Detection of Community Acquired Methicillin Resistant *Staphylococcus aureus* Among *Staphylococcus aureus*.

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By

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INTRODUCTION

Staphylococci

Staphylococci are Gram-positive spherical cocci about 0.5 - 1.0 μm in diameter. They grow in grape- like clusters, pairs and occasionally in short chains. They form bunches because they divide in two planes as opposed to their close relatives streptococci which, although they are similarly shaped, form chains because they divide only in one plane.[1] Nearly all are facultative anaerobes that grow better under aerobic than anaerobic conditions. Most strains grow in presence of 10% sodium chloride and between 18-40^0 C.The organism is susceptible to lyses by lysostaphin but resistant to lyses by lysozyme. [2]

Traditionally, the staphylococci have been divided into two groups according to their ability to clot blood plasma. Coagulase positive staphylococci are members of species *Staphylococcus aureus* (*S. aureus)* they are considered to be opportunistic pathogens in humans & some other mammals the coagulase negative staphylococci are considered to be saprophytes, even though they are occasionally responsible for infection. [2]

Morphology and culture characters

Staphylococci are non-motile, non spore-forming, non flagellate and occasionally capsulated. In liquid media singles, pairs and short chain are also seen. On blood or nutrient agar, incubated in air for 18-24h at the optimal growth temperature of 37^{0}C, it forms colonies 1-3mm in diameter. Colonies are smooth low convex, glistening, densely opaque, and some times narrow zone of haemolysis on blood agar depending on strain. [3]Heavily capsulate colonies may be highly mucoid.[2]

Pigmentation is a characteristic of *S. aureus* species when grow aerobically, and ranges from cream through buff to gold. Pigmentation is enhanced on fatty media by prolonged incubation and by leaving plate at room temperature. [3]

Colonial pigment is variable and influenced by growth condition. Pigment production tends to be enhanced with age or storage at refrigerator temperatures. The ability to form yellow pigment is often lost irreversibly, especially after repeated subculture anaerobically or prolonged culture in broth. Non pigmented variants are more susceptible to drying and to linoleic acid than are parental strains and so would be less likely to survive at carriage sites; the ability to form pigment could not be transduced, but its loss was associated with a reduction in the cellular DNA content.[2]

Metabolism

Some species are mainly respiratory, others predominantly fermentative. Carbohydrates or amino acids, or both, are used as carbon sources. A variety of carbohydrates may be utilized aerobically with the formation of acid. Acetate and CO_2 are the major end products of aerobic glucose metabolism. Growth occurs at 10 and 45^0C, but is usually most rapid between 30 and 37^0C; it occurs over a wide range of pH (4-9) and is optimal at pH 7.0-7, 5. [(2)]

Nutritional requirements are variable. Most species examined require an organic nitrogen source, i.e. certain amino acids, and B-group vitamins for growth. [(2)]

For anaerobic growth, most species also require uracil or a fermentative carbon source such as pyruvate, or both. Growth does not occur in the complete absence of CO_2. [(2)]

Susceptibility to physical and chemical agent

Staphylococci are hardly, being relatively resistant to heat and drying and thus can persist for long period on fomites, which can serve as sources of infection.[(4)]

Staphylococci will grow in media containing high concentration of salt or sucrose, for example 12.5% NaCl. Salt containing media are used for selective isolation of *S. aureus.* Disinfectants such as chlorhexidine, hexachlorophene and phenol kill staphylococci rapidly. [(2)]

Staphylococci are very sensitive to aniline dyes; thus, they are inhibited on blood agar containing 1 in 500,000 crystal violet, which permit the growth of streptococci. Fatty acids are also highly active against staphylococci. [(2)]

