Molecular epidemiology of methicillin-resistant Staphylococcus aureus

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Staphylococcus aureus can cause a wide spectrum of infections. Its strong adaptive power to antibiotics has resulted in the emergence 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). Six SCCmec types (I to VI) have been distinguished. To study the molecular epidemiology of MRSA, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), spa typing and SCCmec typing can be used. Several MRSA clones have emerged and disseminated worldwide. Although, in the past, MRSA strains were mainly hospital-acquired (HA-MRSA), from the late 1990s, community-acquired MRSA (CA-MRSA) has emerged. CA-MRSA harbours SCCmec type IV or V and is often associated with Panton-Valentine leukocidin (PVL). This chapter describes the recent developments concerning the structure of SCCmec, the molecular evolution of MRSA and the methods used to investigate the molecular epidemiology of MRSA.

Keywords MLST, MRSA; PFGE, PVL; SCCmec, spa typing

1. Introduction

Since its discovery in the 1880s, Staphylococcus aureus has been known as a potential pathogenic Gram-positive bacterium. It can cause a broad variety of diseases, ranging from minor infections of the skin to post-operative wound infections. Until the introduction of penicillin in the early 1940s and the treatment of S. aureus infections with this antibiotic, the mortality rate of patients with a S. aureus infection was about 80%. However, shortly after the introduction of penicillin for medical use, the first penicillin-resistant strains were isolated in 1942, first in hospitals, and later on in the community. Since 1960, around 80% of all S. aureus strains are penicillin resistant. In 1962, two years after the introduction of methicillin, S. aureus developed resistance to methicillin through the acquisition of the mecA gene [1, 2].

2. Resistance determinant SCCmec

The resistance of S. aureus to methicillin and other β-lactam antibiotics is caused by the mecA gene, which encodes the 78-kDa penicillin-binding protein (PBP) 2a (or PBP2′) [3]. The 2.1-kb mecA gene is located on a mobile genetic element, the Staphylococcal Cassette Chromosome mec (SCCmec) [4]. Currently, six main types of SCCmec (type I to VI) are distinguished, ranging in size from 20.9 to 66.9 kb (Fig. 1).

SCCmec types I (34.3 kb), IV (20.9 to 24.3 kb), V (28 kb) and VI (20.9 kb) encode for resistance to β-lactam antibiotics only. SCCmec types II (53.0 kb) and III (66.9 kb) determine multi resistance, as these

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elements harbour additional drug resistance genes, which are carried on integrated plasmids, i.e. pUB110, pI258 and pT181, and a transposon (Tn554). Plasmid pUB110 carries the anti(4') gene, encoding for resistance to kanamycin, tobramycin and bleomycin, and pI258 codes for resistance to penicillins and heavy metals, such as mercury. Plasmid pT181 codes for tetracycline resistance, while transposon Tn554 carries the ermA gene, which is responsible for inducible macrolide, lincosamide and streptogramin (MLS) resistance (Fig. 1) [4, 9, 12]. S. aureus can also carry resistance genes on plasmids and on other sites of the chromosome. Recently, it has been shown that SCCmec type III is a composite element, consisting of SCCmec type III and SCCmercury, harbouring ccrC, pI258 and Tn554 [13]. Furthermore, SCCmec harbours insertion sequences, such as IS431, as well as genes responsible for the regulation of mecA transcription, i.e. ΔmecRI (on SCCmec types I, IV, V and VI), or mecRI and mecI (on SCCmec types II and III) [4-7, 9]. Both mecI and mecRI can be truncated by IS431 or IS1272, and this results in a de-repression of mecA [14]. These genes are situated on the so-called mec complexes, of which five major classes have been distinguished (Table 1) [4, 5, 14].

