10

Streptococcus, Enterococcus, and Related Organisms

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Chapter Outline

Streptococcus
β Hemolytic *Streptococcus*
Streptococcus pyogenes (Group A Streptococcus)
Streptococcus agalactiae (Group B Streptococcus)
Miscellaneous β Hemolytic *Streptococcus*
Streptococcus pneumoniae
Viridans Streptococci
Enterococcus
Abiotrophia and *Granulicatella*
Other Catalase-Negative Streptococcal-Like Organisms

Key Terms

Bacitracin test
Bile esculin
Bile solubility Brown's classification
CAMP reaction
M (*emm*) protein
Lancefield antigen grouping
Necrotizing fasciitis
Optochin test
Pneumolysin
Pyrrolidonyl arylamidase (PYR)
Salt tolerant
Streptokinase
Streptococcal pyogenic exotoxins
Streptococcal toxic shock-like syndrome
Streptolysin O
Streptolysin S
Vancomycin Resistant Enterococcus (VRE)
Viridans streptococci

Learning Objectives

Upon successful study and review of this chapter, the learner should be able to:

1. Describe characteristics of the genus *Streptococcus.*
2. List the media typically used to isolate streptococci.
3. List, recognize, and describe the types of hemolysis.
4. Differentiate between streptolysin S and streptolysin O.
5. Give the Lancefield group and tests used to presumptively identify each of the following streptococcal species:
   a. *S. pyogenes*
   b. *S. agalactiae*
   c. *Enterococcus*
   d. *Streptococcus bovis* group
6. List the primary infections and sequelae caused by group A streptococcus (GAS)
7. Discuss the invasive GAS infections and list the associated virulence factors.
8. Describe the M (*emm*) protein and relate its role to GAS infections.
9. Discuss the role of group B streptococcus as a neonatal pathogen.
10. Briefly discuss the significance of the Lancefield groups C, F, and G streptococci.
11. List and describe the infections associated with *Streptococcus pneumoniae*.
12. Discuss important microscopic and morphologic characteristics of *Streptococcus pneumoniae* and describe its identification.
13. Differentiate between viridans streptococcus and *Streptococcus pneumoniae.*

14. State the principle and purpose of the following tests:
    a. Bacitracin
    b. SXT
    c. CAMP reaction
    d. Bile esculin
    e. 6.5% salt broth
    f. Optochin
    g. PYRase
15. Discuss the principle and limitations of rapid antigen tests for group A streptococcus.
16. Differentiate between the *Enterococcus* and *Streptococcus bovis* group.
17. Discuss the infections attributed to viridans streptococcus.
18. Describe important characteristics of the viridans streptococcus
19. Describe the infections associated with the *Enterococcus*.
20. Discuss intrinsic and acquired antibiotic resistance in the *Enterococcus*.
21. Discuss the significance of vancomycin-resistant *Enterococcus* (VRE).
22. List antibiotics effective in the treatment of streptococcal infections.
23. Discuss antibiotic resistance in the streptococci.
24. List important characteristics and the clinical relevance of *Abiotrophia* and *Granulicatella.*
25. Describe important characteristics and the clinical relevance of catalase negative streptococcal like bacteria including *Aerococcus, Gemella, Lactococcus, Leuconostoc*, and *Pediococcus.*

The focus of this chapter is on *Streptococcus* and *Enterococcus*, members of the family Streptococcaceae, which are frequently associated with a variety of human infections. Streptococci have been classified by several criteria. Within the past several years, the taxonomy has been revised based on molecular biology techniques, including 16S rRNA and DNA–DNA reassociation techniques. The *Enterococcus* species were previously classified in the genus *Streptococcus* and have been elevated to the genus level of *Enterococcus*. The genus *Streptococcus* was divided into three genera, which are *Streptococcus*, *Enterococcus*, and *Lactococcus*, according to *Bergey's Manual of Determinative Bacteriology*.

Other catalase-negative, gram-positive cocci that resemble the streptococci include *Abiotrophia*, *Granulicatella, Aerococcus, Gemella, Leuconostoc, and Pediococcus*.

Streptococcus

The genus *Streptococcus* currently contains over 100 species; some are nonpathogenic and normal microbiota for humans, whereas others are important human pathogens. Streptococci are widely distributed in the environment, in dairy products, and as normal flora of the human gastrointestinal tract and oral cavity.

Streptococci are gram-positive cocci arranged in pairs or chains. *Streptococcus* and *Enterococcus* species are catalase negative, which differentiates them from *Staphylococcus*, which is catalase positive. Streptococci have the typical gram-positive cell wall of peptidoglycan and teichoic acid and are nonmotile.

Most strains of *Streptococcus* are fastidious, and isolation requires enriched media, such as sheep blood agar (SBA), Todd-Hewitt broth, or chocolate agar. Growth is poor or absent on trypticase soy agar. Colonies are typically small to medium in size, measuring 0.5 to 2.0 mm in diameter; pinpoint; grey, translucent, or clear; and with wide zones of hemolysis. Some species, such as *S. pneumoniae*, are capnophilic, requiring increased (5% to 10%) carbon dioxide for growth. Because streptococci are facultative anaerobes, growth may be observed both aerobically and anaerobically. However, they are not able to use oxygen for respiration and, thus, may be classified as aerotolerant anaerobes. Important characteristics of the genus *Streptococcus* are shown in **Box 10-1**.

To enhance the recovery of β hemolytic streptococci from clinical specimens, a selective blood agar medium that is inhibitory to other bacteria can be used. For example, blood agar with trimethoprim and sulfamethoxazole can be used for the primary isolation from throat cultures to isolate group A streptococcus.

Classification

Streptococci may be classified according to the type and pattern of hemolysis, physiologic divisions, and antigenic character of a group-specific cell wall

BOX 10-1

CHARACTERISTICS OF *STREPTOCOCCUS*

Gram-positive cocci that arrange in pairs or chains
Catalase negative
Nonmotile
Require supportive or enriched media, such as sheep blood agar (SBA) for growth
Small- to medium-sized colonies on SBA that are translucent or greyish
May be α, β, or nonhemolytic
Non-spore forming
Facultative anaerobes or aerotolerant anaerobes
Some species are capnophilic.

polysaccharide known as the **Lancefield antigen grouping**. Classification based on hemolysis was first described by J. H. Brown in 1919. This is an important phenotypic characteristic in the identification process. Types of hemolysis are affected by the animal source of red blood cells incorporated into the medium and the incubation atmosphere; thus, it is important to know the type or blood agar. Sheep blood agar is used most often in microbiology laboratories in the United States; however, other sources, such as horse and rabbit blood agar, may be used for specific testing. Traditional classifications of hemolytic patterns largely have been established on 5% sheep blood agar, with a trypticase soy agar base. Hemolysis is determined by holding the plate directly in front of a light source. **Box 10-2** presents the hemolytic patterns, known as **Brown's classification**. β hemolysis results from the production of two hemolysins, streptolysin S and streptolysin O. **Streptolysin O** is antigenic, oxygen labile, and observed as subsurface hemolysis; it is produced mainly by group A streptococci. Recent group A streptococcal infections can be diagnosed through the detection of antistreptolysin O, an antibody found in the patient's serum. To demonstrate streptolysin O, the blood agar should be cut or stabbed several times to force some of the organism to grow in the reduced oxygen content. Stabs made approximately half the depth of the agar with the inoculating loop while streaking the culture will allow for the detection of streptolysin O. **Streptolysin S** is nonantigenic and oxygen stable and thus noted as surface hemolysis.

In 1933, Rebecca Lancefield identified several distinct β hemolytic streptococcal groups based on specific carbohydrate group antigens. She named the first five groups A, B, C, D, and E. Today, there are numerous Lancefield groups; those that are clinically significant include groups A, B, C, D, F, and G. Immunochemical methods are available whereby the cell wall antigen can be extracted, and the organism serotyped by reacting with type-specific antisera.

BOX 10-2

BROWN'S CLASSIFICATION OF HEMOLYSIS

Alpha (α): Incomplete or partial hemolysis of red blood cells around the colony; green or brown color surrounding the colony. This pattern is seen with the viridans streptococci and *Streptococcus pneumoniae*; α hemolysis occurs as a result of hydrogen peroxide production by the organism, which destroys the red cells and releases hemoglobin.*

Beta (β): Complete hemolysis of red blood cells; clearing or colorless zone around colony. An example of this β hemolysis is exhibited by *Streptococcus pyogenes*, the agent of streptococcal pharyngitis.

Nonhemolytic: Lack of hemolysis; no apparent change in color of area surrounding colony; has been referred to as gamma (γ) hemolytic. Most *Enterococcus* species of Lancefield group D, causes of urinary tract and wound infections, are nonhemolytic.

Alpha prime (α′): A small zone of α hemolysis surrounds the colony and a zone of β hemolysis surrounds the zone of α hemolysis.

*α hemolysis requires the presence of oxygen and appears as nonhemolytic when there is no oxygen in the system.

NOTE

Classification Systems for Streptococcus

There are three systems to classify *Streptococcus*. *Streptococcus pyogenes* is used as an example to illustrate each system.

Brown's System is based on the type of hemolysis: β hemolytic streptococci

Lancefield's grouping is based on specific cell wall carbohydrate group antigens: Group A streptococcus

Sherman's classification is based on physiologic divisions: Pyogenic division

In 1937, Sherman classified the *Streptococcus* into four physiologic divisions: pyogenic, viridans, lactic, and the *Enterococcus*. He based these divisions on hemolytic reactions, carbohydrate antigens, and phenotype tests. The pyogenic division included the β hemolytic strains, A, B, C, E, F, and G, and the viridans division included those streptococci that were not β hemolytic and not salt tolerant or able to grow at a high pH. The *Enterococcus* division included those streptococci that were salt tolerant and able to grow at a high pH and at a temperature range of 10°C to 45°C. The lactic division included streptococci that were not clinically significant and were associated with the dairy industry.

