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Methicillin-resistant *Staphylococcus aureus* Screening PCR adapted to locally emerging variants—Evaluation of novel SCC*mec* primers

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ABSTRACT

Infections with multi-resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), represent a world-wide health-care problem. The original MRSA Screening TaqMan PCR was based on the detection of the SCC*mec-orf*X-junction as described by the group of Huletsky in 2004. In the recent years, this assay increasingly failed to detect new MRSA variants in swab specimens. In this work, we analyzed the usefulness of 17 additional SCC*mec* primers to increase PCR sensitivity by testing 290 collected samples with negative PCR results and positive MRSA culture in a retrospective analysis, and 380 samples of the daily routine diagnostics. Sequencing of the PCR products revealed that locally new MRSA variants became detectable by nine of these forward primers. Four primers were solely responsible for the detection of 85.4% (117/123) of the PCR products: F13 (n = 76), F11 (n = 6), F14 (n = 15) and F25 (n = 8). These four primers were integrated in the Screening PCR and the novel primer collection was validated by testing 71 MRSA isolates, which covered SCC*mec* types I to VI, 50 MSSA isolates and 100 swab specimens. The sensitivity of MRSA Screening PCR increased from 93% to 98.6% without affecting the detection of the common MRSA strains. Phylogenetic analysis of the PCR products suggests that the adapted MRSA Screening PCR is able to detect SCC*mec* types I–X, including CA- and LA-MRSA variants by the SCC*mec* primers F11 and F25.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), firstly described in 1961 (Jevons, 1961), has become a worldwide healthcare problem by causing high morbidity and mortality rates (Cosgrove et al., 2003; Datta and Huang, 2008; Safdar and Bradley, 2008) and thereby enormous healthcare costs (Köck et al., 2010). Beside hospital-acquired MRSA (HA-MRSA), an increasing number of community-acquired MRSA (CA-MRSA) (Vandenesch et al., 2003) and livestock-associated MRSA (LA-MRSA) has emerged (Nemati et al., 2008). Earliest possible detection of both, MRSA infected and colonized patients, is important to prevent MRSA transmission. MRSA screening methods have therefore become part of an effective infection control strategy (Huang et al., 2006; Lucet et al., 2005).

Before MRSA polymerase-chain-reactions (PCRs) were developed MRSA screening was performed as a culture based method. Nowadays cefoxitin-containing selective chromagar plates are commonly used. Suspicious colonies then require confirmation of *S. aureus* and of methicillin-resistance (e.g. the encoding *mec*A gene) (Becker et al., 2013). As test results are only available after 24–48 h, several nucleic acid-based tests have been developed within the past two decades to identify MRSA within a few hours (Francois et al., 2003; Reischl et al., 2000; Rossney et al., 2008). The majority of the first-line PCR assays were based on the simultaneous detection of an *S. aureus*-specific gene, such as *nuc*, and the methicillin-resistance-mediating *mec*A gene (Fang and Hedin, 2003; Jonas et al., 2002). The *mec*A gene is located on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), and encodes a mutated penicillin binding protein, PBP2a or PBP2′, to which penicillin and other ß-lactam antibiotics have no binding affinity (Hartman and Tomasz, 1984). However, a false-positive PCR result can be obtained by the simultaneous presence of coagulase negative staphylococci (CoNS) containing *mec*A gene (MRCoNS) (Suzuki et al., 1992) and *nuc* positive MSSA in the same specimen.

In 2004 Huletsky et al. described a real-time PCR assay based on the amplification of a genome region in-between the SCC*mec* right extremity (SRE) of SCC*mec* types I–IV and the *S. aureus* specific open reading frame X (*orf*X) (Huletsky et al., 2004). Many commercial MRSA-detection kits and *inhouse* screening PCRs were based on this PCR. For routine MRSA screening at the University Clinic in Düsseldorf, we used an MRSA Screening PCR, MRSA_Sc-I, that contained the five SCC*mec* forward primers and the *orf*X-reverse primer as formerly published (Huletsky et al., 2004), as well as a degenerated *orf*X TaqMan

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Table 1
Primer and probe sequences.

