The Staphylococci

Scientific classification
Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: Staphylococcus
Species: *S. aureus*, *S. epidermidis*, *S. saprophyticus*

Morphology & Identification

A. MORPHOLOGY
Staphylococci are spherical cells about 1 μm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Staphylococci are nonmotile and do not form spores. Under the influence of drugs like penicillin, staphylococci are lysed. Their colonies can be yellow, red, or orange.

B. STAINING
Typical staphylococci appear as gram positive cocci in clusters in Gram-stained smears of pus or sputum.

C. CULTURE AND GROWTH CHARACTERISTICS
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. *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. Various degrees of hemolysis are produced by *S. aureus* and occasionally by other species. *Peptostreptococcus* species, which are anaerobic cocci, often resemble staphylococci in morphology.

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 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene. Staphylococci are variably sensitive to many antimicrobial drugs.

D. VIRULENCE FACTORS

Antigenic Structure
Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. 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.

Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid antibodies detectable by gel diffusion may be found in patients with active endocarditis due to *S. aureus*.

Protein A is a cell wall component of many *S. aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of 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 will agglutinate bacteria that have that antigen (“coagglutination”).
Some *S. aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. Most strains of *S. aureus* have coagulase, or clumping factor, on the cell wall surface; coagulase binds nonenzymatically to fibrinogen, yielding aggregation of the bacteria.

**Enzymes & Toxins:**

1) **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.

2) **coagulase** and clumping factor: *S. aureus* produces 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.

3) **clumping factor** is a surface *S. aureus* compound 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.

4) **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.

5) **exotoxins**: the α-toxin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The α-toxin is a potent hemolysin. 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 refers to three proteins that interact with the two proteins comprising the Panton-Valentine leukocidin 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.

6) **leukocidin**: this toxin of *S. aureus* has two components. It can kill white blood cells of humans and rabbits. The two components act synergistically on the white blood cell membrane as described above for γ toxin. This toxin is an important virulence factor in community associated methicillin resistant *S. aureus* infections.

7) **exfoliative toxins**: these epidermolytic toxins of *S. aureus* are two distinct proteins of the same molecular weight. Epidermolytic toxin A is a chromosomal gene product and is heat-stable (resists boiling for 20 minutes). Epidermolytic toxin B is plasmid-mediated and heat-labile. The epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

8) **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. TSST-1 binds to 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.

9) **enterotoxins**: there are multiple (A–E, G–I, K–M) enterotoxins. Approximately 50% of *S. aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens. The enterotoxins are heat-stable and resistant to the action of gut enzymes. An important cause 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 probly 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.
### S. aureus Virulence Factors

<table>
<thead>
<tr>
<th>Structural Components</th>
<th>Biologic Effects</th>
</tr>
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<tbody>
<tr>
<td>Capsule</td>
<td>Inhibits Chemotaxis and phagocytosis; inhibits proliferation of mononuclear cells; facilitates adherence to foreign bodies</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Provides osmotic stability; stimulates production of endogenous pyrogen (endotoxin-like activity); leukocyte chemoattractant (abscess formation); inhibits phagocytosis</td>
</tr>
<tr>
<td>Teichoic acid</td>
<td>Regulates cationic concentration at cell membrane; binds to fibronectin</td>
</tr>
<tr>
<td>Protein A</td>
<td>Inhibits antibody-mediated clearance by binding IgG, IgG₂, and IgG₄ Fc receptors; leukocyte chemoattractant; anticomplementary</td>
</tr>
<tr>
<td>Cytoplasmic membrane</td>
<td>Osmotic barrier; regulates transport into and out of cell; site of biosynthetic and respiratory enzymes</td>
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</tbody>
</table>

### Toxins

**Cytotoxins (α, β, δ, γ, P-V leukocidin)**
- Toxic for many cells, including leukocytes, erythrocytes, macrophages, platelets, and fibroblasts

**Exfoliative toxins (ETA, ETB)**
- Serine proteases that split the intercellular bridges in the stratum granulosum epidermis

**Enterotoxins (A-E, G-D)**
- Superantigens (stimulates proliferation of T cells and release of cytokines); stimulates release of inflammatory mediators in mast cells, increasing intestinal peristalsis and fluid loss, as well as nausea and vomiting

**Toxic Shock Syndrome Toxin-1**
- Superantigen (stimulates proliferation of T cells and release of cytokines); produces leakage or cellular destruction of endothelial cells

### Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>Coagulase</td>
<td>Converts fibrinogen to fibrin</td>
</tr>
<tr>
<td>Catalase</td>
<td>Catalyzes removal of hydrogen peroxide</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Hydrolyzes hyaluronic acids in connective tissue, promoting the spread of staphylococci in tissue</td>
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<tr>
<td>Fibrinolysin</td>
<td>Dissolves fibrin clots</td>
</tr>
<tr>
<td>Lipases</td>
<td>Hydrolyzes lipids</td>
</tr>
<tr>
<td>Nuclease</td>
<td>Hydrolyzes DNA</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>Hydrolyzes penicillins</td>
</tr>
</tbody>
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### Pathogenesis, Pathology & Clinical Findings

**A. Diseases Caused by Staphylococci**

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) 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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Toxin-mediated (food poisoning, toxic shock syndrome); cutaneous (impetigo, folliculitis, furuncles, carbuncles, wound infections); other (bacteremia, endocarditis, pneumonia, empyema, osteomyelitis, septic arthritis)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Bacteremia; endocarditis; surgical wounds; urinary tract infections; opportunistic infections of catheters, shunts, prosthetic devices, and peritoneal dialysates</td>
</tr>
<tr>
<td><em>Staphylococcus saprophytics</em></td>
<td>Urinary tract infections, opportunistic infections</td>
</tr>
<tr>
<td><em>Staphylococcus capitis</em></td>
<td>Bacteremia, endocarditis, urinary tract infections, wound infections, pneumonia, bone and joint infections, opportunistic infections</td>
</tr>
<tr>
<td><em>Staphylococcus haemolyticus</em></td>
<td>Bacteremia, endocarditis, urinary tract infections, wound infections, and opportunistic infections</td>
</tr>
<tr>
<td><em>Micrococcus spp.</em></td>
<td>Opportunistic infections</td>
</tr>
<tr>
<td><em>Stomatococcus mucilaginosus</em></td>
<td>Bacteremia, endocarditis, opportunistic infections</td>
</tr>
<tr>
<td><em>Alloctococcus olitidis</em></td>
<td>Chronic middle ear infections</td>
</tr>
</tbody>
</table>

**B. SOURCE OF AGENTS OF DIFFERENT STAPHYLOCOCCI DISEASES**

Staphylococci, particularly *S. epidermidis*, are members of the normal flora 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.

