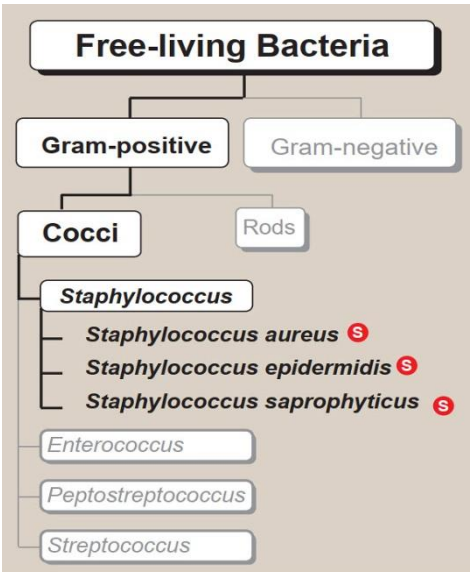
The Staphylococci

Staphylococci and **streptococci** constitute the main groups of medically important gram-positive cocci. Staphylococcal infections range from the trivial to the rapidly fatal. They can be very difficult to treat, especially those contracted in hospitals, because of the remarkable ability of staphylococci to become resistant to antibiotics. Staphylococci are ubiquitous in nature, with about a dozen species occurring as part of human flora. The most virulent of the genus, ***Staphylococcus aureus***, is one of the most common causes of bacterial infections, and is also an important cause of food poisoning and toxic shock syndrome. Among less virulent staphylococcal species, ***Staphylococcus epidermidis*** is an important cause of prosthetic implant infections, whereas ***Staphylococcus saprophyticus*** causes urinary tract infections, especially cystitis in women. Figure summarizes the staphylococci described in this chapter.



General Features

Staphylococci generally stain darkly gram positive. They are round rather than oval and tend to occur in bunches like grapes. Because growth of staphylococci requires supplementation with various amino acids and other growth factors, they are routinely cultured on enriched media containing nutrient broth and/or blood. Staphylococci are facultatively anaerobic organisms.

They produce catalase, which is one feature that distinguishes them from the **streptococci**[The catalase test can be used to differentiate between ***Staphylococcus* spp**. and ***Streptococcus* spp.** It is important for you to remember that virtually all ***Staphylococcus* spp.** are catalase positive (i.e., they produce catalase), whereas all ***Streptococcus* spp.** are catalase negative (i.e., they do not produce catalase)].

The most virulent species of ***staphylococcus*** is ***S. aureus***, almost all isolates of which secrete coagulase, an enzyme that causes citrated plasma to clot. Other species that occasionally cause disease and lack coagulase are often referred to as **coagulasenegative staphylococci**. Staphylococci are hardy, being resistant to heat and drying, and thus can persist for long periods on fomites (inanimate objects), which can then serve as sources of infection.

♦.Classification of Staphylococci

A- Based on pigment production

1- *Staphylococcus aureus* :- Golden-yellow pigmented colonies

2- *Staphylococcus albus* :- White colonies

3- *Staphylococcus citrus* :- Lemon yellow colonies

B-Based on coagulase production

Coagulase is an enzyme that causes the formation of clots. Specifically, coagulase catalyzes the conversion of a plasma protein called fibrinogen into a sticky substance called fibrin. ***Staphylococcus aureus*** is **coagulase positive**, which differentiates it from the other species. ***S aureus*** is a major pathogen for humans. Almost every person will have some type of ***S aureus*** infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections.

The **coagulase-negative staphylococci (CoNS)** are normal human microbiota and sometimes cause infection, often associated with implanted devices, such as joint prostheses, shunts, and intravascular catheters, especially in very young, old, and immunocompromised patients.

Approximately 75% of these infections caused by coagulase-negative staphylococci are caused by ***S epidermidis***; infections caused by ***S lugdunensis***, ***Staphylococcus warneri***, ***Staphylococcus hominis***, and other species are less common. ***S saprophyticus*** is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients. Other species are important in veterinary medicine.

