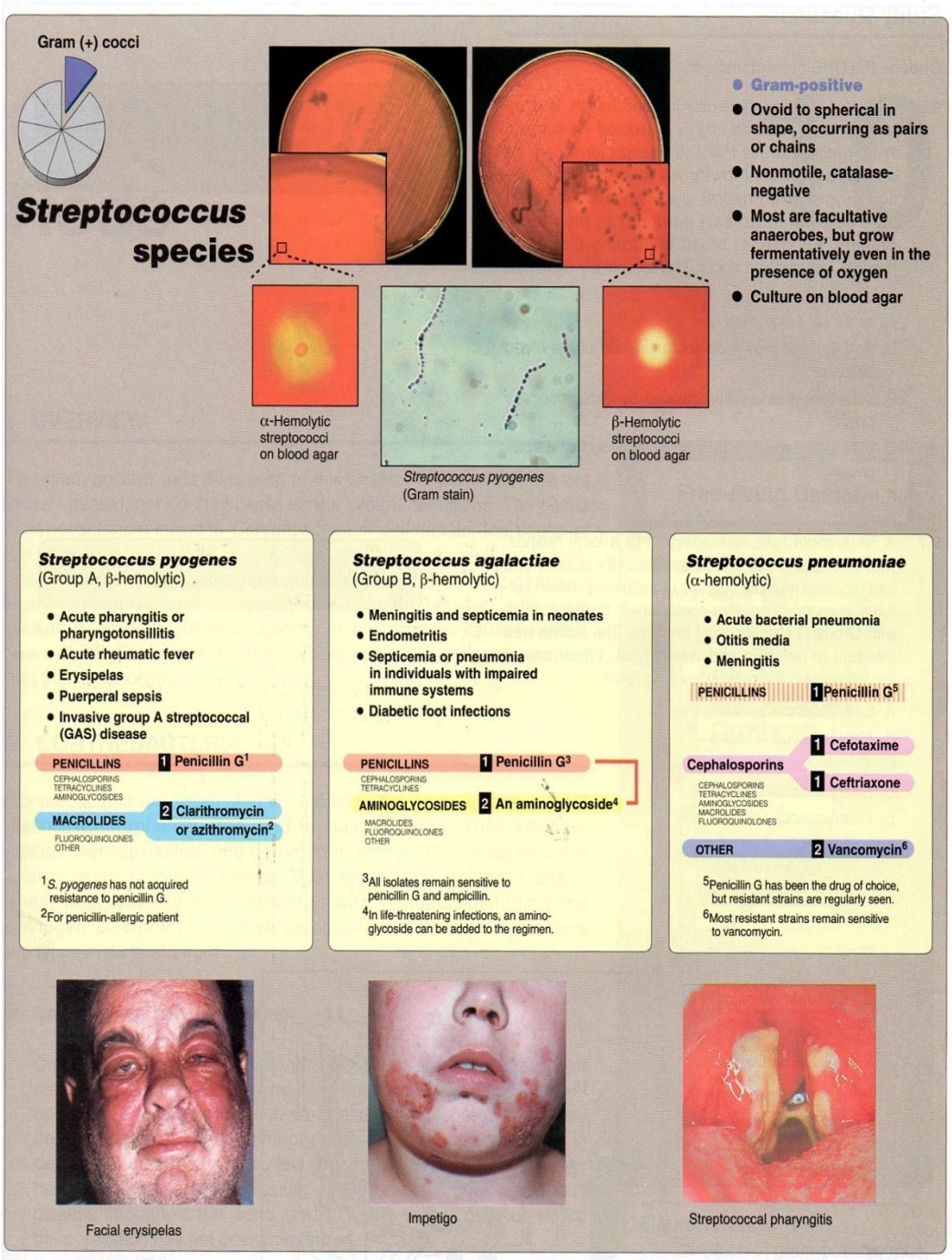

Figure 1
Summary of streptococcal disease.