Huletsky et al. (2004)	MRSA1[a]	GGATCAAACGGCCTGCACA	0,3	–	0,3	0,3	–
	MRSA2[b]	GTCAAAAATCATGAACCTCATTACTTATG	0,3	–	–	–	II, IV(a), VI, VIII
	MRSA3	ATTTCATATAGTAATTCCTCCACATCTC	0,3	–	–	–	III(a), V, VII
	MRSA4	CAAATATTATCTCGTAATTTACCTTGTTC	0,3	–	–	–	III
	MRSA5	CTCTGCTTTATATTATAAAATTACGGCTG	0,3	–	–	–	IVE
	MRSA6	CACTTTTTATTCTTCAAAGATTTGAGC	0,3	–	–	–	II, III, IVa
	MRSA probe	FAM-ACGTCTTACAACGYAGTAACTAYGCA-BHQ1	0,06	–	–	–	
Petersdorf et al. (2015)	IC-probe	HEX-ATGCCTCTTCACATTGCTCCACCTTTCCT-BHQ1	0,02	–	0,02	0,02	
van der Zee et al. (2011)	F1 = MRSA2	GTCAAAAATCATGAACCTCATTACTTATG	–	–	0,2	0,2	II, IV(a), VI, VIII
	F2	AATATTTCATATATGTAATTCCTCCACATCTC	–	–	0,2	0,2	III(a), V, VII
	F3	CTTCAAATATTATCTCGTAATTTACCTTGTTC	–	–	0,2	0,2	III
	F4 = MRSA5	CTCTGCTTTATATTATAAAATTACGGCTG	–	–	0,2	0,2	IVE
	F5	TCACTTTTTATTCTTCAAAGATTTGAGC	–	–	0,2	0,2	II, III, IVa
	F7	CCATTTCTTCCAAAAAATATATTTACTTTAGTC	–	0,2	–	–	V (CA-MRSA)
	F8	TTTCATAATATGTGCTACGCAACCTA	–	0,2	–	–	(I, II, IV)*
	F9	CGAGTTAATTTTTTATTTTAGAGCGCTTAC	–	0,2	–	–	IVc (LA-MRSA)
	F10	CCGCTCCTTTTATATTATACACAACCTATT	–	0,2	0,2	–	?
	F11	GCCATATTAATGCCTCACGAAAC	–	0,2	0,2	0,2	I, II, IV, X
	F12	CATTCATTAACATCGTACTCTGCATTT	–	0,2	–	–	–
	F13	TCCCTTTATGAAGCGGCTGAA	–	0,2	0,2	0,2	I, II, IV(a, c, q), IX (HACO-MRSA)
	F14	AAGCTATAGTTCAGCATTATCGTAAGTTAACT	–	0,2	0,2	0,2	IV(a)
	F15	TGCCAATCACAGTTCAATCAATTATT	–	0,2	–	–	IVa (USA600)
	F16	TCCTTTCTAATTATATTATGCGCAACCT	–	0,2	–	–	I
	F17	ACTCTGATAAGCCATTCATTCATCCA	–	0,2	0,2	–	(N1 − drop out)
	F18	ACAATCCTAACATAAGATTGTGGCTTT	–	0,2	0,2	–	(drop out)
	F20	GCATATTCACTTTGATAAGCCATTCAT	–	0,2	0,2	–	(IV K)
	F21	CGGTTCTGATATCTTTTCAACCATT	–	0,2	–	–	(S.cohnii)
	F23	CCCCTCCCATTAACTCCGTATAT	–	0,2	–	–	IVc (drop out)
	F24	CCCAAACTCTTAACTTTCTTCAATACATT	–	0,2	0,2	–	(S. epidermidis- drop out)
	F25	TTCTAAGGTAGCTTCCCTTTCAATAATTT	–	0,2	0,2	0,2	V (USA 300 CA-MRSA)
	R1	CGTCATTGGCGGATCAAAC	–	0,2	0,2	0,2	
	R2	CGTCATTGGTGGATCAAACG	–	0,2	0,2	0,2	
	probe	FAM-CACAARGAYGTCTTACAACG-MGBNFQ	–	0,1	0,1	0,1	

