3. Review of Literature

*Staphylococcus aureus* is a significant versatile pathogen as it can adapt itself to different environmental conditions and cause a wide range of diseases (1). It can cause infection as simple as superficial skin infection like folliculitis to as complex and life threatening as septicemia (1). This organism normally colonizes skin and mucous membrane of humans but can breach the first line of defense and result in infection (1).

In 1940s, penicillin was introduced for treatment of *S. aureus* infections. However, within few years itself resistance to penicillin developed in *S. aureus* leading to manufacture of other β-lactam antibiotics such as methicillin, oxacillin and ampicillin which were related to penicillin. In 1960s, just two years after the introduction of methicillin, methicillin resistant *S. aureus* (MRSA) emerged (3). This mutated pathogen possessed mecA gene which coded for an altered penicillin binding protein (PBP) PBP-2a resulting in resistance to all β-lactam antibiotics (4).

Since 1960s, MRSA has become a never ending problem in healthcare setting and has resulted in several waves of epidemic and endemic affecting healthcare setting in several countries throughout the world (18-21). Except Scandinavian countries and Netherland the extensive spread of healthcare associated MRSA (HA-MRSA) is now a global endemic and has lead to increase in morbidity and mortality (18-21). The rate of HA-MRSA varies from one geographical location to another. High rate of MRSA (> 50%) is reported from countries like United States of America, Asia and Malta while intermediate rate (25 – 50%) is seen in Africa, China and Europe (18-21).

HA-MRSA has an extensive armamentarium of virulence factors including secreted and structural proteins which helps in its pathogenesis when the host’s defense is breached (22,23). These virulence factors help it to evade the immune response of the host and thereby help it to establish itself as a pathogen. It is one of the common causes of healthcare-associated infections and has also established itself in the community (2). Being
ubiquitous this pathogen is widespread and causes several infections in healthcare settings. Patients with lowered immunity due to preexisting disease, presence of indwelling medical devices and on antibiotics become a ready target for healthcare associated infection (HAI) due to MRSA (24). Most common HAIs due to it include surgical site infection, secondary infection in burn patient, catheter associated bacteremia, ventilator associated pneumonia and urinary tract infection (1).

MRSA was initially considered to be a pathogen which was limited within the boundary of healthcare settings but in 1990s cases of MRSA causing infections in healthy immunocompetent patients with no prior risk factors in the community came into light (2). Community - acquired MRSA (CA-MRSA) mainly causes skin and soft tissue infections (2). Though the emergence of CA-MRSA is a matter of concern, the ever existing HA-MRSA still prevails and has become resistant to several antibiotics overtime. Risk factors for HA-MRSA include previous admission to hospital, surgery, presence of indwelling medical devices, antibiotic usage, lowered immunity and dialysis (24). Also patients colonized with MRSA at time of admission to healthcare settings are at a risk of acquiring healthcare associated infection (HAI). Multidrug resistance among HA-MRSA is a serious concern and has aggravated the problem of antibiotic selection in neonatal and orthopedic wards (25). Emergence of vancomycin intermediate \textit{S.aureus} (VISA) and vancomycin resistant \textit{S.aureus} (VRSA) in several parts of the world and India has forced the clinicians to select expensive and more toxic antibiotics (14,16).

3.1 Definition of HA-MRSA

National Healthcare Safety Network (NHSH) and Centers for Disease Control and Prevention (CDC) had put forth a definition to define healthcare associated infection (HAI) to conduct NHSH/CDC surveillance (26). According to this definition MRSA isolated from patients with localized or systemic condition that resulted from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and was not present or incubating at the time of admission to the healthcare setting but became evident after 48 h or more of
admission, patients with a history of hospitalization, hemodialysis, surgery or stay in a long – term healthcare facility within one year before the isolation of MRSA and patients with indwelling catheter, intravenous line or other percutaneous medical device at the time of culture collection are termed as HA-MRSA (26).

Staphylococcal cassette chromosome (SCC) mec type I, II and III were used in the past as a marker for HA-MRSA. However, recently changes in the molecular epidemiology of HA-MRSA have been reported. The emergence of MRSA having SCCmec type IV and V which are typically present in CA-MRSA have been reported from healthcare settings (7-9). Therefore molecular epidemiology based on SCCmec type is no longer dependable for defining HA-MRSA.

3.2 Epidemiology of HA-MRSA

3.2.1 Origin of HA-MRSA

In 1959, methicillin was used clinically for treating penicillin resistant S.aureus and just within two years S.aureus gained resistance to this antibiotic leading to emergence of first MRSA in 1961 from a hospital in United Kingdom (3). The rate of MRSA infections occurring in healthcare settings was low and did not cause any significant outbreak till 1980s (27). Epidemics due to it started being reported from many developed countries in 1980s which was due to emergence of new HA-MRSA strains which had the ability to spread at a fast pace between hospitals resulting in a significant rise in the rate of HAI (3,28).

MRSA originated when a mobile genetic element called Staphylococcal Chromosome Cassette mecA (SCCmecA) was acquired by methicillin sensitive S.aureus (MSSA) (29). Many studies have been done to determine the source of mecA gene (30-32). According to various studies done, S.murium group is suspected as the source of this gene. Members of S.murium group include S.murium, S.vitulinus, S.fleurettii and S.lentus. Out of all these members mecA gene is absent in S.lentus thereby eliminating it as the suspected
source of this gene (32). Sequence analysis was performed for the other three isolates and mecA nucleotide identities of these isolates were compared with MRSA strain N315. mecA gene of MRSA N315 showed a resemblance of 85-86%, 94% and 99-100% with that of S.sciuri, S.vitulinus and S.fleurettii respectively (32). mecA gene of S.fleurettii designated as mecA_{sf} had 99% nucleotide identity not only with SCCmec II of MRSA strain N315 but also with other strains having SCCmectype II, III and VIII (Fig 3.1) (32). It was thus hypothesized that S.fleurettii was the most probable source of mecA gene (32).

Fig 3.1: Comparison of S. fleurettii mecA_{sf} and SCCmec II of MRSA strain N315. Adapted from Tsubakishita et al 2010 (32)

Kreiswirth et al proposed single clone theory according to which different MRSA clones were believed to have emerged from a common MRSA ancestor (33). However, several other researchers have put forth the multi-clone theory according to which instead of a common MRSA ancestor several S.aureus lineages were involved and SCCmec element was introduced several times in them thus resulting in different clones of HA-MRSA (34,35).

The major HA-MRSA clones which are recognized till date includes Archaic, Berlin (USA600), Brazilian/Hungarian, Iberian, Irish I, New
York/Japan (USA100), Pediatric (USA800), UK-EMRSA-2/6’ (USA500), Southern Germany, UK-EMRSA-3, UK-EMRSA-15 and UK-EMRSA-16 (USA200) (35). These clones are described by their sequence type (ST), clonal complex (CC), lineage type, pulse filed gel electrophoresis (PFGE) type or spa type. HA-MRSA can be grouped into five phylogenetically distinct lineage and the clonal complexes used to describe them are CC8, CC5, CC45, CC22 and CC30 (36). According to their sequence type another HA-MRSA clone is described which is known as ST239 (36).

3.2.2 Incidence of HA-MRSA

The first strain of MRSA was isolated in 1961 from United Kingdom just two years after the introduction of methicillin (3). The first outbreak of MRSA in healthcare setting of United States occurred in 1963 at Boston City Hospital, Massachusetts where MRSA were isolated from 18 patients out of which 15 patients had acquired the infection in the hospital (3). Reports of outbreaks due to MRSA were reported mainly in European countries over the next 10 years which involved United Kingdom, France, Denmark and Switzerland (3). Some outbreaks were also reported from Australia (3). The initial MRSA isolates were mainly of phage group III. Later along with phage group III, mixed phage group I (phage type 29) appeared in United Kingdom, South Africa and Switzerland (35).

After these episodes a reduction in the rate of MRSA in European countries was noted in 1970s (37). However, in the late 1970s and early 1980s a new MRSA strain emerged in United States, the Irish Republic and Australia (37). Also the rate of sporadic infection due to MRSA increased in 1980s in United Kingdom with the emergence of epidemic MRSA (EMRSA 1, phage type 85/88A/932) strain in London hospitals which spread to involve Southeastern England (3). Fifteen other epidemic strains of MRSA have been described and includes EMRSA 2 (phage type 80/85/90/932+), EMRSA 3 (phage type 75/83A/932), EMRSA 4 (phage type 85/90/932), EMRSA 5 (phage type 77/84), EMRSA 6 (phage type 90/932), EMRSA 7 (phage type 85), EMRSA 8 (phage type 83A/83C/932), EMRSA 9 (phage type 77/84/932), EMRSA 10 (phage type 29/75/77/83A/85), EMRSA 11 (phage type 84),
EMRSA 12 (phage type 75/83A/83C/932), EMRSA 13 (phage type 29/83C/932), EMRSA 14 (phage type 29/6/47/54/90/932), EMRSA 15 (phage type 75) and EMRSA 16 (phage type 29/52/75/83A/83C) (6,27,35,38).

In 1991 EMRSA 15 reemerged, and the isolation ward which was used as a preventive measure in previous incidences failed to stop the spread of MRSA. EMRSA 15 spread rapidly in United Kingdom healthcare settings by 2000 and over 60% of healthcare-associated bacteremia by MRSA was due to EMRSA 15 (39). Reports of EMRSA 15 outbreaks were also reported from Australia, Portugal, the Czech Republic, New Zealand, Germany and Singapore, though Asia remained unaffected by this strain (39). EMRSA 16 (ST 36) has over the last 20 years caused several endemics in United Kingdom (39). The rate of MRSA causing bloodstream infection was less than 5% in Estonia, Denmark, Iceland, Finland, Norway, the Netherlands and Sweden (40). In Austria, Slovenia and Luxembourg the rate of MRSA was less than 10% while a rate of 10% - 24% was recorded in eight European countries (Czech Republic, Belgium, Germany, France, Latvia, Hungary, Switzerland and Portland) (40). In Croatia, Bulgaria, Greece, Cyprus, Italy, Israel, Republic of Ireland, Turkey, Spain, Romania and United Kingdom a rate of equal to or greater than 25% of HA-MRSA was recorded (40). A rate of greater than 50% was seen in two European countries (Malta and Portugal) (40).

United States carried out a surveillance program of nosocomial bloodstream infections and reported an increase in the rate of MRSA from 22% in 1995 to 57% in 2001 (41). The rate of MRSA causing infections in intensive care unit (ICU) of NNIS hospitals reported a surge of 3.1% every year from 1992 (42). The rate of HA-MRSA was 35.9% in 1992 which reached to 64.4% in 2003 (42). A national observational study conducted in USA reported 55% incidence rate of HA-MRSA in 2006 (43). However, a recent surveillance done by USA in 2011 has shown a decrease in the rate of MRSA infections in healthcare settings (44).
In the Asian scenario low level resistance to methicillin among *S.aureus* was first reported from Japan in early 1960s (45). In other East Asian countries such as Taiwan and Korea the incidence of MRSA was reported only after 1981 and 1986 respectively (45). From 1980 to 2000 the rate of MRSA increased at a steady rate in East Asia. In Taiwan the rate increased from 20.2% to 69.3% while a nationwide study conducted in Japan reported a rate of 58.6% HA-MRSA among clinical *S.aureus* isolate in 1990 (45). The SENTRY study conducted in 1998 – 2001 showed an increase in the rate of MRSA (46). In Hong Kong, MRSA was reported in early 1980s and a survey conducted in 1984 – 1986 showed the rate of HA-MRSA to be 25 – 30% (45). This rate increased to 73.8% in 1998 – 1999 as shown in SENTRY study (37,46,47). The rate of MRSA in Philippines, Thailand and Vietnam in 2004 – 2006 as per Asian Network for Surveillance of Resistant Pathogens (ANSORP) study was 38.1%, 57% and 74.1% respectively (19). Regional Resistance Surveillance (RRS) program reported HA-MRSA rate of 59% and 53% in Philippines and Thailand respectively in 2011 (48). Four hospitals in Pakistan were involved in a multicentre study in 2006-2008 and the rate of HA-MRSA was documented as 41.9% (45). ANSORP study showed HA-MRSA rate of 86.5% in Sri Lanka while in India the rate was only 22.6% (19). The rate of HA-MRSA reported from different parts of India is not uniform. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) for two years carried out a MRSA survey in 15 study centers throughout India (49). According to this study, in 2008 the mean rate of non ICU patients and ICU patients suffering from MRSA infection was 42 and 43% respectively which increased to 49% and 47% respectively in 2009 (49). In Mangaluru a rise in the rate of HA-MRSA has been reported (50-53). In a tertiary care hospital in Mangaluru, Ha-MRSA rate was 23.9% in 2013, which increased to 30.2% in 2016 (51,52).
3.2.3 Molecular Epidemiology of HA-MRSA

HA-MRSA is prevalent globally and generally exists in all healthcare settings around the world. Different clones of HA-MRSA circulate in different regions of the world. These clones are described by their sequence type (ST), clonal complex (CC), lineage type, pulse filed gel electrophoresis (PFGE) type or spa type. Five phylogenetically distinct lineage and the clonal complexes are used to describe HA-MRSA and accordingly it is grouped in CC8, CC5, CC45, CC22 or CC30 (36,54). Another HA-MRSA clone is described based on its sequence type and is known as ST239 (36,54).

