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MINIREVIEW

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Classification and Typing Methods: an Overview

AGATA TURLEJ[1], WALERIA HRYNIEWICZ[1] and JOANNA EMPEL[2]

[1]Department of Epidemiology and Clinical Microbiology, [2]Department of Molecular Microbiology
National Medicines Institute, Warsaw, Poland

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Abstract

Meticillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causes of hospital-acquired infections, but since late 1990s also the community-acquired. For better understanding of the *S. aureus* epidemiology there is an urgent need for creation of new typing method for SCC*mec* element. The molecular typing of MRSA for epidemiological purposes is investigated by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing and the SCC*mec* type assignment. In last few years not only new type of SCC*mec* (VI to XI) have been identified, but also additional subtypes (*i.e.* IVg-j) and different variants of already existed one (*i.e.* 5C2&5 and 2B2&5) were discovered. The aim of this review is to briefly summarize current knowledge about SCC*mec* classification and to discuss advantages and disadvantages of selected SCC*mec* typing methods.

Key words: *Staphylococcus aureus* (MRSA), SCC*mec* classification and typing

Introduction

Staphylococcus aureus is one of the leading causes of bacterial infections in developed countries and is responsible for a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. Since the introduction of penicillin into medical treatment in early 1940s, the resistance for beta-lactams has started to develop. It was a result of the acquisition of a plasmid, coding for penicillinase, a penicillin-hydrolyzing enzyme, which is able to cleave the beta-lactam ring and thus inactivate antibiotic molecule. Penicillin resistant strains soon spread not only in healthcare facilities, but also in the community. To overcome infections caused by beta-lactamase-producing *S. aureus*, a narrow spectrum semi-synthetic penicillin (meticillin) was introduced. However, soon after that, in 1961, first meticillin-resistant *Staphylococcus aureus* (MRSA) strain was identified. Initially, MRSA strains were encountered only in the hospitals, but in the late 1990s first virulent community-acquired MRSA (CA-MRSA) clones, characterized by the presence of the toxin Panton-Valentine leukocidin (PVL), appeared rapidly and unexpectedly. They quickly spread worldwide, initially only in the community, but later on also in the healthcare facilities, displacing in some countries typical HA-MRSA. For this reason, nowadays, distinction between CA-MRSA and mostly multiresistant HA-MRSA become challenging (Chambers and Deleo, 2009; Deurenberg and Stobberingh, 2008). The resistance of *S. aureus* to meticillin is caused by the presence of the *mecA* gene, encoding for an additional 78-kDa penicillin binding protein 2a, (PBP2a or PBP2'). Compared to other PBP, PBP2a has a low affinity for all beta-lactam antibiotics. As a result of that, even in the presence of a beta-lactam antibiotic, the peptidoglycan layer biosynthesis is not disrupted and the bacterium can survive (Berger-Bachi and Rohrer, 2002; Deurenberg and Stobberingh, 2008). The *mecA* gene is located within a *mec* operon together with its regulatory genes: *mecI* and *mecR1*. (Berger-Bachi and Rohrer, 2002). The *mec* operon is carried by staphylococcal cassette chromosome *mec* (SCC*mec*). The origin of SCC*mec* is still unknown, but it is proposed that it was acquired by *S. aureus* from *S. sciuri* and that the *mecA*-positive coagulase-negative staphylococci (CoNS), especially *S. epidermidis*, may be a potential reservoir for the SCC*mec* element (Mongkolrattanothai *et al.*, 2004; Wu *et al.*, 2001). On the other hand, it is suggested that the main source of SCC*mec* could be MRSA itself (Aires de Sousa and de Lencastre, 2004). There are also suggestions of possible acquisition of the *mecA*

* Corresponding author: Agata Turlej, National Medicine Institute, Chełmska 30/34, 00-725 Warsaw, Poland; phone: (48-22) 841 57 69; fax: (48-22) 8412949; **e-mail: aturlej@cls.edu.pl**

region from *S. fleurettii*, which is a commensal bacterium of animals (Tsubakishita *et al.*, 2010). SCC*mec* typing, which classifies SCC*mec* elements on the basis of their structural differences, is applied in epidemiological studies to distinguish MRSA strains or to define an MRSA clone in combination with the genotype of meticillin-susceptible *S. aureus* (MSSA) strain in which a SCC*mec* element has integrated.

