The molecular evolution of methicillin-resistant *Staphylococcus aureus*

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**ABSTRACT**

*Staphylococcus aureus* is a potentially pathogenic bacterium that causes a broad spectrum of diseases. *S. aureus* can adapt rapidly to the selective pressure of antibiotics, and this has resulted in the emergence and spread of methicillin-resistant *S. aureus* (MRSA). Resistance to methicillin and other β-lactam antibiotics is caused by the *mecA* gene, which is situated on a mobile genetic element, the Staphylococcal Cassette Chromosome mec (SCCmec). To date, five SCCmec types (I–V) have been distinguished, and several variants of these SCCmec types have been described. All SCCmec elements carry genes for resistance to β-lactam antibiotics, as well as genes for the regulation of expression of *mecA*. Additionally, SCCmec types II and III carry non-β-lactam antibiotic resistance genes on integrated plasmids and a transposon. The epidemiology of MRSA has been investigated by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing and SCCmec typing. Numerous MRSA clones have emerged and disseminated worldwide. SCCmec has been acquired on at least 20 occasions by different lineages of methicillin-sensitive *S. aureus*. Although most MRSA strains are hospital-acquired (HA-MRSA), community-acquired MRSA (CA-MRSA) strains have now been recognised. CA-MRSA is both phenotypically and genotypically different from HA-MRSA. CA-MRSA harbours SCCmec types IV or V, and is associated with the genes encoding Panton–Valentine leukocidin. The prevalence of MRSA ranges from 0.6% in The Netherlands to 66.8% in Japan. This review describes the latest developments in knowledge concerning the structure of SCCmec, the molecular evolution of MRSA, the methods used to investigate the epidemiology of MRSA, and the risk-factors associated with CA-MRSA and HA-MRSA.

**Keywords** CA-MRSA, evolution, HA-MRSA, methicillin-resistant *Staphylococcus aureus*, review, *Staphylococcus aureus*

Accepted: 17 July 2006

*Clin Microbiol Infect* 2007; 13: 222–235

**INTRODUCTION**

Since its discovery during the 1880s, *Staphylococcus aureus* has emerged as a potentially pathogenic Gram-positive bacterium that can cause various diseases, ranging from minor infections of the skin to post-operative wound infections, bacteraemia, infections associated with foreign bodies and necrotising pneumonia. Until the introduction of penicillin, the mortality rate of patients infected with *S. aureus* was c. 80%. In the early 1940s, *S. aureus* infections were treated with penicillin, but the first strains resistant to this antibiotic were isolated in 1942, first in hospitals, and later in the community. This resistance resulted from the acquisition of a plasmid that encoded a penicillin-hydrolysing enzyme, i.e., penicillinase. Since 1960, c. 80% of all *S. aureus* isolates have been resistant to penicillin. In addition, *S. aureus* strains have developed resistance to methicillin and vancomycin through the acquisition of the *mecA* and *vanA* genes, respectively [1,2].
STAPHYLOCOCCAL CASSETTE CHROMOSOME mec (SCCmec)

The resistance of *S. aureus* to methicillin is caused by the presence of the *mecA* gene, which encodes the 78-kDa penicillin-binding protein (PBP) 2a (or PBP2'). β-Lactam antibiotics normally bind to PBPs in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of the bacterium. Since β-lactam antibiotics cannot bind to PBP2a, synthesis of the peptidoglycan layer and cell wall synthesis are able to continue. The *mecA* gene is regulated by the repressor MecI and the trans-membrane β-lactam-sensing signal-transducer MecRI, both of which are transcribed divergently. However, in the absence of a β-lactam antibiotic, MecI represses the transcription of both *mecA* and *mecRI–mecI*. In the presence of a β-lactam antibiotic, MecRI is cleaved autocatalytically, and a metallo-protease domain, which is located in the cytoplasmic part of MecRI, becomes active. The metallo-protease cleaves MecI bound to the operator region of *mecA*, which allows transcription of *mecA* and subsequent production of PBP2a [3]. Both *mecI* and *mecRI* can be truncated by insertion sequences IS431 or IS1272, and this results in derepression of the *mecA* gene [4].