Staphylococci are resistant to lysozyme but some micrococci are sensitive to it. Lysozyme causes the cell wall of bacteria to weaken and may cause disruption/lysis of the cell membrane. Lysozymes catalyze the hydrolysis of beta-1, 4-bonds of N-acetylmuramic acid, which is a part of proteoglycans and glucosamineglycans in murein-containing bacterial cell walls. Lysostaphin is a mixture of 3 enzymes active on the cell wall constituents of gram-positive organism and formed by a strain of *S. epidermidis;* the fact that staphylococci are more susceptible to it than micrococci is attributable to the composition of their respective peptidoglycans. Lysostaphin is a zinc metalloenzyme which has a specific lytic action against *S. aureus*. Lysostaphin has activities of three enzymes namely, glycylglycine endopeptidase, endo-β-N-acetyl glucosamidase and N-acteyl muramyl-l-alanine amidase. Glycylglycine endopeptidase specifically cleaves the glycine–glycine bonds, unique to the interpeptide cross-bridge of the *S. aureus* cell wall. Due to its unique specificity, lysostaphin could have high potential in the treatment of antibiotic-resistant staphylococcal infections. [(2)]

Biochemical properties:

Some of the biochemical tests recommended for the separation of staphylococci from micrococci such as: **Glucose fermentation** was developed for separation staphylococci from the non-glucose fermenting micrococci. [2]

Staphylococci produce acid aerobically from glycerol while micrococci usually do not. The growth of the most micrococci is inhibited by erythromycin at low levels (0.2mg/l), while staphylococci were resistance to at least 0.4mg/l. [2]

A medium containing both glycerol and erythromycin was proposed for the rapid separation of staphylococci and micrococci. [2]

Staphylococci are predominantly catalase positive and oxidase negative and reduce nitrate to nitrite. Similarly, few species produce acid from L-arabinose, starch, D-xylose under aerobic conditions. [2]

Staphylococci produce various proteolytic enzymes. Nearly all strains of *S. aureus* and many other staphylococci form a gelatinase, and most *S. aureus* strains digest casein rapidly in the presence of serum. The coagulase negative staphylococci form a number of proteinases that can be distinguished serologically or by their electrophoretic mobility. [2]

In routine laboratory, strains positive in the coagulase test are generally looked upon as *S. aureus*. The rabbit-plasma coagulase test widely employed in human medical bacteriology is quite reliable. However, the ability to clot mammalian plasma is not specific for *S. aureus* and not all the members of this species clot plasma. Furthermore, a pseudocoagulase activity has been reported. Acetoin, α-haemolysin, fibrinolysin, mannitol fermentation and clumping factor production are also common to human *S. aureus* strains. [2]

In addition, Staphylococci can produce both heat–labile and heat resistant nucleases (thermonuclease). The later can cleave either DNA or RNA and is produced by most stains of *S. aureus*. [2]

Table I: Chemical and biochemical characters separating staphylococci from micrococci: [2]

Character	Staphylococcus	Micrococcus
Anaerobic fermentation of glucose	+	□
Susceptibility to Bacitracin (0.04 unit disc)	□	+
Susceptibility to Lysostaphin (200mg/l)	+	□
Peptidoglycan composition (>2 mol glycine:1 mol lysine)	+	□
DNA base composition (mol% G+C)	30-39	65-75

Staphylococcus Aureus

Staphylococcus aureus (Greek staphyle = bunch of grapes, Latin coccus = spherical bacterium, aureus = golden). [5]

S. aureus is a Gram-positive bacterium, it is an opportunistic pathogen, *S. aureus* forms golden yellow colonies with beta-haemolysis of blood agar, and *S. epidermidis* forms white colonies. Individual bacteria are rounded; they have no flagella. They grow in pairs, short chains or clusters. [5]

S. aureus is one of the most common causes of life-threatening bacterial infections. Every year in the United States, roughly 400,000 hospital patients are infected by *S. aureus*. Approximately 100,000 of these patients die from complications due to their infections. [6]

S. aureus is one of about 32 species in the Staphylococcus genus of bacteria. Most of the other species are found only in other mammals and do not infect humans. The origin of *S. aureus* is not well understood, but current theories suggest that it evolved from prehistoric soil bacteria. *S. aureus* was first conclusively described by German physician Anton Rosenbach in 1884. Although *S. aureus* was recognized and described only 125 years ago, it has almost certainly been infecting and killing humans for thousands of years.[6]

