3. Review of Literature

*Staphylococcus* is a versatile organism with several virulent characteristics and resistance mechanisms and was first identified in pus by the surgeon Sir Alexander Ogston in Aberdeen, Scotland in 1883 [Ogston 1883, Fowler Jr, 2006]. The first observation pointing to the endogenous source of bacterial wound infection was made in 1915 by Sir Almroth Wright [Wright 1915]. Humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter. Multidrug resistance is now the norm among the Gram-positive bacteria pneumococci, enterococci and staphylococci. *S. aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections in humans and in various animal species, and its capacity to adapt to different environmental conditions. The evolution of increasingly antimicrobial resistant bacteria stems from a multitude of factors, including the strong selective pressure caused by the widespread and sometimes inappropriate use of antimicrobial agents, the increase in regional and international travel and the relative ease with which antimicrobial-resistant bacteria cross geographic barriers [Lowy, 2003].

The resistance of *Staphylococcus aureus* to phenolics has been described as early as 1925 [Reddish et al 1925]. In 1932, Panton and Valentine described leukocidin as a virulence factor. Panton Valentine leukocidin (PVL) is composed of LukS-PV and LukF-PV [Prevost et al. 1995], and exhibits lytic activity against polymorphonuclear cells, monocytes, and macrophages in humans and rabbits [Cribier et al. 1992]. In 1935, Glenny and Stevens differentiated beta toxin from alpha toxin by antibody neutralization and showed that it lysed sheep, but not rabbit erythrocytes [Arbuthnott 1982]. It is also unique in its ability to cause hot-cold lysis. *S. aureus* beta toxin is encoded by the gene hlb [Projan et al 1989]. Gamma-hemolysin was found to be produced by over 99% of clinical *S. aureus* strains. It is able to lyse erythrocytes from a wide range of mammalian species. In addition, gamma-hemolysin has
leukotoxic properties as it is able to lyse polymorphonuclear cells, monocytes and macrophages (Smith and Price, 1938). Beta toxin is a neutral sphingomyelinase and displays species dependent activity. In 1947, Williams and Harper [1947] proposed the existence of delta toxin which is unique since it is small, heat stable, has surfactive properties and is lytic towards many types of membrane from most animal species including those on erythrocytes, other cells, organelles and even bacterial protoplasts [Williams and Harper, 1947; Arbuthnott 1982].

The first case of penicillinase producing S. aureus was published in 1940, almost a year before penicillin was marketed for clinical use [Abraham 1940]. After the first therapeutical use of penicillin in 1941, ß-lactam antibiotics have been used extensively and successfully as antimicrobials in the treatment of bacterial infections. However, soon reports appeared of penicillin resistant S. aureus strains producing the enzyme ß-lactamase (called penicillinase until 1960), which hydrolyses the ß-lactam ring. The first strain of penicillin G resistant S. aureus was isolated in London, England soon after the introduction of Penicillin G into hospitals. When Kirby's first description of penicillinase-producing strains of S. aureus was published in 1944 [Kirby, 1944], resistance was infrequently encountered, with only a handful of strains available for study. As with MRSA, penicillinase-producing strains first were isolated from hospitalized patients. The prevalence of penicillinase-producing strains of S. aureus within hospitals soon began to rise as penicillin became readily available after World War II. Within a few years, most hospital isolates were resistant to penicillin [Barber et al, 1948]. It has been found previously that penicillinase-producing strains of Staphylococcus aureus each harbor an extrachromosomal element, or plasmid, which apparently carries all the genetic information necessary for penicillinase synthesis.

The earliest known Staphylococcus aureus plasmid encoding multidrug efflux was characterized from a clinical strain dating from 1951 by Paulsen IT et al. Penicillinase stable cephalosporins and semi synthetic penicillins were introduced in the late 1950s. Macrolides, lincosamides and streptogramins were first introduced in 1952, are often referred to as MLS antibiotics. They inhibit protein synthesis by binding to the 50S ribosomal subunit. The glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria. Vancomycin
resistance among staphylococci was developed in laboratories even before the drug was in use clinically (Geraci 1956; Ziegler 1956). The first identified virulence factors of staphylococci were leucocidins that causes formation of pores in the cell membrane [Gladstone 1957]. Approximately one half of all skin infections are caused by *S. aureus* [Elek 1957].

In the early 1960s the introduction of methicillin promised to resolve one of the therapeutic problems associated with infections due to multi resistant, penicillinase producing *S. aureus*. But the first methicillin resistant strain was described in the UK in 1961 [Jevons 1961]. Between 1960 and 1963 a moderate increase in the number of methicillin resistant strains on the order of 5% was noted. Homogenous populations replaced progressively heterogeneous populations of MRSA. Staphylococcal enterotoxin A was first produced in vitro in 1963 [Casman and Bennett 1963]. In Australia, no resistant strains had been identified as of 1964, but 17% of isolates were methicillin resistant in 1965. Between 1965 and 1969 the incidence of methicillin resistant strains increased from 11 to 28% in Zurich, Switzerland hospitals. In France, the incidence of methicillin resistant strains in nine Parisian hospitals was 12% in 1961, 19% in 1963 and 35% in 1966. In Denmark in 1967 about a quarter of Staphylococcal isolates were methicillin resistant. In the United States, a few strains were isolated in 1963. In fact no methicillin resistant strains were isolated in Boston, Massachusetts hospitals between 1960 and 1967. In 1967 however 1.4% of *S. aureus* isolates were methicillin resistant.

The nose is regarded as the major site of *S. aureus* carriage from where the organism can spread to other parts of the body [White 1963; William 1966]. Direct contact transmission involves contact of body surface to body surface and physical transfer of *S. aureus* to the host from an infected or colonized person [Fekety Jr 1964].

Studies by Novick and Richmond in 1965 revealed that a certain amount of variability has been encountered among the penicillinase plasmids harbored by different staphylococcal strains. It was been found that: (i) there are at least three molecular variants of the enzyme itself; (ii) most, but not all, of the penicillinase plasmids carry a genetic determinant of resistance to mercuric ion; (iii) plasmids carried by a very small number of the strains bear a determinant of resistance to erythromycin; (iv) the plasmids determine the fraction of penicillinase excreted into
the medium during growth, and this also varies from strain to strain. Jarlomen et al. showed that Staphylophage 80, propagated on a hospital strain of *Staphylococcus aureus* 80/81, could transduce antibiotic resistance markers such as penicillin and tetracycline to a variety of staphylococcal recipient strains in vitro [Jarlomen et al. 1965].

In 1965 Gravenkemper et al. discovered two strains resistant to methicillin which were very active producers of penicillinase, and exhibited cross-resistance with other antistaphylococcal antibiotics. Resistant cultures showed resistance to methicillin only with large inocula, and consisted of a mixture of cells. The methicillin-resistant strains caused destruction of methicillin and oxacillin in vitro, but the rate of hydrolysis was slow. Antibiotic destruction was probably due to high concentrations of staphylococcal penicillinase, and not to another specific enzyme [Gravenkemper et al. 1965].

Weaver and Pattee examined the resistance of *Staphylococcus aureus* to erythromycin and it was found to possess the characteristics of an inducible enzyme. The induction of resistance to high concentrations of erythromycin in *S. aureus* occurred only after prior exposure to subinhibitory concentrations of erythromycin [Weaver and Pattee 1964]. Initial studies on staphylococcal plasmids were confined to those that gave resistance to penicillin G and other penicillins, but subsequently plasmids conferring resistance to erythromycin (Novick & Richmond 1965), neomycin (Lacey 1971), tetracycline, chloramphenicol (Chabbert et al. 1964), streptomycin (Grubb & O'Reilly 1971) and fusidic acid (Evans & Waterworth 1966) have all been described and methicillin resistance is a multifactorial process, one element of which is specified by a plasmid [Dombusch et al. 1969]. In practice, it is rare to find more than one type of resistance determined by a single staphylococcal plasmid [Richmond 1972]. In 1969 the first clinical *S. aureus* isolate resistant to gentamicin was reported [Lacey & Mitchell 1969]. There was no systematic surveillance for antibiotic resistance among *S. aureus* isolates circulating within communities. The first comprehensive description and accurate assessment of the epidemiology of drug-resistant strains of *S. aureus* were published in 1969 by Jessen et al. [Jessen et al. 1969]. In the early 1970s, physicians were finally forced to abandon their belief that, given the vast array of effective antimicrobial agents, virtually all
bacterial infections were treatable. Their optimism was shaken by the emergence of resistance to multiple antibiotics among pathogens like *S. aureus*.

The successful development of methicillin and cephem antibiotics in the 1960s was quickly followed by emergence and world wide dissemination of methicillin and cephem resistant *S. aureus* in 1970. World wide epidemics of *S. aureus* diseases have been recognized over the years (Klimek 1976; Lacey 1973). But at the beginning of 1970s, a significant decrease in the number of methicillin resistant strains was reported. Although penicillinase-producing strains were universally present in hospitals by the early 1950s, community isolates of *S. aureus* were considered to be largely penicillin susceptible. Penicillin continued to be recommended as an effective anti-staphylococcal agent as late as the early 1970s [Weinstein 1975]. The first methicillin and aminoglycoside resistant strain was described in Australia in the mid 1970s and shortly afterwards throughout the world. Classical genetic experiments had shown that mec is not transferable between *S. aureus* strains by conjugation [Lacey 1972] but is transferable by bacteriophage mediated generalized transduction [Cohen and Sweeny 1970].

In 1976 methicillin and gentamicin resistant strains were described. However a resurgence of methicillin and aminoglycoside resistant strains were noted at the end of 1970s and the beginning of 1980s in France, Ireland, Greece, Australia and South Africa. No vancomycin-resistant staphylococci were reported in the first 20 years the drug was used. Then the first reports of vancomycin resistance in coagulase-negative staphylococci emerged in 1979 and 1983 [Siebert 1979; Tuazon and Miller 1983]. In Greece between 1978 and 1979 about 50% of the strains were methicillin resistant in Athens hospital. From the mid 1970s the number of resistant isolates had increased progressively and epidemics have been described in all regions of US.

The disease Toxic Shock Syndrome (TSS), mediated by toxic shock syndrome toxin (TSST-1) was first described in 1978 and designated TSS by Todd et al. (1978). Toxic shock syndrome is a staphylococcal illness characterized by acute onset of high fever above 104°F, diffuse eryhematosus rash, desquamation of the skin one to two weeks after onset (if not fatal), especially on the palms and soles; hyperemia of mucous membranes hypotension, and involvement of multiple organ systems as evidenced by diarrhea, thrombocytopenia, cardiopulmonary dysfunction or a variety of
other symptoms [Davis et al 1980; Shands et al 1980]. Toxin mediated diseases caused by other staphylococcal toxins included scalded skin syndrome, are known to be infrequent in the United States, but there are a few data available on the prevalence of such diseases in other parts of the world.

