KEYWORDS
Diplococcus
Pneumococcus
Autolysin
Bile solubility test
Optochin susceptibility
Capsule
Quellung reaction
Staphylococcus aureus
Staphylococcus epidermidis
Coagulase positive or Coagulase negative
Alpha, beta, gamma and delta cytotoxins
Leucocidin
Lipase
Exfoliatin
Enterotoxins
Toxic shock syndrome
Toxic shock toxin
Protein A

Figure 1a *Staphylococcus aureus* - MRSA resistant coccoid prokaryote (dividing); causes food poisoning, toxic shock syndrome and skin and wound infections (scalded skin syndrome, scarlet fever, erysipelas, impetigo, etc.) © Dennis Kunkel Microscopy, Inc. Used with permission

BACTERIOLOGY - CHAPTER THIRTEEN

PART 2

STAPHYLOCOCCI

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Staphylococci are facultative anaerobes. They are Gram positive, occur in grape like-clusters and are catalase positive. They are major components of the normal flora of skin and nose in all people.

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus (figure 1) is one of the commoner causes of opportunistic nosocomial and community infections. These infections include pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis, abscesses/boils and other skin infections (figure 2 and 3). *S. aureus* has gained notoriety because of the increased incidence of Methicillin-resistant *Staphylococcus aureus* (MRSA) Infections.

Pathogenesis

Food poisoning

S. *aureus* produces a number of toxins, of which the enterotoxins (A, B, C and D) cause food poisoning. About a third to a half of *S. aureus* strains produce enterotoxins which are heat stable and thus survive cooking (boiling for 30minutes). They are also resistant to proteolysis by intestinal proteases.

Food becomes contaminated with the organism from human contact, grows and produces enterotoxin. The organism does not "infect" the patient on ingestion of contaminated food; rather the pre-existing toxin causes the symptoms which include:

- vomiting
- nausea
- diarrhea (watery and non-bloody, leading to dehydration)
- abdominal pain

Fever is not observed.

Because only the toxin is involved, onset of symptoms occurs within a few hours and recovery occurs within a day. Antibiotic treatment is not indicated because the bacteria are not directly involved in causing the symptoms (and may, anyway, have been killed by cooking).

Enterotoxins are superantigens that lead to cytokine production, T cell activation, neutrophil infiltration with loss of small intestine brush border cells. The release of inflammatory mediators may be the cause of the characteristic *S. aureus* food poisoning-associated vomiting.

Enterocolitis

Figure 1b *Staphylococcus aureus* (Gram-positive) © Copyright Dr Linda M Stannard 1996. Used with permission

Figure 1c *Staphyllococcus aureus* - Acridine-orange leucocyte cytospin test © Bristol Biomedical Image Archive. Used with permission

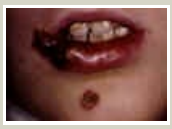

Figure 2 Staphylococcal Infection: Impetigo © Bristol Biomedical Image Archive. Used with permission

Figure 3 Impetigo lesions on forehead caused by *Staphylococcus aureus* bacteria. CDC

Figure 4 Box of Rely tampons. Associated with outbreak of toxic shock syndrome. CDC

The symptoms of enterocolitis are somewhat similar to food poisoning (watery diarrhea and abdominal pain) but also include fever. They are also produced by enterotoxin A and leukotoxin. The cause is the treatment of patients with broad spectrum antibiotics that allow *S. aureus* (which infects almost everyone) to grow in the intestine in preference to the normal bacterial flora. The bacteria can be detected in fecal samples.

Toxic shock syndrome

Toxic shock syndrome is caused by infection with strains of *S. aureus* that produces toxic shock syndrome toxin. It may be associated with a wound in which the bacteria multiply rapidly but became particularly prominent to the public in the 1980's when *S.aureus* infection was found to cause the toxic shock syndrome that was seen after the use of certain tampons such as "Rely" (figure 4). The bacteria were able to divide rapidly within the tampon; they do not disseminate but remain in the vagina. However, the toxin does disseminate and is responsible for the clinical features. This syndrome includes:

- fever
- macular erythrematous rash
- desquamation (all over the body)
- vomiting
- diarrhea

Toxic shock syndrome toxin has the properties of a superantigen, resulting in the production of cytokines, vascular leak and cell toxicity. This results in hypervolemic shock and death as a result of multi-organ failure. Before the cause of toxic shock syndrome was discovered, the mortality rate was high but now is around 5%. There can be recurrent disease if the patient is not treated with the appropriate antibiotic.

