CHAPTER 13

The Staphylococci

The staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal microbiota of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems.

The genus *Staphylococcus* has at least 45 species. The four most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus*. *S aureus* is **coagulase positive**, which differentiates it from the other species. *S aureus* is a major pathogen for humans. Almost every person will have some type of *S aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative staphylococci (CoNS) are normal human microbiota and sometimes cause infection, often associated with implanted devices, such as joint prostheses, shunts, and intravascular catheters, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by **coagulase-negative** staphylococci are caused by *S epidermidis*; infections caused by *S lugdunensis*, *Staphylococcus warneri*, *Staphylococcus hominis*, and other species are less common. *S saprophyticus* is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients. Other species are important in veterinary medicine.

Morphology and Identification

A. Typical Organisms

Staphylococci are spherical cells about 1 µm in diameter arranged in irregular clusters (Figure 13-1). Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram positive; on aging, many cells become gram negative. Staphylococci are nonmotile and do not form spores. Under the influence of drugs such as penicillin, staphylococci are lysed.

Micrococcus species often resemble staphylococci. They are found free living in the environment and form regular packets of four (tetrads) or eight cocci. Their colonies can be yellow, red, or orange. Micrococci are rarely associated with disease.

B. Culture

Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions. They grow most rapidly at 37°C but form pigment best at room temperature (20–25°C). Colonies on solid media are round, smooth, raised, and glistening (Figure 13-2). *S aureus* usually forms gray to deep golden yellow colonies. *S epidermidis* colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of hemolysis are produced by *S aureus* and occasionally by other species. *Peptostreptococcus* and *Peptoniphilus* species, which are anaerobic cocci, often resemble staphylococci in morphology. The genus *Staphylococcus* contains two species, *Staphylococcus saccharolyticus* and *S aureus* subsp. *anaerobius*, which initially grow only under anaerobic conditions but become more aerotolerant on subcultures. This may be seen on rare occasions with some strains of *S epidermidis* as well.

C. Growth Characteristics

The staphylococci produce catalase, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas. Proteolytic activity varies greatly from one strain to another. Pathogenic staphylococci produce many extracellular substances, which are discussed below.

Staphylococci are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 10% sodium chloride but are readily inhibited by certain chemicals (eg, 3% hexachlorophene).

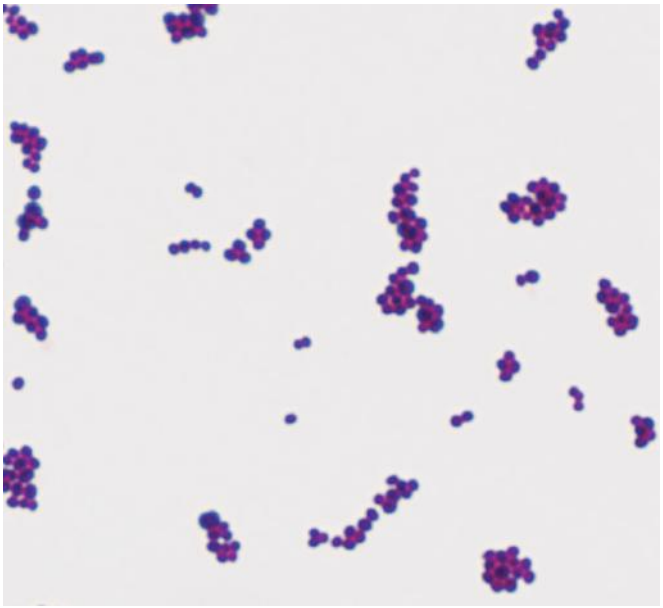


FIGURE 13-1 Gram stain of *Staphylococcus aureus* showing gram-positive cocci in pairs, tetrads, and clusters. Original magnification ×1000. (Courtesy of L Ching.)

Staphylococci are variably susceptible to many antimicrobial drugs. Resistance is caused by several mechanisms:

1. β-Lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins (penicillin G, ampicillin, ticarcillin, piperacillin, and similar drugs). The plasmids are transmitted by transduction and perhaps also by conjugation.
2. Resistance to nafcillin (and to methicillin and oxacillin) is independent of β-lactamase production. Resistance to nafcillin is encoded and regulated by a sequence of genes found in a region of the chromosome called the staphylococcal cassette chromosome *mec* (*SCCmec*). Specifically, the *mecA* and newly described *mecC* genes on this locus encode a low-affinity penicillin-binding protein (PBP2a) that is responsible for the resistance. There are 12 different *SCCmec* types. Types I, II, III, VI, and VIII are associated with hospital-acquired infections (HA-MRSA) and may contain genes that encode resistance to other antimicrobials as well. *SCCmec* type IV has principally been found in community-acquired methicillin-resistant *S aureus* (CA-MRSA) strains that tend to be less resistant, more transmissible, and responsible for outbreaks over the past decade in the United States and some countries in Europe. Types IX and X are associated with animals (livestock-associated MRSA [LA-MRSA]) of which type IX contains *mecC*. The other types have been limited to various geographic locations around the world.
3. In the United States, *S aureus* and *S lugdunensis* are considered to be susceptible to vancomycin if the minimum inhibitory concentration (MIC) is 2 μg/mL or less; of intermediate susceptibility if the MIC is 4–8 μg/mL; and resistant if the MIC is 16 μg/mL or greater. Strains of *S aureus* with intermediate susceptibility to vancomycin have been isolated in Japan, the United States, and several other countries. These are often known as vancomycin-intermediate *S aureus* (VISA). They generally have been isolated from patients with complex infections who have received prolonged vancomycin therapy. Often there has been vancomycin treatment failure. The mechanism of resistance is associated with increased cell wall synthesis and alterations in the cell wall and is not caused by the *van* genes found in enterococci. *S aureus* strains of intermediate susceptibility to vancomycin usually are nafcillin resistant but generally are susceptible to oxazolidinones and to quinupristin–dalfopristin.
4. Since 2002, several isolates of vancomycin-resistant *S aureus* (VRSA) strains (MICs ≥ 16 μg/mL) were isolated from patients in the United States. The isolates contained the vancomycin resistance gene *vanA* likely derived from enterococci (see Chapter 14) and the nafcillin resistance gene *mecA* (see above). Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in *S aureus* is of major concern worldwide.
5. Plasmid-mediated resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs is frequent in staphylococci.
6. "Tolerance" implies that staphylococci are inhibited by a drug but not killed by it—that is, there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug. Patients with endocarditis caused by a tolerant *S aureus* may have a

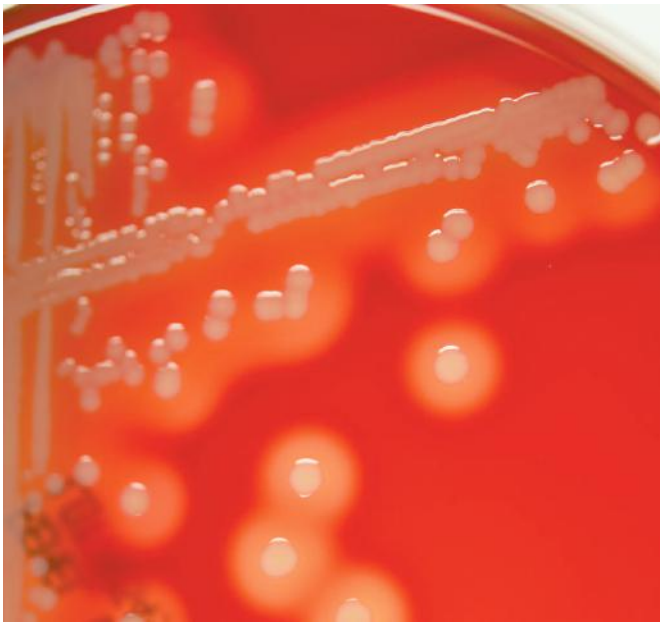


FIGURE 13-2 Colonies of *Staphylococcus aureus* on a blood agar plate after 24 hours incubation. The yellow-gray colonies are 3–4 mm in diameter on the 10-cm plate. The colonies are surrounded by clear zones of hemolysis about 1 cm in diameter. (Courtesy of H Reyes.)

prolonged clinical course compared with patients who have endocarditis caused by a fully susceptible *S aureus*. Tolerance can at times be attributed to lack of activation of autolytic enzymes in the cell wall.

D. Variation

A culture of staphylococci contains some bacteria that differ from the bulk of the population in expression of colony characteristics (colony size, pigment, hemolysis), in enzyme elaboration, in drug resistance, and in pathogenicity. In vitro, the expression of such characteristics is influenced by growth conditions: When nafcillin-resistant *S aureus* is incubated at 37°C on blood agar, one in 10^7 organisms expresses nafcillin resistance; when it is incubated at 30°C on agar containing 2–5% sodium chloride, one in 10^3 organisms expresses nafcillin resistance. Some isolates may develop alterations in phenotypes such as smaller size (pin point colonies) and loss of hemolysis. These are referred to as small colony variants (SCVs) and the variations in phenotypic characteristics enable better survival under intracellular conditions, facilitating persistence and leading to chronic infections.

Antigenic Structure

S aureus has amazing adaptive capacity. Full genome sequencing of numerous isolates (www.ncbi.nlm.nih.gov/genome/genomes/154) has elucidated the evolution of various structures, toxins, and enzymes that this organism has developed over time. *S aureus* has acquired many mobile genetic elements (eg, insertion sequences, transposons, etc) that determine both pathogenicity and antimicrobial resistance (see Regulation of Virulence Determinants).

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a thick polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall and anchors the adhesins (see below). Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of infection: It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement. Peptidoglycan assembly is a target of β-lactam and glycopeptide antimicrobial agents.

Teichoic acids, which are polymers of polyribitol–phosphate, are cross-linked to the peptidoglycan and can be antigenic. They are important in cell wall metabolism. Antiteichoic acid antibodies detectable by gel diffusion may be found in patients with active endocarditis caused by *S aureus*.

Protein A is a cell wall component of *S aureus* strains and is a bacterial surface protein that has been characterized among a group of adhesins called *microbial surface components recognizing adhesive matrix molecules* (MSCRAMMs). Bacterial attachment to host cells is mediated by MSCRAMMs, and these are important virulence factors. Protein A binds to the Fc portion of IgG molecules except IgG3. The Fab portion of the IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic laboratory technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen agglutinates bacteria that have that antigen ("**coagglutination**"). Another important MSCRAMM is clumping factor on the cell wall surface; clumping factor binds nonenzymatically to fibrinogen and platelets, yielding aggregation of the bacteria. The remaining MSCRAMMs, too numerous to mention here (see references), play important roles in establishing *S aureus* colonization and invasion in major infections such as endocarditis.

Most *S aureus* strains of clinical importance have polysaccharide capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. At least 11 serotypes have been identified, with types 5 and 8 responsible for the majority of infections. These capsule types are targets for a conjugate vaccine. Serologic tests have limited usefulness in identifying staphylococci.

Enzymes and Toxins

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, although they may function as enzymes. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extrachromosomal control; and for others, the mechanism of genetic control is not well defined.

A. Catalase

Staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

B. Coagulase and Clumping Factor

S aureus produces an extracellular coagulase, an enzyme-like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential.

Clumping factor is cell wall bound and is another example of an MSCRAMM (see earlier) that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S aureus* forms clumps. Clumping factor is distinct from coagulase. Because clumping factor induces a strong immunogenic response in the host, it has been the focus of vaccine efforts. However, no human vaccines against this factor are available to date.

C. Other Enzymes

Other enzymes produced by staphylococci include a hyaluronidase, or spreading factor—a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase, proteinases, lipases, and β-lactamase.

D. Hemolysins

S aureus possesses four hemolysins that are regulated by *agr* (see Regulation of Virulence Determinants). α-Hemolysin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The β-toxin degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells. The δ-toxin is heterogeneous and dissociates into subunits in nonionic detergents. It disrupts biologic membranes and may have a role in *S aureus* diarrheal diseases. The γ-hemolysin is a leukocidin that lyses white blood cells and is composed of two proteins designated S and F. γ-Hemolysin can interact with the two proteins comprising the Panton–Valentine leukocidin (PVL; see later discussion) to form six potential two-component toxins. All six of these protein toxins are capable of efficiently lysing white blood cells by causing pore formation in the cellular membranes that increase cation permeability. This leads to massive release of inflammatory mediators such as IL-8, leukotriene, and histamine, which are responsible for necrosis and severe inflammation.

E. Panton–Valentine Leukocidin

This toxin of *S aureus* has two components, and unlike the chromosomally encoded hemolysins above, PVL is encoded on a mobile phage. It can kill white blood cells of humans and rabbits. The two components designated as S and F act synergistically on the white blood cell membrane as described for γ-toxin. This toxin is an important virulence factor in CA-MRSA infections.

F. Exfoliative Toxins

These epidermolytic toxins of *S aureus* are two distinct proteins of the same molecular weight. Exfoliative toxin A is encoded by *eta* located on a phage and is heat stable (resists boiling for 20 minutes). Exfoliative toxin B is plasmid mediated and heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are **superantigens** (see Chapter 8).

G. Toxic Shock Syndrome Toxin

Most *S aureus* strains isolated from patients with toxic shock syndrome produce a toxin called **toxic shock syndrome toxin-1** (TSST-1), which is the same as enterotoxin F. TSST-1 is the prototypical **superantigen** (see Chapter 9). TSST-1 binds to major histocompatibility class (MHC) class II molecules, yielding T-cell stimulation, which promotes the protean manifestations of the toxic shock syndrome. The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% of *S aureus* isolates, including MRSA.

H. Enterotoxins

There are 15 enterotoxins (A–E, G–P) that, similar to TSST-1, are superantigens. Approximately 50% of *S aureus* strains can produce one or more of them. The enterotoxins are heat stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S aureus* grows in carbohydrate and protein foods. Ingestion of 25 μg of enterotoxin B results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.

The exfoliative toxins, TSST-1, and the enterotoxin genes are on a chromosomal element called a *pathogenicity island*. It interacts with accessory genetic elements—bacteriophages—to produce the toxins.