The toxin responsible for TSS, known as the toxic shock syndrome toxin (tsst-1) was first identified by Schlievert et al [Schlievert et al 1981] and the criteria for defining TSS cases were established in 1981 [Reingold et al 1982]. TSST-1 was found to be a potent super antigen eliciting a variety of cytokines that along with tumour necrosis factor, contribute a variety of illness [Schlievert et al 1981; Uchiyama et al 1994]. It has also been described in association with many types of S. aureus infections some of which are quite minor [Parsonnet 1996]. It is produced exclusively by S. aureus, and approximately 20% of natural isolates are producers. TSST-1, formerly designated enterotoxin F, was the first toxin shown to be involved in TSS and is accepted as the major toxin associated with this illness, whether menstrual or nonmenstrual, accounting for about 75% of all cases.

VISA isolates were first found in nature more than 25 years ago while investigators were screening isolates for vancomycin susceptibility [Watanakunakorn 1984]. Staphylococci were found to produce diseases in 2 ways: directly by invasion and subsequent tissue destruction whether locally or after having spread via the blood stream and through the effects of toxin [Sheagren 1984]. Staphylococcal food poisoning is the leading cause of food borne microbial intoxication world wide and is usually linked to improper storage of food [Holmberg and Blake 1984]. S. aureus can invade and survive inside epithelial cells including endothelial cells which may also allow it to escape host defenses particularly in endocarditis [Ogawa 1985; Hamill 1986; Arrecubieta 2006; Moreillon 2002]. Time to onset of symptoms after consuming contaminated food averages 4.4 hours [Holmberg 1984].

Ogawa et al [1985] demonstrated that following adherence staphylococci are endocytosed into membrane bound vacuoles in an endothelial cell mediated process. Following internalization there is a fusion of the phagosome with cellular lysosomes and limits any intracellular bacterial replication. Endothelial cells are ineffective phagocytes and Staphylococci are capable of prolonged intracellular survival. Bacteria are released into the medium, spread to the underlying extracellular matrix and cause
progressive cellular damage, ultimately destroying the cell [Hamill et al 1986; Lowy et al 1988; Vann et al 1987]. Virtually all strains of staphylococci of human origin are lipolytic. Genes encoding lipases have been cloned from *S. aureus* (Lee and Iandolo, 1986), and infections include folliculitis, cellulitis, furuncles, carbuncles, hydradenitis suppurativa, mastitis, pyodermas and pyomyositis. Impetigo which involves release of epidermolytic toxins, can range from mild recurrent infections to the more serious bullous impetigo characterized by blisters that continually break and become infected to the potentially life threatening Scalded skin syndrome [Swartz 1987]. It is the leading cause of both soft tissue and bone and joint infections [Maki 1987].

Resistance to trimethoprim (Tp) was found to be mediated by a plasmid-encoded gene in staphylococci. The staphylococcal Tp gene codes for a single protein with DHFR activity that appears to be unrelated to DHFR genes that mediate Tp in members of the Enterobacteriaceae [Coughter et al 1987]. The bifunctional enzyme AAC(6’)/APH(2’’) encoded by aac(6’)-aph(2’’) gene was found to inactivate a broad range of clinically useful aminoglycosides such as gentamycin, tobramycin, netilmicin and amikacin [Lovering et al 1988; Rouch et al 1987]. Genetic and biochemical properties of the tetracycline resistance element of the *Staphylococcus aureus* plasmid pT181 have been studied [Mojumdar and Khan 1988].

In Italy, 6% of isolates were methicillin resistant in 1981 and 6% were resistant in 1986. From 1988 a further increase in these strains was noted. This study also showed that these strains produced large quantities of penicillinase and were resistant to streptomycin and tetracyclines. At the time of their introduction fluoroquinolones showed good activity against methicillin susceptible and resistant *S. aureus* [Gahin-Hausen 1987]. However the sporadic emergence of resistance was soon reported in *S. aureus* isolated from the skin flora of patients during ciprofloxacin therapy [Humphreys 1985], as well as in the skin flora during the treatment of methicillin resistant *S. aureus* carriers [Mulligan 1987].

Studies by Janosi et al 1990, revealed that the determinant for PMS phenotype was located on plasmids, which also encoded beta-lactamase production and cadmium ion resistance, but not arsenate resistance. Three types of plasmid with molecular size of 50 kilobases (kb), 23.8 kb, and 16.8 kb, were found among the strains with PMS
resistance phenotype, and the 50 kb and 23.8 kb plasmids also encoded mercury resistance [Janos et al 1990].

*Staphylococcus aureus* has been pointed out as being the agent responsible for 19% of the nosocomial infections at the Hospital São Paulo, 60% of the isolates being MRSA [Wey et al 1990]. Studies have proven that infection control measures should be accompanied by identification of carriers and subsequent elimination of *S. aureus* carriage [Working Party 1990]. One report mentions the complete control of an outbreak when only simple infection control measures were taken [Guiguet 1990], while several others found that only extensive modifications of local infection control practices were effective [Bitar 1987; Boyce 1981; Kluytmans et al 1995, Murray-Leisure et al 1990].

*S. aureus* has been found to be the second most prevalent organism causing intravascular device associated bacteremia. [Schaberg et al 1991]. Blood stream infections are defined as infections in which no other primary site can be discerned [Banerjee et al 1991]. Regan et al have shown that elimination of nasal carriage by using topical mupirocin also eliminates hand carriage [Regan 1991]. A survey conducted in Central Europe in 1991 showed that the incidence of oxacillin resistant strains of *S. aureus* ranged from 4 to 30.9% depending on the country. The proportion of ciprofloxacin resistant strains ranged from 26 to 47% but is greater than 70% in some countries. The proportion of MRSA rapidly increased from below 5% in the early 1980s to 29% in 1991 [Panlilio et al 1992]. This study also showed that the prevalence generally increased with the size of the hospital. The interspecies conjugation between vancomycin resistant enterococci (VRE) and *S. aureus* has been known to occur since 1992, when Noble demonstrated it by experimental transfer of vancomycin resistance from VRE to *S. aureus* in the laboratory [Noble et al 1992].

In 1992 a mechanism of fluoroquinolone resistance which is the NorA gene was characterized and cloned from chromosomal DNA of a quinolone-and methicillin-resistant *Staphylococcus aureus* strain conferring resistance to hydrophilic quinolones such as norfloxacin, enoxacin, ofloxacin, and ciprofloxacin. The uptake of a hydrophilic quinolone, enoxacin, by *S. aureus* harboring a plasmid carrying the *norA* gene was found to be about 50% of that by the parent strain lacking the plasmid. On
the other hand, the uptake of a hydrophobic quinolone, sparfloxacin, was hardly affected by the norA gene [Yoshida 1992].

Aminoglycosides inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit. The major mechanism of aminoglycoside resistance in staphylococci, is drug inactivation by cellular enzymes such as aminoglycoside acetyltransferases (AAC), aminoglycoside adenylyl transferases (also named aminoglycoside nucleotidyltransferases [ANT]), or aminoglycoside phosphotransferases (APH). A number of loci encoding these enzymes have also been identified in staphylococci (Shaw et al 1993).

Lee et al in 1993 reported the incidence of MRSA strains in South Korea. Between 1964 and 1968 no strain was resistant to methicillin. In 1980, 1985, 1990, 1991 and 1992 the percentages of methicillin resistant strains were respectively 14.1, 22, 43.4, 63 and 61%. Clindamycin, erythromycin, cyclines and Cotrimoxazole were inactive against 88, 97, 86 and 4% of MRSA respectively. In a Spanish survey conducted in November 1991, 15.7% of MRSA strains were resistant to ofloxacin. Use of vancomycin had increased dramatically over the years, because of the increasing prevalence of methicillin resistance in both coagulase-negative staphylococci and Staphylococcus aureus (Ena 1993). In Sweden in 1993, 76% Staphylococcus strains were resistant to piperacillin and between 4.5 and 15% were resistant to ciprofloxacin. Sader et al. [Sader et al 1993] found a prevalence of approximately 70% MRSA among Staphylococcus aureus isolates in some hospitals of the metropolitan area of Sao Paulo. Mortality from S. aureus bacteremia ranges from 11 to 48%, a figure that has increased steadily for a number of years [Mortara et al 1993].

Mulligan et al states that indications for eradication of MRSA are elimination of an outbreak in a health care setting and prevention of recurrent infections in an individual. In settings where MRSA is recurrent elimination of carriage has not been found to be cost effective [Mulligan et al 1993]. In an outbreak situation the first goal is to identify all carriers including patients and health care workers. Then elimination of carriage should be achieved in all identified carriers [Coello et al 1994, Kluytmans et al 1995]. Hand washing remains the single most important measure to reduce the risk of transmitting microorganisms from one person to another [Emori et al 1993,
Garner et al 1996]. In addition to hand washing, gloves play an important role in reducing the risks of transmission of *S. aureus* in health care settings. Containment measures for patients with infections with *S. aureus* usually require standard precautions which include hand washing after touching body fluids or contaminated items whether or not gloves are worn. Restriction from patient care activities or food handling is indicated for personnel who have draining skin lesions with *S. aureus* until they have received appropriate therapy. No work restrictions are needed for colonized personnel unless they have been epidemiologically implicated in *S. aureus* transmission in a facility.

Until 1990s MRSA rarely caused infections among community members without exposure to health care settings. An out break of CA-MRSA infections occurred between 1989 and 1991 among indigenous Australians in Western Australia without health care contact [Udo et al 1993]. Infections were also reported from neighboring regions [Gosbell et al 1993]. The disease spectrum included abscesses, bacteremia, central nervous system infections, endocarditis, osteomyelitis, Urinary tract infections and a host of syndromes caused by exotoxins including bullous impetigo, food poisoning, Staphylococcal scalded skin syndrome and Toxic shock syndrome. Surgical site infections (SSIs) constitute approximately 15% of the infections reported to the NNIS system by hospitals that collected hospital wide surveillance data [Emori and Gaynes 1993; NNIS 1996; Nawas et al 1998]. Transmission of *S. aureus* strains from health care workers to patients have resulted in mediastinitis [Gaynes et al 1991], nosocomial toxic shock syndrome [Kreiswirth et al 1986] and the spread of antimicrobial resistant strains within an institution [Dominguez et al 1994].