The 2.1-kb *mecA* gene is located on a mobile genetic element, designated the Staphylococcal Cassette Chromosome mec (SCCmec) [5]. Currently, five main types of SCCmec (types I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb (Fig. 1). SCCmec types I (34.3 kb), IV (20.9–24.3 kb) and V (28 kb) encode exclusively for resistance to β-lactam antibiotics. In contrast, SCCmec types II (53.0 kb) and III (66.9 kb) determine multiresistance, as these cassettes contain additional drug resistance genes on integrated plasmids (pUB110, pI258 and pT181) and a transposon (Tn554). Plasmid pUB110 carries the *ant(4')* gene, responsible for resistance to kanamycin, tobramycin and bleomycin, and pI258 codes for resistance to penicillins and heavy metals. Plasmid pT181 codes for tetracycline resistance, while transposon Tn554 carries the *ermA* gene, which is responsible for inducible macrolide, lincosamide and streptogramin resistance (Fig. 1) [5,6]. Besides the resistance genes on SCCmec, *S. aureus* can carry resistance genes inserted at other sites of the chromosome and on plasmids. SCCmec also carries insertion sequences, e.g., IS431, as well as genes responsible for the regulation of *mecA* transcription, i.e., ΔmecRI (on SCCmec types I, IV and V) or mecRI and mecl (on SCCmec types II and III) [5,7–9]. These genes are situated in mec complexes. To date, five major classes, A–E, of mec complexes have been distinguished (Table 1) [4,5,7].

For integration into and excision from the chromosome at a specific site (*attB*); at the 3′-end of an open reading frame of unknown function, termed orfX [10]), genes encoding cassette chromosome recombinases (*ccr*) are located within the SCCmec elements. These genes are designated *ccrA1* and *ccrB1* (in SCCmec type I), *ccrA2* and *ccrB2* (in SCCmec types II and IV), *ccrA3* and *ccrB3* (in SCCmec type III), *ccrA4* and *ccrB4* (in SCCmec type IV of methicillin-resistant *Staphylococcus aureus* (MRSA) strain HDE288) and *ccrC* (in SCCmec type V). The regions bordering the mec

![Fig. 1. Schematic arrangement of SCCmec types I–V](https://example.com/fig1.png)
and ccr complexes are designated the J (junkyard) regions (Fig. 1) [4,8,9,11,12].

All SCCmec elements are divided into three regions. The J1 region ranges from the chromosome right junction to the ccr genes, while the J2 region ranges from the ccr genes to the mec complex. The J3 region is located between the mec complex and the left extremity of SCCmec [13,14]. In addition to the five main SCCmec types, several variants of SCCmec have been described (Table 2) [5,12,13,15–19].

Besides MRSA, methicillin-resistant coagulase-negative staphylococci can harbour SCCmec. It has been shown that methicillin-resistant Staphylococcus epidermidis isolates from the 1970s harboured SCCmec types I–IV [20]. Investigations of the SCCmec type among 156 methicillin-resistant Staphylococcus epidermidis isolates from the late 1990s identified SCCmec types I–V among 85 isolates, but the SCCmec type of the other 21 isolates could not be determined [21].

Table 1. Major classes of mec complexes

<table>
<thead>
<tr>
<th>Class</th>
<th>Structure</th>
<th>SCCmec</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mecI–mecRI–mecA–Is431</td>
<td>II, III</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>B</td>
<td>IS2327–mecRI–mecA–Is431</td>
<td>I, IV</td>
<td>S. aureus</td>
</tr>
<tr>
<td>C</td>
<td>Is431–mecRI–mecA–Is431</td>
<td>V</td>
<td>S. aureus</td>
</tr>
<tr>
<td>D</td>
<td>ΔmecRI–mecA–Is431</td>
<td>–</td>
<td>Staphylococcus capnis</td>
</tr>
<tr>
<td>E</td>
<td>ΔmecRI–mecA–Is431</td>
<td>–</td>
<td>S. aureus</td>
</tr>
</tbody>
</table>

*976-bp deletion in mecRI compared to the class D mec complex.