Pathogenesis

Staphylococci, particularly *S epidermidis*, are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S aureus* occurs in 20–50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments.

The pathogenic capacity of a given strain of *S aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs.

Pathogenic, invasive *S aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as *S epidermidis* are coagulase negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. They may be refractory to treatment because of the formation of bioflims. *S lugdunensis* has emerged as a virulent organism causing a disease spectrum similar to *S aureus* with whom it shares phenotypic characteristics such as hemolysis and clumping factor. *S saprophyticus* is typically nonpigmented, novobiocin resistant, and nonhemolytic; it causes urinary tract infections in young women.

Regulation of Virulence Determinants

The expression of staphylococcal virulence determinants is regulated by several systems that sense and respond to environmental signals. The first of these systems consists of two proteins (two-component systems), an example of which is accessory gene regulator (*agr*). The other two systems consist of DNA-binding proteins (eg, Sar proteins) and small regulatory RNAs, respectively (eg, RNAIII), the latter of which have become more appreciated as having major roles in regulation of gene expression. Binding of sensors to specific extracellular ligands, or to a receptor, results in a phosphorylation

cascade that leads to binding of the regulator to specific DNA sequences. This ultimately leads to activation of transcription-regulating functions. There are several well-described two-component regulatory systems in *S aureus*. These include *agr*, the best described, *sae RS*, *srrAB*, *arlSR*, and *lytRS*. A summary of how these systems interact is briefly described below.

The accessory gene regulator (*agr*) is essential in quorum-sensing control of gene expression. It controls the preferential expression of surface adhesins (protein A, coagulase, and fibronectin-binding protein) and production of exoproteins (toxins such as TSST-1) depending upon the growth phase (and hence bacterial density).

At low cell density, the promoter P2 is off, and transcriptions of transmembrane protein, AgrB, peptide precursor, AgrD, transmembrane sensor, AgrC, and transcription regulator, Agr A, are at low levels. As cell density increases during stationary growth phase, the AgrC sensor activates the regulator AgrA. AgrA is a DNA-binding protein that activates promoter P2 and promoter P3. Promoter P3 initiates transcription of δ-hemolysin and an effector called RNAIII, which downregulates the expression of surface adhesins and activates secretion of exoproteins at both the transcriptional and translational levels. *Agr* is also positively controlled by a DNA-binding protein called SarA (encoded by *sar*) and possibly by other regulatory systems.

At least 10 two-component regulatory systems have been shown to affect virulence gene expression and are also involved in metabolic control. Those involved in virulence include: *sae*, *S aureus* exoproteins; *srrAB*, staphylococcal respiratory response; *arlS*, autolysis-related locus sensor; and *lytRS*. *Sae* regulates gene expression at the transcriptional level and is essential for production of α-toxin, β-hemolysins, and coagulase. Its activity is independent from that of *agr*. *SsrAB* is important for regulation of virulence factor expression that is influenced by environmental oxygen. The *arlSR* locus is important to the control of autolysis and decreases the activation of the *agr* locus. The *lytRS* locus is also involved in autolysis. More detailed discussions of the regulation of pathogenesis can be found in the reference by Que and Moreillon.

Pathology

The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Groups of *S aureus* established in a hair follicle lead to tissue necrosis (dermonecrotic factor). Coagulase is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue. Within the center of the lesion, liquefaction of the necrotic tissue occurs (enhanced by delayed hypersensitivity), and the abscess "points" in the direction of least resistance. Drainage of the liquid center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing.

Focal suppuration (abscess) is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body. Suppuration within veins, associated with thrombosis, is a common feature of such dissemination. In osteomyelitis, the primary focus of *S aureus* growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration. *S aureus* may cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. Staphylococci of low invasiveness are involved in many skin infections (eg, acne, pyoderma, or impetigo). Anaerobic cocci (*Peptostreptococcus* species) participate in mixed anaerobic infections.

Staphylococci also cause disease through the elaboration of toxins without apparent invasive infection. Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxins. Toxic shock syndrome is associated with TSST-1.

Clinical Findings

A localized staphylococcal infection appears as a "pimple," hair follicle infection, or abscess. There is usually an intense, localized, painful inflammatory reaction that undergoes central suppuration and heals quickly when the pus is drained. The wall of fibrin and cells around the core of the abscess tend to prevent spread of the organisms and should not be broken down by manipulation or trauma.

S aureus infection can also result from direct contamination of a wound, such as a postoperative staphylococcal wound infection or infection after trauma (chronic osteomyelitis subsequent to an open fracture, meningitis after skull fracture).

If *S aureus* disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other bloodstream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration.

Food poisoning caused by staphylococcal enterotoxin is characterized by a short incubation period (1–8 hours); violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.

Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases. It often occurs within 5 days after the onset of menses in young women who use high-absorbency tampons, but it also occurs in children and men with staphylococcal wound infections. The syndrome can recur. Toxic shock syndrome–associated *S aureus* can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but virtually never in the bloodstream.

Diagnostic Laboratory Tests

A. Specimens

Surface swab pus or aspirate from an abscess, blood, endonasotracheal aspirate, expectorated sputum, or spinal fluid for culture, depending on the localization of the process, are all

appropriate specimens for testing. The anterior nares are frequently swabbed to determine nasal colonization, either by culture or by nucleic acid amplification tests, for epidemiological purposes.

B. Smears

Typical staphylococci appear as gram-positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish nonaureus (eg, *S epidermidis*) from the pathogenic *S aureus* organisms on smears.

C. Culture

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37°C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. *S aureus* but not other staphylococci ferment mannitol. Specimens contaminated with a mixed microbiota can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal microbiota, but not *S aureus*. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers of *S aureus* and to recover *S aureus* from respiratory specimens of patients with cystic fibrosis.

D. Catalase Test

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test result.

E. Coagulase Test

Citrated rabbit (or human) plasma diluted 1:5 is mixed with an equal volume of broth culture or growth from colonies on agar and incubated at 37°C. A tube of plasma mixed with sterile broth is included as a control. If clots form in 1–4 hours, the test result is positive. Rapid latex and agglutination assays are more timely and in some cases more sensitive in the differentiation between *S aureus* and CoNS. These assays detect protein A and clumping factor, and some have monoclonal antibodies against capsular polysaccharides.

Coagulase-positive staphylococci are considered pathogenic for humans; however, coagulase-positive staphylococci of dogs (*Staphylococcus intermedius*) and dolphins (*Staphylococcus delphini*) rarely cause disease in humans. Infections of prosthetic devices can be caused by organisms of the coagulase-negative *S epidermidis* group.

F. Susceptibility Testing

Clinical laboratories adopt methods recommended by the Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) for the performance of susceptibility testing of staphylococci. Broth microdilution using manual or automated commercial methods, or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infections. Resistance to penicillin G can be predicted by a positive test result for β-lactamase; approximately 90% of *S aureus* produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 65% of *S aureus* and approximately 75% of *S epidermidis* isolates. Nafcillin resistance correlates with the presence of *mecA* or *mecC*, the genes that encode for a penicillin-binding protein (PBP2a) not affected by these drugs. These genes can be detected using the polymerase chain reaction (PCR) or other nucleic acid amplification test. Several FDA-cleared systems combine identification and *mecA* resistance marker detection directly from positive blood cultures. The Verigene® assay (Nanosphere, Inc., Northbrook, IL) and the BioFire FilmArray® BCID assay (BioFire Diagnostics, Inc., Salt Lake City, UT) are two examples of such tests but many more are in development. Alternatively, an assay for the *mecA* gene product, PBP2a, is commercially available and is much more rapid than PCR for *mecA* or than testing for resistance using traditional phenotypic methods.

When using disk diffusion to detect nafcillin resistance, the cefoxitin disk test is recommended for testing *S aureus*, *S lugdunensis*, and *S saprophyticus*. Zone sizes < 22 mm indicate resistance. When using broth microdilution, either oxacillin or cefoxitin may be used to detect oxacillin resistance. If the latter drug is tested, then 2% NaCl is added to the media and the test must be incubated for a full 24 hours at 35°C.

An organism that is *mecA* or *mecC* positive or phenotypically is nafcillin, oxacillin, or methicillin resistant is also resistant to all extended spectrum penicillins, carbapenems, and cephalosporins with the exception of ceftaroline, a new cephalosporin with activity against MRSA.

G. Serologic and Typing Tests

Serologic tests for diagnosis of *S aureus* infections have little practical value.

Antibiotic susceptibility patterns may be helpful in tracing *S aureus* infections and in determining if multiple *S epidermidis* isolates from blood cultures represent bacteremia caused by the same strain, seeded by a nidus of infection.

Molecular typing techniques have been used to document the spread of epidemic disease-producing clones of *S aureus*. Pulsed-field gel electrophoresis and multilocus sequence typing are highly discriminatory. *Spa* typing is less discriminatory but easier to perform.

Treatment

Most persons harbor staphylococci on the skin and in the nose or throat. Even if the skin can be cleared of staphylococci (eg, in eczema), reinfection by droplets will occur almost immediately. Because pathogenic organisms are commonly spread from one lesion (eg, a furuncle) to other areas of the skin by fingers and clothing, scrupulous local antisepsis is important to control recurrent furunculosis.

Serious multiple skin infections (acne, furunculosis) occur most often in adolescents. Similar skin infections occur

in patients receiving prolonged courses of corticosteroids. In acne, lipases of staphylococci and corynebacteria liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long-term treatment.

Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy. Many antimicrobial drugs have some effect against staphylococci in vitro. However, it is difficult to eradicate pathogenic staphylococci from infected persons because the organisms rapidly develop resistance to many antimicrobial drugs and the drugs cannot act in the central necrotic part of a suppurative lesion.

It may also be difficult to eradicate the *S aureus* nasal carrier state. Some success has been reported with treatment of colonized individuals with intranasal mupirocin. Literature demonstrates success in reducing postsurgical wounds infections and prevention of bacteremia when treating identified hospitalized patients with 5 days of mupirocin with or without bathing using chlorhexidine, a topical antiseptic.

Acute hematogenous osteomyelitis responds well to antimicrobial drugs. In chronic and recurrent osteomyelitis, surgical drainage and removal of dead bone is accompanied by long-term administration of appropriate drugs, but eradication of the infecting staphylococci is difficult. Hyperbaric oxygen and the application of vascularized myocutaneous flaps have aided healing in chronic osteomyelitis.

Bacteremia, endocarditis, pneumonia, and other severe infections caused by *S aureus* require prolonged intravenous therapy with a β-lactamase-resistant penicillin. Vancomycin is often reserved for use with nafcillin-resistant staphylococci. In recent years, an increase in MICs to vancomycin among many MRSA strains recovered from hospitalized patients has led physicians to seek alternative therapies. Alternative agents for the treatment of MRSA bacteremia and endocarditis include newer antimicrobials such as daptomycin, linezolid, and quinupristin–dalfopristin (see Chapter 28). Also, these agents may be bactericidal and offer alternatives when allergies preclude the use of other compounds or the patient's infection appears to be failing clinically. However, the use of these agents should be discussed with infectious diseases physicians or pharmacists because the side effect profiles and pharmacokinetics are quite unique to each agent. Recently, a novel cephalosporin called ceftaroline, which has activity against MRSA and other gram-positive and some gram-negative bacteria, has been approved for the treatment of skin and soft tissue infections and community-acquired pneumonia. This drug does not yet have an indication for bacteremia. If the infection is found to be caused by non–β-lactamase-producing *S aureus*, penicillin G is the drug of choice, but these *S aureus* strains are rarely encountered.

S epidermidis infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm. *S epidermidis* is more often resistant to antimicrobial drugs than is *S aureus*; approximately 75% of *S epidermidis* strains are nafcillin resistant. Several newer agents that have activity against CoNS and MSSA and MRSA have recently been FDA-cleared for treatment of skin and skin structure infections. These include dalbavancin, a long-acting intravenous lipoglycopeptide; tedizolid phosphate, an intravenous and oral oxazolidinone, similar to linezolid; and oritavancin, a semisynthetic glycopeptide.

Because of the frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs. Resistance to drugs of the erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection. Drug resistance (to penicillins, tetracyclines, aminoglycosides, erythromycins, and so on) determined by plasmids can be transmitted among staphylococci by transduction and perhaps by conjugation.

Penicillin G–resistant *S aureus* strains from clinical infections always produce penicillinase. They constitute more than 95% of *S aureus* isolates in communities in the United States. They are often susceptible to β-lactamase–resistant penicillins, cephalosporins, or vancomycin. Nafcillin resistance is independent of β-lactamase production, and its clinical incidence varies greatly in different countries and at different times. The selection pressure of β-lactamase–resistant antimicrobial drugs may not be the sole determinant for resistance to these drugs: For example, in Denmark, nafcillin-resistant *S aureus* comprised 40% of isolates in 1970 and only 10% in 1980 without notable changes in the use of nafcillin or similar drugs. In the United States, nafcillin-resistant *S aureus* accounted for only 0.1% of isolates in 1970 but in the 1990s constituted 20–30% of isolates from infections in some hospitals. Currently, about 60% of nosocomial *S aureus* among intensive care patients in the United States are resistant to nafcillin. Fortunately, *S aureus* strains of intermediate susceptibility to vancomycin have been relatively uncommon, and the isolation of vancomycin-resistant strains has been rare.

Epidemiology and Control

Staphylococci are ubiquitous human pathogens. The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients may carry antibiotic-resistant staphylococci in the nose or on the skin. Although cleanliness, hygiene, and aseptic management of lesions can control the spread of staphylococci from lesions, few methods are available to prevent the wide dissemination of staphylococci from carriers. Aerosols (eg, glycols) and ultraviolet irradiation of air have little effect.

In hospitals, the areas at highest risk for severe staphylococcal infections are newborn nurseries, intensive care units, operating rooms, and cancer chemotherapy wards. Massive introduction of "epidemic" pathogenic *S aureus* into these areas may lead to serious clinical disease. Personnel with active *S aureus* lesions and carriers may have to be excluded from these areas. In such individuals, the application of topical antiseptics such as mupirocin to nasal or perineal

carriage sites may diminish shedding of dangerous organisms. Rifampin coupled with a second oral antistaphylococcal drug sometimes provides long-term suppression and possibly cure of nasal carriage; this form of therapy is usually reserved for major problems of staphylococcal carriage because staphylococci can rapidly develop resistance to rifampin.

