DEMONSTRATION OF ANTIGENIC AND SPECIFIC WHOLE CELL PROTEINS FOR METHICILLIN-SENSITIVE *Staphylococcus aureus* AND METHICILLIN-RESISTANT *Staphylococcus aureus*

by

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Dissertation Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master Of Pathology (Microbiology)

UNIVERSITI SAINS MALAYSIA
2002

ACKNOWLEDGEMENT

My highest gratitude to God, the Most Gracious, the Most Merciful.

My sincerest appreciation to my supervisor, Dr. Haji Mohd Zaki Salleh, the pillar of strength for this study, for his invaluable guidance, supervision and encouragement without which none of this would be possible.

I am also grateful to Associate Professor Haji Mohd Radzi Johari, Head of the Department of, Professor Dr. Asma Ismail and all the staffs of Medical Microbiology and Parasitology Department and MITD especially to Rosliza, Zaheira, Nasir and Kiren who has helped bring this project to fruition.

A special thank you to Haslizai Hassan and Kadi Tiong who never failed to lend a hand and to my colleagues, Dr Julia, Dr. Habsah, Dr. Azian and Dr. Maraina for their concern, critics and advises.

Last but not least, to my family especially to my mother, Hajjah Rahimah Ismail, for their kind, patience and cooperation during this period of the study preparation and completion.

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ABSTRACT

Staphylococcus aureus is the most common and important cause of life threatening bacterial infection with a high mortality rate. The absence of a positive culture represents a dilemma in diagnosis and treatment, therefore an alternative method that is cheap, user friendly and rapid with high sensitivity and specificity is needed to help in the diagnosis.

This study was done to identify the presence of antigenic and specific proteins of whole cell for *Staphylococcus aureus*, which can be used to develop a rapid serological test. By applying the technique of sodium dodecyl sulphate-polycarylamide gel electrophoresis (SDS-PAGE) and Western blotting, three proteins with molecular weight of 18 kDa, 21. 5 kDa and 28 kDa were found to be antigenic and specific for methicillin-sensitive *Staphylococcus aureus*. These proteins were only specific to IgM antibodies but not to IgA and IgG antibodies. Two proteins were found to be antigenic and specific for methicillin-resistant *Staphylococcus aureus*. The proteins were 57 kDa protein which was specific to IgM antibody only and 40 kDa protein which was only specific to IgG antibody.

ABSTRAK

Jangkitan *Staphylococcus aureus* sering berlaku dan memudaratkan manusia dengan kadar kematian yang tinggi. Dilema dalam diagnosis dan rawatan timbul sekiranya tiada organisma dapat dipencilkan., Oleh itu satu kaedah diagnosis lain yang murah, ringkas dan cepat dengan spesifisiti dan sensitiviti yang tinggi amat diperlukan.

Kajian ini dilakukan untuk mengenalpasti kehadiran protin sel keseluruhan yang sensitif dan spesifik bagi *Staphylococcus aureus* yang boleh digunakan untuk membangunkan ujian serologi yang pantas. Dengan menggunakan kaedah elektroforesis gel natrium dodesil sulfat-poliakrilamida (SDS-PAGE) dan immunoassai blot Western, tiga protein yang mempunyai berat molekul 18 kDa, 21. 5 kDa and 28 kDa telah didapati bersifat antigenik dan spesifik bagi *Staphylococcus aureus* yang sensitif kepada metisilin. Protein ini hanya mepunyai tindakbalas dengan antibodi IgM sahaja dan tidak kepada antibodi IgA and IgG. Untuk *Staphylococcus aureus* yang rintang terhadap metisilin, dua protein yang antigenik dan spesifik telah dikenalpasti. Protein berberat 57 kDa bertindak balas secara spesifik kepada antibodi IgM sahaja dan protein berberat 40 kDa yang bertindakbalas secara spesifik kepada antibodi IgG sahaja.

CHAPTER ONE

INTRODUCTION

1.1 *Staphylococcus* spp.

