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Multiplex PCR Strategy for Rapid Identification of Structural Types and Variants of the *mec* Element in Methicillin-Resistant *Staphylococcus aureus*

Duarte C. Oliveira and Hermínia de Lencastre*

The Rockefeller University, New York, New York 10021, and Instituto de Tecnologia Química e Biológica, ITQB/UNL, 2780 Oeiras, Portugal

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Full characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) requires definition of not only the bacterial genetic background but also the structure of the complex and heterologous *mec* element these bacteria carry, which is associated with drug resistance determinant *mecA*. We report the development, validation, and application of a multiplex PCR strategy that allows quick presumptive characterization of the *mec* element types based on the structural features that were shown to be typical of *mec* elements carried by several MRSA clones. The strategy was validated by using a representative collection of pandemic MRSA clones in which the full structure of the associated *mec* elements was previously determined by hybridization and PCR screenings and also by DNA sequencing. The method was tested together with multilocus sequence typing and other typing methods for the characterization of 18 isolates representative of the MRSA clones recovered during a hospital outbreak in Barcelona, Spain. The multiplex PCR was shown to be rapid, robust, and capable in a single assay of identifying five structural types of the *mec* element among these strains, three major and two minor variants, each one of which has been already been seen among MRSA characterized earlier. This technique should be a useful addition to the armamentarium of molecular typing tools for the characterization of MRSA clonal types and for the rapid tentative identification of structural variants of the *mec* element.

Molecular typing techniques have been used with increasing frequency in studies of the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) and also for a better understanding of the evolutionary relationships among MRSA clones (3, 7, 9, 26). One of the conclusions emerging from these studies was that a complete characterization of MRSA lineages requires not only identification of the genetic background of the bacteria but also identification of the structural types of the large and heterologous *mec* element, which carries methicillin resistance determinant *mecA* (11, 26, 27).

Studies by Ito et al. have elucidated the complete structure of three major *mec* elements, also referred to as the staphylococcal chromosomal cassette (SCC*mec*). Type I (34 kb) was identified in the first MRSA strain isolated in 1961 in the United Kingdom (strain NCTC10442), type II (52 kb) was identified in an MRSA strain isolated in 1982 in Japan (strain N315), and type III (66 kb) was identified in an MRSA strain isolated in 1985 in New Zealand (strain 82/2082) (12, 13). More recently, a smaller fourth *mec* element, SCC*mec* type IV (20 to 24 kb), was independently identified among representatives of the Pediatric clone (26) and in two community-acquired MRSA strains (18).

In a recent report we described the characterization of the structural types of the *mec* elements carried by 28 MRSA isolates belonging to five widespread epidemic clones, which were all characterized by multilocus sequence typing (MLST) as single-locus or double-locus variants of two completely different genetic backgrounds (26). The full structure of each *mec* element carried by these MRSA isolates was determined by Southern blot analysis using three restriction enzymes and several key probes specific for each SCC*mec* type, by PCR detection of several loci covering the entire *mec* element structures, and by DNA sequencing, based on the information described in references 12 and 13.

However, these methods are laborious and time-consuming. In this paper we describe a multiplex PCR strategy which was designed to detect the structural variations observed in the *mec* element in our previous study. Such a method should provide a useful tool for the rapid tentative identification of the structural type of the *mec* elements in MRSA isolates.

MATERIALS AND METHODS

Strain selection. The 26 strains used for the validation of the multiplex PCR were selected from among MRSA isolates previously characterized for MLST profile and for the structural type of the *mec* element (26) (Table 1). Strains COL, N315, and ANS46 were included as controls for the three major structural types of *mec* elements, as previously described (26). Genome data from strain COL (www.tigr.org) indicate that this strain has a *mec* element identical to that of strain NCTC10442, used by Ito et al. for the definition of SCC*mec* I (13). Strain N315 was used in the definition of SCC*mec* II (13). The physical mapping of the *mecA* region of strain ANS46 showed that it carried a *mec* element identical to that of SCC*mec* III (5, 12). After validation, the multiplex PCR was applied to the characterization of 18 representative isolates of MRSA recovered during an outbreak investigation in a hospital in Barcelona, Spain. These strains had been characterized earlier by pulsed-field gel electrophoresis (PFGE) (4).