The first MRSA strain (NCTC 10442), isolated during 1961 in the UK, harboured SCCmec type I, and this so-called archaic clone spread around the world during the 1960s. In 1982, an MRSA strain (N315) with SCCmec type II was discovered in Japan, and this New York/Japan clone also spread worldwide; this was followed by the discovery in 1985 of an MRSA strain (85/2082) harbouring SCCmec type III in New Zealand. MRSA strains harbouring SCCmec IV spread round the world during the 1990s, and at the beginning of the 21st century, the first MRSA strain (WIS) with SCCmec type V was described in Australia [8,24,27].

Although the origin of SCCmec remains unknown, the cassette could originate from staphylococci other than S. aureus. It is believed that Staphylococcus sciuri harboured the ancestor of PBP2a, since a PBP was found in S. sciuri that showed 87.8% amino-acid sequence identity with mecA [22].

Table 2. Structural variants of SCCmec types I–V

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>Structure compared to main SCCmec type</th>
<th>Proposed novel nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>pUB110 integrated downstream of mecA</td>
<td>1B1.2</td>
</tr>
<tr>
<td>IIA</td>
<td>SCCmec type Ivb J1 region; class A4 mec complex</td>
<td>2A.3.1</td>
</tr>
<tr>
<td>IBb</td>
<td>SCCmec type Ivb J1 region; lacks Tn554</td>
<td>2A.3.2</td>
</tr>
<tr>
<td>IIC</td>
<td>SCCmec type Ivb J1 region; class A3 mec complex</td>
<td>2A.3.3</td>
</tr>
<tr>
<td>IID</td>
<td>SCCmec type Ivb J1 region; class A4 mec complex</td>
<td>2A.3.4</td>
</tr>
<tr>
<td>IIE</td>
<td>SCCmec type Ivb J1 region; class A3 mec complex</td>
<td>2A.3.5</td>
</tr>
<tr>
<td>IHb</td>
<td>Lacks pUB110; IS256 inserted upstream of mecA</td>
<td>2A.2</td>
</tr>
<tr>
<td>IIIA</td>
<td>Lacks pT181 and its associated IS431 sequences</td>
<td>3C12</td>
</tr>
<tr>
<td>IIIB</td>
<td>Lacks pT181 and p258, together with IS431; Lacks Tn554</td>
<td>3C13</td>
</tr>
<tr>
<td>IVa/IVb</td>
<td>Different J1 regions when compared to SCCmec type IV; harbours downstream constant region (dcs)</td>
<td>2B1.2/2B.2.1</td>
</tr>
<tr>
<td>IVc</td>
<td>Different J1 region compared to SCCmec type IV; harbours Tn4001 flanked by IS256 sequences</td>
<td>2B.3.1</td>
</tr>
<tr>
<td>IVd</td>
<td>Different J1 region compared to SCCmec type IV</td>
<td>2B.4</td>
</tr>
<tr>
<td>IVa</td>
<td>Harbours pUB110 downstream of mecA</td>
<td>2B.N.2</td>
</tr>
<tr>
<td>IVc</td>
<td>Variant of SCCmec type IV; lacks dcs region; different J1 region</td>
<td>2B.3.3</td>
</tr>
<tr>
<td>IVe</td>
<td>Variant of SCCmec type IV; lacks IS256; different J1 region</td>
<td>2B.2.2</td>
</tr>
<tr>
<td>IVf</td>
<td>Different J1 region composed of 5 ORFs compared to SCCmec type IV</td>
<td>2B.5</td>
</tr>
<tr>
<td>IVg</td>
<td>Harbours ccrC2 variant of ccrC</td>
<td>5C.2</td>
</tr>
</tbody>
</table>

*ORF, open reading frame.
PBP2a. These strains were all susceptible to methicillin, but became resistant to methicillin following growth of the strains in the presence of methicillin because of an increase in the transcription rate of the mecA homologue, subsequent to a point mutation in the promoter. Furthermore, a strain of methicillin-sensitive S. aureus (MSSA) became resistant to methicillin following introduction of this mecA homologue, and could thus be classified as MRSA [28]. It has also been reported that an epidemic MSSA strain and, subsequently, an isogenic MRSA strain were isolated from a neonate who had never been in contact with MRSA. The mecA gene was identical to that found in a S. epidermidis isolate from the same patient. It was concluded that the MRSA strain had emerged in vivo following horizontal transfer of mecA between the two staphylococcal species [29].

