BACTERIOLOGY - CHAPTER TWELVE

STREPTOCOCCI

GROUPS A, B, D AND OTHERS

ENTEROCOCCUS FAECALIS

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KEY WORDS
Lancefield groups
Hemolysis (alpha, beta, gamma)
Group A streptococcus (*S. pyogenes*)
Bacitracin susceptibility test
M, T, R proteins
Streptolysins O and S
Lipoteichoic acid
Rheumatic fever/carditis/arthritis
Glomerulonephritis
Scarlet fever
Toxic shock-like syndrome/bacteremia
"Flesh-eating bacteria"
Erythrogenic (Pyrogenic) toxin
Group B streptococcus (*S.agalactiae*)
Neonatal septicemia/meningitis
CAMP test
Hippurate hydrolysis test
Group D streptococcus
Urinary tract infection/ Endocarditis
Bile-esculin test
Enterococci
Non-enterococci

Streptococci are facultatively anaerobic, Gram-positive organisms that often occur as chains or pairs (figures 1 and 2) and are catalase-negative (in contrast, staphylococci are catalase positive) (figure 3). Streptococci are subdivided into groups by antibodies that recognize surface antigens (figure 4). These groups may include one or more species. The most important groupable streptococci are A, B and D. Among the groupable streptococci, infectious disease (particularly pharyngitis) is caused by group A which is thus emphasized here. *Streptococcus pneumoniae* (a major cause of human pneumonia) and *Streptococcus mutans* and other so-called viridans streptococci (among the causes of dental caries) do not possess group antigens.

Three types of hemolysis reaction (alpha, beta, gamma) are seen after growth of streptococci on sheep blood agar. Alpha refers to partial hemolysis with a green coloration (from production of an unidentified product of hemoglobin) seen around the colonies; beta refers to complete clearing (figure 5) and gamma means there is no lysis. Group A and group B streptococci are beta hemolytic, whilst D are usually alpha or gamma. *Streptococcus pneumoniae* and viridans ("green") streptococci are alpha hemolytic. Thus, the hemolysis reaction is important in grouping streptococci. The hemolysis reaction along with one physiologic characteristic is sufficient for a presumptive clinical identification.

GROUP A STREPTOCOCCUS (*S. PYOGENES*)

Most group A streptococcal infections are relatively mild illnesses but sometimes infection by these bacteria can result in severe and life-threatening diseases. There are several million cases of strep throat and impetigo each year.

Streptococcus pyrogenes frequently causes suppurative, but non-invasive pharyngitis (Strep Throat) (figure 6), and less frequently the skin infection, impetigo. In the middle part of the 1900's, the serious complications of group A streptococcal infections began to decline dramatically and had greatly decreased by the 1970's. Thus, interest in this organism waned. In the 1980's and 1990's, there was an upsurge in classical "rheumatic fever" (a non-suppurative disease of the heart) but also new forms of streptococcal disease which include both "invasive" bacteremia, a toxic shock-like syndrome (as seen with *Staphyllococcus aureus*) and so-called "flesh eating" bacteria.

Group A streptococcal infections affect all ages with peak incidence at 5 to15 years of age. The serious complications (including rheumatic fever and invasive bacteremia) were felt to affect primarily those with some underlying defect in their immune system (including infants, elderly people and those immunocompromised). However, it is clear now that previously healthy children and adults are definitely at risk of serious complications.

Group C, G, F (large colony)
S. anginosus (minute colony)
Viridans streptococci
Dental caries/endocarditis

Strep Throat

Strep throat is an infection in the throat and tonsils caused by group A Streptococci. The disease is spread through contact with aerosols produced in a cough or sneeze of an infected person. It can also be spread by drinking or eating from a utensil used by an infected person. It is also possible to get strep throat from contact with sores from group A strep skin infections.

