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# Evaluation of Three Molecular Assays for Rapid Identification of Methicillin-Resistant *Staphylococcus aureus*▽

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**One home-developed assay and two commercial assays for the rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) were compared by use of a collection of clinical isolates displaying highly diverse genetic backgrounds. Our results suggest that users of *orfX*-staphylococcal cassette chromosome *mec*-based assays should repeatedly monitor the local epidemiology to minimize the risks of detection bias and the omission of emerging MRSA clones.**

*Staphylococcus aureus* is a major pathogen responsible for both nosocomial and community-acquired infections. The rapid detection of inpatients carrying methicillin-resistant *S. aureus* (MRSA) has the potential of minimizing MRSA transmission and may even be cost-beneficial (4, 14). Recently, our group showed that the "same-day detection" of MRSA contributed to the reduction of nosocomial MRSA infections in a medical intensive care unit when detection was linked with appropriate isolation measures (16).

To date, the "gold standard" method for MRSA identification relies on culture (5) and provides results in approximately 48 to 72 h. The *mecA* gene, which originates from a mobile genetic element (named the staphylococcal cassette chromosome *mec* [SCC*mec*]) invariably inserted into the *orfX* gene of methicillin-resistant staphylococci, is the genetic basis of methicillin resistance. Additionally, the high degree of similarity between the *mecA* sequences of the different staphylococcal species precludes identification of MRSA by using *mecA* as a single identification target (1). The molecular composition of SCC*mec* elements allows genotyping (20) and was at the root of PCR assays targeting the insertion of different SCC*mec* elements into *orfX* (18).

The aim of this study was to evaluate the performance of different strategies for the molecular identification of MRSA strains originating from diverse genetic backgrounds. The MRSA collection contained 93 clinical isolates identified between 1993 and 2005 at our institution (P. Francois, S. Harbarth, A. Huyghe, G. Renzi, M. Bento, A. Gervais, D. Pittet, and J. Schrenzel, submitted for publication). All MRSA strains were characterized by SCC*mec* typing (13) and were genotyped by multilocus variable-number tandem-repeat typing (11) or by multilocus sequence typing (MLST). The collection contained 16 strains harboring the toxic shock syndrome toxin 1 gene, whereas the numbers of Panton-Valentine leukocidin- and exfoliatin A-harboring strains were 22 and 11, respectively. Our collection contained isolates with various MLST profiles: sequence type 1 (ST1), ST5, ST8, ST22, ST30, ST72, ST80, ST85, ST88, ST149, ST152, ST228, and ST395 strains harboring SCC*mec* I ($n = 16$), II ($n = 2$), III ($n = 1$), IV ($n = 42$), and V ($n = 16$) elements, with several isolates showing nontypeable cassettes. In addition, strains representative of the intravenous drug user cluster in Zurich, Switzerland, and *nuc*-deficient isolates were analyzed, as were *mecA*-positive and -negative control strains ATCC 33591 and ATCC 25923, respectively. Methicillin-susceptible *S. aureus* (MSSA) isolates ($n = 89$) that originated from different hospital sectors (long-term health care facilities, 50%; medical wards, 40%; surgical intensive care unit, 10%) were tested upon identification.

The strains were subjected to MRSA identification by the IDI-MRSA test (BD-GeneOhm Science). Starting from the isolated colonies, PCR was performed according to the manufacturer's instructions on a SmartCycler II device (Cepheid, Sunnyvale, CA). A PCR-based hybridization assay (GenoType MRSA Direct; Hain Lifescience, Nehren, Germany) was also evaluated. Briefly, the Hain assay consists of a three-step protocol: (i) the isolation of DNA from cultured material (bacteria freshly grown on culture plates; the necessary reagents are not provided with the assay kit), (ii) a multiplex amplification with biotinylated primers, (iii) and a reverse hybridization. The hybridization includes the following steps: chemical denaturation of the amplification products, hybridization of the single-stranded biotin-labeled amplicon to membrane-bound probes, stringent washing, addition of a streptavidin-alkaline phosphatase conjugate, and a staining reaction. All steps were performed according to the manufacturer's instructions by using the HotStart KOD enzyme (Novagen). Detection used two different settings: (i) positive results were recorded for any band corresponding to an MRSA location onto the strip, whatever its intensity (named "Hain 1" in Table 1), and (ii) a positive signal was recorded only for bands showing intensities higher than that for the control MSSA strain run in parallel (named "Hain 2" in Table 1). Finally, a modified triplex quantitative PCR (qPCR) assay (qMRSA) was applied as published previously (12). The sensitivity, specificity, and positive and

