Gram Positive Cocci

Staphylococci and streptococci constitute the main groups of medically important gram-positive cocci.

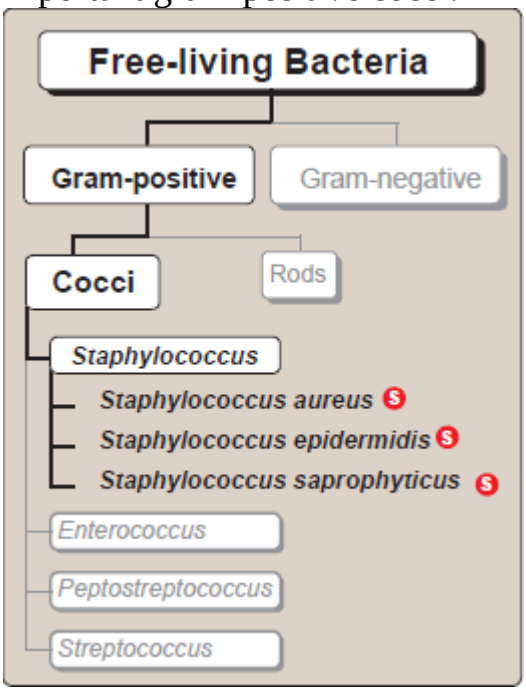

Figure 1: Classification of Gram Positive Cocci.

Staphylococcus

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. They 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 infections.

Staphylococci are **facultatively anaerobic** organisms. They produce **catalase**, which is one feature that distinguishes them from the catalase-negative streptococci. 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. Frequent handwashing before and after contact with food or potentially infected individuals decreases the transmission of staphylococcal disease.

The genus *Staphylococcus* has at least **45 species**. The three most frequently encountered species of clinical importance are ***Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus***. *S. aureus* is **coagulase positive** (an enzyme that causes citrated plasma to

clot), which differentiates it from the other species. The coagulase-negative staphylococci **(CoNS)** are normal human microbiota and sometimes cause infection, 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.

Staphylococcus aureus: Morphology and Structure

In growing cultures, the cells of *S. aureus* are uniformly Gram-positive and regular in size, fitting together in clusters with the precision of **pool balls**. In older cultures, in resolving lesions, and in the presence of some antibiotics, the cells often become more variable in size, and many **lose their Gram positivity.**

The cell wall of *S. aureus* consists of a typical Gram-positive peptidoglycan interspersed with molecules of a **ribitol-teichoic acid**, which is antigenic and relatively **specific for *S. aureus***. In most strains, the peptidoglycan of the cell wall is overlaid with surface proteins; one protein, **protein A**.

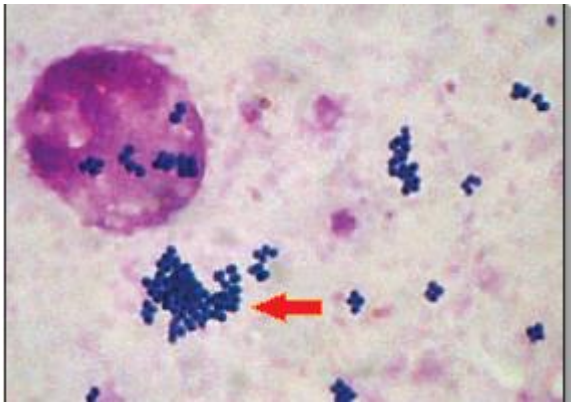

Figure2: Gram stain of *Staphylococcu aureus*

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) 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” toxicoses)

3) a combination of invasive infection and intoxication.

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, or contamination of food, which can then result in food poisoning.

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. [Note: Coagulase activity results in localized clotting, which restricts access by polymorphonuclear neutrophils (PMNs) and other immune defenses.

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.

1. Cell wall virulence factors:

a. Capsule: Most clinical isolates express a polysaccharide "microcapsule" of Types 5 or 8. 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.

2. Cytolytic exotoxins: attack mammalian cell (including red blood cell) membranes, and are often referred to as hemolysins.

3. 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.

4. Superantigen exotoxins: (SAgs) are a class of antigens that cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release.

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).

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.

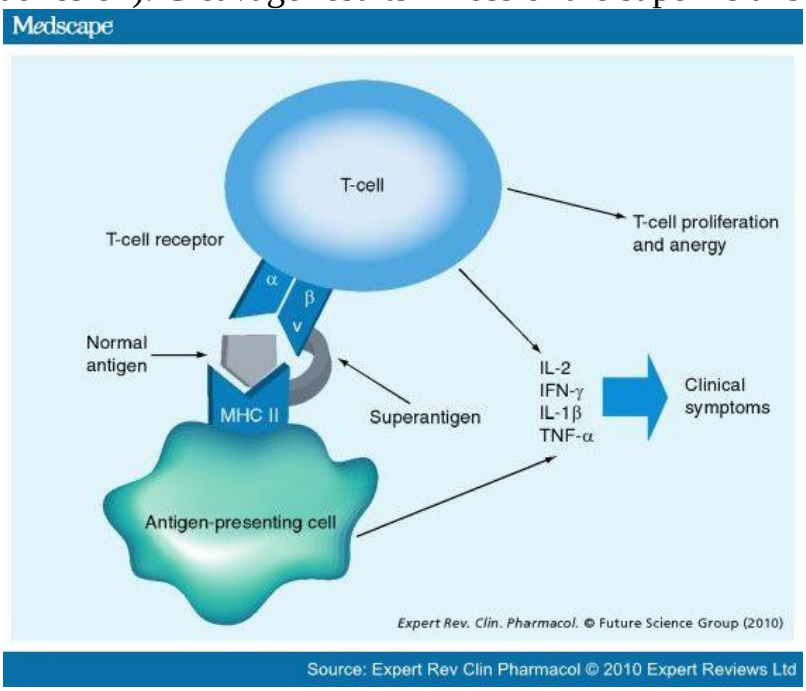

Figure 3: SuperAntigen Mechanism of action

Clinical significance

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.