[a] MRSA1, R1 and R2 = *orf*X-primers.
[b] MRSA2-6, F1-F25 = SCC*mec*-primers.

probe (see Table 1). All samples were tested by culture in parallel. In 2006, at the time of introduction, MRSA_Sc-I was validated with a sensitivity of 94%, specificity of 98%, a positive predicted value (PPV) of 81% and a negative predicted value (NPV) of 99%. Within the last years a growing number of MRSA screening samples showed discrepant results: MRSA_Sc-I was negative while MRSA culture was positive. Until 2015 specificity (98.7%) and NPV (99.6%) remained at the same level, whereas sensitivity and PPV decreased to 88% and 71%, respectively. This finding supported previous reports of increasingly false-negative PCR results using commercial available detection kits due to the emergence of new MRSA variants (Bartels et al., 2009; Laurent et al., 2010).

The goal of this study was to increase the sensitivity of the MRSA Screening PCR MRSA_Sc-I for the local occurring variants. For this purpose, 17 additional SCC*mec*-forward primers, which had been published by the group of van der Zee (van der Zee et al., 2011), were evaluated for detection of novel MRSA variants that appear in the local area of Düsseldorf.

2. Material and methods

2.1. MRSA screening in our institution

MRSA Screening was performed from nasopharyngeal swabs using eSwabs with Amies medium (COPAN Italia S.p.A., Brescia, Italy) (AMIES, 1967). Samples were analyzed by two different methods in parallel: by MRSA Screening PCR, which was performed directly from the Amies medium, and by cultivation, in which the swabs were streaked out on CHROMagar MRSA plates (MAST DIAGNOSTICA GmbH, Reinfeld, Germany) and incubated for 24 h. For every first MRSA isolate of a patient Multiplex PCR for *nuc* and *mec*A detection was performed to confirm a true positive MRSA (McDonald et al., 2005) – independently from the initial result of the MRSA Screening PCR, which was performed directly from swab. Poly-microbial swab samples were never submitted to *nuc/mec*A Multiplex PCR. In case of a negative MRSA Screening PCR from swab, but suspicious colonies on MRSA plate, MRSA isolates were processed by MRSA Screening PCR and Multiplex PCR to confirm the presence of the *S. aureus*-specific *nuc*-gene and the *mec*A gene (McDonald et al., 2005). MRSA isolates, which were false-negative in MRSA Screening PCR but positive in Multiplex PCR for *nuc* and *mec*A, represent novel SCC*mec* variants. Accordingly, the initial swab specimen, from which this isolate derived, contained a novel MRSA variant that was undetectable by the Screening PCR. *S. aureus* isolates, which were *nuc* positive and *mec*A negative in Multiplex PCR, but false-positive in MRSA Screening PCR, represent MSSA strains with SCC*mec* remnants, so called “drop outs”.

2.2. Sample preparations

The MRSA Screening PCR should enable a fast screening of more than 100 patients within a few hours. Thus, a time-consuming DNA-preparation protocol was not feasible and not necessary. Before MRSA-Sc-I PCR was introduced as accredited method, it was validated and proven that heat lysis of swab specimens was sufficient as DNA-preparation method to achieve comparable MRSA detection rates as a (time-consuming) MRSA culturing. To prepare DNA-lysate from swab specimen, 200 µl Amies medium was incubated at 95 °C for 10 min. DNA from a culture derived MRSA isolate was prepared by resuspending a single colony in 200 µl lysis-buffer (1 mM EDTA, 1% (v/v) Triton X-100 and 0.5% (v/v) Tween 20, 10 mM Tris-HCl, pH 8.0), and subsequent incubation at 95 °C for 10 min. After short spin at 10,000 × *g* for 2 min, 100 µl DNA-containing supernatant was trans-