![Fig. 1 Schematic drawing of SCCmec types I to VI [5-9]. The major elements of the six SCCmec types (ccr genes, IS431, IS1272, mecA, mecRI/RI, orfX, pI258, pT181, pUB101 and Tn554) are presented, as are the six loci (A to F) used for SCCmec typing according to the method of Oliviera et al. [8, 10, 11] (Adapted from [11]).](image-url)

<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>S. aureus</td>
</tr>
<tr>
<td>B</td>
<td>IS1272-ΔmecRI-mecA-IS431</td>
<td>I, IV, VI</td>
<td>S. aureus</td>
</tr>
<tr>
<td>C</td>
<td>IS431-ΔmecRI-mecA-IS431</td>
<td>V</td>
<td>S. aureus</td>
</tr>
<tr>
<td>D</td>
<td>ΔmecRI-mecA-IS431</td>
<td>-</td>
<td>S. capryce</td>
</tr>
<tr>
<td>E</td>
<td>ΔmecRI-mecA-IS431a</td>
<td>-</td>
<td>S. aureus</td>
</tr>
</tbody>
</table>

976 bp deletion in mecRI compared to class D mec complex

Genes encoding cassette chromosome recombinases (ccr) are located within the SCCmec elements. Their function is the integration of SCCmec into and excision of SCCmec from the chromosome at a specific site (attBssc) at the 3’ end of an open reading frame (ORF) of unknown function, named orfX [15]). The ccr 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 VI) and ccrC (in...
SCCmec type V). The regions outside the mec and ccr complexes are designated the J (junkyard) regions (Fig. 1) [6, 7, 9, 14, 16, 17].

SCCmec elements are divided into three regions. The J1 region is located between the chromosome right junction to the ccr genes, while the region from the ccr genes to the mec complex is designated the J2 region. The J3 region ranges from the mec complex to the left extremity (orfX) of SCCmec [13, 18]. As shown in table 2, several variants of the major SCCmec elements have been described in S. aureus [4, 10, 17-23].

Table 2 Structural variants of SCCmec I to V (Adapted from [11])

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>Structure compared to main SCCmec type</th>
<th>Proposed nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>pUB110 integrated downstream of mecA</td>
<td>1B.1.2</td>
</tr>
<tr>
<td>IIA</td>
<td>SCCmec type Ivb J1 region; class A mec complex; lacks Tn554</td>
<td>2A.3.1</td>
</tr>
<tr>
<td>IIb</td>
<td>SCCmec type Ivb J1 region; class A3 mec complex; lacks ORFs between Tn554 and mec complex</td>
<td>2A.3.2</td>
</tr>
<tr>
<td>IIc</td>
<td>SCCmec type Ivb J1 region; class A4 mec complex; lacks pUB110 and IS431 at left junction of SCCmec</td>
<td>A.3.3</td>
</tr>
<tr>
<td>IID</td>
<td>SCCmec type Ivb J1 region; class A5 mec complex; lacks pUB110 and IS431 at left junction of SCCmec</td>
<td>2A.3.4</td>
</tr>
<tr>
<td>IIE</td>
<td>SCCmec type Ivb J1 region; class A7 mec complex; lacks region between Tn554 and mec complex; lacks region between pUB110 and IS431 at left junction of SCCmec</td>
<td>2A.3.5</td>
</tr>
<tr>
<td>IIb</td>
<td>lacks pUB110, IS556 inserted upstream of mecI</td>
<td>2A.2</td>
</tr>
<tr>
<td>IIIA</td>
<td>lacks pT181 and its IS431s</td>
<td>3A.1.2</td>
</tr>
<tr>
<td>IIIB</td>
<td>lacks pT181 and p258, together with IS431s; lacks Tn554</td>
<td>3A.1.3</td>
</tr>
<tr>
<td>IVa/IVb</td>
<td>different J1 regions when compared to SCCmec type IV, harbours downstream constant region (dcs) region</td>
<td>2B.1/2B.2.1</td>
</tr>
<tr>
<td>IVc</td>
<td>different J1 region compared to SCCmec type IV, harbours Tn554 flanked by IS256s</td>
<td>2B.4</td>
</tr>
<tr>
<td>IVd</td>
<td>different J1 region compared to SCCmec type IV</td>
<td>2B.3.1</td>
</tr>
<tr>
<td>IVA</td>
<td>harbours pUB110 downstream of mecA</td>
<td>2B.3.2</td>
</tr>
<tr>
<td>IVE</td>
<td>variant of SCCmec type IVc; lacks dcs region; different J3 region</td>
<td>2B.3.3</td>
</tr>
<tr>
<td>IVF</td>
<td>variant of SCCmec type IVd; lacks dcs region; different J3 region</td>
<td>2B.2.2</td>
</tr>
<tr>
<td>IVg</td>
<td>different J1 region composed of 5 ORFs compared to SCCmec type IV</td>
<td>2B.5</td>
</tr>
<tr>
<td>IVh</td>
<td>different J1 region compared to SCCmec type IV, characteristic for the ST22-MRSA-IV clone</td>
<td>2B.6</td>
</tr>
<tr>
<td>Vr</td>
<td>harbours ccrC2 variant of ccrC</td>
<td>3C.2</td>
</tr>
</tbody>
</table>