S. aureus colonizes the skin of many healthy individuals, but most carriers do not become infected. The skin provides a substantial barrier to the entry of bacteria into the body. However, a break in the skin due to injury or surgery provides a point of entry for *S. aureus* already living on the skin. [6]

Morphogenesis of the Staphylococcal Cell Wall

The cell wall is the principal stress-bearing and shape-maintaining element in bacteria, and its integrity is of critical importance to cell viability.[7]

Chemical Composition of the Staphylococcal Cell Wall

The cell wall of *S. aureus* shows the typical features of gram-positive bacterial cell walls. Under the electron microscope it appears as a relatively thick (about 20 to 40 nm) homogeneous structure. [8]

Peptidoglycan (PG), also called murein, (figure 1) is a polymer that consists of long glycan chains that are cross-linked via flexible peptide bridges to form a strong but elastic structure that protects the underlying protoplast from lysing due to the high internal osmotic pressure. [9-12]

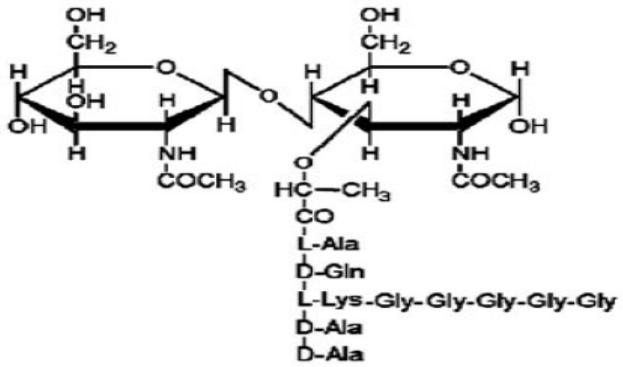

Figure 1: Cell Wall Building Blocks in *Staphylococcus aureus.* [8]

The chemical structure of peptidoglycan, has been known for a long time.[13] This heteropolymer consists of a disaccharide backbone formed by alternating β-1-4-N-acetylglucosamines and N-acetylmuramic acids. The length distribution of glycan chains in *S. aureus* is of 3 to 10 disaccharide units, with a maximum length of at least 23 to 26 units.[14] The average chain length is in the range of 10 disaccharides.[15] Tetrapeptides consisting of L-alanine, D-glutamine, L-lysine, and D-alanine are attached to the N-acetylmuramic acid. This stem peptides are synthesized as pentapeptide chains, containing L- and D-amino acids and one dibasic amino acid, which allows the formation of the peptide cross bridge. The dibasic amino acid is L-lysine which is present in most gram positive bacteria. [7]

About 90% of these stem peptides are cross-linked to the stem peptides of another glycan chain by a pentaglycine group.[16] This pentaglycine is a characteristic feature of the staphylococcal peptidoglycan and connects the ε-amino group of the L-lysine of one stem peptide to the D-alanine of the other one. The stem peptides which are not cross-linked carry an additional D-alanine which is cleaved during the cross-linking reaction. [8]

The long and flexible pentaglycine cross bridge characteristic of *S. aureus* is able to span the distance between stem peptides from different PG layers which

would otherwise be too distant to be crosslinked.[17,18] This permits the very high degree of crosslinking observed in the staphylococcal PG. [19]

The process of cell wall cross-linking is catalyzed by transpeptidases, the penicillin-binding proteins (PBPs). [16] *S. aureus* produces four penicillin-binding proteins, PBP1-4, involved in the cell wall peptidoglycan assembly. [20] The biological activity of these native PBPs is similar to that of serine proteases, and they act as transpeptidases in the crosslinking of the glycan chains. [21, 22] PBP_2 is a bifunctional protein which, in addition to transpeptidase activity, also acts as transglycosylase. [23] PBPs bind effectively to β-lactam antibiotics, and in the presence of these agents, the cell wall assembly is discontinued.

