Research Article

Title: Evaluation of a modular multiplex-PCR methicillin-resistant Staphylococcus aureus (MRSA) detection assay adapted for mecC detection

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Running Title: mecC-MRSA detection

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A mecC (mecA_{GA251})-adapted multiplex PCR-based MRSA detection assay was evaluated using an international, spa-typed Staphylococcus aureus collection comprising 51 mecC-positive MRSA, 240 mecA-positive MRSA and 50 mecA/mecC-negative MSSA isolates. The assay showed 100% sensitivity and specificity for S. aureus species identification as well as for mecA and mecC detection.
Recently, a mecA homologue with 70% identity at DNA level designated mecA<sub>LG251</sub> has been detected as part of a novel SCCmec XI element (1,2). This homologue has now been re-classified as mecC (3) and mecC-harboring methicillin-resistant Staphylococcus aureus (MRSA) strains have been reported in British, Danish, Dutch, French, German, Irish, Norwegian and Swedish studies and case reports (1,2,4-8). Due to nucleic acid sequence divergences in comparison to the original mecA gene, commercial or in-house PCR routine diagnostic protocols fail to detect this homologue, resulting in inconsistent results between molecular-based and phenotypic susceptibility testing (1,2,4). Subsequently, oligonucleotide primer sequences and PCR protocols addressing the mecC detection have been published (1,2,4,5,9). This study tested a commercially available mecC-adapted PCR-based MRSA detection assay against an international collection of known mecC- and mecA-positive MRSA isolates.

A genotypically diverse collection of S. aureus isolates representing 280 different spa-types and one spa non-typeable isolate were used to evaluate a modified version of a modularly designed MRSA detection assay (hyplex<sup>®</sup> MRSA plus; Amplex Diagnostics GmbH, Germany) according to the manufacturer’s recommendations. In this study, the hybridization modules designed for the detection of mecA- and mecC-based MRSA and PVL genes were tested. Briefly, subsequent to DNA extraction by using hyplex<sup>®</sup> lysis buffer, the multiplex PCRs with labeled oligonucleotides targeting S. aureus-species-specific as well as mecA-, mecC- and lukS-PV-lukF-PV sequences were performed. This was followed by the modularly applicable hybridization reactions with specific oligonucleotide probes immobilized on micro titer plate surfaces. Subsequently, the assay’s ELISA approach using a peroxidase conjugate, which binds specifically on the labeled region of the hybridization product, was used as detection procedure.

Overall, 341 S. aureus isolates were included. Of these, 51 mecC-positive isolates (20 spa-types, Suppl. Table 1) from Denmark (n = 22), Germany (n = 18), the Netherlands (n = 1) and the U.K. (n = 10) comprising isolates of human (n = 44) and animal (bovine, bulk milk, n = 5 and ovine, n = 2) origin were used to evaluate the ability of the assay to identify mecC-harboring S. aureus isolates as MRSA.
To establish the specificity and sensitivity of the hyplex® MRSA plus assay for the detection of classical MRSA including PVL-positive isolates, 240 mecA-positive strains (of these 16 lukS-PV-lukF-PV-positive MRSA) comprising 232 different spa-types and 50 methicillin-susceptible S. aureus (MSSA) isolates were included. These control strains comprised 40 spa-types (plus 1 non-typeable isolate) (Suppl. Table 1). MSSA and mecA-based MRSA isolates were representative subsets from German multicenter studies (10,11). For the purposes of sensitivity and specificity calculations, the ‘gold standard’ test for MRSA/MSSA status was the detection of, or failure to detect, a mecA or mecC gene using published genotypic tests (see below). All isolates in the panel were subjected to ‘gold standard’ testing in addition to a number of phenotypic tests for MRSA status. Prior to investigation of the adapted assay, species determination and categorization of control group isolates as either MRSA or MSSA were determined by Pastorex Staph Plus® test (Bio-Rad Laboratories, Hercules, CA), PBP2' latex agglutination test (Oxoid, Basingstoke, U.K.) as well as VITEK® 2 GP and AST 580 cards (bioMérieux, Marcy l'Etoile, France) and additionally examined by DNA-STRIP technology-based GenoType® MRSA assay (HAIN Lifescience, Germany) (12,13). Prior evaluation of mecC possession was done by in house-PCR as recently described (4). The presence of PVL genes was determined by PCR procedure as reported (14) and by GenoType® MRSA assay.