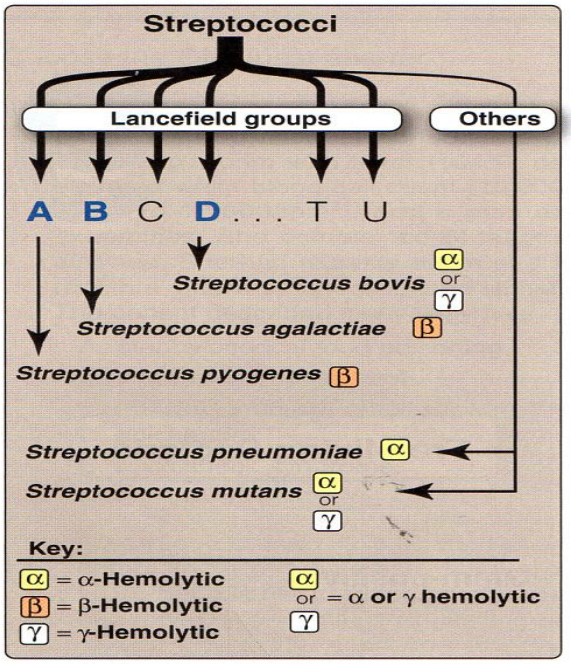

Figure 2
Classification schemes for streptococci.

Antigenic Structure of Streptococci:

Several antigentic substances are formed in the hemolytic *Strep*. cells:

1- **Group-Specific cell wall antigen**

This carbohydrate is contained in the cell wall of many *Streptococci* and forms the basis of serologic grouping (Lancefield A-U)which is determined by an amino sugar which are:

➔ Group A Rhamnose-N- acetylucosamine.
➔ Group B Rhamnose-glucosamine polysaccharide.
➔ Group C Rhamnose-N-acetylgatasamine.
➔ Group D glycerol techoic acid containing D-alanine and glucose.

2- **M-protein**

This protein is associated with virulence of group A and occurs in organisms producing mucoid colonies.This protein resists the phagocytosis.

3- **T- substance**

This antigen has no relationship to virulence of *Strep*. and used to differentiate between certain types of *Strep*. by agglutination with specific antisera.

4- **Nucleoproteins (P-substance)**

That make up most of the *Strep*. cell body and a little of serologic specificity.

Groups of Streptococci

A- β-hemolytic Streptococci

1) Group A *Streptococcus pyogenes*

is the human pathogen of primary importance, produce human respiratory infection, such as Tonsillitis, bronchopneumoniae, scarlet fever, erysipelas, cellulitis, glomerulonephritis and rheumatic fever. Group A are usually bacitracin sensitive.

2) Group B *Streptococcus agalactiae*

are endogenous to the vaginal mucosa and been responsible for puerperal fever, fatal neonatal meningitis and endocarditis.This group is rarely bacitracin sensitive.

3) Group C and G

Causes erysipelas, puerperal fever, throat infection, sinusitis, bacteremia or endocarditis, occur sometimes in pharynx and produce β-hemolysin on sheep blood agar e.g. *Strep. equisimilis* and *Strep.equi*

4) Group D

Includes Enterococci, e.g. *Strep. faecalis* and non-Enterococci,e.g., *Strep. bovis* produces α and γ hemolysin which may causes UTI or endocarditis.

5) Group E, F, H and K, U

Occur primarily in animals others can infect humans.

B- Non β-hemolytic Streptococci

These usually produce α-hemolysis or no hemolysis on blood agar. This group includes:

1- ***Strep. pneumoniae*** **(pneumococci)**

(1) bile soluble , (2) inhibited by optochin disks , (3) Quellung reaction positive (capsule swelling test) (4) can cause pneumomia and other infection processes.

2- *Strep. viridans*

(1) not bile soluble , (2) not inhibited by optochinn disks , (3) Quellung reaction negative, (4) they are normal flora in upper respiratory tract in human and may cause endocarditis.

C- Peptostreptococci

They are members of the normal flora of the gut and female genital tract, grow under aerobic or microaerophilic condition and produce variable hemolysin.

Group A Hemolytic Streptococci :

The most clinically important species of this group is *Strep*. *pyogenes* it can invade in the skin or mucous membrane and cause infection .