DNA isolation. Chromosomal DNAs from three to five isolated colonies were prepared by using the Wizard genomic DNA preparation kit (Promega, Madison, Wis.), with lysostaphin at 0.5 mg/ml and RNase at 0.3 mg/ml for the lysis step.

* Corresponding author. Mailing address: The Rockefeller University, 1230 York Ave., New York, NY 10021. Phone: (212) 327-8278. Fax: (212) 327-8688. E-mail: lencash@mail.rockefeller.edu.

TABLE 1. MRSA strains used for the validation of the multiplex PCR

Strain	Isolation		Clonal type[b]	MLST profile	SCC*mec* type by:	
	Origin[a]	Yr			Full characterization (26)	Multiplex PCR
UK13136	UK	1960	Archaic	3-3-1-1-4-4-16	I	I
BK793	Egypt	1961	Archaic	3-3-1-1-4-4-16	I	I
COL	UK	1965	Archaic	3-3-1-1-4-4-16	I	I
E2125	Denmark	1964	Archaic	3-3-1-12-4-4-16	I	I
PER34	Spain	1989	Iberian	3-3-1-1-4-4-16	IA	IA
HPV107	Portugal	1992	Iberian	3-3-1-12-4-4-16	IA	IA
BK1953	USA	1995	Iberian	3-3-1-12-4-4-16	IA	IA
PER184	Spain	1991	Iberian	3-3-1-12-4-4-16	IA	I
PER88	Spain	1992	Iberian	3-3-1-12-4-4-16	IA	IA
N315	Japan	1982	NY/Japan	1-4-1-4-12-1-10	II	II
BK2464	USA	1996	NY/Japan	1-4-1-4-12-1-10	II	II
JP1	Japan	1997	NY/Japan	1-4-1-4-12-1-10	II	II
ANS46[c]	Australia	1982		2-3-1-1-4-4-3	III	III
R35[c]	USA	1987		2-3-1-1-4-4-3	III	III
HU25	Brazil	1993	Brazilian	2-3-1-1-4-4-3	IIIA	IIIA
HSJ216	Portugal	1997	Brazilian	2-3-1-1-4-4-3	IIIA	IIIA
HDG2	Portugal	1993	Brazilian	2-3-1-1-4-4-3	IIIB	IIIB
HUSA304	Hungary	1993	Hungarian	2-3-1-1-4-4-3	III	III
HU106	Hungary	1996	Hungarian	2-3-1-1-4-4-3	III	III
BM18	USA	1989	Pediatric	1-4-1-4-12-1-10	IV	IV
PL72	Poland	1991	Pediatric	1-4-1-4-12-1-10	IV	IV
POL3	Poland	1992	Pediatric	1-4-1-4-12-1-10	IV	IV
HDE288	Portugal	1996	Pediatric	1-4-1-4-12-1-10	IV	IV
COB3	Colombia	1996	Pediatric	1-4-1-4-12-1-10	IV	IV
BK2529	USA	1996	Clone V[d]	3-3-1-1-4-4-3	IV	IV
BARGII17	USA	1996	Clone V[d]	3-3-1-1-4-4-3	IV	IV

[a] UK, United Kingdom; USA, United States.
[b] Clonal types were defined by a combination of three molecular typing techniques: polymorphisms in the vicinity of *Cla*I::*mecA*; *Cla*I::Tn*554* insertion patterns, and *Sma*I PFGE macrorestriction profiles (see Materials and Methods).
[c] Strains ANS46 and R35 were included as controls for SCC*mec* type III, and they were representatives of an MRSA clone found in Australia and in the United States (5, 6).
[d] Strains BK2529 and BARGII17 are representatives of an MRSA clone detected in hospitals located in the New York metropolitan area characterized by unique *Cla*I::*mecA* pattern V (17, 26, 29).

MLST and *spaA* typing. Molecular typing based on the sequences of seven housekeeping genes (MLST) and the typing of the polymorphic region of protein A (*spaA* typing) were performed according to previously described procedures (7, 25, 33).