The testing of 341 nuc-positive S. aureus isolates using the hyplex® MRSA plus assay resulted in 100% accuracy in terms of S. aureus species detection irrespective of categorization as MRSA or MSSA. All 289 MRSA isolates harboring either mecA or mecC were correctly identified (Table 1). All lukS-PV/lukF-PV-positive isolates (n = 16) were correctly detected by hyplex® MRSA plus.

Variation in the orfX region-neighboring part of the SCCmec elements may expose the weakness of single locus PCR assays for detection of MRSA. Many SCCmec types and subtypes have been described (15-17) that go beyond the SCCmec right extremity sequence diversity targeted by...
Huletsky et al. in their first description of this PCR strategy (18). Those known or unknown variants, not considered by the primer design lead to false-negative single locus PCR assay results, as has been noted even for common SCCmec types (19). Prior to the discovery of mecC, the presence of the mecA gene has been considered the definitive criterion for the MRSA identification. Thus, duplex PCR strategies targeting taxonomic indicator genes such as the nuc or eap genes (20) in parallel with the PBP2a-encoding mecA gene (21,22) have been used as the ‘gold standard’ for verification of presumed MRSA isolates (23). However, the discovery of the mecC homologue reveals the capacity of MRSA to challenge not only clinicians, in terms of prophylaxis and therapy, but also the clinical microbiological laboratory when considering detection and identification. In this study, we have evaluated a test that can be used to establish the genotypic MRSA status that is of particular value when the phenotypic status is ambiguous.

The mecA and mecC genes belong to the PBP2a family of related genes found not only in staphylococci, but also as a possible primordial form in *Macrococcus caseolyticus* designated as mecAm (24) and recently re-classified as mecB (3). Of particular importance, the mecB gene was found to be located on a plasmid (pMCCL2). This first report of a plasmid conveying methicillin resistance, and the subsequent detection of mecC-encoded methicillin resistance, may raise speculation about the existence of further mecA homologues not yet discovered.

Detailed data concerning the true prevalence of mecC-possessing strains are still scarce. In Denmark, national surveillance of MRSA detected 36 mecC MRSA cases out of a total of 1,293 (2.8%) new MRSA cases in 2011 (25). In Germany, a prospective multicenter study comprising more than 30 centers and covering two study periods, 2004/05 and 2010/11, reported carriage of mecC in only 1/1,604 and 1/1,603 (each 0.06%) of MRSA isolates (10). Searches for human MRSA isolates in U.K. yielded 51 isolates that tested mecC-positive from likely candidates of about 120,000 clinical isolates (0.4%) (1). Although relatively uncommon, reducing the impact of the misdiagnosis of MRSA on treatment strategies, health care precautions and on patient outcomes requires accurate identification of phenotypically MRSA irrespective of the underlying genetic nature, *i.e.* mecA homologue-possessing.
MRSA isolates. Similar diagnostic requirements exist in the case of veterinary medicine and food diagnostics.

Here, we evaluated a commercially available MRSA detection assay showing that this assay was able to detect successfully all isolates of a representative collection of mecC-positive isolates comprising almost all currently described mecC-associated clonal lineages. The collection tested included a large set of common MRSA clonal lineages comprising more than 200 spa types to which this assay exhibited 100% sensitivity and 100% specificity in its ability to identify mecA- and mecCpossessing MRSA isolates and MSSA isolates. The mecC-adapted MRSA assay evaluated here provides a commercially available solution to the new diagnostic challenge elicited by the discovery of the beta-lactam resistance-caused by the mecA-homologue mecC.
Acknowledgments

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Table 1. Results of evaluation of hyplex® MRSA plus assay

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of isolates tested (positive/negative)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mecA</td>
<td>mecC</td>
<td>S. aureus-specific target</td>
</tr>
<tr>
<td>MSSA (n = 50)</td>
<td>0/50</td>
<td>0/50</td>
<td>50/0</td>
</tr>
<tr>
<td>meca-MRSA (n = 240)†</td>
<td>240/0</td>
<td>0/50</td>
<td>240/0</td>
</tr>
<tr>
<td>mecc-MRSA (n = 51)</td>
<td>0/51</td>
<td>51/0</td>
<td>51/0</td>
</tr>
</tbody>
</table>

† Sensitivity and specificity are given for determination of MRSA and MSSA. The sensitivity and specificity for the detection of PVL-encoding genes was also 100%.

† Including 16 isolates harboring PVL encoding genes.