Structural Features :

These are involved in the identification of Group A *Streptococci*:

Capsule:

Hyaluronic acid resemble to that found in human connective tissue, it is not recognized as foreign by the body, so it is non-immunogenic.

Cell wall:

Contains no. of clinically important components:

a. Fimbriae: contain the major *Strep*. *pyogenes* virulence factor, the M protein .
b. Group A specific C-carbohydrate: composed of rhamnose & N-acetylglucoseamine (all gp A *Strep*. contain this antigen).
c. Protein F (Fibronectin binding protein): which mediated attachment to fibronectin in pharyngeal epithelium .

Extracellular products play a role in the pathogenesis (Figure3)

More than 20 extracellular products (exotoxins) that are antigenic are produced by group A *Strep*. including :-

1- Streptokinase (Fibrinolysin)

produced by many strains of group A hemolytic *Strep*. Transforms the plasminogen of human plasma into plasmin, which is an active proteolytic enzyme that digests fibrin and other proteins.

2- Streptodornase (DNase)

This E. depolymerizes DNA in exduates and purulent exudates, their viscosity to deoxyribonucleoprotein, both streptokinase and DNase help to liquefy exudates and facilitate removal of pus and necrotic tissue, both enzymes are used for diagnosis test of *Strep.* in general.

3-Hyaluronidase

E. splits hyaluronic acid, an important component of connective tissue. This hyaluronidase aids in spreading infecting microorganisms (Spreading factor), it's antigenic.

4- Diphosphopyridine Nucleotidase

E is kill leukocytes. Proteinase and amylase are produced by some strains.

5-Erythrogenic Toxin (Pyrogenic exotoxins)

It causes the rash in scarlet fever and elaborated by lysogenic *Strep.* (*Strep. pyogenes*) also called **Dick toxin**, and detected by dick test (anti toxin).

6- Hemolysin

In β-hemolytic group A *Strep. pyogenes* elaborates two hemolysins (streptolysins):

a- Streptolysin O= labile and inactivated in O_2 , this antigen is responsible for the produce of Ab called (antistreptolysin O) (ASO) in human following infection and act as a titers for diagnosis if the serum titers of 160-200 units suggests recent infection.

b- Streptolysin S=stable in O_2 and causes hemolytic zones around *Strep*. colonies on blood agar this is not antigenic.

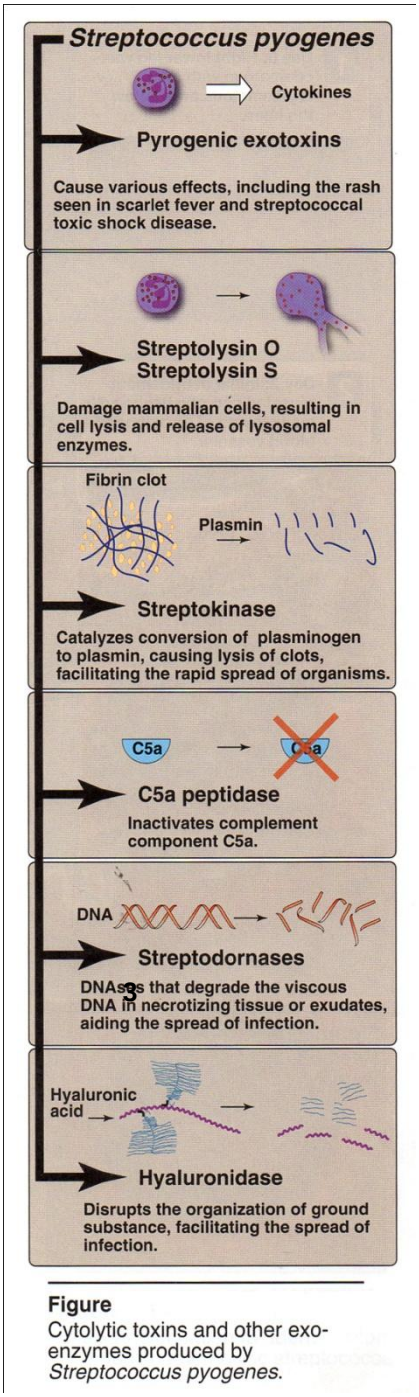

Figure
Cytolytic toxins and other exo-enzymes produced by *Streptococcus pyogenes.*

Clinical Findings

A- **Disease due to invasion :** by β-hemolytic group (A) such as *Strep. pyogenes :* Pathogenicity is determined by (1) portal of entry of the *Strep*., (2) diffusion of spreading of *Strep*. to different parts of the body (3) extend along lymphatic pathways → to the blood stream.