1.1.1 History and taxonomy

Sir Alexander Ogston, a surgeon, was the first to describe staphylococcal disease and its role in sepsis and abscess formation, in 1880 and 1882 (Lowy, 1998). He had chosen the term Staphylococcus, derived from the Greek expression staphyle, which means bunch of grapes because of the characteristic microscopic arrangement in clusters. In 1884, Rosenbach described the two pigmented colony types of *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). *Staphylococcus albus* is now known as *Staphylococcus epidermidis.* Until now, *Staphylococcus aureus (S. aureus)* remains a versatile and important pathogen in human.

Taxonomically, the genus *Staphylococcus* is a member of the *Micrococcaceae* but phylogenetically it is unrelated to any other genera in the family. On the basis of 16s RNA analysis, the genus *Staphylococcus* belongs to the broad *Bacillus-Lactobacillus-Streptococcus* cluster. *Staphylococcus* can be further classified into three main species of clinical importance (i.e. *S. aureus*, *S. epidermidis*, and *S. saprophyticus*). The three species can be distinguished from each other by colony morphology and the following tests – catalase, coagulase, mannitol fermentation, the deoxyribonuclease test and novobiocin resistance tested by disk diffusion with a 5 μg novobiocin disk (Table 1.1).

Table 1.1 Major tests for the differentiation of human staphylococci

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Catalase	+	+	+
Coagulase	+	-	-
Acid production by mannitol fermentation	+	-	Usually positive Occasionally negative
DNAse	+	-	-
Hemolysis	+	Usually negative Occasionally positive	-
Anaerobic growth	+	+	-
Novobiocin	sensitive	sensitive	resistance

1.1.2 Morphology and physiology

Microscopically, *S. aureus* is a gram positive organism characterized by individual cocci with a diameter of 1.5 to 1.7 μm. These cocci occurs singly, in pairs, or in short chains and have a strong tendency to form clusters because cell divisions occurs in three perpendicular planes which does not lead to full separation of the daughter cells. Cluster formation is favored by culturing the organism on solid media. These properties are sometimes missing in clinical

specimens and can lead to erroneous diagnoses. Clustering can also be very limited in liquid media.

Macroscopically, individual colonies are sharply defined, smooth, opaque, and convex, cream-yellow to golden pigmentation with a diameter of 1 to 3 mm within 24 hours with beta-hemolytic properties. Pigment production can be enhanced by further incubation at room temperature and daylight for 24 to 48 hours. Most strains of *S. aureus* produce hemolysis within 24 to 36 hours on horse, sheep, or human blood-agar plates. When encapsulated, the organisms appear as mucoid, sticky colonies.

Staphylococci are non-motile and are facultative anaerob. *S. aureus* is extremely hardy and can survive drying, extremes of environmental temperature, wide ranges of pH and high salt. It can therefore survive in the hospital environment for some time and can be cultured from dried clinical material even after several months (Waldvegal, 2000).

1.1.3 Important Staphylococcal components and products

1.1.3.1 Cell wall

Peptidoglycan is the basic component of the cell wall of *S. aureus* that confers shape and stability to the organism. It represents 50% of cell wall weight. It consists of alternating polysaccharide subunits of N-acetylmuramic acid and N-acetylglucosamine with 1,4-β linkages. The peptidoglycan chains are cross-linked by tetrapeptide chains bound to N-

acetylmuramic acid and by a pentaglycine bridge specific for *S. aureus*. The basic polysaccharide polymer is found in many other organisms as well, whereas the pentaglycine cross-linking chain is specific for *S. aureus* (Schleifer & Kandler, 1972). Peptidoglycan has endotoxin-like activity, it elicits the production of interleukin-1 from human monocytes, is capable of attracting polymorphonuclear leukocytes (PMNs), activates complement, and elicits the production of opsonic antibodies (Kaplan *et al.*, 1982). Differences in the peptidoglycan structure of staphylococcal strains may contribute to variations in their capacity to cause disseminated intravascular coagulation (Kessler *et al.*, 1991)

Another important cell wall components are the teichoic acids, which are polymers of glycerol or ribitol phosphate and they can be antigenic. Ribitol teichoic acid, which is covalently bound to peptidoglycan is the major constituents of the cell wall. Lipoteichoic acid is a glycerol phosphate polymer linked to the lipid of the bacterial cell membrane.