MOLECULAR TYPING OF MRSA
Strategies aimed at preventing the spread of MRSA require a thorough knowledge of both the dissemination and the epidemiology of MRSA strains. For this purpose, various molecular typing techniques have been developed. These techniques include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), SCCmec typing, and typing of the variable tandem repeat region of protein A (spa typing) [30].

PFGE
PFGE is still considered to be the reference standard for typing MRSA isolates, and has been demonstrated to be one of the most discriminative typing methods for studying outbreaks and hospital-to-hospital transmission. PFGE typing of MRSA is based on digestion of purified chromosomal DNA with restriction enzyme SmaI, followed by agarose gel electrophoresis. The PFGE patterns are analysed with the Dice coefficient and unweighted pair-group matching analysis (UPGMA) settings, according to the scheme of Tenover et al. [31]. Significant efforts have been made to harmonise PFGE protocols and to establish a standardised nomenclature. However, these efforts have proved only partially successful when judged in terms of reproducibility, speed and costs of analysis [32–34]. Because of the need for strict adherence to standardised protocols, common databases have been realised only at a national level. At an international level, attempts to produce a common nomenclature have not been successful.

MLST
MLST is an excellent tool for investigating the clonal evolution of MRSA. MLST is based on sequence analysis of 0.5-kb fragments from seven S. aureus housekeeping genes, i.e., arcC, aroE, glpF, gmk, pta, tpi and yqiL. Different sequences are assigned distinct alleles of each housekeeping gene, and each isolate is defined by the alleles of the seven genes. This results in an allelic profile or sequence type (ST). For example, the so-called Iberian clone has the MLST profile 3-3-1-12-4-4–16, which has been defined as ST247 (http://www.mlst.net). Currently, the nomenclature of MRSA strains is based on the ST and the SCCmec type; for example, ST247-MRSA-I is the Iberian clone harbouring SCCmec type I. Clonal complexes (CCs) can be defined with the software package BURST (based upon related sequence types) to analyse evolutionary events (http://www.mlst.net). S. aureus strains are grouped within a single CC when five of the seven housekeeping genes have identical sequences. The ancestor of each CC is the ST with the largest number of single-locus variants. Subgroup founders can be described as single-locus variants or double-locus variants of a founder of a CC that has become prevalent in a population, and that may subsequently have diversified to produce its own set of single-locus variants and double-locus variants [35–38]. A disadvantage of MLST is that it is rather laborious and time-consuming.

SCCmec typing
Four methods are currently available for the characterisation of SCCmec. Oliveira and de Lencastre [17] developed a multiplex PCR for SCCmec types I–IV, in which mecA and six different loci on SCCmec are detected (Fig. 1, Table 3) [17]. A method has also been developed in which parts of the structure of the mec complex (Table 1) and the ccr genes are amplified by PCR (Table 3) [7,39]. However, these methods gave different results when the SCCmec type of the same MRSA strain was characterised [13]. A real-time PCR has also been developed to characterise SCCmec types.
I–IV on the basis of the mec complex and the ccr genes [40]. Zhang et al. [41] developed a multiplex PCR for the characterisation of SCCmec types I–V. This method detects mecA and a single locus on SCCmec. Since the existing methods each determine different structural properties of SCCmec, it would be useful if a single universal method for the classification of this cassette was developed. Chongtrakool et al. [14] have proposed a novel classification scheme for the nomenclature of SCCmec, based on the mec genes (indicated by a number) and the mec complex (indicated by an upper-case letter). Application of this nomenclature results in SCCmec type 1A (type I), type 2A (type II), type 3A (type III), type 2B (type IV) and type 5C (type V). Differences in the J1 region and the J2–J3 regions are then designated with numbers, e.g., SCCmec type 2B.2.1 (type IVb) (Table 2). Finally, the ccr genes and the J regions are numbered in chronological order according to their discovery [14].