1- Erysipelas

If the portal of entry is the skin, erysipelas results with massive brawny edema, and rapidly advancing margin of infection.

2- Puerperal fever

If the *Strep*. enters the uterus after delivery, puerperal fever develops which is essentially a septicemia originating in the infected wound.

3- Sepsis/ bacteremia

Infection of traumatic or surgical wounds with *Strep*. results in bacteremia/ sepsis, which can be fatal rapidly.

B- Diseases due to local infection: by β-hemolytic group (A)

1- Streptococcal sore throat

Patients have intensive nasopharyngitis, tonsillitis and intense redness and edema of the mucous membrane, with purulent exudates, and usually a high fever.If the infecting *Streptococci* produce erythrogenic toxin scarlet fever rash will be produced.

2- Streptococcal pyoderma

Local infection of superficial layers of skin, in children, called "impetigo".

C- Infection of endocarditis

1- Acute endocarditis

Rapid destruction of the valves leads to fatal cardiac failure.

2- Subacute endocarditis

Due to members of the normal flora of respiratory or intestinal tract that have accidently reached the blood.

D- Other infection

Various *Strep*., such as *Enterococci* can cause UTI, *Peptostreptococci* which occurs in the female genital tract, the gut and intestine causes suppurative lesion in the genital tract of the female.

E- Post streptococcal disease

1- Acute glomerulonephritis

occurs after 3 weeks of the infection by *Strep*. group A and about 23% of children with skin infection with type 49 strain develops nephritis or hematuria.

2- Rheumatic fever

This is the most serious of *Strep*. group A infections, because it results in damage to heart muscles and valves, appears after 1-4 weeks of infection, causes a complex infection all parts of the heart (endocardium – mycocardium – pericardium).

Diagnostic laboratory tests

Specimens : depend upon the nature of *Strep*. infection, as swab, pus, blood is obtained for culture. Serum is obtained for Ab determination.

Microscopic examination : G+ spherical or ovoid bacteria that are arranged in chains of varying length, nonsporing , nonmotile ,noncapsulated .

Macroscopic examination: facultative anaerobes require enriched media as blood agar for growth. On blood agar incubated aerobically and anaerobically to show the type of hemolysis and colonial appearance. catalase negative

***Strep*. group A sensitive for bacitracin .**

Serologic test : by using ELISA, or Agglutination test, also antistreptolysin O (anti ASO) in respiratory infections, antihyaluronidase in skin infection. Antistreptokinase, anti-M type – specific Abs and others of these. The anti ASO titer is most widely used.

Treatment

All β-hemolytic group A *Strep*. are still susceptible to penicillin G and most are sensitive to erythromycin.

In endocarditis, which caused by enterococci, a combination of penicillin G and aminoglycoside is used as treatment.

Penicillin prophylacticin is given to person with rheumatic fever, to prevent recurrence of the disease (monthly for several years).

Amoxicillin is used for dental procedure.

Streptococcus agalactiae (group B, β-hemolytic)

Found as normal flora in vagina in 5-25% of woman and in urethral mucous membranes of male carriers as well as in the G.I.T.

Their colonies on blood agar are larger and less hemolysis than Group A β-hemolytic *Strepococci* (*Strep. pyogenes*).

It can transfer from infected mothers to infants at birth and may cause (1) meningitis and septicemia in neonates with high mortality rate, (2) endocarditis endometritis,
(3) septicemia or pneumonia in impaired immune systems individuals .
(4) diabetic foot infections.

