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Detection of *mecA*- and *mecC*-Positive Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates by the New Xpert MRSA Gen 3 PCR Assay

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An advanced methicillin-resistant *Staphylococcus aureus* (MRSA) detection PCR approach targeting SCC*mec-orfX* along with *mecA* and *mecC* was evaluated for *S. aureus* and coagulase-negative staphylococci. The possession of *mecA* and/or *mecC* was correctly confirmed in all cases. All methicillin-susceptible *S. aureus* strains ($n = 98$; including staphylococcal cassette chromosome *mec* element [SCC*mec*] remnants) and 98.1% of the MRSA strains ($n = 160$, including 10 *mecC*-positive MRSA) were accurately categorized.

Rapid methicillin-resistant *Staphylococcus aureus* (MRSA) tests are based upon either the multiple-locus approach, which targets both the resistance determinant *mecA* and an *S. aureus* species-specific target, or the single-locus approach that targets the junction between the staphylococcal cassette chromosome *mec* element (SCC*mec*) and *orfX*. The high diversity of SCC*mec* and its presence in clinically relevant coagulase-negative staphylococcal (CoNS) species may lead to false-positive and/or false-negative results in both approaches, depending on the target structures (1, 2). Hence, combining the two target strategies might help overcome some of the detection and interpretation disadvantages of currently available assays (3). In addition, detection of the recently reported *mecA* homologue, designated *mecC*, should be included (4–8).

This report describes the evaluation of an advanced rapid MRSA assay that includes primers and probes for the detection of *mecA* and *mecC* along with the detection of the SCC*mec-orfX* junction. In addition to the SCC*mec* types I to IV (including subtype IVa), and V, which were already covered by the previous version, the Xpert MRSA Gen 3 assay also detects SCC*mec* types VI to XI (package insert; Xpert MRSA Gen 3, Cepheid, 2014).

Using the GeneXpert automatic system (Cepheid, Sunnyvale, CA), the Xpert MRSA Gen 3 PCR assay (Cepheid) was tested on a total of 308 isolates comprising clinical, type, and reference strains, including 17 staphylococcal species and subspecies (*S. aureus*, $n = 258$; CoNS, $n = 50$) (Table 1). At the time of the study, the Xpert MRSA Gen3 assay was designated research use only (RUO). The current regulatory status of this assay is *in vitro* diagnostic use only in the CE market. All isolates were recovered from clinical specimens during the course of several German and Belgian multicenter studies (9–16). Of these, the *mecA*-positive MRSA strains ($n = 150$) comprised the 50 most prevalent *S. aureus* protein A gene (*spa*) types found in Germany (12) (Table 1). The *mecC*-positive MRSA strains ($n = 10$) were collected in Germany and the Netherlands and exhibited six different *spa* types (6, 12, 17) (Table 1). Additionally, 98 methicillin-susceptible *S. aureus* (MSSA) isolates covering 70 *spa* types were tested, including 10 isolates known to give false-positive results in the previous version of the test (Xpert MRSA assay), and four previously determined SCC*mec* "dropout" strains, i.e., former MRSA strains that had lost major parts of the SCC*mec* element, including the *mec* genes, but still carry short remains, which might serve as a primer target in single-locus PCR approaches (9, 16) (see Table S1 in the supplemental material). Finally, 25 MR-CoNS and 25 MS-CoNS strains comprising 16 species and subspecies were tested (Table 1). Species identification, detection of *mecA* and *mecC*, and SCC*mec* typing were done as described previously (6, 18–21).

To mimic the *in vivo* situation, 1.5×10^4 bacterial cells were used from a fresh overnight culture in 100 μl and transferred to the test cartridge. This was followed by application of the assay protocol as indicated in the Xpert MRSA Gen 3 package insert. The interpretation of the assay results and categorization as MRSA were done as recommended by the manufacturer (with "MRSA detected" meaning that both the SCC*mec-orfX* and *mecA-mecC* targets tested positive, and "MRSA not detected" meaning that one or both of the SCC*mec-orfX* and *mecA-mecC* targets tested negative). To solve discrepant results, whole-genome sequencing (WGS) was performed as recently described (22). The resulting raw reads were mapped to the SCC*mec-orfX* regions of the respective SCC*mec* type of the reference genomes (see Table S2 in the supplemental material) after quality trimming using the BWA algorithm, with default parameters, implemented in the SeqSphere$^+$ software version 2.3 (Ridom, Münster, Germany).