β Hemolytic *Streptococcus*

Currently, 11 species and four subspecies of β hemolytic streptococci are recognized. Some are found in animals other than humans and are not pathogenic in humans. Those species and Lancefield antigens that are associated with human infection include *S. pyogenes* (Lancefield group A), *S. agalactiae* (Lancefield group B), *S. dysgalactiae* (Lancefield groups C and G), *S. equi* (Lancefield groups C and G), and *S. anginosus* (Lancefield groups A, C, F, and G). The identification of these organisms is summarized in **Table 10-1**.

Table 10-1. Identification of β Hemolytic *Streptococcus*

Species	Lancefield antigen	Bacitracin	PYR	CAMP	Hippurate	Bile esculin	Voges-Proskauer
S. pyogenes	A	Susc	+	–	–	–	–
S. agalactiae	B	Res	–	+	+	–	–
S. dysgalactiae subsp. *dysgalactiae* and subsp. *equisimilis*	C and G	Res	–	–	–	V	–
S. equi subsp. *equi* and subsp. *zooepidemicus*	C and G	Res	–	–	–	–	–
S. anginosus	A, C, F, G	Res	–	–	–	+	+

Key:
Susc: Organism is susceptible or sensitive (+)
Res: Organism is resistant (–)
+: Most are positive for the reaction
–: Most are negative for the reaction
V: Reaction is variable

Streptococcus pyogenes (Group A Streptococcus)

Streptococcus pyogenes, or group A streptococcus (GAS), causes bacterial pharyngitis, skin infections, and other invasive diseases. It also is associated with complications or sequelae such as rheumatic heart disease and acute glomerulonephritis. GAS is not considered to be normal flora but may be carried on the skin and in the upper respiratory tract of humans on the nasal and pharyngeal mucosa. Infections are spread through direct person-to-person contact or indirectly in aerosol droplets from coughing or sneezing. Isolation of GAS is almost always regarded as clinically significant.

S. pyogenes is isolated on blood agar, and the incorporation of trimethoprim and sulfamethoxazole (SXT) to the blood agar will enhance its recovery from highly contaminated specimens by suppressing other organisms. After 18 to 24 hours of incubation at 35°C to 37°C, colonies are pinpoint or small (0.5 to 1.0 mm in diameter); transparent, opalescent, or clear; and smooth and white to gray in color. The colonies are surrounded by a wide zone of β hemolysis. **Figure 10-1** illustrates group A streptococcus on sheep blood agar.

On sheep blood agar, group A streptococcal colonies may appear either smooth and glossy or round and mucoid, which indicates the presence of **M (*emm*) protein**. The M protein contains hyaluronic acid and is a surface protein found in encapsulated strains of *S. pyogenes*; its presence is associated with virulence. M protein holds antiphagocytic properties and infection can be established in the absence of type-specific antibody. M protein has been serologically typed by using a capillary precipitin test to subtype the organism. More recently, the *emm* typing system, which sequences the gene that codes for the hypervariable region of the M protein, has been used to type for the *emm* protein. There are over 200 *emm* types according to the Centers for Disease Control and Prevention (CDC). Specific serogroups of M protein are associated with throat infection, rheumatic fever, acute glomerular nephritis skin infections, and invasive diseases. For example, M1 (*emm*1) serotype is most often implicated in pharyngitis. If a person has antibody against a specific emm protein type, there is immunity against that specific *emm* type; however, the individual remains susceptible to all other types of the emm protein.

S. pyogenes is presumptively identified with the **bacitracin test** and the **pyrrolidonyl arylamidase (PYR)**

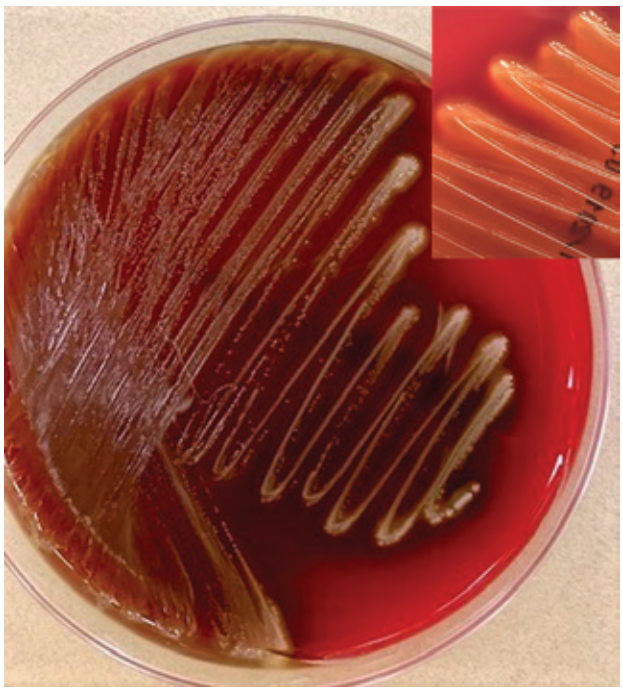


Figure 10-1. Group A streptococcus on sheep blood agar. Courtesy of Maria Delost.

reaction. *S. pyogenes* is susceptible to 0.02 to 0.04 units of bacitracin and is PYR positive. When performing the bacitracin test, the isolate tested must be in pure culture. The bacitracin disk never should be directly placed on a primary isolation plate from the specimen site. Any zone of inhibition is reported as susceptible or as a positive (susceptible) reaction.

Because a small percentage of other β hemolytic streptococci (groups B, C, and G) may also be susceptible to 0.02 to 0.04 unit of bacitracin, other techniques are needed to confirm the identification of group A streptococcus. Additional testing may include the PYR reaction, performing the bacitracin on SXT blood agar or with an SXT disk, and antigen typing for the group A antigen. Group A streptococci and are the *Enterococcus* are PYR positive. PYR can be detected using commercially available PYR disks that contain the substrate L-pyrrolidonyl-β-naphthylamide. Combined with the bacitracin test, PYR is useful in identifying *S. pyogenes*, which is the only β Hemolytic *Streptococcus* that is susceptible to bacitracin and PYR positive. The PYR test is also used to differentiate the *Enterococcus* species, which are PYR positive, from the *S. bovis* group, which are PYR negative, which both carry the Lancefield group D antigen. Organisms resistant to SXT but susceptible to bacitracin can be presumptively identified as group A streptococci. If resistant to both disks, the organism is most likely a group B streptococcus. **Figures 10-2** and **10-3** illustrate the expected susceptible result to bacitracin for or group A, and a resistant result to bacitracin, typical of typical of a streptococcus not belonging to Group A. Groups C, F, and G are susceptible to SXT and resistant to bacitracin.

Rapid antigen detection of throat swabs for rapid screening of group A streptococcus is a common diagnostic procedure. Direct antigen testing can provide a more rapid identification of group A pharyngitis. The group A streptococcal antigen is extracted using nitrous acid or enzymatic methods directly from a throat swab. Once extracted, the group A antigen is detected immunologically using slide agglutination, ELISA (enzyme linked immunosorbent assay), or other optical immunodiagnostic methods. The sensitivity varies on the test method and amount of antigen present in the specimen. Thus, to avoid a false negative, all negative direct antigen tests are confirmed with a throat culture and appropriate testing as discussed above. There are also rapid polymerase chain reaction assays for the detection of GAS on throat swabs.

Box 10-3 provides quick facts about group A streptococcus.

Figure 10-2. Bacitracin susceptible group A streptococcus.
Courtesy of Maria Delost.

Figure 10-3. Bacitracin resistant Not group A streptococcus, possible Group B, C, F, or G.
Courtesy of Maria Delost.

BOX 10-3

GROUP A STREPTOCOCCUS QUICK FACTS

Wide-zone β hemolytic on SBA
Bacitracin susceptible
PYRase positive
SXT resistant
CAMP negative

BOX 10-4

VIRULENCE FACTORS OF *STREPTOCOCCUS PYOGENES*

Streptococcal pyogenic exotoxins A (SpeA), B (SpeB) C (SpeC), F (SpeF) or **superantigens:** Associated with streptococcal toxic shock syndrome; responsible for invasion of soft tissue and necrotizing fasciitis, fever, and alteration of blood–brain barrier; may be associated with organ damage and skin rash of scarlet fever (formerly erythrogenic toxic fever) and induce fever and shock. Superantigens stimulate T cell proliferation by producing cytokines that mediate shock and tissue damage.

Streptolysin O: Lyses red cells, white cells, platelets, and other cells; suppresses neutrophils; oxygen labile; responsible for subsurface hemolysis; very immunogenic and induces an antibody response known as antistreptolysin O (ASO); ASO prevents red cell hemolysis and is a serologic test used to diagnose recent group A infections, especially in complicated cases. An ASO antibody titer of ≥166 Todd units is usually indicative of group A streptococcus infection. Streptolysin O also is produced by Lancefield groups C and G.

Streptolysin S: Oxygen-stable; lyses red cells and platelets in the presence of oxygen; responsible for surface hemolysin; nonimmunogenic; antiphagocytic.

Streptokinase: Fibrinolysin that lyses blood clots, prevents fibrin barrier, and allows spread of infection.

Streptodornase: Enzymes with nuclease activity; degrade host deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA).

Cell Surface Antigens

M protein (*emm*): Associated with virulence; antiphagocytic; interferes with complement activity. Strains lacking M protein are avirulent. Over 200 types of M protein have been identified. Immunity depends on development of antibody to type-specific M protein.

Hyaluronidase: Breaks down hyaluronic acid in connective tissue; spreading factor.

Fibronectin binding protein (Protein F): Surface protein that enables binding to host epithelial cells.

Group A streptococcus produces a variety of virulence factors associated with its pathogenicity, which are summarized in **Box 10-4**.