Multiplex PCR for *mec* element type assignment. The multiplex PCR includes eight loci (A through H) selected on the basis of previously described *mec* element sequences (12, 13, 26) (Table 2). Also included in the protocol as an internal positive control was the *mecA* gene. Locus A is located downstream of the *pls* gene and is specific for SCC*mec* type I; locus B is internal to the *kdp* operon, which is specific for SCC*mec* type II; locus C is internal to the *mecI* gene present in SCC*mec* types II and III; locus D is internal to the *dcs* region, present in SCC*mec* types I, II, and IV; locus E is located in the region between integrated plasmid pI258 and transposon Tn*554*, specific for SCC*mec* type III; locus F, which is also specific for SCC*mec* type III, is located in the region between Tn*554* and the chromosomal right junction (*orfX*). Loci G and H were included to distinguish structural variants IA and IIIA, respectively. Locus G is the left junction between IS*431* and pUB110, and locus H is the left junction between IS*431* and pT181. Primers were designed manually and were commercially obtained (Gibco, Invitrogen Corporation, Carlsbad, Calif.). Primer length and GC contents were kept as uniform as possible in order to minimize differences in the annealing temperature and kinetics, and primer sequences were checked for specificity against available *S. aureus* genomes and SSC*mec* sequences with the BLAST utility available through the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Amplicon sizes ranged from 162 to 495 bp, differing by at least 40 bp in size from one another (Table 2).

The optimization of the multiplex PCR followed the general principles described by Henegariu et al. (10). Each pair of primers was first tested for amplification specificity and robustness at annealing temperatures of 55 and 60°C. For the multiplex PCR, we found it necessary to decrease the annealing temperature, increase the extension time, and adjust primer amounts for some loci. These alterations were tested empirically in small steps. The multiplex PCR was performed in a 50-μl volume with the GeneAmp PCR kit (Applied Biosystems, Foster City, Calif.) containing the following: 1× PCR buffer II; 200 μM (each) deoxynucleoside triphosphate; 400 nM concentrations of primers CIF2 F2, CIF2 R2, MECI P2, MECI P3, RIF5 F10, RIF5 R13, pUB110 R1, and pT181 R1; 800 nM concentrations of primers DCS F2, DCS R2, MECA P4, MECA P7, and IS431 P4; 200 nM concentrations of primers KDP F1, KDP R1, RIF4 F3, and RIF4 R9; 1.25 U of Ampli*Taq*; and approximately 5 ng of template DNA. PCR amplifications were performed in a DNA Thermal Cycler 480 (Applied Biosystems) with the following parameters: predenaturation for 4 min at 94°C; 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min; postextension for 4 min at 72°C; and soaking at 4°C. PCR products (10 μl) were resolved in a 2% SeaKem LE (BioWhittaker Molecular Applications, Rockland, Maine) agarose gel in 0.5× Tris-borate-EDTA buffer (Bio-Rad, Hercules, Calif.) at 100 V and visualized with ethidium bromide.

RESULTS AND DISCUSSION

Multiplex PCR was first developed in 1988 by Chamberlain et al. (2). For staphylococci, this technique has been used to specifically detect MRSA (1, 8, 16, 19, 21, 30, 36), to discriminate between *S. aureus* and coagulase-negative staphylococci together with detection of oxacillin resistance (19, 21, 30), and

TABLE 2. Primers used in the multiplex PCR

Locus	Primer	Oligonucleotide sequence (5′–3′)	Location	Amplicon size (bp)	Specificity[e] (SCC*mec* type)
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	18398–18419[a]	495	I
	CIF2 R2	ATTTACCACAAGGACTACCAGC	18892–18871[a]		
B	KDP F1	AATCATCTGCCATTGGTGATGC	10445–10467[b]	284	II
	KDP R1	CGAATGAAGTGAAAGAAAGTGG	10728–10707[b]		
C	MECI P2	ATCAAGACTTGCATTCAGGC	42428–42447[b]	209	II, III
	MECI P3	GCGGTTTCAATTCACTTGTC	42636–42617[b]		
D	DCS F2	CATCCTATGATAGCTTGGTC	38011–37992[a]	342	I, II, IV
	DCS R1	CTAAATCATAGCCATGACCG	37670–37689[a]		
E	RIF4 F3	GTGATTGTTCGAGATATGTGG	45587–45607[c]	243	III
	RIF4 R9	CGCTTTATCTGTATCTATCGC	45829–45809[c]		
F	RIF5 F10	TTCTTAAGTACACGCTGAATCG	59573–59594[c]	414	III
	RIF5 R13	GTCACAGTAATTCCATCAATGC	59986–59965[c]		
G	IS431 P4	CAGGTCTCTTCAGATCTACG	49963–49982[b]	381	
	pUB110 R1	GAGCCATAAACACCAATAGCC	50343–50323[b]		
H	IS431 P4	CAGGTCTCTTCAGATCTACG	29654–29673[c]	303	
	pT181 R1	GAAGAATGGGGAAAGCTTCAC	29976–29956[c]		
mecA	MECA P4	TCCAGATTACAACTTCACCAGG	1190–1211[d]	162	Internal control
	MECA P7	CCACTTCATATCTTGTAACG	1351–1332[d]		

