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* (SCC*mec*). To date, five SCC*mec* types (I–V) have been distinguished, and several variants of these SCC*mec* types have been described. All SCC*mec* elements carry genes for resistance to β-lactam antibiotics, as well as genes for the regulation of expression of *mecA*. Additionally, SCC*mec* 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 SCC*mec* typing. Numerous MRSA clones have emerged and disseminated worldwide. SCC*mec* 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 SCC*mec* 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 SCC*mec*, 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*

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* (SCC*mec*)

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 IS*431* or IS*1272*, 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* (SCC*mec*) [5]. Currently, five main types of SCC*mec* (types I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb (Fig. 1). SCC*mec* 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, SCC*mec* 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 (Tn*554*). 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 Tn*554* carries the *ermA* gene, which is responsible for inducible macrolide, lincosamide and streptogramin resistance (Fig. 1) [5,6]. Besides the resistance genes on SCC*mec*, *S. aureus* can carry resistance genes inserted at other sites of the chromosome and on plasmids. SCC*mec* also carries insertion sequences, e.g., IS*431*, as well as genes responsible for the regulation of *mecA* transcription, i.e., Δ*mecRI* (on SCC*mec* types I, IV and V) or *mecRI* and *mecI* (on SCC*mec* 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 (*attBscc*; 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 SCC*mec* elements. These genes are designated *ccrA1* and *ccrB1* (in SCC*mec* type I), *ccrA2* and *ccrB2* (in SCC*mec* types II and IV), *ccrA3* and *ccrB3* (in SCC*mec* type III), *ccrA4* and *ccrB4* (in SCC*mec* type IV of methicillin-resistant *Staphylococcus aureus* (MRSA) strain HDE288) and *ccrC* (in SCC*mec* type V). The regions bordering the *mec*
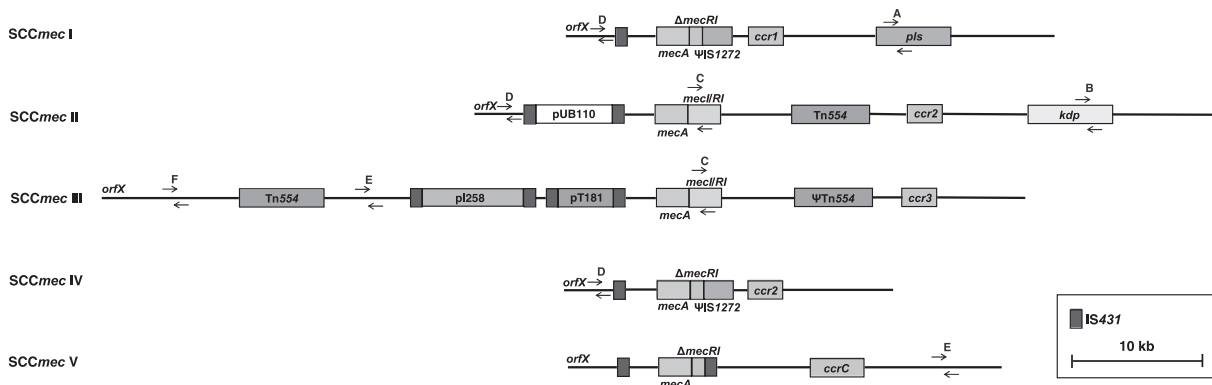

Fig. 1. Schematic arrangement of SCC*mec* types I–V [7–9,131]. The major elements of the five SCC*mec* types (*ccr* genes, IS*431*, IS*1272*, *mecA*, *mecI/RI*, *orfX*, pI258, pT181, pUB101 and Tn*554*) are shown, as are the six loci (A–F) used for SCC*mec* typing according to the method of Oliviera *et al.* [17].

Table 1. Major classes of *mec* complexes

Class	Structure	SCC*mec*	Species
A	*mecI–mecRI–mecA–IS431*	II, III	*Staphylococcus aureus*
B	IS1272–Δ*mecRI–mecA–IS431*	I, IV	*S. aureus*
C	IS431–Δ*mecRI–mecA–IS431*	V	*S. aureus*
D	Δ*mecRI–mecA–IS431*	–	*Staphylococcus caprae*
E	Δ*mecRI–mecA–IS431*[a]	–	*S. aureus*

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

and *ccr* complexes are designated the J (junkyard) regions (Fig. 1) [4,8,9,11,12].

All SCC*mec* 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 SCC*mec* [13,14]. In addition to the five main SCC*mec* types, several variants of SCC*mec* have been described (Table 2) [5,12,13,15–19].