**C. PATHOGENESIS**

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. *S. saprophyticus* is typically nonpigmented, novobiocin-resistant, and nonhemolytic; it causes urinary tract infections in young women.

**D. CLINICAL FINDINGS OF TYPICAL SYMPTOMS OF STAPHYLOCOCCI INFECTION**

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 tends 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, eg, postoperative staphylococcal wound infection or infection following trauma (chronic osteomyelitis subsequent to an open fracture, meningitis following 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 due to 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 tampons, but it also occurs in children or in 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, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

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 saprophytic (S. epidermidis) from 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 flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal flora but not S. aureus. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers of S. aureus and 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.

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 is positive.

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
Broth microdilution 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 for β-lactamase; approximately 90% of S. aureus produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of S. aureus and approximately 75% of S. epidermidis isolates. Nafcillin resistance correlates with the presence of mecA, the gene that codes for a penicillin-binding protein (PBP 2a) not affected by these drugs. The gene can be detected using the polymerase chain reaction. Most clinical laboratories use a phenotypic method such as an oxacillin screening agar plate. Staphylococci that grow on Mueller-Hinton agar containing 4% NaCl and 6 μg/mL of typically are mecA-positive and nafcillin-resistant. Alternatively, an assay for the mecA gene product, PBP 2a, is commercially available and is much
more rapid than PCR for mecA or than testing for resistance using growth on oxacillin-containing salt agar.

G. Serologic and typing tests: Serologic tests for diagnosis of S. aureus infections have little practical value.

Antibiotic susceptibility patterns are helpful in tracing S. aureus infections and in determining if multiple S. epidermidis isolates from blood cultures represent bacteremia due to 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.

**Treatment**

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. 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 due to S. aureus require prolonged intravenous therapy with a β-lactamase-resistant penicillin. Vancomycin is often reserved for use with nafcillin-resistant staphylococci. If the infection is found to be due to non-β-lactamase-producing S. aureus, penicillin G is the drug of choice, but only a small percentage of S. aureus strains are susceptible to penicillin G.

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.

Newer antimicrobial agents such as linezolid, daptomycin, and quinupristin/dalfopristin are generally reserved for patients with serious staphylococcal or enterococcal infections that are resistant to the more traditional agents, who are failing clinically or who are highly allergic.

**Epidemiology, Prevention & Control**

**A. EPIDEMIOLOGY**

Staphylococci are ubiquitous human parasites. 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 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.

**B. PREVENTION**

- Carrier status prevents complete control
- Proper hygiene, segregation of carrier from highly susceptible individuals
- Good aseptic techniques when handling surgical instruments
- Control of nosocomial infections
Scientific classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Streptococcaceae
Genus: Streptococcus
Species: S. agalactiae, S. anginosus, S. bovis, S. mitis

Classification

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 and other cell wall or capsular antigens; (3) biochemical reactions and resistance to physical and chemical factors; and (4) ecologic features.

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 non-hemolytic (sometimes called gamma hemolysis).

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-acetylglactosamine; for group D, it is glycerol teichoic acid containing D-alanine and glucose; and for group F, it is glucopyranosyl-N-acetylglactosamine.

Medically important Streptococci:
- **Group A** (Streptococcus pyogenes)
- **Group B** (Streptococcus agalactiae)
- **Group C and G** (viridans Streptococci: S. mutans)
- **Group D** (Enterococcus faecalis)

C. CAPSULAR POLYSACCHARIDES

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

**Group A: Streptococcus pyogenes**

Morphology & Identification

A. MORPHOLOGY

Individual cocci are spherical or ovoid and are arranged in chains. 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.

Most group A strains produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede phagocytosis. 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. Hair-like 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. Most
streptococci grow in solid media as discoid colonies, usually 1–2 mm in diameter. *S. pyogenes* is β-hemolytic; other species have variable hemolytic characteristics.

**B. STAINING**

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.

**C. CULTURE AND 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% CO₂. Most pathogenic hemolytic streptococci grow best at 37 °C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

**D. VIRULENCE FACTORS**

**Antigenic Structure:**

1. **M protein:** This substance is a major virulence factor of group A *S. pyogenes*. M protein appears as hair-like projections of the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M typespecific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes. *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 many, perhaps 150, types of M protein, a person can have repeated infections with group A *S. pyogenes* of different M types. Both group C and group G streptococci have genes homologous to the genes for M protein of group A, and M protein has been found on group G streptococci.

2. **T substance:** This antigen has no relationship to virulence of streptococci. Unlike M protein, T substance is acid-labile and heat-labile. It is obtained from streptococci by proteolytic digestion, which rapidly destroys M proteins. T substance permits differentiation of certain types of streptococci by agglutination with specific antisera, while other types share the same T substance. Yet another surface antigen has been called R protein.

**Toxins & Enzymes:**

1. **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.

2. **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. Following infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

3. **Pyrogenic exotoxins (erythrogenic toxin):** Pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins: A, B, and C. Exotoxin A 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. Streptococcal pyrogenic exotoxin C may also contribute to the syndrome, while the role for streptococcal pyrogenic exotoxin B is unclear. 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 Vβ 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 due to staphylococcal toxic syndrome toxin-1 and the staphylococcal enterotoxins.