The intracellular environment provides a potential sanctuary protecting staphylococci from host defence mechanisms as well as from the bactericidal effects of antibiotics [Balwit et al 1994; Vesga et al 1996]. Several studies have been devoted to identify the adhesion mechanisms of *Staphylococcus aureus*, which are the most frequent causes of prosthesis-associated infections. *S. aureus* is capable of expressing two fibronectin-binding proteins, FnBPA and FnBPB, encoded by two closely linked genes (Jonsson et al 1991). Recently, in particular for *Staphylococcus aureus*, considerable attention has been given to the host protein receptors as mediators for
bacterial adherence. Fibrinogen binding protein is an important factor that promotes bacterial adhesion. *Staphylococcus aureus* has been shown to interact specifically with fibrinogen. Three different extracellular fibrinogen-binding proteins, two of which have coagulase activity, are produced by *S. aureus* strain. The role of these fibrinogen-binding proteins during staphylococcal colonization and infection has not yet been fully elucidated [Boden and Flock 1994]. As implanted biomaterial always gets coated by a fibronectin-layer, these proteins are suggested to be important in the promotion of foreign body infections (Greene et al 1995).

In a multi centre European study, Voss et al [1994] collected 7333 strains of *S. aureus*, 12.8% of which were resistant to methicillin. The proportion varied according to the country or region, far less from 1% in Scandinavia to more than 30% in Italy, Spain and France. A high level resistant to ciprofloxacin was noted. This study revealed an interesting variation in the geographical distribution of MRSA in Europe according to which the prevalence of MRSA increases significantly from the northern to Southern countries. In India in some of the centres the isolation varied from 20-40 per cent [Geha et al 1994].

In the Netherlands, where MRSA is rare and cross-transmission is therefore readily detected by simple procedures such as phage typing, there was 92% homology in MSSA postsurgical wound infections with the patients’ own nasal isolates, and 86% of MSSA infections were due to a unique strain [Kluytmans et al 1995]. Gamma toxin, leukocidin and other bicomponent toxins are a family of proteins encoded by the hlg and luk PV loci. These toxins contain 2 synergistically acting proteins: one S component and one F component designated on the basis of their mobility. PVL S and F components are LukS-PV and LukF-PV. S and F components of gamma toxin are designated as HlgA and HlgB respectively [Prevost et al 1995].

Morton et al studied conjugative plasmids encoding high-level mupirocin resistance found in *Staphylococcus aureus* isolates from two geographic locations in the United States. Transfer genes on three mupirocin resistance plasmids with different restriction endonuclease profiles were found. The mupirocin resistance gene was flanked by two directly repeated copies of IS431/257 [Hodgson et al 1994; Morton et al 1995].
Out breaks in neonates usually result in skin infections and bacteremia although more serious diseases such as osteomyelitis and meningitis can occur [Haley et al 1995]. The nationwide use of 3rd generation cephalosporins in Japan in the early 1980s coincided with the abrupt and dramatic rise in MRSA detection rates [Tanaka et al 1995]. Although the presence of Oxacillin Resistant *Staphylococcus aureus* (ORSA) in the Asia-Pacific and South Africa (APAC) region has been well recorded, data on the true prevalence of ORSA in the region are limited. Longitudinal data from Australia have been published and show that in hospital isolates from the Eastern Seaboard, the percentage of *S. aureus* strains that were ORSA remained relatively constant at approximately 30% from 1986 to 1994 (Turnidge et al 1996.).

Colonisation particularly of the nares by *S. aureus* leads to hand carriage and from the hands the organism are frequently spread to other areas of the body. Thus Staphylococci follow a nose to hand to wound route of infection. [Fekety Jr 1964; Zimakoff et al 1996]. Contact transmission is the most important and frequent mode of transmission for *S. aureus* [Bolyard et al 1998; Garner 1996].

In India 32.8 per cent MRSA was reported in Mumbai, Delhi and Bangalore in 1996. *Staphylococcus aureus* strains isolated from pus or blood of patients during January 1993 to November 1994 at Vellore were tested for antimicrobial susceptibility and 24% were found to be MRSA. 97 per cent were resistant to trimethoprim-sulphamethoxazole; 85.5 per cent to gentamicin and 45 per cent to amikacin. While over 90 per cent were resistant to norfloxacin and ciprofloxacin, only 53 per cent were resistant to ofloxacin [Mathur et al 1994; Pulimood et al 1996]. In a surveillance study conducted simultaneously at three centres across India, Mehta et al found that from a total 739 cultures of S aureus, 235 (32%) were found to be multiply resistant with the individual figures for resistance being 27% (Bombay), 42.5% (Delhi) and 47% (Bangalore) [Mehta et al 1996].

In 1996 the three phenotypes of resistance to MLS in *S. aureus* has been studied. The first is mediated by 23S-rRNA methylases, encoded by three homologous staphylococcal determinants, *ermA*, *ermB* and *ermC*, which cause a target alteration of the ribosome, thus preventing antimicrobial agents from binding to their ribosomal target site. The second resistance type, mediated by *msrA/msrB* and *vga*, is based on the active efflux of the antimicrobial agent by an ATPdependent pump, thereby
maintaining intracellular concentrations below the level required for binding to ribosomes [Allignet et al 1996].

Virtually any *S. aureus* infection can lead to bacteremia. *S. aureus* causes about 11 to 38% of community acquired bacteremia [Cockerill et al 1997; Kauffman and Bradley 1997]. In a review of data on adult bacteremia from 3 hospitals by Weinstein et al [1997] *S. aureus* was the most common cause of clinically significant bacteremia (18.9%). Approximately 10 to 40% of community acquired cases of *S. aureus* bacteremia progress to endocarditis [Dajani et al 1997; Karchmer 1992; Mortara and Bayer 1993]. Staphylococcal osteomyelitis is classified as either acute or chronic [Waldvogel and Vasey 1980]. Acute hematogenous osteomyelitis is usually a disease of children primarily neonates in whom it affects the long bones of the lower extremity [Rissing 1997].

Antimicrobial agents of the class of tetracyclines bind to the 30S ribosomal subunit, thus resulting in inhibition of protein synthesis. Staphylococci can become resistant to tetracyclines in two different ways. The first mechanism of resistance is based on ribosomal protection and is encoded by the *tet*A(M) gene. The second tetracycline resistance mechanism in staphylococci is active efflux mediated by the *tet*A(K) or *tet*A(L). Sulfonamide resistant *S. aureus* has been reported soon after the introduction of these antimicrobial agents. The chromosomal determinant *sul*A is responsible for an increased production of *p*-aminobenzoic acid. The resistance mechanism encoded by plasmid determinant *sul*B is yet unknown. Chloramphenicol acts as a bacteriostatic antimicrobial agent that binds to the 50S bacterial ribosomal subunit, thus inhibiting the transpeptidation in protein synthesis. Although it has not been widely used as an antistaphylococcal agent, resistance has arisen and is due to the inactivation of the antibiotic by a chloramphenicol acetyltransferase enzyme (CAT), which acetylates the drug [Paulsen et al 1997].

A review of 50574 *S. aureus* nosocomial infections reported to the NNIS system from 1987 to 1997 showed that 29% were resistant to the semi synthetic beta lactam agents oxacillin, nafcillin and methicillin [NNIS 1996]. Several previous reports suggests that the prevalence of *S. aureus* strains resistant to methicillin, nafcillin or oxacillin has increased in the United States and abroad [Aubry-Damon et al 1997; Barrett et al 1998; Irish et al 1998; Struelens et al 1994; Zimakoff et al 1996]
and such strains have the propensity to cause outbreaks [Dominguez et al 1994; Haley et al 1995; Roman et al 1997]. AAD2’-aph6’ and is the most frequently encountered aminoglycoside resistance mechanisms among staphylococcal isolates [Busch-Sorensen et al 1996; Martineau et al 2000].

Overall for the decade 1987 to 1997 the percentage of MRSA isolated from patients in an ICU and patients in non critical care wards were 32% and 26.3% respectively. The development of vancomycin resistance among S. aureus isolates would dramatically impact the ability to control the spread of MRSA in health care settings [Barrett et al 1998; CDC 1997]. Fluoroquinolone resistance emerged very rapidly in HA-MRSA in the years after widespread utilization of agents of this class; at one institution, fluoroquinolone resistance increased from 7% before 1988 to 83% in 1990 [Hershow et al 1998].

S. aureus is an unusual cause of community acquired pneumonia but is a common etiological agent of pneumonia in the hospital setting, frequently as a consequence of influenza [Lowy 1998; NNIS 1998]. In addition to being the leading cause of bacteremia in the United States Staphylococci also are among the four most common causes of food borne illness, surpassing even campylobacteriosis and listeriosis [Armstrong et al 1998]. Typically S. aureus pneumonia develops in elderly patients approximately 4 to 14 days following the onset of influenza. Mortality is approximately 15 to 20%. Chest pain, fever, shortness of breath and signs of pleural effusion are nearly always present [Lowy 1998].

The first clinical isolate of VISA was reported in 1995, which was from a French child who had been receiving vancomycin for an MRSA infection (Ploy et al 1998). The resistance to vancomycin was not easy to induce and so it was thought unlikely to occur in a clinical setting [Moellering 1998]. Though a cause for concern, reports of vancomycin resistance in coagulase negative Staphylococci did not generate a great deal of attention as CoNS are generally considered to be relatively avirulent organisms. A very different response greeted the first report of decreased susceptibility to vancomycin in S. aureus in 1997 (CDC 1997; Hiramatsu 1997). The increase in multiresistant cocci is due to a significant increase in vancomycin use during the last two decades, which evidently served as a selective pressure for the emergence of VISA and VRSA in US hospitals [Kirst et al 1998]. Given the enormous
public health concerns regarding the dissemination of VISA and VRSA, the Hospital
Infection Control Practices Advisory Committee (HICPAC) of the CDC published
infection control guidelines for all staphylococci for which the MIC of vancomycin is
\( \geq 8 \mu g/ml \) in 1997 [CDC 1997].

Studies by Sa Leao et al revealed that over 90% of the MRSA strains belonged
to two clones: the most frequent one, designated the "pediatric clone," was reminiscent
of historically "early" MRSA. Most isolates of this clone were only resistant to beta-
lactam antimicrobials and remained susceptible to macrolides, quinolones,
clindamycin, spectinomycin, and tetracycline. They showed heterogeneous and low-
level resistance to methicillin (MIC, 1.5 to 6 microg/ml). The second major clone was
the internationally spread and multiresistant "Iberian" MRSA with homogeneous and
high-level resistance to methicillin (MIC, >200 microg/ml). [Sa-Leao et al 1999].

In 1996, a wound infection caused by VISA was reported in Japan in a child
receiving vancomycin for an MRSA wound infection [Hiramatsu, Aritaka 1997;
Hiramatsu, Hanaki 1997]. A hVISA strain (Mu3) was first identified in 1996 in Japan
in a 64-year-old patient with MRSA pneumonia whose condition responded poorly to
vancomycin treatment [Hiramatsu 1997]. VISA strains were first reported in UK and
1999; Rotun et al 1999].

