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Update to the Multiplex PCR Strategy for Assignment of *mec* Element Types in *Staphylococcus aureus*▽

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Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is important for the identification and definition of methicillin-resistant *Staphylococcus aureus* clones, and for routine purposes, multiplex PCR assays are the most adequate for SCC*mec* typing. Here, we describe an update to the multiplex PCR strategy for SCC*mec* typing that we described in 2002 so that SCC*mec* types IV and V may be properly identified.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are characterized by the presence of a large heterologous mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*), which includes the *mecA* gene, the central element of methicillin resistance (4). Besides the *mec* gene complex (which comprises the *mecA* gene and its regulators, *mecI* and *mecR1*), SCC*mec* contains the *ccr* gene complex, which encodes recombinases responsible for the mobility of SCC*mec* (7). Several SCC*mec* types have been defined by use of the combination of the class of the *mec* gene complex and the *ccr* allotype (1, 3–5, 9, 16). The remaining parts of SCC*mec* are called J regions (regions J1, J2, and J3), which constitute nonessential components of the cassette, although in some cases these regions carry additional antibiotic resistance determinants. J1 is the region between the chromosomal left junction and the *ccr* complex, J2 is the region between the *ccr* complex and the *mec* complex, and J3 is the region between the *mec* complex and the chromosomal right junction. Therefore, the structural organization of SCC*mec* may be summarized as J1-*ccr*-J2-*mec*-J3. Variations in the J regions within the same *mec-ccr* combination are used to define SCC*mec* subtypes.

In 2002, we described a multiplex PCR strategy for the rapid assignment of SCC*mec* types to MRSA strains. That strategy was able to properly identify SCC*mec* types I to III and some epidemiologically relevant variants (e.g., subtypes IA and IIIA) by probing eight loci scattered through the *mec* elements and generating specific amplification fragments of three to five bands (13). SCC*mec* type IV, which at that time was not yet recognized as an important structural type mainly due to its spread among community-acquired MRSA (CA-MRSA) strains, was not properly identified since it was positive only for the internal positive control and the *dcs* locus, which is also present in SCC*mec* types I and II. Here, we report an update to the previously described "SCC*mec* multiplex PCR strategy" in order to better characterize SCC*mec* type IV and also to include the detection of the recently described SCC*mec* type V.

Table 1 lists the characteristics of the primers used for the updated version of the SCC*mec* multiplex PCR. In order to minimize the complexity of the multiplex PCR, the detection of linearized plasmids pUB110 and pT181 was abandoned. These loci are not critical for SCC*mec* type assignment, and its utility was to discriminate subtypes IA (positive for pUB110) and IIIA (negative for pT181). Eight new primers were added for the detection of *ccrB* allotype 2 (specific for SCC*mec* types II and IV), *ccrC* (specific for SCC*mec* type V), the SCC*mec* type III J1 region, and the SCC*mec* type V J1 region.

The conditions for the multiplex PCR assay were first optimized by using the following prototype strains: COL, SCC*mec* type I (16); N315, SCC*mec* type II (4); ANS46, SCC*mec* type III (16); MW2, SCC*mec* type IVa (1); 8/6-3P, SCC*mec* type IVb (9); Q2314, SCC*mec* type IVc (6); JCSC4469, SCC*mec* type IVd; AR43/3330.1, SCC*mec* type IVE (17); M03-68, SCC*mec* type IVg (8); HAR22, SCC*mec* type IVh (11); WIS, SCC*mec* type V (5); and HDE288, SCC*mec* type VI (15). For validation purposes, a diverse collection of 60 MRSA isolates previously characterized in terms of their genetic backgrounds and SCC*mec* types was tested by use of the updated SCC*mec* multiplex PCR assay (Table 2). All assays were performed in a T1 thermocycler (Biometra, Germany). The optimal cycling conditions were the following: 94°C for 4 min; 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 4 min. Each PCR mixture, in a final volume of 50 μl, contained 5 ng of chromosomal template; 1× PCR buffer with 1.5 mM $MgCl_2$ (Applied Biosystems); 40 μM (each) deoxynucleoside triphosphate (MBI Fermentas, Hanover, MD); 0.2 μM primers kdp F1 and kdp R1; 0.4 μM primers CIF2 F2, CIF2 R2, RIF5 F10, RIF5 R13, SCC*mec* III J1F, SCC*mec* III J1R, SCC*mec* V J1 F, and SCC*mec* V J1 R; 0.8 μM primers mecI P2, mecI P3, dcs F2, dcs R1, mecA P4, mecA P7, ccrB2 F2, ccrB2 R2, ccrC F2, and ccrC R2; and 1.25 U of Amplitaq DNA polymerase (Applied Biosystems). The PCR products (10 μl) were resolved in a 3% Seakem LE (Cambrex, Rockland, ME) agarose gel in 0.5% Tris-borate-EDTA buffer (Bio-Rad, Hercules, CA) at 4 V/cm for 2.5 h and were visualized with ethidium bromide.