To diminish transmission within the hospital setting, high-risk patients, such as those in intensive care units and patients transferred from chronic care facilities where prevalence is high, are frequently surveyed for anterior nares colonization. Patients who test positive by culture or PCR are placed on contact precautions to minimize spread on the hands of health care workers. Health care workers should strictly adhere to infection control policies by wearing gloves and washing hands before and after patient contact.

Until relatively recently, MRSA was confined primarily to the hospital setting. Worldwide dissemination of a few distinct clones of CA-MRSA and now LA-MRSA has resulted in an increase in skin and soft tissue infections and necrotizing pneumonia, primarily in younger patients without known risk factors for MRSA acquisition. These strains appear to be more virulent. CA-MRSA isolates are characterized by the presence of PVL and the presence of staphylococcal cassette chromosome *mec* type IV (see discussion above under "Growth Characteristics"), which may explain the increased susceptibility to other antimicrobial agents compared with health care–associated MRSA strains.

CHAPTER SUMMARY

- Staphylococci are catalase-positive, gram-positive organisms that grow in clusters and are common inhabitants of the skin and mucous membranes of humans and animals.
- The pathogenic staphylococci, most importantly *S aureus*, hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins that make them virulent.
- *S aureus* has complex regulatory systems that respond to environmental stimuli to control the expression of its various virulence genes encoded on pathogenicity islands.
- *S aureus* causes a broad range of invasive and toxigenic diseases; CoNS are less virulent and more often associated with opportunistic infections (*S epidermidis*) or specific syndromes, such as *S saprophyticus* and urinary tract infections.
- Antimicrobial resistance among staphylococci can be quite extensive, encoded by a variety of mechanisms such as β-lactamase production and chromosomal *mecA*, *mecC*, and other resistance determinants.

REVIEW QUESTIONS

1. A 54-year-old woman develops a right shoulder abscess with a strain of *Staphylococcus aureus* that is resistant to nafcillin. She was treated with a 2-week course of intravenous vancomycin and improved. Three weeks later (week 5), the infection recurred, and she was given 2 more weeks of intravenous vancomycin and again improved. Four weeks later (week 11), the infection recurred and the patient was again started on intravenous vancomycin. The MICs for vancomycin for the *S aureus* isolates were as follows: initial isolate (day 1), 1 μg/mL; week 5, 2 μg/mL; and week 11, 8 μg/mL. The patient failed to improve with the third course of vancomycin, and alternative therapy was used. The mechanism that best explains the relative resistance of the patient's strain of *S aureus* to vancomycin is
   (A) Acquisition of the *vanA* gene from another microorganism
   (B) Active transport of vancomycin out of the *Staphylococcus aureus* cell
   (C) Action of β-lactamase
   (D) Increased cell wall synthesis and alterations in the cell wall structure
   (E) Phosphorylation and resultant inactivation of the vancomycin
2. An 11-year-old boy develops a mild fever and pain in his upper arm. A radiograph of his arm shows a lytic lesion (dissolution) in the upper part of the humerus with periosteal elevation over the lesion. The patient is taken to surgery, where the lesion is debrided (dead bone and pus removed). Culture from the lesion yields gram-positive cocci. A test shows that the organism is a *Staphylococcus* and not a *Streptococcus*. Based on this information, you know the organism is
   (A) Susceptible to nafcillin
   (B) β-Lactamase positive
   (C) A producer of protein A
   (D) Encapsulated
   (E) Catalase positive
3. A 36-year-old male patient has an abscess with a strain of *Staphylococcus aureus* that is β-lactamase positive. This indicates that the organism is resistant to which of the following antibiotics?
   (A) Penicillin G, ampicillin, and piperacillin
   (B) Trimethoprim–sulfamethoxazole
   (C) Erythromycin, clarithromycin, and azithromycin
   (D) Vancomycin
   (E) Cefazolin and ceftriaxone
4. Seven days ago, a 27-year-old medical student returned from Central America, where she had spent the summer working in a clinic for indigenous people. Four days ago, she developed an erythematous sunburn-like rash. She also has had headache, muscle aches, and abdominal cramps with diarrhea. Her blood pressure is 70/40 mm Hg. Pelvic examination shows she is having her menstrual period with a tampon in place; otherwise, the pelvic examination is normal. Her kidney function test (serum urea nitrogen and creatinine) results are abnormal, indicating mild renal failure. A blood smear for malaria is negative. Her illness is likely to be caused by which of the following?
   (A) A toxin that results in greatly increased levels of intracellular cyclic adenosine monophosphate (cAMP)
   (B) A toxin that degrades sphingomyelin
   (C) A toxin that binds to the class II major histocompatibility complex (MHC) of an antigen-presenting cell and the Vβ region of a T cell
   (D) A two-component toxin that forms pores in white blood cells and increases cation permeability
   (E) A toxin that blocks elongation factor 2 (EF2)

5. Over a period of 3 weeks, a total of five newborns in the hospital nursery developed *Staphylococcus aureus* infections with *S aureus* bacteremia. The isolates all had the same colony morphology and hemolytic properties and identical antimicrobial susceptibility patterns, suggesting that they were the same. (Later molecular methods showed the isolates were identical.) Which of the following should be done now?
   (A) Prophylactic treatment of all newborns with intravenous vancomycin
   (B) Protective isolation of all newborns
   (C) Closing the nursery and referring pregnant women to another hospital
   (D) Hiring all new staff for the hospital nursery
   (E) Culture using mannitol salt agar of the anterior nares of the physicians, nurses, and others who cared for the infected babies
6. The exfoliative toxins, TSST-1, and the enterotoxins are all superantigens. The genes for these toxins are
   (A) Present in all strains of *Staphylococcus aureus*
   (B) Widely distributed on the staphylococcal chromosome
   (C) On both the staphylococcal chromosome (TSST-1 and exfoliative toxins) and on plasmids (enterotoxins)
   (D) On the staphylococcal chromosome in a pathogenicity island
   (E) On plasmids
7. A 16-year-old bone marrow transplant patient has a central venous line that has been in place for 2 weeks. He also has a urinary tract catheter, which has been in place for 2 weeks as well. He develops fever while his white blood cell count is very low and before the transplant has engrafted. Three blood cultures are done, and all grow *Staphylococcus epidermidis*. Which one of the following statements is correct?
   (A) The *Staphylococcus epidermidis* organisms are likely to be susceptible to penicillin G.
   (B) The *Staphylococcus epidermidis* organisms are likely to be from the surface of the urinary tract catheter.
   (C) The *Staphylococcus epidermidis* organisms are likely to be resistant to vancomycin.
   (D) The *Staphylococcus epidermidis* organisms are likely to be from a skin source.
   (E) The *Staphylococcus epidermidis* organisms are likely to be in a biofilm on the central venous catheter surface.
8. A 65-year-old man develops an abscess on the back of his neck. Culture yields *Staphylococcus aureus*. The isolate is tested and found to be positive for the *mecA* gene, which means that
   (A) The isolate is susceptible to vancomycin.
   (B) The isolate is resistant to vancomycin.
   (C) The isolate is susceptible to nafcillin.
   (D) The isolate is resistant to nafcillin
   (E) The isolate is susceptible to clindamycin.
   (F) The isolate is resistant to clindamycin.
9. Antimicrobial resistance has become a significant problem. Which one of the following is of major concern worldwide?
   (A) Nafcillin resistance in *Staphylococcus aureus*
   (B) Penicillin resistance in *Streptococcus pneumoniae*
   (C) Penicillin resistance in *Neisseria gonorrhoeae*
   (D) Vancomycin resistance in *Staphylococcus aureus*
   (E) Tobramycin resistance in *Escherichia coli*
10. A group of six children younger than 8 years of age live in a semitropical country. Each of the children has several crusted weeping skin lesions of impetigo (pyoderma). The lesions are predominantly on the arms and faces. Which of the following microorganisms is a likely cause of the lesions?
   (A) *Escherichia coli*
   (B) *Chlamydia trachomatis*
   (C) *Staphylococcus aureus*
   (D) *Streptococcus pneumoniae*
   (E) *Bacillus anthracis*
11. Which of the following statements regarding the role of protein A in the pathogenesis of infections caused by *Staphylococcus aureus* is correct?
   (A) It is responsible for the rash in toxic shock syndrome.
   (B) It converts hydrogen peroxide into water and oxygen.
   (C) It is a potent enterotoxin.
   (D) It is directly responsible for lysis of neutrophils.
   (E) It is a bacterial surface protein that binds to the Fc portion of IgG1.
12. Which of the following staphylococcal organisms produces coagulase and has been implicated in infections following a dog bite?
   (A) *Staphylococcus intermedius*
   (B) *Staphylococcus epidermidis*
   (C) *Staphylococcus saprophyticus*
   (D) *Staphylococcus hominis*
   (E) *Staphylococcus hemolyticus*
13. All of the following statements regarding Panton–Valentine leukocidin are correct *except*
   (A) It is a two-component toxin.
   (B) It is commonly produced by community-associated MRSA strains.
   (C) It is an important virulence factor.
   (D) It is identical to one of the staphylococcal enterotoxins.
   (E) It forms pores in the membranes of white blood cells.
14. Which of the following statements best describes the function of the accessory gene regulator in *Staphylococcus aureus*?
   (A) It regulates production of β-hemolysins.
   (B) It is influenced by environmental oxygen.
   (C) It controls the preferential expression of surface adhesins.
   (D) It is important in the control of autolysis.
15. All of the following are important infection control strategies in containing spread of MRSA in hospitals *except*
   (A) Aggressive hand hygiene
   (B) Routine surveillance for nasal colonization among high-risk individuals
   (C) Contact isolation for patients who are colonized or infected with MRSA
   (D) Routine antimicrobial prophylaxis for all patients hospitalized for more than 48 hours
   (E) Aseptic management of skin lesions

Answers

1. D	5. E	9. D	13. D
2. E	6. D	10. C	14. C
3. A	7. E	11. E	15. D
4. C	8. D	12. A	

REFERENCES

Becker K, Ballhausen B, Kock R, Kriegeskorte A: Methicillin resistance in *Staphylococcus* isolates: the "mec alphabet" with specific consideration of mec, C a mec homolog associated with *S. aureus* lineages. *Int J Med Microbiol* 2014;304:794.

Que YA, Moreillon P: Chapter 196, *Staphylococcus aureus* (including staphylococcal toxic shock). In Bennett JE, Dolin R, Blaser MJ (editors). *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, 8th ed. Churchill Livingstone, Elsevier, 2015.

Winn WC, Allen SD, Janda WM, et al (editors): Gram-positive cocci, Part I: staphylococci and related gram-positive cocci. In Winn WC Jr, Allen SD, Janda WM, et al (editors). *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. Lippincott Williams and Wilkins, 2006, p. 623.

CHAPTER 14

The Streptococci, Enterococci, and Related Genera

The streptococci, enterococci, and related organisms are gram-positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human microbiota; others are associated with important human diseases attributable to the direct effects of infection or in other cases to an immunologic response to them. Streptococci elaborate a variety of extracellular substances and enzymes.

The streptococci are a large and heterogeneous group of bacteria, and no one system suffices to classify them. Yet, understanding their taxonomy is key to understanding their medical importance.

CLASSIFICATION OF STREPTOCOCCI

The classification of streptococci into major categories has been based on a series of observations over many years: (1) colony morphology and hemolytic reactions on blood agar, (2) serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens, (3) biochemical reactions and resistance to physical and chemical factors, and (4) ecologic features. More recently, molecular genetics have replaced phenotypic methods in the taxonomic assignment of these organisms. The classification of streptococci of medical importance is summarized in Table 14-1.

A. Hemolysis

Many streptococci are able to hemolyze red blood cells in vitro in varying degrees. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β-**hemolysis**. Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α-**hemolysis**. Other streptococci are nonhemolytic (sometimes called γ- [gamma-] hemolysis).

The hemolysis patterns of the streptococci of medical importance to humans are shown in Table 14-1. The classification of hemolytic patterns is used primarily with the streptococci although other bacteria that cause disease may also typically produce a variety of hemolysins.

B. Group-Specific Substance (Lancefield Classification)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into **Lancefield groups A–H** and **K–U**. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar. For group A streptococci, this is rhamnose-*N*-acetylglucosamine; for group B, it is rhamnose-glucosamine polysaccharide; for group C, it is rhamnose-*N*-acetylgalactosamine; for group D, it is glycerol teichoic acid containing D-alanine and glucose; and for group F, it is glucopyranosyl-*N*-acetylgalactosamine.

Extracts of group-specific antigen for grouping streptococci are prepared by a variety of methods, including extraction of centrifuged culture treated with hot hydrochloric acid, nitrous acid, or formamide; by enzymatic lysis of streptococcal cells (eg, with pepsin or trypsin); or by autoclaving of cell suspensions. These extracts contain the carbohydrate group–specific substance that yields precipitin reactions specific antisera. This permits arrangement of many streptococci into groups A–H and K–U. Typing is generally done only for groups A, B, C, F, and G (see Table 14-1), which cause disease in humans and for which reagents are available that allow typing using simple agglutination or color reactions.