In *Staphylococcus aureus* strains resistance to beta lactams is determined by
*blaZ* gene carried on a plasmid. In contrast to most *S. aureus* isolates in which the
gene for staphylococcal beta-lactamase (*blaZ*) is plasmid borne, isolates typeable by
集团 II bacteriophages frequently carry *blaZ* on the chromosome. Furthermore, the
chromosomal gene encodes the type B variant of staphylococcal beta-lactamase. The
type and amount of enzyme produced by phage group II isolates were studied by
Voladri and Kernodle [1998]. They identified type B and type C enzymes each
encoded on the chromosome and 21-kb plasmid respectively.

The distribution of fluoroquinolone resistance-associated point mutations in
genes encoding the subunits of DNA gyrase and DNA topoisomerase IV was
examined in clinical isolates of *Staphylococcus aureus* and point mutations were
detected by polymerase chain reaction (PCR) and restriction fragment length
polymorphism analysis and mutations were characterized by sequencing of PCR
products. Mutations at Ser84 of gyrA and Ser80 of GrlA were widely distributed among isolates exhibiting various degrees of fluoroquinolone resistance. gyrB and grlB mutants were rare. These studies found that gyrA and grlA mutations impart high levels of fluoroquinolone resistance in *S. aureus* clinical isolates [Takahashi et al 1998].

Mupirocin resistance was correlated with the presence of plasmids in methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in the Rio de Janeiro Federal University Hospital in Brazil, where topical mupirocin has been used extensively since 1990. Strains exhibiting high-level resistance carried a large and relaxable plasmid of about 35 kb. Mupirocin-sensitive derivatives, obtained by growth at 42°C of a strain exhibiting high-level resistance, were devoid of the large plasmid [Bastos et al 1999]. In 1997 a collaborative epidemiological survey carried out in 19 European hospitals showed that 1.6% *S. aureus* showed high level mupirocin resistant.[Schmitz and Jones 1997; Maniatis et al 2002; Udo et al 1999].

The staphylococcal Panton-Valentine leukocidin (PVL) genes, [lukS-PV-lukF-PV], existing on the genome of a temperate bacteriophage phiPVL was isolated from mitomycin C-induced *Staphylococcus aureus* V8 (ATCC 49775) [Kaneko et al 1997]. Gravet et al characterized the staphylococcal bi-component leucotoxins family, LukE (32 kDa) and LukD (34.3 kDa) from *Staphylococcus aureus* strain Newman and found that LukE+LukD was as effective as the Panton-Valentine leucocidin for inducing dermonecrosis when injected in the rabbit skin, but not hemolytic and poorly leucotoxic compared to other leucotoxins expressed by *Staphylococcus aureus* [Gravet et al 1998]. The Panton-Valentine leukocidin (Luk-PVL) belongs to the family of bicomponent toxins and Luk-PVL is associated with skin and soft-tissue infections as well as with more serious infections, e.g., severe necrotizing pneumonia [Diep et al 2004; Lina et al 1999].

Studies by Bayles et al reported staphylococcal internalization by bovine mammary cells. Following entry some bacteria escape the membrane bound vacuoles and induce cellular apoptosis [Bayles et al 1998]. The changes resulting from internalization of staphylococci precipitate a series of cellular changes that contribute to the pathogenesis of life threatening diseases. The events include the ability of *Staphylococcus aureus* to cause (1) endocarditis by adhering to and invading damaged
or undamaged valvular tissue and causing progressive local cardiovascular damage (2) metastatic infections by traversing endothelial or other epithelial cell barriers, elaborating proteolytic enzymes and spreading to adjoining tissues or (3) a sepsis syndrome similar to that produced by endotoxin resulting from endothelial cell injury with the expression of endothelial cell receptors and the release of cellular cytokines [Hamill et al 1986; Ing et al 1997; Lowy 1998].

The molecular epidemiology of MRSA in a university hospital in Italy was studied in a five-month period in 1996, during which all *S. aureus* isolated were collected. The study found that MRSA represented 32.3% of all isolates, with very high percentages from the intensive care units (adult and neonatal). [Villari et al 1998]. Results for the United Kingdom show that the increase in MRSA proportions, reported from 1992 through 1998 (Speller et al 1997, Reacher et al 2000), continued until 2001. In Germany a national surveillance study carried out at regular intervals, reported an increase of MRSA from 2% in 1992 to 21% in 2001 (Kresken and Hafner1999). In India about 32% MRSA was reported in Rohtak and Mangalore in 1999 [Verma et al 2000].

Mutations of the *rpoB* gene conferring resistance to rifampin were analyzed in MRSA isolates obtained from six countries. The majority of clinical isolates showed multiple mutations within *rpoB*. Cross-resistance to rifampin, rifabutin, and rifapentine was demonstrated for all mutants identified. The level of resistance to rifamycins correlated with both the mutation position and type of amino acid substitution [Wichelhaus et al 1999].

Since 2000, an increase in MRSA strains with low cefazolin MICs were observed. It was an important pathogen in human infections causing a wide variety of infections from mild skin infection to more serious and invasive infections [Tenover et al 2000]. 50-70% MRSA was reported from Japan by Takeda et al in 2000 and in Latin America by Gales et al. [Takeda et al 2000; Gales et al 2000]. In other countries such as Tunisia, Malta, Algeria [Kesah et al 2000], Sweden, Switzerland, the Netherlands (SENTRY participants group, 2001) it was low.

Many *S. aureus* strains produce one or more specific staphylococcal exotoxins, including staphylococcal enterotoxins (SEs), staphylococcal exfoliative toxins, and toxic shock syndrome toxin 1 (TSST-1). These toxins cause infections ranging from
relatively mild involvement of the skin and soft tissue to life-threatening sepsis, necrotizing pneumonia, and TSS [Gillet et al 2001; Lina et al 1999; Lowy 1998; McCormick et al 2001; Novick 2000]. In recent years, the existence of new types of SE genes (seg, seh, sei, sej, sek, sel, sem, sen, seo, sep and seq), which belong to the operon of the enterotoxin gene cluster (egc), has been reported [Jarraud et al 1999; Jarraud et al 2001; Nilsson et al 1999; Omoe et al 2002]. In addition, SE-like proteins R, S, T, and U have been identified but remain poorly characterized.

In Poland about 15% of S. aureus were resistant to methicillin and more than 60% were resistant to cyclines but >90% remained susceptible to aminoglycosides. 31% MRSA was observed in Kenya and Cameroon [Kesah et al 2000]. In Australia the frequency of methicillin resistant strains varied according to geographical location: 25.2% in Queensland and 0.4% in the western region of Australia during 1993. Of interest is the significant proportion of ORSA isolates from Australia that were non multi drug resistant. 83% of these isolates were found in a single Western Australian institution. NORSA strains emerged from the remote Kimberley region of Western Australia in the mid-1980s. Since that time, the epidemiology of these strains has been further characterized. They are most commonly found in remote Central and Western Australian Aboriginal communities, where skin colonization rates were as high as 42% and skin sepsis is the most frequent clinical manifestation. While they typically caused community-acquired infections, they have been responsible for hospital outbreaks [Turnidge and Bell 2000; Waldvogel 2000].

The reporting of methicillin resistance for Staphylococcus aureus bacteraemia was made mandatory in England from April 2001 precisely because it was seen as a marker of the prevalence of MRSA infection, which in turn was seen as a marker of infection control practice and rates of hospital-acquired infection. [PHLS Communicable Disease Surveillance Centre.2001] In a survey conducted in the UK and Ireland 2001–2002 by the BSAC found a resistance rate of 42.0% to oxacillin. Oxacillin-resistant S. aureus showed a high prevalence of resistance to ciprofloxacin (95%) and erythromycin (84%). 23% were resistant to clindamycin. There was some resistance to gentamicin (10%, mostly with MICs of 64 mg/L) but little to tetracycline (<2%), and none to vancomycin, teicoplanin or linezolid. 30% resistance to trimethoprim was found.
MRSA is an important cause of infection in hospitalized patients throughout the APAC region. The proportion of *S. aureus* strains resistant to oxacillin in the APAC region (45.9%) was high (ranging from 5.0% in the Philippines to 79.5% in Hong Kong). This proportion was higher than those reported from other geographic regions over the same time period: Latin America (34.9%), United States (34.2%), Europe (26.3%), and Canada (5.7%) (Diekema et al 2001.). A small number of strains, mostly from Western Australia, were nonmultidrug-resistant (NORSA). 33% MRSA was reported from Jeddah, Saudi Arabia [Madani et al 2001] and a much lower prevalence of 12% was reported from the eastern province [Bukharie 2001].

The high prevalence of VRE in US hospitals paved the way for emergence of vanA gene transfer to MRSA. Rapid and steady rise in the number of MRSA and VRE in US hospitals in the past 2 decades is another contributing factor increasing the chance of encounter of 2 species in patient’s bodies [Farr et al 2001]. The significant rise in MRSA and VRE in US hospitals is correlated with the generous use of broad spectrum cephalosporins such as ceftriaxone and ceftazidime [Harbarth et al 2001].

Actual MRSA prevalence is subject to wide geographical variation. In Europe MRSA rates as high as 58% in Italy and 54% in Portugal have been reported [Fluit et al 2001]. In India studies by Vidhani et al reported sensitivity to macrolide group of antibiotics like Erythromycin (17.7%) in MRSA. Amongst the aminoglycosides, maximum sensitivity was seen with amikacin and 26.6% MRSA were sensitive to the same. 67% of MRSA were found to be sensitive to fluoroquinolone group i.e. ofloxacin. All *S. aureus* isolates (MSSA and MRSA) were found to be uniformly sensitive to vancomycin [Vidhani et al 2001].

*S. aureus* has an enhanced ability to produce biofilm on inert polystyrene surfaces and to adhere to and invade epithelial airway cells. The detection of the genes governing the production of extracellular polysaccharide involved in biofilm formation, in particular, the *icaA*, the *icaC* and the *icaD* genes were studied by Aricola et al [2002].

Now MRSA is prevailing both inside and outside hospitals, gradually replacing methicillin susceptible *S. aureus* (MSSA), which is a part of the normal flora of healthy humans [OkumaK 2002]. Bacteraemia reports to the Health Protection
Agency documented a rapid rise in methicillin resistance in *S. aureus* during the 1990s, from under 1.7% of *S. aureus* isolates in 1990, through 4% in 1993, 21% in 1996 and 34% in 1998 to reach 42% in England and Wales in 2000 [Reacher et al 2000; PHLS.2002; Johnson et al 1997]. 42% in 2001 and 43% in 2002 was also reported [Health Protection Agency. 2002, Health Protection Agency.2003.]. Sentinel data from the European Antimicrobial Resistance Surveillance System (EARSS, http://www.earss.rivm.nl) also report a steady level (44%) of methicillin resistance in the UK in 2001 and 2002. *S. aureus* had become the most frequent cause of nosocomial pneumonia [Chastre 2002]. Occasional isolates of *S. aureus* with reduced susceptibility to vancomycin have been reported in the UK in recent years, [Howe et al 1998, Hamilton-Miller 2002.]. In 2002, the first fully vancomycin resistant clinical *S. aureus* strains were isolated in US from a diabetic patient in Michigan, US. Vancomycin resistant *S. aureus* (VRSA) strains contained plasmids carrying vanA gene complex carried on a transposons which had been transferred by conjugation from vancomycin resistant enterococci (VRE) that coexisted in the patient’s body [Bartley 2002].