*a mec complex A4, c mec-I-S182, c mec-I-mecR-mecA, c mec-A3, S182, c mec-I-mecR-mecA

SCCmec type I was harboured in the first MRSA strain (NCTC10442), isolated in 1961 in the United Kingdom (UK), and this so-called Archaic clone disseminated worldwide in the 1960s. SCCmec type II was found in a MRSA strain (N315) isolated in 1982 in Japan, and this New York/Japan clone spread around the world. In 1985, an MRSA strain (85/2082) harbouring SCCmec type III was discovered in
New Zealand. In the 1990s, MRSA strains harbouring SCC\textit{mec} type IV disseminated worldwide. At the beginning of the 21\textsuperscript{st} century, the first MRSA strain (WIS) with SCC\textit{mec} type V was described in Australia. SCC\textit{mec} type VI, represented by MRSA strain HDE288, has until now only been found in MRSA strains from Portugal [6, 9, 24, 25].

3. Typing methods for MRSA

A thorough knowledge of the dissemination and the molecular epidemiology of MRSA strains are required to develop effective strategies to prevent the spread of MRSA. Various molecular typing techniques have been developed to investigate the spread and evolution of MRSA. The most commonly used techniques include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), \textit{spa} typing, and SCC\textit{mec} typing [26].

3.1 Pulsed-field gel electrophoresis

PFGE is still considered to be the golden standard for typing of MRSA isolates, and is one of the most discriminative typing methods. Therefore, PFGE is used to study MRSA outbreaks in hospitals and hospital-to-hospital transmission. PFGE is based on the digestion of chromosomal DNA with the restriction enzyme \textit{SmaI}, followed by agarose gel electrophoresis. The PFGE patterns are analysed with a software package with Dice comparison and unweighted pair group matching analysis (UPGMA) settings according to the criteria of Tenover et al. [27]. Attempts to harmonize PFGE protocols and to establish a standardized nomenclature have proven only partially successful when judged by reproducibility, speed, and costs of analysis. Because of the need for strict adherence to standardized protocols, common databases were only realised on a national level, such as in the USA and The Netherlands. However, on an international level, attempts for a common PFGE nomenclature were not successful [28-30].

3.2 Multilocus sequence typing

MLST is an excellent tool to investigate the clonal evolution of MRSA. MLST is based on sequence analysis of circa 500-bp fragments from seven \textit{S. aureus} housekeeping genes, i.e. \textit{arcC}, \textit{aroE}, \textit{glpF}, \textit{gmk}, \textit{pta}, \textit{tpi} and \textit{yqiL}. The different sequences of each housekeeping gene are assigned as distinct alleles, and each MRSA strain is defined by the alleles of the seven genes. The result is an allelic profile or Sequence Type (ST). The so-called Iberian clone, for example, has MLST profile 3-3-1-12-4-4-16, which has been defined as ST247 (www.mlst.net). The MRSA nomenclature is currently based on MLST and the SCC\textit{mec} type. For example, the Iberian clone is ST247-MRSA-I, harbouring SCC\textit{mec} type I. The software package BURST (Based Upon Related Sequence Types) is used to define clonal complexes (CCs) and to investigate evolutionary events within the MRSA population (www.mlst.net). When 5 of the 7 housekeeping genes have identical sequences, MRSA isolates are grouped within a single CC. The ST with the largest number of single locus variants (SLV) is the ancestor of a CC. Furthermore, subgroup founders can be described as SLVs or double locus variants (DLV) of a founder of a CC that has become prevalent in a population, and may be diversified to produce its own set of SLVs and DLVs [31-34]. A disadvantage of MLST is that it is rather expensive, laborious and time consuming.

