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A Novel Staphylococcal Cassette Chromosome *mec* Type XI Primer for Detection of *mecC*-Harboring Methicillin-Resistant *Staphylococcus aureus* Directly from Screening Specimens

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Methicillin-resistant *Staphylococcus aureus* (MRSA) screening using real-time PCR has decreased in sensitivity due to the emergence of variant staphylococcal cassette chromosome *mec* element (SCC*mec*) types. We have designed and validated a novel SCC*mec* XI primer, which enables for the first time the rapid detection of *mecC*-harboring MRSA directly from nasopharyngeal swabs without prior cultivation.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health problem affecting the therapy of nosocomial, community-acquired, and livestock-associated infections and requiring the implementation of infection control and preventive measures (1). Resistance to β-lactam antibiotics is mediated by expression of penicillin-binding protein PBP2a. The encoding gene, *mecA*, is localized on the mobile staphylococcal cassette chromosome *mec* element (SCC*mec*), which exists in variant types and subtypes (http://www.sccmec.org). A rapid screening for MRSA-colonized patients is possible using a SCC*mec*-*orfX*-based MRSA-screening PCR. The initial TaqMan PCR described in 2004 by Huletsky et al., comprised five SCC*mec* forward primers targeting the right extremity of the main SCC*mec* types and a reverse primer targeting the *S. aureus*-specific *orfX* gene (2), thus amplifying an entity within the MRSA genome sequence, which was detected by a degenerate TaqMan probe. A modified PCR published in 2011 extended the MRSA screening to rare SCC*mec* types (3).

In 2011, a highly divergent *mec* gene, *mecC*, on novel SCC*mec*

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TABLE 1 Primers and probes of MRSA-screening real-time PCR

Primer/probe	Oligonucleotide sequence, 5′ to 3′	μM per PCR	SCC*mec* type[a]	Reference
F1/IC-F[b]	GTCAAAAATCATGAACCTCATTACTTATG	0.2	II, IV, IVa, VI, VIII	2
F2	AATATTTCATATATGTAATTCCTCCACATCTC	0.2	II, IIa, V, VII	3
F3	CTTCAAATATTATCTCGTAATTTACCTTGTTC	0.2	III	3
F4	CTCTGCTTTATATTATAAAATTACGGCTG	0.2	IVe	2
F5	TCACTTTTTATTCTTCAAAGATTTGAGC	0.2	II, IVa	3
F10	CCGCTCCTTTTATATTATACACAACCTATT	0.2		3
F11	GCCATATTAATGCCTCACGAAAC	0.2	I, II, IV, X	3
F13	TCCCTTTATGAAGCGGCTGAA	0.2	I, II, IV, IVa, IVc, IVq, IX	3
F14	AAGCTATAGTTCAGCATTATCGTAAGTTAACT	0.2	IV, IVa	3
F17	ACTCTGATAAGCCATTCATTCATCCA	0.2		3
F18	ACAATCCTAACATAAGATTGTGGCTTT	0.2		3
F20	GCATATTCACTTTGATAAGCCATTCAT	0.2	IVk	3
F24	CCCAAACTCTTAACTTTCTTCAATACATT	0.2		3
F25	TTCTAAGGTAGCTTCCCTTTCAATAATTT	0.2	V	3
R1	CGTCATTGGCGGATCAAAC	0.2		3
R2	CGTCATTGGTGGATCAAACG	0.2		3
Probe	FAM-CACAARGAYGTCTTACAACG-MGBNFQ	0.1		3
IC-R	GGATCAAACGGCCTGCACA	0.2		2
IC probe	HEX-ATGCCTCTTCACATTGCTCCACCTTTCCT-BHQ1	0.02		Adapted to 2
(+/−)				
SCC*mec* XI	GATAACTCTCGCAAAACATAACG	0.2	XI	This study

[a] Defined by BLAST analysis.
[b] F, forward primer; R, reverse primer; (+/−), addition of SCC*mec* XI primer, as indicated.

TABLE 2 MRSA isolates of SCC*mec* type XI in MRSA-screening and MRSA-multiplex PCRs

MRSA strain	SCC*mec* type	*spa* type	Original MRSA screening (C_T[a])	MRSA screening + SCC*mec* XI (C_T)	*nuc* (C_T)	*mecA* (C_T)	*mecC* (C_T)
07-03165	I	t001	22	21	21	19	
10-01267	I	t041	22	22	22	20	
12-00241	I	t001	19	18	19	16	
08-00463	II	t003	20	21	21	18	
14-00535	II	t003	21	20	22	19	
12-02225	II	t003	21	20	21	18	
01-00694	III	t030	17	17	13	15	
12-01196	III	t030	21	20	16	18	
12-00444	III	t037	21	22	15	17	
09-01691	IVa	t008	18	19	15	17	
09-02003	IVa	t008	20	19	15	17	
09-00417	IVa	t008	19	19	15	17	
04-01872	IVc	t019	20	21	17	18	
03-02782	IVc	t008	20	20	16	19	
04-01389	IVd	t036	19	18	15	17	
12-02344	V	t011	20	21	16	18	
12-03117	V	t3091	20	20	16	18	
14-03203	V	t011	19	20	15	17	
13-03522	V	t7656	20	20	20	17	
06-01359	VI	t640	21	21	21	19	
06-00468	VI	t311	22	21	21	18	
EQC	XI	t10009		20	24		25
Sc16837	XI	t1736		23	21		20
Sc26644	XI	t843		25	21		22
DD17[b]	XI	t843		26	19		17
SVA15[b]	XI	t843		27	21		24
DD158[b]	XI	t843		26	20		23
Z4070[b]	XI	t843		24	18		17
Z4183[b]	XI	t843		26	19		18
S130-13[b]	XI	t843		25	19		18
DD44[b]	XI	t5771		25	19		18

[a] C_T, cycle threshold.
[b] MRSA strains according to Schlotter et al. (5).

element XI, was discovered in MRSA strains of domestic and wildlife animals (4–6) and of humans (7–9).