Between April 1998 and December 1999, in the APAC region Oxacillin resistance was detected in 43.1% of blood isolates, 56.9% of respiratory tract isolates, 40.5% of wound isolates, and 57% of urine isolates, the majority of which were multi drug resistant. Multidrug resistance (MORSA) was defined as resistance to penicillin and oxacillin plus three or more of the agents such as erythromycin, clindamycin, rifampin, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, and chloramphenicol. Considerable variation was found in resistance profiles between countries in ORSA strains. The predominant antibiogram for Australia, Singapore, and Taiwan included resistance to erythromycin, clindamycin, ciprofloxacin, gentamicin, tetracycline, and sulfamethoxazole-trimethoprim. The most common profile for Japan, Hong Kong, and mainland China included resistance to erythromycin, clindamycin, ciprofloxacin, tetracycline, and gentamicin for Hong Kong and China, as well as a proportion of Japanese strains (56.5%) [Bell et al 2002].

Linezolid is an important therapeutic option for treatment of infections caused by glycopeptide- and beta-lactam-resistant gram-positive organisms. Linezolid resistance was found to be caused by mutations within the domain V region of the 23S
ribosomal RNA (rRNA) gene, which is present in multiple copies in most bacteria. Pillai et al. found that after serial passage on antibiotic-free medium, the isolate maintained resistance to high concentrations of linezolid and compared with linezolid-susceptible *S. aureus* isolates, the linezolid-resistant *S. aureus* isolate demonstrated no significant differences in in vitro growth characteristics [Pillai et al 2002]. Since 1997 the potential benefits of macrolides therapy in cystic fibrosis patients have been promoted and resistance reached 53% in cystic fibrosis patients compared to 38% in the same geographical area in the general population [Prunier et al 2003].

In India Krishnan et al. studied the predominance of one or more epidemic strains within an endemic situation. A representative selection of 65 isolates of MRSA over a period spanning five years (1994-1998) was subjected to antibiogram comparison, phage-typing and pulsed field gel electrophoresis (PFGE) using SmaI. The antibiogram comparison was not a discerning method of strain discrimination. At least 11 different phage-types were seen among these 65 isolates; 35.4% belonged to phage-types A and SM-A. The study showed that the isolates from India were diverse and distinct from strains of MRSA such as E-MRSA reported from many studies in the UK [Krishnan et al 2002].

In 2003 Salgado et al. reported that according to the NNIS survey MRSA is currently the most commonly identified antibiotic-resistant pathogen in US hospitals and 50% of hospital acquired infections in the ICUs in the USA were due to MRSA [Salgado et al 2003, NNIS 2003]. In North America Hoban et al. reported 43.2% MRSA [Hoban et al 2003]. In Brazil, the frequency of isolation of *Staphylococcus aureus* and its role in nosocomial infection has been greater than in other countries [Trindade et al 2003]. 33% MRSA was reported in Saudi in 2003 [Austin et al 2003]. Several studies have established that the transmission of MRSA between patients within hospital settings occur to a great extent through health care workers (Berthelot et al 2003, Blok et al 2003).

Studies by Orwin et al. identified a novel enterotoxin-like protein that is a member of the new subfamily (group V) of pyrogenic toxin superantigens (PTSAgs) and examined its biochemical and immunobiological properties. They demonstrated that the PTSAg had many of the biological activities associated with SEs, including superantigenicity, pyrogenicity, and ability to enhance endotoxin shock, but lacked
both lethality in rabbits when administered in subcutaneous miniosmotic pumps and emetic activity in monkeys [Orwin et al 2002]. Omoe et al identified and characterized a novel staphylococcal enterotoxin-like putative toxin, which is named SER [Omoe et al 2003].

Analysis of the natural population dynamics and expansion of pathogenic clones of *S. aureus* provided evidence that essentially any *S. aureus* genotype carried by humans can transform into a life-threatening human pathogen but that certain clones are more virulent than others (Melles et al 2004). The incidence and trends of MRSA during a 12-year (1989-2000) period at a university teaching hospital was carried out and the relationship between strain distribution studied by antibiogram and molecular typing. The studies showed the establishment of a dominant MRSA clone (PFGE type A group) in the intensive care, medical, and surgical units and the appearance of a new MRSA strain in 1995 (PFGE type B), which partly explained the rise in incidence of MRSA cases and a disproportionate rise in MRSA bacteremia from 1995 to 2000 in Hong Kong [IP et al 2004].

Surveillance of MRSA in Europe during the period 1999 to 2002 by Tiemersma et al included 50,759 isolates from 428 laboratories serving approximately 500 hospitals. Overall, 20% of these isolates were reported as methicillin resistant. The lowest MRSA prevalence was reported in northern Europe and highest prevalence in southern Europe, Israel, the United Kingdom, and Ireland. MRSA proportions varied almost 100-fold, with the lowest proportion in Iceland (0.5%) and the highest proportion in Greece 44%. Increases in MRSA proportions were significant in Belgium (from 22% in 1999 to 27% in 2002), Ireland (39%–45%), Germany (9%–19%), the Netherlands (0.4%–1%) and the United Kingdom (31%–45%). The proportion of MRSA decreased significantly in Slovenia only, from 22% in 2000 to 15% in 2002. Vancomycin resistance did not occur. Intermediate susceptibility of *S. aureus* (VISA) was only reported for five isolates from France in 2001. [Tiemersma et al 2004]. Studies by Howe et al showed that reduced susceptibility to vancomycin has emerged in many successful epidemic lineages with no clear clonal disposition [Howe et al. 2004].

Characteristics of *Staphylococcus* that help it evade host immune systems has been reviewed by Foster [Foster 2005]. Several epidemiological studies have found
increased morbidity and mortality from nosocomial MRSA compared with those from MSSA [Cosgrove et al 2003; Gastmeier et al 2005; Engemann et al 2003; Reed et al 2005]. Outbreaks have been reported in a variety of settings including hospitals (Mallaval et al 2004), long term care facilities (Larsen et al 2005), outpatient clinics (Lee et al 2005), as well as in the community (Rihn et al 2005). Community MRSA strains have several distinguishing characteristics that may enable them to more readily colonize and infect otherwise healthy hosts [Nour et al 2005].

It was believed that food poisoning in Osaka in 2000 was due to small amounts of staphylococcal enterotoxin A (SEA) in reconstituted milk. Studies by Ikeda et al in 2005 indicated that SEH was also present in the raw material of reconstituted milk, indicating that the food poisoning was caused by multiple staphylococcal enterotoxins [Ikeda et al 2005]. Al Haj Hussein et al [2005] reported a prevalence of 31% MRSA in Saudi in 2005. In Japan nearly 70% of S. aureus blood stream isolates in 2001 were methicillin resistant (Boyce et al 2005). On the other hand Scandinavian countries have consistently reported lower rates of MRSA. In 2005 MRSA prevalence was generally reported to be high in North America (43.7% and 43.2%) [Kuehnert 2005; Bryskier 2005].

Over the past decade, the changing pattern of resistance in S. aureus has underscored the need for new antimicrobial agents. Glycopeptide antibiotics, vancomycin and more recently teicoplanin, often are the last option for treatment of MRSA. They exert their antimicrobial effects by inhibiting synthesis of the S. aureus cell wall. Prevalence of heterogeneous vancomycin-intermediate S. aureus (hVISA) and vancomycin-intermediate S. aureus (VISA) MRSA strains has been reported in Europe, Asia, and the United States [Appelbaum 2006]. MRSA with reduced vancomycin susceptibility (VISA) has been reported from many countries. In subsequent years, close to 100 cases of S. aureus with reduced susceptibility to vancomycin have been reported, with some strains responsible for life-threatening systemic infections [Sancak et al 2005; de Lassence et al 2006]. Until 2006 prevalence of MRSA was low in Australia (14.9%) [Diekema et al 2004; Nimmo et al 2006].

In a study by Baddour et al [2006], the prevalence of MRSA among S. aureus isolates in Saudi hospitals varied from one hospital to another and ranged from 12% to 49.4%. Studies on antibacterial resistance in Kuwait hospitals from 1994 to 2004 by
Udo et al revealed an increasing fusidic acid resistance from 22% in 1994 to 92% in 2004. Erythromycin resistance increased from 66% in 1994 to 88% in 2004 and ciprofloxacin resistance increased from 53% to 92% in 2004 after a peak of 96% in 2002. On the contrary over the period of 10 years trimethoprim resistance declined from 86% to 27% and chloramphenicol decreased from 25% to 2% in 2004. The proportion of isolates resistant to gentamycin decreased from 98% to 77% in 2001. Plasmid DNA was detected in all MRSA strains ranging from 2kb to 38kb [Udo et al 2006].

Rajaduraipandi et al reported 31.1% MRSA from clinical samples and 37.9% from carrier samples in Tamil Nadu. They found 63.6% multidrug resistant strains [Rajaduraipandi et al 2006]. These studies revealed that MRSA is emerging to be a significant problem pathogen in the surgical setting with vancomycin probably the only reliable choice for these infections. VRSA was first reported in India by Tiwari and Sen in 2006. They isolated 2 strains with MICs to vancomycin 32µg/ml and 64µg/ml [Tiwari and Sen 2006].

Outbreaks of staphylococcal food poisoning (SFP) are very common across the world; however, there is hardly any report of SFP from the Indian subcontinent. An outbreak occurred in the state of Madhya Pradesh (India) after the consumption of a snack called "Bhalla" made up of potato balls fried in vegetable oil. More than 100 children and adults who ate the snack suffered from the typical symptoms of SFP and required hospitalization. Studies by Nema et al found that the food and clinical samples were found to contain a large number of enterotoxigenic *Staphylococcus aureus*. All enterotoxigenic isolates produced a combination of SEB and SED enterotoxins and were sensitive to oxacillin and vancomycin. Isolates were characterized by molecular biology tools, viz., SDS-PAGE, amplified ribosomal DNA restriction analysis (ARDRA), randomly amplified polymorphic DNA (RAPD) and nucleotide sequencing of *seb*, *sed*, and 16S rDNA genes. Results of these studies suggested that the isolates, irrespective of their isolation from food or clinical samples, were clonal in origin. Representative isolates from food and clinical samples, were also found to be highly heat resistant [Nema et al 2007].