Toxic shock syndrome toxin is involved in most menstruation-associated toxic shock syndrome. Enterotoxin B is involved in many non-menstruation associated cases of toxic shock syndrome.

Scalded skin syndrome (Ritter disease, pemphigus neonatorum) and bullous impetigo

A minority of *S. aureus* strains produce exfoliative toxins (A and B) and either toxin can cause scalded skin syndrome or bullous impetigo in babies and young children but rarely in adults. These toxins are serine proteases that can digest, among other proteins, some of the proteins found in desmosomes, the structures that link epithelial cells together. For example, the desmosomal protein called desmoglein is digested between the cells of the stratum granulosum epidermis. The process often resolves as the result of the formation of protective neutralizing antibodies. Exfoliative toxins are also superantigens.

Bullous impetigo is a mild form of *S. aureus* disease that usually occurs in newborn infants and young children. It is manifested by large, flaccid bullae and attributed to *S. aureus* strains belonging to phage group II capable of producing exfoliative toxins A and B that separate the stratum corneum from the rest of the epidermis. The more common and milder form of the disease (representing about 10% of all cases of impetigo) differs from non-bullous impetigo in that the vesicles enlarge into flaccid bullae before rupturing . The exposed skin surface is at first moist and red, resembling a small burn. A thin, light-brown, “varnish-like” crust then develops. Unlike the situation with scalded skin syndrome, bacteria can be cultured from the fluid of the bullae and Nikolsky's sign is absent.

The more severe form of disease with greater skin involvement caused by the same staphylococcal strains is known as the staphylococcal scalded skin syndrome. This also usually affects younger children. The disease starts with local peri-oral erythema that spreads over the whole body and progresses to widespread, flaccid bullae that rupture causing exfoliation of the skin that resembles an extensive third-degree burn. There are no organisms that can be cultured from the fluid of the bullae, indicating that the bullae are caused by the toxin and not the bacteria themselves. Before the bullae form, slight pressure on the apparently normal epidermis may separate it at the basal layer. It may be rubbed off when pressed with a sliding motion. This is Nikolsky's sign.

This form of the disease can occur in epidemic form in nurseries, where it is

known as pemphigus neonatorum or Ritter's disease. Fever and other systemic symptoms are usually absent in the more localized forms of the disease but are invariably present in patients with the staphylococcal scalded skin syndrome.

Localized bullous impetigo is self-limited due to the formation of neutralizing anti-toxin antibodies, and this is usually also the case with staphylococcal scalded skin syndrome. However, the latter carries a significant mortality rate (5%) that results from secondary bacterial infections of the areas where the skin surface has been lost. Staphylococcal scalded skin syndrome in adults is rare, and is usually associated with immunosuppression or kidney disease. In this case mortality can be as high as half of the patients.


Figure 5b
A cutaneous abscess on the foot caused by methicillin-resistant *Staphylococcus aureus*. CDC

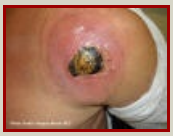
Figure 5c
Cutaneous abscess caused by MRSA. CDC


Figure 5d
Cutaneous abscess caused by MRSA. CDC


Figure 5e
Cutaneous abscess caused by MRSA. CDC


Figure 6
S. epidermidis, the most common cause of blood stream infections in patients with IVCs

Cytotoxins

As noted above, *S. aureus* causes a number of different disease entities associated with production of certain exotoxins. In addition to these "disease-specific" exotoxins, other cell lytic exotoxins (alpha, beta, gamma and delta toxins and leucocidins) may be produced. These are also called cytotoxins because they cause cytolysis as a result of plasma membrane damage. This leads to tissue destruction as a result of lysosomal enzyme release.