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TABLE 1 Number of staphylococcal strains included and results of evaluation the Xpert MRSA Gen 3 PCR assay

Isolate (n)[a]	Results of Xpert MRSA Gen 3 PCR assay				
	No. (% agreement) of isolates tested				
	mecA and/or *mecC*		SCC*mec-orfX*		Interpretation as MRSA (no. [%])[b]
	Positive	Negative	Positive	Negative	
MRSA					
mecA-positive MRSA (150)[c]	150 (100.0)	0	147 (98.0)	3 (2.0)[d]	147 (98.0)
mecC-positive MRSA (10)[e]	10 (100.0)	0	10 (100.0)	0	10 (100.0)
Total (160)	160 (100.0)	0	157 (98.1)	3 (1.9)	157 (98.1)
MSSA (98)[f]	0	98 (100.0)	27 (28.1)	69 (71.9)	0
MR-CoNS (25)[g]	25 (100.0)	0	0	25 (100.0)	0
MS-CoNS (25)[g]	0	25 (100.0)	0	25 (100.0)	0

[a] MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; MR-CoNS, methicillin-resistant coagulase-negative staphylococci; MS-CoNS, methicillin-susceptible coagulase-negative staphylococci.

[b] Interpretation of the assay results and categorization as MRSA as given by the manufacturer: MRSA detected, both SCC*mec-orfX* and *mecA* and/or *mecC* targets tested positive; MRSA not detected, one or both of the SCC*mec-orfX* and *mecA* and/or *mecC* targets tested negative.

[c] Including more frequently encountered (t001, t002, t003, t004, t011, t008, t014, t020, t022, t024, t032, t034, t045, t264, t463, t1227, t2373, t4217, t4881, and t8374, each $n = 6$) and rarely occurring (t012, t015, t030, t037, t038, t041, t044, t063; t114, t127, t151, t223, t318, t379, t437, t481, t504, t535, t578, t634, t651, t785, t849, t1107, t1282, t2369, t4417, t6736, t7391, and t8380, each $n = 1$) MRSA *spa* types in Germany. SCC*mec* types I ($n = 7$), II ($n = 57$), III ($n = 2$), IV ($n = 70$) and V ($n = 13$) were detected; one isolate was nontypeable.

[d] The raw reads of the three isolates (RUO83 [t004], RUO140 [t003], and RUO159 [t004]) were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena/) under the study accession no. PRJEB10686.

[e] Comprising *spa* types t843 ($n = 5$), t978, t1773, t5930, t7189, and t7603.

[f] Includes 4 known SCC*mec* remnant strains and 10 isolates giving false-positive results in the previous version of the Xpert MRSA Gen PCR assay.

[g] Comprising 30 clinical *Staphylococcus* strains (*S. capitis* subsp. *capitis*, $n = 1$; *S. epidermidis*, $n = 10$; *S. haemolyticus*, $n = 10$; *S. hominis* subsp. *hominis*, $n = 8$; and *S. warneri*, $n = 1$) and 20 type and reference strains (*S. auricularis* DSM 20609; *S. cohnii* subsp. *cohnii* DSM 20260; *S. cohnii* subsp. *urealyticus* DSM 6718; *S. haemolyticus* DSM 20263 and DSM 20264; *S. hominis* subsp. *hominis* DSM 20320, DSM 20328, and DSM 20329; *S. hominis* subsp. *novobiosepticus* ATCC 700236; *S. hyicus* DSM 20459; *S. lugdunensis* DSM 4804, DSM 4805, and DSM 6670; *S. saprophyticus* subsp. *saprophyticus* DSM 20229 and DSM 20289; *S. schleiferi* subsp. *schleiferi* DSM 6628; *S. sciuri* subsp. *sciuri* DSM 20345; *S. simulans* DSM 20322; *S. warneri* DSM 20316; and *S. xylosus* DSM 6179).

Overall, 157/160 (98.1% positive agreement) of the MRSA strains were correctly categorized by the novel assay. While all 10 *mecC*-positive strains were detected and classified as MRSA, 3 (2.0%) of the 150 *mecA*-positive MRSA strains were falsely categorized as MSSA due to missing amplification of the SCC*mec-orfX* junction (Table 1). Another assay targeting the SCC*mec-orfX* junction (BD Max MRSA XT kit; BD Diagnostics, Quebec, Canada) likewise failed (not shown). The unequivocal possession of the *mecA* gene of these strains was reviewed by applying a further molecular assay (GenoType MRSA; Hain Lifescience, Nehren, Germany; data not shown). WGS did not show sequence variations within *orfX* compared to published sequences of respective *S. aureus* reference strains. Two of these isolates belonged to *spa* type t004 (sequence type 45 [ST45]) and carried SCC*mec* type IV. The *orfX*-SCC*mec* junction of both isolates showed a 52-kb deletion compared to *S. aureus* CA-347 beginning 326 bp downstream of *orfX*. For the third isolate (*spa* type t003, ST225, SCC*mec* type III), no considerable sequence variants were found within 3.5 kb downstream of *orfX* compared to *S. aureus* strains N315 and MW2.