Central venous catheters have been found to be a major cause of nosocomial bloodstream infections and different attempts have been made to incorporate
antimicrobial agents into catheters, particularly directed at the surface-coating of
devices. To facilitate the antimicrobial adsorption, various cationic surfactants, which
however showed several problems, have been used. On the other hand, impregnated
catheters with only antimicrobials have demonstrated a short-term duration due to the
difficulties to deliver the drug slowly. Ruggeri et al synthesized modified
polyurethanes to introduce different functional groups. Polymers were loaded with two
antibiotics, cefamandole nafate and rifampin (RIF). It was seen that antibiotics
released from various formulations inhibited the bacterial growth and exerted a
synergistic effect when both were present. In particular, PEG10000-containing
polymer was active against the RIF-resistant \textit{S. aureus} strain up to 23 days [Ruggeri
et al 2007].

Genotyping of \textit{seh} gene was recently carried out by Ruzickova V et al in 2008.
SEH-producing \textit{S. aureus} isolates are of high prevalence in staphylococcal food
poisoning cases. Given the unique epidemiological characteristic of these isolates,
SEH and SEA probably are responsible for food poisoning [Sakai et al 2008]. PCR
primers specific for SE like superantigens SEN, SEO, SEP, SEQ, SER, and SEU
gen genes had been developed. The complete SE sequences and their expression potential
for strains positive to \textit{sen}, \textit{seo}, \textit{sep}, \textit{seq}, \textit{ser}, and \textit{seu} specific primers were also
determined [Chiang et al 2008]. Staphylococcal enterotoxin- like genes S and T (SES
and SET), were identified in plasmid pF5, which is harbored by food poisoning-
related \textit{Staphylococcus aureus} strain Fukuoka 5 implicated in a food poisoning
incident in Fukuoka City, Japan, in 1997 [Ono etal2008]. The structural
characterization of alpha hemolysin monomer was also recently carried out by
molecular modeling [Meesters et al 2008]. A recent study reported a new group of
hetero-MRSA strains genetically distinct from those dominant in the same hospital in
the early 1980s might have emerged in the community and started invading the
university hospital, a phenomenon caused by the change in the pattern of antibiotic use
[Kishii et al 2008].

Prevalence of MRSA was extremely high Southern European countries [Voss
and Sri Lanka [Hart et al 1998]. Several reports from India suggest increasing
incidence of MRSA [Pulimood et al 1996; Mathur et al 1994; Pal et al 1991]. In India
a significant part of nosocomial infections are now caused by MRSA and the percentage of resistant strains are ever increasing. Nosocomial MRSA is known to be multidrug resistant and thus difficult to treat [Krishna et al 2007; Shome et al 2008; Rallapalli et al 2008]. Hence accurate and rapid identification of MRSA in a clinical specimen is essential for timely decisions on isolation procedures and effective antimicrobial therapy [Kohner et al 1999; Murakami et al 1991; Sakoulas et al 2001; Unal et al 1994;]. The geographic spread of several MRSA clones between countries and continents has been reported and proven by molecular evidence.

2.1. Typing and molecular characterization

The purpose of speciation is identification of a microorganism as belonging to a basic taxon having a particular clinical significance. Typing is an important epidemiological tool for tracing the spread of particular strains and for identifying the routes of transmission and reservoirs. Typing systems for studying the epidemiology of \textit{Staphylococcus aureus} infections have been investigated intensively. The ideal typing system should be rapid, inexpensive, technically simple, and readily available. Currently, there is no single definitive typing system for distinguishing individual strains of MRSA [Tenover et al 1995]. Classical phenotypical typing methods include biotyping, phagetyping, serotyping and antimicrobial susceptibility testing. Comparative characterization (molecular typing) of isolates within a bacterial species is one of the major problems in microbiology and epidemiology. It is even more difficult to correlate data obtained in various laboratories, because traditional, including molecular, methods employed in typing pathogenic microorganisms cannot be standardized. Due to the disadvantages of phenotypic methods, \textit{S. aureus} typing has recently been dominated by molecular biology techniques based on the variation of DNA sequences in bacterial isolates. A selection of the techniques used in \textit{S. aureus} typing includes RAPD (randomly amplified polymorphic DNA) analysis, PFGE (pulsed-field gel electrophoresis), MLST (multilocus sequence typing), \textit{spa} typing, \textit{coa} typing, \textit{agr}-group typing, MLEE (multilocus enzyme electrophoresis) and MLVA (multiple-locus variable-number tandem repeat analysis). Genotyping procedures can lead the way to elucidating the molecular genetics of colonizing \textit{S. aureus} populations.
The oldest phenotyping method is serotyping which was later replaced by phage typing which has a higher discriminatory power. Most clinical \textit{S. aureus} isolates are lysogenic, containing multiple prophages. These phages rely on bacterial surface components to infect the bacterium. Thus, a particular phagetype is the result of the surface components expressed by a particular \textit{S. aureus} isolate. Lysogeny and other factors including restriction modification systems modulate the susceptibility of a strain to phage infection. This is the basis of phage typing used for epidemiological and evolutionary analysis of \textit{S. aureus} strains. Phage typing provides further subdivision of \textit{S. aureus} strains through identification of bacteriophages to which the bacterium is susceptible. Phage typing is accomplished using bacteriophages of the international typing set for \textit{S. aureus} strains (Parker 1962).

The genome map of \textit{S. aureus} was first developed by Peter A Patter and colleagues at Iowa State University by focusing on \textit{S. aureus} phage group III strain NCTC8235. The first method exploited for genetic analysis of \textit{S. aureus} was transduction [Iandolo 2000]. The work of Lindberg and coworkers [Lindberg 1972] on transformation in \textit{S. aureus} NCTC 8325 along with improvements in chromosomal DNA isolation procedures allowed the analysis of larger fragments of chromosomal DNA.

Studies by Stobberingh had revealed that DNA restriction and modification are the most common mechanism of variation [Stobberingh 1977]. A poorly understood mechanism of genetic exchange named mixed culture transfer or phage mediated conjugation has also been identified in staphylococci. [Lacey 1980]. The mechanism of resistance to methicillin was uncovered in 1981 with the identification of reduced-affinity penicillin binding proteins in MRSA [Hartman and Tomasz 1981].

Phage typing of \textit{S. aureus} has been used since the 1940s and it has been quite valuable in investigating epidemiology of staphylococcal infections [Parker 1983]. But a number of MRSA strains are not typeable with routine phage panels [Archer and Mayhall 1983; Cristino and Pereira 1989; Kerr et al 1990; Khalifa et al 1989; Richardson et al 1988], although use of supplementary phage panels may be helpful. Unfortunately most laboratories are not equipped to handle phage typing and this technique can be used successfully only if it is performed in an experienced reference laboratory [Maslow 1993]. Plasmid profile analysis has been used to identify the
reservoir and mode of transmission of *S. aureus* infections especially in hospitals [Cohen et al 1982]. The phenotypic assays such as antibiogram typing and biotyping are also unreliable. Berger Bachi et al in 1983 showed that additional chromosomally located genes not linked to the *mec* are essential for expression of methicillin resistance [Berger Bachi B 1983; Kornblum et al 1986]. The use of PFGE was introduced in 1984 by Schwarz and Cantor. Meanwhile, PFGE has become by far the most widespread molecular typing tool in developed countries. It is considered to be the method of choice for DNA fingerprinting of *S. aureus*.

Most methicillin resistant *Staphylococcus aureus* isolates contain a DNA segment (greater than 30kb) not present in methicillin sensitive strains. This additional segment of chromosomal DNA is known as *mec*DNA [Beck et al 1986; Skinner et al 1988]. Although DNA can be introduced into staphylococci in the laboratory via transformation, transduction and conjugation, the latter two are found to be significant mediators of natural genetic exchange. Studies have proved that transformation is very inefficient requiring a curious co factor that can be satisfied by a component of phage 55C and is thought to be limited by extracellular nucleases or restriction systems encoded by staphylococci [Lyon 1987; Novick 1990]. Studies by Trees and Iandolo reported that *mec* could be mobilized from the chromosome to a penicillinase plasmid pI524 and suggested the possibility that *mec* may comprise a part of transposable genetic element [Trees and Iandolo 1988].

Berger-Bachi et al cloned and characterized a chromosomally determined gene which encodes a factor essential for the expression of methicillin resistance (*femA*) in *S. aureus*. *femA* mapped in chromosomal segment number 18, genetically very distant from the methicillin resistance determinant (*mec*). The product of *femA* was a protein of an apparent size of 48 kDa. Tn551 mediated inactivation caused a decrease in peptidoglycan associated glycine content of *femA*. Although *FemA* was needed for cell growth in the presence of beta-lactam antibiotics, they found that it had no influence on the synthesis of the low affinity, additional penicillin-binding protein (PBP2') encoded by *mec* and known to be essential for cell wall synthesis in the presence of inhibitory concentrations of methicillin. The nucleotide and amino acid sequence of *femA* showed homologies with ORF419, suggesting that these genes arose by gene duplication. Down stream and adjacent to *femA* lies another factor *femB*
which also affects methicillin resistance levels although to a lesser extend than femA [Berger Bachi et al 1989 Maidhof et al 1991].

Speciation in staphylococci was first based on a data set of morphological characteristics, physiological properties and chemical composition of cell wall [Kloos 1991]. Later DNA-DNA hybridization studies have shown that staphylococci form a well defined genus which can be subdivided into several species group [Schliefer 1990]. Multilocus Enzyme Elecrophoresis is extremely useful for showing clonal relatedness of S. aureus producing toxic hock syndrome toxin1 and also to study the clonal diversity of MRSA [Musser 1990]. The mecA gene, which controls the production of PBP2a, not only confers resistance to b-lactams but also mediates cross-resistance to fluoroquinolones, aminoglycosides, tetracyclines, macrolides, and trimethoprim-sulfamethoxazole. [Chambers 1990; Hiramatsu et al 1992].

Apart from femA and femB genes other fem factors were also found in both methicillin resistant and methicillin susceptible Staphylococcus aureus. Different types of alterations in muropeptide patterns were observed in Tn551 mutants selected for femC genes. femD inactivation results in disappearance of unsubstituted disaccharide pentapeptide monomer from the cell wall [de Jonge et al 1992]. High-level, homogeneously resistant revertants selected by passage of femD mutants in methicillin occur without reversing the femD biochemical defect. FemD mutants produced by Tn551 insertion were found to be deficient in muropeptide1 and muropeptide8 [Jolly et al 1997]. The transposon insertion may be in a regulatory gene controlling peptidoglycan precursor formation. The femF mutation results in a heterogeneous pattern of resistance [Ornelas-Soares et al 1994]. Inactivation of femF causes a block in peptidoglycan precursor synthesis at the lysine addition step.