**spa typing**

Frenay et al. [42] developed a single-locus sequence typing method for *S. aureus* using the sequences of the polymorphic region X of the *S. aureus* protein A (spa) gene. This region consists of a number of mainly 24-bp repeats, with its diversity being attributed mainly to deletions and duplications of the repeats and, more rarely, to point mutations [43,44]. The main advantage of *spa* typing over MLST is its simplicity, since it involves sequencing only a single locus. The discriminatory power of *spa* typing lies between that of PFGE and MLST [45]. In contrast to MLST, both molecular evolution and hospital outbreaks of MRSA can be investigated with *spa* typing [46]. Another advantage of *spa* typing is that several investigators can use ‘in-house’ sequencing platforms and analyse the resulting sequence chromatograms using dedicated software. By this means, decentralised typing is made accessible, i.e., it is not available only in research laboratories. Comparability and a common nomenclature with excellent quality of data are possible [47].

Two major nomenclature systems, described by Koreen et al. [46] and Harmsen et al. [48], respectively, are used worldwide. Unfortunately, this difference in nomenclature systems makes comparison of published *spa* typing data difficult. To date, Ridom StaphType software (Ridom GmbH, Würzburg, Germany) has been used widely for the analysis of *spa* sequences in Europe. Individual laboratory typing data are synchronised via the internet with the central *spa* server (http://www.spaserver.ridom.de), which is curated by the European SeqNet.org initiative (http://www.seqnet.org) to ensure a universal nomenclature and public access to the typing data [48]. The current *spa* server database includes >1200 *spa* types, consisting of a combination of 100 *spa* repeats from >13 000 isolates typed in 36 countries around Europe. Thus, this is one of the largest sequence-based typing databases of *S. aureus*. A further advantage is the possibility of collecting *spa* typing data continuously for infection control purposes and of developing electronic early-warning algorithms for the automatic detection of MRSA outbreaks in regions or hospitals endemic for MRSA, but with heterogeneous circulating *spa* types [49]. Because of the higher discriminatory power, several *spa* types can be found within one ST, as determined by MLST, but they remain within an assigned clonal cluster (Table 4) [37,50]. The implementation of the new clustering algorithm based upon repeat patterns (BURP) into StaphType makes a cluster analysis based on *spa* typing data (*spa* clonal complexes) possible, and future studies should investigate its usefulness and compatibility with CCs established by MLST.

### The Molecular Evolution of MRSA

MRSA emerged within 2 years of the introduction of methicillin in 1959. These strains, which harboured SCCmec type I, were isolated in the UK. During the 1960s, MRSA strains were isolated in other European countries, and then during the
1970s in other parts of the world, e.g., Australia, Japan and the USA. Currently, MRSA is a major cause of nosocomial infections worldwide. The worldwide spread of MRSA is driven by the dissemination of a number of clones with a specific genetic background (Table 4) [30,37,51–82].

Two opposing theories have previously been suggested to describe the relationship between the first MRSA isolates and recent MRSA clones. While the single-clone theory suggests that all MRSA clones have a common ancestor, and that SCCmec have a common ancestor, and that SCCmec types I, II and IV (Fig. 2). Enright et al. [37] investigated 553 MSSA and 359 MRSA isolates, obtained between 1961 and 1999 from 20 countries, using both SCCmec typing and MLST. Five clonal complexes were found among the population, and strains with the same ST harboured different SCCmec types (Table 4). It was demonstrated that the major MRSA clones, defined as groups of isolates from more than one country with the same ST and SCCmec type, belonged to one of five clonal complexes (CC5, 8, 22, 30 and 45). As shown in Table 4, different SCCmec types have been acquired by S. aureus strains with different genetic backgrounds, and this suggests that SCCmec was introduced several times into different S. aureus genetic lineages. Furthermore, ST8-MSSA in CC8 was shown to be the ancestor of the first MRSA strain isolated, i.e., ST250-MRSA-I, with ST250 differing from ST8 by a point mutation in the yqiL gene. ST8-MSSA is a common cause of epidemic MSSA disease, and has acquired SCCmec types I, II and IV (Fig. 2).