All 98 MSSA strains tested showed negative results for *mecA-mecC* amplification (100% negative agreement) and hence were correctly categorized as MSSA (see Table S1 in the supplemental material), similar to in a recent prospective study (23). Twenty-seven MSSA strains showed positive results in SCC*mec-orfX* junction testing, including those previously characterized as SCC*mec* remnant strains, and eight of the 10 isolates tested false positive in the previous version of the Xpert MRSA PCR assay (see Table S1). The four SCC*mec* remnant strains belonged to *spa* types t011, t038, and t068 ($n = 2$). Other SCC*mec-orfX* junction-positive MSSA strains were characterized by *spa* types t002, t008, t216, t364, t369, t5160, and t6752 or belonged to the *spa* clonal complex 127 (*spa*-CC127) (t127, t177, and t948) (see Table S1). The assay detected the *mecA* and *mecC* genes correctly in all CoNS.

The detection of *mecC*-positive MRSA is a major advantage of the Xpert MRSA Gen 3 PCR assay. Diagnostics were challenged by the recent discovery of the $mecA_{LGA251}$ (*mecC*) gene as part of a novel SCC*mec* XI element in *S. aureus* (4, 5) and CoNS (24–26). With the spread of *mecC*-harboring MRSA (4–7, 12, 27–32), the absence of the *mecA* gene alone can no longer be considered a reliable genetic marker to exclude MRSA. The failure in conventional *mecA* detection assays to detect *mecC* results in inconsistent results in comparison to those with phenotypic susceptibility tests (4–6). Besides various in-house PCR procedures (4–6, 27, 33), another commercially available multiplex PCR, based upon a multiple-locus detection strategy, was recently shown to be able to detect *mecC*-positive MRSA (10). Moreover, both the genetic diversity of the strain background and the occurrence of *mecC*-harboring staphylococci in livestock, wildlife, and environmental sources are worrisome (7, 34–39).

The combined detection of *mecA* and *mecC* with the SCC*mec-orfX* junction represents a second major advantage. It overcomes the problem due to *mecA*- and *mecC*-negative remnants of the SCC*mec* element, which may cause false-positive results (16, 40–44). Outbreaks with SCC*mec* remnant MSSA isolates may result in medical and economic burden due to unjustified MRSA precaution measures (45). The inclusion of the *mecA* and *mecC* genes as targets overcomes this source of misinterpretation. All four SCC*mec* remnant strains included were categorized as MSSA.

In the case of the cooccurrence of an MR-CoNS and an

SCC*mec* remnant MSSA in clinical specimens, false-positive results may still arise. Here, the inclusion of another *S. aureus*-specific target gene sequence might clarify this problem (46, 47). The detection of *mecA*-*mecC* amplification along with a negative result for the SCC*mec*-*orfX* junction will be categorized as MRSA not detected, according to the manufacturer's instructions. In this case, the presence of MR-CoNS could be assumed. However, in rare cases, this diagnostic pattern might also indicate a false-negative result if unknown or uncovered nucleic acid variations in the *orfX* region-neighboring part of the SCC*mec* elements hampered the correct identification (48–52). Here, three *mecA*-positive strains were not detected. Two of these strains harbored a deletion close downstream of *orfX* that might explain the failure by a possible loss of the respective primer-binding site; the reason for misidentification of the other strain, determined by another junction-targeting PCR approach, remains unknown. Those strains could remain undetected for a long time, thus necessitating constant monitoring of the local MRSA epidemiology (52–54).

In conclusion, the inclusion of *mecA* and *mecC* as targets closed a gap in the molecular detection of MRSA and minimized the risk of false-positive interpretation as MRSA due to SCC*mec* remnant isolates. The evaluated MRSA assay challenged by a large collection of German and Belgium clonal MRSA lineages was able to detect the *mecA* and *mecC* genes, respectively, of all strains included and correctly categorized the vast majority of MRSA and all non-MRSA strains.

Nucleotide sequence accession number. The raw reads of the three isolates (RUO83 [t004], RUO140 [t003], and RUO159 [t004]) were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena/) under study accession no. PRJEB10686.

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