4. **Diphosphopyridine nucleotidase:** This enzyme is elaborated into the environment by some streptococci. This substance may be related to the organism’s ability to kill leukocytes. Proteinases and amylase are produced by some strains.

5. **Hemolysins:** The β-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins). *Streptolysin O* is a protein (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 is in cuts deep into the medium in blood agar plates. It combines quantitatively with antistreptolysin O, an antibody that appears in humans following 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 antistreptolysin O (ASO) serum titer in excess of 160–200 units is considered abnormally high and suggests either recent infection with \textit{S. pyogenes} or persistently high antibody levels due to 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, but it may be inhibited by a nonspecific inhibitor that is frequently present in the sera of humans and animals and is independent of past experience with streptococci.

**Pathogenesis, Pathology & Clinical Findings**

**A. DISEASES ATTRIBUTABLE TO INVASION BY \textit{S. PYOGENES}, \textit{B}-HEMOLYTIC GROUP A \textit{S. PYOGENES}}**

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, with massive brawny edema and a rapidly advancing 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)—This is infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues. Bacteria other than \textit{S. pyogenes} can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fascitis 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/Sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which rapidly can be fatal. \textit{S. pyogenes} bacteremia can also follow skin infections, such as cellulitis and rarely pharyngitis.

**B. DISEASES ATTRIBUTABLE TO LOCAL INFECTION WITH \textit{S. PYOGENES} AND THEIR BY-PRODUCTS**

1. Streptococcal Sore Throat—The most common infection due to \textit{β}-hemolytic \textit{S. pyogenes} is streptococcal sore throat or pharyngitis. \textit{S. pyogenes} adhere to the pharyngeal epithelium by means of lipoteichoic acid-covered surface pili. 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. \textit{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, eg, influenza or measles, which seem to enhance susceptibility greatly.

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 but do not often lead to rheumatic fever. A clinically identical infection can be caused by S. 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 follow 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)

Following an acute S. pyogenes infection, there is a latent period of 1–4 weeks, 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 represent instead 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 3 weeks after S. pyogenes skin infection (pyoderma, impetigo). Some strains are particularly nephritogenic, principally with M types 12, 4, 2, and 49. Other nephritogenic, M types are 59–61. 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 antigen is probably in the streptococcal protoplast membrane. In acute nephritis, there is 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 recover 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 rheumatic fever is often preceded by S. pyogenes infection 1–4 weeks 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.

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

Rheumatic fever has a marked tendency to be reactivated by recurrent streptococcal infections, whereas 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. 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.

**B. 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% CO2 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 occasionally do 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.

**C. 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 EIA or agglutination tests to demonstrate the presence of the antigen. The tests can be completed minutes to hours after the specimen is obtained. They are 60–90% sensitive, depending upon the prevalence of the disease in the population, and 98–99% specific when compared to culture methods.

**D. SEROLOGIC TESTS**

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

**Treatment**

All *S. pyogenes* are susceptible to penicillin G, and most are susceptible to erythromycin. 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 rheumatic fever patients.

**Epidemiology, Prevention & Control**

**A. EPIDEMIOLOGY**

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 (viridans streptococci, enterococci, etc) are members of the normal flora of the human body. They produce disease only when established in parts of the body.

**B. PREVENTION**

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 some S. pyogenes are 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.

Group B: Streptococcus agalactiae

Morphology & Identification

A. MORPHOLOGY

S. agalactiae is a diplococcal (a pair of cocci, circular, pair) gram-positive, non acid-fast bacterium (~2.0µm) that does not form spores, is not motile, and is catalase-free (catalase is an enzyme that catalyzes the reduction of hydrogen peroxide). It occurs in pairs or short chains and has group B Lancefield antigen present.

B. CULTURE AND GROWTH CHARACTERISTICS

S. agalactiae is a chemoorganotroph that uses glucose as energy source. This bacterium is able to synthesize ATP by oxidative phosphorylation. S. agalactiae is also able to ferment different carbon sources to multiple by-products, lactate, acetate, ethanol, formate or acetoin. 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% CO₂.

C. VIRULENCE FACTORS

Thock peptidoglycan layer in cell wall permits survival on dry surface. Capsule interferes with phagocytosis. Hydrolytic enzymes may facilitate tissue destruction and systemic spread of the bacteria.

Pathogenesis, Pathology & Clinical Findings

Two forms of neonatal disease: early-onset and late-onset; these diseases are characterized by meningitis, pneumonia and bacteremia. Other infections with group B streptococci are endometritis, urinary tract infection, wound infection and bacteremia.

Diagnostic Laboratory Tests

Most strains of S. agalactiae isolated from human sources give a narrow and indistinct zone of beta-hemolysis. Bovine strains are more often nonhemolytic. However, practically all strains, whether hemolytic or not, give a positive CAMP reaction: they produce a diffusible substance that completes the lysis of sheep erythrocytes exposed to a sphingomyelinase C such as staphylococcal b-toxin or the a-toxin of C. perfringens.

Purified CAMP factor is lethal to rabbits when injected intravenously. Furthermore, its role as a virulence factor is supported by its ability to bind immunoglobulins G and M of humans and several animal species via the Fc part. It has also been referred to as protein B.

The hemolysin of S. agalactiae, which has been cloned and sequenced, is not related to the streptolysins of S. pyogenes. It has been considered a virulence factor, but isogenic strains with and
without expression of the hemolysin show no significant difference in virulence in a neonatal rat model.

**Treatment**

Group B streptococci have never been as exquisitely sensitive to penicillin as group A beta-hemolytic streptococci; therefore, the initial therapy for group B streptococcal infection has always been high-dose parenteral penicillin or ampicillin.