3.3 \textit{spa} typing

The sequences of the polymorphic region X of the \textit{S. aureus} protein A (\textit{spa}) gene have been used by Frenay et al. to developed a single-locus sequence typing technique for MRSA [35]. The \textit{spa} locus consists of a number of mainly 24-bp repeats, and its diversity is attributed to deletions and duplications of the repeats, and, more seldom, to point mutations [36, 37]. The discriminative power of \textit{spa} typing lies between that of PFGE and MLST [38], and, in contrast to MLST, \textit{spa} typing can be used to investigate
both the molecular evolution and hospital outbreaks of MRSA [39]. The main advantage of *spa* typing over MLST is its simplicity, since it involves sequencing of only a single locus. Another advantage of *spa* typing is that several laboratories can use different sequencing platforms and analyze the resulting sequence chromatograms using special software. By this means, typing is made accessible not only to reference laboratories, but also to local laboratories. Comparability and a common nomenclature with excellent quality of data are available [40]. Two major nomenclature systems are commonly used, one published by Koreen *et al.* [39] and the other by Harmsen *et al.* [41]. This difference in nomenclature makes comparison of published *spa* typing data difficult. Ridom StaphType software (Ridom GmbH, Würzburg, Germany) is most widely used for the analysis of *spa* sequences in Europe. The laboratory typing data are synchronized with the central *spa* server (www.spaserver.ridom.de), which is curate by the SeqNet.org initiative (www.seqnet.org), ensuring a universal nomenclature and public access to the typing data [42]. Currently, the *spa* server database is one of the largest known sequence-based typing databases of *S. aureus*, and comprises over 2,600 *spa* types consisting of a combination of 170 *spa* repeats from nearly 35,000 isolates typed in 45 countries worldwide. For infection control purposes, it is possible to collect *spa* typing data continuously, and it can easily be adapted for electronic early warning algorithms for the automatic detection of MRSA outbreaks in regions or institutions where MRSA is endemic with a pool of heterogenic circulating *spa* types [43]. Because of its higher discriminatory power, several *spa* types correspond to a single ST as determined with MLST, but they remain within an assigned clonal cluster (Table 4) [33, 44]. The implementation of the clustering algorithm Based Upon Repeat Patterns (BURP) into StaphType makes cluster analysis based on *spa* typing data (spa clonal complexes) possible. A recent study has shown a very good concordance between PFGE, MLST and *spa* typing using BURP analyses [45].

### 3.4 SCC*mec* typing

Three methods are mainly used to characterize SCC*mec*. A multiplex PCR for SCC*mec* type I to IV, in which *mecA* and different loci on SCC*mec* are detected (Fig. 1 and Table 3), was developed by Oliveira *et al.* [10]. Ito *et al.* developed a method in which parts of the structure of the *mec* complex (Table 1) and the *ccr* genes are amplified by PCR (Table 3) [5, 46].

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>B</td>
<td>A, D</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>A2/B2</td>
</tr>
<tr>
<td>III</td>
<td>A</td>
<td>A3/B3</td>
</tr>
<tr>
<td>IV</td>
<td>B</td>
<td>A2/B2</td>
</tr>
<tr>
<td>V</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>VI</td>
<td>B</td>
<td>A4/B4</td>
</tr>
</tbody>
</table>

Locus A (495 bp) is located downstream of the *pls* gene, locus B (284 bp) is internal to the *kdp* operon, locus C (209 bp) is internal to the *mecI* gene, locus D (342 bp) is internal to the *dcs* region, locus E (243 bp) is located in the region between integrated plasmid pI258 and transposon Tn554 and locus F (414 bp) is located in the region between Tn554 and the chromosomal right junction (*orfX*) [10]. Fig. 1 shows a graphic illustration of the different loci on SCC*mec* type I to VI.