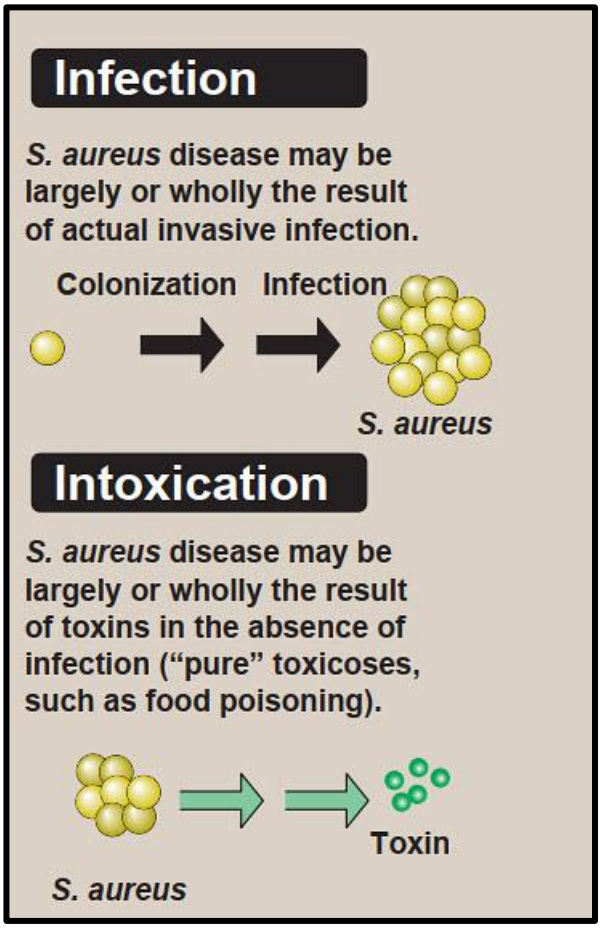
I. *Staphylococcus aureus*

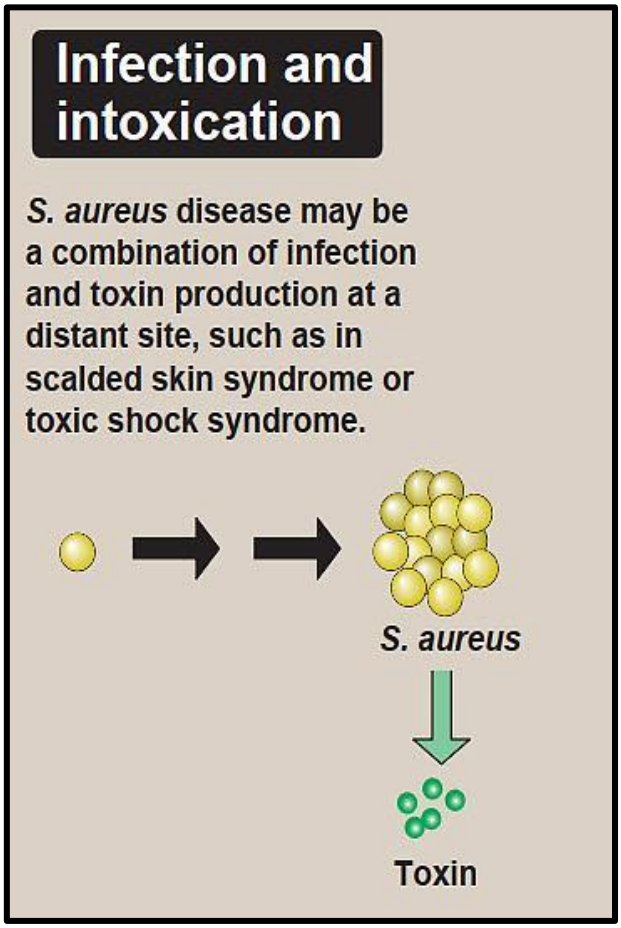
Generally, significant host compromise is required for *S. aureus* infection, such as a break in the skin or insertion of a foreign body (for example, wounds, surgical infections, or central venous catheters), an obstructed hair follicle (folliculitis), or a compromised immune system. *S. aureus* disease may be:

1. largely or wholly the result of actual invasive infection, overcoming host defense mechanisms, and the production of extracellular substances which facilitate invasion;

2. a result of toxins in the absence of invasive infection ("pure" toxinoses); or

3. a combination of invasive infection and intoxication.





A- Epidemiology

S. aureus is frequently carried by healthy individuals on the skin and mucous membranes. Carriers serve as a source of infection to themselves and others; for example, by direct contact, by contamination of fomites (objects such as a doorknob, which in turn can be a source of infection) or contamination of food, which can then result in food poisoning.