**Epidemiology, Prevention & Control**

**A. EPIDEMIOLOGY**

Asymptomatic colonization of the upper respiratory tract and genitourinary tract. Most infections in newborns acquired from mother during pregnancy or at time of birth. Neonates are at higher risk for infection if (1) there is premature rupture of membranes, prolongs labor, preterm birth, or disseminated maternal group B streptococcal disease and (2) mother is without type-specific antibodies and has low complement levels.

Women with genital colonization are at risk for post-partum sepsis. Men and nonpregnant women with diabetes mellitus, cancer, or alcoholism are at increased risk for disease.

**B. PREVENTION**

Intrapartum antibiotic prophylaxis (IAP) is recommended for:

- Women who delivered a previous infant with GBS disease
- Women with GBS bacteriuria in the current pregnancy
- Women with a GBS-positive screening result in the current pregnancy
- Women with unknown GBS status who deliver at less than 37 weeks’ gestation, have an intrapartum temperature of 38°C (100.4°F) or greater, or have rupture of membranes for 18 hours or longer.

Penicillin is the preferred agent for intrapartum antibiotic prophylaxis, and ampicillin is an acceptable alternative.

Simple anti-septic wipes do not prevent mother-to-child transmission. Up to 90% of early-onset GBS infection would be preventable if intravenous antibiotics were offered in labour to all GBS carriers identified by universal sensitive testing late in pregnancy plus to the mothers of babies in the recognised higher risk situations. Early onset GBS infection is most likely to present with breathing problems and pneumonia; late onset GBS infection is more likely to present with meningitis and septicaemia. Once symptoms are present, the condition can be difficult to treat.

**Streptococcus pneumoniae**

**Morphology & Identification**

**A. MORPHOLOGY**

*S. pneumoniae* are Gram-positive bacteria in the shape of a slightly pointed cocci. They are usually found in pairs (diplococci), but are also found singly and in short chains. *S. pneumoniae* are alpha hemolytic (a term describing how the cultured bacteria break down red blood cells for the purpose of classification). Individual bacteria are between 0.5 and 1.25 micrometers in diameter. *S. pneumoniae* do not form spores and are non-motile, though they sometimes have pili used for adherence.

**B. CULTURE AND GROWTH CHARACTERISTICS**

*S. pneumoniae* is a fastidious bacterium, growing best in 5% carbon dioxide. Nearly 20% of fresh clinical isolates require fully anaerobic conditions. In all cases, growth requires a source of catalase (e.g. blood) to neutralize the large amount of hydrogen peroxide produced by the bacteria. In complex media containing blood, at 37°C, the bacterium has a doubling time of 20-30 minutes.

On agar, pneumococci grow as glistening colonies, about 1 mm in diameter. Two serotypes, types 3 and 37, are mucoid. Pneumococci spontaneously undergo a genetically determined, phase variation from opaque to transparent colonies at a rate of 1 in 10^5. The transparent colony type is adapted to colonization of the nasopharynx, whereas the opaque variant is suited for survival in
blood. The chemical basis for the difference in colony appearance is not known, but significant difference in surface protein expression between the two types has been shown.

*S. pneumoniae* is a fermentative aerotolerant anaerobe. It is usually cultured in media that contain blood. On blood agar, colonies characteristically produce a zone of alpha (green) hemolysis, which differentiates *S. pneumoniae* from the group A (beta hemolytic) streptococcus, but not from commensal alpha hemolytic (viridans) streptococci which are co-inhabitants of the upper respiratory tract. Special tests such as inulin fermentation, bile solubility, and optochin (an antibiotic) sensitivity must be routinely employed to differentiate the pneumococcus from *S. viridans*.

*S. pneumoniae* is a very fragile bacterium and contains within itself the enzymatic ability to disrupt and to disintegrate the cells. The enzyme responsible is called an autolysin. The physiological role of this autolysin is to cause the culture to undergo a characteristic autolysis that kills the entire culture when grown to stationary phase. Virtually all clinical isolates of pneumococci harbor this autolysin and undergo lysis usually beginning between 18-24 hours after initiation of growth under optimal conditions. Autolysis is consistent with changes in colony morphology. Colonies initially appear with a plateau-type morphology, then start to collapse in the centers when autolysis begins.

**C. VIRULENCE FACTORS**

The virulence factors of *S. pneumoniae* include a polysaccharide capsule that prevents phagocytosis by the host's immune cells, surface proteins that prevent the activation of complement (part of the immune system that helps clear pathogens from the body), and pili that enable *S. pneumoniae* to attach to epithelial cells in the upper respiratory tract.

The polysaccharide capsule interferes with phagocytosis through its chemical composition, resisting by interfering with binding of complement C3b to the cell's surface. Encapsulated strains of *S. pneumoniae* are found to be 100,000 times more virulent than unencapsulated strains during invasion of mucosal surfaces.

Pili are long, thin extracellular organelles that are able to extend outside of the polysaccharide capsule. They are encoded by the rlrA islet (an area of a genome in which rapid mutation takes place) which is present in only some isolated strains of *S. pneumoniae*. These pili contribute to adherence and virulence, as well as increase the inflammatory response of the host.

**Pathogenesis, Pathology & Clinical Findings**

**A. DISEASES**

*S. pneumoniae* is known to cause bacteremia, otitis media bronchitis, rhinitis, acute sinusitis, conjunctivitis, meningitis, sepsis, osteomyelitis, endocarditis, peritonitis, pericarditis, cellulitis, and brain abscess.

**B. SOURCE OF AGENTS**

*S. pneumoniae* is found in the respiratory tracts of mammals. While it is part of the normal flora of this environment, going unnoticed when present in small densities, it acts as a pathogen toward its host when present in large enough densities.

**C. PATHOGENESIS**

1. **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.

2. **Production of disease:** pneumococci produce disease through their ability to multiply in the tissues. They produce no toxins of significance. 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.

3. **Loss of natural resistance:** since 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:
(a) Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (e.g., allergy), which protect pneumococci from phagocytosis; bronchial obstruction (e.g., atelectasis); and respiratory tract injury due to irritants disturbing its mucociliary function.