However, these methods can give different results when the SCC*mec* type of the same MRSA strain was characterized [18]. Zhang *et al.* developed a multiplex PCR for the characterization of SCC*mec* type I to V. This method detects *mecA* and only a single locus on SCC*mec* [47]. Since these methods each determine different structural properties of SCC*mec*, a single, universal method for the classification of
SCCmec needs to be developed. Recently, Chongtrakool et al. proposed a novel classification scheme for the nomenclature of SCCmec. This scheme is based on the ccr genes (indicated by a number) and the mec complex (indicated by an uppercase letter). Application of this nomenclature results in SCCmec type 1A (type I), type 2A (type II), type 3A (type III), type 2B (type IV), type 4B (type VI) and type 5C (type V). Furthermore, differences in the J1 region and the J2-J3 regions are 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 time of discovery [13].

Kondo et al. developed a PCR scheme using five multiplex PCR reactions for this nomenclature, but this method is not feasible for routine applications due to its relative large number of PCR reactions [48].

4. Molecular epidemiology of HA-MRSA

In 1961, two years after the introduction of methicillin, MRSA strains emerged. During the following years, MRSA strains disseminated to other European countries, whereas in the 1970s, MRSA spread worldwide, e.g. Australia, Japan and the USA. Currently, MRSA is a major cause of nosocomial infections worldwide, and the prevalence ranges from less than 1% in The Netherlands and Scandinavia to over 60% in Japan [49]. The worldwide spread of MRSA is driven by the dissemination of various clones with a specific genetic background [13, 26, 33, 50-84].

Two opposing theories have been suggested to describe the relationship between the first MRSA isolated and the recent MRSA clones. While the single-clone theory suggests that all MRSA clones have a common ancestor and that SCCmec was introduced only once in S. aureus [85], the multi-clone theory suggests that SCCmec was introduced several times into various S. aureus lineages. The latter hypothesis is supported by a number of studies [33, 66, 86, 87].

Enright et al. investigated 359 MRSA and 553 MSSA isolates from 20 countries isolated between 1961 and 1999 with SCCmec typing and MLST. In the MRSA population, five CCs were found, and isolates with the same ST harboured different SCCmec elements (Table 4) [33]. The major MRSA clones, defined as groups of isolates from more than one country with the same ST and SCCmec element, belonged either to CC5, 8, 22, 30, or 45. Table 4 shows that different SCCmec elements have been acquired by S. aureus strains with a different genetic background, and this supports the multi-clone theory described above. Furthermore, it was shown that ST8-MSSA in CC8 is the ancestor of the first MRSA strain isolated, i.e. ST250-MRSA-I; ST250 differs from ST8 by a point mutation in the yqiL locus. ST8-MSSA is a common cause of epidemic MSSA disease and it has acquired SCCmec types I, II and IV (Fig. 2).

Fig. 2 Evolutionary origins of the major MRSA clones and the possible relation between CA-MRSA and HA-MRSA. The arrows indicating either 1) the acquisition of SCCmec, 2) a change of SCCmec, 3) a change of ST, or 4) the acquisition of PVL. The grey coloured 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 a ST8 background [11, 33, 88, 89] (Adapted from [11]).
Table 4 Overview of the major MRSA clones (Adapted from [11]).