[a] Relative to accession no. AB033763, SCC*mec* type I (13).
[b] Relative to accession no. D86934, SCC*mec* type II (12).
[c] Relative to accession no. AB037671, SCC*mec* type III (12).
[d] Relative to accession no. Y00688, *mecA* gene (34).
[e] Loci G and H were included to distinguish variants IA from I and IIIA from III, respectively.

to detect several resistance determinants (19, 20, 24) and staphylococcal toxin genes simultaneously (22, 23, 31, 32).

Strategy for selecting loci included in the multiplex PCR. In choosing the main loci (A through F) for the multiplex PCR we followed three criteria. The first two were to include for each SCC*mec* type one locus located upstream of the *mecA* gene and a second locus located downstream of the *mecA* gene. The third was to select one of the loci to be unique for a single SCC*mec* type.

In addition to those criteria, the genetic organizations of the SCC*mec* types and the similarities among them conditioned the choice of loci (12, 26). SCC*mec* types I and II have very similar downstream region organizations, explaining the common downstream locus D and specific upstream loci A and B. Most of the SCC*mec* type III upstream sequence is similar to that of part of the type II upstream region, whereas the downstream region of type III differs from and is much longer than those of types I and II, explaining the nondifferentiating upstream locus C and the two specific downstream loci, E and F. SCC*mec* type IV is closely related to type I, having the same downstream region and part of the upstream region. Moreover, the specific upstream region (i.e., the one not present in type I) is variable from strain to strain, so that this SCC*mec* type is more accurately defined by the absence of the *pls* region (18 kb) in the *mecA* upstream region (26). Therefore, strains harboring SCC*mec* type IV are positive for downstream locus D and negative for upstream locus A, located in the *pls* region.

To distinguish SCC*mec* type variants IA (from I) and IIIA (from III), primers to amplify the left junctions between IS*431* and integrated plasmids pUB110 (present in variant IA) and pT181 (absent in variant IIIA) were included. The distinction between SCC*mec* types I and IA is important, since type IA is associated with a widely spread MRSA clone, the Iberian clone. In the same way, type IIIA is associated with another widely spread MRSA clone, the Brazilian clone.

Validation of the multiplex PCR strategy. The amplification profiles obtained for the strains included in the validation collection (Table 1) are shown in Fig. 1. All strains were positive for the 162-bp internal fragment of the *mecA* gene, included in the multiplex PCR as an internal positive control. The four major SCC*mec* types are easily distinguishable: type I strains display two bands of 495 and 342 bp; type II strains display four bands of 381, 342, 284, and 209 bp; type III strains display four bands of 414, 303, 243, and 209 bp; and type IV strains display a single band of 342 bp. SCC*mec* variant IA is distinguished from type I by an extra band of 381 bp corresponding to the pUB110 insertion (Fig. 1A, lanes 5 to 7 and 9); SCC*mec* variant IIIA is distinguished from type III by the absence of the 303-bp band corresponding to the pT181 insertion (Fig. 1B, lanes 3 and 4); and SCC*mec* variant IIIB is discriminated from type III by the absence of all downstream bands (Fig. 1B, lane 5). Strain PER184 (Fig. 1A, lane 8) shows, by multiplex PCR, a pattern characteristic of type I. In a detailed previous study, this strain was assigned to variant IA because, in spite of not having the integrated pUB110 copy in the *mecA* downstream vicinity, it has a truncated HVR region (1-kb deletion), which is the specific characteristic of type IA (26), since linearized plasmid pUB110 is also present in SCC*mec*