There is evidence that the function of PBP 1 is the most important one for the survival of staphylococci exposed to β-lactams. [24, 25] PBP 4, in contrast, seems to be responsible for secondary cross-linking, as can be deduced from a low cross-linking rate in PBP 4-defective *S. aureus* mutants. [26]

O acetylation of the muramic acid is another important feature of the staphylococcal peptidoglycan. [27] Due to this, staphylococcal cell walls are rarely degraded by lysozyme, which is sterically hindered in its action. [28]

About 50% of the total mass of the cell wall consist of teichoic acid, a polymer covalently linked to the muramic acid via phosphodiester bonds. Teichoic acids consist of long chains of ribitol phosphate units ;[27] they are usually replaced by ester-linked D-alanine. [29]

Pathogenesis of *S. aureus* infections

S. aureus infections from harmless to life-threatening :

S. aureus causes a wide variety of infections, most of which are localized to the skin and are non fatal. The bacterium produces many superficial skin lesions, such as infections of hair follicles, acne, and sties (a sty is an inflammation of a gland in the eyelid). It also causes boils. In addition to skin conditions, *S. aureus* causes infections in other areas of the body. Swimmer's ear, middle ear infections, and many urinary tract infections can be caused by *S. aureus*. [6]

S. aureus can cause serious internal infections. It is the second leading cause of hospital-acquired pneumonia. It can cause meningitis usually as a result of infection after brain surgery or as a consequence of a *S. aureus* infection in the blood. *S. aureus* also causes a painful infection of joint fluid known as septic or infective arthritis. Most serious of all are the deep-seated infections such as osteomyelitis (that usually occurs in children under 12) and an infection of the heart valves called endocarditis. *S. aureus* is the most common cause of surgical wound infections. Luckily, 60–80% of surgical wound infections is superficial and is easily treated. Deeper wound infections are much more serious and almost always require additional surgery to remove infected tissue. [6]

When *S. aureus* invades the bloodstream, it can be devastating. Any localized infection can generate bacteremia and 40% of patients who get bacteremia do not have an obvious primary infection site. These bloodstream infections often occur in patients who have a surgical wound or are receiving intravenous (IV) medications or supplements, in people undergoing dialysis for kidney failure, in diabetics, and in IV drug users. Overall, the mortality rate associated with *S. aureus* bloodstream infections is about 30%. This rate is significantly higher in the very old, the very young, and people with other complicating factors, such as weakened immune systems from AIDS or other illnesses. [6]

People who have an implanted medical device are at higher risk for developing life-threatening *S. aureus* bacteremia. These devices include IV catheters used for dialysis or other procedures, prosthetic joints, and artificial heart valves. In healthy persons, 1 million bacteria are required to initiate a minor infection. This is because the *S. aureus* attaches to the foreign bodies in such a way that the immune system is unable to combat the infection effectively. In contrast, in people who have an implanted medical device, only 100 bacteria need to be present to start an infection. *S. aureus* is the most common infective agent of prosthetic joints, and these infections almost always require the removal of the joint. The higher risk of infection for this group of patients is likely due in part to the fact that these patients have underlying medical conditions and weakened immune systems. IV drug users are another group at high risk for *S. aureus* infections. [6]

Among IV drug users, 61% of endocarditis and 57% of bloodstream infections are caused by *S. aureus*. [6]

Of the many potential virulence factors produced by *S. aureus*, none can be assigned the single or even primary role contributing to the ability of the bacteria to multiply and cause progressive lesions in tissues. [30]

The fate of the lesion depends on the ability of the host to localize the process, which differs depending on the tissue involved. In the skin, spontaneous resolution of the boil by granulation and fibrosis is the rule. In the lung, kidney, bone, and other organs, the process may continue to spread with satellite foci and involvement of broad areas. In all instances the action of the cytotoxins is highly destructive, creating cavities and massive necrosis with little respect to anatomic boundaries. In the worst cases, the staphylococci are not contained, spreading to the bloodstream and distant organs. Circulating staphylococci may also shed cell wall peptidoglycans, producing massive complement activation, leukopenia, thrombocytopenia, and a clinical syndrome of septic shock. [6, 30]