Common Symptoms of Strep Throat include (CDC):

- Sore throat, usually starting quickly
- Severe pain when swallowing
- Fever (101° F or above)
- Red and swollen tonsils, sometimes with white patches or streaks of pus
- Tiny red spots (petechiae, figure 6) on the soft or hard palate—the area at the back of the roof of the mouth
- Headache
- Nausea and/or vomiting
- Swollen lymph nodes in the neck
- Body aches
- Rash

Rheumatic fever

Rheumatic fever, is an inflammatory disease affecting primarily the heart and joints. Although severe, it can take an extended period of time to develop. The mechanism of chronic immunopathology of rheumatic fever is not resolved. M protein cross-reacts with heart myosin leading to autoimmunity. Also the group A streptococcal cell wall is highly resistant to degradation in the host. These antigens persist for months *in vivo* and experimentally elicit diseases that resemble rheumatic arthritis and carditis. Rheumatic arthritis should not be confused with the most common rheumatic disease - rheumatoid arthritis. Early termination of throat infections with penicillin therapy decreases the incidence of the subsequent development of rheumatic carditis.

Acute glomerulonephritis.

This is an immune complex disease of the kidney.

Scarlet fever

Scarlet fever usually begins with a fever and sore throat which may be accompanied by:

- chills
- vomiting
- abdominal pain
- the tongue may have a whitish coating and appear swollen. It may also have a "strawberry"-like (red and bumpy) appearance
- the throat and tonsils may be very red and sore leading to pain in swallowing

One or two days after the onset of illness, a characteristic red rash appears (although the rash can appear before illness or up to 7 days later). The rash, which is caused by erythrogenic (pyrogenic) toxins that are phage encoded, gives the name: Scarlet Fever. Initially, the rash is seen on the neck, under the arms, and in the groin. It then spreads to other parts of the body. First the rash appears as flat red patches which gradually become fine bumps and feel like sandpaper (figure 7). The cheeks may have a flushed appearance but sometimes there is a pale area around the mouth. Underarm, elbow and groin skin creases may become brighter red than the rest of the rash (Pastia's lines). The rash generally subsides in about a week and the skin may peel around the finger tips, toes, and groin area. This can last up to several weeks.

Treatment is by antibiotics.

Bacteremia, toxic-shock syndrome and necrotizing fasciitis

Normally, infection by group A Streptococci results in mild symptoms. However, these bacteria can also cause a bacteremia resulting in a much more severe disease which can sometimes be fatal. Such diseases include:

- A toxic shock-like disease (including rash, fever and shifting of fluid from the bloodstream to peripheral tissues with resulting edema). This causes blood pressure to drop rapidly and organs (e.g., kidney, liver, lungs) to fail.
- and/or necrotizing myositis and fasciitis. Necrotizing fasciitis (which has earned Group A Streptococci the name "the flesh-eating bacteria") rapidly destroys muscles, fat, and skin tissue.

Production of pyrogenic toxins (A, B and C) are a hallmark of these strains. Pyrogenic toxin is a superantigen (a mitogen) for T cells causing non-specific activation of the immune system. This may be involved in the pathogenesis. This disease is still uncommon but can progress very quickly (a few days) and is life-threatening.

Approximately 9,000 to 11,500 cases of invasive Group A Streptococcal disease occur each year in the United States and lead to 1,000 to 1,800 deaths annually. Thus death occurs in 10%-15% of all invasive cases, approximately 40% of patients with streptococcal toxic shock syndrome and approximately 25% of necrotizing fasciitis cases die from the infection.

CDC list the early signs and symptoms of necrotizing fasciitis. These include:

- Severe pain and swelling, often rapidly increasing
- Fever
- Redness at a wound site

Early signs and symptoms of toxic shock syndrome include:

- Sudden onset of generalized or localized severe pain, often in an arm or leg
- Dizziness
- Flu-like symptoms such as fever, chills, muscle aches, nausea, vomiting
- Confusion
- A flat red rash over large areas of the body (only occurs in 1 in 10 cases)

High dose penicillin and clindamycin are used for treatment of necrotizing fasciitis and toxic shock syndrome along with supportive care in an intensive care unit in very severe cases. Early and aggressive surgery, which may reduce the fatality rate, is often needed to remove damaged tissue and stop disease spread.

General features in pathogenesis


Figure 1 Streptococcus mutans. Gram stain. CDC/Dr. Richard Facklam

The identity of the adhesin allowing adhesion to the respiratory epithelium (via fibronectin) is somewhat controversial. Lipoteichoic acid is localized in the cell membrane of many bacteria. For group A streptococci, much is also present in the fimbriae on the cell exterior. Classical work suggests lipoteichoic acid is the group A streptococcal adhesin although more recently a role for an "F (fibronectin-binding) protein" has been suggested.