Sensitive to penicillin G and ampicilline. ***Strep*. group B resistant for bacitracin .**

Streptococcus pneumonia

Antigenic Structures

It posses a number of Ags:

A- The capsular polysaccharides (SSS)

Specific Soluble Substance in capsulated form. Capsular polysaccharides are used to determine the type specificity of organisms and virulence.Typing can carried out by:

1- Agglutination of cocci with type – specific antisera (Ag + Ab reaction).
2- Precipitation of capsular polysaccharide with specific serum.
3- Capsular swelling reaction (Quellung reaction). Suspension of pneumococci is mixed with specific anti-polysaccharide serum of the same type or the polyvalent antiserum on slide, the capsule swells.This reaction useful for rapid identification and for typing of the microorganism (Figure 4).

B- M- Protein

It's characteristic for each type of *Strep. pneumonia*

C- Group – Specific Carbohydrate

This is common in all pneumococci , which it can be precipitated by (c-reactive proten) in the serum of patients.

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Toxin and Virulence Factors

1- Pneumolysin O

an oxygen sensitive toxin, cytolytic for cells.

2- Neuraminidase

E degrades surface structure of host tissues.

3- Capsular polysaccharide

It's SSS which protects the microorganism from phagocytosis and has association with virulence.

4- C- substance

The cell wall of pneumococci contain teichoic acid (C-sub) that react with a certain antiserum protein, C- reactive protein (CRP) and causes activation of some nonspecific host immune response.

5- Leucocidin

Kills leukocytes W.B.C.

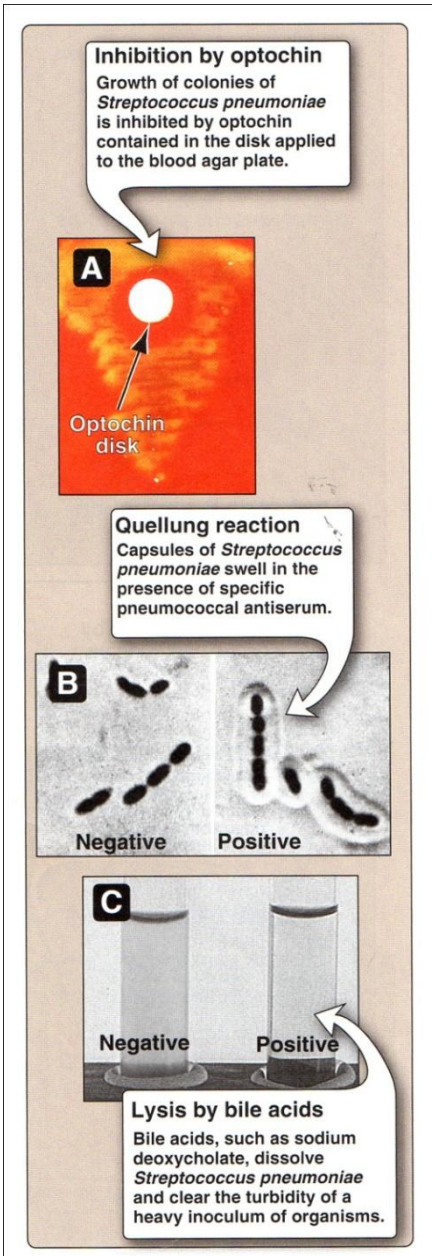

Figure
Laboratory tests useful in the identification of *Streptococcus pneumoniae*. A. Optochin disk test. B. Quellung reaction. C. Lysis by bile acids.

Pathogenesis

It's a normal flora of nasopharynx and oropharynx in 15% of children and 5% of adults.

They produce disease through their ability to multiply in the tissues and the virulence of pneumococci is a function of it's capsule which prevents ingestion by phagocytes.

The normal respiratory mucosa posses natural resistance to the pneuomococci, many factors lower this resistance and predispose to infection, like:

1- Respiratory viral infection, bronchial obstruction, respiratory tract injury, alcohol or drug intoxication .

2- Organism passes from person to another via respiratory secretions and aerosols.

Clinical Findings

The onset of pneumococcal pneumonia is usually sudden with fever, chills and sharp pleural pain, with blood or rusty sputum. In the early disease, when the fever is high, bacteremia is present in about 10-20% of cases, causes severe complication as meningitis, which is the most serious infection common in children, endocarditis, septic arthritis.