(b) Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.

(c) Abnormal circulatory dynamics (e.g., pulmonary congestion, heart failure).

(d) Other mechanisms, e.g., malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency.

D. 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. Serum antibody tests are impractical. 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 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 sputum cultured on blood agar and incubated in CO₂ or a candle jar. A blood culture is also taken.

Immunity. Vaccination

Immunity to infection with pneumococci is type-specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function. It is possible to immunize individuals with type-specific polysaccharides. Such vaccines can probably provide 90% protection against bacteremic pneumonia. This vaccine is appropriate for elderly, debilitated, or immunosuppressed individuals. A pneumococcal conjugate vaccine contains capsular polysaccharides conjugated to diphtheria CRM197 protein. This seven-valent vaccine is recommended for all children aged 2–23 months, to help prevent ear infections, and for selected children aged 24–59 months.

Treatment

Since pneumococci are sensitive to many antimicrobial drugs, early treatment usually results in rapid recovery, and antibody response seems to play a much diminished role. Highdose penicillin G with MICs of 0.1–2 µg/mL appears to be effective in treating pneumonia caused by pneumococci but would not be effective in treatment of meningitis due to the same strains. Some penicillin-resistant strains are resistant to cefotaxime. Resistance to tetracycline and erythromycin occurs also. Pneumococci remain susceptible to vancomycin.
**Epidemiology**

Pneumococcal pneumonia is most common in elderly, debilitated, or immunosuppressed individuals. The disease often sets in after a preceding viral infection damages the respiratory ciliated epithelium; incidence therefore peaks in the winter.

**Viridans streptococci**

The viridans streptococci include *S. mitis*, *S. mutans*, *S. salivarius*, *S. sanguis*, and others. Typically they are α-hemolytic, but they may be nonhemolytic. 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 flora 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, pneumococci, or enterococci 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 a prosthesis can be inserted during antimicrobial therapy.

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 due to members of the normal flora 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 flora, 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 due to 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.
Enterococcus

Scientific classification
Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Enterococcaceae
Genus: Enterococcus
Species: E. faecalis, E. faecium

The enterococci have the group D group-specific substanc and were previously classified as group D streptococci. Because the group D cell wall specific antigen is a teichoic acid, it is not a antigenically good marker enterococci are usually identified by characteristics other than immunologic reaction with group-specific antisera.

Morphology & Identification

A. MORPHOLOGY
Enterococci are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone.

B. CULTURE AND GROWTH CHARACTERISTICS
Enterococci are capable of growing at a range of temperatures from 10-45 degrees Celsius, and can grow in hypotonic, hypertonic, acidic, or alkaline environments. As facultative anaerobes, enterococci can grow under reduced or oxygenated conditions. They are also capable of survival at 60 degrees Celsius for 30 minutes. E. faecalis is able to grow in 6.5% NaCl. Enterococci can also grow in 40% bile salts and over a broad range of pH.
Colonies are usually alpha or gamma hemolytic. Growth on bile-esculin produces a black precipitate derived from esculin; many other bacteria will not grow in the presence of bile. Group D streptococci are divided into those that will grow in 6.5% saline (enterococci) and those that will not (non-enterococci).

C. VIRULENCE FACTORS
1. Colonization factors
   Aggregation substance: hairlike protein embedded in cytoplasmic membrane that facilitates lasid exchange and binding to epithelial cells.
   Carbohydrate adhesions: present in individual bacterium in multiple types; mediate binding to host cells.
2. Secreted factors
   Cytolysin: protein bacteriocin that inhibits of gram-positive bacteria.
   Pheromone: chemotactant for neutrophils that may regulate inflammatory reaction.
   Gelatinase: hydrolyzes gelatin, collagen, hemoglobin.

Diseases caused by Enterococcus
They are a significant cause of urinary tract infections (but much less common than E. coli) and also of opportunistic infections (including intra-abdominal, septicemia and endocarditis).

Diagnostic Laboratory Tests
Enterococci can be isolated using any blood agar base containing 5% They can be isolated from Gram-negative bacteria in a sample using bile-esculin azide, phenylethyl alcohol agar, Columbia colistin-nalidixic acid agar, or other media containing azide. Standard laboratory growing conditions for enterococci is a brain heart infusion, or Todd-Hewitt, broth or agar, supplemented with antibiotics when appropriate, at 35-37 degrees Celsius without aeration.
Esculin hydrolysis.
BEA media.

Treatment
E. faecalis is resistant to many commonly used antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semisynthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole). Resistance to vancomycin in E. faecalis is becoming more
Treatment options for vancomycin-resistant *E. faecalis* include nitrofurantoin (in the case of uncomplicated UTIs), linezolid, and daptomycin, although ampicillin is preferred if the bacteria are susceptible. Quinupristin/dalfopristin can be used to treat *E. faecium* but not *E. faecalis*.

**Epidemiology**

They normally inhabit the bowels of animals, humans included, but they are found in soil, vegetation, and surface water, probably due to contamination by animal excrement.

The enterococci are among the most frequent causes of nosocomial 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.
The Neisseriae

Scientific classification
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Beta Proteobacteria
Order: Neisseriales
Family: Neisseriaceae
Genus: Neisseria
Species: N. gonorrhoeae, N. meningitidis

Morphology & Identification
A. MORPHOLOGY
The typical neisseria is a gram-negative, nonmotile diplococcus, approximately 0.8 μm in diameter. Individual cocci are kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.

B. CULTURE AND GROWTH CHARACTERISTICS
In 48 hours on enriched media (eg, Mueller-Hinton, modified Thayer-Martin), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1–5 mm in diameter. Colonies are transparent or opaque, nonpigmented, and nonhemolytic. Neisseria flavescens, Neisseria subflava, and Neisseria lactamica have yellow pigmentation. Neisseria sicca produces opaque, brittle, wrinkled colonies. M. catarrhalis produces nonpigmented or pinkish-gray opaque colonies.