Figure 2 Streptococcus pyogenes - coccoid prokaryote (dividing); causes pharyngitis, sinusitis, otitis media (middle ear infection), food poisoning, puerperal fever (childbed fever), skin and wound infections (scarlet fever, erysipelas, impetigo) . Group A strep. SEM x56,000

Group A streptococci in the absence of fibrinogen fix complement to the peptidoglycan layer and, in the absence of antibodies, are not phagocytosed. The M protein (also found in fimbriae) binds fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. This allows survival of the organism by inhibiting phagocytosis. However, in immune individuals, neutralizing antibodies reactive with M protein elicit phagocytosis which results in killing of the organism. This is the major mechanism by which immunity is able to terminate group A streptococcal infections. M protein vaccines are thus a major candidate for use against rheumatic fever. The capsule of group A streptococci classically was stated to have limited anti-phagocytic activity. Many of the newly described virulent strains are highly mucoid and the capsules are important in pathogenesis.

Unfortunately, certain M protein types cross-react antigenically with the heart and may be responsible for rheumatic carditis. The fear of autoimmunity has rightly inhibited the use of group A streptococcal vaccines. However, distinct protective versus cross-reactive epitopes have been defined and the availability of a vaccine appears likely. M proteins vary antigenically between strains; thus immunity to one M protein does not imply general immunity to all *S. pyogenes* strains. M typing along with other antigens (T and R) are used for serotyping.

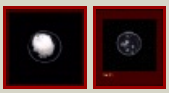
Figure 3 Catalase positive and negative test. In this test, hydrogen peroxide is


Figure 4

converted to oxygen (seen as gas bubbles) © Karen M. Kiser. St Louis Community College, Clinical Laboratory, St. Louis, MO

MOVIE
Catalase test
© The MicrobeLibrary and Neal R. Chamberlain, Department of Microbiology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri

Streptococcus fluorescent antibody stain (digitally colorized). Six groups are in this genus: A, B, C, D, F, and G, which and are often found in pairs or chains
CDC/Dr. M.S. Mitchell


Figure 5
Streptococcus pyogenes on a blood agar plate. These organisms produce a variety of toxins, some of which are capable of lysing or destroying erythrocytes). The result is a clear zone surrounding the bacterial colonies. This complete destruction of the erythrocytes in the agar medium is termed beta-hemolysis. © The MicrobeLibrary and Gloria J. Delisle, Queens University
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Figure 6
Strep throat is caused by group A *Streptococcus* bacteria. These bacteria are spread through direct contact with mucus from the nose or throat of persons who are infected, or through contact with infected wounds or sores on the skin. Note the inflammation of the oropharynx and petechiae, or small red spots on the soft palate caused by Strep Throat. CDC/Dr. Heinz F. Eichenwald


Figure 7
Skin lesions on the chest of a woman with scarlet fever. The rash first appears as tiny red bumps on the chest and abdomen, then spreads all over the body. It resembles a sun burn, and feels like a rough piece of sandpaper. It is usually redder in the axillary and groin areas. CDC


Figure 7a
One way to differentiate beta-hemolytic group A Streptococcus from other beta-hemolytic streptococci is by determination of their sensitivity to bacitracin. *Streptococcus pyogenes* (group A beta-hemolytic) is sensitive to bacitracin and will not grow around the antibiotic- containing disc. The other beta-hemolytic streptococci are not sensitive to bacitracin and will grow next to the antibiotic-containing disc. © The MicrobeLibrary and Neal R. Chamberlain, Department of Microbiology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri

Laboratory diagnosis

1. Direct detection - the antigen is extracted from a throat swab. The antigen extract will then bind with antibody specific to the group A streptococcal carbohydrate. This has classically involved agglutination of antibody coated beads. However, simpler tests have been recently introduced. Results are available within minutes.

2. Lancefield grouping of isolated beta hemolytic colonies (see above).

3. Colonies are beta hemolytic (figure 5) and their growth is inhibited by bacitracin (presumptive diagnosis) (figure 7a).

4. Patient serum shows antibodies to streptolysin O or other streptococcal antigens. This is important if delayed clinical sequelae occur.