C. Capsular Polysaccharides

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

D. Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents. Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed. Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G. For example, the viridans streptococci are α-hemolytic or

TABLE 14-1 Characteristics of Medically Important Streptococci

Name	Group-Specific Substance[a]	Hemolysis[b]	Habitat	Important Laboratory Criteria	Common and Important Diseases
Pyogenic Streptococci					
Streptococcus pyogenes	A	β	Throat, skin	Large colonies (>0.5 mm), PYR[c] test positive, inhibited by bacitracin	Pharyngitis, impetigo, deep soft tissue infections; bacteremia; rheumatic fever, glomerulonephritis, toxic shock
Streptococcus agalactiae	B	β	Urogenital tract, lower GI tract	Hippurate hydrolysis, CAMP-factor positive[d]	Neonatal sepsis and meningitis; bacteremia, UTIs,[e] meningitis in adults
Streptococcus dysgalactiae subspecies *equisimilis*; others	C, G	β (human) infections), α, none	Throat	Large (>0.5 mm) colonies	Pharyngitis, pyogenic infections similar to group A streptococci
Viridans Streptococci					
Streptococcus bovis group[f]	D	None	Colon, biliary tree	Growth in presence of bile, hydrolyze esculin, no growth in 6.5% NaCl, degrades starch	Endocarditis, common blood isolate in colon cancer, biliary disease
Streptococcus anginosus group (*S anginosus*, *Streptococcus intermedius*, *Streptococcus constellatus*)	F (A, C, G) and untypeable	α, β, none	Throat, colon, urogenital tract	Small (<0.5 mm) colony variants of β-hemolytic species; group A are bacitracin resistant and PYR negative; carbohydrate fermentation patterns; arginine, esculin, VP[g] positive	Pyogenic infections, including brain, liver, lung abscesses
Mutans group	Usually not typed	α, none	Oral cavity	carbohydrate fermentation patterns; esculin, VP positive	Dental caries (*S mutans*), endocarditis; abscesses (with many other bacterial species)
Mitis-Sanguinis group					
Streptococcus pneumoniae	None[o]	α	Nasopharynx	Susceptible to optochin; colonies soluble in bile; quellung reaction positive	Pneumonia, meningitis, bacteremia, otitis media, sinusitis
Streptococcus mitis	None	α, none	Oral cavity	VP negative[g]; carbohydrate fermentation patterns	Endocarditis; bacteremia, sepsis in immunocompromised patients; high-level resistance to penicillin
Salivarius group	None	α, none	Oral cavity	VP positive; carbohydrate fermentation patterns	Bacteremia, endocarditis, meningitis

[a] Lancefield classification.

[b] Hemolysis observed on 5% sheep blood agar after overnight incubation.

[c] Hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR).

[d] CAMP, Christie, Atkins, Munch-Peterson.

[e] UTIs, urinary tract infections.

[f] Includes the human species: *Streptococcus gallolyticus* subspecies *gallolyticus*; *Streptococcus gallolyticus* subspecies *macedonicus*; *Streptococcus gallolyticus* subspecies *pasteurianus*; *Streptococcus infantarius* subspecies *infantarius*.

[g] VP, Voges Proskauer; all viridans group streptococci are VP positive except the mitis group.

GI, gastrointestinal.

nonhemolytic and do not react with the antibodies commonly used for the Lancefield classification. Speciation of the viridans streptococci requires a battery of biochemical tests. See Table 14-1. However, because biochemical reactions are labor intensive and often unreliable, laboratories with molecular capabilities, such as gene sequencing or that have implemented mass spectrometry for organism identification (matrix-assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS]), are replacing phenotypic tests with these methods when identification of viridians streptococci is required.

STREPTOCOCCI OF PARTICULAR MEDICAL INTEREST

The following streptococci and enterococci are of particular medical relevance.

STREPTOCOCCUS PYOGENES

Most streptococci that contain the group A antigen are *S pyogenes*. It is a prototypical human pathogen. It is used here to illustrate general characteristics of streptococci and specific characteristics of the species. *S pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders. *S pyogenes* typically produces large (1 cm in diameter) zones of β-hemolysis around colonies greater than 0.5 mm in diameter. They are PYR-positive (hydrolysis of L-pyrrolidonyl-β-naphthylamide) and usually are susceptible to bacitracin.

Morphology and Identification

A. Typical Organisms

Individual cocci are spherical or ovoid and are arranged in chains (Figure 14-1). The cocci divide in a plane perpendicular to the long axis of the chain. The members of the chain often have a striking diplococcal appearance, and rod-like forms are occasionally seen. The lengths of the chains vary widely and are conditioned by environmental factors. Streptococci are gram positive; however, as a culture ages and the bacteria die, they lose their gram positivity and can appear to be gram negative; for some streptococci, this can occur after overnight incubation.

Most group A strains (see Table 14-1) produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede phagocytosis. The hyaluronic acid capsule likely plays a greater role in virulence than is generally appreciated and together with M protein was believed to be an important factor in the resurgence of rheumatic fever (RF) in the United States in the 1980s and 1990s. The capsule binds to hyaluronic-acid-binding protein, CD44, present on human epithelial cells. Binding induces disruption of intercellular junctions allowing microorganisms to remain extracellular as they penetrate the epithelium (see Stollerman and Dale, 2008). Capsules of other streptococci (eg, *S agalactiae* and *S pneumoniae*) are different. The *S pyogenes* cell wall contains proteins (M, T, R antigens), carbohydrates (group specific), and peptidoglycans. Hairlike pili project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with **lipoteichoic acid.** The latter is important in the attachment of streptococci to epithelial cells.

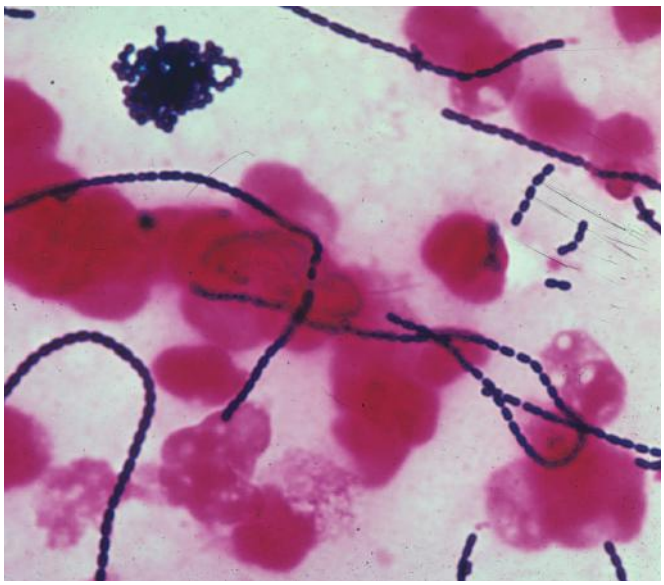


FIGURE 14-1 Streptococci grown in blood culture showing gram-positive cocci in chains. Original magnification × 1000.

B. Culture

Most streptococci grow in solid media as discoid colonies, usually 1–2 mm in diameter. *S pyogenes* is β-hemolytic (Figure 14-2); other species have variable hemolytic characteristics (see Table 14-1).

C. Growth Characteristics

Energy is obtained principally from the utilization of glucose with lactic acid as the end product. Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. The human pathogens are most exacting, requiring a variety of growth factors. Growth and hemolysis are aided by incubation in 10% CO_2. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

D. Variation

Variants of the same *Streptococcus* strain may show different colony forms. This is particularly marked among *S pyogenes* strains, giving rise to either matte or glossy colonies. Matte colonies consist of organisms that produce much M protein

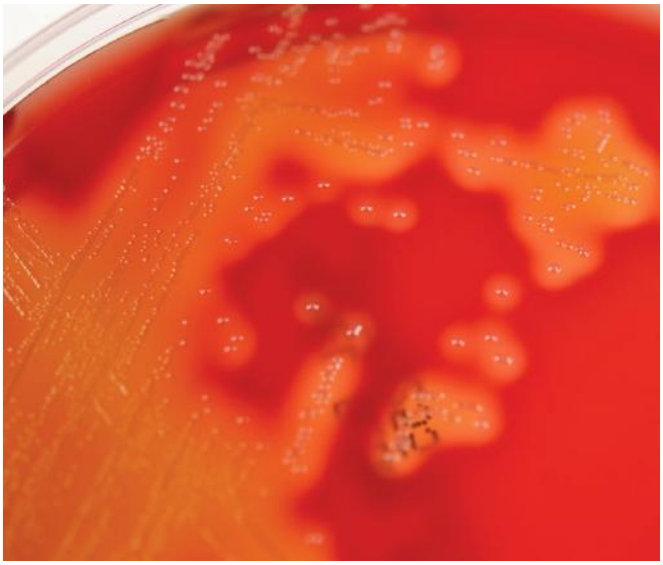


FIGURE 14-2 Group A β-hemolytic streptococci (*Streptococcus pyogenes*) after growth overnight on a 10-cm plate with 5% sheep blood agar. The small (0.5–1 mm diameter) white colonies are surrounded by diffuse zones of β-hemolysis 7–10 mm in diameter. (Courtesy of H Reyes.)

and generally are virulent. The *S pyogenes* in glossy colonies tend to produce little M protein and are often not virulent.

Antigenic Structure

A. M Protein

This substance is a major virulence factor of *S pyogenes*. M protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M type-specific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes by inhibiting activation of the alternate complement pathway. *S pyogenes* that lack M protein are not virulent. Immunity to infection with group A streptococci is related to the presence of type-specific antibodies to M protein. Because there are more than 150 types of M protein, a person can have repeated infections with *S pyogenes* of different M types. Both groups C and G streptococci have genes homologous to the genes for M protein of group A, and M proteins similar to those of group A have been found on groups C and G streptococci.

The M protein molecule has a rodlike coiled structure that separates functional domains. The structure allows for a large number of sequence changes while maintaining function, and the M protein immunodeterminants, therefore, can readily change. There are two major structural classes of M protein, classes I and II.

It appears that M protein and perhaps other streptococcal cell wall antigens have an important role in the pathogenesis of rheumatic fever. Purified streptococcal cell wall membranes induce antibodies that react with human cardiac sarcolemma; the characteristics of the cross-reactive antigens are not clear. A component of the cell wall of selected M types induces antibodies that react with cardiac muscle tissue. Conserved antigenic domains on the class I M protein cross-react with human cardiac muscle, and the class I M protein may be a virulence determinant for rheumatic fever.

Toxins and Enzymes

More than 20 extracellular products that are antigenic are elaborated by *S pyogenes*, including the following.

A. Streptokinase (Fibrinolysin)

Streptokinase is produced by many strains of group A β-hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots. This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase. Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses.

B. Deoxyribonucleases

Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus. The enzymatic activity can be measured by the decrease in viscosity of known DNA solutions. Purulent exudates owe their viscosity largely to deoxyribonucleoprotein. Mixtures of streptokinase and DNases are used in "enzymatic debridement." They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly. An antibody to DNAse develops after streptococcal infections (normal limit, 100 units), especially after skin infections.

C. Hyaluronidase

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source. After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

D. Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by *S pyogenes*. There are three antigenically distinct **streptococcal pyrogenic exotoxins (Spe): A, B, and C**. SpeA has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage. The streptococcal pyrogenic exotoxins have been associated with **streptococcal toxic shock syndrome** and **scarlet fever**. Most strains of group A streptococci isolated from patients with streptococcal toxic shock syndrome either produce Spe A

or have the gene that codes for it; in contrast, only about 15% of group A streptococci isolated from other patients have the gene. Spe C, also encoded by a phage, may contribute to the syndrome. Spe B, a potent protease, interferes with phagocytosis. The group A streptococci associated with toxic shock syndrome are primarily of M protein types 1 and 3.

The pyrogenic exotoxins act as superantigens, which stimulate T cells by binding to the class II major histocompatibility complex in the V_β region of the T-cell receptor. The activated T cells release cytokines that mediate shock and tissue injury. The mechanisms of action appear to be similar to those caused by staphylococcal toxic shock syndrome toxin-1 and the staphylococcal enterotoxins.

E. Hemolysins

The β-hemolytic group A *S pyogenes* elaborates two hemolysins (streptolysins) that not only lyse the membranes of erythrocytes but also damage a variety of other cell types. **Streptolysin O** is a protein (molecular weight [MW], 60,000) that is hemolytically active in the reduced state (available–SH groups) but rapidly inactivated in the presence of oxygen. Streptolysin O is responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates. It combines quantitatively with **antistreptolysin O (ASO)**, an antibody that appears in humans after infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O. This phenomenon forms the basis of a quantitative test for the antibody. An ASO serum titer in excess of 160–200 units is considered abnormally high and suggests either recent infection with *S pyogenes* or persistently high antibody levels caused by an exaggerated immune response to an earlier exposure in a hypersensitive person. **Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum—hence the name streptolysin S. It is not antigenic. Most isolates of *S pyogenes* produce both of these hemolysins. Up to 10% produce only one.

Pathogenesis and Clinical Findings

A variety of distinct disease processes are associated with *S pyogenes* infections. The infections can be divided into several categories.

A. Diseases Attributable to Invasion by *S pyogenes*, β-Hemolytic Group A Streptococci

The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream.

1. Erysipelas—If the portal of entry is the skin, erysipelas results. Lesions are raised and characteristically red. There is massive brawny edema and a rapidly advancing, sharply demarcated margin of infection.

2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions. Pain, tenderness, swelling, and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings: In cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.

3. Necrotizing fasciitis (streptococcal gangrene)—There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia. Bacteria other than *S pyogenes* can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fasciitis have sometimes been termed *flesh-eating bacteria*.

4. Puerperal fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).

5. Bacteremia or sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal. *S pyogenes* bacteremia can also occur with skin infections, such as cellulitis and rarely pharyngitis.

B. Diseases Attributable to Local Infection With *S pyogenes* and Their Byproducts

1. Streptococcal sore throat—The most common infection caused by β-hemolytic *S pyogenes* is streptococcal sore throat or pharyngitis. *S pyogenes* adheres to the pharyngeal epithelium by means of lipoteichoic acid–covered surface pili and by means of hyaluronic acid in encapsulated strains. The glycoprotein fibronectin (MW, 440,000) on epithelial cells probably serves as lipoteichoic acid ligand. In infants and small children, the sore throat occurs as a subacute nasopharyngitis with a thin serous discharge and little fever but with a tendency of the infection to extend to the middle ear and the mastoid. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate; enlarged, tender cervical lymph nodes; and (usually) a high fever. Twenty percent of infections are asymptomatic. A similar clinical picture can occur with infectious mononucleosis, diphtheria, gonococcal infection, and adenovirus infection.

S pyogenes infection of the upper respiratory tract does not usually involve the lungs. Pneumonia, when it does occur, is rapidly progressive and severe and is most commonly a sequela to viral infections, such as influenza or measles,

which seem to greatly enhance the predisposition to bacterial superinfection with this and other pathogens, such as *S pneumoniae*.

