

# Gram positive Cocci/ Streptococcus

Streptococci are gram positive, nonmotile, and catalase negative. Clinically important genera include *Streptococcus* and *Enterococcus* (Figure 1A). They are ovoid to spherical in shape and occur as pairs or chains (Figure 2). Most are aerotolerant anaerobes because they grow fermentatively even in the presence of oxygen. Because of their complex nutritional requirements, blood enriched medium is generally used for their isolation. Diseases caused by this group of organisms include acute infections of the throat and skin caused by group A streptococci (*Streptococcus pyogenes*); female genital tract colonization, resulting in neonatal sepsis caused by group B streptococci (*Streptococcus agalactiae*); pneumonia, otitis media, and meningitis caused by *Streptococcus pneumoniae*; and endocarditis caused by the viridans group of streptococci.

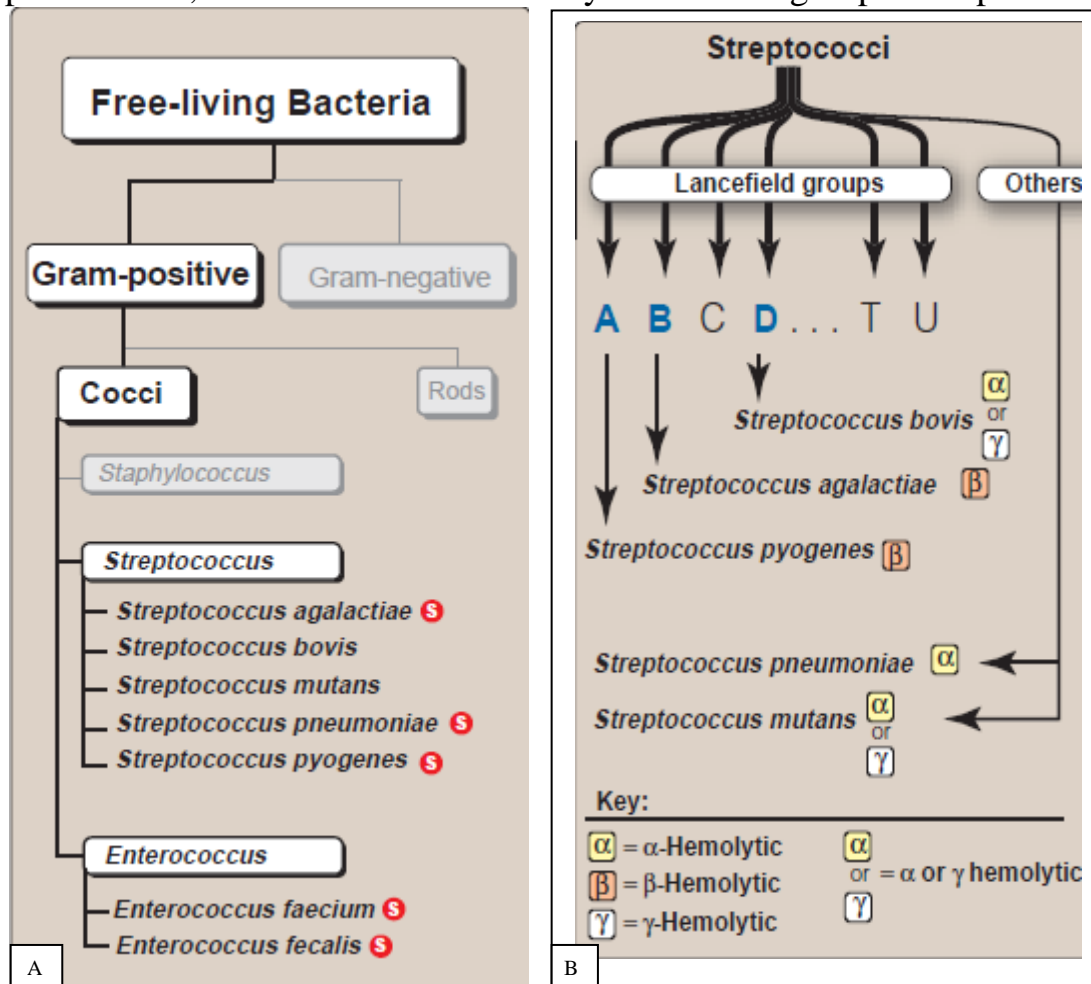


Figure 1: A, Gram Positive Cocci. B, Lancefield groups of Streptococci