SCC*mec* element composition

SCC*mec* elements, detected in almost all MRSA strains, belong to particular type of the staphylococcal mobile genetic elements coding for meticillin-resistance and designated as staphylococcal cassette chromosome *mec* (Katayama *et al.*, 2000). In *S. aureus* strains, SCC*mec* elements always integrate sequence specifically at the unique site of the chromosome, *attBscc* (bacterial chromosomal attachment site). The *attBscc* is located near the origin of replication, at the 3' end of *orfX*, coding for an open reading frame X of unknown function, well conserved among both MRSA and MSSA strains (Hiramatsu *et al.*, 2001; Ito *et al.*, 1999; Ito *et al.*, 2001). The attachment site contains a core 15-bp sequence, called the integration site sequence (ISS), that is necessary for *ccr*-mediated recombination (IWG-SCC, 2009; Katayama *et al.*, 2000). After integration of SCC*mec* into the chromosome, ISS is found in direct repeat sequences at left and right SCC*mec*/chromosomal junctions of the integrated SCC*mec* element. Different SCC*mec* elements share similar backbone structure, that consists of (i) *mec* complex, composed of *mecA* operon, (ii) *ccr* gene complex, composed of cassette chromosome recombinase (*ccr*) gene(s) and (iii) three regions bordering the *ccr* and *mec* complexes, designated as joining (J) regions. The composition of almost all SCC*mec* elements identified so far in *S. aureus* can be presented as follow: (*orfX*)J3-*mec*-J2-*ccr*-J1 (Chongtrakool *et al.*, 2006; Hiramatsu *et al.*, 2002). The exception constitute SCC*mec*VII and a newly described SCC*mec*IX, with the *ccr* gene complex positioned between J3 and J2 regions and the *mec* gene complex between J2 and J1 regions (Berglund *et al.*, 2008; Li *et al.*, 2011). It is noteworthy, that different authors, and sometimes even the same, present the structure of the same SCC*mec* elements in reverse orientations, what creates difficulties or even confusions, especially for these not experienced in the field. The orientation with SCC*mec* located at the right site of *orfX* gene seems to be more correct as it is consistent with direction on genomic maps of *S. aureus* and will be used for needs of this article. Since the structural components mentioned above play a crucial role in classification of SCC*mec* elements they will be presented below in more details.

The *ccr* gene complex. The *ccr* gene complex is composed of the *ccr* gene(s) surrounded by *orfs*. The *ccr* genes encode for DNA recombinases of the invertase-resolvase family, enzymes that can catalyze precise excision of the SCC*mec* as well as its integration, both site- and orientation-specific, into staphylococcal chromosome, being thus responsible for mobilization of the cassette (Katayama *et al.*, 2000). Based on the composition of *ccr* genes, two distinct *ccr* gene complexes have been reported to date, one carrying two adjacent *ccr* genes, *ccrA* and *ccrB*, and the second carrying *ccrC*. The *ccrA* and *ccrB* genes identified to date in *S. aureus* strains have been classified into four and five allotypes respectively, resulting in six *ccr* gene complex types, designated as type 1 (*ccrA1B1*), type 2 (*ccrA2B2*), type 3 (*ccrA3B3*), type 4 (*ccrA4B4*), type 7 (*ccrA1B6*) and type 8 (*ccrA1B3*). All identified so far *ccrC* variants have shown high nucleotide similarity and are assigned to only one allotype, *ccrC1*, constituting type 5 of *ccr* gene complex (Chongtrakool *et al.*, 2006; IWG-SCC, 2009) (http://www.sccmec.org/).

