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 ß-lactam antibiotics is caused by the *mecA* gene, which is situated on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Six SCC*mec* 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 SCC*mec* 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 SCC*mec* type IV or V and is often associated with Panton-Valentine leukocidin (PVL). This chapter describes the recent developments concerning the structure of SCC*mec*, the molecular evolution of MRSA and the methods used to investigate the molecular epidemiology of MRSA.

Keywords MLST, MRSA; PFGE, PVL; SCC*mec*, *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 SCC*mec*

The resistance of *S. aureus* to methicillin and other ß-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* (SCC*mec*) [4]. Currently, six main types of SCC*mec* (type I to VI) are distinguished, ranging in size from 20.9 to 66.9 kb (Fig. 1).

SCC*mec* 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. SCC*mec* 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 (Tn*554*). Plasmid pUB110 carries the *ant(4')*

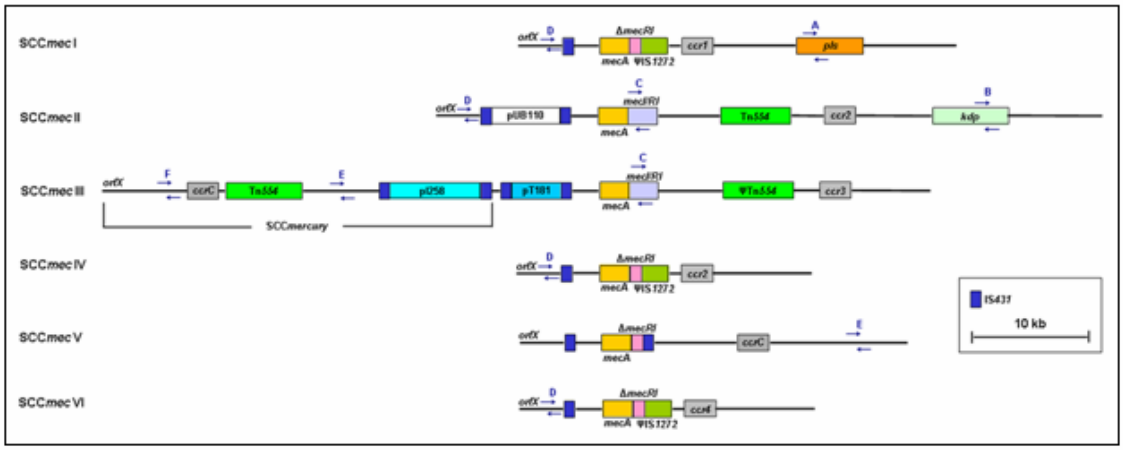

Fig. 1 Schematic drawing of SCC*mec* types I to VI [5-9]. The major elements of the six SCC*mec* types (*ccr* genes, IS*431*, IS*1272*, *mecA*, *mecI*/*RI*, *orfX*, pI258, pT181, pUB101 and Tn*554*) are presented, as are the six loci (A to F) used for SCC*mec* typing according to the method of Oliviera *et al*. [8, 10, 11] (Adapted from [11]).

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 Tn*554* 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 SCC*mec* type III is a composite element, consisting of SCC*mec* type III and SCC*mercury*, harbouring *ccrC*, pI258 and Tn*554* [13]. Furthermore, SCC*mec* harbours insertion sequences, such as IS*431*, as well as genes responsible for the regulation of *mecA* transcription, i.e. Δ*mecRI* (on SCC*mec* types I, IV, V and VI), or *mecRI* and *mecI* (on SCC*mec* types II and III) [4-7, 9]. Both *mecI* and *mecRI* can be truncated by IS*431* or IS*1272*, 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].

Table 1 Major classes of *mec* complexes (Adapted from [11]).

Class	Structure	SCC*mec*	Species
A	*mecI-mecRI-mecA*-IS*431*	II, III	*S. aureus*
B	IS*1272*-Δ*mecRI-mecA*-IS*431*	I, IV, VI	*S. aureus*
C	IS*431*-Δ*mecRI-mecA*-IS*431*	V	*S. aureus*
D:	Δ*mecRI-mecA*-IS*431*	-	*S. caprae*
E	Δ*mecRI-mecA*-IS*431*[a]	-	*S. aureus*

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

Genes encoding cassette chromosome recombinases (*ccr*) are located within the SCC*mec* elements. Their function is the integration of SCC*mec* into and excision of SCC*mec* from the chromosome at a specific site (*attBscc*; 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 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 VI) and *ccrC* (in

SCC*mec* type V). The regions outside the *mec* and *ccr* complexes are designated the J (junkyard) regions (Fig. 1) [6, 7, 9, 14, 16, 17].