**Classification of Streptococci:** Streptococci can be classified by several schemes

## A. Hemolytic properties on blood agar

- **$\beta$ - hemolysis:** complete RBCs destruction Clear zone around the colony (*S. pyogenes*, *S. agalactiae* )
- **$\alpha$ - hemolysis:** partial RBCs destruction result in Greenish discoloration of agar (*S.pneumoniae*, Viridans streptococci)
- **$\gamma$ - hemolysis:** No obvious changes around the colony (No hemolysis).(*Enterococci* and *non-enterococcal streptococci* (*S.bovis*).

## B. Serologic (Lancefield) groupings

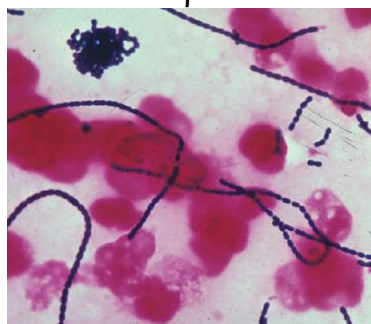
Many species of streptococci have a polysaccharide in their cell walls known as C-substance (Figure 1B).

## C. Capsular Polysaccharides

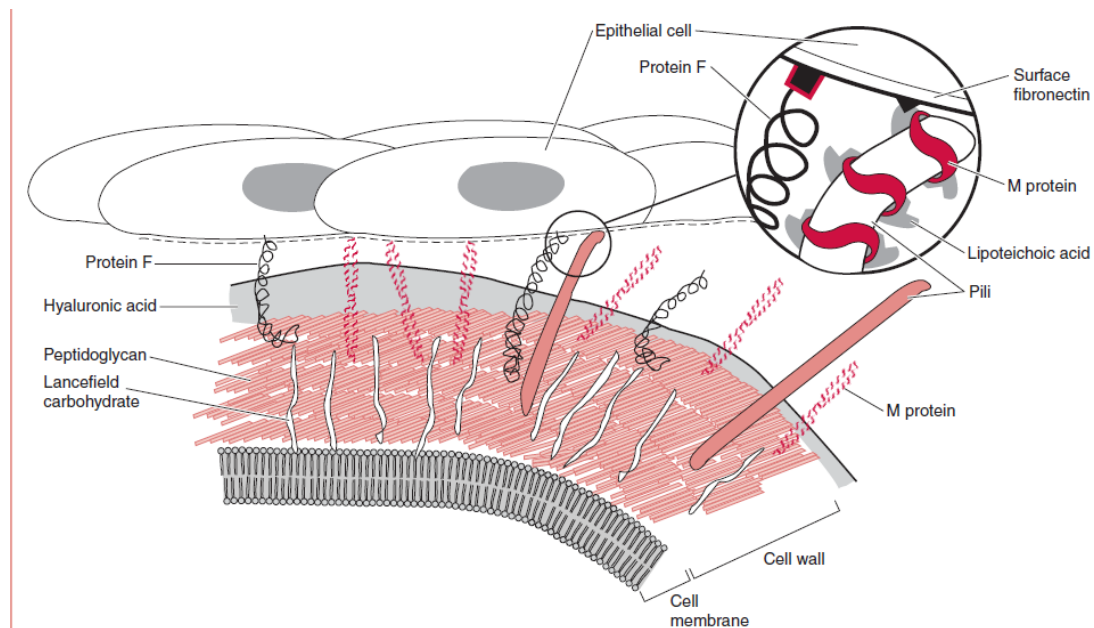
The antigenic specificity of the capsular polysaccharides is used to classify *Streptococcus pneumoniae* into more than 90 types and to type the group B streptococci (*Streptococcus agalactiae*).