Most staphylococci produce microcapsule or a loosely associated slime layer. 11 types of microcapsular polysaccharide serotypes have been identified and types 5 and 8 account for 75 percent of human infections. Most methicillin-resistant *S. aureus* isolates are type 5. This polysaccharide can be released during focal infection and be detected in the serum of infected animals (Arbeit & Dunn, 1987).

S. aureus has many surface proteins including major adhesins such as laminin, fibronectin-binding proteins and clumping factor (Hawiger *et al.*, 1982) to promote attachment to host protein. An adhesin that promote attachment to collagen has been found in strains that cause osteomyelitis and septic arthritis (Patti *et al.*, 1994a). Interaction with collagen may also be important in promoting bacterial attachment to damaged tissue where the underlying layers have been exposed. Several of these related proteins bind extracellular-matrix molecules and have been designated microbial-surface components recognizing adhesive matrix molecules (MSCRAMM). Recent studies suggest that these proteins play an important part in the ability of staphylococci to colonize host tissue (Patti *et al.*, 1994b).

Protein A is present in various amounts in most strains of *S. aureus*. It has antiphagocytic properties based on its ability to bind to the Fc terminal of all human IgG subclasses except IgG_3 and can also activates complement.

1.1.3.2 Enzymes

Staphylococci produce various enzymes and toxins that have been implicated in the pathogenesis of the disease, however their specific effects are often disputed because it is difficult to purify them because of instability.

Protease, lipase and hyaluronidase are enzymes that can destroy tissue. These bacterial products may facilitate the spread of infection to adjoining tissues, although their role in the pathogenesis is not well defined.

Hydrogen peroxide is produced by all staphylococcal strains and is converted into nontoxic H_2O and O_2 by the action of catalase. Because staphylococcal phagocytic killing is mediated by toxic oxygen radicals produced by PMNs, it has been proposed and shown that catalase production, by counteracting host defense mechanisms, correlates with pathogenicity (Mandell, 1975).

Coagulase is a prothrombin activator, converts fibrinogen to fibrin The reaction is used to differentiate *S. aureus* from coagulase-negative staphylococci. Its contribution to bacterial virulence is uncertain.

S. aureus forms clumps when mixed with plasma through an interaction between fibrinogen and a bacterial cell surface compound called clumping factor (Hawiger *et al.*, 1982). This receptor is also responsible for the adherence of *S. aureus* to fibrinogen and fibrin.

Beta-lactamase is an enzyme that inactivates penicillin. Penicillin-binding proteins are enzymes located in the cytoplasmic membrane that are involved in cell-wall assembly. A novel penicillin-binding protein is

responsible for staphylococcal resistance to the penicillinase-resistant penicillin and cephalosporin.

1.1.3.3 Toxins

S. aureus produces numerous toxins. Some of them express their detrimental effect by enzymatic action and others such as toxic shock syndrome toxin and enterotoxins are potent cytokines inducer that act as superantigen.

There are five membrane-damaging toxins produced by *S. aureus* – α-toxin, β-toxin, δ-toxin, γ-toxin and leukocidin. α-toxin is the most potent membrane-damaging toxin. Susceptible cells have a specific receptor for α-toxin, which allows the toxin to bind causing small pores. After binding, a complex series of secondary reaction occurs causing release of cytokines and trigger the production of inflammatory mediators. The consequent cellular damage may contribute to manifestations of the sepsis syndrome. β-toxin produces its cytotoxic effect on sphingomyelin and therefore active on variety of cells including human erythrocytes, leukocytes and fibroblast. The majority of human isolates of *S. aureus* do not express β-toxin. γ-toxin lyses erythrocytes by unknown mechanism and δ-toxin disrupts biologic membrane by a detergent-like action. Panton-Valentine leukocidin, is a leukocytolytic toxin that has been epidemiologically associated with severe cutaneous infection. It consists

of two components, which act together to damage membrane by forming pores, which leads to increased permeability to cations.

The exfoliative toxins, including epidermolytic toxins A and B, causes skin erythema and separation as seen in scalded skin syndrome. Recent data suggest that these toxins are serine proteases and act as superantigen (Marrack & Kappler, 1990).

Toxic shock syndrome toxin-1 (TSST-1), a pyrogenic exotoxin, is the cause of toxic shock syndrome (TSS) which is characterized by fever, desquamative skin rash, hypotension and multisystem involvement. It is structurally similar to Enterotoxin B and C. The gene for toxic shock syndrome toxin 1 is found in 20 percent of *S. aureus* isolates.