Figure 1 illustrates the amplification patterns obtained for the prototype strains. SCC*mec* types I to III generate specific amplification patterns of three to five bands. SCC*mec* type IV generates an amplification pattern of three bands (*mecA*,

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TABLE 1. Primers used in the updated version of SCC*mec* multiplex PCR

Primer name	Primer sequence (5′→3′)	Primer specificity (SCC*mec* type, region)	Amplicon size (bp)	Reference
CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	I, J1 region	495	13
CIF2 R2	ATTTACCACAAGGACTACCAGC			13
ccrC F2	GTACTCGTTACAATGTTTGG	V, *ccr* complex	449	This study
ccrC R2	ATAATGGCTTCATGCTTACC			This study
RIF5 F10	TTCTTAAGTACACGCTGAATCG	III, J3 region	414	13
RIF5 R13	ATGGAGATGAATTACAAGGG			13
SCCmec V J1 F	TTCTCCATTCTTGTTCATCC	V, J1 region	377	This study
SCCmec V J1 R	AGAGACTACTGACTTAAGTGG			This study
dcs F2	CATCCTATGATAGCTTGGTC	I, II, IV, and VI, J3 region	342	13
dcs R1	CTAAATCATAGCCATGACCG			13
ccrB2 F2	AGTTTCTCAGAATTCGAACG	II and IV, *ccr* complex	311	This study
ccrB2 R2	CCGATATAGAAWGGGTTAGC			This study
kdp F1	AATCATCTGCCATTGGTGATGC	II, J1 region	284	13
kdp R1	CGAATGAAGTGAAAGAAAGTGG			13
SCCmec III J1 F	CATTTGTGAAACACAGTACG	III, J1 region	243	This study
SCCmec III J1 R	GTTATTGAGACTCCTAAAGC			This study
mecI P2	ATCAAGACTTGCATTCAGGC	II and III, *mec* complex	209	13
mecI P3	GCGGTTTCAATTCACTTGTC			13
mecA P4	TCCAGATTACAACTTCACCAGG	Internal positive control	162	13
mecA P7	CCACTTCATATCTTGTAACG			13

ccrB2, and *dcs*) for subtypes a to d, g, and h; and subtypes IVE and IVF generate only two bands, since they are negative for the *dcs* locus. SCC*mec* type V also originates a pattern of three bands (*mecA*, *ccrC*, and J1). SCC*mec* type VI, which appears to be an "exotic" local variant (14), generates only two bands (*mecA* and *dcs*), and its assignment must be confirmed by *ccrAB* allotyping (*ccrAB4*). Note that SCC*mec* types IV and VI previously could not be discriminated since both were positive only for *mecA* and *dcs*.

As illustrated in Table 2, the updated SCC*mec* multiplex PCR assay performed well in assigning SCC*mec* types to the 60 diverse MRSA isolates. As expected, subtypes IA and IIIA, defined by the presence of pUB110 and the absence of pT181, respectively, could not be detected, whereas the sporadically occurring subtype IIIB, defined as being negative for the J3 region specific locus, was still detected. Strains PL72 and POL3 were incorrectly assigned to SCC*mec* type VI. These sporadically occurring strains were first tentatively classified as SCC*mec* type IV (13) and after *ccrB* sequencing (14) were classified as new type I variants with a partial deletion within the J1 region, which includes the J1-specific locus of SCC*mec* type I included in the multiplex PCR assay. This unique example of an incorrect assignment reinforces the importance of confirming multiplex PCR results by *ccr* gene complex typing, either by conventional PCR allotyping (12) or by DNA sequencing (15), at least for a subset of strains belonging to the same clonal type or whenever a new clone is detected.