The pandemic, epidemic and sporadic HA-MRSA clones from different parts of the world are described as below:

A. Clonal complex 5 (CC5)

It is a widespread clonal complex and consists of many different MRSA strains. Enterotoxin gene cluster $egc(seg, sei, sem, sen, seo \text{ and } seu/y)$ is carried by CC5 isolates, sometimes partial deletion of $egc$ have been recorded. CC5 carries many different SCC mec types along with variable virulence or resistance associated genes (54).

a. ST228-MRSA-I belongs to CC5 lineage and carries SCC mec I. This strain unlike other CC5 strains lacks $fnbB$ gene which is responsible for encoding fibronectin-binding protein (54). This strain is commonly known as South German Epidemic strain, Italian clone, Spanish PFGE types E6/9/15/17/18 (55). The strain occurs exclusively in Germany but has also been isolated from Hungary, Italy, Slovenia and Switzerland (18). Its variant ST111, ST221 and ST5 have been reported from Croatia and Paraguay (54). Variants of this clone have been frequently observed but the spread of this strain is limited. In the last trimester of 2008, an unusual dissemination of a strain belonging to ST228 was reported which affected more than five hundred patients in less than two years (56).

b. ST5-MRSA-II/ST225-MRSA-II is another MRSA strain belonging to CC5 family and carries SCC mec type II. It is also known as New York-Japan clone, UK-EMRSA-3, Irish AR11, Irish AR07.3, Irish AR07.4, Rhine-Hesse Epidemic strain, Canadian MRSA-2 and USA100 (57). spa types t002, t003
or t045 is exhibited by ST5-MRSA-II (54). Aminoglycoside resistance gene \textit{aadD} and \textit{erm(A)} are usually present and the strain commonly carries \textit{tst1}, \textit{sea}, \textit{sea-N315}, \textit{sec}, \textit{sed}, \textit{sej}, \textit{sel} and \textit{ser} virulence gene (54,58). It has been reported from Croatia, Austria, Hungary, Hong-Kong, Japan, Taiwan, Ireland, Portugal, Germany, the UK and the USA (54,59-62). ST5-MRSA-II is the second most prevalent MRSA strain isolated in Dresden/Saxony and is most frequently isolated from its ICU setting (54). Transmission of this strain from New York to Australia was reported in a healthcare worker of Australia who underwent surgery in a New York hospital (63).

c. CC5-MRSA-IV also known as the Paediatric clone, USA800, WA MRSA-03 (ST5), WA MRSA-39 (ST526), WA MRSA-50 (ST73) and Marseille Cystic Fibrosis clone carries SCC\textit{mec} IV and is globally distributed (64). Complete genome hybridization and multilocus assay showed this strain to be closely related to Mu50 strain (64). Isolates of this group exhibit multidrug resistance even though they carry SCC\textit{mec} type IV (64).

d. CC5-MRSA-VI was initially considered as Paediatric clone with SCC\textit{mec} IV but later it was found to carry SCC\textit{mec} VI element (mec gene complex class B and \textit{ccrAB} type 4) (65). It was first isolated in Portugal in 1992 and was renamed as New Paediatric clone in France (65,66). It has also been isolated from Colombia, Portugal, the USA and Argentina and has been sporadically identified in Germany and Australia (54). This isolate possesses \textit{agr-2} allele, ST5 and a unique \textit{spa} type t777 (66). The \textit{sed}, \textit{sem}, \textit{seo}, and \textit{ser} genes are frequently present in this strain (66). It exhibit resistance to fluoroquinolones in addition to \textit{β} - lactam drugs and is susceptible to aminoglycosides (66).

B. Clonal Complex 8 (CC8)

It is a pandemic MRSA lineage like CC5. Its core genome genes like protease, microbial surface components recognizing adhesive matrix molecules (MSCRAMM) and \textit{ssl/set} genes are mostly in accordance with the sequenced genome though deletions of genes like \textit{clfA}, \textit{bbp} or \textit{sdrD} has been occasionally observed (54). Presence of exotoxins and \textit{β}-toxin-converting phages is variable (54).

a. ST250-MRSA-I was the first known MRSA and hence is also called as Early or Ancestral MRSA (54). It is also known as Irish AR02 or Irish
Phenotype I and II (54). This clone is becoming extinct but rare cases have been reported from Australia (one in 4000 isolates tested is positive for this clone) (67).

b. ST247-MRSA-I was isolated from UK in 1971. It is also called as UK-EMRSA-5,-8 and -17, North German Epidemic strain, Spanish PFGE type E1, Rome clone, Irish New02, Irish AR22 and Iberian clone (54). The incidence of this isolate is decreasing in Portugal and Spain and has not been isolated from Dresden, Saxony since 1997 or Ireland since 1999 (54,61,68). It has been isolated from UK, Australia, Croatia, the Czech Republic, Italy and the Netherlands (54,69). It has a hybridization profile similar to ST250-MRSA-I but carries a complete set of ssl/set genes (54).

c. ST8/ST576-MRSA-IV lacks PVL and enterotoxin genes. It includes UK-EMRSA-14 and WA MRSA-5, -6 and -31 (54). Lyon clone or UK-EMRSA-2 is another PVL negative CC8-MRSA-IV strain which is usually isolated in France (54). It is also found in UK, Germany, Ireland, the Netherlands, Australia and Norway (54). UK-EMRSA-6 also belongs to this group and resembles UK-EMRSA-2 (54). UK-EMRSA-12 and -13 also belongs to this group and is found in Northern Ireland, the UK, Australia and Norway (70,71).

d. ST254-MRSA also known as UK-EMRSA-10 or Hannover Epidemic strain has spa type t009 or t036 (54). This strain was previously isolated frequently from German hospitals but has decreased since 2000 (54). It is a multidrug resistant strain prevalent in healthcare settings (54).

e. ST239-MRSA-III is the oldest pandemic MRSA strain documented. Though it belongs to CC8 lineage, integration of a CC30 DNA fragment near the oriC has resulted in a different MLST profile, hybridization profile and spa types (54). This strain is widespread and has been reported from several European countries like Greece, Italy, Croatia, the Czech Republic, Malta, Portugal, the UK and Spain (54,71). It was the most commonly isolated MRSA in Hungary but has now been replaced by ST228-MRSA-I (54). It is frequently isolated from Turkey, Saudi Arabia, Iran, China, Hong Kong, Singapore and Taiwan (59,72-74). A trauma patient from Iraq introduced this strain to Ireland where it has now become the prominent HA-MRSA (73). It has also been reported from Argentina, Brazil, Chile, Egypt, Korea,
India, Mongolia, Malaysia, New Zealand, Pakistan, Russia, Paraguay, Thailand, South Africa and Uruguay (54,75-80).

ST239-MRSA-III can be divided into 3 clades: a European, a South American and an Asian one (54). ST239-MRSA-III is colloquially called as Vienna, Czech, Portuguese, Brazilian, Hungarian clone, AUS-EMRSA-2 or -3, UK-EMRSA-1, -4, -7, -9 or -11, Irish AR01, -09, -15 or -23, Irish phenotype III, Canadian MRSA-3 or Canadian MRSA-6 (54).

C. Clonal complex 22 (CC22)

ST22-MRSA-IV belongs to this lineage and is called as Barnim Epidemic strain, UK-EMRSA-15, Canadian MRSA-8, Spanish PFGE E13 or Irish AR06 (54). This strain accounted for 50%, 54%, 66% and more than 80% of MRSA in Dresden, Portugal, Malta and Ireland respectively (54). It is extensively isolated from the UK and has been reported to be the cause of 85% bacteremia due to MRSA (81). β-lactamase and \( \text{erm}(C) \) are the common resistance markers while virulence markers \textit{sea}, \textit{sec}, \textit{set}, IEC genes (\textit{sak}, \textit{chp}, \textit{scn}) and \textit{tst1} are present (54).

Another variant of ST22-MRSA-IV is present in Dublin, Ireland and has ACME-locus and carries resistance markers like β-lactamase, \textit{erm}(C), \textit{lnu}(A), \textit{aacA-aphD}, \textit{aadD} and \textit{mupA} (54,81). A PVL positive ST22-MRSA-IV resulted in a nosocomial outbreak in Bavaria, Germany and has also been reported from patients in Germany who had relatives in Turkey (82,83). PVL positive ST22-MRSA-IV having \textit{tst1} gene has also been reported from India (84). The resistance markers of this isolate are β-lactamase, \textit{erm}(C), \textit{aacA-aphD}, \textit{aadD}, \textit{dfrA} and Q6GD50 (54).

D. Clonal Complex 30 (CC30)

It is another clonal complex from which both HA-MRSA and CA-MRSA has originated and is represented by Sanger MRSA 252 genome sequence (54). Core genomic marker includes MSCRAMM and \textit{ssl/set} genes which are present as allelic variants different from other CCs (54). CC30 strain has \textit{egc}, \textit{can}, \textit{bbp}, \textit{fnbB} and \textit{sdrD} (54). CC30-MRSA-I carries SCC\textit{mecI} and was reported in 1980 from Italy (55). ST36/39-MRSA-II is another widespread HA-MRSA strain from this lineage (85). It is commonly known as UK-EMRSA-16, USA200, Spanish PFGE type E12, Irish AR7.0/AR07.2 or Canadian MRSA-4.
(85). This strain carries SCCmec II, β-lactamase operon, aadD integrated into pUB110 and _erm(A)_ inserted into Tn554 (54).

**E. Clonal Complex 45 (CC45)**

The strains from this lineage is separated into two distinct groups, one which belongs to _agr_ group I and another which are present in _agrB_ or _agrC_ group (48). This lineage does not produce PVL but enterotoxin _sec_ and _sel_ are occasionally present (54). ST45-MRSA-II also called as USA600-MRSA-II, USA600 or Canadian MRSA-I is a member of this clonal complex (54,57). It is mainly restricted to North America where it has been reported to result in a high mortality rate in blood stream infections (86). It has been sporadically reported from Hong Kong and Australia (54,59).

ST45-MRSA-IV or Berlin Epidemic strain also called as USA600-MRSA-IV or WA MRSA-75 is another important strain belonging to this lineage (54). This strain has been reported from Saxony, Dresden, Belgium, the UK, the Netherlands, Switzerland, Australia and Croatia (87,88).

**Asian Scenario**

The molecular epidemiology of HA-MRSA isolates throughout Asia differs. ST254 with SCCmec IV and ST239 with SCCmecIII were the major HA-MRSA isolated from Taiwan in 1990s (48). ST239 or ST241 with SCCmec III/IIIA became prominent in 2000 (2). The molecular epidemiology of HA-MRSA in Taiwan has changed overtime. ST5-SCCmec II and CA-MRSA clone ST59 have been reported as the major cause of nosocomial bloodstream infections due to MRSA in Taiwan (74).

Since 1996 ST5 and ST239 are the major MRSA clones in Korean healthcare setting (77). ST5-SCCmec-II was widespread in Japanese and Korean hospitals in 1990s from where it slowly spread to other Asian countries like Hong Kong, Taiwan and China (59,77,89-91). ST5 has a low rate of antibiotic resistance and are susceptible to trimethoprim/sulfamethoxazole while ST239 MRSA has a high rate of resistance to ciprofloxacin, erythromycin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole and tetracycline (91). As the rate of CA-MRSA increased the dominant CA-MRSA genotype ST72-SCCmec-IV/IVA became a major cause of HA-MRSA infection in Korean healthcare settings (92).
ST5-SCCmec II is the major HA-MRSA isolated in Japan and accounts for >95% of clinical MRSA strains in their healthcare settings (89). In Hong Kong ST239-SCCmec III is the major HA-MRSA clone (48). ST5-SCCmec II and ST45 are the other 2 minor clones found in Hong Kong hospitals (19).

**Indian Scenario**

MRSA is prevalent in Indian healthcare settings (49-53,93,94). Studies on genotypic characterization of HA-MRSA reveal that ST239 is the predominant MRSA in India (8,76,84,93-106). A study involving patients and staffs in a burn unit of a single healthcare setting in Bangaluru showed that HA-MRSA strains present in their setting were different from epidemic MRSA strains of UK (98). HA-MRSA strains in India mainly harbor SCCmec III/IIIA (94-102). Typing by PFGE, MLST, spa and SCCmec revealed that Indian HA-MRSA were different from UK EMRSA strains but had genetic similarity with Hungarian and Brazilian MRSA isolates (95).

A hospital in Vellore reported a novel SCCmecIII element in its HA-MRSA isolates. These MRSA had lower minimum inhibitory concentration to methicillin as compared to typical SCCmec III isolates (100). Studies from New Delhi have reported ST239-MRSA-III to be the predominant HA-MRSA in their settings, these strains were closely related to UK-EMRSA-I and were multidrug resistant (8,101). Recent studies have shown that ST22-MRSA-IV and ST772-MRSA-V which are normally considered as CA-MRSA have infiltrated into healthcare settings and are slowly becoming the cause of healthcare-associated infections in India (8,76,102).