Another clone that is related closely to ST250 is ST247-MRSA-I (Fig. 2) is one of the major MRSA clones isolated currently in European hospitals.

Another major ST within CC8 is ST239-MRSA-III, which corresponds to the Brazilian clone. This clone has evolved by the transfer, through homologous recombination, of a 557-kb fragment of the chromosome of ST30 into ST8-MRSA-III (Fig. 2).
Fig. 2. Evolutionary origin of the major methicillin-resistant *Staphylococcus aureus* (MRSA) clones and the possible relationship between community-acquired (CA)-MRSA and hospital-acquired (HA)-MRSA. The arrows indicate either the acquisition of SCCmec, a change of SCCmec, a change of sequence type (ST), or the acquisition of the genes encoding Panton–Valentine leukocidin (PVL). The grey-shaded circles represent the MRSA clones from CC30, while the white circles represent the MRSA clones from CC8. ST239-MRSA-III from CC8 has evolved by the transfer of a 557-kb fragment from the chromosome of ST30 into an ST8 background [37,88,112]. MSSA, methicillin-sensitive *Staphylococcus aureus*.

[50]. It was further shown that CC5, 22, 30 and 45 were all derived from epidemic MSSA lineages that have acquired SCCmec, since they differed from each other, and from ST8, at six or seven loci (Table 4). Furthermore, MLST analyses showed that some of the first vancomycin-intermediate *S. aureus* isolates have emerged from ST5-MRSA-II, a pandemic MRSA clone known as the New York/Japan clone [37]. It has also been shown that multiple lineages of *S. aureus* harbour different SCCmec types among hospitalised patients in Australia [86].

A study of 147 MRSA isolates with geographically diverse origins indicated that MRSA has emerged at least 20 times following acquisition of SCCmec, and that the acquisition of SCCmec by MSSA was four-fold more common than the replacement of one SCCmec with another. Interestingly, SCCmec type IV was found in twice as many MRSA clones as other SCCmec types, suggesting that most clones arise by acquisition of SCCmec type IV by *S. aureus* [87]. This is probably a result of the smaller size of SCCmec type IV compared with other SCCmec types, which may facilitate transfer of the cassette among staphylococcal species [88]. Furthermore, it has been shown that MRSA strains that belong to the major CCs (1, 5, 8, 22, 30 and 45) are easier to transform with *mecA*-expressing plasmids than are strains belonging to minor CCs. This indicates that the genetic background of *S. aureus* may be important for the stability of SCCmec [89].

Besides the major clones shown in Table 4, MRSA strains are also isolated in single hospitals (minor clones) or from single patients (sporadic isolates) [30]. Two studies have been published in which clonal evolution within a single hospital was described. ST30-MRSA-IV was present in a Mexican hospital between 1997 and 2000, but this clone was replaced by ST5-MRSA-II during 2001, with the latter clone predominating in 2002 [57]. Similarly, a study in Spain showed that ST247-MRSA-I was replaced by ST36-MRSA-II between 1998 and 2002 [56]. Although most MRSA strains are isolated in hospitals, community-acquired (CA) strains have now emerged.

**THE EMERGENCE OF CA-MRSA**

The worldwide emergence of CA-MRSA is a threat to individuals in both the community and the hospital environment, since these strains are known to be more virulent than hospital-acquired (HA)-MRSA strains [90,91]. Furthermore, CA-MRSA strains have started to replace HA-MRSA in healthcare settings [92,93].

Although at least eight definitions for CA-MRSA exist [94,95], a general and international agreement has now been reached on a universal definition of CA-MRSA, i.e., strains isolated in an
outpatient setting, or from patients within 48 h of hospital admission. Furthermore, such patients must have no history of MRSA infection or colonisation, and no history in the previous year of either hospitalisation, admission to a nursing home, dialysis or surgery. Moreover, the patient should not have permanent indwelling catheters or medical devices that pass through the skin.