SCC*mec* 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 SCC*mec* [13, 18]. As shown in table 2, several variants of the major SCC*mec* elements have been described in *S. aureus* [4, 10, 17-23].

Table 2 Structural variants of SCC*mec* I to V (Adapted from [11]).

SCC*mec*	Structure compared to main SCC*mec* type	Proposed nomenclature
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 Tn*554*	2A.3.2
IIC	SCC*mec* type IVb J1 region; class A3 *mec* complex[b]; lacks ORFs between Tn*554* and *mec* complex	A.3.3
IID	SCC*mec* type IVb J1 region; class A4 *mec* complex[a], lacks pUB110 and IS*431* at left junction of SCC*mec*	2A.3.4
IIE	SCC*mec* type IVb J1 region; class A3 *mec* complex[b]; lacks region between Tn*554* and *mec* complex; lacks region between pUB110 and IS*431* at left junction of SCC*mec*	2A.3.5
IIb	lacks pUB110; IS*256* inserted upstream of *mecI*	2A.2
IIIA	lacks pT181 and its IS*431*s	3A.1.2
IIIB	lacks pT181 and pI258, together with IS*431*s; lacks Tn*554*	3A.1.3
IVa/IVb	different J1 regions when compared to SCC*mec* type IV; harbours downstream constant region (*dcs*) region	2B.1/2B.2.1
IVc	different J1 region compared to SCC*mec* type IV; harbours Tn*4001* flanked by IS*256*s	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
IVh	different J1 region when compared to SCC*mec* type IV, characteristic for the ST22-MRSA-IV clone	2B.6
V_T	harbours *ccrC2* variant of *ccrC*	5C.2

[a] *mec* complex A4, Δ*mecI*-IS*1182*-Δ*mecI-mecRI-mecA*
[b] *mec* complex A3, IS*1182*-Δ*mecI-mecRI-mecA*

SCC*mec* 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. SCC*mec* 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 SCC*mec* type III was discovered in

New Zealand. In the 1990s, MRSA strains harbouring SCC*mec* type IV disseminated worldwide. At the beginning of the 21st century, the first MRSA strain (WIS) with SCC*mec* type V was described in Australia. SCC*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), *spa* typing, and SCC*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 *Sma*I, 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 e*t 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 *S. aureus* housekeeping genes, i.e. *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *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*mec* type. For example, the Iberian clone is ST247-MRSA-I, harbouring SCC*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, sub-group 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 *spa* typing

The sequences of the polymorphic region X of the *S. aureus* protein A (*spa*) gene have been used by Frenay *et al.* to developed a single-locus sequence typing technique for MRSA [35]. The *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 *spa* typing lies between that of PFGE and MLST [38], and, in contrast to MLST, *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 3 Methods for SCC*mec* typing (Adapted from [11]).

SCC*mec*	Method of Ito *et al.* [5]		Method of Oliveira *et al.* [10]
	mec complex	*ccr* genes	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
VI	B	A4/B4	D

[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*) [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

SCC*mec* needs to be developed. Recently, Chongtrakool *et al.* proposed a novel classification scheme for the nomenclature of SCC*mec*. 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 SCC*mec* 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. 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 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 (Table 4) [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 SCC*mec* was introduced only once in *S. aureus* [85], the multi-clone theory suggests that SCC*mec* 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 SCC*mec* typing and MLST. In the MRSA population, five CCs were found, and isolates with the same ST harboured different SCC*mec* elements (Table 4) [33]. The major MRSA clones, defined as groups of isolates from more than one country with the same ST and SCC*mec* element, belonged either to CC5, 8, 22, 30, or 45. Table 4 shows that different SCC*mec* 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 SCC*mec* types I, II and IV (Fig. 2).