Beta hemolysis is caused by two hemolysins O and S; the former is inactive in the presence of oxygen. Thus, stabbing of the plate increases the intensity of the hemolysis reaction.

GROUP B STREPTOCOCCUS

Streptococcus agalactiae

Group B Streptococci, which are common in the alimentary tract, cause illness in people of all ages. In adults, group B streptococci most commonly cause invasive bloodstream infections (bacteremia), pneumonia, skin and soft-tissue infections, and bone and joint infections.

In newborns, these bacteria can cause sepsis (septicemia), pneumonia and sometimes neonatal meningitis. The neonatal meningitis and septicemia occur after transmission from the normal vaginal flora of the mother. Antibiotics given during labor can be very effective at preventing transmission.

According to CDC, about 19,800 cases occur each year in the United States in all age groups; approximately 7,600 cases occurred in newborns before recent prevention strategies. The rate of early-onset infection decreased from 1.7 cases per 1,000 live births in 1993 to 0.28 cases per 1,000 live births in 2008. Since active prevention began in the mid 1990s, the rate of group B strep disease among newborns in the first week of life has declined by 80%. The incidence among blacks approximately twice that of non-blacks for all age groups.

Adult infections

The rate of invasive disease is about 7 cases per 100,000 non-pregnant adults and increases with age with an average age in non-pregnant adults of about 60 years. The rate is highest among adults 65 years and older (20 to 25 cases per 100,000). Most adult group B disease occurs in adults with other medical conditions including:

- diabetes mellitus
- cardiovascular disease
- congestive heart failure
- cancer
- obesity

Serious group B strep infections in adults can be fatal. On average, 8% of adults with invasive group B strep infections (infections where the bacteria have entered a part of the body that is normally not exposed to bacteria) die. Risk of death is lower among younger adults, and adults who do not have other medical conditions.

The most common problems caused by group B streptococci in adults are:

- Bloodstream infections
- Pneumonia
- Skin and soft-tissue infections
- Bone and joint infections

Group B streptococci can also lead to rare cases of meningitis.

The cause of adult infections is unknown but it may be from fecal contamination. Diagnosis is as used with newborns and treatment is with antibiotics (penicillin). On some occasions, infections of bone and soft tissue require surgery.

Newborns

Most newborns with early-onset disease (less than 7 days old) have symptoms on the day of birth. Babies who develop late-onset disease (7 to 90 days old) may appear healthy at birth and develop symptoms of group B strep disease after the first week of life.

Symptoms include (CDC):

- Fever
- Difficulty feeding
- Irritability, or lethargy (limpness or hard to wake up the baby)
- Difficulty breathing
- Blueish color to skin

Late-onset disease sometimes also results from mother to baby transmission, but sometimes the bacteria come from another source. For a baby whose mother does not test positive for group B strep, the source of infection for late-onset disease is often unknown. The fatality rate in newborns is about 5%.

Diagnosis

The disease is diagnosed when the bacteria are grown from samples of the infants blood or spinal fluid. The organism can be identified on the basis of beta hemolysis, hydrolysis of hippurate and the CAMP reaction (figure 8). CAMP is an abbreviation for the names of the four individuals who originally described the test. Group B streptococci produce a factor that increases beta hemolysis of an *S. aureus* indicator strain.

Risk Factors

Some pregnant women are at higher risk of having a baby with early-onset disease. Risk factors include (CDC):

- Testing positive for group B strep late in the current pregnancy (35 to 37 weeks gestation)
- Detecting group B strep in urine during the current pregnancy
- Delivering early (before 37 weeks gestation)
- Developing fever during labor
- Having a long period between water breaking and delivering
- Having a previous infant with early-onset disease

Late-onset disease is more common among premature babies (less than 37 weeks). Babies with group B strep positive mothers also have a higher risk of late onset disease.