2. Streptococcal pyoderma—Local infection of superficial layers of skin, especially in children, is called **impetigo**. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted. It spreads by continuity and is highly communicable, especially in hot, humid climates. More widespread infection occurs in eczematous or wounded skin or in burns and may progress to cellulitis. Group A streptococcal skin infections are often attributable to M types 49, 57, and 59–61 and may precede glomerulonephritis (GN) but do not lead to rheumatic fever.

A clinically identical infection can be caused by *Staphylococcus aureus* and sometimes both *S pyogenes* and *S aureus* are present.

C. Invasive Group A Streptococcal Infections, Streptococcal Toxic Shock Syndrome, and Scarlet Fever

Fulminant, invasive *S pyogenes* infections with **streptococcal toxic shock syndrome** are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients. The infections tend to occur after minor trauma in otherwise healthy persons with several presentations of soft tissue infection. These include necrotizing fasciitis, myositis, and infections at other soft tissue sites; bacteremia occurs frequently. In some patients, particularly those infected with group A streptococci of M types 1 or 3, the disease presents with focal soft tissue infection accompanied by fever and rapidly progressive shock with multiorgan failure. Erythema and desquamation may occur. The *S pyogenes* of the M types 1 and 3 (and types 12 and 28) that make pyrogenic exotoxin A or B are associated with the severe infections.

Pyrogenic exotoxins A–C also cause **scarlet fever** in association with *S pyogenes* pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities. Streptococcal toxic shock syndrome and scarlet fever are clinically overlapping diseases.

D. Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

After an acute *S pyogenes* infection, there is a latent period of 1–4 weeks (mean 7 days), after which nephritis or rheumatic fever occasionally develops. The latent period suggests that these poststreptococcal diseases are not attributable to the direct effect of disseminated bacteria but instead represent a hypersensitivity response. Nephritis is more commonly preceded by infection of the skin; rheumatic fever is more commonly preceded by infection of the respiratory tract.

1. Acute glomerulonephritis—This sometimes develops 1–5 weeks (mean 7 days) after *S pyogenes* skin infection (pyoderma, impetigo) or pharyngitis. Some strains are particularly nephritogenic, principally with M types 2, 42, 49, 56, 57, and 60 (skin). Other nephritogenic M types associated with throat infections and glomerulonephritis are 1, 4, 12, and 25. After random streptococcal skin infections, the incidence of nephritis is less than 0.5%.

Glomerulonephritis may be initiated by antigen—antibody complexes on the glomerular basement membrane. The most important antigens are thought to be SpeB and a nephritis-associated plasmin receptor. In acute nephritis, the patient has blood and protein in the urine, edema, high blood pressure, and urea nitrogen retention; serum complement levels are also low. A few patients die, some develop chronic glomerulonephritis with ultimate kidney failure, and the majority recovers completely.

2. Rheumatic fever—This is the most serious sequela of *S pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens.

The onset of acute rheumatic fever (ARF) is often preceded by *S pyogenes* pharyngitis 1–5 weeks (mean 19 days) earlier, although the infection may be mild and may not be detected. In general, however, patients with more severe streptococcal sore throats have a greater chance of developing rheumatic fever. Rheumatic fever is not associated with cutaneous streptococcal infections. In the 1950s, untreated streptococcal infections were followed by rheumatic fever in up to 3% of military personnel and 0.3% of civilian children. In the 1980s through 2000 a resurgence of ARF occurred in the United States. M types 1, 3, 5, 6, and 18 were most frequently involved. Since that time, the incidence has once again declined. Rheumatic fever occurs up to 100 times more frequently in tropical countries and is the most important cause of heart disease in young people in developing countries.

Typical symptoms and signs of rheumatic fever include fever, malaise, a migratory nonsuppurative polyarthritis, and evidence of inflammation of all parts of the heart (endocardium, myocardium, and pericardium). The carditis characteristically leads to thickened and deformed valves and to small perivascular granulomas in the myocardium (Aschoff bodies) that are finally replaced by scar tissue. Patients may develop severe and progressive congestive heart failure. Sydenham's chorea is another manifestation of ARF and is characterized by involuntary, uncoordinated movements and associated muscle weakness. It has been hypothesized that other types of neurobehavioral conditions may also follow streptococcal infections. These are referred to as PANDAS—*p*ost-streptococcal *a*utoimmune, *n*europsychiatric *d*isorders *a*ssociated with *s*treptococci. More research is required to definitely establish a link to *S pyogenes* infections.

Erythrocyte sedimentation rates, serum transaminase levels, electrocardiograms, and other tests are used to estimate rheumatic activity.

Whereas rheumatic fever has a marked tendency to be reactivated by recurrent streptococcal infections, nephritis does not. The first attack of rheumatic fever usually produces only slight cardiac damage, which, however, increases with each subsequent attack. It is therefore important to protect such patients from recurrent *S pyogenes* infections by prophylactic penicillin administration.

Diagnostic Laboratory Tests

A. Specimens

Specimens to be obtained depend on the nature of the streptococcal infection. A throat swab, pus, cerebrospinal fluid or other sterile body fluid, or blood is obtained for culture. Serum is obtained for antibody determinations.

B. Smears

Smears from pus often show single cocci or pairs rather than definite chains. Cocci are sometimes gram negative because the organisms are no longer viable and have lost their ability to retain the blue dye (crystal violet) and be gram positive. If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected. Smears of throat swabs are rarely contributory because viridans streptococci are always present and have the same appearance as group A streptococci on stained smears.

C. Culture

Specimens suspected of containing streptococci are cultured on blood agar plates. If anaerobes are suspected, suitable anaerobic media must also be inoculated. Incubation in 10% CO_2 often speeds hemolysis. Slicing the inoculum into the blood agar has a similar effect because oxygen does not readily diffuse through the medium to the deeply embedded organisms, and it is oxygen that inactivates streptolysin O.

Blood cultures will grow hemolytic group A streptococci (eg, in sepsis) within hours or a few days. Certain α-hemolytic streptococci and enterococci may grow slowly, so blood cultures in cases of suspected endocarditis may not turn positive for a few days.

The degree and kind of hemolysis (and colonial appearance) may help place an organism in a definite group. *S pyogenes* can be identified by rapid tests specific for the presence of the group A-specific antigen and by the PYR test. Streptococci belonging to group A may be presumptively identified by inhibition of growth by bacitracin, but this should be used only when more definitive tests are not available.

D. Antigen Detection Tests

Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, then use enzyme immunoassay (EIA) or agglutination tests to demonstrate the presence of the antigen. The tests can be completed in minutes to hours after the specimen is obtained. They are 60–90% sensitive, depending on the prevalence of the disease in the population, and 98–99% specific compared with culture methods. More sensitive assays that use DNA probes or nucleic acid amplification techniques are now available and are beginning to replace the earlier antigen detection tests, although they remain more costly.

E. Serologic Tests

A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include ASO, particularly in respiratory disease; anti-DNase B and antihyaluronidase, particularly in skin infections; antistreptokinase; anti-M type-specific antibodies; and others. Of these, the anti-ASO titer is most widely used.

Immunity

Resistance against streptococcal diseases is M type specific. Thus, a host who has recovered from infection by one group A streptococcal M type is relatively immune to reinfection by the same type but fully susceptible to infection by another M type. Anti-M type-specific antibodies can be demonstrated in a test that exploits the fact that streptococci are rapidly killed after phagocytosis. M protein interferes with phagocytosis, but in the presence of type-specific antibody to M protein, streptococci are killed by human leukocytes.

Antibody to streptolysin O develops after infection; it blocks hemolysis by streptolysin O but does not indicate immunity. High titers (>250 units) indicate recent or repeated infections and are found more often in rheumatic individuals than in those with uncomplicated streptococcal infections.

Treatment

All *S pyogenes* are susceptible to penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin-allergic patients and for patients with necrotizing fasciitis. However, resistance to macrolide antibiotics has been increasing in Europe and the United States. Some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever. In acute streptococcal infections, however, every effort must be made to rapidly eradicate streptococci from the patient, eliminate the antigenic stimulus (before day 8), and thus prevent poststreptococcal disease. Doses of penicillin or erythromycin that result in effective tissue levels for 10 days usually accomplish this. Antimicrobial drugs are also very useful in preventing reinfection with β-hemolytic group A streptococci in patients with rheumatic fever.

Epidemiology, Prevention, and Control

Although humans can be asymptomatic nasopharyngeal or perineal carriers of *S pyogenes*, the organism should be considered significant if it is detected by culture or other means. The ultimate source of group A streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons via droplets from the respiratory tract or skin. The nasal discharges of a person harboring *S pyogenes* are the most dangerous source for spread of these organisms.

Many other streptococci (eg, viridans streptococci, enterococci) are members of the normal microbiota of the human body. They produce disease only when established in parts of the body where they do not normally occur (eg, heart valves). To prevent such accidents, particularly in the course of surgical procedures on the respiratory, gastrointestinal, and urinary tracts that result in temporary bacteremia, antimicrobial agents are often administered prophylactically to persons with known heart valve deformity and to those with prosthetic valves or joints. Guidelines published by the American Heart Association and other professional societies have clarified some of these recommendations (see Wilson et al, 2007).

Control procedures are directed mainly at the human source:

1. Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci. Prompt eradication of streptococci from early infections can effectively prevent the development of poststreptococcal disease. This requires maintenance of adequate penicillin levels in tissues for 10 days (eg, benzathine penicillin G given once intramuscularly). Erythromycin is an alternative drug, although many *S pyogenes* are now resistant.
2. Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever. This involves giving one injection of benzathine penicillin G intramuscularly every 3–4 weeks or daily oral penicillin or oral sulfonamide. The first attack of rheumatic fever infrequently causes major heart damage; however, such persons are particularly susceptible to reinfections with streptococci that precipitate relapses of rheumatic activity and give rise to cardiac damage. Chemoprophylaxis in such individuals, especially children, must be continued for years. Chemoprophylaxis is not used in glomerulonephritis because of the small number of nephritogenic types of streptococci. An exception may be family groups with a high rate of poststreptococcal nephritis.
3. Eradication of *S pyogenes* from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operating rooms, classrooms, or nurseries. Unfortunately, it is often difficult to eradicate β-hemolytic streptococci from permanent carriers, and individuals may occasionally have to be shifted away from "sensitive" areas for some time.

Concept Checks

- Streptococci are a large group of gram-positive organisms that are catalase negative and tend to grow in pairs and long chains.
- No one system accurately classifies all streptococci, and the taxonomy continues to evolve. Major classifications include the type of hemolysis (α, β, or no hemolysis) conditions for growth, and capacity to cause disease.
- Streptococci will grow well on 5% sheep blood agar and other media that support the growth of gram-positive cocci.
- *S pyogenes* (group A β-hemolytic streptococcus) is the most virulent pathogen in the *Streptococcus* family. It elaborates numerous proteins, hemolysins, enzymes, and toxins responsible for the broad range of suppurative (eg, cellulitis) and immunologic diseases (poststreptococcal GN, RF) associated with this organism.

STREPTOCOCCUS AGALACTIAE

These are the **group B streptococci**. They typically are β-hemolytic and produce zones of hemolysis that are only slightly larger than the colonies (1–2 mm in diameter). The group B streptococci hydrolyze sodium hippurate and give a positive response in the so-called CAMP (Christie, Atkins, Munch-Peterson) test.

Group B streptococci are part of the normal vaginal flora and lower gastrointestinal tract in 5–30% of women. Group B streptococcal infection during the first month of life may present as fulminant sepsis, meningitis, or respiratory distress syndrome. Substantial reductions in the incidence of early-onset neonatal group B streptococcal infections have been observed after the 1996 recommendations for screening pregnant women at 35–37 weeks of pregnancy. This is done by using either broth-enriched culture or molecular methods on rectal and vaginal swabs obtained at the time of screening. Intravenous ampicillin given to mothers who are colonized with group B streptococci and are in labor prevents colonization of their infants and subsequent group B streptococcal disease. Group B streptococcal infections are increasing among nonpregnant adults. Two expanding populations, namely elderly adults and immunocompromised hosts, are most at risk for invasive disease. Predisposing factors include diabetes mellitus, cancer, advanced age, liver cirrhosis, corticosteroid therapy, HIV, and other immunocompromised states. Bacteremia, skin and soft tissue infections, respiratory infections, and genitourinary infections in descending order of frequency are the major clinical manifestations.

GROUPS C AND G

These streptococci occur sometimes in the nasopharynx and may cause pharyngitis, sinusitis, bacteremia, or endocarditis. They often look like group A *S pyogenes* on blood agar

medium and are β-hemolytic. They are identified by reactions with specific antisera for groups C or G. Groups C and G streptococci have hemolysins and may have M proteins analogous to those of group A *S pyogenes*. Poststreptococcal sequelae of acute glomerulonephritis (AGN) and RF have been rarely reported.

GROUP D STREPTOCOCCI

The group D streptococci have undergone recent taxonomic changes. There are eight species in this group, many of which do not cause infections in humans. The *Streptococcus bovis* group is of most importance to human disease and is further classified into biotypes (old classification), which are important epidemiologically, and more recently into four DNA clusters. Animal species in the bovis group have been assigned to the species *Streptococcus equinus* (DNA cluster I). Biotype I (in DNA cluster II) isolates ferment mannitol and are now designated as *Streptococcus gallolyticus* subspecies *gallolyticus*. This organism causes human endocarditis and is frequently epidemiologically associated with colon carcinoma. DNA cluster II also includes *S gallolyticus* subspecies *pasteurianus* (formerly *S bovis* biotype II.2) and *S gallolyticus* subspecies *macedonius*. *S bovis* biotype II.1 is now in DNA cluster III and has the species name *Streptococcus infantarius*, which includes two subspecies (subsp. *infantarius* and subsp. *coli*). Biotype II bacteremias are often associated with biliary sources and less frequently with endocarditis. Finally, DNA cluster IV has one species, *Streptococcus alactolyticus*. Because of the confusing taxonomy and the failure of most automated or kit systems to discriminate to the subspecies level, most diagnostic microbiology laboratories will likely continue to refer to these organisms as either the *S bovis* group or group D non-enterococci. All group D streptococci are nonhemolytic and PYR negative. They grow in the presence of bile and hydrolyze esculin (bile esculin positive) but do not grow in 6.5% NaCl. They are part of the normal enteric microbiota of humans and animals.