The neisseriae grow best under aerobic conditions, but some will grow in an anaerobic environment. They have complex growth requirements. Most neisseriae ferment carbohydrates, producing acid but not gas, and their carbohydrate fermentation patterns are a means of distinguishing them. The neisseriae produce oxidase and give positive oxidase reactions; the oxidase test is a key test for identifying them. When bacteria are spotted on a filter paper soaked with tetramethylparaphenylenediamine hydrochloride (oxidase), the neisseriae rapidly turn dark purple.

Meningococci and gonococci grow best on media containing complex organic substances such as heated blood, hemin, and animal proteins and in an atmosphere containing 5% CO₂ (eg, candle jar). Growth is inhibited by some toxic constituents of the medium, eg, fatty acids or salts. The organisms are rapidly killed by drying, sunlight, moist heat, and many disinfectants. They produce autolytic enzymes that result in rapid swelling and lysis in vitro at 25 °C and at an alkaline pH.

Neisseria gonorrhoeae

Gonococci ferment only glucose and differ antigenically from the other neisseriae. Gonococci usually produce smaller colonies than those of the other neisseriae. Gonococci that require arginine, hypoxanthine, and uracil (Arg−, Hyx−, Ura− auxotype) tend to grow most slowly on primary culture. Gonococci isolated from clinical specimens or maintained by selective subculture have typical small colonies containing piliated bacteria. On nonselective subculture, larger colonies containing nonpiliated gonococci are also formed. Opaque and transparent variants of both the small and large colony types also occur; the opaque colonies are associated with the presence of a surface-exposed protein, Opa.

Virulence factors
N. gonorrhoeae is antigenically heterogeneous and capable changing its surface structures in vitro—and presumably in vivo—to avoid host defenses. Surface structures include the following.
1. Pili (fibriae)
Pili are the hair-like appendages that extend up to several micrometers from the gonococcal surface. They enhance attachment to host cells and resistance to phagocytosis. They are made up of stacked pilin proteins (MW 17,000–21,000). The amino terminal of the pilin molecule, which contains a high percentage of hydrophobic amino acids, is conserved. The amino acid sequence near
the mid portion of the molecule also is conserved; this portion of the molecule serves in attachment to host cells and is less prominent in the immune response. The amino acid sequence near the carboxyl terminal is highly variable; this portion of the molecule is most prominent in the immune response. The pilins of almost all strains of *N. gonorrhoeae* are antigenically different, and a single strain can make many antigenically distinct forms of pilin.

2. Por

Por protein extends through the gonococcal cell membrane. It occurs in trimers to form pores in the surface through which some nutrients enter the cell. Por proteins may impact intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion. The molecular weight of Por varies from 34,000 to 37,000. Each strain of gonococcus expresses only one of two types of Por, but the Por of different strains is antigenically different. Serologic typing of Por by agglutination reactions with monoclonal antibodies has distinguished 18 serovars of PorA and 28 serovars of PorB.

3. Opa proteins

These proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cells, especially cells that express carcinoembryonic antigens (CD66). One portion of the Opa molecule is in the gonococcal outer membrane, and the rest is exposed on the surface. The molecular weight of Opa ranges from 24,000 to 28,000. A strain of gonococcus can express no, one, two, or occasionally three types of Opa, though each strain has eleven or twelve genes for different Opas.

4. Rmp (protein III)

This protein (MW about 33,000) is antigenically conserved in all gonococci. It is a reduction-modifiable protein (Rmp) and changes its apparent molecular weight when in a reduced state. It associates with Por in the formation of pores in the cell surface.

5. Lipooligosaccharide (los)

In contrast to the enteric gram-negative rods, gonococcal LPS does not have long O-antigen side chains and is called a lipooligosaccharide. Its molecular weight is 3000–7000. Gonococci can express more than one antigenically different LOS chain simultaneously. Toxicity in gonococcal infections is largely due to the endotoxic effects of LOS. In a form of molecular mimicry, gonococci make LOS molecules that structurally resemble human cell membrane glycosphingolipids. The gonococcal LOS and the human glycosphingolipid of the same structural class react with the same monoclonal antibody, indicating the molecular mimicry. The presence on the gonococcal surface of the same surface structures as human cells helps gonococci evade immune recognition.

6. Other proteins

Several antigenically constant proteins of gonococci have poorly defined roles in pathogenesis. Lip (H8) is a surface- exposed protein that is heat-modifiable like Opa. The Fbp (iron-binding protein), similar in molecular weight to Por, is expressed when the available iron supply is limited, eg, in human infection. Gonococci elaborate an IgA1 protease that splits and inactivates IgA1, a major mucosal immunoglobulin of humans. Meningococci, *Haemophilus influenzae*, and *S. pneumoniae* elaborate similar IgA1 proteases.

**Pathogenesis, Pathology & Clinical Findings**

Gonococci exhibit several morphologic types of colonies, but only piliated bacteria appear to be virulent. Opa protein expression varies depending on the type of infection. Gonococci that form opaque colonies are isolated from men with symptomatic urethritis and from uterine cervical cultures at mid cycle. Gonococci that form transparent colonies are frequently isolated from men with asymptomatic urethral infection, from menstruating women, and from invasive forms of gonorrhea, including salpingitis and disseminated infection. Antigenic variation of surface proteins during infection allows the organism to circumvent host immune response.

Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis. In males, there is usually urethritis, with yellow, creamy pus and painful urination. The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be
asymptomatic. In females, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis. Chronic gonococcal cervicitis or proctitis is often asymptomatic.

Gonococcal bacteremia leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis. Gonococcal endocarditis is an uncommon but severe infection. Gonococci sometimes cause meningitis and eye infections in adults; these have manifestations similar to those due to meningococci. Complement deficiency is frequently found in patients with gonococcal bacteremia. Patients with bacteremia, especially if recurrent, should be tested for total hemolytic complement activity.