Study from South India proclaims that HA-MRSA ST239 still remains the major cause of healthcare-associated infections (96). Studies have shown ST772- MRSA-III and ST22-MRSA-V as the major strain causing both mild and severe eye infections while another study has shown ST1-MRSA-II and ST88-MRSA-II to be associated with keratitis (97,105). ST22-MRSA-IV carrying PVL was the cause of post-partum breast abscess outbreak recently in Mumbai caused by community-onset HA-MRSA (106).
Fig 3.2: Important HA-MRSA lineages or clones around the world. Adapted from Otto 2010 (107)
<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>Conventional name (s)</th>
<th>Sequence type</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC5</td>
<td>USA100, New York/Japan clone</td>
<td>ST5</td>
<td>SCC\textit{mec} II, most common healthcare-associated MRSA in the USA</td>
</tr>
<tr>
<td></td>
<td>USA800, paediatric clone</td>
<td>ST5</td>
<td>SCC\textit{mec} IV, mostly isolated in Argentina, Colombia and the USA</td>
</tr>
<tr>
<td></td>
<td>HDE288, New Paediatric clone</td>
<td>ST5</td>
<td>SCC\textit{mec} VI, isolated from Colombia, Portugal, the USA, Argentina, Germany and Australia</td>
</tr>
<tr>
<td></td>
<td>UK-EMRSA-3</td>
<td>ST5</td>
<td>SCC\textit{mec} II, isolated from Croatia, Austria, Hungary, Hong-Kong, Japan, Taiwan, Ireland, Portugal, Germany, the UK and the USA</td>
</tr>
<tr>
<td>CC8</td>
<td>UK-EMRSA-1</td>
<td>ST239</td>
<td>SCC\textit{mec} III, In 1980s resulted in epidemic in Eastern Australia</td>
</tr>
<tr>
<td></td>
<td>AUS-2 and AUS-3</td>
<td>ST239</td>
<td>SCC\textit{mec}III, a common multidrug-resistant clone of the early 2000s prevalent in Australia</td>
</tr>
<tr>
<td></td>
<td>Brazilian or Hungarian clone</td>
<td>ST239</td>
<td>SCC\textit{mec}III/IIIA</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Strain Type</th>
<th>ST</th>
<th>SCC mec Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA500</td>
<td>ST8</td>
<td>SCC mec IV, commonly isolated</td>
<td>from France, Germany, Ireland, the UK, the Netherlands, Australia and Norway</td>
</tr>
<tr>
<td>Irish-1, Irish AR05, AR13, AR14, Irish New03, Irish AR43, Irish-02, UK-EMRSA-12/-13</td>
<td>ST8</td>
<td>SCC mec II, predominant in Ireland</td>
<td></td>
</tr>
<tr>
<td>Iberian clone, UK-EMRSA-5,-8 and -17, North German Epidemic strain, Spanish PFGE type E1, Rome clone, Irish New02 and Irish AR22</td>
<td>ST247</td>
<td>SCC mec IV, predominant in Northern Ireland, also isolated from Norway, the UK and Australia</td>
<td>The incidence of this isolate is decreasing in Portugal and Spain and has not been isolated from Dresden, Saxony since 1997 or Ireland since 1999</td>
</tr>
<tr>
<td>Archaic</td>
<td>ST250</td>
<td>SCC mec I, First known MRSA</td>
<td></td>
</tr>
<tr>
<td>UK-EMRSA-10, Hannover Epidemic strain</td>
<td>ST254</td>
<td>Irregular SCC mec element, frequently isolated from German hospitals in 1990s</td>
<td></td>
</tr>
<tr>
<td>CC22, UK-EMRSA-15, Barnim Epidemic strain, Canadian MRSA-8, Spanish PFGE E13, Irish AR06</td>
<td>ST22</td>
<td>SCC mec IV, a pandemic international clone that is prominent in Europe and Australia</td>
<td></td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>CC30</th>
<th>UK-EMRSA-16, USA200, Spanish PFGE type E12, Irish AR7.0/AR07.2, Canadian MRSA-4</th>
<th>ST36</th>
<th>SCCmecII. It is the single most abundant cause of MRSA infections in UK hospitals and was the second most common cause of MRSA infections in US hospitals in 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC45</td>
<td>USA600, Canadian MRSA-I Berlin clone, USA600-MRSA-IV, WA MRSA-75</td>
<td>ST45</td>
<td>SCCmec II [SCC\text{mec} \text{II}] [SCC\text{mec} \text{II}]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST45</td>
<td>SCCmec IV [SCC\text{mec} \text{IV}] [SCC\text{mec} \text{IV}]</td>
</tr>
</tbody>
</table>

**Reference:** (54)

### 3.3 Difference between HA-MRSA and CA-MRSA

HA-MRSA was initially limited to healthcare settings while CA-MRSA was found only in the community. The appearance of CA-MRSA in the healthcare setting and spillage of HA-MRSA into community indicates the possible circulation of CA-MRSA in the healthcare setting and HA-MRSA in the community. However, by evaluating the epidemiological and molecular profile of HA-MRSA and CA-MRSA it is evident that both the strains are different from each other in terms of antibiotic resistance pattern, epidemiology, pathogenesis, virulence, genotypic pattern and treatment (108). The major differences between HA-MRSA and CA-MRSA are summarized in Table 3.2.
Table 3.2: Comparison of HA-MRSA and CA-MRSA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection setting</td>
<td>Healthcare setting</td>
<td>Community</td>
</tr>
<tr>
<td>Susceptible group</td>
<td>Older age patients and neonates, patients admitted in hospitals and other health-care facilities, Immunocompromised individuals</td>
<td>Healthy, immunocompetent young adults, people residing in overcrowded areas such as prisoners, army recruits living in barracks</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>Healthcare associated risk factors like long duration of hospital stay, exposure to antibiotics, presence of indwelling medical devices, dialysis or surgery is always present</td>
<td>No healthcare risk factors associated. Close skin to skin contact, broken skin, unhygienic conditions and overcrowding predispose to CA-MRSA infection</td>
</tr>
<tr>
<td>Clinical Presentation</td>
<td>Surgical site infection, bloodstream infection, infection of burn wound, respiratory tract infection and urinary tract infection</td>
<td>Skin and soft tissue infections, necrotizing pneumonia</td>
</tr>
<tr>
<td>Antibiotic susceptibility</td>
<td>Multidrug resistant</td>
<td>Usually resistant to only β-lactam antibiotics. Is usually sensitive to chloramphenicol, clindamycin and trimethoprim/sulphamethoxazole</td>
</tr>
</tbody>
</table>

Continued on next page
SCCmec type | SCCmec I, II and III, larger in size with integrated genes which confers resistance to other non β-lactam antibiotics | SCCmec IV and V, smaller in size | Rarely present in less than 5% of the isolates | Usually present in >95% of the isolates | USA100, USA200 | USA300, USA400

3.4 Molecular mechanism of methicillin resistance in HA-MRSA

MRSA genome is composed of a complex mixture of genes and is located on Staphylococcus Cassette Chromosome (SCC) which is a mobile genetic element 20.9 to 66.9 kb in length (4,35). SCC carries mecA and other antibiotic resistance determinants. mecA encodes a 78-kDa modified penicillin binding protein, PBP2a which determines penicillin resistance (4,35).

Two important clusters of genes i.e. mec gene complex which encodes methicillin resistance and cassette chromosome recombinase (ccr) gene complex which encodes site specific recombinase for movement of the element are integrated at the 3' end of orfX at a site specific location (aatBscc). SCCmec is demarcated by specific direct repeats (DR) and inverted repeats (IR) which contain integration site sequence (110). SCCmec elements are large with pseudogenes or truncated copies of transposons and insertion sequences present, this area is called as junkyard, joining or J region and its sequence is used for subtyping of different SCCmec types (111). At present 11 types of SCCmec elements have been described (Fig 3.3).
Fig 3.3: The structures of 11 types of SCCmec. Adapted from Hiramatsu et al 2013 (110)

Three major classes of mec gene complexes based on the genomic organization of mecA vicinity have been defined as follows (111):

a) Type I – having mec class B + ccrAB allotype 1 (1B)
b) Type II - having mec class A + ccrAB allotype 2 (2A)
c) Type III - having mec class A + ccrAB allotype 3 (3A)
d) Type IV - having mec class B + ccrAB allotype 2 (2B)
e) Type V - having mec class C + ccrC (5C)
f) Type VI - having mec class B + ccrAB allotype 4 (4B)

HA-MRSA contains the larger SCCmec I, II, III, VI, or VIII elements that may encode resistance determinants in addition to mecA while CA-MRSA strains typically carry SCCmec IV, V, or VII elements, whereas (112,113).

Ito et al in 2001 carried out a detailed structural study of SCCmec type I, II and III (114). The size of type I, II and III SCCmec is 34,364bp, 53,017bp and 66,896bp respectively. The differences in size of these 3 SCCmec was due to the presence of a type-specific DNA region in addition to the essential structures of SCCmec. Regions commonly shared by type I and type II SCCmec are located at the right extremities of the elements between the rightmost IS431 and the right junction point (114). Two ORFs of unknown function are present in the region and are identical between the two types of SCCmec. The nucleotide sequence of the region is seen to be extremely well conserved between the two types with only 1 base substitution in 2,120 bases (114). SCCmec type II has a unique additional 102 bases sequence in the very end of SCCmec (114). The regions common to type II and type III SCCmec are located between the ccr and mec complexes (114).

In SCCmec type III, another copy of Tn554 is present downstream in addition to a ccr-complex-like structure towards the right of the mec complex (114). The ccr complex of SCCmec III is composed of a ccrB homologue and three adjacent ORFs (114). The amino acid sequences had greater than 30% identity to the corresponding ORFs in the SCCmec type II ccr complex (114).

No antibiotic resistance gene except for mecA is present in SCCmec type I (114). In contrast, type III SCCmec contains multiple antibiotic resistance genes which includes transposon C, Tn554 encoding cadmium resistance inserted between ccr and mec complexes, an integrated copy of plasmid pT181 which encodes tetracycline resistance, and transposon,
Tn554 which harbors the *ermA* gene that codes erythromycin and spectinomycin resistance (114). The latter three are present downstream (to the right) of the *mec* complex, and pT181 and the *mer* operon are bracketed by a pair of IS431 copies. Integrated plasmid pUB110 harbors the *ant(4')* gene responsible for resistance to several aminoglycosides, e.g. kanamycin, tobramycin and bleomycin (114). Resistance to penicillins and heavy metals, such as mercury, is encoded by pl258 (114). A unique ORF of SCC*mec* type III is Z059 which is flanked by IS431, the deduced amino acid sequence of it shows a high similarity to HsdR of *Klebsiella pneumonia* and *Salmonella enteric*. HsdR is a catalytic subunit of the restriction-modification system (114). Hence it can be deduced that SCC*mec* type III is a composite element having a combination of SCC*mec* type III and SCC*mercury* which contains ccrC, pl258 and Tn554. Structure of SCC*mec* I/II/III is shown in Fig 3.4 (113).

Fig 3.4: Structures of SCC*mec* type I (A), SCC*mec* type II (B), and SCC*mec* type III (C). Adapted from Ma et al (113)
3.5 Risk Factors and transmission of HA-MRSA

Risk factors associated with infection due to HA-MRSA include advanced age, previous hospitalization, long duration of hospital stay, admission to intensive care unit (ICU), chronic medical illness, prior antibiotic usage, surgery, patient undergoing dialysis, presence of indwelling medical devices and exposure to colonized or infected patients.

Readmission of individuals who are colonized with MRSA during their previous hospital admission may act as a reservoir and result in sporadic transmission of MRSA in healthcare settings (115). MRSA from infected patients or healthcare workers can spread in the same nursing unit by direct or indirect contact (115,116). Healthcare providers colonized with MRSA can spread the infection by direct skin to skin contact while administering patient care. This scenario is particularly common in paediatric units where infants are highly vulnerable. Burn and orthopedic units are other healthcare units where high incidence of MRSA is recorded. In transplant units approximately 5% of the patients are infected with MRSA (117). Detection of MRSA on patient's palm enhances the possibility of MRSA contamination of the surrounding environmental surfaces. Mobile reservoirs like healthcare personnel or medical devices played an important role. Healthcare workers may acquire MRSA after caring for MRSA infected/carrier patients even after isolation precautions. Hence healthcare workers play a critical role in transmission of MRSA from one patient to another in a healthcare setting (116).

Environment and hygiene of healthcare setting plays an important role in the transmission of HA-MRSA infection (118). In healthcare settings medical equipments like stethoscope, blood pressure cuffs and finger probes may become contaminated. Preoperative nurse should take precaution while marking the surgical site as MRSA can even contaminate skin markers resulting in surgical site infections (119). MRSA transmission in intensive care units (ICUs) is a major concern as the patients admitted to ICU have a suppressed immunity. Oztoprak et al conducted a study to analyze the risk factors for ICU acquired MRSA infection (120). They found that insertion of
central venous catheter and total parenteral nutrition was an important risk factor for MRSA infections in ICU.

Contamination of parenteral nutrition solutions may occur either during the assembly or during administration via a contaminated feeding tube (120). Factors that contribute to the contamination of parenteral feedings include the composition of the feeding solution, duration of administration and the number of manipulations in the feeding process. Anterior nares are the most important carrier site for *S. aureus*. Nasal colonization is a significant risk factor for HA-MRSA infections as 57% of the infected patient in the study had nasal MRSA colonization (120).

The healthcare reservoir for MRSA includes patient carriers, infected or colonized patients, healthcare workers colonized with MRSA and hospital environment. In various settings the development of infection has been attributed to *S. aureus* carriage. Coello et al showed that 50% of patients infected with MRSA also yielded MRSA from 1 or more carriage sites (121). Treatment of the carrier was found to be directly proportional to the decrease in number of newly infected patients and more than 900 patients were affected by the control of large hospital outbreak (121). Pujol et al have showed that MRSA nasal carriers admitted to ICU had a high risk of developing bacteremia due to MRSA (122). MRSA colonized patients develop postoperative infection almost twice as fast as those who were not colonized with MRSA. Thus concluding that autoinfection in patients with nasal MRSA carriage or cross infection was the most common mode of acquiring MRSA infection. Cross infection via staff hands through portal of entry such as broken skin or intravascular devices can also result in MRSA infection (122).

Length of hospitalization, invasive procedure, previous antibiotic usage, mechanical ventilation, central venous catheter insertion and total parenteral nutrition are important risk factors for ICU acquired MRSA infection (123-125). Grudmann et al showed that risk factors for acquiring HA-MRSA in ICU are head trauma, neurosurgery, renal replacement therapy, tracheostomy, patient exposed to relative staff deficit, urgent admission, surgery, transfer from other healthcare settings, patient who received airway management or underwent
bronchoscopy (126). The rate of contact between medical staff and intubated patient is more than that for other patients as an intubated patient requires frequent nursing care such as postural change, oral care and suctioning of secretions. This thus increases the chance of MRSA transmission. Albrabial et al studied the risk factors for HA-MRSA infection in paediatric patient and found that patients who were under one year of age had the highest rate of admission due to HA-MRSA (127). Population based study conducted in Wales have shown that the highest incidence of invasive HA-MRSA infection occurs in men with age ≥ 75 years but other controlled studies have not identified age as a significant risk factor (122,127).

Previous hospital admission is an important risk factor for colonization with MRSA at the time of admission (128). Patients already colonized with MRSA during their previous stay are more prone to develop MRSA infection as the colonized pathogen can easily result in clinical disease whenever a breach in skin occurs or a medical device is inserted. Patients who acquire bacteremia in healthcare setting have more exposure to surgery, indwelling medical devices, are undergoing hemodialysis or are usually debilitated (129). High rate of congestive heart failure and underlying heart valve disease has been considered as a risk factor for HA-MRSA bacteremia (130).

Development of invasive HA-MRSA infection or HA-MRSA bacteremia involves acquisition of organism and colonization of skin or superficial surface by HA-MRSA which after sometime enters the host through breached skin or mucous membrane, evades the host defenses and invades the bloodstream (129). Central or peripheral vascular lines are the most important risk factors for direct entry of HA-MRSA into the bloodstream (129). A strong association between MRSA bacteremia and insertion of urinary catheter at time of admission has been reported (131). It is possible that the insertion and/or presence of a urinary catheter is an important risk factor for HA-MRSA bacteremia. Surveillance of device related hospital acquired bacteremia shows that 11% of total bacteremia and 5% of bacteremia due to MRSA is related to urinary catheter associated infection (132). Yamakawa et al in their study showed four independent risk factors associated with HA-MRSA
infection in the ICU (24). The risk factors included intubation, presence of open wound, treatment with antibiotics and administration of steroid, all occurring within 24 hours of ICU admission (24).