Primary infections caused GAS include pharyngitis, tonsillitis, or "strep throat." GAS is the most common causes of bacterial pharyngitis and is spread by droplets and close contact with an infected person. Following an incubation period of 1–4 days, there is an abrupt onset of sore throat, fever, swollen and tender lymph nodes, and malaise. Strep throat is primarily an infection of children aged 5 to 15 years, but may also cause infections in young adults in colleges and on military bases. Streptococcal pharyngitis occurs more often in the fall and winter. Symptoms generally subside in 3–5 days unless there are complications, such as peritonsillar abscess, which is a deeper infection of the head and neck and is usually a combination of both aerobic and anaerobic bacteria. Symptoms of peritonsillar abscess include fever, throat pain, and difficulty in opening the mouth, and complications may include airway blockage or aspiration pneumonitis.

Scarlet fever or scarlatina may occur 1–2 days after the primary GAS infection, generally after streptococcal pharyngitis. Pyogenic exotoxins are responsible for the symptoms of scarlet fever, which include a characteristic bright red rash that covers most of the body. The rash resembles a sunburn and typically first appears on the face or neck and then spreads to the upper chest and trunk, arms, and legs. The tongue appears red and bumpy and is covered with a yellow-white coating with red papillae and is described as a "strawberry tongue." There is also a high fever, headache, sore throat, difficulty swallowing, enlarged and tender cervical lymph nodes, and nausea and vomiting. The rash lasts about 1 week, which is followed by desquamation or peeling of the skin. Scarlet fever occurs most often in children aged 5 to 15 years and was once viewed as a serious childhood illness; antibiotics have lessened its severity. If not treated, complications of the heart and kidneys may occur.

GAS is also the cause of skin or pyodermal infections, which include impetigo, cellulitis, wound infections, and erysipelas. Impetigo is a localized, superficial, very contagious skin infection that occurs most often in infants and children. It generally first appears as red sores around the child's face and on the hands and feet, which rupture and ooze and then form a honey-colored crust. GAS may also penetrate the epidermis through abrasions and invade the subcutaneous tissues. Erysipelas, one type of cellulitis, is most commonly seen in the elderly and is characterized by erythematous lesions of the skin with edema, most commonly on the face or legs. Cellulitis may progress to bacteremia, septicemia, or gangrene. These skin infections also may become associated with the GAS sequela of acute glomerulonephritis. Other infections

associated with GAS include otitis media, pneumonia, and bacteremia.

GAS Sequelae

Sequelae of GAS infections include acute rheumatic fever (ARF) and acute glomerulonephritis (AGN). These sequelae are believed to result from a cross-reacting antibody that attacks cardiac or renal tissue while attempting to destroy streptococcal antigens. Rheumatic fever arises as a delayed sequela to GAS pharyngitis and may occur 1 to 5 weeks after pharyngitis. Symptoms include inflammation of the heart, joints, skin, and nervous system. Rheumatic fever was once a common cause of heart disease in children in the United States. While its incidence has declined in the United States, ARF remains a major worldwide healthcare concern in underdeveloped countries. Specific *emm* protein types and host susceptibility factors contribute to the development of rheumatic fever. The most common expression of rheumatic fever is arthritis, while carditis is its most serious manifestation. Carditis may lead to chronic rheumatic heart disease and progressive degeneration of the heart valves. Increased antibodies against streptolysin O and anti-DNase B antibody levels are observed in rheumatic fever.

AGN may occur in patients following streptococcal pharyngitis or skin infections. AGN is observed more often in children and young adults and is characterized by edema of the extremities and face, hypertension, hematuria, proteinuria, and red blood cell casts in the urine. Circulating immune complexes deposit in the glomeruli of the kidneys with complement fixation and a subsequent inflammatory response leading to renal damage. There is a latency period of 1 to 2 weeks following the primary GAS infection. ASO titers are elevated in AGN, occurring after pharyngitis. For AGN occurring after skin infection, there is a longer latency period of 3 to 6 weeks following the primary infection and the ASO titers may be low. Specific M protein serogroups are associated with AGN.

S. pyogenes strains that produce pyogenic exotoxins and other virulence factors are associated with invasive infections, including streptococcal toxic shock syndrome and **necrotizing fasciitis**. Virulence factors of these invasive diseases include streptococcal pyogenic exotoxins A (SpeA), B (SpeB) C (SpeC), and F (SpeF), or superantigens. These infections are most frequently found in individuals with underlying medical conditions, including surgery, skin trauma, burns, and disruptions to the skin barrier. The syndrome known as **streptococcal toxic shock-like syndrome** (TSLS) is a toxin-mediated disease that causes hypotension and multiple organ failure. Other symptoms of TSLS include fever, erythema, swelling, tachycardia, acute respiratory distress, renal impairment, and shock. There also may be severe pain and rapid necrosis of skin and cutaneous tissues; the mortality rate has been estimated to be as high as 50%. Specific M protein serotypes have been associated with TSLS.

Necrotizing fasciitis (NF), another invasive GAS disease, is also associated with specific M protein serogroups that produce SpeA and SpeB. NF is a soft tissue infection with severe necrosis of the fascia. The disease has a rapid progression and has become known as the "flesh eating bacteria" because of this rapid tissue destruction. It is believed that the bacteria enter through breaks in the skin, although this is not always the case. NF can lead to sepsis, shock, and organ failure; amputation of the limbs or other surgery to remove the infected tissue may also be needed. Even with treatment, according to the CDC, about one-third of those with NF will die; for individuals with both NF and TSLS, the mortality rate is 60%. NF is a rare disease and those with an underlying medical disorder or weakened immune system such as diabetes, renal disease, cancer, or liver disease may be at a higher risk. NF is not contagious. Proper wound care, including cleaning all cuts and scrapes with soap and water, cleaning and covering draining wounds, and having wounds assessed by a healthcare provider are all important prevention measures. Those with open wounds should avoid hot tubs; swimming pools; and natural bodies of water such as lakes, rivers, and the ocean. According to the CDC, since 2010, approximately 700 to 1,200 cases of NF occur each year in the United States.

Puerperal sepsis, a once-common infection, is infrequently attributed to group A streptococcus today.

S. pyogenes is generally treated with penicillin, and alternatives include macrolides such as erythromycin, azithromycin, or clarithromycin, as well as certain cephalosporins. Vancomycin can be used for serious infections in those who are allergic to penicillin. Currently, there has been no resistance to penicillin, cephalosporins, or vancomycin, but resistance to macrolides has occurred.

NOTE

GAS Infections and Diseases

- Primary infections: Streptococcal pharyngitis, scarlet fever, skin infections
- Invasive infections: Streptococcal toxic shock-like syndrome, necrotizing fasciitis
- Sequelae: Acute rheumatic fever, acute glomerulonephritis

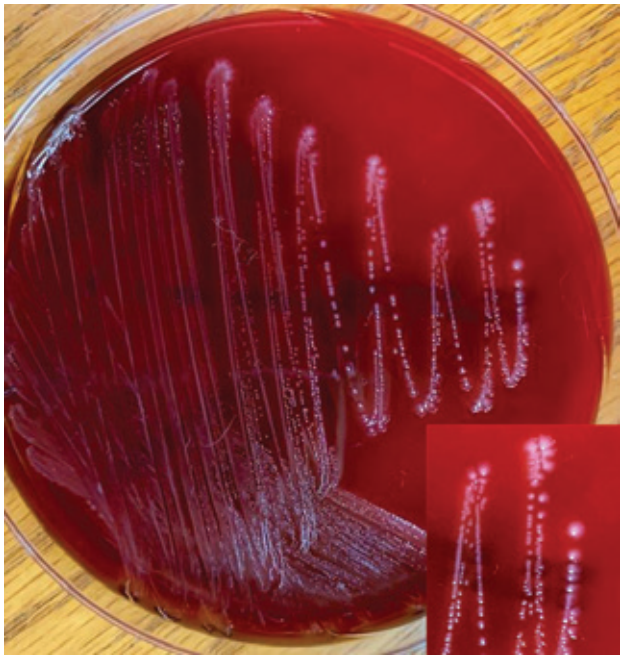


Figure 10-4. *S. agalactiae* on sheep blood agar.
Courtesy of Maria Delost.

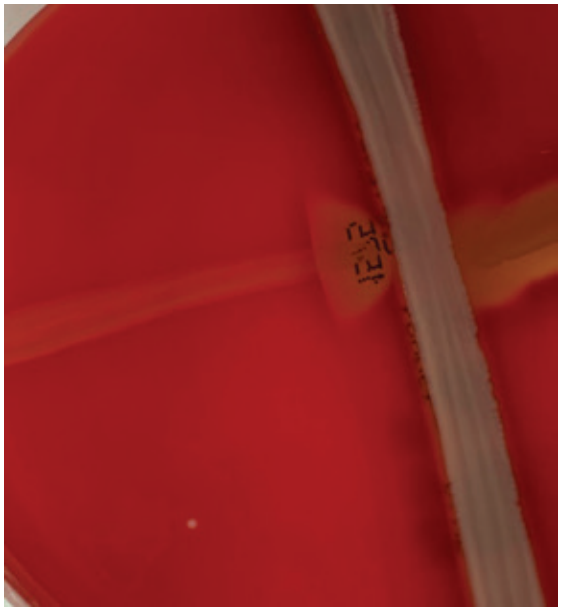


Figure 10-5. CAMP reaction.
Courtesy of Maria Delost.

Streptococcus agalactiae (Group B Streptococcus)

Streptococcus agalactiae or group B streptococcus (GBS) is isolated on sheep blood agar. Colonies are medium in size, and usually larger than group A Streptococcus flat, greyish-white, and translucent or opaque. Most often, the colonies show narrow-zone β hemolysis (95% to 99% of clinical isolates); however, nonhemolytic colonies may be occasionally observed. **Figure 10-4** shows *S. agalactiae* on sheep blood agar.