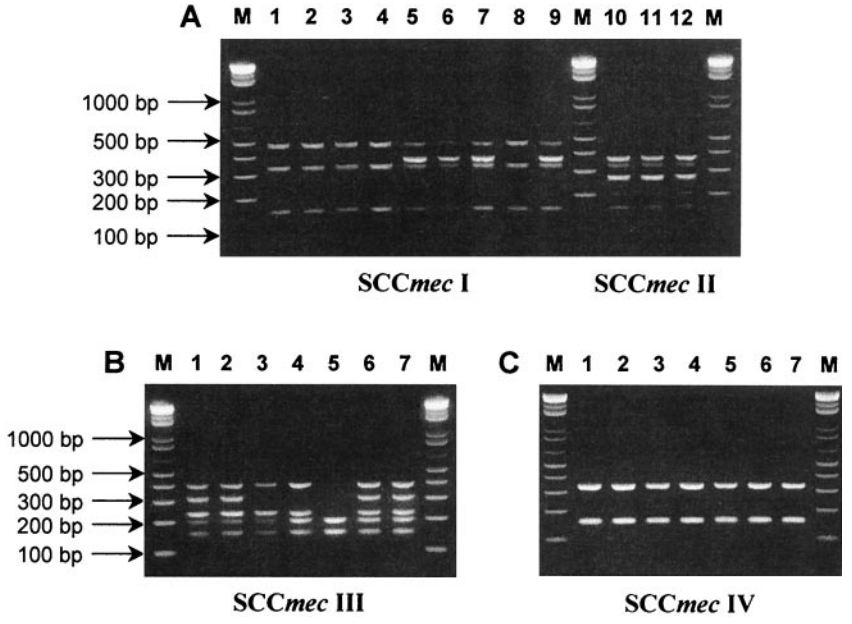

FIG. 1. Validation of the SCC*mec* multiplex PCR strategy. Major SCC*mec* types are given at the bottom. (A) SCC*mec* type I (lanes 1 to 4 and 8), variant IA (lanes 5 to 7 and 9), and SCC*mec* type II (lanes 10 to 12). (B) SCC*mec* type III (lanes 1, 2, 6, and 7), variant IIIA (lanes 3 and 4), and variant IIIB (lane 5). (C) SCC*mec* type IV. Lane numbers are correlated with the order of strains (from top to bottom) in Table 1 (e.g., panel A, lane 1 represents strain UK13136 and panel B, lane 1 represents strain ANS46). M, DNA molecular size marker (1-kb DNA Ladder Plus; Gibco BRL, Invitrogen Corporation).

type II (13). The distinction between SCC*mec* types I and IA by the multiplex strategy is based on the detection of a pUB110-specific fragment, so the multiplex PCR fails to discriminate these types. However, the absence of pUB110 in SCC*mec* type IA strains is rare and can be detected on the basis of *Cla*I::*mecA* vicinity polymorphisms (28).

The primary criteria originally used for the classification of the *mec* element into three structural types were the structure of the *mecA* complex (*mecA* gene with the upstream regulatory region) and the nature of the *ccrAB* allele (12), the function of which is associated with the precise insertion and excision mechanisms of the *mec* element at the specific chromosomal site of *orfX* (15). The multiplex PCR method described here was designed to provide maximum resolution for the various structural variants of the *mec* element that we have identified in association with various clones of MRSA (26). This strategy did not include identification of the *ccrAB* alleles. However, PCR primers to confirm the presence of the specific *ccrAB*

TABLE 3. Application of the multiplex PCR to an outbreak collection

Strain	PFGE pattern[a]	No. of band differences in relation to pattern A1	*SpaA* typing	MLST profile	SCC*mec* type	Clonal type
PER88	B7	6	YHFGFMBQBLO	3-3-1-12-4-4-16	IA	Iberian
PER4	A1	0	YHFGFMBQBLO	ND[b]	IA	Iberian
PER90	A2	2	YHFGFMBQBLO	ND	IA	Iberian
PER110	B1	4	YHFGFMBQBLO	ND	IA	Iberian
PER135	B2	6	YHFGFMBQBLO	ND	IA	Iberian
PER87	B3	4	YHFGFMBQBLO	ND	IA	Iberian
PER170	B4	6	YHFGFMBQBLO	ND	IA	Iberian
PER138	B5	6	YHFGFMBQBLO	ND	IA	Iberian
PER128	B6	4	YHFGFMBQBLO	ND	IA	Iberian
PER46	B8	8	YHFGFMBQBLO	ND	IA	Iberian
PER184	D	6	YHFMBQBLO	3-3-1-12-4-4-16	I	Iberian
PER92	E	6	YHFGFMBQBLO	3-3-1-12-4-4-16	IA	Iberian
PER222	H	8	YHFGFMBQBLO	3-3-1-12-4-4-16	IA	Iberian
PER178	F	10	WGKAOMQ	2-3-1-1-4-4-3	IIIA	Brazilian
PER127	G	>10	$WGQQL_2LO$	2-2-2-2-6-3-2	IV	ST30/UK
PER2	I	>10	WGKAKAOMQQ	2-2-2-2-6-3-2	IVA	ST30/UK
PER220	J	>10	WGKAKAOMQQ	2-2-2-2-6-3-2	IV	ST30/UK
PER205	C	8	$UJGFGMDMGGML_2$	1-4-1-8-4-4-3	IVA	New

