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 assay targeting SCCmec-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 [SCCmec] 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 (SCCmec) and orfX. The high diversity of SCCmec 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 SCCmec-orfX junction. In contrast to the SCCmec types I to IV (including subtype IVa), and Vc, which were already covered by the previous version, the Xpert MRSA Gen 3 assay also detects SCCmec 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 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 SCCmec “dropout” strains, i.e., former MRSA strains that had lost major parts of the SCCmec element, including the mec genes, but still carry short remnants, 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 SCCmec typing were done as described previously (6, 18–21). To mimic the in vivo situation, 1.5 × 10⁴ 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 SCCmec-orfX and mecA-mecC targets tested positive, and “MRSA not detected” meaning that one or both of the SCCmec-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 SCCmec-orfX regions of the respective SCCmec 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).
**TABLE 1 Number of staphylococcal strains included and results of evaluation the Xpert MRSA Gen 3 PCR assay**

<table>
<thead>
<tr>
<th>Isolate (n)*</th>
<th>Results of Xpert MRSA Gen 3 PCR assay</th>
<th>Interpretation as MRSA (no. [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>meca and/or mecC</td>
<td>SCC mec-orfX</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>MRSA</td>
<td>150 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td>meca-positive MRSA (150)†</td>
<td>150 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td>mecC-positive MRSA (10)†</td>
<td>10 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td>Total (160)</td>
<td>160 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td>MSSA (98)‡</td>
<td>0</td>
<td>98 (100.0)</td>
</tr>
<tr>
<td>MR-CoNS (25)‡</td>
<td>25 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td>MS-CoNS (25)‡</td>
<td>0</td>
<td>25 (100.0)</td>
</tr>
</tbody>
</table>

*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.

† 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.

‡ Including more frequently encountered (t012, t015, t030, t037, t038, t041, t044, t063; t114, t127, t151, t223, t318, t379, t437, t481, t504, t535, t678, t634, t651, t785, t849, t1107, t1128, t1236, t1417, 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.

‡ 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.

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.

Comprising spa types t843 (n = 5), t978, t1773, t5930, t1879, and t7603.

Includes 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 DSM 20260; S. cohnii subsp. auricularis DSM 6718; S. haemolyticus DSM 20263 and DSM 20264; S. hominis subsp. hominis DSM 20328; DSM 20329; S. hominis subsp. novobiosepticus ATCC 700236; S. hyicus DSM 20459; S. lugdunensis DSM 4804; DSM 4805; and DSM 6676; S. saprophyticus subsp. saprophyticus DSM 20229 and DSM 20289; S. schleiferi subsp. schleiferi DSM 6628; S. sciuri subsp. sciuri DSM 20343; 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 meca-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 5.3 kb downstream of orfX compared to *S. aureus* strain 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 t1948) (see Table S1). The assay detected the meca and mecC genes correctly in all CoNS.

The detection of meca-positive MRSA is a major advantage of the Xpert MRSA Gen 3 PCR assay. Diagnostics were challenged by the recent discovery of the meca-SCC mec XI element in *S. aureus* (4, 5) and CoNS (24–26). With the spread of mecC-carrying 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 meca 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-carrying 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
SCCmec 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 SCCmec-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 SCCmec 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 mis-identification 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-negative interpretation as MRSA due to SCCmec 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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