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Evaluation of a Modular Multiplex-PCR Methicillin-Resistant *Staphylococcus aureus* Detection Assay Adapted for *mecC* Detection

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A *mecC* (*mecA*$_{LGA251}$)-adapted multiplex PCR-based methicillin-resistant *Staphylococcus aureus* (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*- and *mecC*-negative methicillin-susceptible *S. aureus* (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 the DNA level designated *mecA*$_{LGA251}$ has been detected as part of a novel SCC*mec* XI element (1, 2). This homologue has now been reclassified 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 molecularly based and phenotypic susceptibility testing (1, 2, 4). Subsequently, oligonucleotide primer sequences and PCR protocols addressing *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 m*ecA*-positive MRSA isolates.

A genotypically diverse collection of *S. aureus* isolates representing 280 different *spa* types and one *spa*-nontypeable isolate were used to evaluate a modified version of a modularly designed MRSA detection assay (hyplex MRSA Plus; Amplex Diagnostics GmbH, Munich, Germany) according to the manufacturer's recommendations. In this study, the hybridization modules designed for the detection of *mecA*- and *mecC*-based MRSA and Panton-Valentine leukocidin (PVL) genes were tested. Briefly, subsequent to DNA extraction by using hyplex 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 microtiter plate surfaces. Subsequently, the assay's enzyme-linked immunosorbent assay (ELISA) approach, using a peroxidase conjugate that 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; see Table S1 in the supplemental material) from Denmark ($n = 22$), Germany ($n = 18$), The Netherlands ($n = 1$), and the United Kingdom ($n = 10$) comprising isolates of human ($n = 44$) and animal (bovine, bulk milk, $n = 5$; ovine, $n = 2$) origins 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 (16 *lukS-PV–lukF-PV*-positive MRSA isolates) comprising 232 different *spa* types and 50 methicillin-susceptible *S. aureus* (MSSA) isolates were included. These control strains comprised 40 *spa* types (plus 1 nontypeable isolate) (see Table S1). 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 or MSSA status was the detection of or failure to detect a *mecA* or *mecC* gene by 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 performed with the Pastorex Staph Plus test (Bio-Rad Laboratories, Hercules, CA), PBP2′ latex agglutination test (Oxoid, Basingstoke, United Kingdom), ands Vitek 2 GP and AST 580 cards (bioMérieux, Marcy l'Etoile, France) and additionally examined by the 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 as reported previously (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*- or *lukF-PV*-positive

Received 9 January 2013 Returned for modification 2 February 2013
Accepted 16 March 2013
Published ahead of print 20 March 2013
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00075-13.

doi:10.1128/JCM.00075-13

TABLE 1 Results of evaluation of the hyplex MRSA Plus assay

Isolate type (*n*)	No. of isolates testing positive/negative				Sensitivity (%)[a]	Specificity (%)[a]
	mecA	*mecC*	*S. aureus*-specific target	*lukS-PV–lukF-PV*		
MSSA (50)	0/50	0/50	50/0	0/50	100	100
mecA-MRSA (240)[b]	240/0	0/50	240/0	16/224	100	100
mecC-MRSA (51)	0/51	51/0	51/0	0/51	100	100

[a] Sensitivity and specificity are given for determination of MRSA and MSSA. The sensitivity and specificity for the detection of PVL-encoding genes were also 100%.
[b] Including 16 isolates harboring PVL-encoding genes.

isolates ($n = 16$) were correctly detected by the hyplex MRSA Plus assay.

Variation in the *orfX* region-neighboring part of the SCC*mec* elements may expose the weakness of single-locus PCR assays for detection of MRSA. Many SCC*mec* types and subtypes have been described (15–17) that go beyond the SCC*mec* 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 SCC*mec* types (19). Prior to the discovery of *mecC*, the presence of the *mecA* gene had 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 $mecA_m$ (24) and recently reclassified 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 to 2005 and 2010 to 2011, 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 the United Kingdom 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 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 *mecC*-possessing 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 β-lactam resistance caused by the *mecA* homologue *mecC*.

ACKNOWLEDGMENTS

We are grateful to M. Schulte and A. Hassing for excellent technical assistance.

This work was supported in part by a grant from the Bundesministerium für Bildung und Forschung (BMBF), Germany "Interdisciplinary Research Network MedVet-Staph" to K.B., R.K., A.W.F., and G.P. (01KI1014A). The hyplex MRSA Plus assay compounds were provided free of charge by Amplex Diagnostics GmbH.

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