Gonococcal ophthalmia neonatorum, an infection of the eye of the newborn, is acquired during passage through an infected birth canal. The initial conjunctivitis rapidly progresses and, if untreated, results in blindness. Gonococci that produce localized infection are often serum-sensitive (killed by antibody and complement).

### Diagnostic laboratory tests

**A. SPECIMENS**

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. Blood culture is necessary in systemic illness, but a special culture system is helpful, since gonococci (and meningococci) may be susceptible to the polyanethol sulfonate present in standard blood culture media.

**B. SMEARS**

Gram-stained smears of urethral or endocervical exudate reveal many diplococci within pus cells. These give a presumptive diagnosis. Stained smears of the urethral exudate from men have a sensitivity of about 90% and a specificity of 99%. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Cultures of urethral exudate from men are not necessary when the stain is positive, but cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

**C. CULTURE**

Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium) and incubated in an atmosphere containing 5% CO₂ (candle extinction jar) at 37 °C. To avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs (eg, vancomycin, 3 μg/mL; colistin, 7.5 μg/mL; amphotericin B, 1 μg/mL; and trimethoprim, 3 μg/mL). If immediate incubation is not possible, the specimen should be placed in a CO₂-containing transport- culture system. Forty-eight hours after culture, the organisms can be quickly identified by their appearance on a Gram-stained smear, by oxidase positivity, and by coagglutination, immunofluorescence staining, or other laboratory tests. The species of subcultured bacteria may be determined by fermentation reactions. The gonococcal isolates from anatomic sites other than the genital tract or from children should be identified as to species using two different confirmatory tests because of the legal and social implications of the isolates.

**D. NUCLEIC ACID AMPLIFICATION TESTS**

Several Food and Drug Administration-cleared nucleic acid amplification assays are available for direct detection of *N. gonorrhoeae* in genitourinary specimens. In general, these assays have excellent sensitivity and specificity in symptomatic, high-prevalence populations. Advantages include better detection, more rapid results, and the ability to use urine as a specimen source. Disadvantages include poor specificity of some assays due to cross reactivity with nongonococcal *Neisseria* species. These assays are not recommended for use for the diagnosis of extragenital gonococcal infections or for infection in children.

**E. SEROLOGY**

Serum and genital fluid contain IgG and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci in vitro. In infected individuals, antibodies to gonococcal pili and outer membrane proteins can be detected by
immunoblotting, radioimmunoassay, and ELISA (enzyme-linked immunosorbent assay) tests. However, these tests are not useful as diagnostic aids for several reasons: gonococcal antigenic heterogeneity; the delay in development of antibodies in acute infection; and a high background level of antibodies in the sexually active population.

**Immunity**
Repeated gonococcal infections are common. Protective immunity to reinfection does not appear to develop as part of the disease process, because of the antigenic variety of gonococci. While antibodies can be demonstrated, including the IgA and IgG on mucosal surfaces, they either are highly strain-specific or have little protective ability.

**Treatment**
Since the development and widespread use of penicillin, gonococcal resistance to penicillin has gradually risen, owing to the selection of chromosomal mutants, so that many strains now require high concentrations of penicillin G for inhibition (MIC ≥ 2 μg/mL). Penicillinase-producing *N. gonorrhoeae* (PPNG) also have increased in prevalence (see above). Chromosomally mediated resistance to tetracycline (MIC ≥ 2 μg/mL) is common. High-level resistance to tetracycline (MIC ≥ 32 μg/mL) also occurs. Spectinomycin resistance as well as resistance to fluoroquinolones has been noted. Additional therapy with doxycycline, orally twice a day for 7 days, is recommended for the possible concomitant chlamydial infection; erythromycin base, orally four times a day for 7 days, is substituted for doxycycline in pregnant women. Modifications of these therapies are recommended for other types of *N. gonorrhoeae* infection. Since other sexually transmitted diseases may have been acquired at the same time as gonorrhea, steps must also be taken to diagnose and treat these diseases (see discussions of chlamydiae, syphilis, etc).

**Epidemiology, Prevention & Control**
Gonorrhea is worldwide in distribution. Gonorrhea is exclusively transmitted by sexual contact, often by women and men with asymptomatic infections. The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20–30% for men and even greater for women. The infection rate can be reduced by avoiding multiple sexual partners, rapidly eradicating gonococci from infected individuals by means of early diagnosis and treatment, and finding cases and contacts through education and screening of populations at high risk. Mechanical prophylaxis (condoms) provides partial protection. Chemoprophylaxis is of limited value because of the rise in antibiotic resistance of the gonococcus.

PPNG first appeared in 1976. These totally penicillin-resistant gonococcal strains have appeared in many parts of the world. Areas with a high incidence of PPNG include Singapore, parts of sub-Saharan Africa, and focal areas in the United States. Focal outbreaks of disease due to PPNG have occurred in many areas of the United States and elsewhere, and endemic foci are being established.

Gonococcal ophthalmia neonatorum is prevented by local application of 0.5% erythromycin ophthalmic ointment or 1% tetracycline ointment to the conjunctiva of newborns. Although instillation of silver nitrate solution is also effective and is the classic method for preventing ophthalmia neonatorum, silver nitrate is difficult to store and causes conjunctival irritation; its use has largely been replaced by use of erythromycin or tetracycline ointment.

**Neisseria meningitidis**
The bacterium *N. meningitidis*, the meningococcus, is identical in its staining and morphological characteristics to *N. gonorrhoeae*. However, at the ultrastructural level, *N. meningitidis* has a prominent antiphagocytic polysaccharide capsule. *N. meningitidis* strains are grouped on the basis of their capsular polysaccharides, into 12 serogroups, some of which are subdivided according to the presence of outer membrane protein and lipopolysaccharide antigens.