The first CA-MRSA strain was reported in Western Australia during 1993 in patients from remote communities with no known risk-factors for MRSA colonisation [96]. CA-MRSA is both phenotypically and genotypically distinct from HA-MRSA. In contrast to HA-MRSA, CA-MRSA strains are generally susceptible to antibiotics other than β-lactams. PFGE analyses have shown that CA-MRSA strains belong to clonal types unrelated to clones isolated in hospitals [97,98]. Furthermore, MLST has shown that CA-MRSA has greater clonal diversity than HA-MRSA [37,39]. Although CA-MRSA strains mainly harbour SCCmec types IV or V [5,8,9], two reports have described some CA-MRSA strains harbouring SCCmec types I, II or III [52,99], while another report described HA-MRSA strains with SCCmec type IV [100]. This shows that the distinction between CA-MRSA and HA-MRSA based on the SCCmec type is beginning to blur.

There are conflicting reports as to whether a relationship exists between SCCmec type IV and production of Panton–Valentine leukocidin (PVL). PVL is a S. aureus-specific exotoxin, encoded by two co-transcribed genes designated lukF-PV and lukS-PV, and is associated with skin and soft-tissue infections and severe necrotising pneumonia [27,101]. Vandenesch et al. [27] showed that CA-MRSA was characterised by SCCmec type IV, and that PVL was a stable genetic marker for CA-MRSA. The relationship among CA-MRSA, SCCmec type IV and PVL was confirmed by a study in the USA by Shukla et al. [58]. However, O’Brien et al. [59] did not find a relationship between CA-MRSA, SCCmec type IV and PVL in Australia. Further studies showed PVL-positive CA-MRSA strains harbouring SCCmec types I and III in The Netherlands [99] and PVL-positive HA-MRSA strains in Algeria [73]. In general, <5% of MRSA strains harbouring SCCmec types I–III carry PVL, and 40–90% of MRSA strains that harbour SCCmec type IV also carry PVL [102]. Further studies are needed to investigate the possible relationship between SCCmec type IV (and type V) and PVL in CA-MRSA strains. Recently, Müller-Premru et al. [103] described the first detection of PVL in MRSA with ST5 (spa t002), and CA-MRSA with ST152 (spa t454), associated with a clinically significant outbreak of infections among members of a football team in Slovenia. The emergence of PVL in MRSA with ST5 (spa t002) is of particular concern because of its epidemic potential [103].

Worldwide, CA-MRSA strains differ in their SCCmec type, PFGE pattern, and MLST and spa profiles, with ST30 (spa t012, t018, t019, t021, t138, t268, t276, t318, t338, t391) in Australian and South American isolates, ST80 (spa t044) in European and Middle Eastern isolates, and ST1 (spa t127, t128, t174, t176, t386, t558), ST8 (spa t008, t024, t064, t190, t206, t211) and ST59 (spa t199, t216, t444) in USA isolates [27,104]. Although many CA-MRSA strains harbour SCCmec type IV, CA-MRSA strains with the ST80 genetic background harbour SCCmec type IVc, and CA-MRSA strains with ST1 harbour SCCmec type IVa [105]. Furthermore, European CA-MRSA strains carry the far1 gene, encoding fusidic acid resistance, and have a unique spa type. This European CA-MRSA clone has spread through Belgium, Finland, France, Germany, Norway, Scotland, Sweden and Yugoslavia [65,106–108].

CA-MRSA strains harbouring SCCmec type V are present among isolates from Australia with ST5, ST8, ST45, ST59, ST152, ST573 and ST577, from Taiwan with ST59, from Finland with ST8 and ST27, from Uruguay with ST45, from Singapore with ST1, ST7, ST8, ST45, ST59, ST88, ST188, ST524 and ST573, from France with ST377, and from Kosovo with ST152 [18,69–72,81,86,109,110]. These studies showed that CA-MRSA strains harbouring SCCmec type V have a diverse genetic background.

