Streptococci and Enterococci

**General Description of Streptococci and Enterococci**

The genus *Streptococcus* includes a heterogeneous group of different Gram positive species (>30) that are identified on Gram stain by their spherical or ovoid shape and their tendency to grow in pairs and chains. They are nonmotile, non-spore forming, and mostly facultatively anaerobic. Streptococci are a heterogeneous group that includes a variety of species capable of causing a diverse array of different diseases ranging from minor soft tissue infections to life-threatening sepsis. The most important pathogens in this group are *Streptococcus pyogenes* (group A streptococcus), *Streptococcus agalactiae* (group B streptococcus) and *Streptococcus pneumoniae* (pneumococcus). DNA homology studies have established *Enterococcus* (formerly streptococci) as a separate genus, distinct from *Streptococcus*. This lecture will cover only two of these pathogens, *S. pyogenes* and *S. pneumoniae*. Although not covered in the lecture you are also responsible for *S. agalactiae*, enterococci and viridans streptococci.

The different streptococcal species have different sites of colonization and cause different types of infections. These are described in the table below.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites of colonization</th>
<th>Common Sites of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em></td>
<td>oropharynx, nose</td>
<td>lungs, sinuses, middle ear, meninges</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>nares, pharynx, rectum</td>
<td>pharynx, skin, soft tissue</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>gastrointestinal tract</td>
<td>urinary tract, biliary tract, peritoneum, heart valves</td>
</tr>
<tr>
<td><em>E. faecalis, faecium</em></td>
<td>genitourinary tract</td>
<td>neonatal, bloodstream, lung and meninges, genitourinary tract</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viridans streptococcus</td>
<td>oropharynx</td>
<td>dental caries, heart valves, bloodstream</td>
</tr>
</tbody>
</table>

**Classification**: There are a number of different classification systems for streptococci and enterococci. The most commonly used, because of its simplicity, is the hemolytic pattern of the different species -- alpha (green hemolysis), beta (clear lysis) and gamma (no hemolysis) on blood agar plates. Lancefield grouping, based on the cell wall carbohydrate antigens, is also commonly used to differentiate the streptococcal species.

The different streptococcal species are all catalase negative and are generally identified in the laboratory by a number of biochemical tests that are summarized in the table below (+ = positive, – = negative, S = susceptible, R = resistant).

<table>
<thead>
<tr>
<th>Species</th>
<th>Optochin disk</th>
<th>Bacitracin disk</th>
<th>Growth in 6.5% NaCl</th>
<th>Hydrolyze Bile-Esculin</th>
<th>Hippurate hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em></td>
<td>S</td>
<td>R</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>R</td>
<td>S</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>E. faecalis, faecium</em></td>
<td>R</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>R</td>
<td>R</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Viridans streptococcus</td>
<td>R</td>
<td>R</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Streptococcus pyogenes (Group A Streptococcus)

Originally identified by Billroth in patients with wound infections in 1874, Group A Streptococci (GAS) are remarkable pathogens. They cause a variety of infections, utilizing a large number of different pathogenetic mechanisms. They cause infections that vary in severity ranging from minor soft tissue to life-threatening sepsis.

Structural components of GAS: The outer surface of GAS consists of a unique hyaluronic acid capsule that interferes with phagocytosis. The cell wall consists of a large number of different antigenic molecules that include alternating units of N-acetyl glucosamine and N-acetyl muramic acid, group specific carbohydrate antigens, lipoteichoic acid and proteins that extend to the cellular surface and are involved in adherence and invasion. These surface proteins facilitate attachment to molecules found in the extracellular matrix and on host cells.

M protein is the major virulence factor of GAS. The protein interferes with phagocytosis and strains defective in M protein are avirulent. The M proteins appear to promote colonization of tissue surfaces. M proteins are also used to classify GAS based on their variation in structure and sequence. The structure is filamentous and is an alpha-helical coiled-coiled structure. Immunity to infection with GAS is type-specific and is based on the antiphagocytic moiety of the M protein. The antigenically variable regions of the protein are found at the amino terminus and are the most distal. M protein has also been implicated in the pathogenesis of rheumatic fever.

Protein F1 and lipoteichoic acid mediate GAS binding to fibronectin, a host extracellular matrix molecule found on the surface of epithelial cells.

Protein G binds to the Fc portion of immunoglobulin (similar to protein A in S. aureus).

Secreted Products of GAS:

Enzymes produced by GAS. Streptolysins O and S are responsible for the hemolysis seen on blood agar plates. Their role as virulence determinants has not been established. Other enzymes, including DNases, hyaluronidases and streptokinase may contribute to tissue breakdown and pus formation. Antibodies to these enzymes have also been used to diagnose recent streptococcal infections.