Besides MRSA, methicillin-resistant coagulase-negative staphylococci can harbour SCC*mec*. It has been shown that methicillin-resistant *Staphylococcus epidermidis* isolates from the 1970s harboured SCC*mec* types I–IV [20]. Investigations of the SCC*mec* type among 106 methicillin-resistant *Staphylococcus epidermidis* isolates from the late 1990s identified SCC*mec* types I–V among 85 isolates, but the SCC*mec* 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 SCC*mec* type. Moreover, other studies have found novel SCC*mec* 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 SCC*mec* type I, and this so-called archaic clone spread around the world during the 1960s. In 1982, an MRSA strain (N315) with SCC*mec* 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 SCC*mec* type III in New Zealand. MRSA strains harbouring SCC*mec* IV spread round the world during the 1990s, and at the beginning of the 21st century, the first MRSA strain (WIS) with SCC*mec* type V was described in Australia [8,24,27].

Although the origin of SCC*mec* 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

SCC*mec*	Structure compared to main SCC*mec* type	Proposed novel nomenclature [14]
IA	pUB110 integrated downstream of *mecA*	1B.1.2
IIA	SCC*mec* type IVb J1 region; class A4 *mec* complex[a]	2A.3.1
IIB	SCC*mec* type IVb J1 region; lacks Tn554	2A.3.2
IIC	SCC*mec* type IVb J1 region; class A3 *mec* complex[b]; lacks ORFs between Tn554 and *mec* complex	2A.3.3
IID	SCC*mec* type IVb J1 region; class A4 *mec* complex[a]; lacks pUB110 and IS431 at left junction of SCC*mec*	2A.3.4
IIE	SCC*mec* type IVb J1 region; class A3 *mec* complex[b]; lacks region between Tn554 and *mec* complex; lacks region between pUB110 and IS431 at left junction of SCC*mec*	2A.3.5
IIb	Lacks pUB110; IS256 inserted upstream of *mecI*	2A.2
IIIA	Lacks pT181 and its associated IS431 sequences	3C12
IIIB	Lacks pT181 and pI258, together with IS431; lacks Tn554	3C13
IVa/IVb	Different J1 regions when compared to SCC*mec* type IV; harbours downstream constant region (*dcs*)	2B.1/2B.2.1
IVc	Different J1 region compared to SCC*mec* type IV; harbours Tn4001 flanked by IS256 sequences	2B.3.1
IVd	Different J1 region compared to SCC*mec* type IV	2B.4
IVA	Harbours pUB110 downstream of *mecA*	2B.N.2
IVE	Variant of SCC*mec* type IVc; lacks *dcs* region; different J3 region	2B.3.3
IVF	Variant of SCC*mec* type IVd; lacks *dcs* region; different J3 region	2B.2.2
IVg	Different J1 region composed of 5 ORFs compared to SCC*mec* type IV	2B.5
V$_T$	Harbours *ccrC2* variant of *ccrC*	5C.2

[a]*mec* complex A4, Δ*mecI*–IS1182–Δ*mecI*–*mecRI*–*mecA*.
[b]*mec* complex A3, IS1182–Δ*mecI*–*mecRI*–*mecA*.
ORF, open reading frame.

Table 2. Structural variants of SCC*mec* types I–V

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), SCC*mec* 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 *Sma*I, 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 SCC*mec* type; for example, ST247-MRSA-I is the Iberian clone harbouring SCC*mec* 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.

SCC*mec* typing

Four methods are currently available for the characterisation of SCC*mec*. Oliveira and de Lencastre [17] developed a multiplex PCR for SCC*mec* types I–IV, in which *mecA* and six different loci on SCC*mec* 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 SCC*mec* type of the same MRSA strain was characterised [13]. A real-time PCR has also been developed to characterise SCC*mec* types

Table 3. Methods for SCC*mec* typing

	Method of Ito *et al.* [7]		
SCC*mec*	*mec* complex	*ccr* genes	Method of Oliveira *et al.* [17] Loci[a]
I	B	A1/B1	A, D
II	A	A2/B2	B, C, D
III	A	A3/B3	C, E, F
IV	B	A2/B2	D
V	C	C	E

[a] 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 Tn*554*; and locus F (414 bp) is located in the region between Tn*554* and the chromosomal right junction (*orfX*) [17]. Fig. 1 shows a schematic illustration of the different loci on SCC*mec* types I–V.