B. Pathogenesis

Virulence factors are the genetic, biochemical, or structural features that enable an organism to produce disease. The clinical outcome of an infection depends on the virulence of the pathogen and the opposing effectiveness of the host defense mechanisms. *S. aureus* expresses many potential virulence factors (Figure). For the majority of diseases caused by *S. aureus*, pathogenesis depends on the combined actions of several virulence factors, so it is difficult to determine precisely the role of any given factor.

Cellwall associated structures	Extracellular toxins	Enzymes
• Peptidoglycan • Capsule • Protein A • Clumping factor (bound coagulase)	• Haemolysin • Panton-Valentine leukocidin • Enterotoxin • TSST • Exfoliatin toxin	• Coagulase • Catalase • Staphylokinase • DNAase • β-lactamase • lipase • Phospholipase • hyaluronidase • proteinase

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

S. aureus produces an extracellular coagulase, an enzymelike 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.

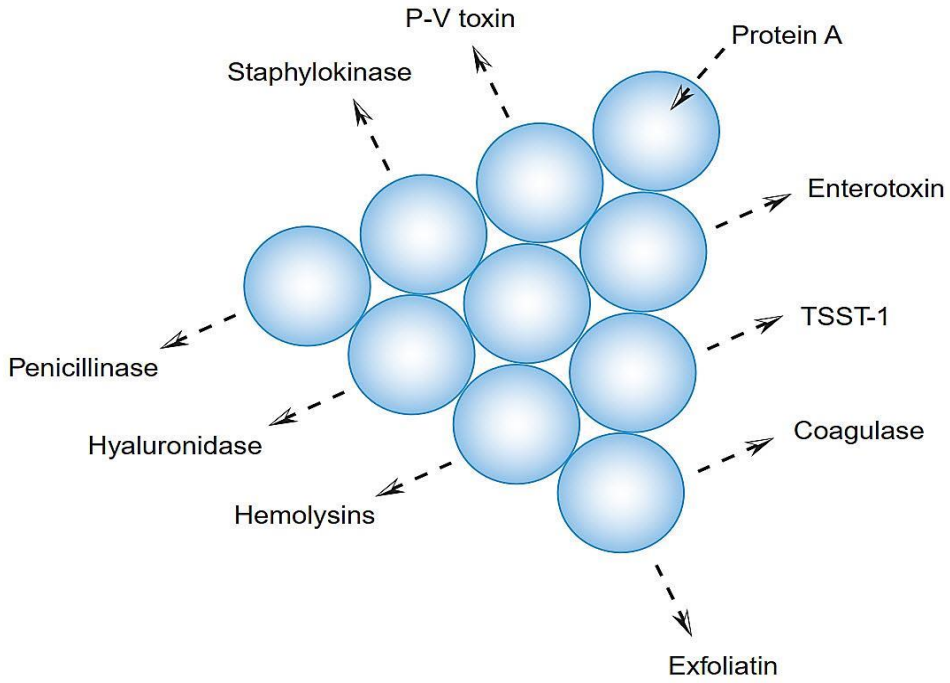


Figure. Virulence factors **of *Staphylococcus aureus*.** P-V, Panton-Valentine; TSST-1, toxic shock syndrome toxin 1.