[a] As described in the original reference (4) and according to criteria by Goering et al. (9).
[b] ND, not determined.

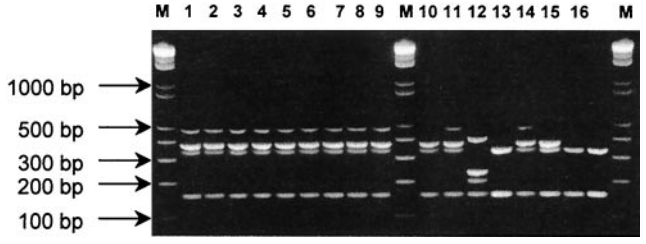

FIG. 2. Application of the SCC*mec* multiplex PCR strategy to an outbreak collection including the sporadic clones. Lanes 1 to 9, PFGE patterns A1, A2, B1 to B6, and B8, respectively; lane 10, pattern C; lanes 11 to 16, patterns E through J, respectively. M, DNA molecular size marker (1-kb DNA Ladder Plus).

alleles associated with SCC*mec* types I, II, and III, identified by the multiplex PCR strategy, are available (12). Identification of the recently described type IV *mec* element is more problematic. This new *mec* element has a structure identical to that of *mec* element I in its *mecA* complex and in the downstream region but showed variability, from one strain to another, further upstream of the *mecA* complex (18, 26). The *ccrAB* allele of type IV strains differed from the alleles associated with *mec* element types I through III. Moreover, different strains carrying the type IV *mec* element showed variations in *ccrAB*. In the multiplex PCR described here the type IV *mec* element is defined through the absence of the *pls* region (characteristic of SCC*mec* type I) and a positive reaction for the downstream *dcs* region.

Overall, the multiplex PCR strategy was able to discriminate the four major *mec* element types and some of its previously detected variants, such as SCC*mec* IA and IIIA, associated with widespread MRSA clones (Iberian and Brazilian MRSA clones, respectively). There was a correct assignment of SCC*mec* types to the strains for which the *mec* element had been previously extensively characterized except for strain PER184 (see above) (Table 1).

Application of the multiplex PCR strategy. We tested the multiplex PCR method as part of a protocol for the complete genetic characterization of a group of MRSA isolates recovered in a hospital outbreak in Barcelona, Spain (4). A preliminary, relatively rapid characterization of the genetic background of strains was done by *spaA* typing, which was shown to be predictive of MLST results in many of the cases (26). Confirmation of the genetic background was done by MLST, and tentative identification of the *mec* elements was done by multiplex PCR. The strains had already been characterized by PFGE (5). The results of this study are summarized in Table 3.

The application of the multiplex PCR to this collection of strains allowed rapid presumptive assignment of the SCC*mec* types (Fig. 2). The outbreak clone (Iberian clone), classified as having PFGE patterns A and B, carried SCC*mec* type IA (Fig. 2, lanes 1 to 9). Representative isolates of what appeared to be sporadic lineages, found by MLST and *spaA* typing to be related to the Iberian clone, also carried SCC*mec* type IA (Fig. 2, lanes 11 and 14). The single isolate belonging to the epidemic Brazilian clone was shown to have the characteristic SCC*mec* type IIIA (Fig. 2, lane 12). The isolates representing clone ST30/UK, a single-locus variant of clone E-MRSA 16 (epidemic MRSA clone 16) (7), were assigned to SCC*mec* type IV or IVA, which differs from type IV by the presence of a 381-bp band due to the integration of pUB110 (Fig. 2, lanes 13, 15, and 16). The new clone, classified as having PFGE pattern C, also carried SCC*mec* type IVA (Fig. 2, lane 10).