**Virulence factors**
**Antigenic Structure**
At least 13 serogroups of meningococci have been identified by immunologic specificity of capsular polysaccharides. The most important serogroups associated with disease in humans are A, B, C, Y, and W-135. The group A polysaccharide is a polymer of N-acetylmannosamine phosphate,
and that of group C is a polymer of N-acetyl-O-acetyleneuraminic acid. Meningococcal antigens are found in blood and cerebrospinal fluid of patients with active disease. Outbreaks and sporadic cases in the Western Hemisphere in the last decade have been caused mainly by groups B, C, W-135, and Y; outbreaks in southern Finland and São Paulo, Brazil, were due to groups A and C; those in Africa were due mainly to group A. Group C and, especially, group A are associated with epidemic disease.

The outer membrane proteins of meningococci have been divided into classes on the basis of molecular weight. All strains have either class 1, class 2, or class 3 proteins; these are analogous to the Por proteins of gonococci and are responsible for the serotype specificity of meningococci. They help form pores in the meningococcal cell wall. As many as 20 serotypes have been defined; serotypes 2 and 15 have been associated with epidemic disease. The Opa (class 5) protein is comparable to Opa of the gonococci. Meningococci are piliated, but unlike gonococci, they do not form distinctive colony types indicating piliated bacteria. Meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease.

**Pathogenesis, Pathology & Clinical Findings**

Humans are the only natural hosts for whom meningococci are pathogenic. The nasopharynx is the portal of entry. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia; the symptoms may be like those of an upper respiratory tract infection. Fulminant meningococcemia is more severe, with high fever and hemorrhagic rash; there may be disseminated intravascular coagulation and circulatory collapse (Waterhouse-Friderichsen syndrome).

Meningitis is the most common complication of meningococcemia. It usually begins suddenly, with intense headache, vomiting, and stiff neck, and progresses to coma within a few hours.

During meningococcemia, there is thrombosis of many small blood vessels in many organs, with perivascular infiltration and petechial hemorrhages. There may be interstitial myocarditis, arthritis, and skin lesions. In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate.

It is not known what transforms an asymptomatic infection of the nasopharynx into meningococcemia and meningitis, but this can be prevented by specific bactericidal serum antibodies against the infecting serotype. *Neisseria bacteremia* is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency (C5, C6, C7, or C8). Meningococci are readily phagocytosed in the presence of a specific opsonin.

**Diagnostic laboratory tests**

**A. SPECIMENS**

Specimens of blood are taken for culture, and specimens of spinal fluid are taken for smear, culture, and chemical determinations. Nasopharyngeal swab cultures are suitable for carrier surveys. Puncture material from petechiae may be taken for smear and culture.

**B. SMEARS**

Gram-stained smears of the sediment of centrifuged spinal fluid or of petechial aspirate often show typical neisseriae within polymorphonuclear leukocytes or extracellularly.

**C. CULTURE**

Culture media without sodium polyanethol sulfonate are helpful in culturing blood specimens. Cerebrospinal fluid specimens are plated on “chocolate” agar and incubated at 37 °C in an atmosphere of 5% CO₂ (candle jar). Freshly drawn spinal fluid can be directly incubated at 37 °C if agar culture media are not immediately available. A modified Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin) favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures. Presumptive colonies of neisseriae on solid media, particularly in mixed culture, can be identified by Gram stain and the oxidase test. Spinal fluid and blood generally yield pure cultures that can be further identified by carbohydrate fermentation reactions and agglutination with type-specific or polyvalent serum.
D. SEROLOGY

Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity. These tests are done only in reference laboratories.

Immunity

Immunity to meningococcal infection is associated with the presence of specific, complement-dependent, bactericidal antibodies in the serum. These antibodies develop after subclinical infections with different strains or injection of antigens and are group-specific, type-specific, or both. The immunizing antigens for groups A, C, Y, and W-135 are the capsular polysaccharides. For group B, a specific antigen suitable for use as a vaccine has not been defined; however, group B vaccines with mixtures of antigens have been used in many parts of the world. Currently there are two vaccine types against serogroups A, C, Y, and W-135. A polysaccharide tetravalent vaccine in which each dose consists of four purified bacterial capsular polysaccharides is poorly immunogenic in children under 18 months, does not confer long-lasting immunity, and does not cause a sustainable reduction in nasopharyngeal carriage. A newly approved (2005) tetravalent conjugate vaccine (Encarta, Sanofi Pasteur, Inc.) is licensed for use in persons 11–55 years of age. It contains capsular polysaccharide conjugated to diphtheria toxoid. The advantage of this vaccine is that a T-cell–dependent response to vaccine is induced. This enhances primary response among infants and substantially reduces asymptomatic carriage.

Treatment

Penicillin G is the drug of choice for treating meningococcal disease. Either chloramphenicol or a third-generation cephalosporin such as cefotaxime or ceftriaxone is used in persons allergic to penicillins.

Epidemiology, Prevention & Control

Meningococcal meningitis occurs in epidemic waves (eg, in military encampments, in religious pilgrims, and in sub-Saharan Africa; in Brazil, there were more than 15,000 cases in 1974) and a smaller number of sporadic interepidemic cases. Five to 30% of the normal population may harbor meningococci (often nontypeable isolates) in the nasopharynx during interepidemic periods. During epidemics, the carrier rate goes up to 70–80%. A rise in the number of cases is preceded by an increased number of respiratory carriers. Treatment with oral penicillin does not eradicate the carrier state. Rifampin, 600 mg orally twice daily for 2 days (or minocycline, 100 mg every 12 hours), can often eradicate the carrier state and serve as chemoprophylaxis for household and other close contacts. Since the appearance of many sulfonamide-resistant meningococci, chemoprophylaxis with sulfonamides is no longer reliable.

Clinical cases of meningitis present only a negligible source of infection, and isolation therefore has only limited usefulness. More important is the reduction of personal contacts in a population with a high carrier rate. This is accomplished by avoidance of crowding. Specific polysaccharides of groups A, C, Y, and W-135 can stimulate antibody response and protect susceptible persons against infection. Such vaccines are currently used in selected populations (eg, the military and in civilian epidemics).