The CAMP factor is an extracellular, thermostable, antigenic protein produced by group B streptococcus and is an acronym for Christie, Atkins, and Munch-Petersen. These individuals first identified the synergistic hemolytic pattern between GBS and β hemolytic *S. aureus*. In this reaction, an arrowhead-shaped zone of hemolysis forms when GBS is streaked perpendicularly to a β hemolytic strain of *S. aureus* (**Figure 10-5**). Because a small percentage of GAS also may give a positive **CAMP reaction**, the bacitracin, PYRase, and SXT reactions should also be used to differentiate group A from group B streptococcus.

The hippurate reaction also may be used to identify GBS. In a positive reaction, GBS hydrolyzes sodium hippurate to benzoic acid and glycine. Benzoic acid is then detected with ferric chloride. Alternatively, glycine may be detected with ninhydrin reagent.

Group B streptococci are resistant to bacitracin and SXT and are bile-esculin negative. Definitive identification can be made with immunochemical reactions, such as agglutination using anti-B antisera bound to latex beads to detect the group B antigen. **Box 10-5** provides quick facts for GBS.

Group B streptococcus is normal flora of the gastrointestinal tract and vaginal tract and may also colonize the mucous membranes of the genitourinary, upper respiratory, and gastrointestinal tracts. GBS is an important cause of neonatal infections, including pneumonia, meningitis, and sepsis. Mothers colonized with GBS may transmit the organism through the amniotic fluid during pregnancy or labor and the infant may aspirate the organism into the lungs. GBS also may invade the blood or central nervous system, causing bacteremia and meningitis. Infections, generally pneumonia and sepsis, acquired within the first week of life, are termed early-onset infections. Those infections, usually meningitis and sepsis, acquired 1 week to 3 months after birth are known as late-onset infections. Currently, the CDC recommends screening

BOX 10-5

GROUP A STREPTOCOCCUS QUICK FACTS

β hemolytic (narrow zone) or nonhemolytic on SBA
CAMP positive
Hippurate positive
Bacitracin resistant
SXT resistant
PYRase negative
Bile-esculin hydrolysis negative

all pregnant women for vaginal and rectal colonization between 35 and 37 weeks of gestation using an enrichment broth, such as Todd-Hewitt broth with nalidixic acid. After incubation for 18–24 hours, the broth is subcultured onto blood agar. A selective enrichment broth, such as Strep B Carrot Broth™ (Hardy Diagnostics), uses chromogenic pigments and provides enhanced sensitivity and specificity and decreased incubation time to isolate GBS. Broths that show the production of orange, red, or brick-red pigment are unique for GBS. Prophylactic antibiotics then can be administered to pregnant women who are carrying GBS to prevent infection in the baby. Direct antigen testing for the group B antigen also can be performed on the infant's spinal fluid or blood. There is also nucleic acid amplification for nonenriched clinical specimen.

Adult group B streptococcal infections include skin and soft tissue infections, pneumonia, septic arthritis, bacteremia, urinary tract infections, and endocarditis. These infections are often healthcare-associated infections and often involve an immunosuppressed host.

GBS is treated with penicillin and an aminoglycoside may be added, as well as ceftriaxone or cefotaxime. Vancomycin can be used in those allergic to penicillin. There has been no resistance to penicillin, cephalosporins, or vancomycin reported.

Miscellaneous β Hemolytic *Streptococcus*

Other clinically significant β hemolytic streptococci include Lancefield groups C, F, and G. These organisms are found as normal microbiota of the skin, nasopharynx, gastrointestinal tract, and genital tract. Endogenous strains may gain access to a sterile site and infections may be spread through direct person-to-person contact. Infections resemble those caused by GAS and GBS, but usually involve immunocompromised patients. The Lancefield group C antigen is found in several species of *Streptococcus,* which include *S. equi* subsp. *zooepidemicus*, *S. equi* subsp. *equi*, *S. dysagalactiae* subsp. *equisimilis*, and *S. dysagalactiae* subsp. *dysagalactiae*. *S. dysagalactiae* causes infections like those of GAS, including pharyngitis, skin and soft tissue infections, and bacteremia. The organism is β hemolytic; gives negative reactions for the CAMP factor, PYR, and bile-esculin hydrolysis; and is susceptible to SXT. *S. dysagalactiae* may alternatively carry the Lancefield group F antigen.

S. anginosus generally carries the Lancefield group F antigen and is usually considered as normal flora in the oropharynx and urogenital tracts. It may be the cause of cellulitis, abscesses, and bacteremia. Strains of *S. anginosus* also may have A-, C-, or G-type Lancefield antigen, while other strains do not have any Lancefield antigen. Colonies are typically small, and may be α, β, or nonhemolytic.

To identify Lancefield groups C, F, and G, biochemical reactions and antigen testing are required. To identify the group antigen, the organism is first isolated on blood agar, and the cell wall antigen is extracted. The extract is then combined with antisera specific for each Lancefield group, which have been tagged with latex beads to aid visualization. Agglutination should occur with a single antiserum type, thus identifying the specific Lancefield group.

These infections are treated with penicillin or vancomycin for penicillin-allergic patients.

Streptococcus pneumoniae

S. pneumoniae appears as gram-positive diplococci that are lancet shaped and generally arrange in chains. The Gram stain reaction becomes gram variable with age, and some cells may appear as gram negative. *S. pneumoniae* can be isolated on sheep blood agar but requires 5% to 10% CO_2. Thus, primary isolation plates should be incubated in a candle jar or CO_2 chamber. At 24 hours of incubation at 35°C to 37°C, encapsulated strains produce small, round, glistening, dome-shaped colonies that are transparent, with an entire edge. The colonies may run together. Mucoid colonies indicate the presence of a capsule. Colonies show a wide zone of α hemolysis after 24 hours of incubation, as shown in **Figure 10-6.** This α hemolysis may be attributed to

Figure 10-6. *S. pneumoniae* on SBA.
Courtesy of Maria Delost.

the production of **pneumolysin** by *S. pneumoniae*. As the colonies age, autolysis occurs, and the colonies collapse and appear umbilicated, leaving an outer elevated margin and a centrally depressed region when observed following 48 hours of incubation. Autolysis may make it difficult to keep the organism viable, which requires subculture every few days.

S. pneumoniae is identified using the **optochin test** (P disk). Optochin contains ethylhydrocupreine hydrochloride and selectively inhibits the growth of *S. pneumoniae* at low levels. A zone of inhibition of at least 14 mm around a 5-µg optochin disk indicates a positive susceptibility test, identifying *S. pneumoniae*. Other α hemolytic streptococci, including viridans streptococci and enterococci, are resistant to optochin. **Figure 10-7** illustrates the optochin test, showing the susceptibility of *S. pneumoniae* and the resistance of viridans streptococci.

S. pneumoniae also can be identified using **bile solubility** tests. Surface active agents or bile salts, such as sodium deoxycholate and sodium taurocholate, can lyse *S. pneumoniae* when grown in culture. On agar, colonies of the organism will lyse or break down when exposed to surface active agents while there is no observable effect for other α hemolytic streptococci.

The Quellung reaction, or capsular swelling test, has been used in the past to identify *S. pneumoniae* directly in body fluids, such as cerebrospinal fluid, synovial fluid, or sputum. Equal amounts of the specimen are mixed with *S. pneumoniae*–specific capsular antisera. If the reaction is positive, the antisera will bind to the capsular antigen, making the capsule swell and become more prominent. In the Quellung reaction, the organism can be both identified and serotyped. There are also coagglutination and agglutination methods for direct antigen testing for the pneumococcal capsular polysaccharide. In agglutination tests, polyvalent antisera, which reacts with most serogroups of *S. pneumoniae*, is tagged with latex particles. Agglutination will occur in the presence of the corresponding capsular antigen. These tests are rapid and may be used on broth or isolated colonies on agar or directly on blood cultures and other clinical specimens.

Extracellular products or antigenic compounds associated with *S. pneumoniae* that contribute to its virulence include capsular polysaccharide antigen, M protein, pneumolysin, and autolysin. These are summarized in **Box 10-6**.

Because *S. pneumoniae* may be a part of the normal flora of the upper respiratory tract, it may be difficult to interpret its recovery from sputum and lower respiratory tract cultures. Also, because of autolysis, it is sometimes problematic to recover *S. pneumoniae* from clinical specimens. Therefore, some patients who are positive for infection may be culture negative. For that reason, the direct Gram stain is an important diagnostic tool. Bartlett's classification scheme can be used to evaluate the suitability of the sputum sample and the presence of infection. The direct Gram stain from sputum specimens from individuals with pneumococcal infections of the lower respiratory tract show a predominance of *S. pneumoniae,* with a decrease or lack of normal oropharyngeal flora. A moderate to large number of neutrophils (PMNs) may be present, and the sputum may be rust tinged or bloody.

S. pneumoniae is transmitted via person-to-person contact through respiratory droplets of asymptomatic carriers or from individuals who are infected with the organism. It may colonize the nasopharynx epithelium, especially in the presence of viral infections or allergies and in those who smoke. The bacteria may then travel to anatomically connected areas, such as the middle ear and sinuses, and cause infections in these sites.

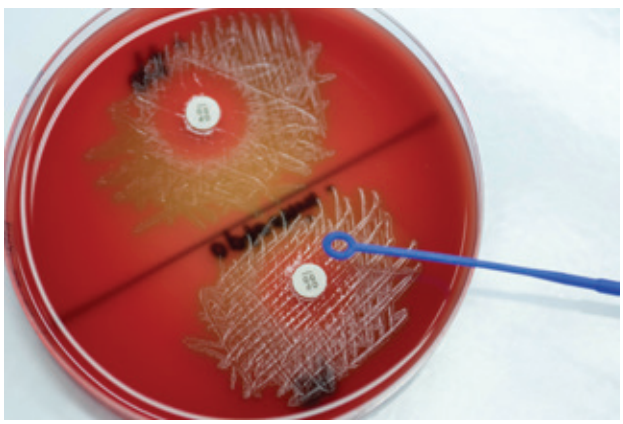


Figure 10-7. Optochin (P) disk. *S. pneumoniae* is shown on the top part of the plate with a zone of inhibition indicating its susceptibility to optochin. Alpha hemolytic streptococci are shown on the bottom part of the plate, with no zone of inhibition or a resistant result.