Toxins produced by GAS. GAS produce a family of pyrogenic exotoxins that have some limited sequence similarity to the staphylococcal exotoxins but are capable of producing superantigen mediated toxic shock syndrome that is similar to the syndrome produced by S. aureus. These toxins -- streptococcal pyrogenic exotoxins (SPE A, B, C) -- are also responsible for Scarlet fever.

Epidemiology: Humans are the natural reservoir for GAS. Asymptomatic carriage can occur in the nares, pharynx or less commonly the rectum. Spread of infection primarily occurs by droplets produced by sneezing or coughing, or direct contact with secretions. Although GAS survives on environmental surfaces, these sources are rare as a cause of infection. Skin and soft tissue infections are the result of minor trauma to colonized skin or to skin that is inoculated with GAS (e.g., by hospital personnel).

Diseases caused by GAS. GAS cause a variety of diseases that can be divided into infectious, toxin-mediated and post-infectious. The infections include pharyngitis, impetigo, erysipelas, puerperal infections, sepsis and myonecrosis. The different toxin-mediated diseases include scarlet fever and streptococcal toxic shock syndrome. Finally the poststreptococcal diseases include glomerulonephritis and rheumatic fever that have an entirely different pathogenetic mechanism.
Pharyngitis the most common of the streptococcal infections is characterized by fever, lymphadenopathy, swollen, erythematous tonsils often with a visible purulent exudate. Infections are generally self-limited however there are both infectious and noninfectious sequelae. It is often difficult to distinguish this bacterial infection from viral pharyngitis. Clinical findings are not always diagnostic. Serologic assays including the anti-streptolysin O (ASO) test are helpful in diagnosing recent streptococcal infection.

Rheumatic fever is one of the nonsuppurative sequela of Group A streptococcal infections. It is characterized by carditis, polyarthritis, subcutaneous nodules, chorea and a skin rash called erythema marginatum. Because the presentation is a syndrome, a set of criteria – the Jones Criteria – is used to diagnose this illness.

Pathogenesis of disease:

Pharyngitis.  GAS are the most common bacterial infection of the throat in children, especially ages 5-15. They appear capable of causing infections at this site because of the ability of GAS to adhere to and perhaps be internalized by oropharyngeal epithelial cells. This occurs via specific adhesin – receptor interactions (some are noted above).

Toxin-related diseases. GAS can elaborate three different exotoxins that function as superantigens in much the same way as staphylococcal enterotoxins. For further discussion of the mechanism of superantigen-mediated disease see the staphylococcus lecture. The severity of the disease and its clinical manifestations are in part determined by the presence or absence of antibody against these exotoxins as well as antibody against the M protein for the causative streptococcal strain. What is different about the clinical presentation of streptococcal toxic shock syndrome is the frequency with which there is evidence of clinical infection, often severe, present in these cases versus staphylococcal toxic shock where infection is unusual.

Treatment and Prevention of GAS Infection:

Treatment:  GAS remain exquisitely sensitive to penicillins and these drugs continue to be the drugs of choice for infections caused by GAS. Other than slightly reducing the length of acute illness, one of the primary reasons for treating streptococcal pharyngeal infections is to reduce the chance of poststreptococcal sequelae such as rheumatic fever (pharyngitis). It is not clear that treatment of GAS skin infections prevents the development of glomerulonephritis when the strain is nephritogenic.

Prevention:  Several different approaches have been taken in the hopes of developing a vaccine to prevent GAS infection, primarily pharyngitis. One is to develop a vaccine directed against the immunity inducing type-specific epitopes from the terminal region of the M protein. The second approach is to use highly conserved regions of the protein and the third is to design a vaccine directed at the proteins that mediate adherence to pharyngeal epithelial cells. A concern about these vaccines is the possibility that they will induce a rheumatic fever like response or that the epitopes selected might induce a superantigen–type reaction. Of interest, recent work has identified a region of the M protein that cross-reacts with myosin and sarcolemma membrane. This concern is in addition to the difficulties of inducing protection either at the mucosal or the systemic level.

Streptococcus pneumoniae (pneumococcus)

The pneumococcus was first recognized in the 1880’s in France by Pasteur and by Sternberg in the United States. This pathogen continues to be the most commonly identified cause of bacterial pneumonia and is among the most common causes of mortality worldwide (3-5
With the widespread development of multidrug-resistant pneumococci in South Africa in the late 1970's therapy of these infections has become increasingly problematic.

**Microbiology and Structure.** Pneumococci are identified on Gram stain as lancet-shaped diplococci. On blood agar plates they form white to clear colonies that may dimple with time. As noted above in the table they are the only species that is susceptible to the optochin disk. The most important virulence determinant of pneumococci is the **polysaccharide capsule**. This capsule interferes with phagocytosis and confers type-specific immunity but does not elicit an inflammatory response. There are 84 different serotypes. The capsule formed the basis for the first polyvalent pneumococcal vaccine. The **cell wall (peptidoglycan)** is believed to contribute to initiating the inflammatory response following infection. This includes recruitment of polymorphonuclear leukocytes, initiating the coagulation pathway and inducing cytokine production. There is a limited amount of information available on the role of pneumococcal surface proteins in the pathogenesis of these infections. There are a number of secreted products of pneumococcus. The most studied is pneumolysin.