About half of all strains of *S. aureus* isolated produce enterotoxins (A to E, G and H) (Archer, 2000). These heat-stable toxins are major causes of food poisoning and increase intestinal peristalsis, possibly by sympathetic activation. A central nervous system effect is also suggested by the intensity of vomiting in food poisoning. When expressed systematically, enterotoxins can also cause toxic shock syndrome. In fact, enterotoxin B and C cause 50% of non-menstrual cases of TSS.

TSST-1, enterotoxins, and exfoliative toxins belong to the family of superantigens, which are molecules that share the characteristics: they

bind with high affinity to major histocompatibility complex class II receptors of monocyte-macrophages at sites distinct from the classic antigen-binding groove (Kim *et al.*, 1994). This complex is recognized by the variable V_β region of the T-cell receptor of some subsets of T lymphocytes, and superantigen-receptor interaction causes prolific activation or, under certain circumstances, inhibition of T-cell functions, including liberation of interleukin-1, tissue necrosis factor and interferon-gamma (Marrack *et al.*, 1990). Small foci of superantigen-producing *S. aureus*, at a concentration of 10^{-13} to 10^{-14} mol/l, can lead to dramatic cytokine liberation which lead to major systemic effects such as fever, hypotension, skin lesions, shock, multiorgan failure and death.

1.1.4 Antibiotic susceptibility

Shortly after penicillin G became available, Spink and Ferris in 1945 reported the isolation of a resistant *S. aureus* strain that produced a beta-lactamase, a serine protease (penicillinase) that inactivated the antibiotic (Waldvegal, 2000). It rapidly spread to many *S. aureus* isolates and stimulated the development of semisynthetic penicillinase-resistant compounds; methicillin, the isoxazolyl penicillins (oxacillin, cloxacillin, etc.), and nafcillin between 1960 and 1964. They solved the resistance problem only temporarily. Isolation of methicillin-resistant strains was reported in 1961 by Barber (Waldvegal, 2000).

Methicillin resistance or intrinsic resistance has created major therapeutic, management, and epidemiologic problems throughout the world. This high level

of resistance requires the presence of the mecA gene that encodes penicillin-binding protein (PBP) 2a. PBP 2a has low affinity for all beta-lactams, conferring resistance not only to methicillin and other penicillin but also cephalosporin and carbepenams. The mecA genes probably originated from a different species of staphylococci. Although many methicillin-resistant strains appear to be descendants of a limited number of clones, some appear to be monoclonal in origin, suggesting the horizontal transfer. The expression of resistance to methicillin is often heterogenous, and the percentage of a bacterial population that expresses the resistance phenotype varies according to the environmental conditions. The mecA gene, which has been cloned and sequenced, can be detected by DNA probes or by polymerase chain reaction (PCR).

Methicillin resistance is defined as an oxacillin MIC of 4 mg/liter or greater or a methicillin MIC of 16 mg/liter or greater. Several methods are available to detect methicillin resistance, which is improved by supplementing the media with sodium chloride, a low temperature of 30°C, and a high inoculum. When disk diffusion is used, an oxacillin disk charged with 1 μg should be applied to Mueller-Hinton agar containing 5% sodium chloride and incubated at 37°C or without sodium chloride and incubated at 30°C with an inoculum of 10^6 cfu/ml.

MRSA frequently is cross resistant to aminoglycosides, lincosamides, macrolides, tetracyclines, trimethoprim and sulfonamides. Aminoglycoside-resistant strains have been described with increasing frequency however the

percentage seems to be lower for fusidic acid. Rifampin, which is remarkably active against *S. aureus*, cannot be used as a single agent because of a high one-step mutation rate of 10^{-7} to 10^{-8} to resistance. Resistance to fluoroquinolones has been found in methicillin-sensitive (Kaatz *et al.*, 1991) and methicillin-resistant strains (Murakami & Tomasz, 1989) and is becoming a major epidemiologic problem.