Alpha toxin

This singler polypeptide toxin interacts directly with the plasma membrane of many cells, embedding itself in the lipid bilayer and forming pores that allow ions to pass into and out of the cell. In particular, potassium ions are lost and sodium and calcium enter the cell. This leads to osmoticlysis. Alpha toxin is made by most *S. aureus* strains.

Beta toxin

Beta toxin also damages cell membranes by degrading specific lipids, sphingomyelin and lysophosptidyl choline. The toxin is a sphingomyelase C and is also a single polypeptide that is made by most *S. aureus* strains. It appears that the degree of toxicity depends on the concentrations of these lipids in the cell, both of which are found primarily in the outer monolayer of the plasma membrane bilayer.

Gamma toxins and P-V leukocidin

Gamma toxins and Panton-Valentine leukocidin consist of two polypeptide chains, an S chain and an F chain, which together form pores in the plasma membranes of susceptible cells. So far, three S chains and two F chains have been found which can combine to form a number of different toxins that are cytolytic to neutrophils and macrophages. The gamma toxins are also hemolytic whereas P-V leukocidin is not. The gamma toxins are also made by most *S. aureus* strains whereas P-V leukocidin is made by only a minority of strains. It has been particularly associated with virulent Methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Delta toxin

This is a small protein that is cytotoxic to many cells. It may act like a detergent, damaging cell membrane bilayers resulting in cytolysis.

Other diseases caused by *S. aureus*

Respiratory disease

Aspiration pneumonia can result from entry of oral secretions into the lungs. The bacteria can cause local abscesses and infiltrates. The disease is found in the very young, the very old and patients with pulmonary disease. There can also be spread of blood-borne organisms to the lungs, causing hematogenous pneumonia. People with MRSA can get necrotizing pneumonia which has a very high fatality rate.

Empyemia is an accumulation of pus in a cavity of the body such as the lungs and is sometimes seen in pneumonia patients. Many of these cases are the result of *S. aureus* infections.

Bacteremia

S. aureus is found on the skin of most people and can enter the body in wounds; however, many cases are nosocomial and result from surgery or catheter use. The bacteria may disseminate throughout the body.

Endocarditis

Endocarditis is an inflammation of the endocardium (the inner layer of the heart) and usually involves the heart valves (native or prosthetic valves). *S. aureus*-associated endocarditis can have a high mortality rate.

Urinary tract infections

Complicated urinary tract infections occur in specific clinical settings. Renal abscess can result from hematogenous seeding of the renal cortex (most often due to *S. aureus*) or from ascending infection leading to severe pyelonephritis (most often due to gram-negative rods).

Dissemination to other parts of the body

S. aureus bacteremia can disseminate via the bloodstream to other parts of the body causing disease. Such sites include bone giving rise to *S. aureus* osteomyelitis resulting in pain, fever and sometimes a Brodie abscess and septic arthitis.

Skin disease

Folliculitis

Folliculitis, by which is meant pyoderma involving the hair follicles and apocrine glands, affects nearly everyone at one time or another but is usually self-limited. Occasionally, folliculitis evolves into larger lesions known as furuncles and carbuncles.
S. aureus is the usual cause of folliculitis in non-immunocompromised patients, the infection probably arising from prior nasal colonization by this bacterium.

Furuncles, carbuncles and skin abscesses

The familiar furuncle or “boil” is thought to arise from folliculitis. The term furunculosis refers to multiple boils or to frequent recurrences. Carbuncles are more extensive and difficult-to-treat lesions that often require surgical intervention. Skin abscesses, although similar to carbuncles histologically, are usually deeper infections that do not originate in hair follicles.