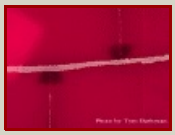


Figure 8a
CAMP positive reaction


Figure 8b
CAMP negative reaction

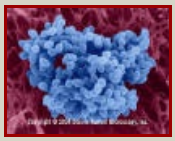


Figure 9
Streptococcus faecalis - coccoid prokaryote (dividing); a pathogen causing skin and wound infections

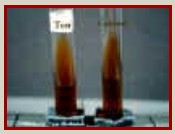



Figure 10
Bile esculin test. Group D streptococci are positive in this test (Above: Positive. Below: Negative)



Figure 11
Positive growth in 6.5% sodium chloride (top) and no growth in a similar medium (bottom)

ENTEROCOCCUS

GROUP D STREPTOCOCCUS

Now classified as an Enterococcus. The most common is *E. faecalis*

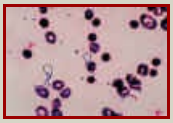
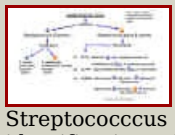


Figure 12
The bacterium *Streptococcus viridans*, is responsible for approximately half of all cases of bacterial endocarditis, but is found in the mouth as normal

Enterococci are distantly related to other streptococci and have been moved into the genus *Enterococcus*; the most commonly isolated is *E. (S.) faecalis* (figure 9). As the name implies enterococci are found in the gut flora where they are usually harmless commensals and infection often follows from fecal contamination. They are a significant cause of urinary tract infections (but much less common than *E. coli*) and also of opportunistic infections (including intra-abdominal, septicemia and endocarditis). There are a number of virulence

oral bacterial flora. CDC/Dr. Mike Miller



Streptococccus identification scheme

factors that may contribute to *E. faecalis* infections.

- A plasmid-encoded hemolysin (cytolysin)
- A plasmid-encoded factor (aggregation substance)

The cytolysin in combination with high-level gentamicin resistance is associated with a five-fold increase in risk of death in human bacteremia patients.

E. faecalis can cause serious human nosocomial infections in humans. This is because the organisms shows high levels of antibiotic resistance. It is often found in teeth after root canal operations with a prevalence from 30% to 90% of the cases. It is resistant to many common antibiotics such as aminoglycosides, aztreonam, cephalosporins, clindamycin, the semisynthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole. Resistance to vancomycin is becoming more common.

When the bacteria are vancomycin-resistant, the patient with a urinary tract infection may be treated with nitrofurantoin. Other options include ampicillin, linezolid and daptomycin. In root canal treatments sodium hypochlorite and chlorhexidine are used before isolating the canal.

Colonies are usually alpha or gamma hemolytic. Growth on bile-esculin produces a black precipitate derived from esculin (figure 10); many other bacteria will not grow in the presence of bile. Group D streptococci are divided into those that will grow in 6.5% saline (enterococci) and those that will not (non-enterococci) (figure 11).

OTHER BETA HEMOLYTIC GROUPS

Groups C and G (and rarely group F) occasionally cause human disease (particularly pharyngitis).

Group C streptococci includes:

- *Streptococcus equi*, which causes a disease in horses
- *S. zooepidemicus* which causes infections in cattle and horses among other animals
- *S. dysgalactiae*

Group G streptococci includes

- *S. canis*. This is normally found in a number of animals but can also cause infection in humans.

Group H streptococci cause infections in dogs and rarely cause illness in humans unless the person has direct contact with the mouth of an infected dog. This can occur by "kissing" a dog or from saliva after being licked by an infected dog.

MINUTE COLONY STREPTOCOCCI

The normal human flora contains organisms that may be group A, C, F or G or are non-groupable (*Streptococcus anginosus, Streptococcus milleri*). Their role in human disease is unclear but *Streptococcus anginosus* can cause diseases including brain and liver abscesses under certain circumstances, particularly in immuno-deficient individuals.

VIRIDANS STREPTOCOCCI

These are a diverse group of commensal species commonly found orally (including *S. mutans*) and cause endocarditis after release into the bloodstream from tooth extraction (figure 12). *S. mutans* is responsible for approximately half of all cases of bacterial endocarditis. They can synthesize dextrans from glucose. This allows them to adhere to fibrin-platelet aggregates at damaged heart valves. Thus, they have the ability to cause sub-acute valvular heart disease following their introduction into the bloodstream (such as by tooth extraction).

These bacteria are also involved in dental caries and pericoronitis, an inflammation of the soft tissues surrounding the crown of a partially erupted tooth.

They are either alpha or non- hemolytic and negative for other tests described above. They produce a green color on blood agar plates (Viridis, Latin: Green). Viridans streptococci can be differentiated from *S. pneumoniae* using an optochin test. Viridans streptococci are

optochin-resistant.

They are non-groupable.

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