BOX 10-6

VIRULENCE COMPOUNDS OF *STREPTOCOCCUS PNEUMONIAE*

Capsular antigens: Antigenic polysaccharides that enable organism to resist phagocytosis and stimulate antibody production. There are over 90 serogroups based on the capsular antigen.

M protein: Type-specific protein antigens

Pneumolysin: Cytotoxic protein that accumulates in cell and is released upon cell lysis; antiphagocytic and hemolytic and activates complement

Autolysin: Breaks down organism at end of its growth cycle and aids in release of pneumolysin

The bacteria may also travel to the alveoli in the lungs, often through aspiration, where lobar pneumonia may develop. If the host lacks antibodies to the capsule, invasive strains can enter the lung lymphatics and the blood and then seed target organs.

Diseases of *S. pneumoniae* include pneumonia, bacteremia, septicemia, sinusitis, endocarditis, pericarditis, osteomyelitis, and meningitis. Pneumococcal pneumonia is a leading cause of community-acquired pneumonia and otitis media in infants and small children. There are over 90 serotypes of the organism based on the capsular antigen; strains without capsules are not virulent.

If the patient lacks the corresponding antibody to the capsular antigen, lobar pneumonia may occur. Predisposing factors for lobar pneumonia include alcoholism, malnutrition, viral infections in the upper respiratory tract, and anesthesia. Symptoms of lobar pneumonia include the sudden onset of fever, chills, dyspnea, and cough. Bacterial meningitis from *S. pneumoniae* may occur in all age groups and may follow pneumonia, ear or sinus infections. Because the pneumococcus is also isolated from cases of endocarditis, bacteremia, and peritonitis, blood cultures are often collected to diagnose pneumococcal infection.

Most isolates of *S. pneumoniae* remain susceptible to penicillin, which remains the drug of choice. Other antibiotic options include ceftriaxone, cefotaxime, macrolides, trimethoprim sulfamethoxazole, and quinolones such as levofloxacin and moxifloxacin. However, some *S. pneumoniae* have shown resistance to penicillin, cephalosporins and macrolides, although vancomycin resistance has not been documented. In these cases, erythromycin or chloramphenicol may be used. Resistance to antibiotics, especially penicillin, varies with the geographic region and time period, so susceptibility testing that provides a minimal inhibitory concentration (MIC) for β-lactam antibiotics is recommended. Penicillin resistance is due to altered penicillin-binding proteins and strains resistant to penicillin may also show resistance to other β-lactams, macrolides and tetracyclines. Inducible clindamycin resistance should be tested for in those isolates that show resistance to erythromycin. Multidrug resistant *S. pneumoniae* refers to those strains that show in vitro resistance to two or more antimicrobial classes and include erythromycin, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol.

Infection may be prevented through the pneumococcal conjugate vaccine, which is recommended for those at higher risk of infection. These include the elderly and debilitated; children younger than 2 years of age; and those with underlying medical conditions, including diabetes mellitus, leukemia, lymphoma, human immunodeficiency virus, sickle cell anemia, and other immunocompromising conditions. Asplenic patients are especially susceptible because they cannot remove the antibody-coated organism. There is a pneumococcal conjugate vaccine, which is recommended for infants and young children, that protects against 13 types of pneumococci. There is also a 23-valent polysaccharide vaccine, which is recommended for adults who are 65 years of age or older and for those who are 2 years and older and at increased risk of infection.

Viridans Streptococci

The **viridans streptococci** are a diverse group of streptococcal species that are α hemolytic on sheep blood agar. There are many classification strategies used for the viridans streptococci, which currently contains over 30 species and 5 phenotypic groups. Some species are not classified. **Table 10-2** summarizes one classification system based on phenotypic characteristics. Some species may possess specific Lancefield antigens.

Members of the viridans streptococci include *S. mutans*, *S. salivarius*, *S. anginosus*, *S. mitis*, *S. sanguinis*, *S. constellatus*, and *S. intermedius*. Most are normal flora of the human oropharynx, gastrointestinal tract, and female genital tract and are opportunistic pathogens. The viridans streptococci are not very invasive, but infections usually occur through the endogenous route after surgical or dental procedures. The viridans streptococci are the most frequent cause of subacute bacterial endocarditis (SBE), which occurs as a result of a transient bacteremia, where the bacteria enter the blood following dental or genitourinary procedures. Those with damaged valvular heart tissue, such as a prolapsed mitral valve or resulting from rheumatic fever, as well as individuals who have prosthetic heart valves are at risk of SBE. *S. mitus* is the most common streptococcal species in SBE resulting from valve disorders. Viridans streptococci are also involved in oral infections, such as dental caries and gingivitis. *S. mutans* produces the enzyme glucosyl transferase, which breaks down sucrose, which then binds to glucose to form the complex polysaccharides glucan and dextran. These facilitate the attachment of *S. mutans* to tooth enamel, thus playing a role in dental caries. Viridans streptococci may cause bacteremia and septicemia, especially in patients who are immunocompromised (including those who are neutropenic), as well as meningitis, abscesses, and osteomyelitis. Meningitis may occur following trauma.

Members of the *S. anginosus* group are generally considered to be normal flora of the oral cavity and gastrointestinal tract, but may be associated with abscesses in the oropharynx, brain, and abdominal cavity. *S. salivarius*

Table 10-2. Phenotype Categories of Viridans Streptococci

Phenotypic Group	Representative or Significant Species	Important Biochemical Reactions						Comments
		Arginine	Voges Proskauer	Mannitol	Sorbitol	Esculin	Urea	
S. mutans group	*S. mutans* and *S. sorbinus*	–	+	+	+	+	–	May be α, β, or nonhemolytic; *S. mutans* is associated with dental carries
S. salivarius group	*S. salivarius, S. vestibularius*, and *S. infantarius*	–	+/V	–	–	V	+/V	Related to *S. bovis* group based on rRNA
S. anginosus group	*S. anginosus, S. constellatus*, and *S. intermedius*	+	+	–/V	–	+	–	May be α, β, or nonhemolytic; β strains may have Lancefield antigens A, C, F, or G. α strains are more common. Normal flora in oral cavity, oropharynx, gastrointestinal tract, and vaginal tract
S. mitis group	*S. mitis, S. sanguis*, and *S. oralis*	–	–	–	–	–/V	–/V	Found in oral cavity; frequent cause of subacute bacterial endocarditis
S. bovis group	*S. equinus, S. infantarius*, and *S. gallolyticus*		+	V	–	+	–	*S. bovis* and *S. equinus* are now a single species (*S. equinus*).
								May be α or nonhemolytic; have Lancefield group D antigen

+: Most are positive for the reaction.
–: Most are negative for the reaction.
V: Reaction is variable.
+/V: Reaction is usually positive for most, but may be variable.
–/V: Reaction is usually negative for most; but may be variable.

has been isolated as the cause of bacteremia, meningitis, and endocarditis.

The level of identification of the viridans streptococci depends on the specimen source and immune status of the patient. While it is sometimes acceptable to report viridans streptococci, it may be necessary to identify the specific streptococcal species when isolated from blood cultures or in cases of endocarditis, as well as the *S. bovis* group when found are blood cultures.

Viridans streptococci infections are treated with penicillin or ceftriaxone; an aminoglycoside may be added. Vancomycin is used in cases of allergy to penicillin or β-lactam resistance. There has been resistance to β-lactam antibiotics and the cephalosporins by the viridans streptococci, especially *S. mitus*, so susceptibility testing that provides an MIC is recommended for these antibiotics.

The viridans streptococci grow as tiny, pinpoint, grey, smooth or matte colonies on sheep blood agar, as shown in **Figure 10-8.** Although, the name "viridans" means "greening" and refers to α hemolysis, some species may be nonhemolytic or β hemolytic. Viridans streptococci may have a butterscotch odor when grown on chocolate

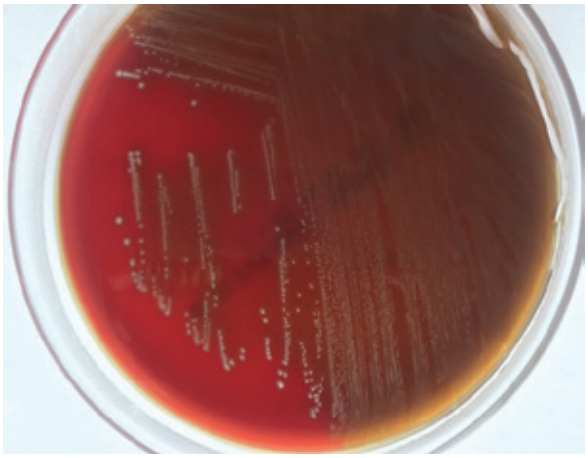


Figure 10-8. Viridans streptococci on sheep blood agar.

agar. All viridans streptococci are resistant to surface active agents, such as bile, sodium deoxycholate, and optochin. In fact, viridans streptococci are often identified by exclusion, which means that negative results are obtained for most presumptive tests used to identify the other streptococci. The organisms may be grouped phenotypically based on specific biochemical reactions, such as fermentation of mannitol and sorbitol, production of urease, and the VP reaction. Commercially available identification kits are also available, as are automated procedures to identify the viridans streptococci to the species level.