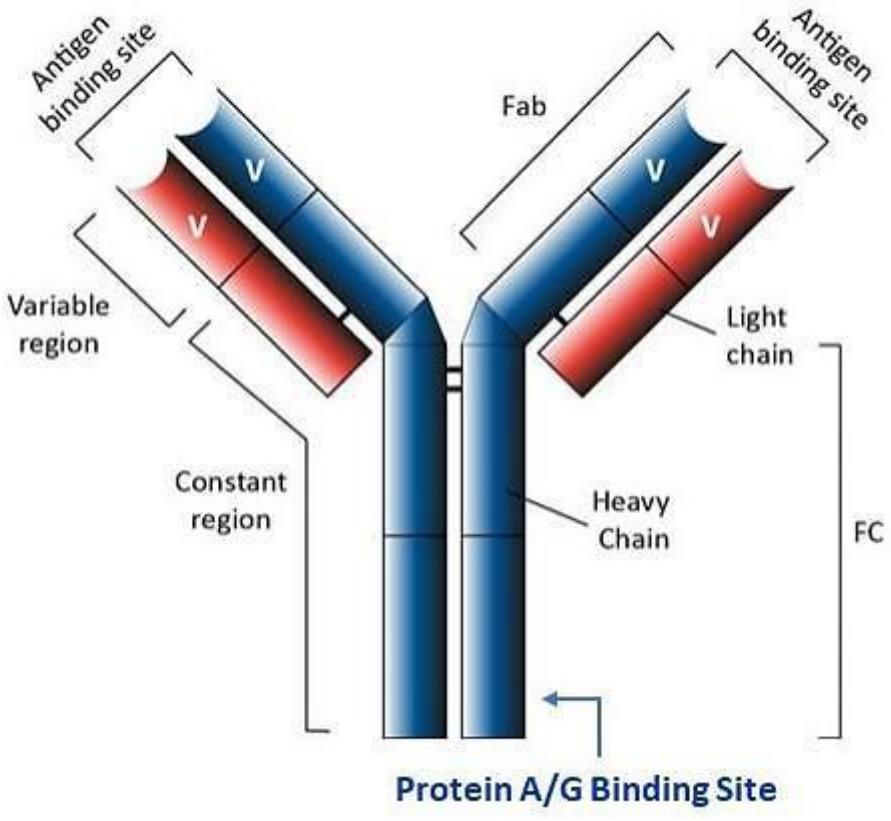
3. Cell wall virulence factors:

a. Capsule

The capsule layer is very thin but has been associated with increased resistance to phagocytosis. Clinical isolates produce capsule but expression is rapidly lost upon in vitro cultivation.

b. Protein A

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



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

d. Clumping factor A: is a virulence factor from *S. aureus* that binds to fibrinogen but does not convert fibrinogen to fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase. Because clumping factor induces a strong immunogenic response in the host, it has been the focus of vaccine efforts. However, no human vaccines against this factor are available to date.

4. Hemolysins (Cytolytic exotoxins): *S. aureus* possesses four hemolysins that are regulated by ***agr***. α, β, γ, and δ Toxins attack mammalian cell (including red blood cell) membranes, and are often referred to as hemolysins. α Toxin is the best

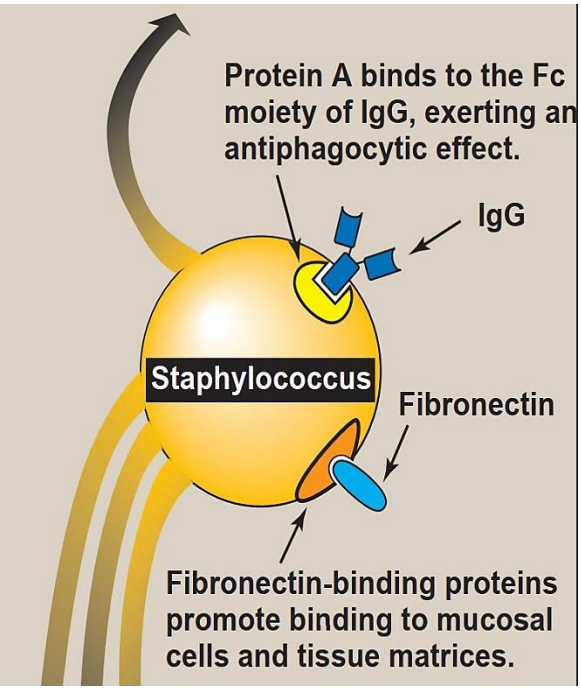
studied, and is chromosomally encoded. It polymerizes into tubes that pierce membranes, resulting in the loss of important molecules and, eventually, in osmotic lysis.

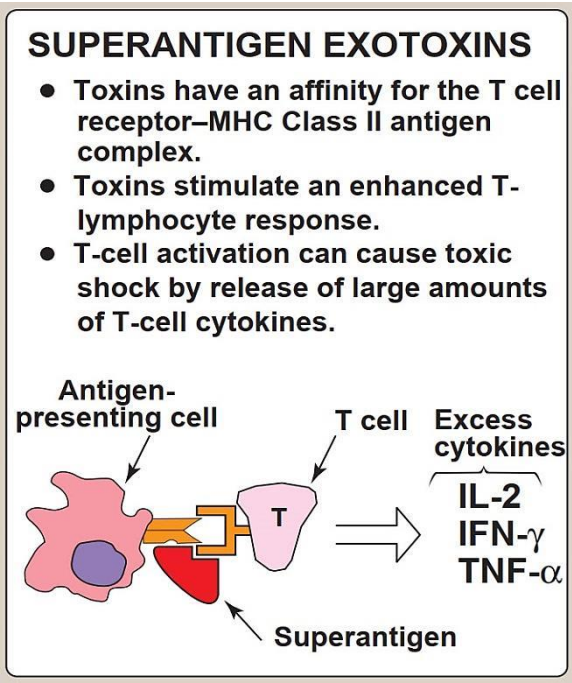
5. Other Enzymes

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**.