The *mec* gene complex. Two evolutionary different lineages of *mec* gene complexes have been described in *S. aureus*. The first one, which encompasses the vast majority of known and well characterized *mec* gene complexes, have been observed in MRSA isolates of human origin since the nineties. The prototype of this lineage is the *mec* gene complex designated as class A, composed of an intact *mec* operon, the hyper-variable region (HVR) and the insertion sequence IS*431* (Ito *et al.*, 2001; Katayama *et al.*, 2001). The *mec* operon includes *mecA* gene and located upstream of *mecA* its regulatory genes: *mecR1* and *mecI*, coding for the signal transducer and the repressor, respectively. Differences between class A *mec* gene complex and other *mec* gene complexes of this lineage, described to date, result mainly from insertions of IS elements, IS*1272* or IS*431*, into the region of *mecA* regulatory genes, causing complete deletion of *mecI* and, different in size, partial deletions of *mecR1*. Depending on the structural diversity of *mecI-mecR1* region, five major classes of *mec* gene complexes, of the said lineage, have been defined by IGW-SCC (IWG-SCC, 2009):

- Class A, which contains intact *mec* gene complex: IS*431-mecA-mecR1-mecI;*
- Class B, where *mecR1* is truncated by insertion sequence IS*1272*: IS*431-mecA*-Δ*mecR1*-IS*1272*;
- Class C1, where *mecR1* is truncated by insertion sequence IS*431* having the same direction as the IS*431* downstream of *mecA*: IS*431-mecA*-Δ*mecR1*--IS*431*;
- Class C2, where *mecR1* is truncated by insertion sequence IS*431* having the reverse direction to the IS*431* downstream of *mecA*: IS*431-mecA*-Δ*mecR1*-IS*431*; and

Table I
Additional resistance genes located on mobile elements within the SCC*mec* elements

Genetic element	SCC*mec* type/subtype	Gene	Resistance
pUB110	I, II, IVA	*ble*	bleomycin
		ant4'	tobramycin
Tn*554*	II, SCCHg,VIII	*ermA*	erythromycin
		aad9/*spc*	streptomycin / spectinomycin
SCC_{Hg}	–	*mer*	mercury
pT181	III	*tet*	tetracycline
ΨTn*554*	III	*cad*	cadmium
Tn4001	IV (IVc and 2B&5)	*aac*A-*aph*D	aminoglycosides

- Class D, where *mecR1* is partly deleted but there is no IS element downstream of Δ*mecR1*: IS*431*-*mecA*-Δ*mecR1*. This class has been observed in *S. caprae* only (Katayama *et al.*, 2001).

Recently, data from genome sequencing project of the bovine *S. aureus* isolate LGA251, have revealed a *mec* gene complex of the second evolutionary lineage (McCarthy and Lindsay, 2010)) (http://www.sanger.ac.uk/pathogens). This new complex, depicted as: bla Z-$mecA_{LGA251}$-$mecR1_{LGA251}$-$mecI_{LGA251}$, constitutes the sixth defined major class, assigned as class E (http://www.sccmec.org/).

Besides the major classes of the *mec* gene complex several variants within the classes have also been distinguished, for example: class A_3, where *mecI* is truncated by insertion sequence IS*1182*: IS*431*-*mecA*-*mecR1*-Δ*mecI*-IS*1182* and class A_4, where *mecI* is disrupted by insertion sequence IS*1182*: IS*431*-*mecA*-*mecR1*-Δ*mecI*-IS*1182*-Δ*mecI* (Shore *et al.*, 2005) or class B_2, which has an insertion of the transposone Tn*4001* upstream of *mecA* in Δ*mecR1*: IS*431*-*mecA*-Δ*mecR1*- Tn*4001*-IS*1272* (Heusser *et al.*, 2007).

The joining (J) regions. Apart from the *ccr* and *mecA* gene complexes, essential for the SCC*mec* biological functions, the cassette comprises also three joining regions (J1-J3), previously called "junkyard" regions (Hiramatsu *et al.*, 2002). J regions from different SCC*mec* elements are arranged in the same order. The J1 region is located at right site of the cassette, the J2 region between the *ccr* and the *mec* complexes and the J3 region at the left chromosomal junction adjacent to *orfX* (IWG-SCC, 2009). Although considered as less important in terms of SCC*mec* functions, these regions are epidemiologically significant since they may serve as targets for plasmids or transposons, carrying additional antibiotic and heavy metal resistance determinants. Acquisition and accumulation of resistance genes by mobile elements like SCC*mec* enables their dissemination and in consequence leads to emerge of multidrug resistance strains. Examples of antibiotic resistance determinants, that may be carried within J regions are summarized in Table I (Ito *et al.*, 2003; Malachowa and DeLeo, 2010). Sequence analysis of J regions from different SCC*mec* elements revealed that they are unique to particular types of *ccr-mec* gene complex combinations and that variations of these regions within the same *ccr-mec* gene complex combination are specific for SCC*mec* subtypes (Hisata *et al.*, 2005; Ito *et al.*, 2003; IWG-SCC, 2009; Kwon *et al.*, 2005; Ma *et al.*, 2002; Ma *et al.*, 2006; Milheirico *et al.*, 2007b; Oliveira *et al.*, 2001; Oliveira and de Lencastre, 2002; Shore *et al.*, 2005).