Co-morbidities are consistently found associated with surgical site infections (SSI). The most frequently considered co-morbidity includes diabetes, chronic obstructive pulmonary disease (COPD), coronary heart disease, congestive heart failure, acute myocardial infarction, renal insufficiency, hypertension and osteoporosis (133-135). The relationship between SSI and co-morbidities was assessed in several studies. Studies have reported a statistically significant association between SSI and increasing number of co-morbidities (136,137). Graffunder et al showed that the factors which were independently associated with MRSA infection included previous hospitalization (within the last 12 months), longer length of stay before infection, previous surgery, parenteral feedings, macrolide use and levofloxacin use (137). In the study based on the results of the three models the role of macrolides was found to be debatable (137). The most significant risk factors included parenteral feedings and use of levofloxacin (137).

Previous hospitalization and longer length of hospital stay before infection are well known risk factors for acquisition of HA-MRSA infection and antibiotic resistance as these factors represent chronic illness and previous exposure to antibiotics (137). Previous hospitalization or longer duration of hospital stay provides ambient opportunities for MRSA colonization in patient. Surgery has previously been identified as a risk factor for MRSA infection and may represent a breakdown of the normal host defense, surgical technique or post-operative care (137). Surgical site infections were found to be 50% more prominent in the MRSA colonized population (137). Antibiotic usage has historically been associated with MRSA infection as it creates a selective pressure propagating MRSA spread and infection (137).
3.6 Virulence factors of HA-MRSA

HA-MRSA has a wide range of virulence factors which helps it to evade host immune defense and establish itself as a successful pathogen resulting in a wide range of infection. It carries both structural and secreted virulence factors which helps it in its pathogenesis (23). Production of biofilm and several toxins enhances the virulence of HA-MRSA. Host immune status which is usually lowered due to existing co-morbidities in case of hospital admission helps in the progression of HA-MRSA infection (23). Various virulence factors associated with HA-MRSA as shown in Table 3.3 and Fig 3.5.

Table 3.3: Virulence factor of HA-MRSA and its activity

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell wall polymers</strong></td>
<td></td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Activity similar to endotoxin. Provides osmotic stability to bacterium, inhibits inflammatory response</td>
</tr>
<tr>
<td>Teichoic acid</td>
<td>Adsorption of phage. Stores bound divalent cations</td>
</tr>
<tr>
<td>Capsular polysaccharides</td>
<td>Colonization of mucosal surfaces, inhibits phagocytosis</td>
</tr>
<tr>
<td><strong>Cell surface proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Microbial surface components recognizing adhesive matrix molecules (MSCRAMM)</td>
<td>Binds to host molecules such as collagen, fibronectin, and fibrinogen</td>
</tr>
<tr>
<td>Staphylococcal protein A (SpA)</td>
<td>Binds to Fc portion of immunoglobulin G, inhibits phagocytosis</td>
</tr>
<tr>
<td>Fibronectin-binding proteins (FnbpA and FnbpB)</td>
<td>Attachment to fibronectin</td>
</tr>
<tr>
<td>Collagen-binding protein</td>
<td>Mediates adherence to collagenous tissues and cartilage</td>
</tr>
<tr>
<td>Clumping factor</td>
<td>Binds to fibrinogen and promotes adherence to tissue</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th><strong>Exotoxins</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>α toxin</td>
<td>Results in impairment of membrane permeability, has cytotoxic effects on tissue cells and phagocytes</td>
</tr>
<tr>
<td>β toxin</td>
<td></td>
</tr>
<tr>
<td>γ toxin</td>
<td></td>
</tr>
<tr>
<td>δ toxin</td>
<td></td>
</tr>
<tr>
<td>Panton Valentine Leukocidin (PVL) toxin</td>
<td>Lysis of leukocytes, dermonecrotic</td>
</tr>
<tr>
<td>Staphylococcal enterotoxins</td>
<td>Superantigen effects, induces diarrhea and vomiting</td>
</tr>
<tr>
<td>Toxic shock syndrome toxin-1 (TSST-1)</td>
<td>Superantigen effects, Results in T-cells and macrophages activation, organ dysfunction, induces fever</td>
</tr>
<tr>
<td>Epidermolytic toxins</td>
<td>Superantigen effects, Results in T-cells and macrophages activation, cause blistering of skin</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
</tr>
<tr>
<td>Coagulase</td>
<td>Converts fibrinogen into fibrin, prevents phagocytosis, prevents entry of antibiotics into the lesion</td>
</tr>
<tr>
<td>Lipase</td>
<td>Degrades lipid</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>Breaks down deoxyribonucleic acid</td>
</tr>
<tr>
<td>Protease</td>
<td>Degrades protein and tissue peptides</td>
</tr>
<tr>
<td>Staphylokinase</td>
<td>Dissolves fibrin clots</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Degrades intercellular cementing substance and promotes spread of Staphylococci</td>
</tr>
</tbody>
</table>

**Reference:** (23,138,139)
Peptidoglycan and teichoic acid causes the release of tumor necrosis factor which results in septic shock (23). Teichoic acid with its attached substituent helps the bacterial cell surface to maintain its charge and hydrophobicity which thus affects binding of extracellular molecules and protects the bacteria from adverse conditions (140). When the wall teichoic acid is absent bacterium is sensitive to high temperatures and is unable to grow in high salt media which indicates that teichoic acid is involved in temperature tolerance and osmotic stress (140). Teichoic acid deficient cells are more susceptible to lysis by human antibacterial fatty acids (AFAs), as hydrophobic AFAs have the ability to penetrate the less hydrophilic mutant cell wall easily, it can efficiently bind to the cell membrane of the bacteria.
resulting in their lysis (140). HA-MRSA which lack D-alanine has increased susceptibility to phagocytes, killing by neutrophils, lysostaphin and lysozyme (140).

### 3.6.2 Microbial surface components recognizing adhesive matrix molecules (MSCRAMM)

HA-MRSA has numerous surface proteins which help it to establish an infection. These proteins are called as “microbial surface components recognizing adhesive matrix molecules” (MSCRAMMs). These proteins help it to mediate adherence to host tissues (23). MSCRAMMs helps it to bind to host molecules such as collagen, fibronectin, and fibrinogen (23). Different MSCRAMMs of *S. aureus* may help it to bind to the same host-tissue component (23). MSCRAMMs are predicted to play an important part in the initiation of endovascular infections, prosthetic-device infections and bone and joint infections (23). Different constellations of MSCRAMMs may be present in different *S. aureus* strains thus predisposing it to cause different kinds of infections (23).

### 3.6.3 Staphylococcus Protein A (SpA)

Hong et al showed that spa expression in HA-MRSA ST239 was higher compared to that of CA-MRSA ST398 (141). They also discovered that nasal colonization and cell adhesion in ST239 is enhanced by increased production of staphylococcal protein A (spa). HA-MRSA ST239 resists phagocytosis and induces apoptosis of B cells through expression of surface-anchor and release of protein A, it thereby defends itself against the adaptive immune response of host (141).

This protein also plays an important role in subverting the host immune response by its ability to induce early shedding of tumor necrosis factor (TNF)-α receptor1 (TNFR1) from phagocytic cells. Neutralization of circulating TNF-α and impairment of host inflammatory response occur with increased levels of soluble TNFR1 (141). Hong et al tested the possibility of protein A as a virulence factor in bacteremia model in mice and found that protein A
contributed to the durative tissue damage of abscess formation sites in infection caused by HA-MRSA ST239 (141). These functions of protein A benefit HA-MRSA ST239 in its pathogenesis and results in its widespread infection (141).

3.6.4 Fibronectin binding protein

Fibronectin binding is a very common characteristic of HA-MRSA isolates. Most HA-MRSA isolates express two related fibronectin-binding proteins; they are FnbpA and FnbpB which are coded by two closely linked genes (142). Previous studies showed that these proteins are responsible for mediating bacterial attachment to immobilized fibronectin in vitro and also contributes to the attachment of S. aureus cells to plasma clots and ex vivo biomaterial surface which has been in long-term contact with the host. Fibronectin binding proteins are hence important factors which contribute to the initiation of prosthetic device related infections (142). FnbpA and FnbpB proteins have a structural organization similar to other fibronectin binding proteins present in different species of streptococci (142).

3.6.5 Collagen-binding protein (Cna)

Cna mediates bacterial adherence to collagen and collagenous tissues (143). The presence of Cna is mandatory for MRSA to adhere to cartilage inside the host body (143). Binding to collagen and adherence of bacteria to cartilage is blocked by antibodies produced against Cna. A septic arthritis model showed that virulence of MRSA without Cna is significantly lower than the one expressing Cna (143). It has also been noted that introduction of can gene into a MRSA isolate lacking this gene increases its virulence (143).

3.6.6 Fibrinogen-binding protein

Until recently, it was believed that the ability of MRSA to adhere to fibrinogen-containing substrates and to clump in the presence of fibrinogen occurred due to clumping factor ClfA (143). ClfA mediates attachment of MRSA to plasma clots formed in vitro and to plastic biomaterial exposed to canine and human blood for < 6 h (143). Thus fibrinogen binding proteins are
most probably a significant factor in wound and foreign body infection (143). The clfA and clfB genes are distinct genes and not allelic variants. ClfA is present on the surface of MRSA at all stages of growth whereas ClfB is present only on cells which grow aerobically during early exponential phase and is not present on the cell surface when the organism is in stationary phase of growth or in absence of air (143).

3.6.7 Arginine catabolic mobile element (ACME)

It is a large mobile genetic element which plays an important role in the growth, transmission and pathogenesis of CA-MRSA. This virulent factor was identified through genomic sequencing of FPR3757, a multidrug-resistant USA300 MRSA strain (144). Cases of USA300 causing healthcare-associated infection has been reported (145). The origin of ACME is *Staphylococcus epidermidis*, as a high prevalence of ACME has been seen in this organism and so predicts that ACME confers a selective advantage for colonization of human skin. The two main gene clusters identified in ACME include the arc genes (*arcA, arcB, arc* and *arcD*) and the opp genes (*opp-3A, opp-3B, opp-3C, opp3-D* and *opp3-E*) and are homologues of genes which are recognized as virulence factors (144).

The USA300 strain and other strains which have been identified to carry ACME gene includes ST5 (USA100) with SCCmec type II which is a predominant nosocomial MRSA strain mainly found in the United States and ST59 (USA1000) with SCCmec type IVa (146). The isolates with ACME have superior fitness compared to isogenic mutant that lack ACME and SCCmec element (176,181).ACME was initially limited to MRSA USA300 genome downstream of SCCmec (146). Recent study has shown the presence of ACME located upstream of SCCmec between direct repeats (DRs) 2 and 3 of an HA-MRSA strain isolated from Denmark (t024-ST8) clonally related to the USA300 strain (147).

Another study has also identified presence of ACME type II in the ST22-MRSA-IV clone which is a predominant HA-MRSA isolate in healthcare settings of Ireland (148). This isolate had a high-level resistance to mupirocin
and this property along with ACME increases host tissue colonization by preventing nasal decolonization by mupirocin therapy. Another finding was that the novel ACME/SCC\textit{mec}-CI element isolated from Ireland was located upstream of SCC\textit{mec}, similar to the t024-ST8 strain from Denmark which shows that ACME could have been inserted into the chromosome before SCC\textit{mec} (148). Another potential benefit of ACME for its bacterial host is polyamine resistance which exerts bactericidal effects on \textit{S.aureus} when present at a physiological concentration (139).

3.6.8 Phenol soluble modulin (PSM)

Phenol soluble modulins are a class of secreted \(\alpha\)-helical peptides produced by different species of staphylococci. According to Otto et al genes for PSMs are found in every strain of \textit{S.aureus} and there is no any significant difference among strains (149). PSM has the ability to recruit, activate and lyse human neutrophils (139). Mutant strains found with a deletion in the operon of PSMs have been shown to have a reduced capacity to cause SSTIs and bacteraemia in animal models (150). Another study recently described PSM gene and showed that \textit{psm-mec}, was the first to get localized within a SCC\textit{mec} mobile genetic element (MGE) (150). The production of PSM-\textit{mec} peptide was seen to be considerably more in two strains of HA-MRSA i.e, USA100 and USA200 (151). Previous study has shown that the \textit{psm-mec} gene is linked to class A me\textit{c} gene complex present in SCC\textit{mec} types II, III and VIII (151). It has a conserved location located next to the me\textit{c} type I gene (151).

3.6.9 Biofilm

Infections caused by HA-MRSA are further complicated by their ability to form biofilm which is thus a very important virulence mechanism. Infections especially those in which foreign materials like catheters or prosthetic devices are involved get complicated. Several studies have reported formation of biofilm in MRSA isolated from prosthetic device related infections (152-155). Biofilm is defined as surface-attached communities of cells which are encased in an extracellular polymeric matrix which is mostly resistant to antibiotics
The enclosed bacteria are latent and are ineffective to antibiotic therapy as well as are protected against the host's immune response (139). The most common treatment of infections in which chances of biofilm formation is present involves removal of the infected device but the process can be challenging if the patient is elderly or debilitated with limited alternative options such as venous access in individuals requiring chronic indwelling intravenous catheters or if the device is attached to a permanent fixture like in case of a pacemaker or prosthetic implant (139).

Biofilm formation by MRSA occurs in multiple steps. The process starts with the adherence of the pathogen either directly to artificial surface or by host factors such as fibrinogen or fibronectin which acts as bridging molecules (139). Bacteria can colonize by adhering to components of the extracellular matrix of host tissues. Adherence in MRSA is promoted by microbial surface components recognizing adhesive matrix molecules (MSCRAMM) (139). The next step in biofilm formation involves multiplication of the bacteria and accumulation of it into a biofilm requiring intercellular adhesion (139). The previous step is supported by polysaccharide intercellular adhesin (PIA), the adhesin is synthesized by gene products which is encoded by the icaADBC operon. The bhp homologue gene also encodes biofilm-associated protein which helps in biofilm accumulation. This protein was detected in a strain of MRSA which was isolated from a burn unit (139). The transition of MRSA from planktonic stage to biofilm stage occurs through quorum sensing (QS) and is defined as a multicellular response. It helps in a population density dependent manner to coordinate expression of genes required for biofilm formation (Fig 3.6) (139).
Matrix composition of MRSA biofilm involves extracellular DNA (eDNA) (139). Mann et al showed that chromosomal DNA is believed to be the source of eDNA and is released through cell lysis (156). The previous study also showed that staphylococcal thermonuclease helps to break down eDNA and promotes dispersal of biofilm (156).