STREPTOCOCCUS ANGINOSUS GROUP

Other species names in the *S anginosus* group are *Streptococcus constellatus* and *Streptococcus intermedius*. These streptococci are part of the normal microbiota of the throat, colon, and urogenital tract. They may be β-, α-, or nonhemolytic. *S anginosus* group includes β-hemolytic streptococci that form minute colonies (<0.5 mm in diameter) and react with groups A, C, or G antisera and all β-hemolytic group F streptococci. Those that are group A are PYR negative. *S anginosus* are Voges-Proskauer test positive. They may be classified as viridans streptococci. These organisms are frequently associated with serious infections such as brain, lung, and liver abscesses. They can be easily detected in the laboratory by their characteristic butterscotch or caramel odor.

GROUPS E, F, G, H, AND K–U STREPTOCOCCI

These streptococci occur primarily in animals. One of the multiple species of group G streptococci, *Streptococcus canis*, can cause skin infections of dogs but uncommonly infects humans; other species of group G streptococci infect humans.

Concept Checks

- Streptococci that have Lancefield antigens other than group A are a diverse group of organisms that include other pyogenic streptococci (groups B, C, and G), streptococci that occur primarily in animals (E, H, and K–U), the *S bovis* group (group D) and small colony variant members of the *S anginosus* group (primarily group F).
- *S agalactiae* (group B streptococci) are important pathogens among pregnant women and their neonates. Rectal and vaginal screening at 35–37 weeks of pregnancy and treatment of colonized moms during labor with penicillin has significantly reduced the incidence of early-onset neonatal group B streptococcal infections.
- Groups C and G streptococci cause infections similar to those of group A streptococci, including rare reports of poststreptococcal sequelae such as AGN and RF.
- The *S bovis* group (group D non-enterococci) has undergone significant taxonomic reclassification. These organisms are PYR negative and bile esculin positive but do not grow in 6.5% NaCl. They are associated with bacteremia and endocarditis in patients with significant biliary tract disease or colon pathology, including carcinoma.
- Members of the *S anginosus* group (also includes *S intermedius*, *S constellatus*) may be β-hemolytic, can possess Lancefield antigens A, C, F, G; tend to be small colony variants (<0.5 mm); and are associated with brain, lung, and liver abscesses.

VIRIDANS STREPTOCOCCI

The many species of the viridans streptococci are classified into groups and include the *Streptococcus mitis* group, *S anginosus* group (see above), *Streptococcus mutans* group, *Streptococcus salivarius* group, and *S bovis* group (see above). Typically they are α-hemolytic, but they may also be nonhemolytic. As discussed earlier, members of the *S anginosus* group can be β-hemolytic. Their growth is not inhibited by optochin, and colonies are not soluble in bile (deoxycholate). The viridans streptococci are the most prevalent members of the normal microbiota of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves. Some viridans streptococci (eg, *S mutans*) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

In the course of bacteremia, viridans streptococci or enterococci and rarely pneumococci, may settle on normal or previously deformed heart valves, producing **acute endocarditis**. Rapid destruction of the valves frequently leads to fatal cardiac failure in days or weeks unless surgery can be performed to place a prosthetic valve during antimicrobial treatment or following therapy. More frequently, the viridans streptococci are associated with a subacute course.

Subacute endocarditis often involves abnormal valves (congenital deformities and rheumatic or atherosclerotic lesions). Although any organism reaching the bloodstream may establish itself on thrombotic lesions that develop on endothelium injured as a result of circulatory stresses, subacute endocarditis is most frequently caused by members of the normal microbiota of the respiratory or intestinal tract that have accidentally reached the blood. After dental extraction, at least 30% of patients have viridans streptococcal bacteremia. These streptococci, ordinarily the most prevalent members of the upper respiratory microbiota, are also the most frequent cause of subacute bacterial endocarditis. The group D streptococci (enterococci and *S bovis*) also are common causes of subacute endocarditis. About 5–10% of cases are caused by enterococci originating in the gut or urinary tract. The lesion is slowly progressive, and a certain amount of healing accompanies the active inflammation; vegetations consist of fibrin, platelets, blood cells, and bacteria adherent to the valve leaflets. The clinical course is gradual, but the disease is invariably fatal in untreated cases. The typical clinical picture includes fever, anemia, weakness, a heart murmur, embolic phenomena, an enlarged spleen, and renal lesions.

α-Hemolytic streptococci and enterococci vary in their susceptibility to antimicrobial agents. Particularly in bacterial endocarditis, antibiotic susceptibility tests are useful to determine which drugs may be used for optimal therapy. Aminoglycosides often enhance the rate of bactericidal action of penicillin on streptococci, particularly enterococci.

NUTRITIONALLY VARIANT STREPTOCOCCI

The nutritionally variant streptococci (NVS) are now classified in the genus *Abiotrophia* (*Abiotrophia defectiva* is the sole species) and the genus *Granulicatella* (two species *Granulicatella adiacens* and *Granulicatella elegans*). They have also been known as "nutritionally deficient streptococci" and "pyridoxal-dependent streptococci." They require pyridoxal or cysteine for growth on blood agar and may grow as satellite colonies around colonies of staphylococci and other bacteria that produce pyridoxal. Routinely supplementing blood agar medium with pyridoxal allows recovery of these organisms. They are usually α-hemolytic but may also be nonhemolytic. MALDI-TOF MS has been shown to reliably differentiate them from streptococci and other catalase negative gram-positive cocci. NVS are part of the normal microbiota and occasionally cause bacteremia or endocarditis and can be found in brain abscesses and other infections. Clinically, they are very similar to the viridans streptococci.

PEPTOSTREPTOCOCCUS AND RELATED GENERA

These streptococci grow only under anaerobic or microaerophilic conditions and variably produce hemolysins. They are part of the normal microbiota of the mouth, upper respiratory tract, bowel, and female genital tract. They often participate with many other bacterial species in mixed anaerobic infections (see Chapter 21). Such infections may occur in wounds, in the breast, in postpartum endometritis, after rupture of an abdominal viscus, in the brain, or in chronic suppuration of the lung. The pus usually has a foul odor.

STREPTOCOCCUS PNEUMONIAE

S pneumoniae (pneumococci) is a member of the *S mitis* group (see Table 14-1) and are indistinguishable from them on the basis of 16SrRNA. Pneumococci are gram-positive diplococci, often lancet shaped or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are readily lysed by surface-active agents, which probably remove or inactivate the inhibitors of cell wall autolysins. Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, peritonitis, and other infectious processes.

Morphology and Identification

A. Typical Organisms

The typical gram-positive, lancet-shaped diplococci (Figure 14-3) are often seen in specimens of young cultures. In sputum or

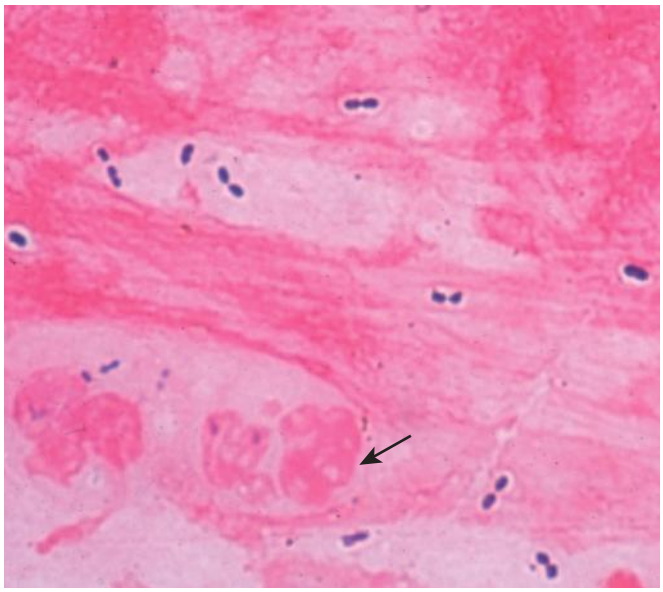


FIGURE 14-3 *Streptococcus pneumoniae* in sputum are seen as lancet-shaped gram-positive diplococci. Degenerating nuclei of polymorphonuclear cells are the large darker irregular red shapes (*arrow*). Mucus and amorphous debris are present in the background. Original magnification ×1000.

pus, single cocci or chains are also seen. With age, the organisms rapidly become gram negative and tend to lyse spontaneously. Autolysis of pneumococci is greatly enhanced by surface-active agents. Lysis of pneumococci occurs in a few minutes when ox bile (10%) or sodium deoxycholate (2%) is added to a broth culture or suspension of organisms at neutral pH. Viridans streptococci do not lyse and are thus easily differentiated from pneumococci. On solid media, the growth of pneumococci is inhibited around a disk of optochin; viridans streptococci are not inhibited by optochin (Figure 14-4).

Other identifying points include almost uniform virulence for mice when injected intraperitoneally and the "capsule swelling test," or quellung reaction (see below).

B. Culture

Pneumococci form small round colonies, at first dome-shaped and later developing a central depression with an elevated rim. Other colonies may appear glistening because of capsular polysaccharide production. Pneumococci are α-hemolytic on blood agar. Growth is enhanced by 5–10% CO_2.

C. Growth Characteristics

Most energy is obtained from fermentation of glucose; this is accompanied by the rapid production of lactic acid, which limits growth. Neutralization of broth cultures with alkali at intervals results in massive growth.

D. Variation

Pneumococcal isolates that produce large amounts of capsules appear as large mucoid colonies. Capsule production is not essential for growth on agar medium, and capsular production is therefore lost after a small number of subcultures. The pneumococci will, however, again produce capsules and have enhanced virulence if injected into mice.

Antigenic Structure

A. Component Structures

The pneumococcal cell wall has peptidoglycan and teichoic acid, similar to other streptococci. The capsular polysaccharide is covalently bound to the peptidoglycan and to the cell wall polysaccharide. The capsular polysaccharide is immunologically distinct for each of the 91 types. C-polysaccharide that is found in the cell wall of all *S pneumoniae* can be detected in the urine and cerebrospinal fluid (CSF) as useful diagnostic tests for pneumococcal infections.

B. Quellung Reaction

When pneumococci of a certain type are mixed with specific antipolysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by cross-linking of the antibodies (see Figure 14-4C). This reaction is useful for rapid identification and for typing of the organisms, either in sputum or in cultures. The polyvalent antiserum, which contains antibody to all of the types ("omniserum"), is a good reagent for rapid microscopic determination of whether or not pneumococci are present in fresh sputum. This test is rarely used because of the high reagent costs and the expertise required in assay performance and interpretation.

Pathogenesis

A. Types of Pneumococci

In adults, types 1–8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all fatalities in pneumococcal bacteremia; in children, types 6, 14, 19, and 23 are frequent causes.

B. Production of Disease

Pneumococci produce disease through their ability to multiply in the tissues. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes. A serum that contains antibodies against the type-specific polysaccharide protects against infection. If such a serum is absorbed with the type-specific polysaccharide, it loses its protective power. Animals or humans immunized with a given type of pneumococcal polysaccharide are subsequently immune to that type of pneumococcus and possess precipitating and opsonizing antibodies for that type of polysaccharide.

C. Loss of Natural Resistance

Because 40–70% of humans are at some time carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus. Among the factors that probably lower this resistance and thus predispose to pneumococcal infection are the following:

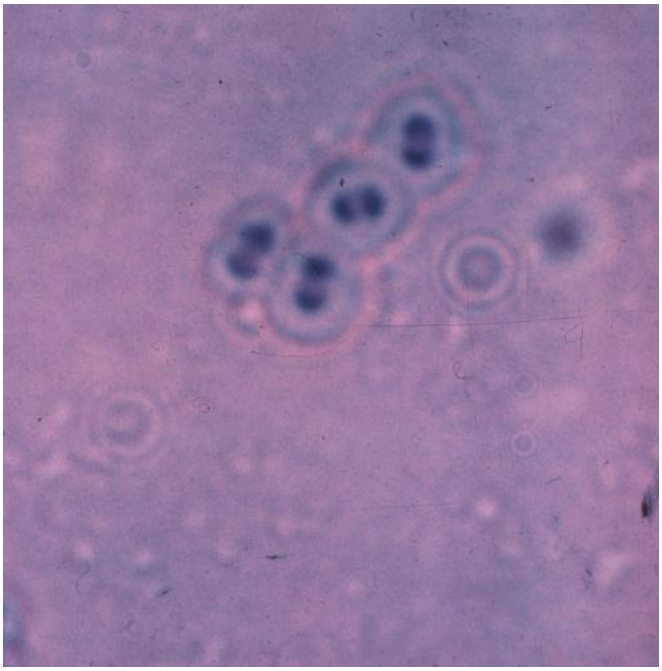
1. Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (eg, allergy), which protect pneumococci from phagocytosis; bronchial obstruction (eg, atelectasis); and respiratory tract injury caused by irritants disturbing its mucociliary function.
2. Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.
3. Abnormal circulatory dynamics (eg, pulmonary congestion, heart failure)
4. Other mechanisms, such as malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency

Pathology

Pneumococcal infection causes an outpouring of fibrinous edema fluid into the alveoli followed by red blood cells and leukocytes, which results in consolidation of portions of the lung. Many pneumococci are found throughout this exudate,

A

B



C

FIGURE 14-4 **A:** Optochin inhibition and bile solubility of *Streptococcus pneumoniae*. The *S pneumoniae* were grown overnight on 5% sheep blood agar. The optochin (ethyl hydrocupreine HCl) or P disk was placed when the plate was inoculated. The pneumococci are α-hemolytic with greening of the agar around the colonies. The zone of inhibition around the P disk is larger than 14 mm, indicating that the organisms are pneumococci rather than viridans streptococci. A drop of desoxycholate ("bile") solution was placed on the overnight growth just to the right of the P disk area (*arrow*); after about 20 minutes at room temperature, the colonies of pneumococci were solubilized (bile soluble). **B:** The growth of viridans streptococci appears similar to the growth of pneumococci, but growth of the viridans streptococci is not inhibited by optochin. **C:** *S pneumoniae* quellung reaction: a small amount of growth is mixed with saline, antisera against the capsule polysaccharide, and methylene blue stain. After incubation at room temperature for 1 hour, the reaction is observed under the microscope. The organisms are outlined in light blue. A positive reaction shows clumping because of cross-linking of the antibodies and pneumococci. The halo effect around the pneumococci is apparent capsular swelling. A negative control would show no clumping or capsular swelling. (Courtesy of H. Reyes.)

and they may reach the bloodstream via the lymphatic drainage of the lungs. The alveolar walls remain normally intact during the infection. Later, mononuclear cells actively phagocytose the debris, and this liquid phase is gradually reabsorbed. The pneumococci are taken up by phagocytes and digested intracellularly.