<table>
<thead>
<tr>
<th>Clone</th>
<th>MLST profile</th>
<th>ST</th>
<th>CC</th>
<th>SCC mec</th>
<th>spa type</th>
<th>Geographical spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic</td>
<td>3-3-1-1-4-4-16</td>
<td>250</td>
<td>8</td>
<td>I</td>
<td>008, 005, 4194</td>
<td>Art, Den, Ger, Svi, Uga, UK, USA</td>
</tr>
<tr>
<td>Southern Germany</td>
<td>1-4-1-4-12-24-29</td>
<td>223</td>
<td>5</td>
<td>I</td>
<td>001, 025, 0411, 0186</td>
<td>Bel, Den, Ger, Ita, Svi, Spa, Svi</td>
</tr>
<tr>
<td>UK EMRSA-3</td>
<td>1-4-1-4-12-1-10</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>001, 002, 003, 010, 0105, 0103, 062, 1214, 178, 185, 1216, 011, 6319, 6589, 6443</td>
<td>Arg, Nor, Pol, Svi, UK</td>
</tr>
<tr>
<td>Iberian</td>
<td>3-3-1-1-4-4-16</td>
<td>247</td>
<td>8</td>
<td>I</td>
<td>008, 061, 053, 056, 000</td>
<td>Bel, Can, Den, Fin, Fra, Ger, Ita, Net, No, Pol, Por, Svi, Spa, Swe, Swe, US, USA</td>
</tr>
<tr>
<td>Irish-1</td>
<td>3-3-1-1-4-4-3</td>
<td>8</td>
<td>8</td>
<td>II</td>
<td>008, 064, 066, 0190, 0206, 0211</td>
<td>Ire, UK, UK, USA</td>
</tr>
<tr>
<td>New York/Japan</td>
<td>1-4-1-4-12-1-10</td>
<td>5</td>
<td>5</td>
<td>II</td>
<td>001, 002, 003, 010, 045, 053, 062, 0107, 0131, 0139, 0685, 0443</td>
<td>Art, Bel, Can, Den, Fra, Ger, Ire, Jap, Kor, Mex, Nor, SA, Swe, Tai, Tai, US, UK, UK, USA</td>
</tr>
<tr>
<td>UK EMRSA-16</td>
<td>2-2-2-2-3-3-2</td>
<td>36</td>
<td>36</td>
<td>II</td>
<td>018, 075, 0419</td>
<td>Art, Bel, Den, Fra, Ger, Ita, Net, Nor, Spa, Swe, Swe, US, UK, USA</td>
</tr>
<tr>
<td>Brazilian/Hungarian</td>
<td>2-3-1-1-4-4-3</td>
<td>239</td>
<td>8</td>
<td>III</td>
<td>030, 037, 0206, 0211</td>
<td>Arg, Alg, Aut, Aus, Bel, Can, Che, Cze, Fra, Ger, Gre, Ind, Irl, Eus, Mls, Net, Por, Pol, Por, SA, Swe, Svi, Spa, Swe, Swe, Tai, Tai, US, UK, USA</td>
</tr>
<tr>
<td>Berlin</td>
<td>10-1-4-4-8-10-3-2</td>
<td>45</td>
<td>45</td>
<td>IV</td>
<td>004, 015, 026, 021, 036, 050, 056, 0206, 0200, 0130</td>
<td>Art, Bel, Can, Fra, Ger, Ire, Nor, Por, Por, Spa, Swe, Swe, Tai, Tai, UK, USA, Vla</td>
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<tr>
<td>Pediatric</td>
<td>1-4-1-4-12-1-10</td>
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<td>5</td>
<td>IV</td>
<td>001, 002, 003, 010, 045, 053, 062, 0107, 0131, 0139, 0685, 0443</td>
<td>Arg, Alg, Aut, Aus, Bra, Col, Den, Fra, Kor, Nor, Pol, Por, Spa, Swe, Swe, Tai, Tai, UK, USA</td>
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<td>UK EMRSA-2A-6</td>
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<td>Art, Bel, Fra, Ger, Ire, Nor, Tai, UK, USA</td>
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<td>UK EMRSA-15</td>
<td>7-4-1-5-8-9-6</td>
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<td>22</td>
<td>IV</td>
<td>005, 022, 0222, 0209, 01310, 0617, 0420</td>
<td>Arg, Bel, Cza, Den, Ger, Ire, Kav, NZ, Nor, Por, Swe, Swe, Swe, Swe, Tai, Tai, US, UK, USA</td>
</tr>
</tbody>
</table>

*Sequence Type
Clonal Complex
*spa types according to spa server (most prevalent spa type in bold)

