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

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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].

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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 mecI (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 SCC; 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 [7–9,131]. The major elements of the five SCCmec types (ccr genes, IS431, IS1272, mecA, mecRI–mecI, orfX, pI258, pT181, pUB101 and Tn554) are shown, as are the six loci (A–F) used for SCCmec typing according to the method of Oliviera et al. [17].
and ccr complexes are designated the J (junkyard) 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 106 methicillin-resistant S. 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]. Hanssen et al. [22] studied 39 methicillin-resistant coagulase-negative staphylococcal isolates, of which 22 had a novel SCCmec type. Moreover, other studies have found novel SCCmec types [13,23,24], or SCC elements without mecA, which could be a reservoir for antibiotic resistance islands, in S. aureus [25,26].

### THE HISTORY OF MRSA

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 (Wisconsin) 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 **Proposed novel nomenclature** [14].

<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>1B.1.2</td>
</tr>
<tr>
<td>IIA</td>
<td>SCCmec type Ib–J1 region; class A4 mec complex*</td>
<td>2A.3.1</td>
</tr>
<tr>
<td>IIB</td>
<td>SCCmec type Ib–J1 region; lacks Tn554</td>
<td>2A.3.2</td>
</tr>
<tr>
<td>IIC</td>
<td>SCCmec type Ib–J1 region; class A3 mec complex*; lacks ORFs between Tn554 and mec complex</td>
<td>2A.3.3</td>
</tr>
<tr>
<td>IID</td>
<td>SCCmec type Ib–J1 region; class A4 mec complex*; lacks region between Tn554 and mec complex</td>
<td>2A.3.4</td>
</tr>
<tr>
<td>IIE</td>
<td>SCCmec type Ib–J1 region; class A3 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>Ib</td>
<td>Lacks pUB110; IS556 inserted upstream of mecI</td>
<td>2A.2</td>
</tr>
<tr>
<td>IIA</td>
<td>Lacks pT181 and its associated IS431 sequences</td>
<td>3C12</td>
</tr>
<tr>
<td>IIB</td>
<td>Lacks pT181 and p258, together with IS431; lacks Tn554</td>
<td>3C13</td>
</tr>
<tr>
<td>IVA/VB</td>
<td>Different J1 regions when compared to SCCmec type IV; harbours downstream constant region (dcs)</td>
<td>2B.1/2B.2.1</td>
</tr>
<tr>
<td>IVc</td>
<td>Different 1J region compared to SCCmec type IV; harbours Tn601 flanked by IS256 sequences</td>
<td>2B.3.1</td>
</tr>
<tr>
<td>IVd</td>
<td>Different 1J region compared to SCCmec type IV</td>
<td>2B.4</td>
</tr>
<tr>
<td>IVa/Vb</td>
<td>Harbours pUB110 downstream of mecA</td>
<td>2B.5/2B.6</td>
</tr>
<tr>
<td>IVE</td>
<td>Variant of SCCmec type 1Vc; lacks 2J region; different J3 region</td>
<td>2B.3.3</td>
</tr>
<tr>
<td>IVF</td>
<td>Variant of SCCmec type 1Vd; lacks 3J region; different J3 region</td>
<td>2B.2.2</td>
</tr>
<tr>
<td>IVg</td>
<td>Different 1J region composed of 5 ORFs compared to SCCmec type IV</td>
<td>2B.5</td>
</tr>
<tr>
<td>Vr</td>
<td>Harbours ccrC2 variant of ccrC</td>
<td>5C.2</td>
</tr>
</tbody>
</table>

*ORF, open reading frame.

**Table 2. Structural variants of SCCmec types I–V**

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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...
shows a schematic illustration of the different loci on SCCmec the region between Tn554 internal to the dcs to the kdp

Table 3. Methods for SCCmec typing

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>mec complex</th>
<th>cer genes</th>
<th>Method of Ito et al. [7]</th>
<th>Method of Oliveira et al. [17]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>B</td>
<td>A1/B1</td>
<td>A, D</td>
<td>Loca*</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>A2/B2</td>
<td>B, C, D</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>A</td>
<td>A3/B3</td>
<td>C, E, F</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>B</td>
<td>A2/B2</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>C</td>
<td>C</td>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

*a Locus A (495 bp) is located downstream of the pfl gene; locus B (284 bp) is internal to the kdp operon; locus C (209 bp) is internal to the meca gene; locus D (342 bp) is internal to the dcs region; locus E (243 bp) is located in the region between integrated plasmid pT28 and transposon Tn554; and locus F (414 bp) is located in the region between Tn554 and the chromosomal right junction of50 [17].