Name	Group-Specific Substance <sup>a</sup>	Hemolysis <sup>b</sup>	Habitat	Important Laboratory Criteria	Common and Important Diseases
<b>Pyogenic Streptococci</b>					
<i>Streptococcus pyogenes</i>	A	$\beta$	Throat, skin	Large colonies (>0.5 mm), PYR <sup>c</sup> test positive, inhibited by bacitracin	Pharyngitis, impetigo, deep soft tissue infections; bacteremia; rheumatic fever, glomerulonephritis, toxic shock
<i>Streptococcus agalactiae</i>	B	$\beta$	Urogenital tract, lower GI tract	Hippurate hydrolysis, CAMP-factor positive <sup>d</sup>	Neonatal sepsis and meningitis; bacteremia, UTIs, <sup>e</sup> meningitis in adults
<b>Viridans Streptococci</b>					
<i>Streptococcus bovis</i> group <sup>f</sup>	D	None	Colon, biliary tree	Growth in presence of bile, hydrolyze esculin, no growth in 6.5% NaCl, degrades starch	Endocarditis, common blood isolate in colon cancer, biliary disease
<i>Streptococcus pneumoniae</i>	None <sup>g</sup>	$\alpha$	Nasopharynx	Susceptible to optochin; colonies soluble in bile; quellung reaction positive	Pneumonia, meningitis, bacteremia, otitis media, sinusitis

## GROUP A $\beta$ -HEMOLYTIC STREPTOCOCCI



**Structure:** The structure of group A streptococci is illustrated in Figure



*S. pyogenes* cells usually form long chains when recovered from liquid culture, but may appear as individual cocci, pairs, or clusters of cells in Gram stains of samples from infected tissue. Structural features involved in the pathology or identification of group A streptococci include:

**1. Capsule:** Hyaluronic acid, identical to that found in human connective tissue, forms the outermost layer of the cell. This capsule is not recognized as foreign by the body and, therefore, is nonimmunogenic. The capsule is also antiphagocytic.

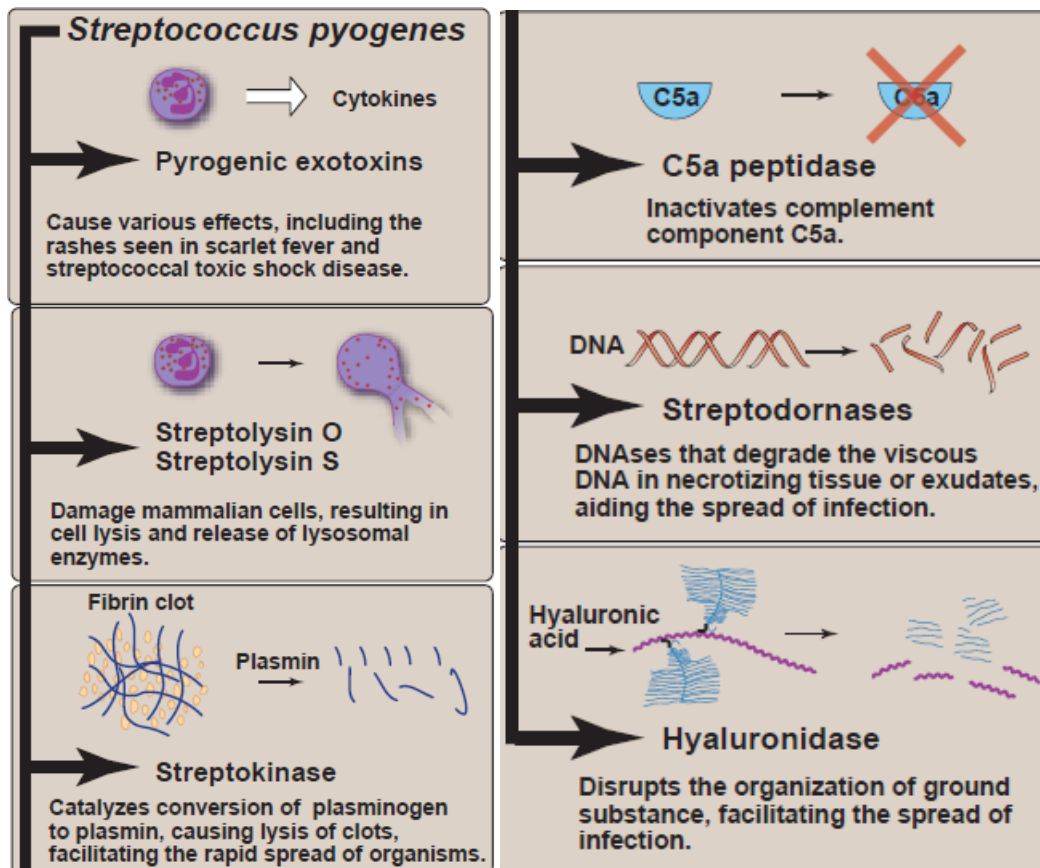
**2. Cell wall:** The cell wall contains a number of clinically important components. Beginning with the outer layer of the cell wall, these components include the following:

**a. M protein:** *S. pyogenes* is not infectious in the absence of M protein. M proteins are highly variable resulting in over 150 different antigenic types. Thus, individuals may have many *S. pyogenes* infections throughout their lives as they encounter new M protein types for which they have no antibodies. M proteins are antiphagocytic and they form a coat that interferes with complement binding.

**b. Group A-specific C-substance:** This component is composed of rhamnose and N acetylglucosamine.

**c. Protein F (fibronectin-binding protein)** mediates attachment to fibronectin in the pharyngeal epithelium.

### 3. Extracellular products



**Note:** Streptolysin O: The O stands for oxygen labile as it is inactivated by oxygen. This enzyme destroys red and white blood cells. This enzyme is also antigenic. Following pharyngeal or systemic beta hemolytic group A streptococcal infection, anti-streptolysin O (ASO) antibodies develop. Streptolysin S: The S stands for oxygen stable. This is also responsible for beta-hemolysis but is not antigenic.

### Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by *S pyogenes*. There are three antigenically distinct **streptococcal pyrogenic exotoxins (Spe): A, B, and C**. SpeA has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage.

The streptococcal pyrogenic exotoxins have been associated with **streptococcal toxic shock syndrome** and **scarlet fever**. The pyrogenic exotoxins act as superantigens

Pathogenesis:

Inhalation of droplet, attachment to the pharyngeal mucosa via actions of protein F, lipoteichoic acid, and M protein. The bacteria may simply replicate sufficiently to maintain themselves without causing injury in which case the patient is then considered colonized. Alternatively, bacteria may grow and secrete toxins, causing damage to surrounding

cells, invading the mucosa, and eliciting an inflammatory response with attendant influx of white cells, fluid leakage, and pus formation. The patient then has streptococcal pharyngitis.

Spreading of microbe through bloodstream resulting in septicemia and/or seeding of distant sites, where cellulitis (acute inflammation of subcutaneous tissue), fasciitis (inflammation of the tissue under the skin that covers a surface of underlying tissue), or myonecrosis (death of muscle cells) may develop rapidly.

### **Clinical significance**

*S. pyogenes* is a major cause of cellulitis. Other more specific syndromes include:

**1. Acute pharyngitis or pharyngotonsillitis:** Pharyngitis is the most common type of *S. pyogenes* infection. *S. pyogenes* pharyngitis (“strep throat”) is associated with severe, purulent inflammation of the posterior oropharynx and tonsillar areas.

[Note: If a sun-burn like rash develops on the neck, trunk, and extremities in response to the release of pyrogenic exotoxin to which the patient does not have antibodies, the syndrome is designated scarlet fever.]