Now, a new threat has emerged: the first case of vancomycin-intermediate *S. aureus* (VISA) was reported in 1996 from Japan (Hiramatsu *et al.*, 1997) followed by various other strains in the United States, and several other countries. The mechanism of resistance in these isolates is not known but is not due to van resistance genes present in vancomycin-resistant enterococci. The suggested mechanisms are increased cell-wall synthesis and alterations in the cell wall that prevent vancomycin from reaching sites of cell-wall synthesis.

The emergence of intermediately vancomycin-resistant *S. aureus* has created considerable concern in the medical community and are likely to pose a major therapeutic challenge in the future.

1.1.5 Epidemiology

1.1.5.1Colonization and Infection

Humans are natural reservoir of *S. aureus*. Shortly after birth, many neonates are colonized by *S. aureus* by their immediate human surroundings either on the skin, the umbilical stump, the perineal area

and sometimes the gastrointestinal tract. Later on most children and adults will become intermittently colonized by *S. aureus* and harbor the organism either in their nasopharynx or occasionally on their skin and clothing, rarely in the vagina (an important prerequisite in toxic shock syndrome), or exceptionally in the rectum or perineal area. Thirty to 50 percent of healthy adults are colonized, with 10 – 20 percent persistently colonized (Casewell & Hill, 1986). Both methicillin-sensitive and methicillin-resistant isolates are persistent colonizers. In a recent study done in the community of New York City, the prevalence of *S. aureus* nasal carriage was 35% in children and 28% for guardians. (Shopsin *et al.*, 2000)

Some groups of population are more prone to colonization with *S. aureus*. For example higher percentage of carriers are found in physicians, nurses and hospital ward attendants compared to general population. Rates of staphylococcal colonization are also high among patients with Type 1 diabetes, intravenous drug users, patients undergoing hemodialysis, patients with acquired immunodeficiency syndrome (Archer, 2000) and in surgical patients (Kluytmans *et al.*, 1995).

Persons colonized with *S. aureus* are at risk for subsequent infections (Wenzel & Perl, 1995). The mucous membranes and the skin offer a very efficient mechanical barrier against local tissue invasion. If this barrier is

breached by trauma or surgery, *S. aureus* may gain access to the underlying tissue and create a characteristic local abscess lesion. Toxin liberation to skin and other organs can cause various types of skin rash and general symptoms. At any time, multiplying bacteria can overcome local phagocytic mechanisms and gain access to the lymphatic channels and the blood stream. The ensuing staphylococcal bacteremia is a dreaded complication, and it can lead to metastatic infections (e.g., endocarditis, pneumonia, or osteomyelitis) and to the patient's demise.

MRSA strains are usually introduced into an institution by an infected or colonized patient (nasopharynx, tracheostomy site, wound) or by a colonized health care worker. Transfer from one patient to another via the colonized hands of health personnel or the inanimate environment (Mulligan *et al.*, 1993) has led to major epidemics in tertiary care hospitals, as well as in chronic care facilities. Risk factors for the acquisition of MRSA include the administration of multiple antibiotics. Colonization of the anterior nares by MRSA carries a significantly greater risk for infection than does colonization by sensitive strains (Muder *et al.*, 1991). Thus, epidemiologic surveys and control measures are particularly important regarding MRSA.

1.1.5.2 Transmission

Persons colonized with *S. aureus* strains are at increased risk of becoming infected with these strains. From the anterior nares, carriers transfer the organisms to their skin. Trauma provides a portal of entry for

the organism, with subsequent local and possibly generalized infection. In case of infection, the organism is often of endogenous origin, whereas in other cases, it is transmitted by hospital personnel or a family member. Most cases of nosocomial infection are acquired through exposure to the hands of health care worker after they have been transiently colonized with staphylococci from their own reservoir or from contact with an infected patient.

1.1.5.3 Temporal trends in S. aureus disease

The numbers of both community acquired and hospital-acquired infections have increased in the past 20 years. This trend parallels the increased use of intravascular devices. During the period from 1992 through 1997, *S. aureus* was the most common cause of nosocomial cases of pneumonia and the third most common cause (after coagulase-negative staphylococci and enterococci) of nosocomial bloodstream infections according to data from the National Nosocomial Infections Surveillance system of the Centers For Disease Control and Prevention (Richards *et al.*, 1999). In St. Thomas' Hospital, United Kingdom, *S. aureus* accounted for 11% of bacteremic organisms from year 1986 to 1995 (Edgeworth *et al.*, 1999).