It is unclear whether SCCmec elements in CA-MRSA have been acquired by MSSA strains in the community, or whether they have been derived from HA-MRSA. Okuma et al. [39] showed that CA-MRSA strains represent novel acquisitions of SCCmec type IV in the community. Another study raised the possibility that some CA-MRSA strains may originate in hospitals, since several similarities were found between CA-MRSA and HA-MRSA strains [111]. A recent study has suggested that an HA-MRSA and a CA-MRSA clone have a common ancestor. During the 1950s, a penicillin-resistant S. aureus clone (phage type 80/81)
emerged worldwide in hospitals and the community, but was largely eliminated following the introduction of penicillinase-resistant β-lactam antibiotics during the 1960s. It has been shown that this clone was ST30-MSSA and harboured PVL. The clone re-emerged and acquired SCCmec type IV to become the main CA-MRSA clone found in Australia (ST30-MRSA-IV). In addition, ST30-MSSA has also acquired SCCmec type II, possibly via several intermediate steps, such as the acquisition of SCCmec type IV, to become ST36-MRSA-II, the pandemic EMRSA-16 clone (Fig. 2) [112].

PREVALENCE AND RISK-FACTORS

A high prevalence of MRSA in hospitals has been associated with increased patient mortality and higher healthcare costs. The SENTRY antimicrobial surveillance programme found that the prevalence of MRSA in hospitals between 1997 and 1999 was 22.4% in Australia, 66.8% in Japan, 34.9% in Latin America, 40.4% in South America, 32.4% in the USA and 26.3% in Europe [113,114]. The prevalence of MRSA in Europe varies among countries; e.g., the prevalence of MRSA in the northern European countries (c. 0.6% in Scandinavian countries and The Netherlands) is significantly lower than in other European countries (up to 44.7%). The low prevalence of MRSA in The Netherlands and Scandinavia has been attributed to a low antibiotic selection pressure and screening of ‘high-risk’ patients for MRSA before or at the time of admission to a hospital (‘search-and-destroy’ policy) [115]. More effective disinfection procedures and hand hygiene guidelines could, in part, help to prevent the spread of MRSA in the hospital environment [116]. Strict implementation of the above rules in Denmark, beginning at a time when the prevalence of MRSA was as high as 30%, have decreased the MRSA prevalence to <1% [117]. Risk-factors for MRSA colonisation include previous exposure to one or several antibiotics, prolonged duration of therapy, stay in an intensive care or burns unit, severe underlying illness, invasive procedures, surgical wounds or burns, and contact with patients colonised with MRSA [118–123].

The prevalence of CA-MRSA is currently low worldwide, but appears to be increasing [91,95]. The prevalence of CA-MRSA is <0.5%, but HA-MRSA strains are also circulating in the community [124–126]. Salgado et al. [95] performed a global analysis of 57 studies on CA-MRSA prevalence among hospitalised patients and individuals in the community, and found that most individuals with CA-MRSA had at least one risk-factor for MRSA. This study suggested that the prevalence of CA-MRSA among individuals without risk-factors is 0.24% [95]. A recent study showed that the prevalence of CA-MRSA in Europe is 0.03–1.5% [115]. Among >13 275 entries in the spa database, spa type t044 (ST80), which is highly associated with PVL, accounted for 685 (5.16%) isolates and was the fifth most frequent spa type. However, more frequent typing of CA-MRSA strains with a higher rate of infection may contribute to over-representation in this database. The higher rates of CA-MRSA colonisation among Australian aboriginals (76%) or native Americans (62%) are probably associated with risk-factors for spread in the community, e.g., skin infections and the use of broad-spectrum antibiotics [97,127].

A number of risk-factors associated with CA-MRSA colonisation have been identified. These include gastrointestinal disease, intravenous drug use, direct contact with an individual who has a skin infection with CA-MRSA, indirect contact with contaminated objects, such as shared soap bars and towels in sport facilities and jails, and close contact among military recruits. Furthermore, recent medication, other than antibiotics, also seems to be a risk-factor for CA-MRSA colonisation [90,128–130]. Nevertheless, further studies to investigate the true prevalence of CA-MRSA and the risk-factors for CA-MRSA colonisation are required.

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