Streptococcus bovis Group

The *Streptococcus bovis* group has undergone many changes in taxonomy, resulting in four DNA clusters. *S. bovis* and *S. equinus* have been determined to be a single species and are currently known as *S. equinus*. Thus, *S. bovis* is no longer a valid species name. Other important species in this group include *S. gallolyticus*, *S. infantarius*, and *S. alactolyicus*. They are usually nonhemolytic on sheep blood agar, but some strains may show α hemolysis. The *S. bovis* group has the Lancefield group D antigen and, in the past, were categorized as group D *Streptococcus*—nonenterococcus. The *S. bovis* group and enterococci both possess the Lancefield group D antigen and are **bile-esculin** positive. However, members of the *S. bovis* group are PYR negative and unable to grow in 6.5% NaCl broth, whereas the enterococci are PYR positive and able to grow in 6.5% NaCl broth. Bacteria in the *S. bovis* group are found as agents of bacteremia, septicemia, and endocarditis. There has been an association of the isolation of *S. gallolyticus* subsp. *gallolyticus* with gastrointestinal cancer when isolated from blood cultures. The relationship between bacteremia due to *S. bovis* and colon cancer is well established. Those diagnosed with *S. bovis* bacteremia should be screened for gastrointestinal pathologies. **Figure 10-9** shows a member of the *S. bovis* group on sheep blood agar.

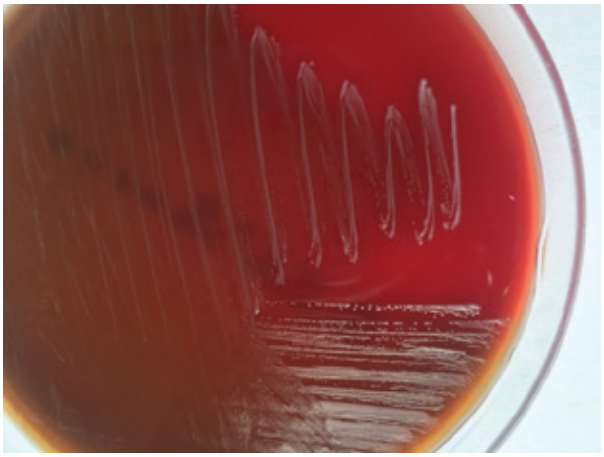


Figure 10-9. *Streptococcus bovis* group on sheep blood agar. Courtesy of Maria Delost.

Enterococcus

The *Enterococcus* were previously classified in the genus *Streptococcus* and were distinguished by possessing the Lancefield group D antigen and by their increased resistance to chemical and physical agents. Because of molecular techniques, including DNA–DNA reassociation and rRNA sequencing, these organisms were classified into the genus *Enterococcus* in 1984. Along with the *S. bovis* group, the enterococci possess the Lancefield group D antigen and can grow in 40% bile and hydrolyze esculin. The *Enterococcus* grow as small, cream, grey, or white, smooth colonies that are 1 to 2 mm in diameter after 24 hours of incubation on sheep blood agar and are generally either alpha-hemolytic or nonhemolytic, and rarely beta hemolytic, **Figure 10-10** shows the typical colonies of the *Enterococcus* on sheep blood agar after 24 hours of incubation.

There are over 35 species of *Enterococcus*, which are grouped according to phenotypic characteristics. *E. faecalis* causes almost 80% to 90% of human enterococcal infections, followed by *E. faecium*, which is implicated in 5% to 10% of enterococcal infections. There has been an increase in hospital-acquired infections caused by *E. faecium* in recent years. Other species include *E. durans*, *E. avium*, *E. casseliflavus*, *E. gallinarum*, *E. dispar*, and *E. canis*.

Enterococci are found throughout the environment in foods, plants, and oil, and in many animals, including birds, reptiles, horses, cattle, and other mammals. In humans, the enterococci are found as normal flora

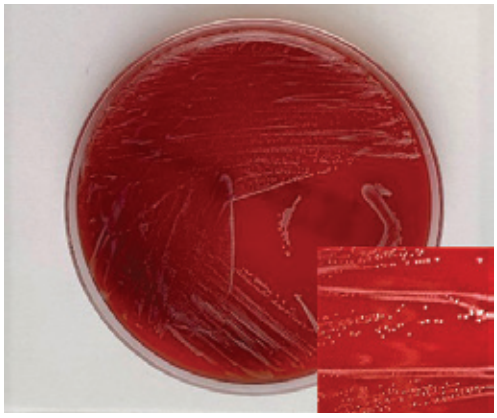


Figure 10-10. *Enterococcus* on sheep blood agar. Courtesy of Maria Delost.

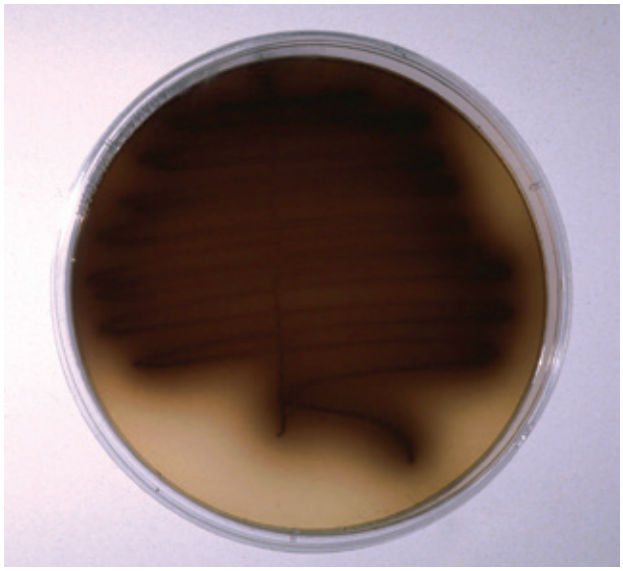


Figure 10-11. Bile-esculin positive.

of the gastrointestinal tract; on the skin; and in the oral cavity, oropharynx, female genital tract, and genitourinary tract. *E. faecalis* is the most common species found colonizing the human gastrointestinal tract. **Figure 10-11** illustrates enterococci on bile esculin agar, showing growth and positive hydrolysis.

The enterococci can grow in 6.5% NaCl broth (are **salt tolerant**) and are PYR positive, which is used to differentiate the *Enterococcus* from the *S. bovis* group (formerly nonenterococcus), which also carry the Lancefield group D antigen. **Figure 10-12** illustrates positive and negative reactions for 6.5% NaCl broth, and **Figure 10-13** shows the PYR reaction. **Table 10-3** summarizes the differentiation of the *Enterococcus* from the *S. bovis* group.

Enterococci are opportunistic pathogens and are the agents of healthcare-associated infections, including

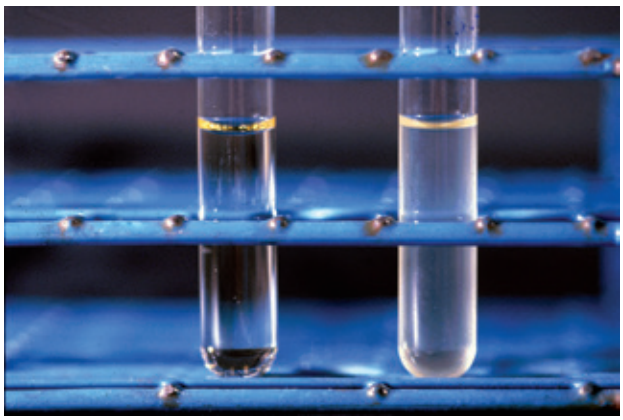


Figure 10-12. Negative (left) and positive (right) reactions for 6.5% NaCl broth.

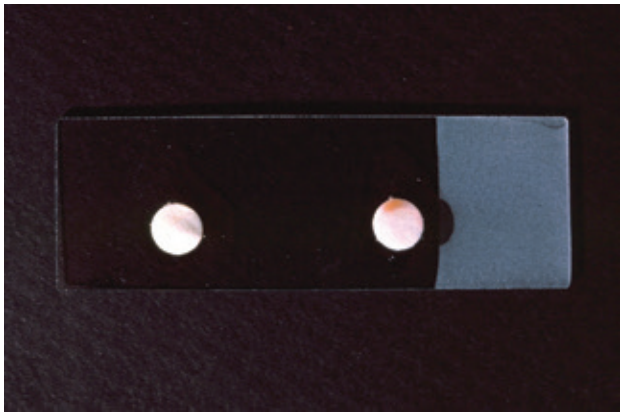


Figure 10-13. PYRase reaction: Negative on left and positive on right.

urinary tract infections, wound infections, endocarditis, and bacteremia. Polymicrobic enterococcal infections include wound infections and intra-abdominal and pelvic abscesses. Predisposing factors for enterococcal infections include immunosuppression, prolonged antibiotic therapy, and invasive procedures.

Enterococcal strains are intrinsically resistant to β-lactam antibiotics, including the penicillins and cephalosporins, and to the aminoglycosides. Intrinsic resistance is inherent and coded in the chromosomes of the organism and is found in most enterococci. To overcome intrinsic resistance, treatment of enterococcal infections has included a combination therapy of a β-lactam (such as penicillin, ampicillin, or vancomycin) with an aminoglycoside, such as gentamycin or streptomycin. Linezolid or daptomycin may also be used for serious infections. For urinary tract infections, ampicillin, nitrofurantoin, tetracyclines, or quinolones can be used.

There is widespread and frequent acquired resistance in the *Enterococcus*, including resistance to linezolid and daptomycin; the macrolides; aminoglycosides; tetracycline; and the glycopeptides, especially vancomycin. Acquired resistance occurs as a result of mutations

Table 10-3. Differentiation of Group D Streptococci and *Enterococcus*

	Bile-esculin Media	6.5% NaCl broth	PYRase
Group D *Enterococcus*	+	+	+
Streptococcus bovis group Group D non-*Enterococcus*	+	–	–
Not group D	–	–	–

+: Most are positive for the reaction
–: Most are negative for the reaction

in DNA or plasmids and transposons. **Vancomycin Resistant Enterococci (VRE)** were first reported in 1986, approximately 30 years after vancomycin was used as an antibiotic. VRE continues to increase as a public health issue. There are six types of vancomycin resistance based on genetic and phenotypic characteristics, of which VanA, VanB, and VanC phenotypes are most significant. The VanA phenotype is encoded by the *vanA* gene and shows inducible high-level resistance to vancomycin and teicoplanin. The VanB phenotype, encoded by the *vanB* gene, shows a variable amount of resistance to vancomycin alone. The VanC phenotype is associated with noninducible low-level resistance to vancomycin and is coded for by the *vanC* genes.