Manifestations: A- primary infection

1-Furuncle and Carbuncle

The furuncle or boil is a superficial skin infection that develops in a hair follicle, sebaceous gland, or sweat gland. Blockage of the gland duct with inspissation of its contents causes predisposition to infection. Furunculosis is often a complication of acne vulgaris. The infected patient is often a carrier of the offending Staphylococcus, usually in the anterior nares. The course of the infection is usually benign, and the infection resolves upon spontaneous drainage of pus. No surgical or antimicrobic treatment is needed. Infection can spread from a furuncle with the development of one or more abscesses in adjacent subcutaneous tissues. This lesion, known as a carbuncle, occurs most often on the back of the neck but may involve other skin sites. Carbuncles are serious lesions that may result in bloodstream invasion. [30]

2-Chronic Furunculosis

Some individuals are subject to chronic furunculosis, in which repeated attacks of boils are caused by the same strain of *S. aureus*. There is little, if any, evidence of acquired immunity to the disease; indeed, delayed-type hypersensitivity to staphylococcal products appears responsible for much of the inflammation and necrosis that develops. Chronic staphylococcal disease may be associated with factors that depress host immunity, especially in patients with diabetes or congenital defects of polymorphonuclear leukocyte function. However, in most instances, predisposing disease other than acne is not present. [6, 30]

3-Impetigo

S. aureus is most often seen as a secondary invader in group A streptococcal pustular impetigo, but it can produce the skin pustules of impetigo on its own. Strains of *S. aureus* that produce exfoliatin cause a characteristic form called bullous impetigo, characterized by large blisters containing many staphylococci in the superficial layers of the skin. Bullous impetigo can be considered a localized form of scalded skin syndrome. [6, 30]

B-Deep Lesions

S. aureus can cause a wide variety of infections of deep tissues by bacteremic spread from a skin lesion that may be unnoticed. These include infections of bones, joints, deep organs, and soft tissues, including surgical wounds. More than 90% of the cases of acute osteomyelitis in children are caused by *S. aureus*. Staphylococcal pneumonia is typically secondary to some other insult to the lung, such as influenza, aspiration, or pulmonary edema. At deep sites, the organism has the same tendency to produce localized, destructive abscesses that it does in the skin. All too often the containment is less effective, and spread with multiple metastatic lesions occurs. Bacteremia and endocarditis can develop. (30)