6. Panton-Valentine leukocidin: This pore-forming toxin lyses PMNs. Production of this toxin makes strains more virulent. This toxin is produced predominantly by community-acquired **methicillin-resistant *S. aureus*** (MRSA) strains.

7. Superantigen exotoxins: These toxins have an affinity for the T- cell receptor-major histocompatibility complex Class II antigen complex. They stimulate enhanced T-lymphocyte response (as many as 20 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- γ (IFN- γ), and tumor necrosis factor-a (TNF- α).





a. Enterotoxins: Enterotoxins (six major antigenic types: A, B, C, D, E, and G) are produced by approximately half of all ***S. aureus*** isolates. When these bacteria contaminate food and are allowed to grow, they secrete enterotoxin, ingestion of which can cause food poisoning. [Note: The toxin stimulates the vomiting center in the brain by binding to neural receptors in the upper gastrointestinal (GI) tract.] Enterotoxins are superantigens that are even more heat-stable than ***S. aureus***. Therefore, organisms are not always recovered from incriminated food but the toxin may be recovered.

b. Toxic shock syndrome toxin (TSST-1): This is the classic cause of toxic shock syndrome (TSS). Because of similarities in molecular structure, it is sometimes referred to as staphylococcal enterotoxin F, although it does not cause food poisoning when ingested.

c. Exfoliatin (exfoliative toxin, ET) is also a superantigen. It causes scalded skin syndrome in children. The toxin cleaves desmoglein 1, which is a component of desmosomes (cell structures specialized for cell-to-cell adhesion). Cleavage results in loss of the superficial skin layer.

C. Clinical significance

S. aureus causes disease by infecting tissues, typically creating abscesses and/or by producing toxins (Figure). A common entry point into the body is a break in the skin, which may be a minute needlestick or a surgical wound. Another portal of entry is the respiratory tract. For example, staphylococcal pneumonia is an important complication of influenza.

The localized host response to staphylococcal infection is inflammation, characterized by swelling, accumulation of pus, and necrosis of tissue. Fibroblasts and their products may form a wall around the inflamed area, which contains bacteria and leukocytes. This creates a characteristic pus-filled boil or abscess. Serious consequences of staphylococcal infections occur when the bacteria invade the bloodstream. The resulting septicemia (the presence and persistence of pathogenic microorganisms or their toxins in the blood) may be rapidly fatal. Bacteremia (the presence of viable bacteria circulating in the bloodstream) may result in seeding internal abscesses, skin lesions, or infections in the lung, kidney, heart, skeletal muscle, or meninges.

1. Localized skin infections: The most common ***S. aureus*** infections are small, superficial abscesses involving hair follicles (folliculitis) or sweat or sebaceous glands (see Figure 8.12). For example, the common sty (external hordeolum) is created by infection of an eyelash follicle. 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*, or both organisms together. Human staphylococcal infections usually remain localized at the portal of entry by normal host defenses.

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). [Note: Septic joints are medical emergencies because pus can rapidly cause irreparable cartilage damage. They must be treated promptly with drainage and an antibiotic.]

3. Acute endocarditis: Generally associated with intravenous drug abuse, acute endocarditis 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. An abscess in any organ or tissue is cause to suspect ***S. aureus***, although many other bacteria can cause abscesses.

4. Septicemia is a generalized infection with sepsis or bacteremia that may be associated with a known focus (for example, a septic joint) or not (an occult focus).

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-associated infections, often of wounds (surgical, decubital) or bacteremia associated with catheters (see Figure 8.10). Progression to septicemia is often a terminal event.