Abiotrophia and *Granulicatella*

Abiotrophia* and *Granulicatella were previously known as nutritionally variant streptococci and resemble viridans streptococci, but require cysteine or pyridoxal (vitamin B_6) for growth. These organisms also are referred to as "satelliting," thiol-requiring or pyridoxal-requiring, or cell wall–deficient streptococci. When in a mixed culture with *S. aureus*, these bacteria "satellite" around the colonies of *S. aureus*, which provides pyridoxal. Important species include *A. adjacens, A. defectivus* and *G. adiacens*. The organisms are normal flora of the human oral cavity and have been implicated as endogenous agents in endocarditis, bacteremia, and otitis media.

Other Catalase-Negative Streptococcal-Like Organisms

There are other catalase-negative organisms that resemble streptococci and are found as normal flora on the skin and in the gastrointestinal tract or oral cavity. These are generally rare human pathogens but may be associated with infections in immunocompromised hosts. They also may be mistaken for or misidentified as other streptococci. *Aerococcus* is an airborne organism and may be rarely isolated as a cause of endocarditis, bacteremia, and meningitis in immunosuppressed individuals. *Leuconostoc* species have been infrequently isolated from several clinical sites, including the blood, wounds, and abscesses. *Pediococcus* is normal flora of the lower gastrointestinal tract and has been isolated occasionally from abscesses. *Leuconostoc* and *Pediococcus* both have exhibited resistant to vancomycin. *Lactococcus* and *Gemella* are rare human pathogens. These organisms are summarized in **Box 10-7**.

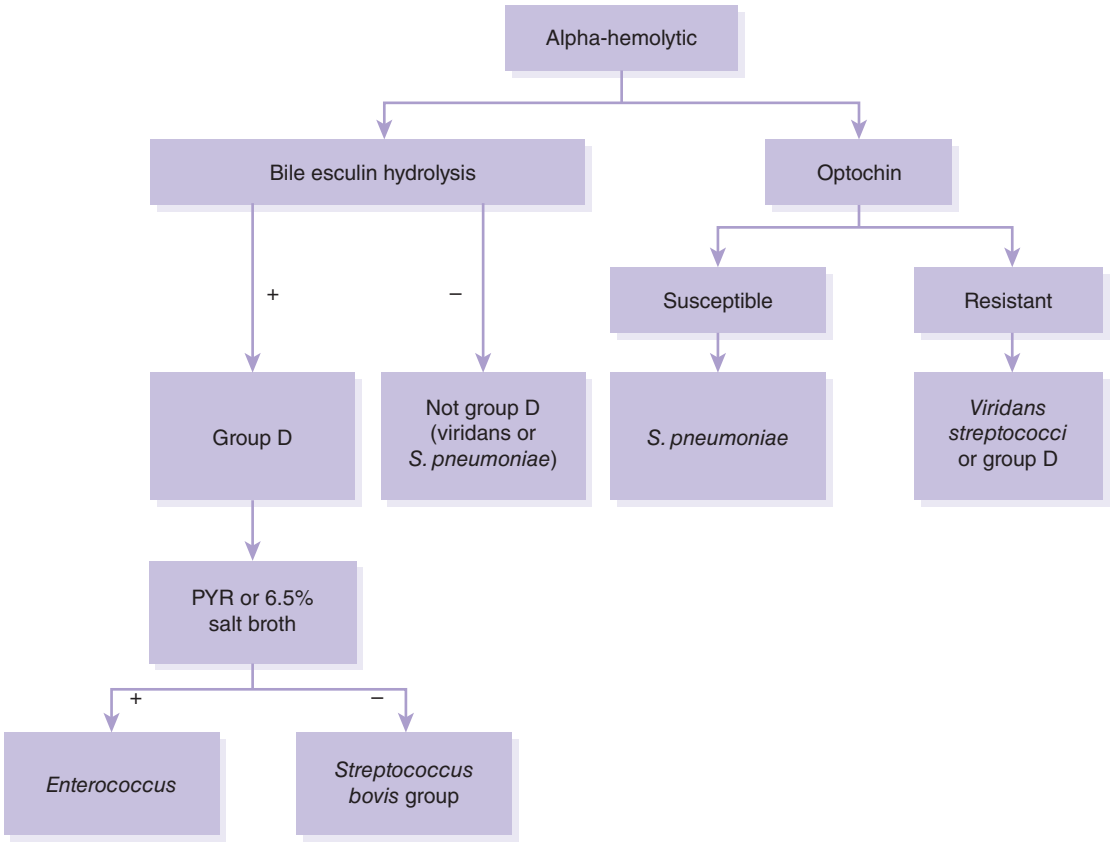
BOX 10-7

OTHER CATALASE-NEGATIVE STREPTOCOCCAL-LIKE BACTERIA

Organism	Characteristics	Clinical Significance
Aerococcus	Resemble viridans streptococci on sheep blood agar; gram-positive cocci in clusters or tetrads May give weak positive catalase reaction May be confused with enterococci because of growth in 6.5% NaCl and some species give positive bile-esculin reaction	Airborne; found with increasing frequency in immunosuppressed patients causing bacteremia, endocarditis, meningitis, and urinary tract infections
Gemella	Resembles viridans streptococci; may decolorize and appear as gram-negative cocci in pairs, chains, tetrads, or clusters	Rare human pathogen causing endocarditis, respiratory, and wound infections and abscesses
Lactococcus	Gram-positive cocci occurring singly and may resemble *Enterococcus,* but produce acid from carbohydrates; α or nonhemolytic on sheep blood agar; previously classified as group N streptococci	Rare human pathogen causing endocarditis and urinary tract infections
Leuconostoc	Irregular arrangement of gram-positive cocci; intrinsically resistant to vancomycin May cross-react with Lancefield group D antigen	Opportunistic infections such as meningitis, bacteremia, urinary tract infections, and pulmonary infections
Pediococcus	Intrinsically resistant to vancomycin; resemble and may be misidentified as viridians streptococci or *Enterococcus*	Normal flora of lower gastrointestinal tract and causes infections in those who have preexisting gastrointestinal or abdominal surgery or abnormalities; may cause bacteremia, meningitis, or abscess infections

Identification

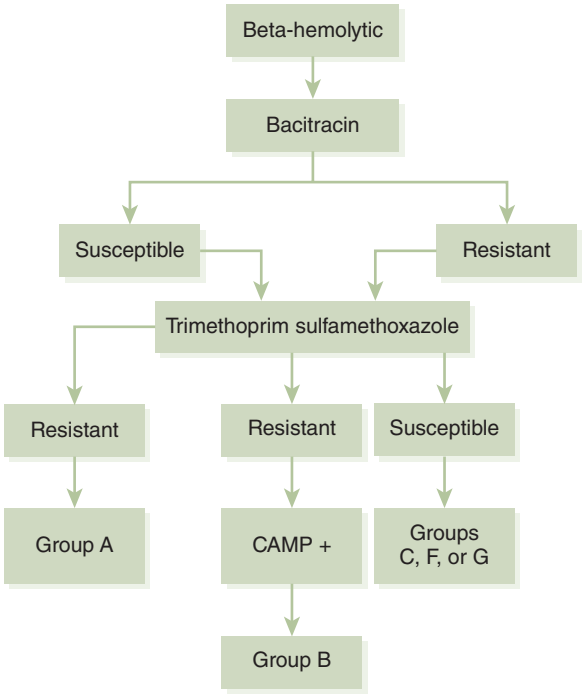
To identify *Streptococcus*, *Enterococcus* and similar organism, it is important to identify the type of hemolysis on blood agar. Identification schemes are shown in **Figures 10-14** to **10-16** Automated methods are available and molecular diagnostic and MALDI-TOF MS identification methods continue to be developed.



Group	Optochin	Bile esculin	6.5% salt broth	PYR
D-enterococcus	R (–)	+	+	+
D-*S. bovis* group	R (–)	+	–	–
S. pneumoniae	S (–)	–	–	–
Viridans streptococci groups	R (–)	–	V	–

R = resistant S = susceptible V = variable

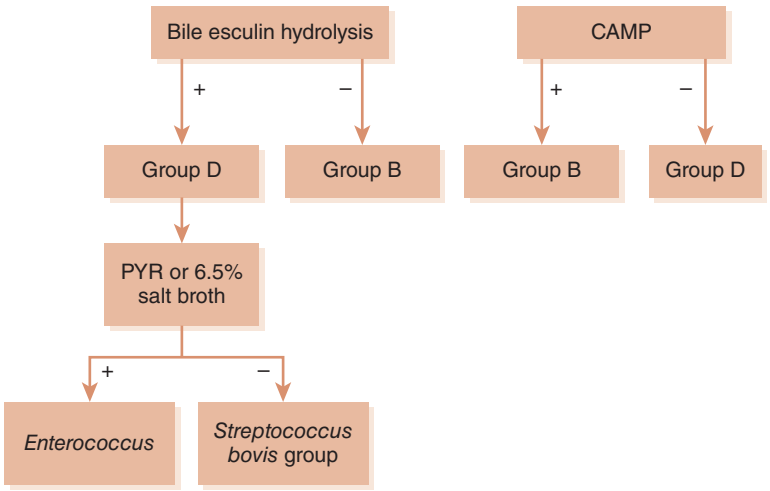
Figure 10-14. Identification of α hemolytic streptococci.