Many strep throats are mild, and many sore throats caused by viruses are severe. Hence, laboratory confirmation is important for accurate diagnosis and treatment of streptococcal pharyngitis, particularly for the prevention of subsequent acute rheumatic fever and rheumatic heart disease.

**2. Impetigo:** Although *S. aureus* is recovered from most contemporary cases of impetigo, *S. pyogenes* is the classic cause of this syndrome. The disease begins on any exposed surface (most commonly, the legs). Typically affecting children, it can cause severe and extensive lesions on the face and limbs.

**3. Puerperal sepsis:** This infection is initiated during, or following soon after, the delivery of a newborn. It is caused by exogenous transmission (for example, by nasal droplets from an infected carrier or from contaminated instruments) or endogenously, from the mother’s vaginal flora. This is a disease of the uterine endo -metrium in which patients experience a purulent vaginal discharge and are systemically ill.

**4. Invasive group A streptococcal disease:**

Patients may have a deep local invasion either without necrosis (cellulitis) or with it (necrotizing fasciitis/myositis), [Note: The latter disease led to the term “flesh-eating bacteria.”]

**5. Streptococcal toxic shock syndrome:** This syndrome is defined as isolation of group A  $\beta$ -hemolytic streptococci from blood or another normally sterile body site in the presence of shock and multiorgan failure. The syndrome is mediated by the production of streptococcal pyrogenic

exotoxins that function as superantigens causing massive, nonspecific T-cell activation and cytokine release.

## **6. Post-streptococcal sequelae**

**a. Acute rheumatic fever:** This autoimmune disease occurs 2 to 3 weeks after the initiation of pharyngitis. It is caused by cross reactions between antigens of the heart and joint tissues, and the streptococcal antigen (especially the M protein epitopes).

### **b. Acute glomerulonephritis:**

This is an antibody-mediated inflammatory disease of the glomeruli of the kidney. It occurs about one week after infection of either the **pharynx OR skin** by **nephritogenic** (having the ability to cause glomerulonephritis) strains of beta-hemolytic group A streptococci. Certain antigens from these nephritogenic streptococci induce an antibody response. The resulting antigen-antibody complexes travel to and are deposited in the glomerular basement membrane, where they activate the complement cascade. This leads to local glomerular destruction in the kidney

### **Lab Dx for *S.pyogenes*:**

**Specimens:** depend on the nature of infection, e.g. sputum, throat swab, nasopharyngeal swab, blood, CSF...etc.

**Gram stain:** G+ve cocci, arrange in in short chains, but also in pairs and singly. Long chains are formed in fluid cultures.

**Culture:** on **blood agar** under 5-10% CO<sub>2</sub> → pinpointed, Grayish white, translucent, with large zone of β- hemolysis.

**Sensitive to Bacitracin disc** (inhibition zone more than 15mm).

### **Biochemical test:**

**catalase test:** streptococci (negative)

**PYR +ve:** rapid test, pink is positive.

**Serology:** 1) The rapid strep test (ELISA-based), 2) Lancefield grouping, 3) M-protein serotyping and 4) ASO test: Ab Titer: Normal < 200 > significance result

## **Group B Streptococci:**

Represented by the pathogen *S. agalactiae*, gram-positive, catalase-negative organisms. *S. agalactiae* is found in the vaginocervical tract of female carriers, and the urethral mucous membranes of male carriers as well as in the gastrointestinal (GI) tract.

*S. agalactiae* can be transmitted sexually among adults and from an infected mother to her infant at birth.

Group B streptococci are a leading cause of **meningitis and septicemia in neonates**, with a high mortality rate.

They are also an occasional cause of infections in postpartum women (endometritis) and individuals with impaired immune systems, in whom the organism may cause septicemia or pneumonia.

### **Pathogenesis and virulence factors**

Capsule is the major organism factor. The sialic acid moiety of the capsule has been shown to bind serum factor H, which in turn accelerates degradation of C3b before it can be effectively deposited on the surface of the organism.