A second trend is the worldwide increased of infections caused by methicillin-resistant *S. aureus* (MRSA) (Panlilio *et al.*, 1992). MRSA are also increasingly found in the community (Moreno *et al.*, 1995) Data

from the National Nosocomial Infections Surveillance system of USA from the period 1987 to 1997 show the number of methicillin-resistant *S. aureus* infections in intensive care units has continued to increase. A study on the susceptibility pattern of *S. aureus* isolated in Malaysian hospital showed that 40% of *S. aureus* are methicillin-resistant (Rohani *et al.*, 1999). Methicillin-resistant strains have also become resistant to other antimicrobial agents (Speller *et al.*, 1997). The same 10-year CDC survey showed proportion of methicillin-resistant isolates with sensitivity only to vancomycin increased from 22.8 percent in 1987 to 56.2 percents in 1997.

1.1.6 **Pathogenesis**

The disease syndromes caused by *S. aureus* can be divided into two different mechanisms. The organisms can become locally or systematically invasive by producing a variety of components that can thwart host defence mechanisms, or it can elaborate toxins that cause disease without the need for the organism itself to invade tissue.

Colonization by *S. aureus* requires initial adherence to host cells. Adherence to nasal mucosal cells is mediated by the teichoic acid component of *S. aureus* and by a variety of other cell-associated ligands on *S. aureus* It is increased in chronic staphylococcal carriers and after vaccination or infection with influenza A (Davidson & Sanford, 1982). *S. aureus* binding to mucin may also be critical for colonization of the nasopharyngeal mucosa (Shuter *et al.*, 1996).

The factors that result in initial *S. aureus* infections are not clear, normal individuals seem to be fairly resistant to local infection. Intact cornified squamous epithelium is normally a barrier both to colonization and infection by *S. aureus.*

Infections are initiated when a breach of the skin or mucosal barrier allows staphylococci access to adjoining tissues or the bloodstream. Whether an infection is contained or spreads depends on a complex interplay between *S.aureus* virulence determinants and host defense mechanisms.

Patients with certain congenital defects are predisposed to *S. aureus* infection. These includes chemotaxis defects as in Job's syndrome, Chediak–Higashi syndrome and Wiskott-Aldrich syndrome, opsonization defects, staphylocidal defects of polymorphonuclear leukocytes (Waldvegal, 2000).

The risk of infection is also increased by the presence of foreign material. Elek and Conen first demonstrated the ability of sutures to reduce the threshold for infection (Walgvegal, 2000). Several factors contribute to the increased susceptibility to infection. Phagocytic function in the presence of foreign material is seriously impaired. Devices such as intravenous catheters are rapidly coated with serum constituents, such as fibrinogen or fibronectin, which enable staphylococci to adhere through MSCRAMM-mediated mechanisms and to elaborate glycocalices that further facilitate colonization. Intravenous catheters are frequently implicated in the pathogenesis of nosocomial endocarditis. The

introduction of long-term indwelling catheters has led to cases of nosocomial endocarditis. The catheter traumatizes the valvular surface, creating a nonbacterial thrombus on the cardiac valve that facilitates subsequent bacterial adherence.

1.1.6.1 Local infection

The hallmark of localized staphylococcal infection is an abscess. An abscess consists of central necrosis and liquefaction and containing cellular debris and multiplying bacteria surrounded by a layer of fibrin and intact phagocytic cells. The abscess may be superficial, in skin (furuncle), or deep, in organs (renal carbuncle) as a result of bacteremic dissemination.

1.1.6.2 Invasive infections

A small percentage of local infections progress to dissemination, where *S. aureus* gains access to the blood. Staphylococcal bacteremia may be complicated by endocarditis, metastatic infection, or the sepsis syndrome. The endothelial cell is central to these pathogenic processes. Staphylococci avidly adhere to endothelial cells and bind through adhesin-receptor interactions. In vitro studies demonstrate that after adherence, staphylococci are phagocytized by endothelial cells. The intracellular environment protects staphylococci from host defense mechanisms as well as the bactericidal effects of antibiotics. These factors may enhance bacterial survival and contribute to the development of persistent or recurrent infections.