7. **Toxinoses**: These 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:

a. Toxic shock syndrome: TSS results in high fever, rash (resembling a sunburn, with diffuse erythema followed by desquamation), vomiting, diarrhea, hypotension, and multiorgan involvement (especially GI, renal, and/or hepatic damage). An outbreak of TSS occurred in the late 1970s among menstruating women. It was shown to be related to the use of hyperabsorbant tampons by women who happened to be vaginally colonized by toxic shock syndrome toxin-(TSST)-positive strains of *S. aureus*. [Note: These tampons stimulated TSST expression, resulting in entry of the toxin into the circulation in the absence of true infection.] The incidence has decreased markedly since such tampons were removed from the market. Of the few cases of TSS that occur currently, approximately half are associated with ordinary *S. aureus* infections. Of the remainder, many result from a circulating enterotoxin rather than TSST. Figure 8.6 shows the desquamation (peeling or scaling of the skin) seen in TSS.

b. Staphylococcal gastroenteritis: This 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 or cream pastry) or salty, like ham (*S. aureus* is salt tolerant), and improperly refrigerated. These heat-resistant toxins are able to withstand subsequent reheating. Symptoms, such as nausea, vomiting, and diarrhea, are acute following a short incubation period (less than 6 hours) and are triggered by local actions of the toxin on the GI tract rather than from infection. The short incubation period of staphylococcal food poisoning occurs because the toxin in the food has already been formed by the staphylococci before the food is ingested.

c. Scalded skin syndrome: This 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 (see Figure 8.12). The bullae may be infected or may result from toxin produced by organisms infecting a different site.

D. Laboratory identification

Identification of an isolate as a staphylococcus relies largely on microscopic and colony morphology and catalase positivity. Bacteria stain strongly gram-positive, and are frequently seen in grapelike clusters. *S. aureus* is distinguished from the coagulase-negative staphylococci primarily by coagulase positivity. In addition, S. aureus colonies tend to be yellow (hence "aureus," meaning golden) and hemolytic (see Figure 8.12), rather than gray and nonhemolytic like the coagulase-negative staphylococci. *S. aureus* is also distinguished from most coagulase-negative staphylococci by being mannitol-positive.

E. Immunity

S. aureus infections do not elicit strong or long-lasting immunity, as demonstrated by the continuing susceptibility of individuals to *S. aureus* infections throughout life.

F. Treatment

Serious *S. aureus* infections require aggressive treatment, including incision and drainage of localized lesions, as well as systemic antibiotics. Choice of antibiotics is complicated by the frequent presence of acquired antibiotic resistance determinants. Virtually all community and hospital-acquired *S. aureus* infections are now resistant to penicillin G due to penicillinase-encoding plasmids or transposons. This has required the replacement of the initial agent of choice, penicillin G, by β-lactamase-resistant penicillins, such as methicillin or oxacillin. However, increased use of methicillin and related antibiotics has resulted in *S. aureus* that is resistant to a number of β-lactam antibiotics, such as methicillin, oxacillin and amoxicillin (Figure 8.8). These strains are known as methicillin-resistant *S. aureus* (MRSA).

1. Hospital-acquired methicillin-resistant *S. aureus* (MRSA)

In recent decades, a high percentage (often in the range of 50 percent) of hospital *S. aureus* isolates has been found to be also resistant to methicillin or oxacillin. Antibiotic resistance is caused by chromosomal acquisition of the gene for a distinct penicillin¬binding protein, PBP-2a. This protein codes for a new peptidoglycan transpeptidase with a low affinity for all currently available β-lactam antibiotics, and thus renders infections with MRSA unresponsive to β-lactam therapy. Compared with methicilllin-sensitive *S. aureus*, MRSA infections are associated with worse outcomes, including longer hospital and

intensive care unit stays, longer durations of mechanical ventilation, and higher mortality rates. MRSA strains are also frequently resistant to many other antibiotics, some being sensitive only to glycopeptides such as vancomycin.

2. Community-acquired MRSA (CA-MRSA)

Community acquired MRSA infections were documented in the mid-1990s, occurring in individuals who had no previous risk factors for MRSA infections, such as exposure to hospital. The most common clinical manifestations of CA-MRSA are skin and soft tissue infections such as abscesses or cellulitis (Figure). Less commonly, CA-MRSA can also cause severe diseases such as necrotizing pneumonia, osteomyelitis, and septicemia.