Previous studies have determined the host’s adaptive immune response to MRSA biofilm by using MRSA-M2, an ST30, spa type T019 and agr type III strain in a mouse model and showed that response by T helper2 (Th2) cell and T regulatory cell (Th2/Treg) protected the host against biofilm formation, while response by Th1/Th17 helped in the development of chronic implant infection (157).
3.6.10 Alpha-toxin (α-toxin)

It is a pore-forming leukocytic toxin and has been well described as a virulence factor in many MRSA strains (158). α-toxin does not cause lysis of neutrophils but results in lysis of other immune cells like macrophages and lymphocytes (139). It can change platelet morphology resulting in increased thrombotic events which is associated with S. aureus sepsis (158). α-toxin monomers secreted by MRSA gets integrated into the cell membrane of the target cells and form cylindrical heptamers (159). This oligomeric form of α-toxin is capable of destroying eukaryotic cells which is the most important property of α–toxin (159).

α-toxin is dermonecrotic and neurotoxic in action and can be very lethal in a variety of animal systems which concludes that this toxin is important in several disease conditions caused by MRSA. Formation of thromboxane and prostacyclin due to activation by metabolic pathway results in vasoconstriction. Furthermore, osmotic swelling results in breakdown of cell integrity which results in an increased vascular permeability and leads to pulmonary edema or adult respiratory distress syndrome in the host (159). α-toxin may also result in edema as a result of endothelial cell contraction leading to leakage of adenosine triphosphate (ATP) along with calcium ion influx, which causes formation of leaky gaps between cells (159). Calcium influx may trigger cellular nucleases which can ultimately lead to apoptotic cell death (159). Destruction of myelin sheaths surrounding rabbit nerves and in the murine cerebral cortex due to α-toxin has been observed (159). An effect on macrophages has also been observed which leads to neurotoxicity (159).

3.6.11 Beta-toxin (β - toxin)

Beta-toxin (β – toxin) was first identified in 1935 by Glenny and Stevens who showed that it was highly hemolytic for sheep erythrocytes but not rabbit erythrocytes and was not dermonecrotic in guinea pigs or lethal in mice (159). The hemolytic activity of this toxin is enhanced when incubated below 10°C after treatment at 37°C thus giving the name “hot-cold” hemolysin. The hlb gene is located on a 4-kb Clal DNA fragment (159). β – toxin was shown to
have phosphorylaseC activity which required the presence of magnesium ion and has specificity for sphingomyelin and lyso-phosphatidylcholine. (159). Differing susceptibility of erythrocytes in different organisms to β – toxin may be because of different sphingomyelin content present in their erythrocytes (159). Hot-cold hemolysis may occur at 20°C, cohesive forces present within the membrane are able to keep the hemolytic products together, but during cooling phase separation may occur resulting in bilayer collapse. Though the role of β – toxin in disease pathogenesis has not been clearly explained, however, high level of expression indicates that β – toxin producers have some selective advantage from secretion of toxin as its production enhances S. aureus growth in the murine mammary glands compared to an isogenic hld knockout organism (159).

3.6.12 Delta-toxin (δ – toxin)

It is a 26-amino-acid peptide which results in membrane damage in a variety of mammalian cells. Human isolates of S. aureus produce delta-toxin which has a nine amino acid difference from the canine strain even though both are 26 residues long (159). hld gene encodes a 514-nucleotide transcript which finally results in the production of delta-toxin (159). Even though delta-toxin may cause a wide range of cytotoxic effects but its importance in disease etiology is yet unclear. Delta-toxin has dermonecrotic activity and causes lethality in experimental animals when given in high concentrations (159). Delta-toxin activity is inhibited by presence of phospholipids in the hosts. Using hydrophobic and hydrophilic domains on opposite sides, α-helix is formed by delta-toxin (159). It thus disrupts the cell membrane of the host by acting as a surfactant (159). Delta-toxin has the ability to lyse erythrocytes and other mammalian cells, it also lyses subcellular structures like membrane-bound organelles, spheroplasts and protoplasts (159).
3.6.13 Gamma-toxin and Panton Valentine Leukocidin (Two-Component Toxins)

These toxins are two types of bicomponent toxins produced by MRSA (159). Both these toxins are made as two non associated secreted proteins and are referred to as S and F components (based on slow- and fast-eluting proteins as seen in an ion-exchange column) (159). Gamma-toxin (γ-toxin) is produced by virtually all S. aureus isolates but Panton Valentine Leukocidin (PVL) is produced only by 2 to 3% of strains. These toxins affect neutrophils and macrophages, and γ-toxin also lyses many varieties of mammalian erythrocytes. γ-toxin cannot be identified on blood agar plates as the agar exhibits inhibitory effect on toxin activity (159).

PVL causes pore formation in the membranes of leukocytes resulting in leukocytosis (139). A study involving 1055 S. aureus isolates from the USA showed that 36% were positive for lukSF-PV genes (160). PVL has been seen to be produced mainly in CA-MRSA isolates worldwide (54). A combination of clonal expansion and horizontal transfer is responsible for PVL genes spread among S. aureus strains (139). There are lineage-specific relationships seen among the type of PVL phage lysogenized in the genome of CA-MRSA strains (139).

Panton and Valentine in 1932 first associated PVL with SSTIs (139). Clinical and experimental results have shown that PVL-producing strains are linked with necrotizing pneumonia and severe, necrotizing skin infections (Fig 3.7) (139,161). The appearance of CA-MRSA in healthcare settings raises a serious concern over the presence of this virulence factor as the immunity of patients is already compromised (54).
PVL enhanced the ability of the USA300 isolate to cause lung necrosis, pulmonary oedema, alveolar haemorrhage, haemoptysis and death (139). Purified PVL when injected directly into the lung results in lung injury by recruitment and lysing of neutrophils which in turn damages lung tissue by releasing cytotoxic granules (139).

PVL was not associated with higher risk of clinical failure or mortality in patients with hospital-acquired pneumonia (HAP) caused by MRSA (139). Studies have shown that patients with HAP and ventilator associated pneumonia (VAP) caused by MRSA are not associated with PVL production (162,163). Since 2000, presence of PVL genes in HA-MRSA strains have been described (19,164). A study conducted in China between 2005 and 2006 showed that out of 702 MRSA isolates from 18 teaching hospitals in 14 cities 2.3% of the MRSA carried PVL genes with all PVL-positive isolates being HA-MRSA as per patient’s medical records (90). Another study conducted in a teaching hospital in Wenzhou showed that 11.9% of the HA-MRSA isolates were PVL positive (165). A study conducted in Tunisian hospitals reported
that 51% of HA-MRSA strains were positive for PVL while another study showed that 5.7% of the HA-MRSA isolates were PVL positive in Asian countries (19,164). The risk of the emerging PVL-positive HA-MRSA strain is a serious concern as it may lead to circulation of multidrug-resistant HA-MRSA isolates with increased virulence in healthcare settings which may worsen MRSA infection.

3.6.14 HA-MRSA superantigens (Epidermolytic toxin, Enterotoxins, Toxic Shock Syndrome Toxin -1)

Superantigens of MRSA include toxic shock syndrome toxin-1 (TSST-1), Epidermolytic toxin and enterotoxins. These superantigens produce a cytokine storm which results in a sepsis-like syndrome (159). Superantigens are mostly small secreted proteins with a size of 20–28 kDa and have similar biochemical and structural properties (159,166). Mobile genetic element encodes all S. aureus superantigens. SEIX is a core-genome-encoded superantigen which modulates the immune response in both human and animal models of disease pathogenesis (166).

The virulence mechanism of these superantigens is explained by many researchers. Superantigens when produced in combinations by strains are more toxic than production of a single superantigen (166). Combinations of superantigens results in over activation of multiple T cell populations (166). Gamma interferon (IFN-γ) production by over activated CD4 T cells results in suppression of antibody production by superantigens (166). It is seen that excessive production of tumor necrosis factor (TNF), a proinflammatory cytokine, by immune cells exposed to superantigens in turn reduces normal phagocytic cell infiltration into infection sites (166). The major mechanism of immunity towards MRSA is believed to be neutralization of toxins by antibodies along with opsonization by antibody-complement system and microbial killing by phagocytic cells; the presence of superantigens interfere with these activities by mugging up antibody production and phagocytic cell chemotaxis. Secondly, production of cytotoxins acts locally to kill immune
cells which reach the infection sites even when superantigens are present (166).

**Epidermolytic toxin**

Epidermolytic toxins (ETs) of *S. aureus* are serine proteases and are responsible for staphylococcal scalded skin syndrome (SSSS), a disease which mainly affects neonates and infants. Adult patients with immune deficiency and renal impairment are also prone to this infection (167). ETs attack the protein desmoglein 1 and break this protein to destroy desmosomal cell attachments resulting in epidermal detachment of skin epidermis (168). Disruption of epidermal layer in turn facilitates the pathogenesis of infection. ETs as a superantigen are milder in comparison to other superantigens such as TSST-1 (169). Occasional reports of SSSS due to MRSA in hospitalized patients are available (170).

**Enterotoxins and Toxic Shock Syndrome Toxin (TSST-1)**

Enterotoxins and TSST-1 are short secreted proteins which are soluble in water and saline solutions. Both the toxins share similar structural and biochemical properties and are thermoresistant. The potency of these toxins can only be gradually decreased by prolonged boiling or autoclaving. Enterotoxins unlike toxic shock syndrome toxin-1 (TSST-1) are highly stable and resistant to most proteolytic enzymes (171). Their biological properties include induction of high fever in host, lethal shock due to excessive intravenous doses in animals, increased host susceptibility to endotoxin lethality, production of cytokine and polyclonal T-lymphocyte proliferation (171).

A study conducted in France showed that a strain of MRSA which carries the *tst* gene encoding toxic shock syndrome toxin 1 (TSST-1) was present in their setting (172). It was called the ST5 Geraldine clone and carried SCC*mec* type I gene. A large prospective study involving 104 laboratories in France showed that the ST5 Geraldine clone of MRSA was more prevalent than the European ST80 clone (173). Infections by ST5 Geraldine clone were equally distributed in the hospital and community and had a wide range of clinical
manifestations than the ST80 clone (173). However, the cases of toxic shock syndrome were relatively few which implied that TSST-1 is not one of the major virulence determinants present in ST5 Geraldine clone but is just an epidemiological marker for this isolate. The ST5 Geraldine clone is resistant to fusidic acid, kanamycin and tobramycin (174).

The Staphylococcal enterotoxins (SEs), SEA to SEI except for SEF are produced by many HA-MRSA. It is the cause of staphylococcal food poisoning but SEB and SEC have also been implicated as a cause of nonmenstrual toxic shock syndrome (159). Eight major staphylococcal enterotoxins have been identified which includes SEA to SEI. SEC is further divided into 3 major antigenic subtypes, SEC1 to SEC3. Many SEC variants have been isolated from bovine and ovine mastitis, they were immunologically identical but very different in function (159).

TSST-1 is believed to be restricted to USA200 and related strains of *S. aureus*, however SEB and SEC are present in both USA200 and USA400 strains. As many as 15 to 30% toxic shock syndrome occurs due to USA200 which produces TSST-1 as well as SEC, MRSA strains usually never produce TSST-1 and SEB together, however rare strains may be present (174). USA400 clone of MRSA produces either SEB or SEC and some rare strains may produce SEB and SEC together. These isolates are the most common cause of all forms of nonmenstrual TSS and account for up to 50% of cases (175,176).

In a previous study it was shown that USA100, USA200, USA400, and USA600 isolates contained the enterotoxin gene cluster or encoded SEC and/or TSST-1 (177). These strains were isolated from different infection sites which included lungs, skin, blood, and vagina. All these isolates were capable of causing infective endocarditis (177). Researchers detected SEG, SEI, staphylococcal enterotoxin-like M (SEl-M), SEl-N, SEl-O, and SEl-U genes, collectively known as the enterotoxin gene cluster (egc) in all isolates from the USA100, USA200, and USA600 lineages (177). SEC was found to be not just high in USA400 isolates but was also present in approximately 60% of USA600 strains and in about 10% of USA100 and USA200 strains (177).
USA300 strains which were frequently associated with invasive disease lacked SAgs. The majority of USA300 strains encoded SEl-K, SEl-Q, and SEl-X (177). The results of the study thus showed that the high frequency of SAg genes in *S. aureus* is usually associated with invasive and life-threatening diseases (177).

### 3.6.15 Lipase

Lipolytic activity in staphylococci was shown as early as in 1901. Lipolytic activity in MRSA is associated with release of fatty acids like linoleic acid in human plasma. The lipase responsible for such reaction includes extracellular lipase which is secreted into the medium (178). The glycerol ester hydrolase (Geh), a lipase of *S. aureus* is released as a 72 kDa precursor enzyme (proGeh), it is then processed into mature form of 42 kDa (Geh). The function of this lipase is to catalyze the hydrolysis of ester bonds between glycerol and fatty acids, which form triglycerides and is believed to help the pathogen by contributing to the breakdown of host tissue, subsequently liberating nutrients. Geh plays an important role in MRSA pathogenesis by interfering with the host granulocyte function and increases the survival of bacteria against the host defense by neutralizing bactericidal lipids (179). Mutation in the lipase genes result in decreased peritoneal abscess formation (179).