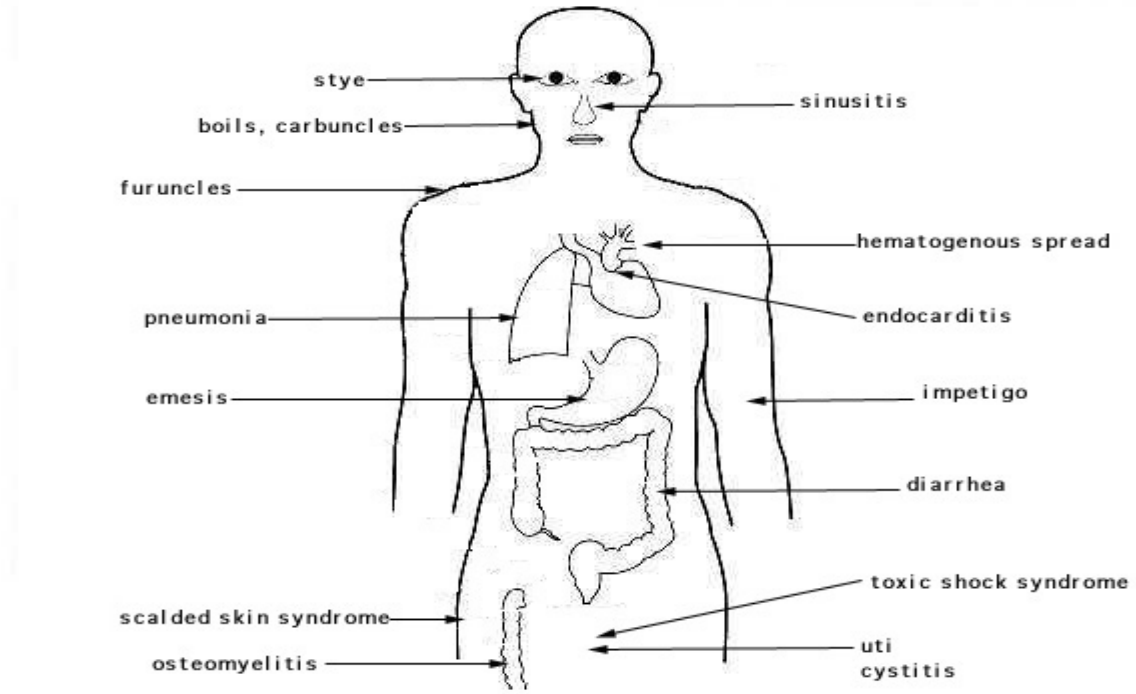

Figure 2: Sites of infection and diseases caused by *S. aureus.* (38)

C-Specific diseases caused by *S. aureus* toxins

S. aureus bacteria grow and damage tissue at the site of infection. But *S. aureus* causes another set of diseases through a different mechanism. The bacteria establish an infection in one part of the body and then release toxins into the bloodstream. These toxins then cause disease, sometimes in remote parts of the body. (6)

1-Scalded Skin Syndrome (SSS):

One of the diseases caused by *S. aureus* toxins is a serious skin condition called scalded skin syndrome (SSS). People with SSS develop a rash over most of their body and their skin becomes extremely sensitive. The disease was named "scalded skin syndrome" because the skin of the affected person looks as if it has been burned. SSS is caused by infection with a *S. aureus* strain that produces toxins that cause extensive areas of the patient's upper layer of skin peel off, especially on the hands and feet. (6)

SSS occurs most often in children, but it can also affect adults. Skin peeling occurs at places on the body other than the primary infection site. The *S. aureus* bacteria at the infection site produce the toxin that then travels through the bloodstream to other areas of the body. By an unknown mechanism, the toxin targets the skin. The primary infections that are associated with SSS can be relatively mild in children, such as an ear infection or conjunctivitis (pinkeye). Although the progression the disease is traumatic both physically and visually, there is a relatively low mortality rate. Only about 3% of children who are treated appropriately with antibiotics die from SSS, and many die not from SSS itself but from secondary infections that arise as a result of the skin loss. In adults, SSS is most often associated with a more serious primary infection, such as pneumonia or bacteremia. In addition, it mainly affects the very old or people with an underlying chronic condition such as diabetes or kidney failure, or people with a weakened immune system. As a result, adults who develop SSS have a much poorer prognosis than children (more than 50% of adults die), even with antibiotic treatment. [6]

2-Toxic Shock Syndrome

TSS is an acute and potentially fatal illness that is characterized by a high fever, diffuse erythematous rash, desquamation of the skin 1 to 2 weeks after onset (if not fatal before this time), hypotension, and involvement of three or more organ systems [31-35]. The illness was initially brought to the attention of the medical community in 1978 by Todd et al. [33], who recognized TSS as a major systemic illness associated with noninvasive *S. aureus* infections in children.

TSS is characterized by a broad spectrum of clinical and histopathological findings. [32, 35, 36] TSS is considered a capillary leak syndrome that is manifested clinically as hypotension, hypoalbuminemia, and generalized non-pitting edema. Many of the signs and symptoms of TSS appear to result from severe hypotension, but some sequelae appear to involve other pathogenic processes. [37]

The acute respiratory distress syndrome and disseminated intravascular coagulation are common and potentially life threatening complications of TSS. [35, 36]

3-Food Poisoning

S. aureus food poisoning is different from other bacteria that cause food poisoning, however, as with TSS and SSS, it is not the bacteria themselves that make the patient sick, but rather the toxins that the bacteria release into the food. In fact, in food poisoning caused by *S. aureus*, there is no infection in the body at all, and the food poisoning does not even require the person who gets sick to ingest live bacteria.[6]