Clinical Findings

The onset of pneumococcal pneumonia is usually sudden, with fever, chills, and sharp pleural pain. The sputum is similar to the alveolar exudate, being characteristically bloody or rusty colored. Early in the disease, when the fever is high, bacteremia is present in 10–20% of cases. With antimicrobial therapy, the illness is usually terminated promptly; if drugs are given early, the development of consolidation is interrupted.

Pneumococcal pneumonia must be differentiated from pulmonary infarction, atelectasis, neoplasm, congestive heart failure, and pneumonia caused by many other bacteria. Empyema (pus in the pleural space) is a significant complication and requires aspiration and drainage.

From the respiratory tract, pneumococci may reach other sites. The sinuses and middle ear are most frequently involved. Infection sometimes extends from the mastoid to the meninges. Bacteremia from pneumonia has a triad of severe complications: meningitis, endocarditis, and septic arthritis. With the early use of chemotherapy, acute pneumococcal endocarditis and arthritis have become rare.

Diagnostic Laboratory Tests

Blood is drawn for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. CSF and urine can be used to detect pneumococcal C-polysaccharide by rapid immunochromatographic membrane assays. Serum antibody tests are impractical. All specimens should be sent to the microbiology laboratory as soon as possible after collection because pneumococci tend to autolyse and delay will significantly impact recovery by culture. Sputum may be examined in several ways.

A. Stained Smears

A Gram-stained film of rusty-red sputum shows typical organisms, many polymorphonuclear neutrophils, and many red blood cells.

B. Capsule Swelling Tests

Fresh emulsified sputum mixed with antiserum causes capsule swelling (the quellung reaction) for identification of pneumococci.

C. Culture

The culture is created by inoculating sputum to blood agar and incubating the plate in CO_2 at 37°C. A blood culture is also usually obtained.

D. Nucleic Acid Amplification Tests

Several manufacturers have included *S pneumoniae* on panels for identification of positive blood culture bottles and several of these assays are FDA cleared. Also, in development are panel tests for meningitis and separate molecular panels for direct detection of *S pneumoniae* in respiratory samples obtained from specimens in patients suspected of having community acquired or health care–associated pneumonia.

E. Immunity

Immunity to infection with pneumococci is type specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function. Vaccines can induce production of antibodies to capsular polysaccharides (see later discussion).

Treatment

Over the last several decades, pneumococci have become increasingly more resistant to a broad range of antimicrobial agents. Penicillin G can no longer be considered the empiric agent of choice. Around 15% of pneumococci from nonmeningeal sources are penicillin resistant (minimum inhibitory concentration [MIC] ≥8 μg/mL). High-dose penicillin G appears to be effective in treating pneumonia caused by pneumococci with MICs to penicillin below 8 μg/mL (resistant breakpoint) but would not be effective in treatment of meningitis caused by the same strains. Some penicillin-resistant strains are resistant to cefotaxime. Resistance to tetracycline, erythromycin, and fluoroquinolones also occurs. Pneumococci remain susceptible to vancomycin. Because resistance profiles are not predictable, routine susceptibility testing using a method that can determine MIC values for isolates from sterile sites should be performed for all pneumococcal infections.

Epidemiology, Prevention, and Control

Pneumococcal pneumonia accounts for about 60% of all bacterial pneumonias. In the development of illness, predisposing factors (see earlier discussion) are more important than exposure to the infectious agent, and a healthy carrier is more important in disseminating pneumococci than a sick patient.

It is possible to immunize individuals with type-specific polysaccharides. Such vaccines can probably provide 90% protection against bacteremic pneumonia. A polysaccharide vaccine containing 23 types (PPSV23) is licensed in the United States. A pneumococcal conjugate vaccine contains capsular polysaccharides conjugated to diphtheria CRM_{197} protein. The current conjugate vaccine is a 13-valent one (PCV13, Prevnar 13, Wyeth Pharmaceuticals). PCV13 contains the polysaccharide conjugates of the serotypes found in the PCV7 vaccine (4, 6B, 9V, 14, 18C, 19F, 23 F) plus serotypes 1, 3, 5, 6A, 7F, and 19A. It is recommended for all children as a four-dose series at 2, 4, 6, and 12–15 months of age. Children younger than 24 months of age who began their vaccination with PCV-7 and who have received one or more doses can complete the series with PCV-13. Older children and those

with underlying medical conditions who were fully vaccinated with PCV-7 should receive a single dose of PCV-13.

Adults 19 years of age or older with immunocompromising conditions should receive both PPSV23 and PCV13. The schedule for vaccine administration depends upon the timing and type of prior vaccination. The reader is referred to the latest recommendations published by the Centers for Disease Control and Prevention for current guidelines and schedules (http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-combined-schedule.pdf). In 2014, in addition to the existing recommendation to receive PPSV23, persons more than 65 years of age should now also receive one dose of PCV13. See above-mentioned guidelines for complete information.

ENTEROCOCCI

The enterococci have the group D group-specific substance and were previously classified as group D streptococci. Because the group D cell wall–specific antigen is a teichoic acid, it is not an antigenically good marker; enterococci are usually identified by characteristics other than immunologic reactions with group-specific antisera. They are part of the normal enteric microbiota. They are usually nonhemolytic but are occasionally α-hemolytic or rarely β-hemolytic. Enterococci are PYR positive. They grow in the presence of bile, hydrolyze esculin (bile esculin positive) and in contrast to non-enterococcal group D streptococci, they grow well in 6.5% NaCl. Whereas enterococci grow well at between 10 and 45°C, streptococci generally grow at a much narrower temperature range. They are more resistant to penicillin G than the streptococci. Many isolates are vancomycin resistant.

There are at least 47 species of enterococci, but less than one-third of these are associated with disease in humans. *Enterococcus faecalis* is the most common and causes 85–90% of enterococcal infections; *Enterococcus faecium* causes 5–10%. The enterococci are among the most frequent causes of health care–associated infections, particularly in intensive care units, and are selected by therapy with cephalosporins and other antibiotics to which they are resistant. Enterococci are transmitted from one patient to another primarily on the hands of hospital personnel, some of whom may carry the enterococci in their gastrointestinal tracts. Enterococci occasionally are transmitted on medical devices. In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood. Enterococci may cause meningitis and bacteremia in neonates. In adults, enterococci can cause endocarditis. However, in intra-abdominal, wound, urine, and other infections, enterococci usually are cultured along with other species of bacteria, and it is difficult to define the pathogenic role of the enterococci in these clinical circumstances.

Antibiotic Resistance

A major problem with the enterococci is that they can be very resistant to antibiotics. *E faecium* is usually much more antibiotic-resistant than *E faecalis*.

A. Intrinsic Resistance

Enterococci are intrinsically resistant to cephalosporins, penicillinase-resistant penicillins, and monobactams. They have intrinsic low-level resistance to many aminoglycosides, are of intermediate susceptibility or resistant to fluoroquinolones, and are less susceptible than streptococci (10- to 1000-fold) to penicillin and ampicillin. Enterococci are inhibited by β-lactams (eg, ampicillin) but generally are not killed by them. High-level resistance to penicillin and ampicillin is most often due to altered penicillin-binding proteins; β-lactamase producing strains have been rarely identified.

B. Resistance to Aminoglycosides

Therapy with combinations of a cell wall–active antibiotic (a penicillin or vancomycin) plus an aminoglycoside (streptomycin or gentamicin) is essential for severe enterococcal infections, such as endocarditis. Although enterococci have intrinsic low-level resistance to aminoglycosides (MICs <500 μg/mL), they have synergistic susceptibility when treated with a cell wall–active antibiotic plus an aminoglycoside. However, some enterococci have high-level resistance to aminoglycosides (MICs >500 μg/mL) and are not susceptible to the synergism. This high-level aminoglycoside resistance is due to enterococcal aminoglycoside-modifying enzymes. The genes that code for most of these enzymes are usually on conjugative plasmids or transposons. The enzymes have differential activity against the aminoglycosides. Resistance to gentamicin predicts resistance to the other aminoglycosides except streptomycin. (Susceptibility to gentamicin does not predict susceptibility to other aminoglycosides.) Resistance to streptomycin does not predict resistance to other aminoglycosides. The result is that only streptomycin or gentamicin (or both or neither) is likely to show synergistic activity with a cell wall–active antibiotic against enterococci. Enterococci from severe infections should have susceptibility tests for high-level aminoglycoside resistance (MICs >500 μg/mL for gentamicin and >1000 μg/mL for streptomycin in broth media) to predict therapeutic efficacy.

C. Vancomycin Resistance

The glycopeptide vancomycin is the primary alternative drug to a penicillin (plus an aminoglycoside) for treating enterococcal infections. In the United States, enterococci that are resistant to vancomycin have increased in frequency. These enterococci are not synergistically susceptible to vancomycin plus an aminoglycoside. Vancomycin resistance has been most common in *E faecium*, but vancomycin-resistant strains of *E faecalis* also occur.

There are multiple **vancomycin resistance phenotypes.** The VanA phenotype is manifested by inducible high-level resistance to vancomycin and teicoplanin. VanB phenotypes are inducibly resistant to vancomycin but susceptible to teicoplanin. VanC strains have intermediate to moderate resistance to vancomycin. VanC is constitutive in the less commonly isolated species, *Enterococcus gallinarum* (VanC-1) and *Enterococcus casseliflavus* (VanC-2/VanC-3). The VanD

phenotype is manifested by moderate resistance to vancomycin and low-level resistance or susceptibility to teicoplanin. The VanE phenotype is classified as low-level resistance to vancomycin and susceptibility to teicoplanin. VanG and VanL isolates (usually *E faecalis*) have low-level resistance to vancomycin and are susceptible to teicoplanin.

Teicoplanin is a glycopeptide with many similarities to vancomycin. It is available for patients in Europe but not in the United States. It has importance in investigation of the vancomycin resistance phenotype of enterococci.

Vancomycin and teicoplanin interfere with cell wall synthesis in gram-positive bacteria by interacting with the D-alanyl-D-alanine (D-Ala-D-Ala) group of the pentapeptide chains of peptidoglycan precursors. The best-studied vancomycin resistance determinant is the VanA operon. It is a system of genes packaged in a self-transferable plasmid containing a transposon closely related to Tn*1546* (Figure 14-5). There are two open reading frames that code for transposase and resolvase; the remaining seven genes code for vancomycin resistance and accessory proteins. The *vanR* and *vanS* genes are a two-component regulatory system sensitive to the presence of vancomycin or teicoplanin in the environment. *vanH*, *vanA*, and *vanX* are required for vancomycin resistance. *vanH* and *vanA* encode for proteins that manufacture the depsipeptide (d-Ala-d-lactate) rather than the normal peptide (d-Ala-d-Ala). The depsipeptide, when linked to UDP-muramyl-tripeptide, forms a pentapeptide precursor that vancomycin and teicoplanin will not bind to. *vanX* encodes a dipeptidase that depletes the environment of the normal d-Ala-d-Ala dipeptide. *vanY* and *vanZ* are not essential for vancomycin resistance. *vanY* encodes a carboxypeptidase that cleaves the terminal d-Ala from the pentapeptide, depleting the environment of any functional pentapeptide that may have been manufactured by the normal cell wall building process. *vanZ*'s function is unclear.

Similar to *vanA*, *vanB*, and *vanD* code for d-Ala-d-Lac, but *vanC* and *vanE* code for d-Ala-d-Ser.

Because enterococci that are resistant to vancomycin frequently carry plasmids that confer resistance to ampicillin and the aminoglycosides, newer agents such as daptomycin, linezolid, quinupristin–dalfopristin, and tigecycline (among others) are used for treatment of vancomycin-resistant enterococci (VRE) infections (see Chapter 28).

D. Trimethoprim-Sulfamethoxazole Resistance

Enterococci often show susceptibility to trimethoprim–sulfamethoxazole by in vitro testing, but the drugs are not effective in treating infections. This discrepancy is because enterococci are able to utilize exogenous folates available in vivo and thus escape inhibition by the drugs.

OTHER CATALASE-NEGATIVE GRAM-POSITIVE COCCI

There are nonstreptococcal gram-positive cocci or coccobacilli that are causing disease with increasing frequency (Table 14-2). These organisms have many growth and morphologic characteristics similar to viridans streptococci. They may be α-hemolytic or nonhemolytic. Most of them are catalase negative; others may be weakly catalase positive. *Pediococcus* and *Leuconostoc* are the genera whose members are **vancomycin resistant**. Lactobacilli are anaerobes that can be aerotolerant and α-hemolytic, sometimes forming coccobacillary forms similar to the viridans streptococci. Most **lactobacilli** (80–90%) are vancomycin resistant. Other organisms that occasionally cause disease and should be differentiated from streptococci and enterococci include *Lactococcus*, *Aerococcus*, and *Gemella*, genera that generally are **vancomycin susceptible**. *Rothia mucilaginosa* was previously considered a *Staphylococcus*, but it is catalase negative; colonies show a distinct adherence to agar.

Concept Checks

- Viridans streptococci and enterococci are part of the normal microbiota of the human oral and gastrointestinal tracts, but they can be associated with serious infections, such as bacteremia and endocarditis under certain conditions.
- *S pneumoniae* is α-hemolytic; optochin susceptible; and virulent largely because of its polysaccharide capsule, which inhibits phagocytosis.