The SCC*mec* element classification

The first SCC*mec* element was identified in Japanese *S. aureus* strain, N315 in 1999 and shortly after two additional SCC*mec* from different MRSA strains were determined (Ito *et al.*, 1999; Ito *et al.*, 2001). Based on detailed structural analysis these three SCC*mec* elements were classified as types I to III (Ito *et al.*, 2001). In time, both new types of SCC*mec*, such as SCC*mec*IV (Ma *et al.*, 2002), SCC*mec*V (Ito *et al.*, 2004), SCC*mec*VI (Oliveira *et al.*, 2006a), SCC*mec*VII (Berglund *et al.*, 2008), SCC*mec*VIII (Zhang *et al.*, 2009), SCC*mec*IX, SCC*mec*X (McCarthy and Lindsay, 2010), SCC*mec*XI (http://www.sccmec.org/) and many new variants of already known SCC*mec* types have been reported (Boyle-Vavra *et al.*, 2005; Cha *et al.*, 2005; Chlebowicz *et al.*, 2010; Heusser *et al.*, 2007; Higuchi *et al.*, 2008; Hisata *et al.*, 2005; Kwon *et al.*, 2005; O'Brien *et al.*, 2005; Oliveira and de Lencastre, 2002; Shore *et al.*, 2005; Shukla *et al.*, 2004; Stephens *et al.*, 2007; Takano *et al.*, 2008). With growing number of SCC*mec* types, subtypes or variants published in the literature it became obvious that without approved international rules of nomenclature system it would be difficult in the nearest future to keep in order suitable naming of new emerged SCC*mec* elements. To meet the urgent need the International Working Group on Classification of Staphylococcal Cassette Chromosome (SCC) Elements

Table II
Reference strains for SCC*mec* types, which have been described up to date

SCC*mec* type/subtype	Strain	GenBank accession no	Origin	Isolation date	Description
I (1B)	NCTC 10442	AB033763	UK	1961	(Ito *et al.*, 2001)
II (2A)	N315	D86934	Japan	1982	(Ito *et al.*, 1999)
III (3A)	85/2082	AB037671	New Zealand	1985	(Ito *et al.*, 2001)
IVa (2B)	CA05	AB063172	USA	1999	(Ma *et al.*, 2002)
IVb (2B)	8/6-3P (JCSC1978)	AB063173	USA	1996	(Ma *et al.*, 2002)
IVc (2B)	81/108 (MR108)	AB096217	Japan	NA*	(Ito *et al.*, 2003)
IVd (2B)	JCSC4469	AB097677	Japan	1982	(Ma *et al.*, 2006)
IVg (2B)	M03-68	DQ106887	Korea	2003	(Kwon *et al.*, 2005)
IVh (2B)	HAR22	NA	Finland	2002	(Milheirico *et al.*, 2007b)
IVi (2B)	JCSC6668	AB425823	Sweden	1999	(Berglund *et al.*, 2009)
IVj (2B)	JCSC6670	AB425824	Sweden	1990	(Berglund *et al.*, 2009)
V (5C2)	WIS (JCSC3624)	AB121219	Australia	1999	(Ito *et al.*, 2004)
VI (4B)	HDE 288	AF411935	Portugal	1996	(Oliveira *et al.*, 2006a)
VII (5C1)	JCSC6082	AB373032	Sweden	2002	(Berglund *et al.*, 2008)
VIII (4A)	C10682	FJ670542	Canada	2003	(Zhang *et al.*, 2009)
IX 1(C2)	JCSC6943	NA	Thailand	2006	(Li *et al.*, 2011)
X (7C1)	JCSC6945	NA	Canada	2006	(Li *et al.*, 2011)
XI (8E)	LGA251	Na	NA	NA	http://www.sccmec.org/