S. aureus is the usual cause of both furuncles and carbuncles, and is also the sole or predominant pathogen in about 50% of skin abscesses. Predisposing factors to recurrent furuncles (furunculosis) include obesity, corticosteroid therapy, disorders of neutrophil function, and possibly diabetes mellitus. Immunoglobulin levels are usually normal in patients with furunculosis (low IgM levels have been demonstrated in some patients but this is of uncertain significance and, in contrast to IgG deficiency, replacement therapy is impractical). Most patients with recurrent furuncles have no obvious predisposing factors other than being nasal carriers of *S. aureus* nasal carriers. Outbreaks of furunculosis have been described in families, athletic teams, and in village residents who took steam baths together. Skin abscesses can result from minor trauma, injecting drug use (the practice of subcutaneous and intramuscular injection is known as “skin popping”), or bacteremia. Congenital immunodeficiency syndromes such as the hyperimmunoglobulin E-recurrent infection syndrome (Job’s syndrome) are sometimes present in patients with recurrent skin abscesses. Rarely, skin abscesses are self-inflicted (factitious abscess), in which case Gram’s stain and culture may reveal “mouth flora” bacteria.

For more information see: Infectious disease - skin and bone

Other secreted enzymes

S. aureus strains secrete a number of tissue-degrading enzymes that may result in tissue damage. These include lipases, nucleases, hyaluronidase, coagulase and plasmin. One form of coagulase is bound to the *S. aureus* surface and converts fibrinogen to fibrin. This insoluble protein causes the bacteria to aggregate. The other coagulase is secreted and combines with coagulase-reacting factor in the serum resulting in the formation of staphylothrombin that, like normal thrombin, also forms insoluble fibrin. This may also be anti-phagocytic.

Protection against phagocytosis

In addition, to the toxins and enzymes that directly damage cells and tissues described above, *S. aureus* strains produce other proteins involved in pathogenesis. For example, these bacteria have two mechanisms that protect them against phagocytosis by polymorphonuclear leukocytes and other phagocytic cells.

- Although the bacteria are opsonised by proteins in serum, the capsule and slime layer protect the cells against phagocytosis.
- Protein A is found on the surfaces of most *S. aureus* strains. It binds to immunoglobulin G and complement, blocking Fc and complement receptors and is thus anti-phagocytic.

Identification

- *S. aureus* is beta-hemolytic on sheep blood agar
- Ferments mannitol (figure 9)
- Is often golden pigmented (hence the name *aureus*)
- Is coagulase-positive
- Presence of protein A

In reference laboratories phage-typing is used.

Methicillin-resistant *Staphylococcus aureus* (MRSA) Infections

Methicillin-resistant *Staphylococcus aureus (*MRSA) is defined as any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics (such as penicillins) and cephalosporins. This results from the production of a phage-coded penicillinase that degrades beta lactam antibiotics. Some strains also have modified penicillin binding proteins.

Many healthy people carry MRSA asymptomatically. Patients with compromised immune systems are at a significantly greater risk of symptomatic infections. Apparently healthy people may have simple topical skin infections (noted above) but in some people MRSA may progress rapidly within a day or two of initial topical symptoms. In these patients, after about 72 hours, MRSA may invade tissues and become resistant to treatment.

The majority of community-associated MRSA infections are localized to skin and soft tissue and usually can be treated effectively but some strains exhibit enhanced virulence and spread into the tissues, causing illness much more severe than traditional nosocomial MRSA infections.

At first, MRSA is characterized by small red pimples and there may be fever and a rash. As the infection progresses over a period of a few days, the pimples increase in size and become more painful. Eventually, they form deep, pus-filled boils (figure 5b-e). The infection can disseminate throughout the body (sepsis) and vital organs may be affected. This can lead to toxic shock syndrome, and necrotizing pneumonia, some times referred to as "flesh eating" pneumonia. In hospitals, there can be surgical site infections.

Epidemiology

Two per cent of people carry MRSA. Currently, in the United States, there are about 75,000 cases of invasive MRSA Infections per year, of which about 14,000 are in dialysis patients. Nosocomial invasive MRSA infections declined 54% between 2005 and 2011, with 30,800 fewer severe MRSA infections. In addition,

there were 9,000 fewer deaths in hospital patients in 2011 versus 2005.

MRSA is usually spread by direct contact with an infected wound or from contaminated hands, usually those of healthcare providers. People who carry MRSA but do not have signs of infection can spread the bacteria to others and potentially cause an infection.