Recently, MRSA screening cultures of two patients in Düsseldorf revealed two MRSA isolates, Sc16837 and Sc26644, with unusual resistance patterns (oxacillin susceptible, cefoxitin screening positive), arousing the suspicion of *mecC*-positive MRSA strains (10). Both strains were susceptible to all tested antibiotics other than β-lactams, as described for *mecC*-positive MRSA strains from human and animals in Europe (11–15). An in-house MRSA multiplex PCR detected the *S. aureus*-specific *nuc* gene but did not detect the *mecA* nor the PVL gene (Tables 1 and 2) (16). The *spa* typing revealed *spa* types t843 and t1736. Type t843 has already been associated with *mecC*-positive MRSA strains in humans and cattle in northern Europe (6, 17). Presence of the *mecC* gene was verified with *mecC* real-time PCR (6) with subsequent sequencing and BLAST analysis of the 357-bp PCR products. The embedded regions of the primers showed 100% sequence homology to respective *mecC* gene regions of *S. aureus* LGA251 (GenBank accession no. FR821779) and other SCC*mec* type XI strains (e.g., GenBank accession nos. LK024544, HF569116, and JN794592).

To date, the detection of MRSA with the SCC*mec* type XI element is rare in humans. Nevertheless, its occurrence might have been underestimated, as it depends on strain cultivation. A rapid molecular screening approach by the commonly used SCC*mec*-*orfX*-based MRSA-screening real-time PCR has been hindered by the high variability of the SCC*mec* type XI element (2, 3). Although the *mecC*-carrying MRSA strains Sc26644 and Sc16837 presented here derived from routine screening of asymptomatic patients, other clinical *mecC*-derived MRSA isolates that cause severe soft tissue infections, osteomyelitis, and bacteremia have been described (8, 18, 19). Thus, rapid detection of *mecC*-derived MRSA is necessary, and methods have recently been developed to resolve this deficiency. Commercial systems have been upgraded by the addition of *mecC* detection. Becker et al., published in 2013 a modified version of a commercial, modular multiplex-PCR MRSA detection assay, in which labeled oligonucleotides were used for the detection of *nuc* as the *S. aureus* species-specific gene, *mecA*, and *mecC* (20). The group of Nijhuis et al., developed a high-throughput screening approach by the use of combined real-time PCR assays which were based on overnight selective broth enrichment (21).

All of these methods require isolation of the strain prior to testing, as they have a common potential source of error: The simultaneously amplified *S. aureus* and *mecC* gene regions may not necessarily be located on the same genome but may be derived from a mixture of oxacillin-susceptible *S. aureus* (MSSA) and *mecC*-positive coagulase-negative staphylococcus (CoNS) if the test is performed directly from swab sample.

At the University Clinic of Düsseldorf, the MRSA-screening

real-time PCR was customized with respect to locally occurring MRSA variants. This TaqMan PCR was carried out on a CFX96 real-time PCR machine (Bio-Rad, Munich, Germany) in a total volume of 25 μl containing 2.5 μl DNA lysate of a swab specimen in NoROX PCR mastermix (Qiagen, Hilden, Germany) with primers and probes concentrations given in Table 1. Thermal cycling conditions were as follows: 10 min at 95°C for initial denaturation followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. Fifty copies of an internal control (IC) plasmid were added as an inhibition control prior to extraction, consisting of an artificial 128-bp insert with the IC-R primer binding site (bp 1 to 19), a genomic sequence of *Drosophila melanogaster* as the target of the IC probe (bp 49 to 77), and the F1-primer binding site (bp 100 to 128).

Based on an alignment of the 2.25-kb SCC*mec* regions of *mecC*- and *mecA*-carrying MRSA strains, which comprised the regions from the binding site of the *mecC* forward primer to that of the *orfX* reverse primer, we designed a novel SCC*mec* primer, SCC*mec* XI (5′-GATAACTCTCGCAAAACATAACG-3′) which targeted all published SCC*mec* type XI right extremities and which leads to an SCC*mecC-orfX* PCR product of 224 bp. As summarized in Table 2, supplementation of the MRSA-screening PCR (3) with this primer enabled detection of the *mecC*-positive MRSA strains Sc26644 and Sc16837, of a *mecC*-carrying MRSA strain analyzed for external quality control, and of seven animal *S. aureus* strains harboring *mecC* (5) without affecting the detection of MRSA strains of SCC*mec* types I to VI. Fifty MRSA isolates from our strain collection (including 12 different *spa* types) and 50 methicillin-susceptible *S. aureus* isolates (consecutively collected from routine specimens) were tested in the SCCmec XI-supplemented MRSA-screening PCR, revealing 100% sensitivity and 100% specificity. Fifty consecutive nasopharyngeal swabs tested MRSA positive in routine screening diagnostics, and 50 consecutive nasopharyngeal swabs with MRSA-negative results in routine testing were additionally analyzed. No difference in *mecA*-carrying MRSA detection compared to the original PCR (3) was observed, indicating that the robustness of the MRSA-screening PCR is not affected by addition of the SCC*mec* XI primer. It should be noted that the test may perform differently in different clinical settings due to different population characteristics.

This is, to our knowledge, the first description of an SCC*mec* XI primer which now allows the rapid detection of *mecC*-positive MRSA by MRSA-screening real-time PCR without former cultivation of MRSA directly from nasopharyngeal swabs.

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