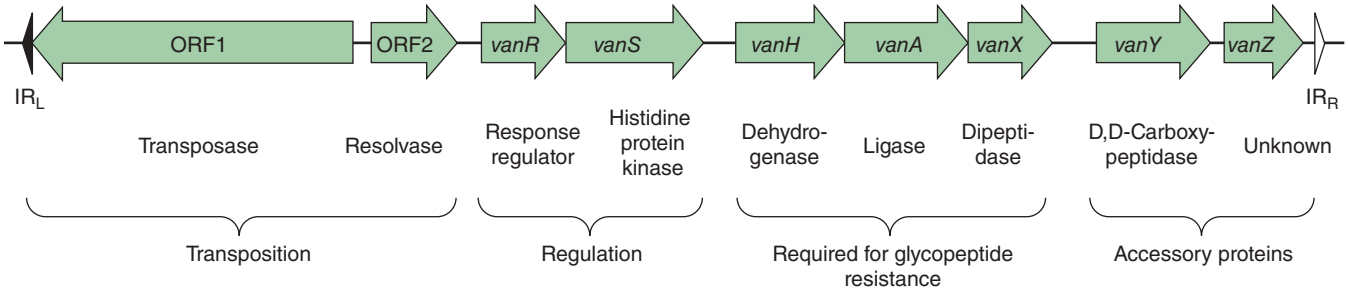


FIGURE 14-5 Schematic map of transposon Tn*1546* from *Enterococcus faecium* that codes for vancomycin resistance. IR_L and IR_R indicate the left and right inverted repeats of the transposon, respectively. (Adapted and reproduced with permission from Arthur M, Courvalin P: Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* 1993;37:1563.)

TABLE 14-2 Most Frequently Encountered Nonstreptococcal Catalase-Negative Gram-Positive Cocci and Coccobacilli

Genus[a]	Catalase	Gram Stain	Vancomycin Susceptibility	Comments
Abiotrophia[b] (nutritionally variant streptococcus)	Negative	Cocci in pairs, short chains	Susceptible	Normal microbiota of oral cavity; isolated from cases of endocarditis
Aerococcus	Negative to weakly positive	Cocci in tetrads and clusters	Susceptible	Environmental organisms occasionally isolated from blood, urine, or sterile sites
Enterococcus faecalis (and other enterococci)	D	None, α Rarely β	Some are resistant, mostly *Enterococcus faecium*	Abdominal abscess, urinary tract infection, endocarditis
Gemella	Negative	Cocci in pairs, tetrads, clusters, and short chains	Susceptible	Decolorize easily and may look gram negative; grow slowly (48 hours); part of normal human microbiota; occasionally isolated from blood and sterile sites
Granulicatella[b] (nutritionally variant streptococcus)	Negative	Cocci in chains, clusters	Susceptible	Normal microbiota of oral cavity; isolated from cases of endocarditis
Leuconostoc	Negative	Cocci in pairs and chains; coccobacilli, rods	Resistant	Environmental organisms; look like enterococci on blood agar; isolated from a wide variety of infections
Pediococcus	Negative	Cocci in pairs, tetrads, and clusters	Resistant	Present in food products and human stools; occasionally isolated from blood and abscesses
Lactobacillus	Negative	Coccobacilli, rods in pairs and chains	Resistant (90%)	Aerotolerant anaerobes generally classified as bacilli; normal vaginal flora; occasionally found in deep-seated infections

[a]Other genera in which isolates from humans are rare or uncommon include, *Dolosicoccus*, *Dolosigranulum*, *Facklamia*, *Globicatella*, *Helcococcus*, *Ignavigranum*, *Lactococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*.

[b]Require pyridoxal for growth.

- *S pneumoniae* is the major cause of community-acquired pneumonia but can also disseminate via the bloodstream to the central nervous system. Invasive disease is preventable through vaccination using either the 23-valent polysaccharide vaccine (adults) or the 13-valent conjugate vaccine (children). Drug resistance has become a problem in certain geographic regions.
- Enterococci are remarkable for the varieties of resistance determinants they have evolved that include β-lactam agents, glycopeptides, and aminoglycosides, among others. Newer agents such as linezolid and tedizolid are used for treatment of VRE infections. These organisms play a prominent role in health care–associated infections.

REVIEW QUESTIONS

1. A 48-year-old alcoholic man is admitted to a hospital because of stupor. He is unkempt and homeless and lives in an encampment with other homeless people, who called the authorities when he could not be easily aroused. His temperature is 38.5°C, and his blood pressure 125/80 mm Hg. He moans when attempts are made to arouse him. He has positive Kernig and Brudzinski signs, suggesting meningeal irritation. Physical examination and chest radiography show evidence of left lower lobe lung consolidation. An endotracheal aspirate yields rust-colored sputum. Examination of a Gram-stained sputum smear shows numerous polymorphonuclear cells and numerous gram-positive lancet-shaped diplococci. On lumbar puncture, the cerebrospinal fluid is cloudy and has a white blood cell count of 570/μL with 95% polymorphonuclear cells; Gram stain shows numerous gram-positive diplococci. Based on this information, the likely diagnosis is
   (A) Pneumonia and meningitis caused by *Staphylococcus aureus*
   (B) Pneumonia and meningitis caused by *Streptococcus pyogenes*
   (C) Pneumonia and meningitis caused by *Streptococcus pneumoniae*
   (D) Pneumonia and meningitis caused by *Enterococcus faecalis*
   (E) Pneumonia and meningitis caused by *Neisseria meningitidis*
2. The patient in question 1 is started on antibiotic therapy to cover many possible microorganisms. Subsequently, culture of sputum and cerebrospinal fluid yields gram-positive diplococci

with a minimum inhibitory concentration to penicillin G of greater than 2 μg/mL. The drug of choice for this patient until further susceptibility testing can be done is

(A) Penicillin G
(B) Nafcillin
(C) Trimethoprim–sulfamethoxazole
(D) Gentamicin
(E) Vancomycin

3. This infection (question 1) might have been prevented by
   (A) Prophylactic intramuscular benzathine penicillin every 3 weeks
   (B) A 23-valent capsular polysaccharide vaccine
   (C) A vaccine against serogroups A, C, Y, and W135 capsular polysaccharide
   (D) A vaccine of polyribosylribitol capsular polysaccharide covalently linked to a protein
   (E) Oral penicillin twice daily
4. The pathogenesis of the organism causing the infection (question 1) includes which of the following?
   (A) Invasion of cells lining the alveoli and entry into the pulmonary venule circulation
   (B) Resistance to phagocytosis mediated by M proteins
   (C) Migration to mediastinal lymph nodes where hemorrhage occurs
   (D) Lysis of the phagocytic vacuole and release into the circulation
   (E) Inhibition of phagocytosis by a polysaccharide capsule
5. A 13-valent capsular polysaccharide protein conjugate vaccine for the pathogen in question 1 is recommended
   (A) For children up to age 18 years and for selected adults
   (B) Only on exposure to a patient with disease caused by the organism
   (C) For all children ages 2–23 months plus selected older children and adults with immunocompromising conditions
   (D) For children ages 24–72 months
   (E) For all age groups older than age 2 months
6. An 8-year-old boy develops a severe sore throat. On examination, a grayish-white exudate is seen on the tonsils and pharynx. The differential diagnosis includes group A streptococcal infection, Epstein-Barr virus infection, severe adenovirus infection, and diphtheria. (*Neisseria gonorrhoeae* pharyngitis would also be included, but the patient has not been sexually abused.) The cause of the boy's pharyngitis is most likely
   (A) A catalase-negative gram-positive coccus that grows in chains
   (B) A single-stranded positive-sense RNA virus
   (C) A catalase-positive gram-positive coccus that grows in clusters
   (D) A catalase-negative gram-positive bacillus
   (E) A double-stranded RNA virus
7. A primary mechanism responsible for the pathogenesis of the boy's disease (question 6) is
   (A) A net increase in intracellular cyclic adenosine monophosphate
   (B) Action of M protein
   (C) Action of IgA1 protease
   (D) Action of enterotoxin A
   (E) Inactivation of elongation factor 2
8. A 40-year-old woman develops severe headache and fever. Her neurologic examination findings are normal. A brain scan shows a ring-enhancing lesion of the left hemisphere. During surgery, a brain abscess is found. Culture of the abscess fluid grows an anaerobic gram-negative bacillus (*Fusobacterium nucleatum*) and a catalase-negative gram-positive coccus that on Gram stain is in pairs and chains. The organism is β-hemolytic and forms very small colonies (<0.5 mm in diameter). One person thought it smelled like butterscotch. It agglutinates with group F antisera. The organism most likely is
   (A) *Streptococcus pyogenes* (group A)
   (B) *Enterococcus faecalis* (group D)
   (C) *Streptococcus agalactiae* (group B)
   (D) *Streptococcus anginosus* group
   (E) *Staphylococcus aureus*
9. Important methods for classifying and speciating streptococci are
   (A) Agglutination using antisera against the cell wall group-specific substance
   (B) Biochemical testing
   (C) Hemolytic properties (α-, β-, nonhemolytic)
   (D) Capsular swelling (quellung) reaction
   (E) All of the above
10. An 8-year-old girl develops Sydenham's chorea ("St. Vitus dance") with rapid uncoordinated facial tics and involuntary purposeless movements of her extremities, strongly suggestive of acute rheumatic fever. She has no other major manifestations of rheumatic fever (carditis, arthritis, subcutaneous nodules, skin rash). The patient's throat culture is negative for *Streptococcus pyogenes* (group A streptococci). However, she, her brother, and her mother all had sore throats 2 months ago. A test that if positive would indicate recent *S pyogenes* infections is
    (A) Antistreptolysin S antibody titer
    (B) Polymerase chain reaction for antibodies against M protein
    (C) ASO antibody titer
    (D) Esculin hydrolysis
    (E) Antihyaluronic acid antibody titer
11. All of the following statements regarding the hyaluronic acid capsule of *S pyogenes* are correct *except*
    (A) It is responsible for the mucoid appearance of the colonies in vitro.
    (B) It is antiphagocytic.
    (C) It binds to CD44 on human epithelial cells.
    (D) It is an important virulence factor.
    (E) A vaccine against the capsule is currently available.
12. Enterococci can be distinguished from nonenterococcal group D streptococci on the basis of which of the following characteristics?
    (A) γ-Hemolysis
    (B) Esculin hydrolysis
    (C) Growth in 6.5% NaCl
    (D) Growth in the presence of bile
    (E) Gram stain morphology
13. Which of the following statements regarding the *Streptococcus bovis* group is correct?
    (A) They possess Lancefield group D antigen.
    (B) Some strains are vancomycin resistant.
    (C) Infections caused by these organisms are benign.
    (D) All subspecies are PYR positive.
    (E) All subspecies are β-hemolytic.

14. Which of the following genera requires pyridoxal for growth?
    (A) *Aerococcus*
    (B) *Granulicatella*
    (C) *Enterococcus*
    (D) *Leuconostoc*
    (E) *Pediococcus*
15. Which of the following genera is typically resistant to vancomycin?
    (A) *Aerococcus*
    (B) *Gemella*
    (C) *Pediococcus*
    (D) *Streptococcus*
    (E) *Abiotrophia*

Answers

1. C	5. C	9. E	13. A
2. E	6. A	10. C	14. B
3. B	7. B	11. E	15. C
4. E	8. D	12. C	

REFERENCES

Arias CA, Murray BE: *Enterococcus* species, *Streptococcus gallolyticus* group, and *Leuconostoc* species. In Bennett JE, Dolin R, Blaser ME (editors). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8th ed. Elsevier, 2015.

Bryant AE, Stevens DL: *Streptococcus pyogenes*. In Bennett JE, Dolin R, Blaser ME (editors). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8th ed. Elsevier, 2015.

Cunningham MW: Pathogenesis of group A streptococcal infections and their sequelae. *Adv Exp Med Biol* 2008;609:29.

Edwards MS, Baker CJ: *Streptococcus agalactiae* (group B streptococcus). In Bennett JE, Dolin R, Blaser ME (editors). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8th ed. Elsevier, 2015.

Paradiso PR. Advances in pneumococcal disease prevention: 13-valent pneumococcal conjugate vaccine for infants and children. *Clin Infect Dis* 2011;52:1241.

Ruoff KL, Christensen JJ: *Aerococcus, Abiotrophia*, and other catalase-negative gram positive cocci. In Jorgensen JH, Pfaller MA, Carroll KC (editors). *Manual of Clinical Microbiology*, 11th ed. ASM Press, 2015.

Schlegel L, Grimont F, Ageron E, et al: Reappraisal of the taxonomy of the *Streptococcus bovis*/*Streptococcus equinus* complex and related species: description of *Streptococcus gallolyticus* subsp. *gallolyticus* subsp. nov., *S. gallolyticus* subsp. *macedonicus* subsp. nov. and *S. gallolyticus* subsp. *pasteurianus* subsp. nov. *Int J Syst Evol Microbiol* 2003;3:631–645.

Sendi P, Johansson L, Norrby-Teglund A: Invasive group B streptococcal disease in non-pregnant adults. *Infection* 2008;36:100.

Shulman ST, Bisno AL: Nonsuppurative poststreptococcal sequelae: Rheumatic fever and glomerulonephritis. In Bennett JE, Dolin R, Blaser ME (editors). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8th ed. Elsevier, 2015.

Spellberg B, Brandt C. *Streptococcus*. In Jorgensen JH, Pfaller MA, Carroll KC, Funke G, et al (editors). *Manual of Clinical Microbiology*, 11th ed. ASM Press, 2015.

Stollerman GH, Dale JB: The importance of the group A streptococcus capsule in the pathogenesis of human infections: A historical perspective. *Clin Infect Dis* 2008;46:1038.

Teixeira LM, et al: *Enterococcus*. In Jorgensen JH, Pfaller MA, Carroll KC, Funke G, et al (editors). *Manual of Clinical Microbiology*, 11th ed. ASM Press, 2015.

Wilson W, Taubert KA, Gewitz M, et al: Prevention of infective endocarditis: Guidelines from the American Heart Association: A guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;116:1736–1754.