* NA – information not available

(IWG-SCC) was established in 2009. The main objectives of the group was to define consensus rules of a uniform nomenclature system for SCC*mec* elements, determine minimum requirements for the description of the new SCC*mec* elements and establish guidelines for the identification of SCC*mec* elements for epidemiological studies (IWG-SCC, 2009). In published guidelines IWG-SCC decided to retain the previous nomenclature of SCC*mec* with additional information about combination of *ccr* complex type and class of *mec* complex present in the element. Thus, classification of SCC*mec* element into the types (SCC*mec* typing), should be based on the combination of the type of *ccr* gene complex and the class of the *mec* gene complex present in the cassette, while variants within SCC*mec* types (SCC*mec* subtyping) should be defined by differences in their J regions, as it was proposed earlier by Hiramatsu group (Chongtrakool *et al.*, 2006; IWG-SCC, 2009). Accordingly, SCC*mec* type I, was described additionally as 1B, what indicates the SCC*mec* element harboring the type 1 *ccr* and a class B *mec* gene complexes. To date, eleven SCC*mec* types have been defined. The other known SCC*mec* types are designated type II (2A), type III (3A), type IV (2B), type V (5C2), type VI (4B), type VII (5C1), type VIII (4A), type IX (1C2), type X (7C1) and type XI (8E). They all are summarized in Table II.

The improved way of SCC*mec* classification allowed also to assign the mosaic variants of SCC*mec*. For example, SCC*mec* element from ZH47 strain, harboring type 2 *ccr* gene complex and additional *ccrC1* in combination with *mec* class B_2 was designated type IV (2B&5), while SCC*mec* element from TSGH17 and PM1 strains, carrying two different *ccrC1* allels, 2 and 8, that was previously reported as SCC*mec* type VII or Taiwanese SCC*mec* type V (SCC*mec*V_T), was designated type V (5C2&5) (Boyle-Vavra *et al.*, 2005; Heusser *et al.*, 2007; Higuchi *et al.*, 2008; IWG-SCC, 2009; Takano *et al.*, 2008).

Due to the increasing diversity among SCC*mec* subtypes, IWG-SCC proposed the preparation of a computerized system, which will be able to characterize and assign certain SCC*mec* subtype based on the occurrence of specific elements within the J regions.

Together with the discovery of new SCC*mec* types, also a need for new more complex SCC*mec* typing methods has emerged.

Available SCC*mec* typing methods

It had already been observed that the worldwide spread of MRSA is driven by the dissemination of a number of clones with a specific genetic background. Epidemiological studies revealed that for proper clone assignment not only the multilocus sequence typing (MLST) and *spa* typing is required, but also SCC*mec* typing is needed (Deurenberg *et al.*, 2007). Since that

time, it has become necessary to find an easy and robust method for SCC*mec* element identification and typing. The more complex our knowledge about SCC*mec* elements is, the more challenging invention of the typing method becomes and the more relevant becomes the question, how accurate should we be in assigning SCC*mec* element type for epidemiological purposes. The SCC*mec* typing methods has been developed along with the new SCC*mec* types descriptions and appearance of the novel techniques or approaches for their analysis. Three different schemes of SCC*mec* typing can be distinguished: methods based on the restriction enzymes digestion, methods based on PCR or multiplex PCR (M-PCR) and methods based on real-time PCR.

First SCC*mec* typing methods. The first methods for detecting polymorphisms in the *mecA* vicinity were based on hybridizations of the *mecA* probe and Tn*554* probe with *Cla*I-digested genomic DNA from the analyzed isolates (Leski *et al.*, 1998). This method was very useful for epidemiological studies before the SCC*mec* element's structure was described. Nowadays there are some concepts on using restriction enzymes digestion in combination with PCR, like in multienzyme multiplex PCR-amplified fragment length polymorphism (ME-AFLP) or in SCC*mec* typing method by PCR amplification of the *ccrB* gene in combination with restriction fragment length polymorphism (RFLP) employing endonucleases HinfI and BsmI (van der Zee *et al.*, 2005; Yang *et al.*, 2006).