We applied the SCC*mec* multiplex PCR to MRSA isolates collected in the Barcelona outbreak as a kind of field test of the multiplex PCR method. Beyond proving its rapidity and fidelity, the study also yielded some interesting new information. First, some of the "sporadic isolates" were related to the major clone responsible for the outbreak, and others were related to highly epidemic MRSA clones, such as the Brazilian and E-MRSA 16 clones (14, 35). Second, the previously detected presence of SCC*mec* type IV in different genetic backgrounds

TABLE 4. Presence of SCC*mec* type IV in different genetic backgrounds[b]

Strain	Origin	MLST profile[a]	Clonal type	SCC*mec* type	Reference(s), source
PER34	Spain	3-3-1-1-4-4-16	Iberian	IA	4
BK2529	USA	3-3-1-1-4-4-3	Clone V	IV	26
BARGII17	USA	3-3-1-1-4-4-3	Clone V	IV	26
N315	Japan	1-4-1-4-12-1-10	NY/Japan	II	13, 26
BM18	USA	1-4-1-4-12-1-10	Pediatric	IV	26
PL72	Poland	1-4-1-4-12-1-10	Pediatric	IV	26
POL3	Poland	1-4-1-4-12-1-10	Pediatric	IV	26
HDE288	Portugal	1-4-1-4-12-1-10	Pediatric	IV	26
COB3	Colombia	1-4-1-4-12-1-10	Pediatric	IV	26
E-MRSA 16	UK	2-2-2-2-3-3-2	E-MRSA 16	II	7, this study
PER127	Spain	2-2-2-2-6-3-2	ST30/UK	IV	4, this study
PER2	Spain	2-2-2-2-6-3-2	ST30/UK	IVA	4, this study
PER220	Spain	2-2-2-2-6-3-2	ST30/UK	IV	4, this study
PER205	Spain	1-4-1-8-4-4-3	PFGE pattern C	IVA	4, this study
E-MRSA 15	UK	7-6-1-5-8-8-6	E-MRSA 15	IV	4, this study
	USA	ND	CA-MRSA	IV	18
	Japan	ND	CNS	IV	11

[a] Single-locus variants are underlined.
[b] Abbreviations: ND, not determined; CA-MRSA, community-acquired MRSA; CNS, coagulase-negative staphylococci; UK, United Kingdom; USA, United States.

was observed, which is compatible with the suggested enhanced mobility of this *mec* element (18, 27), perhaps because it is smaller than the other SCC*mec* types. In previous studies, this SCC*mec* type was found to be associated with MRSA of several different genetic backgrounds, each one of which was also found in association with other SCC*mec* types (26). Recent data indicate the presence of SCC*mec* type IV also in community-acquired MRSA (18) and in coagulase-negative staphylococci (11). In this study, SCC*mec* type IV was found in two distinct MRSA clones: the new clone classified as having PFGE pattern C and clone ST30, which is related to clone E-MRSA 16. A representative strain of clone E-MRSA 16 is being sequenced, and analysis of its preliminary genome sequence indicates that it harbors SCC*mec* type II, which was confirmed by the multiplex PCR strategy described in this study (data not shown). Another important MRSA clone circulating in British hospitals and also present in Germany, E-MRSA clone 15, was also found to carry SCC*mec* type IV by SCC*mec* multiplex PCR (data not shown). It seems that SCC*mec* type IV is or was a mobile version of the *mec* element, and SCC*mec* multiplex PCR has provided more evidence for this mobility (Table 4).

The protocol for the multiplex PCR developed in this study was kept as simple as possible in order to allow rapid presumptive assignment of SCC*mec* types to MRSA strains. This strategy allows the presumptive classification of the SCC*mec* type resident in each MRSA isolate in a single PCR, a technique nowadays commonly available in clinical microbiology laboratories. The routine assignment MRSA isolates to SCC*mec* types, now feasible with the SCC*mec* multiplex PCR typing tool, may provide researchers with important information concerning the origin of MRSA clones and the evolutionary relationships among them, since it probes the genetic organization of the *mec* element, which harbors the central genetic component of methicillin resistance.

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