### 3.6.17 Protease

MRSA encodes 4 major extracellular proteases which includes a metalloproteinase (Aur, aureolysin), a serine glutamylendopeptidase (SspA, serine protease) and two cysteine proteases, staphopain A (ScpA) and staphopain B (SspB) (180). Co-transcription of SspA and SspB takes place in the *sspABC* operon, it also has a third open reading frame *sspC*, which encodes a cytoplasmic inhibitor of SspB (181). The SspA, SspB and Aur proteases are formed as proenzymes, which are then activated into Staphylococcal proteolytic cascade pathway (Fig 3.8) (182).
Aureolysin (a secreted protease) belongs to the M4 metalloproteinase family of enzymes and is a known MRSA virulence factor. Aureolysin breaks down many host proteins which also includes peptides involved in immune response. Experiments have demonstrated that aureolysin breaks some plasma proteinase inhibitors and also activates prothrombin in human plasma (183). \textit{agr} and \textit{sarA} (Staphylococcal accessory regulator) both regulate aureolysin production. \textit{sarA} is responsible for repression of extracellular protease transcription while \textit{agr} results in its stimulation (184). The \textit{agr} gene system produces RNA III, which in turn stimulates the expression of many exoprotein genes. RNA III interacts with other regulatory proteins instead of directly binding to the promoter site of target genes. RNA III neutralizes a repressor of \textit{aur}, the Rot protein formed by \textit{rot} gene, a global gene regulator thus helping in the regulation of \textit{agr}. \textit{sarA} is a direct repressor of \textit{aur} (185).

The mature SspA helps in moderating \textit{S. aureus} adhesion to fibronectin, as it cleaves cell surface of fibronectin binding proteins and so contributes to invasive infection, it also helps in the activation of proSspB (185). The end

\textbf{Fig 3.8: Staphylococcal proteolytic cascade - Autocatalyzation of aureolysin occurs by induction of the staphylococcal proteolytic cascade which results in its mature form which finally results in mature SspA with subsequent activation of SspB. Adapted from Arsic et al 2012 (182)}
product of the staphylococcal proteolytic pathway is the mature form of Staphopain B cysteine protease. The mature SspB protease has many roles which includes moderating the adhesive functions of the cell, cleavage of fibronectin to release the N-terminal portion. The proteases of \textit{S.aureus} staphylococcal cascade helps in MRSA virulence by breaking plasma molecules, degrading molecules of the immune system, enabling immune evasion, controlling adhesion and activation of other enzymes (180-185).

\textbf{3.7 Infections caused by HA-MRSA}

\textbf{3.7.1 Surgical site infection}

HA-MRSA is an important cause of surgical site infections (SSIs). Surgical wounds are a prime target for infection by MRSA, especially in hospitalized patients (186). In some surgical wards, this problem can become endemic and challenging requiring effective steps for possible eradication (187). Decubitus ulcers and possibly any chronic cutaneous ulcers can also be colonized and infected by MRSA. Patients in medical institutions and long-term care facilities are on top of the list for these types of infections (188).

The rate of wound infection varies from one surgeon to another, from one healthcare setting to another setting, from one surgical procedure to another and mainly from one patient to another. \textit{S. aureus} from the environment or from the patient’s own skin flora is the usual cause of infections associated with clean surgical procedures in which the gastrointestinal, gynecologic and respiratory tracts have not been entered. According to the data obtained from the National Nosocomial Infections Surveillance system, the incidence and distribution of the pathogens isolated from SSIs in 1990s has undergone very little change (189).

A study conducted in Mangaluru showed a prevalence of 32.2\% \textit{Staphylococcus aureus} and 9.6\% MRSA in surgical site infections (190). In 2008, a study reported a rate of 37\% \textit{S.aureus} while in USA the incidence rate of 39\% has been reported (191,192). In USA, Weigelt et al have shown an incidence of 20.6\% MRSA in SSIs while an even higher rate of 45\% and
58.2% MRSA have been reported by Eagye et al and Keith et al (192-194). Sisirak et al in their study involving infections of surgical wounds by MRSA showed that the surgical departments dominated by MRSA included plastic surgery with a MRSA rate of 24.4%, orthopaedic surgery with a rate of 24.1% and MRSA rate of 12.8% in the neurosurgery (195).

3.7.2 Bacteremia

Out of all the infections caused by MRSA, bacteremia is the one which is associated with relatively high morbidity and mortality. A study of mortality at 2 British hospitals from 1997 through 2003 was conducted which showed that MRSA added to the baseline MSSA infection rates and is slowly replacing methicillin-susceptible *S. aureus* (MSSA) as the cause of nosocomial *S. aureus* bacteremia (196). Two meta-analytical studies have shown that MRSA bacteremia was associated with a higher risk of death than in cases of MSSA infection in both analyses (197,198). Multifactorial reasons maybe involved with increased mortality associated with MRSA infection and are not completely understood. The most probable explanations of this may include higher virulence of MRSA, clinical differences between patients infected such as MRSA infection in immunocompromised and old age patients, decreased efficacy of MRSA therapy than MSSA therapy and delay in initiation of proper treatment. Delay in initiation of appropriate antibiotic therapy has been associated with an almost 2-fold increase in mortality associated with MRSA infection (199).

In 2013 the European Centre for Disease Prevention and Control conducted a *S. aureus* bacteremia (SAB) surveillance program, the mean percentage of methicillin resistant *S. aureus* in the study was seen to be 18.0% (200). Among the European countries included in the study, 0% MRSA was recorded in Iceland while 64.5% was recorded in Romania (200,201). In Australian Staphylococcal Sepsis Outcome Programme (ASSOP) 2014, 18.8% of the 2,206 SAB episodes were found to be methicillin resistant which was almost similar to 19.1% in ASSOP 2013 (202). The northern European countries (Germany, France and the United Kingdom) noted a decrease in the
percentage of MRSA causing SAB than that reported in ASSOP 2014 (203-205).

### 3.7.3 Osteomyelitis

Osteomyelitis can involve either a single section of bone or can include cortex, marrow, periosteum and the nearby surrounding soft tissue (201). Osteomyelitis can present itself with a variety of symptoms and signs which may include an open wound with exposed fractured bone or an indolent draining fistula to even local swelling associated with bone pain but no skin lesion (206).

The increase in rate of MRSA and isolation of MRSA with reduced susceptibility to vancomycin has created a therapeutic challenge for the treatment of osteomyelitis (207). *S. aureus* is the most common organism causing osteomyelitis (206). The capacity of *S. aureus* to invade and enter cells may explain its capability to colonize tissues and persist after incidence of bacteremia. A previous study has demonstrated the invasion of cultured osteoblasts by *S. aureus* thus explaining its persistence (208). Both *S. aureus* and *S. epidermidis* can produce biofilm that acts as a diffusion barrier against entry of antibiotics and nutrients (209). One antibiotic therapy is mostly considered to be sufficient for treatment, though nowadays use of combination therapy is in trend, antibiotics such as vancomycin or a β-lactam with rifampin, for the treatment of chronic osteomyelitis and infections involving prosthetic joints (206,210). Prospective clinical studies demonstrating the effect and safety of the newer antibiotics for the treatment of patients with osteomyelitis caused by MRSA are limited but preclinical models of infection show that daptomycin may have good activity in orthopedic-related infections such as osteomyelitis, endocarditis, and foreign body infections (211-213).

An osteomyelitis rate of 7.5% among invasive infections caused by HA-MRSA was documented in the United States (214). Clerc et al showed that in 233 episodes of septic arthritis occurring in their study setting in Switzerland between 1999 and 2008, *S. aureus* caused 115 episodes, of which 11 (9.6%)
were MRSA (215). Another study conducted in United Kingdom which involved 58 adult patients with hematogenous septic arthritis during the study period June 2000 to June 2005 showed that 15 (25.9%) of the clinical isolates were methicillin-resistant (216). Another retrospective analysis which included 53 adults with septic arthritis in Japan from 1955 to 2005, 22% MRSA from *S. aureus* arthritis cases were identified (217). In another study conducted in Taiwan a MRSA rate of 40.9% among cases of septic arthritis was identified (218).

### 3.7.4 Urinary tract infections

Although it is extremely rare but dissemination of MRSA to the urinary tract may occur via the blood stream or can ascend from the urethral meatus (219). In patients with MRSA-induced bacteremia, a positive urine culture is mainly associated with hematogenous spread or ascending infection (219). Catheter-associated urinary tract infection is a frequent cause of significant morbidity (220). A study reported 10 cases of glomerulonephritis due to HA-MRSA, the patients also had sepsis thus indicating hematogenous spread of MRSA (221).

### 3.7.5 Respiratory tract infections

Lower respiratory tract infections with MRSA are commonly seen nowadays. It may affect healthy individuals, but is mainly seen in persons with chronic respiratory conditions such as cystic fibrosis, bronchiectasis and immune-compromised patients (222). One of the severe and serious presentations of lower respiratory tract infection is necrotizing pneumonia. It is mostly a very serious condition and the patient proceeds rapidly to respiratory failure and mortality (223). Prolonged intubation in patients or patients undergoing tracheostomy are at a high risk of developing HA-MRSA respiratory tract infections, this scenario is common in intensive care and long-term healthcare facility settings (224).

MRSA accounts for 20%–40% of all hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). Most of the MRSA strains which cause healthcare–associated pneumonia (HCAP), HAP, and VAP contained
the SCCmec type I/II/III (225,226). Two MRSA strains in Russia has been identified to cause pneumonia and includes ST239<sub>Kras</sub> and ST8<sub>Kras</sub> which are multidrug resistant and has clonally and widely spread causing fatal cases of HAP bacteremia. The study showed that MRSA associated with large epidemics, has a unique set of multiple virulence factors and fatal cases of ST239<sub>Kras</sub> HAP resulted by unique combination of TSST-1, PSM<sub>α</sub>/Hld, Hla, SEK/SEQ, SAK/SCIN, and Cna. ST239<sub>Kras</sub> was found to carry a completely unique phage and mobile DNA, and exhibited unique virulence phenotypes; therefore, ST239<sub>Kras</sub> represented a new (Siberian Russian) clade of the ST239 lineage, which was created by regional stepwise evolution during its possible Brazil-Europe-Russia transmission (227).

### 3.8 Antibiotic resistance in HA-MRSA

HA-MRSA exhibits resistance to many antibiotics. Table 3.4 shows antibiotic resistance mechanism with genes for antibiotic resistance in HA-MRSA.

#### Table 3.4: Antibiotic resistance mechanism in HA-MRSA with associated genes

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Genes</th>
<th>Resistance Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td>blasZ</td>
<td>β-lactamases – inactivate antibiotics</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>mecA</td>
<td>Altered penicillin-binding protein (PBP2a) targets</td>
</tr>
<tr>
<td>Monobactams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbapenems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>gene unknown</td>
<td>VISA – cell wall thickens</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>vanA, vanB– from enterococci</td>
<td>VRSA – modified target</td>
</tr>
<tr>
<td></td>
<td>vanA– from enterococci</td>
<td>GRSA – modified target</td>
</tr>
</tbody>
</table>

Continued on next page
Lipopeptides
Daptomycin  

<table>
<thead>
<tr>
<th><strong>Aminoglycosides</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td><em>aac</em>– plasmid</td>
</tr>
<tr>
<td>Gentamicin</td>
<td><em>ant</em>– plasmid</td>
</tr>
<tr>
<td>Tobramycin</td>
<td><em>aph</em>– plasmid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Tetracycline</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocycline</td>
<td><em>tetK</em>– plasmid</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td><em>tetM</em>– plasmid</td>
</tr>
<tr>
<td>Tigecycline</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Chloramphenicol</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>cat</em> – plasmid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Macrolides, Lincosamides and Streptogramins</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td><em>ermA, ermB,</em></td>
</tr>
<tr>
<td>Clindamycin</td>
<td><em>ermC</em>– plasmid</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Oxazolidinones</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td><em>rrn</em></td>
</tr>
<tr>
<td></td>
<td><em>cfr</em>– plasmid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Fluoroquinolones</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td><em>gyrA</em></td>
</tr>
<tr>
<td>Norfloxacine</td>
<td></td>
</tr>
<tr>
<td>Levofloxacine</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td><em>norA</em></td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td><em>grlA</em></td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td><em>TMP – dhfr</em>,</td>
</tr>
<tr>
<td></td>
<td><em>SMZ – dhps</em></td>
</tr>
</tbody>
</table>

| **Reference:** | (228,229) |

**Lipopeptide (Daptomycin)**

The lipopeptide is involved in depolarization of bacterial cell membrane. Daptomycin is the only antibiotic which is at present marketed for this group (230). Resistance mechanisms have been studied for this antibiotic and mutation in the *mprF* gene is the most common of them. Mutations in *mprF* which is responsible for encoding lysylphosphatidylglycerol synthetase, *yygG* which codes sensor histidine kinase along with *rpoB* and *rpoC* which codes the β and β’ subunits, respectively, of RNA
polymerase is present in *S. aureus* isolates having daptomycin MICs more than the susceptible limit. Mutation in *mprF* occurs early in the selection process, whereas mutation in *rpoB* and *rpoC* occurs later. The *mprF* gene codes an enzyme which adds lysine to cell membrane components and changes the negatively charged peptidoglycan to a positively charged moiety. This change in charge reduces the ability of daptomycin to bind to cell membrane of bacteria (231).

Majority of cases have been described where daptomycin resistant MRSA have emerged in patients with recalcitrant endocarditis in whom vancomycin therapy was given before changing to daptomycin. Patients with relapsing *S. aureus* osteomyelitis during daptomycin treatment have also shown evolution of daptomycin resistant isolates on treatment (232). Cases of daptomycin resistance have been observed to evolve both in MRSA, as well as MSSA strains (232). Kaatz *et al* reported daptomycin resistant MRSA in a patient with right-sided endocarditis after just four days of daptomycin therapy and the authors proposed that use of dosage below the optimal level may have resulted in the emergence of daptomycin resistant isolate (233). Sharma *et al* documented emergence of daptomycin resistant strain after daptomycin therapy in a large cohort of *S. aureus* bacteremia cases (230).