In the first one, the obtained typing patterns were found to cluster together according to the SCC*mec* type of the strain, with the discriminatory power comparable to PFGE. However, it is just a pattern based typing, which might be an interesting method for prescreening of a large strain collection, but is by far not sensitive enough to proper assign SCC*mec* type for epidemiological purposes, since it does not recognize any characteristic features for already described SCC*mec* elements. The second method is simple and time-effective, but far not elaborate enough, because it focuses only on the *ccrB* typing. Since the *ccrC* genes were also described, it is not complex enough to be useful, concerning the current knowledge of SCC*mec* types. Moreover, for proper SCC*mec* assignment the *mec* class description in combination with *ccr* type is necessary. It seems that the scheme of SCC*mec* typing based on restriction enzymes digestion is no longer superior. The most common methods used nowadays for SCC*mec* typing based on PCR are summarized below.

PCR based SCC*mec* typing methods. During the past several years, a number of SCC*mec* typing methods based on multiplex PCR (M-PCR) have been developed (Boye *et al.*, 2007; Hisata *et al.*, 2005; Kondo *et al.*, 2007; Milheirico *et al.*, 2007a; Oliveira and de Lencastre, 2002; Zhang *et al.*, 2005). Two different approaches were applied in this methods; one was focused on analysis of J regions, whereas the other determine mainly *mec* class and *ccr* type. The first M-PCR method was described by Oliveira *et al.* (Oliveira and de Lencastre, 2002). At that time it was innovative technique that enabled to increase analysis scale and exchange the information about SCC*mec* types all over the world. It was based on identification of specific genes or motifs located mostly in the J regions of particular cassettes. Potentially, this method should detected SCC*mec* type I–IV, but in practice detection of SCC*mec* type III was problematic as in fact the primers were designed not to SCC*mec* type III but to so-called SCC*mercury*, which at that time was believed to be the integral part of the element. For more details see Chongtrakool *et al.* (Chongtrakool *et al.*, 2006). This SCC*mec* typing strategy also did not discriminate SCC*mec* type IV and VI. (Oliveira *et al.*, 2006a). In 2007, the same group published an update for Oliveira's method, which improved the detection of SCC*mec* type I to IV and includes the structure determination of the SCC*mec* type V and VI. However, the SCC*mec*VI was suggested to be confirmed by *ccrB* sequencing, which is costly and time-consuming (Milheirico *et al.*, 2007a; Oliveira *et al.*, 2006b) (http://www.ccrbtyping.net). The most significant advantage of this method is that it is a quick, single-tube M-PCR reaction for all detectable by this method SCC*mec* types. Unfortunately, the method is still based on markers located within the J regions. This can cause some problems, as for example in our practice we sometimes see the pattern for SCC*mec* type I similar to type VI, which is confusing. Almost at the same time Zhang *et al.* has described a complex method for SCC*mec* I to V typing and SCC*mec* type IV subtyping in a multiplex PCR reactions (Zhang *et al.*, 2005). This method include three M-PCR reactions and one single target PCR reaction with sets of primers specific to *mec*, *ccr* and J1 region. First M-PCR reaction uses a set of primers specific for SCC*mec* type I to V, with SCC*mec* subtypes IVa to IVd, the second M-PCR reaction uses primers for assigning *mec* class A and B and third M-PCR reaction uses primers for type 1–3 *ccr* described previously by Ito *et al.* For detection of type 5 *ccr* the authors propose PCR reaction with single pair of primers (Ito *et al.*, 2001; Zhang *et al.*, 2005). The proposed approach seems to be not useful for large-scale analysis, since four separate reactions should be done. Moreover, it does not allow to detect neither *mec* class C1 and C2 nor type 4 *ccr*. On the other hand, this method allows to detect Taiwanese SCC*mec* type V (5C2&5). This mosaic variant of SCC*mec* type V gives one extra band of 1599 bp, compared to the reference type V (5C2). Another SCC*mec* typing method was developed in 2005 by Hisata *et al.* who proposed another set of primers (Hisata *et al.*, 2005). The method allowed