In short, the updated version of the previously described SCC*mec* multiplex PCR strategy enables the rapid presumptive assignment of all known SCC*mec* types to MRSA strains. Whereas the previous version enabled the prompt identification of only SCC*mec* types I to III, since SCC*mec* type IV was poorly identified and SCC*mec* type V was not detected, the means for the proper identification of SCC*mec* types IV and V was added to this updated version. The prompt detection of SCC*mec* types IV and V is particularly relevant for the characterization of the recent threat of CA-MRSA, mostly characterized by these two structural variants of the *mec* element. In practical terms, changes to the previous protocol were kept to a minimum, and so the implementation of this SCC*mec* typing strategy will be straightforward, particularly for those laboratories currently using the previously described SCC*mec* multiplex PCR strategy.

The importance of SCC*mec* typing is well illustrated by the fact that the proposal by Enright and colleagues (2) that MRSA clones be named according to their multilocus sequence types and SCC*mec* types (e.g., clone ST5-MRSA-II), which was agreed to by a subcommittee of the International Union of Microbiology Societies in Tokyo, Japan, in 2002, is currently consensual in the specialized literature. In this context, rapid and easy assays for the detection of SCC*mec* types, such as the multiplex PCR typing strategy described in this study, are critical tools for the proper characterization of MRSA clones. The SCC*mec* element, which carries the determinant for "broad-spectrum" beta-lactam resistance in staphylococci, is a critical epidemiological marker for MRSA clones. However, besides being an important tool for surveillance studies, SCC*mec* typing of large international collections of isolates has contributed dramatically to the elucidation of the

TABLE 2. Prototype strains and representative collection used for validation of updated SCC*mec* multiplex PCR assay

Strain[a]	Origin	Isolation date	MLST (ST)[b]	SCC*mec* type	*ccrAB* type	Multiplex PCR assignment	Reference[c]
COL	United Kingdom	1965	250	I	1	I	16
UK13136	United Kingdom	1960	250	I	1	I	16
BK793	Egypt	1961	250	I	1	I	16
E2125	Denmark	1964	247	I	1	I	16
PER34	Spain	1989	250	IA	1	I	16
HPV107	Portugal	1992	247	IA	1	I	16
BK1953	United States	1995	247	IA	1	I	16
PER184	Spain	1991	247	IA	1	I	16
PER88	Spain	1992	247	IA	1	I	16
HAR40	Belgium	1995	228	I	1	I	15
HAR21	Finland	2002	5	I	1	I	15
PL72	Poland	1991	5	I-VAR[d]	1	VI	15
POL3	Poland	1992	5	I-VAR	1	VI	15
N315	Japan	1982	5	II	2	II	4
USA100	United States	1995–2003	5	II	2	II	10
BK2464	United States	1996	5	II	2	II	16
JP1	Japan	1987	5	II	2	II	16
JP205	Japan	1998	5	II	2	II	15
USA200	United States	1995–2003	36	II	2	II	10
HAR24	Finland	2002	36	II	2	II	15
USA600	United States	1995–2003	45	II	2	II	10
ANS46	Australia	1982	239	III	3	III	16
R35	United States	1987	239	III	3	III	16
HU25	Brazil	1993	239	III	3	III	16
HUSA304	Hungary	1993	239	III	3	III	16
HU106	Hungary	1996	239	III	3	III	16
LHH1	United States	1994	239	III	3	III	16
BK2421	United States	1996	239	III	3	III	16
HSJ216	Portugal	1997	239	IIIA	3	III	16
HDG2	Portugal	1993	239	IIIB	3	IIIB	16
MW2	United States	1998	1	IVa	2	IV	1
CA05	Chicago	1999	256	IVa	2	IV	9
8/6-3P	Chicago	1996	770	IVb	2	IV	9
Q2314	Dallas, TX	1996	5	IVc	2	IV	6
JCSC4469	Japan	1982	5	IVd	2	IV	AB097677
AR43/3330.1	Ireland	1988–2002	8	IVE	2	IV	17
M03-68	Korea	2003	5	IVg	2	IV	8
HAR22	Finland	2002	22	IVh	2	IV	11
USA400	United States	1995–2003	1	IV	2	IV	10
USA800	United States	1995–2003	5	IV	2	IV	10
BM18	United States	1989	5	IV	2	IV	16
COB3	Colombia	1996	5	IV	2	IV	16
FFP311	Portugal	1996	5	IV	2	IV	14
VNG17	Portugal	1992–1993	5	IV	2	IV	14
RJP17	Portugal	1992–1993	5	IV	2	IV	14
HSA49	Portugal	1993	5	IV	2	IV	15
HSA74	Portugal	1993	5	IV	2	IV	14
ARG9	Argentina	1996	5	IV	2	IV	14
USA300	United States	1995–2003	8	IV	2	IV	10
USA500	United States	1995–2003	8	IV	2	IV	10
BK2529	United States	1996	8	IV	2	IV	16
BargII17	United States	1996	8	IV	2	IV	16
GRE120	Greece	1993	8	IV	2	IV	15
HAR36[e]	Greece	2002	254	IV-VAR	2	IV-VAR	15
HGSA146	Portugal	2003	22	IVh	2	IV	11
HGSA163	Portugal	2003	22	IVh	2	IV	11
DEN2946	Denmark	2001	30	IV	2	IV	15
DEN2294	Denmark	2001	30	IV	2	IV	15
HAR38	Belgium	1995	45	IV	2	IV	15
USA700	United States	1995–2003	72	IV	2	IV	10
GRE14	Greece	1998	80	IV	2	IV	15
DEN2949	Denmark	2001	80	IV	2	IV	15
DEN114	Denmark	2001	80	IV	2	IV	15
DEN1451	Denmark	2001	153	IV	2	IV	15
WIS	Australia			V	*ccrC*	V	5
HT0087	Australia	2005	377	V	*ccrC*	V	18
HT0184	Greece	2005	377	V	*ccrC*	V	18
HT0350	The Netherlands	2003	377	V	*ccrC*	V	18
HT0388	Switzerland	2003	377	V	*ccrC*	V	18
HT0826	France	2003	377	V	*ccrC*	V	18
HDE288	Portugal	1996	5	VI	4	VI	14
HUC136	Portugal	1995	5	VI	4	VI	14
IPO92	Portugal	2001	5	VI	4	VI	14