Alg-Algeria, Arg-Argentina, Aus-Australia, Aut-Austria, Bel-Belgium, Brz-Brazil, Can-Canada, Che-Chile, Chin-China, Col-Colombia, Cro-Croatia, Cze-Czech Republic, Den-Denmark, Fin-Finland, Fra-France, Ger-Germany, Gre-Greece, Hun-Hungary, Ind-India, Ise-Indonesia, Ire-Ireland, Isr-Israel, Ita-Italy, Jap-Japan, Kor-Korea, Kmr-Korea, Mec-Mexico, Mon-Mongolia, Net-Netherlands, NZ-New Zealand, Nor-Norway, Pol-Poland, Por-Portugal, SA-Saudi Arabia, Sin-Singapore, Slo-Slovenia, Sri-Sri Lanka, Spa-Spain, Swe-Sweden, Swe-Switzerland, Tai-Taiwan, Tha-Thailand, Uga-Uganda, UK-United Kingdom, Uru-Uruguay, USA-United States of America, Vie-Vietnam
Another ST250-related clone is ST247-MRSA-I (Iberian clone). These STs differ from each other by a single point mutation at the gmk locus. ST247-MRSA-I is one of the major MRSA clones currently isolated in European hospitals (Table 4). ST239-MRSA-III (Brazilian clone) is another major MRSA clone within CC8. It has evolved by the transfer through homologous recombination of a 557-kb fragment of the chromosome of ST30 into ST239-MRSA-III (Fig. 2) [44]. Furthermore, it has been shown that CC5, 22, 36 and 45 were all derived from epidemic MSSA lineages that acquired SCCmec, since they differed from each other and from ST8 at six or seven MLST loci (Table 4). MLST analyses also showed that some of the first vancomycin-intermediate S. aureus (VISA) isolates have emerged from ST5-MRSA-II, a pandemic MRSA clone known as the New York/Japan clone [33].

As shown by a study characterizing 147 geographically diverse MRSA strains, MRSA has emerged at least twenty times upon acquisition of SCCmec. That study also demonstrated that the acquisition of SCCmec by MSSA was four times more common than the replacement of one SCCmec element with another element. Furthermore, SCCmec type IV was found in twice as many MRSA clones than other SCCmec elements. This suggests that most clones arise by acquisition of SCCmec type IV in MSSA [90]. This is probably due to the smaller size of SCCmec type IV element compared to the other SCCmec elements, which may facilitate transfer of the cassette between staphylococcal species [89]. Furthermore, it has been shown that MRSA strains that belong to CC1, 5, 8, 22, 30, and 45, were easier to transform with mecA-expressing plasmids, compared to strains belonging to other CCs. This suggests that the genetic background of S. aureus is important for the stability of SCCmec [91].

Besides the major clones presented in table 4, certain MRSA strains are isolated in single hospitals (minor clones), or from single patients (sporadic isolates) [26]. Recently, several studies have described the clonal evolution within one hospital, such as in Mexico [56]. Although the majority of the MRSA strains are isolated in hospitals, CA-MRSA is an emerging problem outside the hospital.

5. Worldwide emergence of CA-MRSA

The emergence of CA-MRSA is a worldwide threat to both the community and the hospital environment since these strains are more virulent than HA-MRSA strains [92, 93]. Furthermore, CA-MRSA strains have started to replace HA-MRSA in health-care settings [94].

Since many definitions for CA-MRSA exist [95, 96], a general and international agreement has been reached on a universal definition of CA-MRSA. The Center for Disease Control and Prevention (CDC) defines CA-MRSA as strains isolated in an outpatient setting, or from patients within 48 hours of hospital admission. Furthermore, these patients must have no medical history of MRSA infection or colonisation, and no medical history of MRSA infection or colonisation in the past year of either hospitalisation (e.g. surgery), admission to a nursing home, or dialysis. Moreover, the patient should not have permanent indwelling catheters or medical devices that pass through the skin.

In 1993, the first CA-MRSA strain was reported from patients in remote communities in Western Australia [97]. Interestingly, CA-MRSA strains were isolated from patients who have no known risk factors for MRSA colonisation. CA-MRSA is both phenotypically and genotypically different from HA-MRSA. In contrast to HA-MRSA, CA-MRSA strains are mainly susceptible to antibiotics other than β-lactam antibiotics. PFGE and MLST have shown that CA-MRSA strains belong to clonal types unrelated to clones isolated in hospitals [98, 99], and that CA-MRSA has greater clonal diversity than HA-MRSA [33, 46]. Although CA-MRSA mainly harbour SCCmec type IV or V [4, 6, 7], two reports described CA-MRSA isolates with SCCmec type I, II or III [51, 100].