to detect SCC*mec* type I, IIa, IIb, III and IVa to IVd and was based mainly on *mec* class and *ccr* type assignment. Unfortunately, it was not possible to perform the analysis in a single M-PCR reaction and the method did not become popular widely. Another recently developed technique was presented by Boye *et al.* (Boye *et al.*, 2007). It is a quick and easy to interpret method based on single-tube M-PCR reaction, using primers for specific detection of both *mec* class and *ccr* type of SCC*mec* type I to V. It seems to be very useful for first screening of large amount of strains, but for the detection of *SCCmec* type I to III it is not complex enough. For example, to detect SCC*mec* type III only *ccrC* specific pair of primers is used, which confirms just the presence of SCC*mercury* (Boye *et al.*, 2007). Moreover, this method also misclassifies SCC*mec* type I with type VI. However, this method can also be used for confirmation of doubtful SCC*mec* types. The most complex and promising system for SCC*mec* assignment, especially in the light of the new guidelines for SCC*mec* elements classification was developed by Kondo *et al.* (Kondo *et al.*, 2007). This PCR scheme combines six M-PCR reactions: M-PCR 1 for amplification of *ccr* type (1–4) along with *mecA* gene; M-PCR 2 for amplification of *mec* class (A, B and C2); M-PCR 3 for amplification of ORFs from J1 region of SCC*mec* type I and IV; M-PCR 4 for amplification of ORFs from J1 region of SCC*mec* type II, III and V; M-PCR 5 and 6 for amplification of gene alleles located in J2 and J3 region of SCC*mec* elements, respectively. M-PCRs 5 and 6 are used for the identification of integrated copies of transposons (Tn*554* or ΨTn*554*) and plasmids (pUB110 or pT181). The most significant advantage of this method is its flexibility, since it does not detect any particular SCC*mec* type, but only crucial loci, which in combination gives at the end the SCC*mec* type. This approach potentially allows detection of SCC*mec* types I to IX except SCC*mec*VII and X since primers specific for *mec* class C1 have not been included to this system yet. Using M-PCRs 3 to 6 it is possible also to identify variety of SCC*mec* subtypes. However, since this method requires a relatively large number of PCR reactions to determine the structure of SCC*mec* it is quite complicated and time consuming. That is why it is suggested to perform just M-PCR 1 and 2 to assign type of SCC*mec* elements and in most cases, it may be enough for epidemiological purposes. Recently new SCC*mec* type V variants, which are getting epidemiologically important, were also described (Chlebowicz *et al.*, 2010; Higuchi *et al.*, 2008). It turned out that despite the high similarity among *ccrC1* genes, its' specific alleles 1, 2, 8, 9 and 10 typing could be also important for SCC*mec* type V precise characterization. For detection of mosaic SCC*mec*V (5C2&5) variant, Higuchi *et al.* provides a set of primers specific for two types 5 *ccr* (*ccrC1* allel 2 and *ccrC1* allel 8), two characteristic ORFs (*orf33* and *orf35*) and the *mec* class C2 variant, in which nucleotide substitution in IS*431* results in truncated transposase in ΨIS*431* in *mec* complex C2 (Higuchi *et al.*, 2008). The last method we would like to mention here concerns detection of recently discovered SCC*mec* type VIII (McClure *et al.*, 2010; Zhang *et al.*, 2005). It is based on single-tube M-PCR reaction using a set of primers specific for detection of characteristic features of this particular of SCC*mec* element, namely type 4 *ccr*, *mec* class A, and a unique junction within the J region as well as internal controls. Regarding the growing number of MRSA clones harbouring different SCC*mec* IV subtypes one of the most important issue become also invention of a robust method for SCC*mec* type IV subtyping. In our opinion dedicated to solve this problem is method published by Milheirico *et al.*, which is to our knowledge the most complex among available methods and allow to detect SCC*mec* type IV subtypes from a to h (Milheirico *et al.*, 2007b). We also use subtypig descriebed by Zhang *et al.*, but we find that quite often it is difficult to detect SCC*mec* type IVc using this method, while we never had such problems when using Milheirico *et al.* method.The PCR based methods for SCC*mec* typing are the most common and the easiest to implement in laboratories, since they do not require additional expensive equipment, but on the other hand, they are labor and time-consuming, compared to real-time PCR based analysis.