A study was conducted for two and a half year from 2004–2006 and the authors included all patients who were treated with DAP for a minimum of two days. Out of 18 daptomycin treated *S. aureus* bacteremic patients, 10 patients had persistent bacteremia, with parental and post daptomycin therapy isolate pairs included in the study. In 9 out of 10 cases daptomycin was prescribed for persistent bacteremia despite vancomycin therapy in 7 cases. Eight cases of endovascular infection was recorded, most common of which were intravascular catheter-related or endocarditis-related bacteremia. Out of 7 patients with both pre-daptomycin therapy and during post-therapy, isolates were tested for minimum inhibitory concentration, 4 isolates at the later stage exhibited an increase in daptomycin MIC in the range 2-4 µg/ml (234).
Fowler et al conducted a seminal multicenter and multinational randomized study in 2006 to investigate the clinical experience in the use of daptomycin as a primary therapy for \emph{S. aureus} bacteremic cases (235). In this study \emph{S. aureus} bacteremia or right-sided endocarditis cases were included, 120 patients were treated with daptomycin of which 77% had complicated or uncomplicated bacteremia and 16% had right-sided endocarditis while 7% of the patients had left-sided endocarditis. Out of these patients 6 patients had clinical failure which coincided with emergence of daptomycin resistant isolates having a MIC of 2–4 µg/ml (236).

**Aminoglycosides**

Resistance to aminoglycosides is seen mainly because of aminoglycoside-modifying enzymes such as acetyltransferases [AACs], phosphoryltransferases [APHs], and adenylyltransferases [ANTs], decreased intracellular antibiotic accumulation due to outer membrane alterations or by mutation in ribosomal proteins or RNA (236). Aminoacyl site of 16S rRNA gets methylated by 16S rRNA methyltransferases resulting in resistance to aminoglycosides like amikacin, tobramycin, and gentamicin (236).

Gentamicin resistance pattern varies in HA-MRSA isolated from one healthcare setting to another. Indian studies have shown the rate of gentamicin resistance to vary from 17.3% to 58.3% (8,49,51,52). Increase in the rate of gentamicin resistance has been seen over the years. In a study setting within a period of 4 years the rate of resistance to gentamicin has increased by 24.4% (51,52). A study conducted by Asian Network for Surveillance of Resistant Pathogens (ANSORP) reported gentamicin resistance rate in HA-MRSA to be 78.6% while a study from Spain has reported gentamicin resistance rate among MRSA isolated from their healthcare setting to be 0% (19,237).

**Tetracyclines**

Tetracyclines are bacteriostatic antibiotics which bind reversibly to the ribosome 30S subunit, thus inhibiting protein synthesis; these antibiotics enter by active transport or passive diffusion inside the bacterial cell. The main
resistance mechanism includes efflux systems which are encoded by the *tet* genes (*tetA, tetB*) (280). HA-MRSA isolated from Poland is usually resistant to tetracycline (234). Healthcare settings where heterogeneous MRSA was prevalent, the rate of tetracycline resistance was seen to be approximately 50%, while in settings where homogeneous MRSA prevails the rate of tetracycline resistance was recorded to be >95% (238-240).

HA-MRSA from Bulgaria and Turkey also show tetracycline resistance to be a common feature (241,242). Till mid-1990s high rate of tetracycline resistance was a common occurrence in MRSA isolated from healthcare settings of England and Wales, after that decrease in the rate of tetracycline resistance has been recorded which could be associated with the emergence and spread of epidemic MRSA strains (EMRSA-15 and -16) susceptible to tetracycline (38,243). ANSORP study involving 8 Asian countries showed the rate of tetracycline resistance in HA-MRSA to be 72.2% (19).

**Oxazolidinones**

Linezolid belongs to this group. Linezolid resistance in *Staphylococcus aureus* occurs by mutations in the V domain region of one or more of the 5 or 6 copies of the 23S rRNA gene, plasmid –mediated ribosomal methyltransferase *cfr* gene is acquired and deletions or mutations occurs in the ribosomal protein L3 of the peptidyltransferase area (244). Substitutions in L4 ribosomal protein of the peptidyltransferase area have also been seen in linezolid resistant strains (244). Resistance to linezolid occurs due to a point mutation called G2576T in which a thymine base replaces guanine in base pair 2576 of the genes coding for 23S ribosomal RNA and is the most common linezolid resistance mechanism in *staphylococci* (245). Resistance to linezolid in *Staphylococcus aureus* may be acquired after long term exposure to linezolid (246).

In pediatric surgical intensive care unit high linezolid resistance has been observed (246). Previous studies have shown clonal dissemination of linezolid resistant strains within or across healthcare settings (245,246). The first case of linezolid resistant *Staphylococcus aureus* was reported in 2001 (247). The
first outbreak of linezolid resistant MRSA infections was reported from a Spanish hospital (248). Global surveillance studies have reported, <1% of linezolid resistance in *Staphylococcus aureus* (247). In India occasional reports of linezolid resistance among HA-MRSA has been reported (51,249,250). Long term linezolid use resulting in linezolid resistance in HA-MRSA has been reported from London and Japan (244,251-253).

**Macrolide,Lincosamid and Streptogramin B**

MRSA resist macrolide and lincosamid by three ways: methylation or mutation of target-site which prevents antibiotic binding to its target site, by efflux of the agent and by antibiotic inactivation (254-256). Methylation of the ribosomal target site for binding of antibiotic results in cross-resistance to macrolides, lincosamides, and streptogramins B, resulting in MLS\(_B\) phenotype (255). MLS\(_B\) resistance can either be constitutive or inducible. In case of inducible resistance, MRSA produces an inactive mRNA which does not code methylase. The mRNA only becomes active when a macrolide inducer is present (257). In constitutive resistance active methylase mRNA is produced. Induction is associated with the presence of an attenuator upstream from the structural *erm* gene for the methylase. Post transcription induction occurs in *erm*(A-C) derminants (257). In presence of an inducer, rearrangement of mRNA takes place resulting in ribosomal methylation. The strains which carry an inducible *erm* gene are resistant to the inducers but remain susceptible to non inducer macrolides and lincosamides. The *erm*(A) and *erm*(C) determinants are predominant in staphylococci (258). The *erm*(A) gene is mainly found in MRSA strains and is carried by transposons related to Tn554,whereas *erm*(C) gene mainly results in erythromycin resistance in methicillin-susceptible strains and is plasmid borne (258).

The other mechanism of resistance is by efflux pump encoded by plasmid borne *msr*(A) genes (257). The *msr*(A) gene codes for a protein with 2 ATP-binding domains characteristic of ABC transporters. The efflux system is multicomponent in nature and involves *msr*(A) and chromosomal genes to form a completely functional efflux pump which has specificity for 14- and 15-membered macrolides and type B streptogramins and the isolates having this
type of resistance pattern are called the MS\(_B\) phenotype (257). This resistance is inducible. Erythromycin and other 14- and the 15-membered macrolides are inducers but not streptogramin B. Hence the strains become resistant to streptogramin B only after induction with erythromycin. As clindamycin is neither an inducer nor a substrate for the pump so the strains remain fully susceptible to this antibiotic (257).

The third mechanism involves antibiotic modification and is brought about by different enzymes secreted by MRSA (257). Clinical isolates of MRSA produces phosphotransferases which is encoded by \(mph(C)\) gene (259). \(Inu(A)\) and \(Inu(B)\) encodes lincosamide nucleotidyltransferase which inactivates only lincosamides (259). In India the rate of cMLS\(_B\) varies from 11.4% to 46% in MRSA isolated from healthcare settings while the rate of iMLSB varies from 18% to 44.8% (51,52,260-265). The increase in the rate of iMLSB phenotype is a serious concern as these isolates escape detection in routine antibiotic susceptibility testing and when treated with clindamycin could result in treatment failure.

**Flouroquinolone**

Resistance to quinolone occurs by stepwise acquisition of chromosomal mutations. The quinolones work by acting on DNA gyrase which then stops DNA supercoiling, and also inhibits topoisomerase IV, which separates DNA strands. Amino acid changes in important regions of the enzyme-DNA complex called as quinolone resistance–determining region (QRDR) attains reduce quinolone affinity for both of its targets. The \(GrlA\) subunit of topoisomerase IV and the \(GyrA\) subunit in gyrase are the most common affected sites which gets mutated thus resulting in resistance; topoisomerase IV mutation is the most important of both as it is the primary antibiotic target in staphylococci (266,267). \(NorA\) multidrug resistance efflux pump is another mechanism which confers quinolone resistance in \(S.aureus\). Enhanced expression of this pump in \(S. aureus\) can mediate low-level quinolone resistance (268).
Flouroquinolone consumption is considered as a significant risk factor associated with acquisition of HA-MRSA infections (137). Ciprofloxacin was one of the first flouroquinolones to be introduced for the treatment of MRSA infections and also the first against which MRSA developed resistance. Several studies have shown high rate of resistance to ciprofloxacin. Studies from Vellore, Central India, Varanasi and Mangaluru have reported ciprofloxacin resistance in HA-MRSA to be 90%, 84%, 75.6% and 75% respectively (52,249,269,270). ANSORP study showed the overall ciprofloxacin resistance in HA-MRSA from Asian countries to be 77.6% (19). MRSA has attained resistance to even the latest introduced flouroquinolones. A study showed resistance to ofloxacin, gatifloxacin, levofloxacin, sparfloxacin and moxifloxacin in 80.4%, 53.3%, 49.5%, 45.8% and 39.3% respectively in MRSA isolates included in the study (271).

**Glycopeptide**

Teicoplanin and vancomycin are two glycopeptides most commonly used for the treatment of HA-MRSA infections (272,273).

Increase in the rate of HA-MRSA infections has led to the extensive use of vancomycin for treating these conditions. However, extensive use of this antibiotic has led to the emergence of *S. aureus* strains with reduced susceptibility to vancomycin. Hiramatsu et al from Japan reported the first clinical strain of MRSA with reduced susceptibility to vancomycin. The strain was named Mu50 and was isolated from the pus sample obtained from the sternal incision site of a 4-month-old male infant affected with pulmonary atresia (12). The definitions of VISA and VRSA are clearly defined based on the value of MICs obtained by standard CLSI methods while heterogeneous VISA (hVISA) definition has not yet been clearly stated as a standardized method for its MIC determination is not yet available.

In 2006, as a result of increased vancomycin treatment failure CLSI revised the vancomycin MIC breakpoint. At present according to CLSI guidelines *S. aureus* with a MIC of vancomycin of ≤ 2 µg/ml is considered as VSSA while VISA has a vancomycin MIC of 4 – 8µg/ml (15). As vancomycin
MIC results may differ based on the testing methods used, hence CLSI recommended broth macro or microdilution or agar dilution should be performed before confirming that the isolate is VISA (274). Other definitions like S. aureus with reduced vancomycin susceptibility (SA-RVS), is also used to describe VISA (275). The definition of vancomycin resistant S. aureus is slight confusing as different cutoff values are used in different countries to classify vancomycin susceptibility. CLSI in 2006 has revised the vancomycin MIC used for defining VRSA. Accordingly, vancomycin MIC value of ≥ 32µg/ml was revised to a MIC of ≥ 16µg/ml for defining VRSA (15). CLSI guidelines with the above MIC values are used in United States and several other countries for classifying VRSA. However in Japan S. aureus with MIC of vancomycin ≥ 8 µg/ml is described as VRSA (15,276).

hVISA isolates have two population of cell; population’s major part is susceptible to vancomycin (MIC ≤ 2µg/ml) while a smaller part is resistant with a MIC of 8µg/ml. The minor population is present in a very small rate of 10^{-5} to 10^{-6} therefore they escape detection by the recommended CLSI method for vancomycin MIC determination which uses an inoculum of 5 X 10^4 CFU/well in case of broth dilution or 1 X 10^4 CFU/spot in case of agar dilution (277). Population analysis profile/ area under the curve (PAP/AUC) is a diagnostic method used for detecting hVISA even when S. aureus strains have a vancomycin MIC as less as 0.5 to 1µg/ml (278).

It is believed that VISA has developed from hVISA strain as a result of prolonged exposure to glycopeptides (16,279). Decreased vancomycin susceptibility has been attributed to increase in the thickness of S. aureus cell wall. Regulatory systems which control bacterial cellular physiology gets mutated or modulated, thus resulting in enhanced cell wall metabolism which in turn leads to increased production of D-ala-D-ala residues (16). More production of murein monomers and peptidoglycan layers increases the thickness of S. aureus cell wall. The vancomycin gets entrapped in the thickened outermost layer of cell wall and the amount of vancomycin which now reaches the target site is very low and this mechanism of vancomycin entrapment is known as “affinity trapping” (Fig 3.9) (280,281).
Fig 3.9: Affinity trapping in MRSA with reduced susceptibility to vancomycin. Adapted from Sieradzki et al 1999 (281)

The entrapped vancomycin disrupts the outer peptidoglycan layer and in turn blocks further movement of vancomycin to the inner section of cell wall resulting in “clogging phenomenon” (280). The activity of peptidoglycan hydrolase enzyme (an enzyme responsible for shedding the old outer layer of peptidoglycan) is blocked due to binding of vancomycin to cell wall and so the autolytic activity also decreases (282).

*agr* operon has been associated with reduced vancomycin susceptibility (283). Heteroresistance to glycopeptides in *S.aureus* is increased due to isogenic mutation in *agr* group II polymorphism (282). Mutations associated with *walkKR*, *vraSR*, *rpoB*, *yvqF/vraSR* genes mainly constitute the genetic makeup of VISA strains (284). These genes are either directly or indirectly associated with synthesis or metabolism of cell wall (284). Mechanism of resistance to vancomycin in VRSA resembles that seen in vancomycin resistant Enterococci (VRE) (280). An 11-kb mobile genetic element called
Transposon Tn1546 is responsible for vancomycin resistance in *S. aureus* (280). It is from Tn3 family of transposons and codes 9 polypeptides ORF1 and ORF2 which results in transposition. Of these VanR and VanS are responsible for expression of vancomycin resistance (280). Two genetic pathways are involved in vancomycin resistance, they are plasmid transfer during conjugation with Enterococcus species or by transposition by insertion of Tn1546 from Enterococcus species to plasmid or chromosome of *S. aureus* (280). The Tn1546 which gets inserted confers vanA type resistance by producing D-alanine-D-lactate (D-Ala-D-Lac) in place of D-Ala-D-Ala which has very low binding affinity for vancomycin (280).