Thus, it is not possible to prevent infection by heating contaminated food to kill the bacteria because the toxins are unaffected by heat. The most common foods that cause *S. aureus*-induced food poisoning include cream-filled baked goods, meat and seafood, potato and egg salads, and cheese. Of those who eat contaminated food, 80–100% will develop symptoms of food poisoning. A common cause is the unintentional contamination of food by food workers who are colonized with S.

aureus. If the food is not kept at proper temperatures, the bacteria then grow and produce toxins. [6]

The bacteria produce a family of toxins called enterotoxins, which are potent emetic agents. Scientists propose that these toxins bind to specific emesis receptors in the gut and induce vomiting very quickly. Thus, bacteria that produce enterotoxins can quickly escape the hostile environment of the digestive system. In fact, *S. aureus* induced food poisoning typically has a rapid onset within 30 minutes to 7 hours after eating contaminated food. The person suffers from an abrupt onset of severe cramps, nausea, vomiting, and diarrhea. The symptoms usually go away within 24 hours. [6]

S. aureus virulence factors: [38]

(1) **Surface proteins** that promote colonization of host tissues & Invasins that promote bacterial spread in tissues (**leukocidin**, **kinases**, **and hyaluronidase**).
(2) Surface factors that inhibit phagocytic engulfment (**capsule**, **Protein A**).
(3) Biochemical properties that enhance their survival in phagocytes (**catalase production**).
(4) Immunological disguises (**Protein A**, **coagulase**, **clotting factor**).
(5) Membrane-damaging toxins that lyse eukaryotic cell membranes (**hemolysins**, **leukotoxin**, **leukocidin**.
(6) Exotoxins that damage host tissues or otherwise provoke symptoms of disease (**SEA-G**, **TSST**, **ET**).
(7) Inherent and acquired **resistance to antimicrobial agents**.

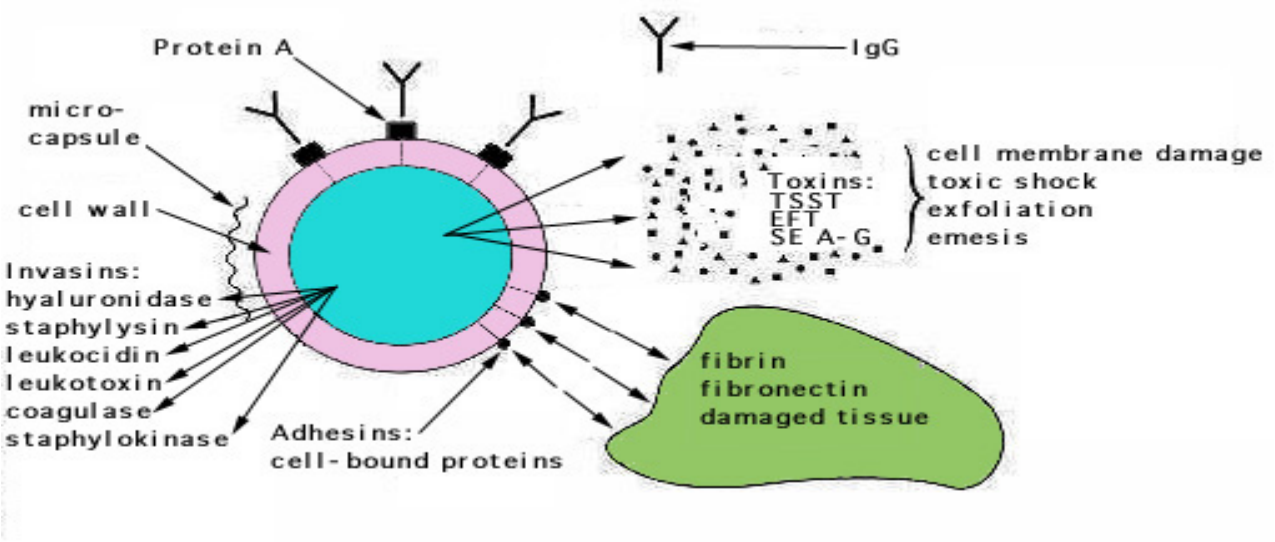

Figure 3: Virulence determinants of *Staphylococcus aureus*. [38]