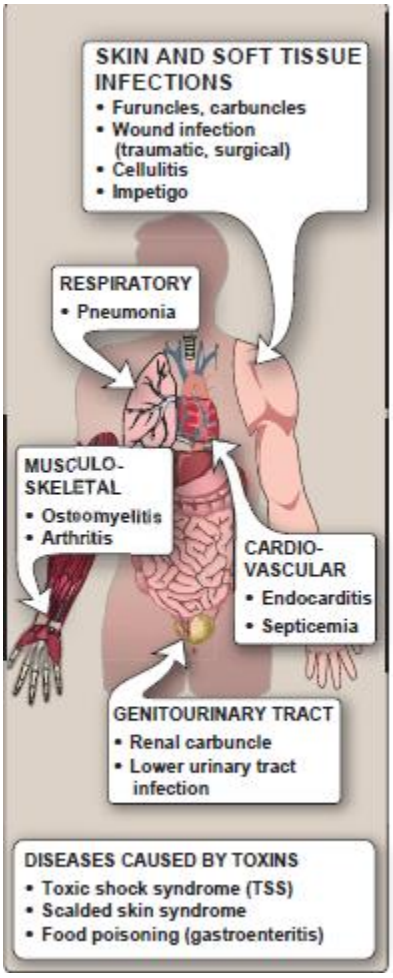

Figure 4: Diseases caused by *s aureus*

LABORATORY FEATURES

Specimens: Pus and swabs from infected sites, sputum, cerebrospinal fluid, blood for culture.

Faeces, vomit and the remains of food when food poisoning is suspected.

Morphology

Staphylococci are Gram positive cocci of uniform size, occurring characteristically in groups but also singly and in pairs (see colour plate 24). They are non-motile.

Blood agar, chocolate (heated blood) agar: *S. aureus* produces yellow to cream or occasionally white 1–2 mm in diameter colonies after overnight incubation.

Mannitol salt agar: A useful selective medium for recovering *S. aureus* from faecal specimens when investigating staphylococcal food-poisoning. It can also be used to screen for nasal carriers. *S. aureus* ferments mannitol and is able to grow on agar containing 70–100 g/l sodium chloride. Mannitol salt agar containing 75 g/l sodium chloride (plus 4 mg/l methicillin) is recommended, particularly for isolating MRSA strains.

Biochemical tests

● Coagulase positive.

● DNA-ase positive
● Catalase positive

Antimicrobial susceptibility

Antibiotics with activity against *S. aureus* include:
Penicillins,* Vancomycin, Macrolides, Cephalosporins, Fusidic acid
*Most strains of *S. aureus* (particularly hospital strains) are resistant to penicillin due to the production of plasmid-coded *beta*-lactamase.

MRSA (methicillin resistant S. aureus): These strains are resistant to methicillin and related penicillins and are particularly difficult to treat because they are also resistant to most other common antibiotics. Vancomycin is often needed to treat MRSA infections.

Other pathogenic *Staphylococcus* species

_ *Staphylococcus saprophyticus:* Causes urinary tract infections in sexually active women.

_ *Staphylococcus epidermidis:* May cause endocarditis and bacteraemia following infection of cannulae, indwelling catheters, or other appliances positioned in the body. Infections are difficult to treat due to the resistance of *S. epidermidis* to many antimicrobials. Virulence factors is production of an exopolysaccharide "slime". The slime layer around the bacterial cell wall:

· promotes adherence to plastic surfaces
· increase resistance to phagocytosis
· inhibits enterance of antibiotics to the cell.

Microscopically, *S. saprophyticus* and *S. epidermidis* resemble *S. aureus*. **Culturally** the colonies of *S. epidermidis* are white and usually non-haemolytic. The colonies of *S. saprophyticus* may be white or yellow. They are non-haemolytic. Growth may not occur on MacConkey agar. *S. saprophyticus* and *S. epidermidis* are coagulase negative.

Biochemical reactions that differentiate *S. epidermidis* and *S. saprophyticus* from *S. aureus*

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	–	–
DNA-ase	+	+ Weak	–
Mannitol*	+	–	+
Trehalose*	+	–	+
Sucrose*	+	+	+
Novobiocin (5 μg disc)	S	S	R

Further Readings:

***SHERRIS MEDICAL MICROBIOLOGY:* AN INTRODUCTION TO INFECTIOUS DISEASES**, 4TH EDITION *by KENNETH J. RYAN, MD C. GEORGE RAY, MD*

Lippincott's Illustrated Reviews: Microbiology, Third Edition, Copyright © 2013 Lippincott Williams & Wilkins, a Wolters Kluwer business.

District Laboratory Practice in Tropical Countries, Part 2, Second Edition by Monica Cheesbrough, Cambridge University Press

CASES IN MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES, FOURTH EDITION, ASM Press Washington, DC, by Peter H. Gilligan, Ph.D. Daniel S. Shapiro, M.D. and Melissa B. Miller, Ph.D.