Real-time PCR based SCC*mec* typing methods. In parallel to PCR based methods for SCC*mec* typing, the methods based on real-time PCR have also been developed. A multiplex scheme based on a real-time PCR targeting the *ccrB* regions of SCC*mec* types I to IV was published in 2004 by Francois *et al.*, but with current knowledge about SCC*mec* types this method is not elaborate enough (Francois *et al.*, 2004). Recently, however, a new approach using *ccr*-specific padlock probes and tag microarray analysis for simultaneous probing of core genome diversity and identification of SCC*mec* was developed. However, the set of padlock probes includes only oligonucleotides targeting diagnostic regions in the *mecA*, *ccrB* and *ccrC* genes without *mec* class recognition (Kurt *et al.*, 2009). A significant disadvantage of these methods is that they detect only *ccr* locus and ignore the *mec* complex, which may result in misclassification and do not allow the detection of novel combinations of the *mec* and *ccr* complex in SCC*mec* elements. On the other hand, in the same year, a very interesting method for SCC*mec* element typing was published, based on a rapid molecular beacon real-time PCR (MB-PCR) assay (Chen *et al.*, 2009). The design of the system is based on the definition of SCC*mec* types as a combination of the *ccr* allotype along with the *mec* class complex. The assay con-

sists of two multiplex panels, the combination of which results in two targets (*mec* class, *ccr*) for each SCC*mec* type. MB-PCR panel I targets *mecA*, *ccrB2*, *mecI*, and the Δ*mecR1*-IS*1272* junction (*mec* class B) and thus can definitively identify SCC*mec* types II and IV. MB-PCR panel II detects *ccrC*, *ccrB1*, *ccrB3*, *ccrB4*, and the Δ*mec*R1-IS431 junction (*mec* class C2) and is therefore capable of identifying SCC*mec* types I, III, V, and VI in combination with panel I. This method can also detect the recently described novel SCC*mec* type VIII (*ccrAB4* with *mec* class A). The authors of this method ascertain that it is possible to easily classify isolates within 3 to 4 h, including DNA isolation, PCR cycling and analysis, which is extremely quick. However, in this analysis it is impossible to detect SCC*mec* subtypes, since there are no primers designed for J-regions and this method does not detect the *mec* class C1, which is characteristic for SCC*mec* type VII and X.

The most significant advantage of real-time PCR based methods is the small amount of time and labor required for the analysis. They do not combine many preparatory steps and are easy to interpret. On the other hand, they require special equipment and reagents, which are very expensive.

Conclusion

A variety of different methods for SCC*mec* typing are now available. To cope with the increasing diversity of SCC*mec* elements being reported, methods for their detection should be elaborated and flexible. Conventional PCR assays using commonly up to ten primer pairs in a single-tube assay can give various sensitivities, depending on the template quality and may be easily contaminated. On the other hand, the real-time PCR assay requires expensive reagents and instruments, which can limit its use in many microbiological laboratories. Unfortunately, there is still no method available for SCC*mec* type VII and X–XI typing. High heterogenicity and variability of SCC*mec* elements make them complicated as a markers for epidemiological clone assignment. In our opinion, up to date, the best way of assigning the SCC*mec* type is to prepare the M-PCR 1 and 2 according to Kondo *et al.* or real-time PCR according to Chen *et al.*, for *ccr* type and *mec* class detection. For SCC*mec* type IV subtyping we recommend method by Milheirico *et al.* (Milheirico *et al.*, 2007b). This proceeding should be enough in most cases. For more accurate subtyping we suggest using the other M-PCRs described by Kondo *et al.* and if necessary also methods established by Higuchi *et al.* and McClure *et al.* Recent work done by IWG-SCC clarified the classification of the major SCC*mec* elements type, but there is still a lot of ambiguity regarding naming of the SCC*mec* variants, which requires further investigations and agreements. This includes answering the question, how accurate should we be in assigning SCC*mec* element type/variant for epidemiological purposes.

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