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 SCC*mec* types I–V. This method detects *mecA* and a single locus on SCC*mec*. Since the existing methods each determine different structural properties of SCC*mec*, 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 SCC*mec*, based on the *ccr* genes (indicated by a number) and the *mec* complex (indicated by an upper-case letter). Application of this nomenclature results in SCC*mec* 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., SCC*mec* 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 SCC*mec* 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 *Staphylcoccus aureus* (MRSA)

Clone	MLST profile	ST	CC	SCCmec	spa type[a]	Geographical spread[b]
Archaic	3-3-1-1-4-4-16	250	8	I	**t008**, t009, t194	Ast, Den, Ger, Swi, Uga, UK, USA
Southern Germany	1-4-1-4-12-24-29	228	5	I	**t001**, t023, t041, t188, t201	Bel, Den, Ger, Ita, Slo, Spa, Swi
UK EMRSA-3	1-4-1-4-12-1-10	5	5	I	**t001**, **t002**, t003, t010, t045, t053, t062, t105, t178, t179, t187, t214, t311, t319, t389, t443	Arg, Nor, Pol, Slo, UK
Iberian	3-3-1-12-4-4-16	247	8	I	**t008**, **t051**, t052, t054, t200	Bel, Cro, Cze, Den, Fin, Fra, Ger, Ita, Net, Pol, Por, Slo, Spa, Swe, Swi, UK, USA
Irish-1	3-3-1-1-4-4-3	8	8	II	**t008**, t024, t064, t190, t206, t211	Ast, Ire, UK, USA
New York/Japan	1-4-1-4-12-1-10	5	5	II	**t001**, **t002**, t003, t010, t045, t053, t062, t105, t178, t179, t187, t214, t311, t319, t389, t443	Ast, Bel, Can, Den, Fin, Fra, Ger, Ire, Jap, Kor, Mex, Sin, Swe, Uru, UK, USA
UK EMRSA-16	2-2-2-2-3-3-2	36	36	II	**t018**, t253, t418, t419	Ast, Bel, Can, Den, Fin, Gre, Ire, Mex, Nor, Spa, Swe, Swi, UK, USA
Brazilian/Hungarian	2-3-1-1-4-4-3	239	8	III	t030, **t037**, t234, t387, t388	Alg, Arg, Ast, Aus, Bra, Chi, Chn, Cze, Fin, Ger, Gre, Ind, Ids, Kor, Mon, Net, Pol, Por, Sin, Slo, Spa, Sri, Swe, Tha, UK, Uru, USA, Vie
Berlin	10-14-8-6-10-3-2	45	45	IV	**t004**, t015, t026, t031, t038, t050, t065, t204, t230, t390	Arm, Ast, Bel, Fin, Ger, Hun, Net, Nor, Spa, Swe, Swi, USA
Paediatric	1-4-1-4-12-1-10	5	5	IV	**t001**, **t002**, t003, t010, t045, t053, t062, t105, t178, t179, t187, t214, t311, t319, t389, t443	Alg, Arg, Ast, Bra, Col, Den, Fra, Kor, Nor, Pol, Por, Spa, Swe, Uru, UK, USA
UK EMRSA-2/-6	3-3-1-1-4-4-3	8	8	IV	**t008**, t024, t064, t190, t206, t211	Ast, Bel, Fin, Fra, Ger, Ire, Net, Nor, Tai, UK, USA
UK EMRSA-15	7-6-1-5-8-8-6	22	22	IV	t005, t022, **t032**, t223, t309, t310, t417, t420	Ast, Bel, Cze, Den, Ger, Ire, Kuw, NZ, Nor, Por, Sin, Spa, Swe, UK

[a]spa types according to spa server (most prevalent spa type in bold).
[b]Alg, Algeria; Arg, Argentina; Arm, Armenia; Ast, Australia; Aus, Austria; Bel, Belgium; Bra, Brazil; Can, Canada; Chi, Chile; Chn, China; Col, Colombia; Cro, Croatia; Cze, Czech Republic; Den, Denmark; Fin, Finland; Fra, France; Ger, Germany; Gre, Greece; Hun, Hungary; Ind, India; Ids, Indonesia; Ire, Ireland; Ita, Italy; Jap, Japan; Kor, Korea; Kuw, Kuwait; Mex, Mexico; Mon, Mongolia; Net, The Netherlands; NZ, New Zealand; Nor, Norway; Pol, Poland; Por, Portugal; Sin, Singapore; Slo, Slovenia; Sri, Sri Lanka; Spa, Spain; Swe, Sweden; Swi, Switzerland; Tai, Taiwan; Tha, Thailand; Uga, Uganda; Uru, Uruguay; Vie, Vietnam.