VISA and hVISA were identified for the first time in 1997 in Japan from a 4 month infant who underwent heart surgery and a 64 year male patient suffering with pneumonia respectively (12,285). Since then infection due to *S. aureus* with decreased susceptibility to vancomycin has been reported from different parts of the globe (16,286). Japan, France, Brazil, United States, South Korea, Hong Kong, Australia, South Africa, Scotland, Thailand and Israel are some of the many which have reported these incidences (16). The rate of VISA ranges from < 0.1% - 44.9% in Asian countries while in America the rate ranges from 0% - 28.6% and a rate of 0.1% - 31.7% has been observed in European countries (10,286,287). A systematic review was conducted by Zhang et al which included data from Asia, Europe, Australia and America from studies published from 1997 to 2014, these studies revealed that the rate of hVISA has gradually increased from 4.7% to 7% over the years (10). Similarly the rate of VISA has also increased from 2.1% to 7.9% at present (10). According to this study the rate of VISA was 3.4% in Asia and 2.8% in Europe/America while hVISA had a rate of 6.8% in Asia and 5.6% in Europe/America (10). First case of hVISA from Australia was reported in 2001 and an increase in the rate of hVISA and VISA has been reported since then (10). In India VISA has been reported from Hyderabad, Pondicherry, Chandigarh, Mangaluru and Varanasi (11,52,287-290).

Thirty six vanA positive VRSA cases have been documented worldwide till date (280). Investigators have reported 7 VRSA isolates from Hyderabad.
healthcare settings (11). A study reported 28.86% of VRSA and 45.11% VISA from nosocomial sources while in ICU and NICU the rate of VRSA and VISA was 16.80% and 45.17% respectively (287). Ever since the discovery of vancomycin-resistant Enterococcus in the late 1980s, concern over the emergence of vancomycin resistance in MRSA isolates through transfer of plasmids was there. A vancomycin-resistant S.aureus (VRSA), however, emerged only in 2002 and was reported from the United States of America (17). This strain had acquired the vancomycin resistance gene cluster vanA from vancomycin-resistant enterococci. The acquisition of resistance plasmid was the cause of vancomycin resistance in most cases of VRSA. In several cases, VRE strains have been isolated along with the VRSA strains from the same infectious site of the patients which thus supports the theory that the Tn1546 plasmid which carries the vanA gene cluster found in VRSA is acquired from VRE (284,291).

Depending on the level of resistance to glycopeptides i.e., vancomycin, and teicoplanin, two resistance phenotypes are defined for VRSA isolates. High-level resistant VRSA (HLR VRSA) includes those VRSA strains which has high-level resistance to both vancomycin and teicoplanin (MIC > 256 μg/ml and > 32 μg/ml). Till date most of the isolates have HLR vancomycin resistance while just two of the VRSA isolates with moderate level of resistance to vancomycin (MIC 32 μg/ml and 64 μg/ml) and low-level resistance to teicoplanin have been isolated and termed as low-level-resistant VRSA (LLR VRSA) (292).

### 3.9 Treatment options for HA-MRSA

Vancomycin is the gold standard for treating invasive and serious infections due to HA-MRSA. It is used as first line therapy for the treatment of bacteremia, endocarditis, central nervous system infections (subdural empyema, brain abscess, spinal epidural abscess), SSTIs, endophthalmitis and prosthetic joint infections (293). Higher vancomycin MIC values of 1.5 or 2 μg/ml is associated with bad prognosis and hence an alternative treatment is recommended (293). Treatment of MRSA infection with vancomycin
involves complications due to side effect of this antibiotic like toxicity and nephrotoxicity (294,295).

Teicoplanin is another glycopeptide which is used in the treatment of gram positive infections, especially infections caused by MRSA. Many previous studies have demonstrated that teicoplanin is as effective as vancomycin for the treatment of bacteremia, bone and joint infections and is usually tolerated better with lesser adverse effects (296,297). Teicoplanin is available for intravenous as well as intramuscular administration and only a single dose is usually recommended. Teicoplanin is similar in its structure to vancomycin which has a similar activity spectrum but a longer half-life (297). As the oral absorption of vancomycin and teicoplanin is very less, these agents must therefore be administered intravenously for controlling systemic infections and rapid activity (295).

Daptomycin is a cyclic lipopeptide antibiotic which is frequent used to treat multidrug resistant MRSA especially vancomycin resistant MRSA (298). It has rapid bactericidal activity against MRSA (298). Daptomycin binds to the cytoplasmic cell membrane resulting in cell death. It exhibits concentration based bactericidal activity and its unique mechanism of action prevents the production of any cross resistance (298). In a study conducted by Fowler et al therapeutic effect of daptomycin was found to be similar to standard therapy used for the treatment of MRSA bacteremia with or without infective endocarditis (298). Elevated creatine kinase is an adverse effect of daptomycin with cases of rhabdomyolysis being reported hence, creatine kinase should be measured weekly so as to avoid progressive myopathy (299). Other serious side effects reported are nephropathy, peripheral neuropathies and hepatotoxicity (300). Since pulmonary surfactant inactivates it so daptomycin should not be used in cases of pneumonia (301).

Tigecycline is a glycylcycline antibiotic having a bacteriostatic effect on gram positive bacteria including S. aureus. Tigecycline is used for treating complicated cases of skin/skin structure and intra abdominal infections along with infection due to MRSA (302). Gardiner et al conducted a study which compared the effect of tigecycline in the treatment of patients with secondary
bacteremia with standard therapy and found that cure rate with tigecycline was 81.1% compared to 78.5% by standard therapy (303). It has not been associated with toxicity of any organ or any severe side effects and dose adjustment for hemodialysis dependence is not required. Common side effects include nausea and vomiting (303).

Linezolid is the first member of an oxazolidinone class of antibiotic. Linezolid inhibits ribosomal protein synthesis thereby inhibiting bacterial growth it is bacteriostatic against staphylococci. Advantage of linezolid includes high oral bioavailability as well as intravenous administration (304). Studies have shown that linezolid is comparable to standard antibacterial therapy in the treatment of pneumonia and SSTIs caused by HA-MRSA (305,306). Wilcox et al conducted a study which raised some concerns over the use of linezolid in the treatment of catheter associated bloodstream infections as higher mortality was associated with linezolid therapy than during treatment with vancomycin in patients with catheter related bloodstream infection (307). Another observational Korean study documented that linezolid had utility in the setting of persistent MRSA bacteremia (308). Linezolid is associated with reversible myelosuppression, mainly thrombocytopenia, when treatment is prolonged. Patients with linezolid treatment should be closely monitored along with complete blood counts. Other side effects include lactic acidosis, optic and peripheral neuropathy and a serotonin like syndrome which can be elicited by the administration of certain antidepressant medications simultaneously (309). Most side effects get completely or partially reversed when the treatment is stopped but peripheral neuropathy may persist even after antibiotic discontinuation (309).

Telavancin is a lipoglycopeptide used once daily with efficacy against MRSA, VISA, and VRSA strains which is attributed to its dual mechanism of action (293). Stryjewski et al in a randomized, double blind study showed that telavancin was as effective as vancomycin for the treatment of complicated skin and skin structure infections caused by MRSA and had a clinical cure rate of 90.6% than that of 84.4% seen in vancomycin (310). Side effects of
telavancin include metallic taste, vomiting, nausea, dizziness, headache, rash and decrease in platelet count (311).

Quinupristin-dalfopristin is a combination of two streptogramin antibiotics. The site of action is the bacterial ribosome, and collectively it acts synergistically for protein synthesis inhibition. A previous multicenter study compared it and vancomycin in the treatment of nosocomial pneumonia by gram positive pathogens and found it to have same clinical success with both antibiotics (312). The data suggests that quinupristin-dalfopristin is not a better option than vancomycin as both had limited efficacy against MRSA pneumonia in the study (312). Side effect of this antibiotic includes severe myalgia and hence is not frequently used by many practitioners (295).

Perioperative prophylaxis with nasal mupirocin is used to prevent and reduce the rate of MRSA skin and soft tissue infections after orthopaedic surgery due to reduction in nasal MRSA carriage in the endemic setting and no selection of mupirocin resistance takes place (295).

Dalbavancin and oritavancin are other lipoglycopeptides having bactericidal activity against MRSA (313). Phase II randomized clinical trial with dalbavancin has shown it to be superior to vancomycin for treating catheter related bloodstream infections whereas oritavancin was similar to standard therapy for the treatment of skin and soft tissue infections and bacteremia (313). Dalbavancin has a long half life hence weekly intravenous administration is sufficient while oritavancin is administered once daily by intravenous route (314).

Ceftobiprole and ceftaroline are newer antibiotics having bactericidal activity against MRSA (315). None of these antibiotics have however been tested for treating *S.aureus* bacteremia. Two unpublished phase III studies have tested ceftobiprole efficiency against community associated and healthcare associated pneumonia, respectively. In both studies ceftobiprole was found to be similar in its effect to the comparator treatment whereas ceftaroline was inferior in the treatment of ventilator associated pneumonia (313,315). Both ceftobiprole and ceftaroline are efficient in the treatment of
skin and soft tissue infections due to *S. aureus* (315) Ceftobiprole has a strong affinity for the penicillin binding protein PBP2a, thus resulting in its activity against MRSA (316).

### 3.10 Laboratory diagnosis of HA-MRSA

Laboratory identification of HA-MRSA from clinical sample helps in identification of the cause of infection, for obtaining antibiotic susceptibility data for treatment and to study the epidemiology of infection (317). Based on the site of infection, specimens like pus, exudates, blood, urine, sputum and other specimens are collected under aseptic precautions and transported to laboratory where it is processed without delay (317). To culture the specimen and isolate *S. aureus* blood agar is used. Standard tests and procedures are used to identify *S. aureus*; these procedures include colony morphology, gram stain, catalase and coagulase test (317).

According to CLSI, cefoxitin disk (30µg) is recommended for detection of methicillin resistance (318). Oxacillin agar screening (using Mueller-Hinton agar plate with 6µg/ml Oxacillin and NaCl 4%) can also be used as an alternatively for the detection of methicillin resistance (319). Organisms expressing heteroresistance grow at a slower rate therefore isolates tested for oxacillin/methicillin should be incubated at 33°C-35°C for 24 h as recommended by CLSI (318,319). *mecA* gene that encodes PBP-2a can be detected by polymerase chain reaction (320). For epidemiological purposes HA-MRSA is typed by various methods which include SCC*mec* typing, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and X region encoding protein A (*spa*), the *mec* associated hypervariable region (*dru*) and the accessory gene regulator (*agr*) typing (5,6). Latest development in identification of MRSA is the introduction of an integrated modular-based microfluidic system, it carries out multistep assay in a single disposable fluidic cartridge and is capable of identifying strains within 40 min, it also helps to differentiate HA-MRSA from CA-MRSA on the basis of absence or presence of PVL gene (320).
3.11 Prevention of HA-MRSA

Prevention of HA-MRSA is very important in healthcare setting as HA-MRSA is a major cause of increased morbidity and mortality in patients. Prevention strategies for MRSA wherever applied in the healthcare setting till date have mainly focused on prevention of cross-transmission and have included hand hygiene practices, disinfection and environmental cleaning, timely identification of MRSA-colonized patients and management of either MRSA infected or colonized patients under isolation precautions (321). Active surveillance culture for detection of MRSA is regularly performed in healthcare settings of the United States due to formed legislation (321). Active surveillance culture includes culture of specimens obtained from patients’ nares at the time of hospital admission so as to detect MRSA carriers and, thus, to implement isolation precautions to reduce the chances of MRSA transmission to other patients. In Illinois healthcare setting it is mandatory for screening of all persons at high risk of infection for MRSA who get admitted to the hospital, and similar legislations have been introduced or approved in several other states (321).

Decolonization of *S. aureus* for a short term is usually successful, but patients often get recolonized with usually the same strain, suggesting that there is involvement of either non nasal or exogenous sources (322-325). A meta-analysis conducted in 2003 investigated the effects of topical and systemic antibiotics on MRSA carriers but found insufficient evidence which supported the use of topical or systemic antibiotic therapy used for eradication of nasal or extra nasal MRSA, and no study showed that either topical therapy, systemic therapy, or a combination of both types of therapy was superior (325).

Jernigan et al have divided prevention of HA-MRSA into two strategies which includes core and supplemental strategies (326). Core prevention strategies includes assessing of hand hygiene practices, implementation of contact precautions, recognition of previously colonized patients, rapid reporting of MRSA laboratory results and providing MRSA education to healthcare providers (326). Easy access to soap and water/alcohol based
hand gels with regular education regime for healthcare providers on the proper use of it should be implemented, particularly around high risk patients that are either carriers of MRSA or infected with MRSA (326). Contact precaution involves use of gown and gloves prior to entry of room and removal before exiting the room (326). The colonized/infected patients should be cohorted and use of dedicated non-essential items such as stethoscopes, IV poles and pumps or blood pressure cuffs might help to decrease transmission within the hospital (326). Previously colonized patients should be detected and decolonized as part of supplemental prevention strategy (326). Regular surveillance/tracking of MRSA should be carried out to evaluate changes in the rate of invasive MRSA cases in the healthcare settings.

Management of antibiotic resistant organisms in healthcare settings 2006 guidelines by CDC MDRO suggested seven categories of interventions which are standard precautions, contact precautions, environmental measures, education, decolonization, judicious use of antibiotics and administrative support (326). In case of outbreak if there is implication of transmission in such cases the healthcare workers (HCWs) should be tested for carriage of MRSA (326). Nares and or other appropriate sites such as open wound should be sampled and cultured (326). If HCW is found to be infected in such cases the HCW should be counseled and information pertaining to infection control measures should be imparted to them. Any deficiency in the technique of HCW should be corrected immediately (326). Decolonization of carrier HCW should be done and if these initial steps fail the HCW should be removed from care of high risk patients. In an outbreak if initial methods of control fail to limit it then patients should be cohorted and cohorting staff i.e., same staff should be assigned to care for this particular group till the outbreak ends, equipments too should be dedicated to the cohorted area (326).

An outbreak of MRSA in France neonatal ICU was controlled by implementation of sustainable prevention strategy which included extensive HCW involvement, continuous hand-hygiene training, education along with active MRSA colonization surveillance (327). In Canada an outbreak in ICU occurred and initial control measures such as HCW education, patient
cohorting, enhanced surveillance and enhanced environmental cleaning were employed. Even then three more cases were reported the hospital then implemented contact isolation for patients, HCW screening and used cohorted nursing staff. All positive patients were decolonized. After five weeks with no further cases identified the outbreak was declared to be over (328).