[a] Strains in boldface are the prototype strains for SCC*mec* types and subtypes.
[b] MLST, multilocus sequence type; ST, sequence type.
[c] Reference for SCC*mec* type assignment and *ccr* type.
[d] VAR, variant.
[e] Strain HAR36 is characterized by SCC*mec* type IV and is also positive for the J3 locus specific for SCC*mec* type III.

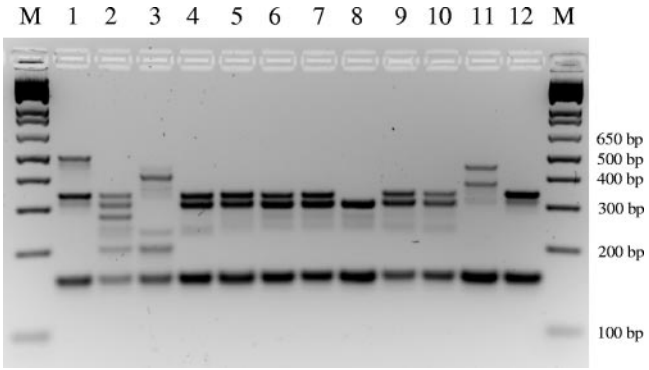

FIG. 1. Amplification patterns for the prototype strains obtained with the updated SCC*mec* multiplex PCR strategy. Lane 1, SCC*mec* type I (strain COL); lane 2, type II (N315); lane 3, type III (ANS46), lane 4, type IVa (MW2); lane 5, type IVb (8/6-3P); lane 6, type IVc (Q2314); lane 7, type IVd (JCSC4469); lane 8, type IVE (AR43/3330.1); lane 9, type IVg (M03-68); lane 10, type IVh (HAR22); lane 11, type V (WIS); lane 12, type VI (HDE288); lanes M, DNA molecular size marker (1-kb DNA Ladder Plus; Invitrogen Life Technologies, Carlsbad, CA).

origin(s) and evolutionary history of contemporary MRSA clones.

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