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 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, i.e., the Iberian clone. These STs differ from each other by a single point mutation at 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)
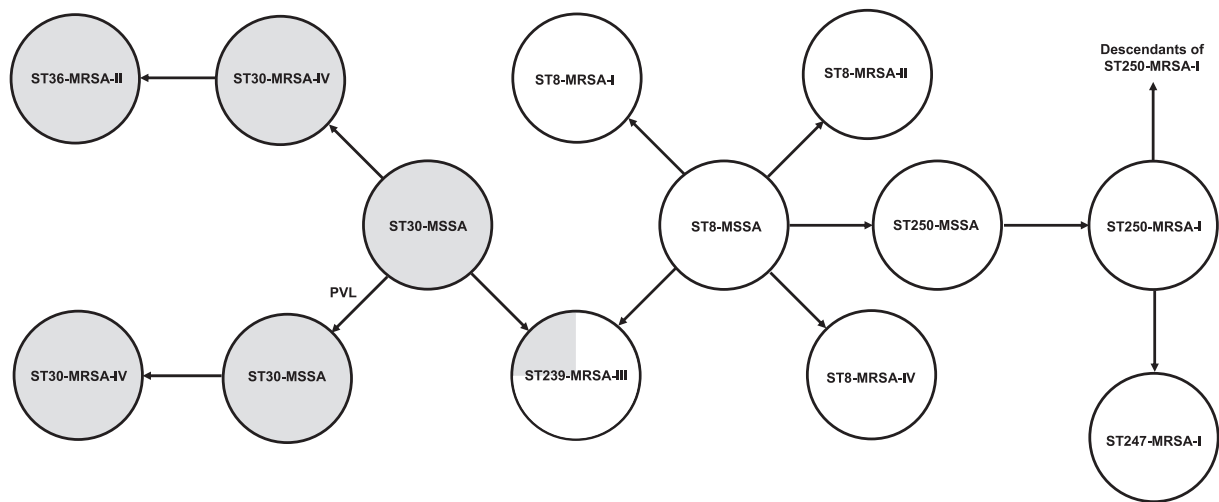
Fig. 2. Evolutionary origin of the major methicillin-resistant *Staphylococcus aureus* (MRSA) clones and the possible relationship between community-acquired (CA)-MRSA and hospital-acquired (HA)-MRSA. The arrows indicate either the acquisition of SCC*mec*, a change of SCC*mec*, a change of sequence type (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 SCC*mec*, 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 SCC*mec* 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 SCC*mec*, and that the acquisition of SCC*mec* by MSSA was four-fold more common than the replacement of one SCC*mec* with another. Interestingly, SCC*mec* type IV was found in twice as many MRSA clones as other SCC*mec* types, suggesting that most clones arise by acquisition of SCC*mec* type IV by *S. aureus* [87]. This is probably a result of the smaller size of SCC*mec* type IV compared with other SCC*mec* 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 SCC*mec* [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 SCC*mec* types IV or V [5,8,9], two reports have described some CA-MRSA strains harbouring SCC*mec* types I, II or III [52,99], while another report described HA-MRSA strains with SCC*mec* type IV [100]. This shows that the distinction between CA-MRSA and HA-MRSA based on the SCC*mec* type is beginning to blur.

There are conflicting reports as to whether a relationship exists between SCC*mec* 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 SCC*mec* type IV, and that PVL was a stable genetic marker for CA-MRSA. The relationship among CA-MRSA, SCC*mec* 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, SCC*mec* type IV and PVL in Australia. Further studies showed PVL-positive CA-MRSA strains harbouring SCC*mec* types I and III in The Netherlands [99] and PVL-positive HA-MRSA strains in Algeria [73]. In general, <5% of MRSA strains harbouring SCC*mec* types I–III carry PVL, and 40–90% of MRSA strains that harbour SCC*mec* type IV also carry PVL [102]. Further studies are needed to investigate the possible relationship between SCC*mec* 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 SCC*mec* 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 SCC*mec* type IV, CA-MRSA strains with the ST80 genetic background harbour SCC*mec* type IVc, and CA-MRSA strains with ST1 harbour SCC*mec* 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 SCC*mec* 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 SCC*mec* type V have a diverse genetic background.

It is unclear whether SCC*mec* 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 SCC*mec* 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 SCC*mec* type IV to become the main CA-MRSA clone found in Australia (ST30-MRSA-IV). In addition, ST30-MSSA has also acquired SCC*mec* type II, possibly via several intermediate steps, such as the acquisition of SCC*mec* 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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