**Microbiology**

**Medical bacteriology**

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**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 genus *Staphylococcus* has at least **45 species**. The four most frequently encountered species of clinical importance are *Staphylococcus aureus*, *S. epidermidis*, *S. lugdunensis*, and *S. saprophyticus*.
* *S aureus* is **coagulase positive**, which differentiates it from the other species. *S aureus* is a major pathogen for humans. The coagulase-negative staphylococci (**CoNS**) are normal human microbiota and sometimes cause infection, often associated with implanted devices, such as 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*; *S saprophyticus* is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients.

**Morphology and Identification**

**A. Typical Organisms**

Staphylococci are spherical cells about 1μm in diameter arranged in irregular clusters. 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.

**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. *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.

**C. Growth Characteristics**

1- The staphylococci produce catalase, which differentiates them from the streptococci.

2- Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas.

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

4- 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).

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*). The *mecA* and newly described *mecC* genes on this locus encode a low-affinity penicillin-binding protein (PBP2a) that is responsible for the resistance.

3. In the United States, *S aureus* and *S lugdunensis* are considered to be susceptible to vancomycin if (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. 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.

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 and the nafcillin resistance gene *mecA*. 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. Patients with endocarditis caused by a tolerant *S aureus* may have a 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.

**Antigenic Structure**

*S aureus* has amazing adaptive capacity. *S aureus* has acquired many mobile genetic elements (insertion sequences, transposons) that determine both pathogenicity and antimicrobial resistance. Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure.

**Cell wall virulence factors:**

Protein A is a major component of the S. aureus cell wall. It binds to the Fc moiety of IgG, exerting an antiopsonin (and therefore strongly antiphagocytic) effect.

Fibronectin-binding protein (FnBP) and other staphylococcal surface proteins promote binding to mucosal cells and tissue matrices.

* \*Peptidoglycan,

\* Teichoic acids,

 \*Most *S aureus* strains of clinical importance have polysaccharide capsules.

**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.

**A. Catalase**

Staphylococci produce catalase, which converts H2O2 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.

**Clumping factor** is cell wall bound and 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.

**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. 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 RBC. The δ-toxin is heterogeneous, 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.

**E. Panton–Valentine Leukocidin**

PVL is encoded on a mobile phage. It can kill white blood cells of humans and rabbits.

**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. Exfoliative toxin B is plasmid mediated and heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome (SSSS) by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are **superantigens.**

**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). TSST-1 is the prototypical **superantigen**. The gene for TSST-1 is found in about 20% of *S aureus* isolates, including MRSA.

\*Superantigen exotoxins: These toxins have an affinity for the T cell receptor MHC Class II antigen complex. They stimulate enhanced T lymphocyte response (as many as twenty percent of T cells respond, compared with 0.01 percent responding to the usual processed antigens). This difference is a result of their ability to recognize a relatively conserved region of the T cell receptor. This major T cell activation can cause toxic shock syndrome, primarily by release into the circulation of inordinately large amounts of T cell cytokines, such as interleukin-2 (IL-2), interferon, and tumor necrosis factor.

**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.

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

**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.
* 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).
* 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**

1. Localized skin infections: The most common S. aureus infections are small, superficial abscesses involving hair follicles (folliculitis) or sweat or sebaceous glands. Subcutaneous abscesses called furuncles (boils) often form around foreign bodies, such as splinters. These generally respond to local therapy, that is, removal of the foreign body, soaking, and drainage as indicated. Carbuncles are larger, deeper, multiloculated skin infections that can lead to bacteremia and require antibiotic therapy and debridement. Impetigo is usually a localized, superficial, spreading crusty skin lesion generally seen in children. It can be caused by S. aureus, although more commonly by Streptococcus pyogenes (see p. 80), or both organisms together.

*\*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).

2.Deep, localized infections: These may be metastatic from superficial infections or skin carriage, or may result from trauma. *S. aureus* is the most common cause of acute and chronic infection of bone marrow. *S. aureus* is also the most common cause of acute infection of joint space in children (septic joint).

3. Acute endocarditis, generally associated with intravenous drug abuse, is caused by injection of contaminated preparations or by needles contaminated with *S. aureus*. *S. aureus* also colonizes the skin around the injection site, and if the skin is not sterilized before injection, the bacteria can be introduced into soft tissues and the bloodstream, even when a sterilized needle is used.

4- Septicemia is a generalized infection with sepsis or bacteremia.

5- Pneumonia: *S. aureus* is a cause of severe, necrotizing pneumonia

6-Nosocomial infections: *S. aureus* is one of the most common causes of hospital-acquired infections, often of wounds (surgical) or bacteremia associated with catheters. Progression to septicemia is often a terminal event.

7-Toxinoses are diseases caused by the action of a toxin, frequently when the organism that secreted the toxin is undetectable. Toxinoses caused by *S. aureus* include:

\*Toxic shock syndrome, which results in high fever, rash (resembling a sunburn, with diffuse erythema followed by desquamation), vomiting, diarrhea, hypotension, and multi-organ involvement (especially renal, and/or hepatic damage).

 \*Staphylococcal gastroenteritis is caused by ingestion of food contaminated with enterotoxin-producing *S. aureus*. Often contaminated by a food-handler, these foods tend to be protein-rich (for example, egg salad, cream pastry) and improperly refrigerated. Symptoms, such as nausea, vomiting, and diarrhea, are acute following a short incubation period (1–8 hours). There is no fever.

\*Scalded skin syndrome involves the appearance of superficial bullae resulting from the action of an exfoliative toxin that attacks the intercellular adhesive of the stratum granulosum, causing marked epithelial desquamation. The bullae may be infected or may result from toxin produced by organisms infecting a different site.

**Diagnostic Laboratory Tests**

**A. Specimens**

Surface swab pus or aspirate from an abscess, blood, endonasotracheal aspirate, sputum, or spinal fluid for culture, depending on the localization of the process. The anterior nares are frequently swabbed to determine nasal colonization.

**B. Smears**

Typical staphylococci appear as gram + cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish non-pathogenic (*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 no 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*.

**D. Catalase Test**

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% H2O2 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.

**G. Serologic and Typing Tests**

Serologic tests for diagnosis of *S aureus* infections have little practical value. Molecular typing techniques have been used to document the spread of epidemic disease-producing clones of *S aureus*.

**Treatment**

* The multiple skin infections (acne, furunculosis) occur most often in adolescents. Tetracyclines are used for long-term treatment.
* Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy.
* 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.
* Alternative agents for the treatment of MRSA bacteremia and endocarditis include newer antimicrobials such as daptomycin, linezolid, and quinupristin–dalfopristin.
* *S epidermidis* is more often resistant to antimicrobial drugs than is *S aureus*; approximately 75% of *S epidermidis* strains are nafcillin resistant. dalbavancin, a longacting intravenous lipoglycopeptide; tedizolid phosphate, an intravenous and oral oxazolidinone, similar to linezolid; and oritavancin, a semisynthetic glycopeptide.