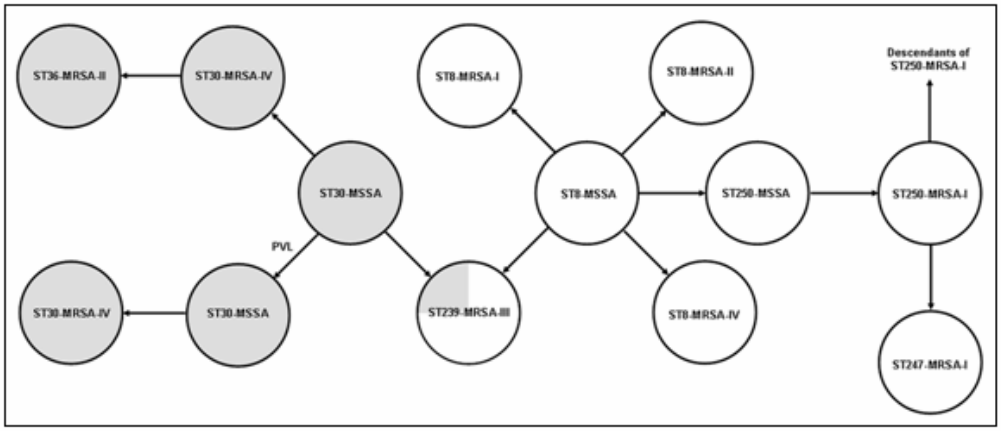

Fig. 2 Evolutionary origins of the major MRSA clones and the possible relation between CA-MRSA and HA-MRSA. The arrows indicating either 1) the acquisition of SCC*mec*, 2) a change of SCC*mec*, 3) a change of ST, or 4) the acquisition of PVL. The grey coloured circles represent the MRSA clones from CC30, 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]).

Clone	MLST profile	ST[a]	CC[b]	SCC*mec*	*spa* type[c]	Geographic spread[d]
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, Isr, Ita, Net, Nor, 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, Isr, Jap, Kor, Mex, Nor, SA, Sin, Swe, Tai, 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, Nor, Pol, Por, SA, Sin, Slo, Spa, Sri, Swe, Tai, 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
Pediatric	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] Sequence Type
[b] Clonal Complex
[c] *spa* types according to *spa* server (most prevalent *spa* type in bold)
[d] 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, Isr-Israel, Ita-Italy, Jap-Japan, Kor-Korea, Kuw-Kuwait, Mex-Mexico, Mon-Mongolia, Net-Netherlands, NZ-New Zealand, Nor-Norway, Pol-Poland, Pro-Portugal, SA-Saudi Arabia, Sin-Singapore, Slo-Slovenia, Sri-Sri Lanka, Spa-Spain, Swe-Sweden, Swi-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 SCC*mec*, 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 SCC*mec*. That study also demonstrated that the acquisition of SCC*mec* by MSSA was four times more common than the replacement of one SCC*mec* element with another element. Furthermore, SCC*mec* type IV was found in twice as many MRSA clones than other SCC*mec* elements. This suggests that most clones arise by acquisition of SCC*mec* type IV in MSSA [90]. This is probably due to the smaller size of SCC*mec* type IV element compared to the other SCC*mec* 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 SCC*mec* [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 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 SCC*mec* type IV or V [4, 6, 7], two reports described CA-MRSA isolates with SCC*mec* type I, II or III [51, 100].

The various reports are conflicting as to whether there is a relation between SCC*mec* 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 SCC*mec* type IV and that PVL was a stable genetic marker for CA-MRSA [25]. The relationship between CA-MRSA, SCC*mec* 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, SCC*mec* type IV and PVL [58]. Further studies showed PVL–positive CA-MRSA strains

harbouring SCC*mec* 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 SCC*mec* type IV carry PVL and less than 5% of the MRSA strains harbouring SCC*mec* type I to III carry PVL [102]. Further studies are needed to investigate the possible relation between SCC*mec* type IV (and V) and PVL in CA-MRSA strains. Recently, Müller-Premru *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 SCC*mec* type IV, the ST1 clone harbours SCC*mec* type IVa and the ST80 clone harbours SCC*mec* type IVc [106]. Furthermore, the ST80 clone harbours the *farI* gene, coding for resistance to fusidic acid.

SCC*mec* 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 SCC*mec* type V harbouring CA-MRSA isolates have a diverse genetic background.

Whether SCC*mec* 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 SCC*mec* 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 ß-lactam antibiotics in the 1960s, this clone has disappeared. This PVL-harbouring ST30 clone re-emerged and acquired SCC*mec* type IV to become the ST30-MRSA-IV CA clone found in Australia. ST30-MSSA has also acquired SCC*mec* type II, possible through several intermediate steps, such as the acquisition of SCC*mec* 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.

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