Diagnosis

This can be done by growth of the organism in the laboratory. There are more rapid tests available such as quantitative PCR.

Treatment

Intravenous vancomycin and teicoplanin are used to treat MRSA but some new MRSA strains are resistant to these antiboitcs also. Daptomycin is often used to treat these strains

STAPHYLOCOCCUS EPIDERMIDIS

Staphylococcus epidermidis (figure 6) is a major component of the normal skin flora and thus commonly a contaminant of cultures in laboratories. It is a less common cause of opportunistic infections than *S. aureus*, but is still significant. Normally, infections are nosocomial. The bacteria form biofilms on catheters, shunts, artificial heart valves and other surgical devices and can cause endocarditis and sepsis.

The formation of biofilms is import in the virulence of the bacteria. It is likely that the bacteria bind blood proteins and extracellular matrix proteins to their surface. The bacteria also produce a sulfated polysaccharide extracellular coat called polysaccharide intercellular adhesion. Other bacteria bind to this surface coat making a multilayer biofilm. The cells within the biofilm become partially metabolically inactive and this, together with the difficulty in penetrating the biofilm with antibiotics, makes it difficult to treat the infection. In addition, *S. epidermidis* strains are often antibiotic-resistant (including penicillin, amoxicillin, and methicillin).

Since antibiotics are largely ineffective in clearing biofilms, the usual tratment is to chnage the infected medical device. The drug of choice is usually vancomycin, to which rifampin or aminoglycoside can be added.

Identification

- *Staphylococcus epidermidis* is non-hemolytic on growth on sheep blood agar
- Does not ferment mannitol (figure 7)
- Is non-pigmented
- Is coagulase-negative.

Figure 7 Two different species of *Staphylococcus* growing on mannitol salt agar (MSA). MSA is selective because it contains 7.5% salt–a high salt concentration that promotes the growth of some organisms while discouraging the growth of others. MSA is a differential medium because it contains the sugar mannitol and the pH indicator phenol red. Organisms that can ferment mannitol produce acid by-products, causing a color change. Phenol red is a cherry red color above pH 8.5, yellow-red from pH 6.9 to 8.5, and bright yellow at pH 6.9 or lower. Although both *Staphylococcus epidermidis* and *Staphylococcus aureus* can tolerate the high salt content of MSA, only *S. aureus* can ferment mannitol, causing the phenol red in the medium to turn yellow. © Margaret (Peg) Johnson, Mesa Community College, Mesa, Arizona and The MicrobeLibrary

MOVIE
Catalase Test

Cultures of Staphylococcus aureus (left) and Streptococcus pyogenes (right) were grown on blood agar plates for 16 h at 37 degrees. A colony from each plate was placed on a glass slide. A drop of 3% hydrogen peroxide was placed on both organisms. The catalase- producing organism catalyzes the breakdown of H2O2 to oxygen and water. O2 is released as bubbles. The catalase test is used to differentiate Staphylococcus sp. from Streptococcus

sp. Staphylococcus sp. are positive for catalase production. Streptococcus sp. are negative for catalase production.

STAPHYLOCOCCUS SAPROPHYTICUS

This organism is a Gram-positive, coagulase-negative bacterium and a significant cause of urinary tract infections, usually in young women who are sexually active. It is not usually differentiated from *S. epidermidis* clinically.

It occurs in the normal flora of the female genital tract and perineum and in females in the 17 to 27 years old age group, it is the second most common cause of urinary tract infection (after E. coli). Symptoms include dysuria and pyuria. Sexual activity increases the risk of infection of the urinary tract because bacteria are transferred from the vagina and perineum into the urethra. Most cases occur within a day of sexual intercourse and the infection is sometimes known as "honeymoon cystitis". *S. saprophyticus* has the capacity to selectively adhere to human urothelium. The adhesin for S. saprophyticus is a lactosamine structure. S. saprophyticus produces no exotoxins.

 Return to the Bacteriology Section of Microbiology and Immunology On-line

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