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

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Staphylococcal cassette chromosome mec (SCCmec) 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 SCCmec typing. Here, we describe an update to the multiplex PCR strategy for SCCmec typing that we described in 2002 so that SCCmec 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 (SCCmec), 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), SCCmec contains the ccr gene complex, which encodes recombinases responsible for the mobility of SCCmec (7). Several SCCmec 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 SCCmec 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 SCCmec may be summarized as J1-ccr-J2-mec-J3. Variations in the J regions within the same mec-ccr combination are used to define SCCmec subtypes.

In 2002, we described a multiplex PCR strategy for the rapid assignment of SCCmec types to MRSA strains. That strategy was able to properly identify SCCmec types I to III and also to discriminate subtypes IA (positive for pUB110) and IIA (negative for pUB110). Eight new primers were added for the detection of ccrB allotype 2 (specific for SCCmec types II and IV), ccrC (specific for SCCmec type V), the SCCmec type III J1 region, and the SCCmec type V J1 region.

In 2002, we described a multiplex PCR strategy for the rapid assignment of SCCmec types to MRSA strains. That strategy was able to properly identify SCCmec 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). SCCmec 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 SCCmec types I and II. Here, we report an update to the previously described “SCCmec multiplex PCR strategy” in order to better characterize SCCmec type IV and also to include the detection of the recently described SCCmec type V.

Table 1 lists the characteristics of the primers used for the updated version of the SCCmec 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 SCCmec type assignment, and its utility was to discriminate subtypes IA (positive for pUB110) and IIA (negative for pT181). Eight new primers were added for the detection of ccrB allotype 2 (specific for SCCmec types II and IV), ccrC (specific for SCCmec type V), the SCCmec type III J1 region, and the SCCmec type V J1 region.

In the conditions for the multiplex PCR assay were first optimized by using the following prototype strains: COL, SCCmec type I (16); N315, SCCmec type II (4); ANS46, SCCmec type III (16); MW2, SCCmec type IVA (1); 8/6-3P, SCCmec type IVb (9); Q2314, SCCmec type IVc (6); JCSC4469, SCCmec type IVd; AR43/3330.1, SCCmec type IVE (17); M03-68, SCCmec type IVg (8); HAR22, SCCmec type IVh (11); WIS, SCCmec type V (5); and HDE288, SCCmec type VI (15). For validation purposes, a diverse collection of 60 MRSA isolates previously characterized in terms of their genetic backgrounds and SCCmec types was tested by use of the updated SCCmec 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 MgCl₂ (Applied Biosystems); 40 μM (each) deoxyribonucleoside 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, SCCmec type III J1F, SCCmec type III J1R, SCCmec V J1 F, and SCCmec V J1 R; 0.8 μM primers mecI P1, mecI P3, dcs F2, dcs R1, mecA P4, mecA P7, ccrB F2, ccrB 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. SCCmec types I to III generate specific amplification patterns of three to five bands. SCCmec type IV generates an amplification pattern of three bands (mecA,
TABLE 1. Primers used in the updated version of SCCmec multiplex PCR

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<tr>
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<th>Primer specificity (SCCmec type, region)</th>
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The importance of SCCmec 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 SCCmec 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 SCCmec types, such as the multiplex PCR typing strategy described in this study, are critical tools for the proper characterization of MRSA clones. The SCCmec 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, SCCmec typing of large international collections of isolates has contributed dramatically to the elucidation of the

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

- C1F2 F2: TTCGAGTTGCTGATGAAGAAGG, ATTACCAACAAGACTACCCACG  
  - I, J1 region: 495 bp (13)
- C1F2 R2: GTACTCGTTTACATTTGGG, ATAAATGCTCATGCTTACC  
  - V, ccr complex: 449 bp (This study, This study)
- R1F5 F10: TTTTAAGTACAGCCTAATCG, ATGGAGAGTAAATTACAGGG  
  - III, J3 region: 414 bp (13, 13)
- SCCmec V J1 F: TTCCATTCTTTGTTCATCC, AGAAACTCTGACTTAAAGTGG  
  - V, J1 region: 377 bp (This study, This study)
- des F2: CATCCTATGATAGCTTGGTC, CTAAATCATAGGCGATGCG  
  - I, II, IV, and VI, J3 region: 342 bp (13, 13)
- ccrB2 F2: AGTTTCTCAGAATCGACCG, CGCATATGAAAAWGGTTAGC  
  - II and IV, ccr complex: 311 bp (This study, This study)
- kdp F1: AATACATCTGATCAGTTAGCG, CGAATGAAGTGAAGAAAGTGG  
  - II, J1 region: 284 bp (13, 13)
- SCCmec III J1 F: CATTGTGAACAGTACAGCG, GTTATTGAGACTCCTAAGG  
  - III, J1 region: 243 bp (This study, This study)
- SCCmec III J1 R: ATCAAGCTTGGATTCAAGGC, CGGTTTTCAATTCACTTGTCC  
  - II and III, mec complex: 209 bp (13, 13)
- mecA P4: TCCAGATTTAACAATCCACCAG, CCACCTCATATCTTGTACC  
  - Internal positive control: 162 bp (13, 13)

The importance of SCCmec 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 SCCmec 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 SCCmec types, such as the multiplex PCR typing strategy described in this study, are critical tools for the proper characterization of MRSA clones. The SCCmec 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, SCCmec typing of large international collections of isolates has contributed dramatically to the elucidation of the
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a Strains in boldface are the prototype strains for SCCmec types and subtypes.
b MLST, multilocus sequence type; ST, sequence type.
c Reference for SCCmec type assignment and ccr type.
d VAR, variant.
e Strain HAR36 is characterized by SCCmec type IV and is also positive for the J3 locus specific for SCCmec type III.
We thank T. Ito, D. C. Coleman, R. Daum, K. T. Park, W. B. Grubb, J. Etienne, and A. Tomasz for having kindly given some of the prototype strains.

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REFERENCES
ERRATUM

Update to the Multiplex PCR Strategy for Assignment of mecl Element Types in Staphylococcus aureus

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Volume 51, no. 9, p. 3374–3377, 2007. Page 3375, Table 1: The sequence for primer RIF5 R13 should read “GTC ACA GTA ATT CCA TCA ATG C.”