Staphylococcal strains that cause endocarditis adhere to both damaged and undamaged native valvular surfaces, are resistant to platelet microbicidal proteins and elaborate proteolytic enzymes that facilitate spread to adjacent tissues. The adherence of staphylococci to the platelet-fibrin thrombus that forms on damaged valvular surfaces may involve the adherence MSCRAMM proteins to exposed matrix molecules. Staphylococcal endocarditis also occurs on undamaged valves. The invasion of endothelial cells by *S.aureus* may initiate the cellular alterations, including the expression of tissue factor, that promote the formation of vegetations.

The cellular events leading to septic shock are similar in staphylococcal infection and infection with gram-negative bacteria. In both cases, monocytes and macrophages have a central role, although polymorphonuclear leukocytes, endothelial cells, and platelets also play a part. The monocytes release tumor necrosis factor-α and interleukin-1, interleukin-6, and interleukin-8 after contact with intact staphylococci, peptidoglycan, or lipoteichoic acid. In contrast, the expression of interleukin-1 and interleukin-6 by endothelial cells requires bacterial phagocytosis. As a result of cytokine and cellular activation, the complement and coagulation pathways are activated, arachidonic acid is metabolized, and platelet-activating factor is released. These events, in turn, cause fever, hypotension, capillary leak, disseminated intravascular coagulopathy, depression of myocardial function, and multiorgan

dysfunction. Several staphylococcal components appear to be capable of initiating the sepsis syndrome. Peptidoglycan, especially when combined with lipoteichoic acid, reproduces many of the physiologic responses of endotoxin in animal models of sepsis. Alpha toxin alone reproduces many of the findings of sepsis, including hypotension, thrombocytopenia, and reduce oxygenation, in animal models.

1.1.6.3 Toxin-mediated disease

S. aureus produces toxins, that produce specific syndromes without the need for the organism itself to invade and disseminate. Enterotoxins, exfoliative toxin and toxic shock syndrome toxin (TSST-1) have superantigen activity. Superantigens stimulate T cells non-specifically without normal antigenic recognition. Up to one in five T cells may be activated, whereas only 1 in 10,000 are stimulated during a usual antigen presentation. Cytokines are released in large amounts, causing the symptoms of TSS. Superantigens bind directly to class II major histocompatibility complexes of antigen-presenting cells outside the conventional antigen-binding groove. This complex recognizes only the V_β element of the T cell receptor. Thus any T cell with the appropriate V_β element can be stimulated, whereas normally antigen specificity is also required in binding.

Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. TSST-1 is expressed

systemically and is the cause of toxic shock syndrome (TSS). When expressed systemically, enterotoxins can also cause toxic shock syndrome. TSS can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically and the host lacks appropriate neutralizing antibodies.

Exfoliatin toxin (ET) causes the scalded skin syndrome in neonates, which results in widespread blistering and loss of the epidermis. There are two antigenically distinct forms of the toxin, ETA and ETB. The toxins have a specific esterase activity, but it is not clear how this would causes epidermal splitting. There is some evidence that the toxins have protease activity, so it is also possible that the toxins target a very specific protein, which is involved in maintaining the integrity of the epidermis.

1.1.6.4 Host response to infection

The typical pathological finding of staphylococcal disease is abscess formation. Leukocytes are the primary host defense against *S. aureus* infection (Verdrengh & Tarkowski, 1997). The migration of leukocytes to the sites of infection results from the expression of adhesion molecules on endothelial cells. This cytokines mediated process is triggered by bacteria and tissue-based macrophages. After infection, cytokines are first demonstrable within vessels, extending into tissues as inflammatory cells migrate to the sites of infection.

The presence of opsonizing antibody directed against capsule, peptidoglycan, or complement facilitates phagocytosis in vitro (Peterson *et al.*, 1978; Karakawa *et al.*, 1988). The role of antibody in vivo is less certain, since the titer of antistaphylococcal antibodies is not correlated with protection from infection, except in which the presence of anti-toxic shock syndrome toxin-1 is protective (Freedman & Beer, 1991).