1- Acute bacterial pneumonia

A leading causes of death in the aged and those whose resistance is impaired. This disease caused mostly by *Strep. pneumonia*. Pneumonia is preceded by an upper or middle respiratory viral infection, which predisposes to *Strep. pneumonia* infection of pulmonary parenchyma, by the mechanisms of:

1) increased volume and viscosity of secretions that are more difficult to clear,
2) Secondary, inhibition of the action of bronchial cilia by viral infection.

2- Otitis media

Which is the most common bacterial infection of children, is most caused by pneumococcus,followed by *Haemophils influenza* and *Moraxella catarrhalis*

The traditional treatment of it with a β-lactam antibiotics (with or without a penicillinase – inhibitor) has been threatened by the spread of penicillin – resistant pneumococci.

3- Bacteremia/ sepsis

In the absence of a focus of infection is commonly caused by pneumococcus, especially in splenectomized individuals.

4- Meningitis

H. influenzae was leading cause of bacterial meningitis in U.S. After a vaccine was developed against this organism, *Strep. pneumonia* became the most common cause, which is high mortality rate even when treated.

Laboratory identification (Table 1) :

Specimens → nasopharyngeal swab, pus, sputum or spinal fluid.

Microscopic examination : show many polymorphonuclear neutrophiles and many RBC. (1) G + , (2) lancet shaped (3) diplococci, (4) nonmotile, (5) nonsporing, (6) capsulated.

Macroscopic examination : need enriched media with blood or serum for growth, produce α-hemolysin on blood agar growth is enhanced by 5-10% CO_2. On solid media → form a small round colonies dome-shaped at first, few hours later it undergoes some autolysis in the center of the colony and become flat while the edges are arised.

Biochemical tests →

1- Optochin sensitivity
2- Lysis the cell by bile acids
3- Capsular swelling (the Quellung reaction, see figure 4) is observed when sputum treated by type– specific antisera as immunological test.
4- Inuline fermentation to different from *Sterp. viridans*

Animal pathogenicity → mice are most susceptible to pneumococcal infection and used for diagnosis, animal will die in 18-48h.

Treatment and Prevention

Pneumococci are sensitive to sulfonamides and penicillin. Amoxicillin, cephalosporins, erythromycin, cortimoxazole ,vancomycin and penicillin G. Recently some drug resistance has appeared, e.g. tetracycline, erythromycin and lincomycin.

Prevention by pneumococcal polysaccharides vaccine ppv, immunizes against 23 serotype of *Sterp. pneumoniae* and is indicated for the protection of high risk individuals older than 2 years, for 85-90% of infections, including prominent penicillin-resistant strains.

Pneumococcal conjugate vaccine pcv7, is effective in infants (6 weeks – 5 years of age). Its made up of 7 pneumococci Ags conjugated to CRM 197 (a mutant nontoxic diphtheria toxin). This vaccine protect invasive pneumococcal disease, in older children and adults, effects on

pneumococci transmission (indirect effects on herd immunity) than through it's direct effect of protecting vaccinated children.

Streptococcus viridans

Include species *: Sterp. nitis , Sterp. mutans and Sterp. salivarins*

There growth is not inhibited by optochin disk , not soluble in bile salt and not ferment Inuline (see table 1).

It's a members of the normal flora of the upper respiratory tract, it cause endocarditis, some like *Strep. mutans* produce large polysaccharide which contribute to the genus of dental caries.

Table 1: Differentiation between *Streptococcus pneumococcus* and *Streptococcus viridans*

	Strepto. pneumonia	***Strepto viridans***
Microscopical morphology	Capsulated flame-shaped diplococci	Non capsulated oval or round arranged in chains
Culture colonies	Initially dome-shaped later flated colonies	Dome – shaped
Quelling test	+	-
Growth in liquid media	Uniform turbidity	Granular turbidity and powdery deposits
Bile solubility	+	-
Inuline fermentation	+	-
Optochin sensitivity	Sensitive	Resistant
Intraperitoneal inoculation in mice	Cause fatal infection	non pathogenic