All bacterial genomes harbour repetitive sequences that can be used for epidemiological typing. For S. aureus intragenic repetitive regions have been found in the coagulase (coa) and protein A (spa) genes. The coagulase protein is an important virulence factor of S. aureus. coa has a polymorphic repeat region that can be used for differentiating S. aureus isolates. The variable region of coa is comprised of 81-bp tandem short sequence repeats (SSRs) that are variable in both number and sequence, as determined by restriction fragment length polymorphism analysis of PCR products [Goh et al 1992].
In 1993 microlitre techniques for plasmid isolation was introduced [Goering 1993]. The value of plasmid profile analysis is limited however since plasmids can be spontaneously lost or acquired. Some MRSA strains lack plasmids and are therefore nontypeable by plasmid profiling. Restriction enzyme analysis of chromosomal DNA identifies the epidemic strains, but due to the numerous overlapping bands that are produced, it is difficult to interpret small variations in the restriction profiles. Application of repetitive extragenic palindromic PCR and enterobacterial repetitive intergenic consensus sequence analysis for the discrimination of MRSA strains have proven to be useful [Lessing et al 1995].

The transformation of colonizing MSSA to MRSA has been found to occur very rapidly (i.e., 24–48 hours) in hospitalized patients and this strong evidence against importation and cross transmission of MRSA [Chetchotisakd et al 1994], reveals that antibiotic selective pressure might play a larger role in the genesis of endemic MRSA than previously suspected. Thus MRSA is not often imported. Rather, it is typically homegrown. Improved typing systems are important for implementing appropriate infection control measures and for the clinical management of MRSA infections, particularly in evaluating the efficacy of therapy for infected or colonized patients [Emmerson 1994].

Although the polymorphism of coaA gene leads to amplimers of different length it has not been as discriminative as smalI macrorestriction patterns [Schwarzkopf 1994]. In contrast spa gene shows more sensitivity than necessary and generates more than one pattern within a set of obviously clonal strains. While spa-typing does not have the resolving power of PFGE subtyping, it is fast, easy to use and interpret, and compatible for building relational databases. Most importantly, DNA sequence analysis of the proteinA repeat region provides an unambiguous, portable dataset that simplifies information sharing between laboratories and facilitates creating a large-scale database for studying global and local epidemiology [Freney et al 1994].

PFGE is a molecular typing technique that does not have the same limitations as bacteriophage typing, since the bacterial genome is more stable than most protein markers. PFGE offers advantages over other DNA-based S. aureus strain-typing techniques (Prevost et al 1992; Tenover et al 1994]. For example, plasmid typing offers only moderate reproducibility, restriction fragment length polymorphism typing
requires the analysis of complex banding patterns, and ribotyping is highly labor-intensive and time-consuming. To date, all *S. aureus* isolates are typeable by PFGE. Because of its great discriminatory power, high degree of specimen typeability, excellent reproducibility and its good correlation with epidemiologically linked data, it is still accepted as the gold standard for the molecular typing of *S. aureus* isolates [Nawas et al 1998]. Ichiyama compared genomic DNA fingerprinting patterns among isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) by using pulsed-field gel electrophoresis (PFGE). Chromosomal fragment profiles digested with Sma I was found most suitable for discriminating between isolates which could not be differentiated by phenotypic methods. [Ichiyama S 1994].

Data published by Kluytmans et al. [Kluytmans et al 1995] from an analysis of strains from an outbreak examined by PFGE and PCR-based fingerprinting of bacterial DNA with five different primers have revealed that both PFGE and arbitrarily primed-PCR techniques were equally good in discriminating outbreak-related strains. The majority of these strains came from a point source food-borne outbreak, and therefore, only a limited range of types was to be expected. PFGE identified more subclones than did PCR typing. The most common application of RFLP is ribotyping. Although ribotyping was found to be less discriminative than SmaI macrorestriction patterns it was successfully used for the discrimination of MRSA from different continents and can also be used for speciation [Hiramatsu 1995].

Although generically termed *mec* region, it was found that there is considerable variation in both composition and size (20-60kb) of *mec* regions from different strains. Nucleotide sequencing has revealed the presence of terminal repeats at the end of *mec* region and confirmed an identical insertion site in different *S. aureus* strains [Hiramatsu 1995]. The *mec* region acts as a chromosomal hotspot for the insertion of additional antimicrobial resistance determinants in association with transposable elements. The expression of cell wall and extracellular proteins in *Staphylococcus aureus* is controlled by global regulatory systems, including *sar* and *agr*. *sarA* and the adjacent upstream DNA are essential to the expression of a DNA-binding protein(s) with specificity for the RNAII promoter, thereby controlling agr-related transcription [Heinrichs et al 1996]. The two recombinase genes were found
mediating the excision and circularization of the cassette structure and also mediating site and orientation specific insertion into the chromosome of a plasmid carrying them and a mec region attachment sequence.

mecA gene is seen as a part of SCCmec flanked by cassette chromosome recombinase genes (ccrA/ccrB or ccrC) that permit intra and inter species horizontal transmission of SCCmec. Study of early isolates of MRSA showed that the key genetic component responsible for resistance, mecA, is not native to the S. aureus genome. The ubiquitous carriage of mecA homologue by Staphylococcus sciuri has led to the suggestion that this represents the origin of mec determinants found in other species [Wu et al 1996]. Evidence suggests that the mec regions have been introduced into S. aureus on a limited number of occasions [Hiramatsu et al 1996]. Couto et al suggested that the mecA gene may have arisen in Staphylococcus sciuri or a related commensal of wild animals [Couto et al 1996].

DNA typing procedures either PCR mediated or PFGE of large DNA restriction fragments have frequently been applied and proven quite valuable for assessing the clonality among many given strains of S. aureus. [Van Belkum 1995, Cookson BD 1996]. Pulsed-field gel electrophoresis (PFGE) has been recommended as a highly discriminatory method for typing MRSA isolates because it can distinguish among several concurrent epidemic strains although it is a time-consuming and expensive typing method not well suited for screening large number of isolates by a diagnostic laboratory. PFGE has been used for the investigation of MRSA and has been compared with other methods in several studies [Bannerman et al 1995; Chiou et al 2000; Prasanna Kumari et al 1997; Van belkum et al 1995; Struelens et al 1996; Tenover et al 1996; Yoshida et al 1997] and has been successfully been used to study the epidemiology of S. aureus nosocomial infection and methicillin resistance [Kumari DN et al 1997; Yoshida T et al 1997]. For S. aureus the enzyme SmaI has been most useful, yielding 8 to 20 fragments ranging from 8-800 kb in size. Staphylococcal isolates are regarded as different strains if their SmaI macrorestriction patterns differ in four or more bands [Tenover et al 1997].

In 1997 the femAB operon was described which encodes two closely linked, cytoplasmic 49-kDa proteins that are required for formation of the pentaglycine interpeptide bridge that serves as the cross-link of peptidoglycan [de Jonge et al 1993;
Maidhof et al 1991]. \textit{femB} mutants produced cross links with only three glycines. \textit{femA} mutants do not incorporate the second and third glycines into the bridge. Disruption of these genes reduces the level of resistance to nearly susceptible levels. Production of PBP 2a and other PBPs is unaffected. A study in which the entire \textit{femAB} operon was eliminated by allelic replacement resulted in mutants which produced peptidoglycan with muropeptide subunits carrying only monoglycyl substituents. They were fully viable but showed morphological abnormalities, decreased growth rate and radically reduced methicillin resistance [Standen et al 1997].

Although homologous recombination is considered to play a role in the integration of \textit{mec} region in some strains the studies also indicate that the \textit{mec} region can behave as a site specific mobile genetic element. The size and features of the \textit{mec} region suggests that it could be considered as a resistance island [Hacker et al 1997]. A complete characterization of MRSA requires not only identification of the genetic background of the bacteria but also identification of the structural types of Staphylococcal Cassette Chromosome \textit{mec} element (SCC\textit{mec}), which carries methicillin resistance determinant \textit{mecA}.

In 1998, Maiden et al. proposed multilocus sequence typing (MLST); through which alleles of several housekeeping genes are directly assessed by nucleotide sequencing, each unique allele combination determining a sequence type of a strain. The advantages of this approach was that the culturing of pathogenic microorganisms is avoided, as their gene fragments are amplified directly from biological samples, and that the sequencing data are unambiguous, easy to standardize, and electronically portable. MLST is a highly discriminatory method of characterizing bacterial isolates on the basis of the sequences of internal fragments of seven housekeeping genes. For each gene fragment, the different sequences are assigned as distinct alleles, and each isolate is defined by the alleles at each of the seven housekeeping loci. It was first developed by using \textit{Neisseria meningitidis} as the model species (Enright and Spratt, 1999). The MLST approach is too labor-intensive, time-consuming, and costly to use in a clinical setting. More than 2,500 bp must be compared for each isolate. In addition, for certain recent subpopulations, such as MRSA, genetic variability in the
housekeeping targets will likely be limited and discrimination will be restricted [Maiden et al 1998].

However, a single-locus target, if discriminating, provides an inexpensive, rapid, objective, and portable genotyping method to subspeciate bacteria. Using a single target depends on finding a region for sequencing that is sufficiently polymorphic to provide useful strain resolution. Loci with short sequence repeat (SSR) regions may have suitable variability for discriminating outbreaks [Van Belkum et al 1998]. Two *S. aureus* genes conserved within the species, protein A (*spa*) and coagulase (*coa*), have variable SSR regions constructed from closely related 24- and 81-bp tandem repeat units, respectively. In both genes, the in-frame SSR units are degenerative, variable in number, and variable in the order in which repeat units are organized.

In 1999 a previously unrecognized penicillin binding protein (PBP) gene, *pbpF*, was also identified in *S. aureus* encoding a protein of 691 amino acid residues with an estimated molecular mass of 78 kDa, a molecular mass is very close to that of *S. aureus* PBP2 (81 kDa), and the protein was tentatively named PBP2B. The purified rPBP2B was shown to have penicillin binding activity [Komatsuzawa et al 1999]. The protein PBP2a is encoded by *mecA* gene and the PBP2a enzyme restores cell wall biosynthesis in the presence of beta lactams. The *mecA* gene expression was found to be controlled by 2 regulatory proteins encoded by the upstream genes *mecR1* and *mecI* [Hiramatsu et al 2001].