IgG antibody response has been reported (Wheat, *et al.*, 1978). High levels of IgG antibody were present in 70% - 90% of patients with staphylococcal endocarditis or bacteremia complicated by secondary sites of infection but not in patients with uncomplicated staphylococcal bacteremia. To study the IgM response to staphylococcal infections, Wheat *et al.* in 1981 used solid-phase radioimmunoassay on ultrasonic extract of the Wood 46 strain of S. aureus. High levels of IgM were less frequent than high levels of IgG antibodies in patients with staphylococcal infection and both IgG and IgM were present in healthy control subjects but at lower levels than in infected patients. Concomitant elevations of levels of IgM and IgG antibodies to staphylococci was highly suggestive of staphylococcal endocarditis or complicated bacteremia.

1.1.7 Clinical and pathological features

S. aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. The infection can either be trivial, superficial, localized skin

infections such as folliculitis, stye and furuncles (boils) or more serious infections, such as pneumonia, mastitis, phlebitis, meningitis, urinary tract infection, and deep-seated infections such as osteomyelitis and endocarditis. *S.aureus* is a major cause of hospital-acquired infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of pyrogenic extoxins into the blood stream.

1.1.7.1 Skin and soft tissue infections

The most common *S. aureus* infections are folliculitis and the furuncle, or boil. These infections involve a single hair follicle or a localized area of the epidermis and dermis. Furuncles can coalesce and spread causing a more extensive and serious infection called carbuncle. Carbuncles are most common over the upper back and back of the neck, where they can form multiple draining sinuses; bacteremia occurs in approximately one fourth of patients. A boil or furuncle also may be called a skin abscess if it becomes large but remains circumscribed, confined to one area, and fluctuant. *S. aureus* can also cause cellulitis, particularly common in individuals with pre-existing chronic skin disease such as stasis dermatitis and diabetic, trophic, or decubitus ulcers.

Impetigo is a very superficial staphylococcal skin infection that affects mostly children, usually on exposed areas of the body (e.g., on the face and the legs). Although most of these infections are due to *S. aureus*,

about 10% are due to *Streptococcus pyogenes* and another 10% will yield both organisms on culture

The most common nosocomial *S. aureus* skin and soft tissue infection is the wound infection, in which surgical or catheter exit-site wounds are contaminated with *S. aureus* and become erythematous, draining purulent or serosanguineous fluid. *S. aureus* is the most common and most serious cause of hospital-acquired wound infections, leading to local, deep-wound infections and systemic, metastatic infections due to bacteremia.

1.1.7.2 Respiratory infections

S. aureus is not a common cause of pneumonia in healthy, unhospitalized adults, accounting for less than 10% of community-acquired pneumonia. However, following influenza A infections, the incidence of *S. aureus* pneumonia markedly increases. Chest radiographs of patients with community-acquired *S. aureus* pneumonia may show abscesses and thin-walled cysts, resembling the pneumatoceles seen in infants.

In contrast to community-acquired pneumonia, *S. aureus* is a prominent cause of nosocomial pneumonia, particularly in intubated patients on mechanical ventilation. Cultures obtained from intubated patients by techniques designed to minimize contamination of specimens by organisms colonizing the upper airway have found *S. aureus* in up to a

third of patients. Pneumonia in ventilator-dependent patients is a particularly lethal event, with one fourth to one half of the patients dying as a direct result of their pulmonary infection. The radiographic appearance of nosocomial *S. aureus* pneumonia cannot be differentiated from that of pneumonia due to other nosocomial pathogens.

1.1.7.3 Bacteremia

The most common life-threatening manifestation of *S aureus* infection is bacteremia. A substantial proportion of *S. aureus* bacteremia appears to be of endogenous origin since they originate from colonies in the nasal mucosa (Von Eiff *et al.*, 2001).

An increasing percentage of bacteremic infections are related to catheterization. In about one third of cases of septicemia, no initial focus of infection could be detected. The consequences of of nosocomial infection are usually fever and malaise, but they can include endocarditis, osteomyelitis, metastatic abscesses in various organs, and death from overwhelming sepsis.

Use of injecting drugs is another contributing factor to the increased incidence of *S aureus* bacteremia. Users of injecting drugs often have nasal colonization with *S aureus* and likely transmit the organism to the skin, and eventually to the bloodstream, following unsterile injection practices. In users of injecting drugs, *S aureus* bacteremia is often accompanied by tricuspid valve endocarditis.