This makes alternate pathway-mediated mechanisms of opsonophagocytosis relatively ineffective. Thus, complement-mediated phagocyte recognition requires specific antibody and the classical pathway.

Newborns will have this antibody only if they receive it from their mother as transplacental IgG.

GBS have also been shown to produce a peptidase that inactivates C5a, the major chemoattractant of PMNs.

### **LABORATORY FEATURES**

*Specimens:* Include cerebrospinal fluid, ear swab, and blood for culture from neonates. High vaginal swab is required from women with suspected sepsis.

#### **Morphology**

Group B streptococci are Gram positive cocci, occurring characteristically in short chains but also in pairs and singly. The organisms are non-motile. Most strains are capsulated.

#### **Culture**

*Blood agar:* Most strains of *S. agalactiae* produce grey mucoid colonies about 2 mm in diameter, surrounded by a small zone of *beta* haemolysis. About 5% of strains are nonhaemolytic. Placing discs of penicillin and gentamicin on the plate can help to identify these strains (penicillin sensitive, gentamicin resistant).

*MacConkey agar:* Most strains grow on this medium.

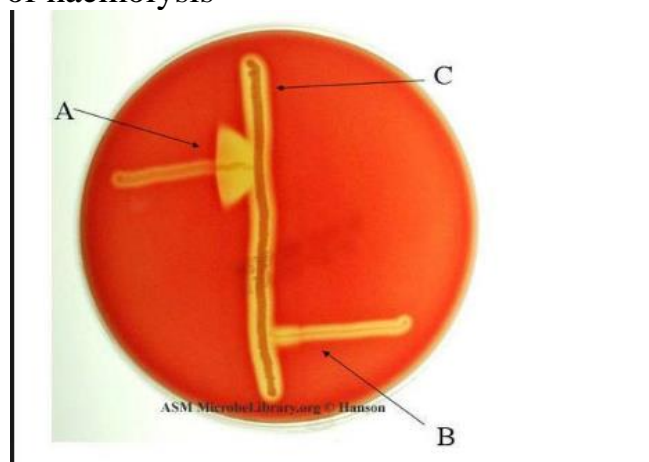
*Neomycin blood agar:* A useful selective medium for isolating *S. agalactiae* from urogenital specimens.

**Orange pigment:** Produced by *S. agalactiae* when cultured on serum starch agar anaerobically.

### **CAMP (Christie, Atkins, Munch, Peterson) test to identify presumptively *S. agalactiae***

This test requires the use of a *beta*-lysin producing strain of *S. aureus* to detect the CAMP factor, i.e. extracellular diffusible protein produced by *S. agalactiae*. This protein interacts with the staphylococcal *beta*-lysin on sheep (or ox) blood agar producing enhanced haemolysis.

The test is performed by streaking a known *beta*-lytic *Staphylococcus* strain across a 10% blood agar plate and then inoculating the test organism at right angles to it. The test organism must not touch the staphylococcal inoculum. An *Enterococcus* species is also inoculated as a negative control. After overnight incubation at 35–37 °C, there is an arrow-head shaped area of haemolysis



A: *S agalactiae*, B: *Enterococcus* spp., C: *S aureus*

**Latex agglutination tests** can also demonstrate the presence of group B antigen in these samples.

#### **Further Readings:**

**SHERRIS MEDICAL MICROBIOLOGY: AN INTRODUCTION TO INFECTIOUS DISEASES**, 4TH EDITION by **KENNETH J. RYAN, MD C. GEORGE RAY, MD**

**Lippincott's Illustrated Reviews: Microbiology**, Third Edition, Copyright © 2013 Lippincott Williams & Wilkins, a Wolters Kluwer business.

**District Laboratory Practice in Tropical Countries**, Part 2, Second Edition by Monica Cheesbrough, Cambridge University Press

**CASES IN MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES**, FOURTH EDITION, ASM Press Washington, DC, by Peter H. Gilligan, Ph.D. Daniel S. Shapiro, M.D. and Melissa B. Miller, Ph.D.