The various reports are conflicting as to whether there is a relation between SCCmec type IV and Panton-Valentine leukocidin (PVL). PVL is a S. aureus-specific exotoxin, which is encoded by two co-transcribed genes, designated lukF-PV and lukS-PV, and is associated with skin and soft tissue infections, and severe necrotizing pneumonia [25, 101]. Vandenesch et al. showed that CA-MRSA was characterised by SCCmec type IV and that PVL was a stable genetic marker for CA-MRSA [25]. The relationship between CA-MRSA, SCCmec type IV and PVL was confirmed in a study by Shukla et al. in the USA [57]. However, another study by O’Brien et al. in Australia did not find a relationship between CA-MRSA, SCCmec type IV and PVL [58]. Further studies showed PVL–positive CA-MRSA strains
harbouring SCCmeA type I and III in The Netherlands [100] and PVL-positive HA-MRSA strains in Algeria [72]. In general, 40 to 90% of the MRSA strains that harbour SCCmeA type IV carry PVL and less than 5% of the MRSA strains harbouring SCCmeA type I to III carry PVL [102]. Further studies are needed to investigate the possible relation between SCCmeA type IV (and V) and PVL in CA-MRSA strains. Recently, Müller-Preunru et al described the first detection of PVL in MRSA with ST5 (spa t002), and CA-MRSA with ST152 (spa t454) in Slovenia associated with a clinical significant outbreak within members of a football team. The emergence of PVL in MRSA with ST5 is of particular concern, because of its epidemic potential with high capacity to spread [103].

Five main PVL-positive CA-MRSA clones are isolated worldwide. The ST1 clone [spa t127, t128, t174, t176, t386, t558] is found in Asia, Europe and the USA, the ST8 clone [spa t008, t024, t064, t190, t206, t211] in Europe and the USA, the ST30 clone [spa t012, t018, t019, t021, t138, t268, t276, t318, t338, t391] is found in Australia, Europe and South America, the ST59 clone [spa t199, t216, t437, t444] in Asia and the USA, and the ST80 clone [spa t044, t131] in Asia, Europe and the Middle-East [25, 104, 105]. Although these CA-MRSA clones harbour SCCmeA type IV, the ST1 clone harbours SCCmeA type IVa and the ST80 clone harbours SCCmeA type IVc [106]. Furthermore, the ST80 clone harbours the farI gene, coding for resistance to fusidic acid.

SCCmeA type V is present in Australian CA-MRSA strains with ST5, 8, 45, 59, 152, 573 and 577, in Taiwanese isolates with ST59, in Finish strains with ST8 and 27, in strains from Uruguay with ST45, in Singaporean strains with ST1, 7, 8, 45, 59, 88, 188, 524 and 573, in Australian, Dutch, French, Greek and Swiss strains with ST377, and in strains from Kosovo with ST152 [21, 68-71, 80, 105, 107-109]. This shows that SCCmeA type V harbouring CA-MRSA isolates have a diverse genetic background.

Whether SCCmeA of CA-MRSA has been acquired by MSSA strains in the community, or that CA-MRSA is derived from HA-MRSA remains unclear. Okuma et al showed that CA-MRSA are novel acquisitions of SCCmeA type IV in the community [46]. Another study by Aires de Sousa et al. raised the possibility that some CA-MRSA strains may originate in hospitals, since several similarities between CA-MRSA and HA-MRSA strains were found [110]. A recent study showed that a HA-MRSA and a CA-MRSA clone have a common ancestor. In the 1950s, a penicillin-resistant S. aureus clone (phage type 80/81) emerged worldwide in hospitals and the community, but since the introduction of penicillinase-resistant β-lactam antibiotics in the 1960s, this clone has disappeared. This PVL-harbouring ST30 clone re-emerged and acquired SCCmeA type IV to become the ST30-MRSA-IV CA clone found in Australia. ST30-MSSA has also acquired SCCmeA type II, possible through several intermediate steps, such as the acquisition of SCCmeA type IV, to become ST36-MRSA-II, the pandemic EMRSA-16 clone (Fig. 2) [88]. This paragraph shows that there are still a number of questions to be answered about the molecular epidemiology of CA-MRSA.

References

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