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 cer 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 reference 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
Table 4. Overview of the major clones of methicillin-resistant *Staphylococcus aureus* (MRSA)

<table>
<thead>
<tr>
<th>Clone</th>
<th>MLST profile</th>
<th>ST</th>
<th>CC</th>
<th>SCCmec</th>
<th>spa type</th>
<th>Geographical spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archaic</td>
<td>3-3-1-1-4-4-16</td>
<td>250</td>
<td>8</td>
<td>I</td>
<td>0008, 0309, 0194</td>
<td>Ast, Den, Ger, Sth, Uga, UK, USA</td>
</tr>
<tr>
<td>Southern Germany</td>
<td>1-4-1-4-12-24-29</td>
<td>228</td>
<td>5</td>
<td>I</td>
<td>0001, 0231, 0401, 0188</td>
<td>Bel, Den, Ger, Ita, Sth, Spa, Swi</td>
</tr>
<tr>
<td>UK EMRSA-3</td>
<td>1-4-1-4-12-1-10</td>
<td>5</td>
<td>5</td>
<td>I</td>
<td>0001, 0002, 0003, 0101, 0345, 0553, 0662, 0105, 0178, 0179, 0187, 0214, 0311, 0319, 0389, 0443</td>
<td>Arg, Nor, Pol, Slo, UK</td>
</tr>
<tr>
<td>Iberian</td>
<td>3-3-1-12-4-4-16</td>
<td>247</td>
<td>8</td>
<td>I</td>
<td>0005, 0551, 0552, 0554, 0200</td>
<td>Ast, Bel, Can, Den, Din, Fra, Ger, Ita, Net, Pol, Por, Sth, Spa, Sve, Swi, UK, 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>0005, 0201, 0349, 0190, 0206, 0111</td>
<td>Ast, Ire, 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>0001, 0002, 0003, 0101, 045, 0553, 0662, 0105, 0178, 0179, 0187, 0214, 0311, 0319, 0389, 0443</td>
<td>Arg, Bel, Can, Din, Fra, Ger, Ire, Jap, Kor, Mex, Sin, Sve, Swi, 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>0255, 0253, 0141, 0119</td>
<td>Ast, Bel, Can, Din, Fin, Gre, Ire, Mex, Nor, Spa, Sve, Swi, 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>0300, 0397, 0234, 0387, 0388</td>
<td>Alg, Arg, Ast, Aus, Bra, Chi, Chn, Cze, Fin, Ger, Gre, Ind, Ids, Kor, Mon, Nor, Pol, Por, Sin, Slo, Spa, Sth, USA, UK, USA</td>
</tr>
<tr>
<td>Berlin</td>
<td>10-14-8-6-10-3-2</td>
<td>45</td>
<td>45</td>
<td>IV</td>
<td>0004, 0105, 026, 031, 0328, 0305, 0307, 0204, 0230, 0190</td>
<td>Arm, Ast, Bel, Fin, Ger, Hun, Net, Nor, Spa, Sve, Swi, USA, Vie</td>
</tr>
<tr>
<td>Paediatric</td>
<td>1-4-1-4-12-1-10</td>
<td>5</td>
<td>5</td>
<td>IV</td>
<td>0001, 0002, 0003, 0101, 0359, 0551, 0662, 0105, 0178, 0179, 0187, 0214, 0311, 0319, 0389, 0443</td>
<td>Alg, Arg, Ast, Bra, Col, Den, Fra, Kor, Nor, Pol, Por, Spa, Sve, Swi, UK, USA</td>
</tr>
<tr>
<td>UK EMRSA-2/6</td>
<td>3-3-1-1-4-4-3</td>
<td>8</td>
<td>8</td>
<td>IV</td>
<td>0008, 0204, 0349, 0200, 0111</td>
<td>Ast, Bel, Fin, Fra, Ger, Ire, Nor, Tai, UK, USA</td>
</tr>
<tr>
<td>UK EMRSA-15</td>
<td>7-6-1-5-8-8-6</td>
<td>22</td>
<td>22</td>
<td>IV</td>
<td>0005, 0202, 0302, 0223, 0309, 0310, 0417, 0420</td>
<td>UK, USA</td>
</tr>
</tbody>
</table>

*spa types according to spa server (most prevalent spa type in bold).

**Table 4.** Overview of the major clones of methicillin-resistant *Staphylococcus aureus* (MRSA)

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 was introduced only once into *S. aureus* [83], the multi-clone theory hypothesises that SCCmec was introduced several times into different *S. aureus* genetic lineages. The latter theory has now been supported by several studies [37,67,84,85].

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 single point mutation in the *gmk* locus. 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).
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, 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].

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 Pant–Valentine leucocidin (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, ST73 and ST777, 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 (spa t454) 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 and ST73 and ST77, 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 (spa t454). 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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