Recently, some new genotyping methods, such as the microarray technique, degenerate high-performance liquid chromatography, real-time PCR, TaqMan assay, and Invader assay, have been developed [Cooksey et al 2000; Fang et al 2003]. MLST was successfully adapted to *S. aureus* in 2000 (Enright et al 2000). MLST makes it possible to generate an expandable global database for each species at an Internet site, in order to use it for the purposes of genotyping pathogenic bacteria and other infectious agents [Platonov et al 2000]. The earliest strain of MRSA in which SCC*mec* type IV has been identified was isolated in 1981 [Ito et al 2001]. As in the work of Enright et al, Crisostomo et al [Crisostomo et al 2001] identified probable recipient MSSA strains for early MRSA strains in another collection of isolates.
Molecular analysis of the SCCmec region revealed that they carried cassette chromosome recombinases ccrA and ccrB which encode recombinases of the invertase resolvase family [Ito et al 2001]. SCCmec is a group of mobile DNA elements of 21 to 67 kb that is integrated into the chromosome of MRSA at unique site (attBScc) located near the S. aureus origin of replication [Hiramatsu et al 2001]. Resistance in S. aureus was found to be either constitutive due to class B complex or inducible due to class A complex. These are enclosed in a large element named the Staphylococcal Cassette Chromosome. The Staphylococcal Cassette Chromosome mec (SCCmec) has been characterized as a novel, mobile resistance element that differs from both transposons and bacteriophages [Berger-Bachi 2002].

A multiplex PCR strategy that allows quick presumptive characterization of the mec element types based on the structural features that were shown to be typical of mec elements carried by several MRSA clones was developed by Oliveira and Lencastre. Their strategy was validated by using a representative collection of pandemic MRSA clones in which the full structure of the associated mec elements was previously determined by hybridization and PCR screenings and also by DNA sequencing. This method was found to be rapid, robust, and capable in a single assay of identifying five structural types of the mec element among these strains, three major and two minor variants, each one of which has been already been seen among MRSA characterized earlier [Oliveira and Lencastre, 2002].

Gilot et al. (2002) developed a typing system for S. aureus based on the genetic variability in the accessory gene regulator (agr) locus. The agr system is a quorum-sensing system that downregulates the transcription of genes encoding some surface proteins and upregulates the transcription of certain extracellular toxins during the transition from the exponential to the stationary phase of growth. Based on this agr typing system, S. aureus strains can be divided into four major groups. Jarraud et al. (2002) observed a relationship between genetic background, agr group, and disease type for several toxin-mediated diseases.

Using multilocus sequence typing Enright et al demonstrated that MRSA clones evolved from 5 different groups of related genotypes or clonal complexes each arising from a distinct ancestral genotype. The earliest MRSA isolates evolved from sequence type ST-8 MSSA which after a point mutation evolved into ST 250 MSSA.
This MSSA was likely the first recipient of SCC mec to yield the first MRSA labeled ST 250 MRSA, a strain originating in Denmark and possessing SCCmec type I, most extant isolates of which were obtained in the 1960s [Enright et al 2002]. The genetic basis of MRSA is a crucial point [Eady & Cove 2003].

Hiramatsu et al found that SCCmec types I, II and III are poorly transferable being too large for bacteriophage transduction. Type IV being smaller is easily transferable by bacteriophages. Melter et al studied the molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated in 21 hospitals in the Czech Republic in the period 2000-2002 and compared with previous results from 1996-1997, using molecular techniques such as PFGE of SmaI digests, ribotyping of HindIII digests hybridized with a 16S-23S DNA probe multilocus sequence typing and cassette chromosome typing. The prevalence of the most clinically important macrolide (ermA, ermB, ermC, and msrA) and aminoglycoside (aph3', ant4', and aac6'-aph2") resistance genes was also evaluated. Type III cassette was found to be the most predominant and a correlation existed between the distribution of aminoglycoside resistance genes and MRSA clonal types [Melter et al 2003]. Naimi et al. [Naimi et al 2003] conducted a prospective study of 1100 MRSA infections and found 131 (12%) CA-MRSA and 937 (85%) HA-MRSA strains (32 could not be classified). The CA-MRSA isolates were more likely to be more susceptible to ciprofloxacin, clindamycin, erythromycin, and gentamicin than were the HA-MRSA isolates. There is a worldwide spread of community-acquired methicillin-resistant S. aureus isolates carrying the PVL locus (Vandenesch et al., 2003). Thus, the PVL locus is now considered to be a stable molecular marker for these strains worldwide [Vandenesch et al 2003].

A rapid and simplified approach for the strain characterization of Staphylococcus aureus on the basis of multilocus sequence typing (MLST) in which sequence variations in the MLST housekeeping gene loci are detected by restriction fragment pattern analysis rather than sequencing was developed by Diep et al known as the multilocus restriction fragment typing (MLRFT). In this study PCR amplification of each of the seven MLST housekeeping gene loci were carried out by using the same primer pairs used in MLST and the amplicons were then digested
directly with one or two restriction enzymes and the restriction fragments are resolved by agarose gel electrophoresis [Diep et al 2003].

There are at least 5 SCCmec types (types I–V), varying in size from \( \geq 20 \) kb to 68 kb, that have been identified so far [Ito et al 2001]. The mecA genes of type I, II and III SCCmec are associated with hospital acquired infection and that of type IV is associated with CA-MRSA. The smallest of these—SCCmec types I, IV, and V—contain only recombinase genes and the structural and regulatory genes for resistance to methicillin and lack the transposable elements and genes encoding resistance to non-\( \beta \)-lactam antibiotics carried by types II and III [Ma et al 2002]. SCCmec types I-IV contain alleles of ccrA and ccrB, whereas type V, which has to date been identified in a small number of Australian CA-MRSA isolates, contains a novel ccr designated ccrC [Ito et al 2004]. Two possible additional SCCmec types have recently been identified among Australian CA-MRSA strains [O’Brien et al 2004].

Variable-number tandem repeats (VNTRs) have been shown to be a powerful tool in the determination of evolutionary relationships and population genetics of bacteria. The sequencing of a number of Staphylococcus aureus genomes has allowed the identification of novel VNTR sequences in S. aureus, which are similar to those used in the study of the evolution of Mycobacterium tuberculosis clades. Seven VNTRs, termed staphylococcal interspersed repeat units (SIRUs), distributed around the genome are described, occurring in both unique and multiple sites, and varying in length from 48 to 159 bp. Variations in copy numbers were observed in all loci, within both the sequenced genomes and the UK epidemic methicillin-resistant S. aureus (EMRSA) isolates. Clonally related UK EMRSA isolates were clustered using SIRUs, which provided a greater degree of discrimination than multi-locus sequence typing [Hardy et al 2004].

The DNA microarray technique could have the ability to simultaneously analyze multiple-point mutations or deleted and/or inserted regions. In particular, the newly developed PamChip microarray (Pam-Gene International, Hertogenbosch, Netherlands) is a unique three-dimensional (3-D) flowthrough platform for kinetic hybridization reactions [Van Beumingen et al 2001]. Unlike general microarrays that employ a two-dimensional (2-D) substrate such as a glass slide, the PamChip microarray has long branching capillaries that bind probe DNA molecules onto a solid
3-D structure which ensures an optimal solution-driven reaction on the PamChip microarray by repeated pumping of the sample. [Maekawa et al 2004].

HA-MRSA isolates rarely carry the gene for PVL. In contrast, types IV and V are associated with CA-MRSA isolates, and at least type IV frequently carries PVL [Deresinski 2005]. Molecular characterization of MRSA in Tunisia revealed that the strains were predominantly (SCCmec) type IV and IVA [Ben Nejma et al 2006]. In a study by Kerttula et al on the nationwide trends in molecular epidemiology of methicillin resistant Staphylococcus aureus in Finland an annual rise in incidence of MRSA was noted i.e. from 2.3 notifications per 100000 populations in 1997 to 27.9 in 2004. They observed 253 different PFGE patterns and more than half of the strains carried SCCmec type IV or V [Kerttula et al 2007].

Denmark has been a low prevalence country for MRSA since the mid-1970s, but has in recent years experienced an increasing number of CA-MRSA cases. In a study by Larsen et al MRSA specimen and corresponding clinical data during 1999-2006 was investigated. Molecular typing showed >60 circulating clones and 81.2% carried SCCmec IV. All the CA MRSA strains carried type IV cassette chromosome and were positive for PVL genes [Larsen et al 2008]. Molecular characterization of methicillin-resistant Staphylococcus aureus isolates from three hospitals in Israel was carried out by Chmelnitsky et al in which 11 distinct genetic clones were identified by pulsed-field gel electrophoresis. Molecular typing identified four different SCCmec types-I, II, IV, and V-and nine spa types [Chmelnitsky et al 2008].

Kilic et al studied the SCCmec types and occurrence of the PVL gene by using TaqMan real-time PCR method, and correlated these with phenotypic antibiotic susceptibility patterns for MRSA strains collected during 4 years study period from Turkey. Their study revealed SCCmec types-I, II, III, IV, V, nontypeable and PVL occurrence were detected in 11 (2.8%), 3 (0.8%), 316 (82.1%), 20 (5.1%), 20 (5.1%), 15 (3.9%) and 5 (1.3%) isolates, respectively. SCCmec-III MRSA strains that do not carry the PVL gene were the predominant MRSA strains in the hospital setting in Ankara, capital of Turkey and SCCmec-I/II/III MRSA strains were involved in serious infections in surgical departments especially those with intensive care units [Kilic et al 2008]. In a study by Luczak-Kadłubowska A et al to assess the proportion of methicillin-resistant Staphylococcus aureus (MRSA) strains among hospital-acquired
isolates and to determine the clones of MRSA currently circulating in Poland, 208 isolates of MRSA were analysed. SCCmec type III clones were predominant and the emergence of an MRSA clone with SCCmec type V was observed [Luczak-Kadłubowska et al 2008].

Recently Spence et al [2008] developed and validated a novel, cost-effective multiwell microarray for the identification and characterization of Staphylococcus aureus comprising of 84 gene targets, including species-specific, antibiotic resistance, toxin, and other virulence-associated genes, and is capable of examining 13 different isolates simultaneously, together with a reference control strain.

Staphylococcus aureus is now a major pathogen responsible for both nosocomial and community-acquired infections. Although S. aureus isolates displaying resistance to methicillin were reported in the early 1960s, endemic strains of multi drug resistant MRSA became a worldwide nosocomial problem in the early 1980s. Recent efforts in the field of high-throughput sequencing resulted in the release of several MRSA genome sequences enabling the development of sophisticated tools to study clinical isolates of MRSA. Microarrays covering whole genomes and high-throughput sequencing devices are the two main techniques currently utilizable for whole-genome characterization. These tools not only provide information for the development of genotyping assays but also allow evaluation of potential virulence of the strains, by enumerating genetic-encoded resistance markers and toxin content. This will help in understanding the epidemiology of MRSA and the relationship between genome content and also virulence potential of the strains. In addition, sequence information is mandatory for the development of molecular tests allowing the rapid identification, genotyping and characterization of clinical isolates.