Group	Bacitracin	Trimeth/sulfa	CAMP	PYRase
A	S (+)	R(–)	–*	+
B	R (–)	R(–)	+	–
C, F, G	R (–)**	S (+)	–	–

TS = Trimethoprim sulfamethoxazole
* A small percentage of Group A give an intermediate CAMP reaction
** A small percentage of Groups C, F, and G are susceptible to bacitracin

Figure 10-15. Identification of β hemolytic streptococci.



Group	CAMP	Bile esculin	6.5% salt broth	PYR
D-*enterococcus*	–	+	+	+
D-*S. bovis* group	–	+	–	–
B	+	–	–	–

Figure 10-16. Identification of nonhemolytic streptococci.

HERE AND NOW

Invasive Group A Streptococcal Diseases

Most GAS diseases are self-limited cases of pharyngitis and skin infections and are estimated to account for over 10 million cases annually in the United States, although the number of cases is not tracked by the CDC. However, the CDC estimates that approximately 11,000 to 24,000 cases of invasive group A strep disease occur each year in the United States. These invasive diseases include cellulitis with blood infection, necrotizing fasciitis, pneumonia, and streptococcal toxic-shock like syndrome (STSS). Each year between 1,200 and 1,900 people die due to invasive group A strep disease. STSS and necrotizing fasciitis (NF) account for 6% to 7% of these invasive diseases. The mortality rate of necrotizing fasciitis ranges from 24% to 34% and for STSS is over 35%. The mortality rate increases to 60% when there is coincident necrotizing fasciitis and streptococcal toxic shock syndrome (STSS).

Invasive GAS infections are most commonly associated with M serogroups M1, M3, M12, and M18, which produce pyogenic exotoxins A and B. Pyogenic exotoxins cause fever and induce shock by decreasing the threshold to exogenous endotoxin.

STSS is associated with shock and multi-organ failure. Complications include bacteremia, soft tissue infection, shock, acute respiratory distress, and acute renal and hepatic failure. The primary infection site is rarely the pharynx, but instead a site of minor local, nonpenetrating skin trauma. In other instances, viral infections, including varicella and influenza, provide the site of entry. The most common clinical sign is severe and rapid onset of pain in an extremity, but also may resemble peritonitis, pneumonia, or myocardial infarction. There also may be influenza - like symptoms of fever, nausea, vomiting, and diarrhea. Signs of soft tissue infection, such as swelling and erythema, may be observed, which may result in myositis or necrotizing fasciitis.

In STSS, hypotension, or a drop in blood pressure, quickly develops. Laboratory parameters show hemoglobinuria, elevated serum creatinine, and decreased albumin and calcium. The serum creatine kinase is elevated, and there is a mild leukocytosis, with immature white cells seen. Blood cultures are positive in approximately 60% of the cases. Shock quickly may develop, and complications include renal failure, respiratory failure, coagulopathies, and death.

NF is a soft tissue infection with severe necrosis of the fascia, which can be caused by a variety of bacteria, but most commonly GAS. NF occurs when the bacteria invade the subcutaneous tissues and then spread into the superficial and deep fascia. The infection may be polymicrobic or an infection that is associated with many species of bacteria. NF progressively destroys the fascia and fat, but not always the skin and muscle. The disease was first described in 1848 and was later given the name necrotizing fasciitis; it is sometimes called the "flesh-eating bacteria" because of its rapid and progressive destruction of tissue. There are different types of NF: Type I is a polymicrobic infection; type II is associated with GAS; and type III is known as saltwater NF, which is associated with *Vibrio* bacteria. NF may occur following a variety of medical or surgical procedures. Bacterial toxins and enzymes; host factors such as poor blood circulation and oxygen to the issue; and other host factors such as chronic disease, surgery, and immunosuppression can promote the spread of the disease. Other symptoms of NF include respiratory, renal, and liver failure. *S. pyogenes* is generally isolated from a normally sterile site. The CDC reports that approximately 700 to 1,200 cases of NF occur each year in the United States.

GAS has been an important human pathogen for hundreds of years. Historians report that there were epidemics of scarlet fever in Europe in the 1600s as well as in the American colonies in 1736. In the 19th century, there were severe epidemics of invasive and severe GAS disease. In the 1880s, one-fourth of all children with scarlet fever died, yet this figure dropped dramatically to less than 2% by the turn of the century, although this cannot be explained by the use of antibiotics or improved living conditions. Perhaps, this can be attributed to a decreased expression of the virulence factors or improved host immunity within the affected communities. Outbreaks of rheumatic fever occured during World War II. The severity of GAS disease declined until the 1980s, when severe GAS disease seemed to reemerge. The expression of different virulence factors and the lack of immunity to these factors may explain the resurgence of severe GAS infection. These extremely virulent strains (M1, M3, M12, and M18) have spread globally in this latest occurrence of severe GAS diseases.

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WHAT WOULD YOU DO NEXT?

1. An elderly male with a cough, chills, fever, and difficulty breathing produces a rust-tinged sputum. How would you process this specimen?
2. The Gram stain showed a moderate amount of gram-positive, lancet-shaped diplococci and many PMNs per oil immersion field. What test(s) would you do next?
3. After incubation, the primary isolation plates showed the following:

   SBA: Few, small, mucoid colonies that were α hemolytic
   CNA: Similar growth as SBA
   Chocolate: Small, mucoid colonies
   MAC: No growth
   Do these growth results confirm what was seen in the Gram stain?
4. An optochin test was performed. There was a 15-mm zone of inhibition around the P disc after incubation for 24 hours in increased CO_2. How would you interpret this reaction? Based on the information provided, what organism do you suspect?

Review Questions

Matching

Match the Lancefield group to the correct species:

1. ***S. bovis* group**

2. ***S. agalactiae***

3. ***E. faecalis***

4. ***S. pyogenes***

5. ***E. faecium***
   a. A
   b. B
   c. C
   d. D

Multiple Choice

6. **Lancefield's classification of *Streptococcus* is based on:**
   a. type of hemolysis.
   b. cell wall polysaccharide.
   c. capsular antigens.
   d. M protein serotype.

7. **Streptolysin O is:**
   a. oxygen stable.
   b. antigenic.
   c. observed as surface hemolysis.
   d. All of these are correct.

8. **Which of the following reactions is incorrect for *Enterococcus*?**
   a. Growth in 6.5% salt broth
   b. Positive hydrolysis of bile-esculin media
   c. Negative catalase reaction
   d. PYR negative

9. **Colonies of *Streptococcus pneumoniae*, at 24 hours, are typically:**
   a. mucoid and α hemolytic.
   b. autolysed and α hemolytic.
   c. mucoid and β hemolytic.
   d. None of these is correct.

10. **β hemolytic streptococci were isolated from the throat culture of a 15-year-old male. Which of the following is the *best* group of tests to identify this organism?**
    a. Bacitracin and PYRase
    b. Bacitracin and CAMP
    c. Bile esculin and PYRase
    d. Optochin and CAMP

11. **Which β hemolytic streptococci are PYR negative, CAMP negative, resistant to bacitracin, and susceptible to sulfamethoxazole?**
    a. Group A streptococci
    b. Group A streptococci
    c. Lancefield group C, F, or G
    d. *Enterococcus*

12. **The nutritionally variant streptococci:**
    a. are classified as Lancefield group A.
    b. require vitamin B_6 for growth.
    c. are now classified in the genus *Lactococcus*.
    d. do not need pyridoxal for growth.

13. **Which of the following statements are correct about enterococci?**
    a. They have intrinsic resistance to penicillin and the aminoglycosides.
    b. They show acquired resistance to many antibiotics, including vancomycin.
    c. They are healthcare-associated pathogens.
    d. All of these are correct.

Short Answer

14. **Complete the chart for the expected reactions of the β hemolytic streptococci. Use "S" for susceptible, indicating a positive reaction, and "R" for resistant, indicating a negative reaction.**

Species	Bacitracin	SXT	CAMP
Streptococcus pyogenes			
Streptococcus agalactiae			
Groups C, F, and G			

15. Complete the chart for the expected reactions of α hemolytic and nonhemolytic streptococci.

Species	Optochin	Bile Esculin	6.5% Salt Broth	PYRase
Group D *Enterococcus*				
Group D— *S. bovis* group				
Streptococcus pneumoniae				
Viridans streptococci				

16. Is antibiotic susceptibility testing routinely performed on GAS isolated from throat cultures? Why or why not?

17. Name two instances when it is essential to identify viridans streptococci to the species level.

18. What are two invasive diseases associated with GAS? What are the virulence factors associated with these diseases?

19. What are two sequelae associated with GAS infections? What are the mechanisms of these diseases?

20. Why is it necessary to subculture *S. pneumoniae* frequently? How does this play a role in isolation of the organism in clinical cultures?

21. What are some of the infections and antibiotic resistance concerns associated with *Enterococcus*?

22. Design an identification scheme for nonhemolytic streptococci isolated from a blood culture of a patient who has been hospitalized for treatment of leukemia.

23. How would you work up β hemolytic streptococci isolated from the blood culture of a newborn?

Case Studies

1. An ambulatory patient with a temperature of 101°F and painful urination visits her physician, who orders a urine culture. A clean-catch midstream urine sample reveals numerous gram-positive cocci in chains in the Gram stain. The specimen is plated on blood agar and MacConkey. At 24 hours of incubation, flat, gray, nonhemolytic colonies are found on the blood agar. No growth is observed on MacConkey. Further testing revealed that the bile-esculin agar exhibited a black precipitate and the PYRase, a pink color.
   Identify the organism: _____

2. A sputum sample is collected from a 40-year-old man with a cough, fever, and symptoms of an upper respiratory tract infection. The Gram stain shows an abundance of gram-positive cocci in chains, a moderate amount of squamous epithelial cells, and no PMNs. The blood agar plate grew small, greyish, α hemolytic colonies. The P disc was performed, and the organism grew up to the disc. Does this sample seem to show a pathogen or contamination? Why? What organism do you suspect?

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