DRUG	HA-MRSA (Hospital strain)	CA-MRSA (Community strain)
Characteristics of patients	Patients are typically elderly, debilitated, and/or chronically ill.	Patients are typically young and healthy. Children students, athletes, and military service personnel are at risk.
Infection site	Bacteremia commonly occurs with no obvious infection site. Infection of surgical wounds, open ulcer, intravenous line, and urinary catheters often occur.	Infections often occur in skin and soft tissues, producing cellulitis and abscesses. Infections include necrotizing community pneumonia, septic shock, and bone and joint infections.
Transmission	Transmission occurs within health care settings. Only rarely is transmission among household contacts.	Transmission occurs in the community. May spread in families, sport teams, and other risk groups.
Medical history	Infections more likely in patients with a history of MRSA infections, recent surgery, admission to a hospital or nursing home. Antibiotic use, dialysis and permanent indwelling catheters are risk factors.	Patients show no significant medical history or health care contact.
Virulence of infecting strain	Spread of infection in the community is limited. PVL genes are usually absent.	Spread of infection in the community readily occurs. PVL genes are often present, predisposing to necrotising soft tissue or lung infections.
Antibiotic susceptibility	Multidrug antibiotic resistance often occurs, resulting in a limited choice of effective therapeutic agents.	CA-MRSA strains are often more virulent than HA-MRSA, but they tend to be susceptible to a broader array of antibiotics.

Figure Comparison of **hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA)** with **community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). PVL** = Panton-Valentine leukocidin.

Community-acquired MRSA has a number of characteristics that help distinguish it from hospital- associated MRSA. For example, CA-MRSA has a characteristic pattern of DNA fragments obtained upon enzymic cleavage and electrophoresis, and it produces specific toxins. CA-MRSA also exhibits a unique antibiotic

resistance pattern, that is, CA-MRSA is sensitive to many antibiotics that do not show much activity against hospital-associated MRSA. These antibiotics include **ciprofloxacin** and **clindamycin**, with some CA-MRSA even sensitive to **erythromycin**, **gentamicin**, **rifampin**, **tetracycline**, and/or **trimethoprim-sulfamethoxazole**.

Note: Emerging antibiotic-resistant strains of ***S. aureus*** that infect otherwise healthy individuals (community-acquired infections) are often more virulent than the more common strains that originate in hospitals.

3**. Vancomycin resistance**

Vancomycin has been the agent of choice for empiric treatment of life-threatening MRSA S. aureus infections. Unfortunately, in 1997, several MRSAs were isolated that had also acquired low-level vancomycin resistance. The incidence of vancomycin resistance has increased steadily, prompting the use of alternative drugs such as **quinupristin-dalfopristin**, **linezolid**, and **daptomycin**. These agents have good in vitro activity against MRSA and most other clinically important gram-positive bacterial pathogens.

G. Prevention

There is no effective vaccine against ***S. aureus***. Infection control procedures, such as barrier precautions and disinfection of hands and fomites, are important in the control of nosocomial *S. aureus* epidemics.

II. Coagulase-Negative Staphylococci

Of 12 coagulase-negative staphylococcal species that have been recovered as normal commensals of human skin and anterior nares, the most abundant and important is *S. epidermidis*. For this reason some clinical laboratories designate all coagulase-negative staphylococci as *S. epidermidis*, a practice that is not encouraged. The second most important coagulase-negative staphylococcus is *S. saprophyticus*, which has a special medical niche. Coagulase-negative staphylococcal species are important agents of hospital-acquired infections associated with the use of implanted prosthetic devices and catheters.

A. *Staphylococcus epidermidis*

S. epidermidis is present in large numbers as part of the normal flora of the skin. As such, it is frequently recovered from blood cultures, generally as a contaminant from skin. Despite its low virulence, it is a common cause of infection of implants such as heart valves and catheters (Figure). Acquired drug resistance by *S. epidermidis* is even more frequent than by *S. aureus*. Vancomycin sensitivity remains the rule, but vancomycin-resistant isolates have been reported. *S. epidermidis* produces an extracellular polysaccharide material called polysaccharide intercellular adhesin (sometimes called "slime"), that facilitates adherence to bioprosthetic material surfaces, such as intravenous catheters, and acts as a barrier to antimicrobial agents.

B. *Staphylococcus saprophyticus*

This organism is a frequent cause of cystitis in women, probably related to its occurrence as part of normal vaginal flora. It tends to be sensitive to most antibiotics, even penicillin G. *S. saprophyticus* can be distinguished from *S. epidermidis* and most other coagulase-negative staphylococci by its natural resistance to novobiocin (Figure). [Note: Urinary coagulase-negative staphylococcus is often presumed to be *S. saprophyticus*; but **novobiocin** resistance can be used for confirmation.].

Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation	Novobiocin resistance
S. aureus	**Common**	+	**Golden Yellow**	+	-
S. epidermidis	**Common**	-	**White**	-	-
S. saprophyticus	**Occasional**	-	**Variable**	-	+

Figure Summary of various species of staphylococci.

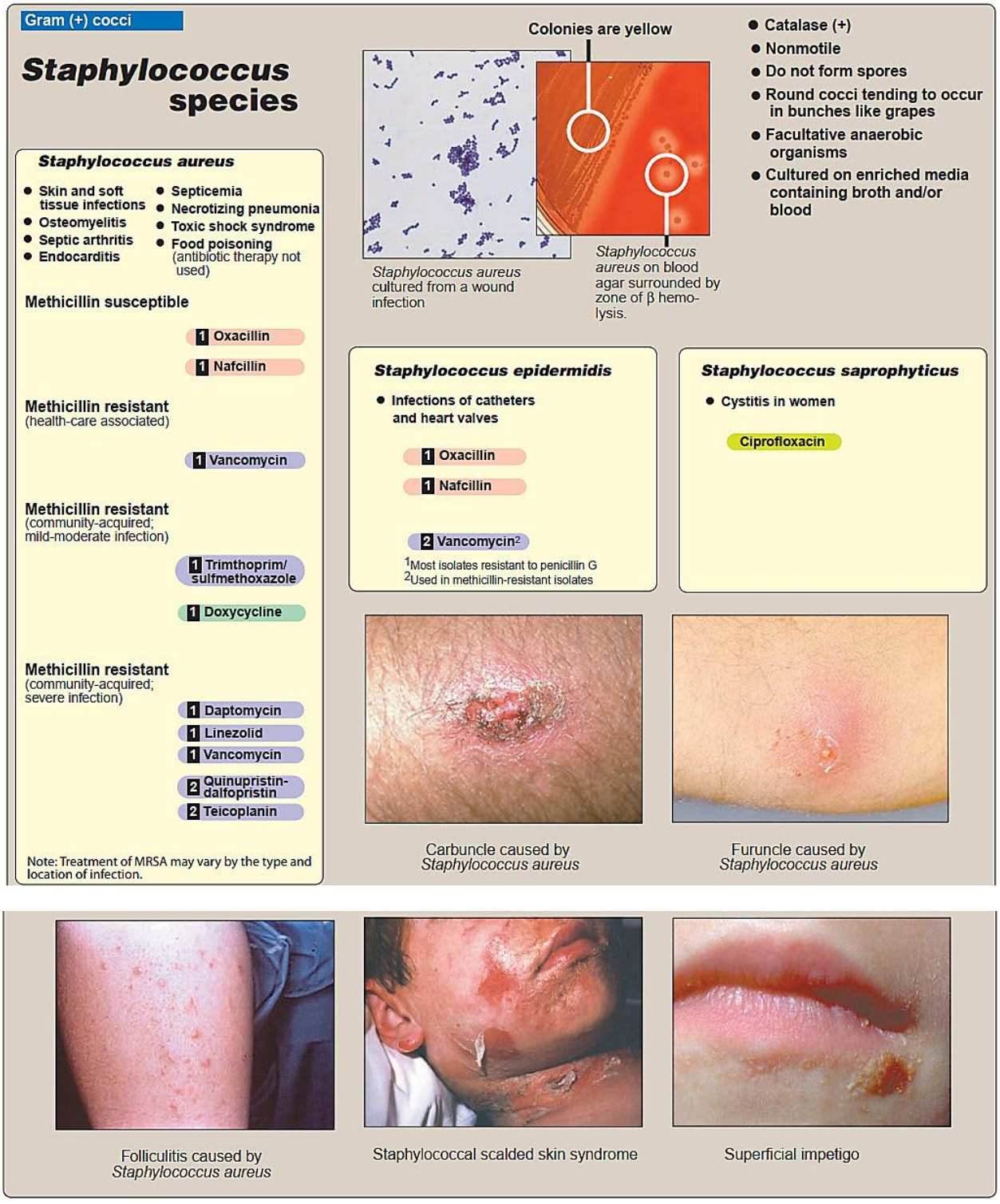


Figure -Summary of staphylococcal disease. 1 Indicates first-line drugs; 2 indicates alternative drugs.

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