Pneumococci are “competent” and are therefore naturally transformable bacteria and can acquire DNA from the environment. This process was critical in the seminal studies of Avery and Griffith on the role of DNA. They are also responsible for the acquisition of genes conferring antimicrobial resistance.

**Epidemiology** Humans are the natural reservoir for pneumococci. Their site of colonization in the host is the nasopharynx. Twenty to 40% of children (especially young children) may be colonized at any particular time. Colonization is seasonal perhaps because of antecedent viral infections. Pneumococcal infection tends to occur at the extremes of age. These infections also tend to occur in individuals with defects in humoral immunity such as patients with hypogammaglobulinemia, multiple myeloma or sickle cell disease.

**Diseases caused by pneumococcus** Pneumococcus causes a diversity of infections that range from sinusitis, otitis to bacterial pneumonia, arthritis, septicemia and meningitis. These infections can be self-limited or life threatening.

**Pathogenesis of disease** Pneumonia serves as a prototype of pneumococcal infection. Colonization of the nasopharynx results from specific adhesin-receptor interactions. Strains with increased quantities of phosphoryl choline appear to have an increased capacity to bind nasopharyngeal cells. Infection develops as a result of aspiration of material from the oropharynx into the lungs. Interference with normal respiratory clearance mechanisms will increase the risk of infection. The ability to evade phagocytosis is a principal means of allowing infection to progress. Once in the alveoli, pneumococci adhere to the Type II alveolar cells. Much of the cascade of events that are initiated in the lung including the initial accumulation of fluid, subsequent consolidation and eventual resolution is the result of the inflammatory response initiated by components of the cell wall. Resolution of infection does not occur until type specific antibody develops. Individuals develop type-specific immunity to the capsular type causing infection.

**Clinical Manifestations of Pneumococcal Disease**

**Pneumonia.** The classic presentation of pneumococcal pneumonia is the sudden onset of fever, chills, pleuritic chest pain and cough associated with rusty colored sputum. With the advent of the AIDS era this presentation in young adults has often been somewhat more atypical. On chest x-ray the infiltrate is usually lobar. Up to a third of infections are associated with bacteremia. These
are the cases that may be complicated by the development of meningitis, arthritis or endocarditis \((i.e., \text{the pneumococcus seeds another organ}).\)

**Treatment and Prevention of Pneumococcal Infection:**

**Treatment:** Penicillin remains the drug of choice for the treatment of penicillin susceptible pneumococcal infections. Unfortunately as noted above, multidrug resistance (including to penicillin), has emerged. In some areas this has necessitated the use of alternative less active agents.

**Prevention:** A polyvalent polysaccharide anticapsular vaccine is currently available. It contains antigens of 23 of the most commonly encountered serotypes and has been effective in reducing invasive pneumococcal disease. A new 7-valent protein conjugate vaccine with efficacy in infants has been recently released. In contrast with the 23-valent vaccine it can be used in infants \(\leq 2\) year of age and appears to reduce nasopharyngeal carriage of pneumococcus.

**Streptococcus agalactiae (Group B streptococcus)**

Group B streptococci cause a narrow band of beta-hemolysis on blood agar plates. They are distinguished from other streptococci because of their ability to hydrolyze hippurate. Although they can cause disease in other groups, they are most commonly associated with infections of the newborn. Newborns are at particular risk of Group B sepsis or meningitis if their mother is vaginally colonized with Gp B streptococcus, lacks type-specific antibody, and there is prolonged ruptured membranes. These infections are life-threatening and can also result in permanent disability to the infant. Chemoprophylaxis with penicillin is recommended for all pregnant women who are colonized or are at high risk.

**Viridans streptococci**

This is a heterogeneous group of streptococci that includes 24 different species. They were originally grouped on the basis of the alpha hemolysis that is present (for the most part) when they are grown on blood agar plates. They are part of the oropharyngeal flora and some species can also be found in the gastrointestinal tract. They are relatively avirulent organisms however they do cause dental caries \((S.\ mutans)\) and are the most common cause of subacute infective endocarditis.

**Enterococci**

Formerly classified as streptococci, DNA analysis has resulted in the separation of these Gram positives into their own species. They grow as white colonies on blood agar plates and are generally nonhemolytic (gamma hemolysis). They are part of the normal gastrointestinal flora in part because of their resistance to bile salts. They are relatively common causes of infective endocarditis and have emerged, in part due to their increasing resistance to many antimicrobials, as important nosocomial pathogens.

**References**

Material covered in this lecture can be found in Murray - chapters 23, pages 237-258 and Chapter 24, pages 259-263.