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TABLE 1. Results obtained by standard growth-based and molecular assays with a collection of 93 MRSA and 89 MSSA isolates[a]

| Assay or protocol | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|
| Culture | 1 | 1 | 1 | 1 |
| qMRSA | 1 | 1 | 1 | 1 |
| BD | 0.94 | 0.64 | 0.71 | 0.92 |
| Hain 1 | 0.90 | 0.53 | 0.66 | 0.84 |
| Hain 2 | 0.67 | 0.57 | 0.61 | 0.63 |

[a] Three MRSA strains from our collection were found to be negative by qMRSA after they were thawed. These isolates were tested by standard growth-based methods and identified as MSSA. The excision of the *mecA* gene obviously yields negative results when this gene is targeted. These isolates generated false-positive results when they were tested by the two other molecular assays. The data for these three isolates were therefore not included in the analysis presented here.

negative predictive values were evaluated by comparing the results to those obtained by the reference culture-based method (Table 1).

The study described in this report attempted to determine the diagnostic accuracies of three molecular assays and their abilities to identify MRSA isolates of various genetic backgrounds. The evaluation was performed with collections of clinical isolates containing clonal lineages that are prevalent in community-associated and nosocomial MRSA infections observed in Europe, North America, and Asian countries. It is important to stress here that community-acquired European strains display a broad diversity of genetic backgrounds (15), in contrast to the epidemiologic situation in the United States (21).

Faced with a diversity of *S. aureus* molecular backgrounds and with the tremendous sequence variations observed in the SCC*mec-orfX* insertion (19, 20), one alternative consists of identifying the *mecA* gene and its origin, especially in samples containing mixed flora. Our qPCR assay consists of the multiplex PCR amplification of the *mecA* and the *femA* genes from *S. aureus* and *S. epidermidis* and allows the identification of the origin of the *mecA* signal. Obviously, this triplex PCR was applied to isolated MRSA and MSSA isolates to assess its ability to reliably detect the *S. aureus femA* gene and, simultaneously, to potentially excise the *mecA* gene after the isolates are thawed (23). The results of the assay were in complete concordance with the results obtained by standard culture-based methods (Table 1).

The accuracy of MRSA identification was significantly different among the commercial tests targeting the SCC*mec-orfX* region. The Hain and BD-GenOhm assays showed 90 and 94% sensitivities, respectively, as well as 53 and 64% specificities, respectively. Overall, the specificities of these assays were surprisingly low. Samples found to have false-negative results by the BD-GeneOhm tests revealed nontypeable SCC*mec* elements ($n = 5$) and included two SCC*mec* type IV isolates as well as one isolate from the Zurich intravenous drug user cluster, SCC*mec* type III ($n = 1$), and SCC*mec* type V ($n = 3$).

Compared with the BD-GeneOhm platform, which is a closed system (i.e., there is no ability to visually evaluate PCR amplification curves), the Hain assay is potentially sensitive to users, as the readout consists of enzymatic coloration on solid strips. Thus, two different methods were used to evaluate the test commercialized by Hain. The Hain test failed to detect 9 and 30 MRSA strains by use of the Hain 1 and Hain 2 protocols, respectively. Among the misidentified strains we found SCC*mec* type IV isolates ($n = 5$ and 10 by protocols Hain 1 and Hain 2, respectively), some SCC*mec* type I isolates ($n = 1$ and 9 by protocols Hain 1 and Hain 2, respectively), SCC*mec* type V isolates ($n = 3$ and 5 by protocols Hain 1 and Hain 2, respectively; the version of the Hain test used in this study contained primers specific only for SCC*mec* types I to IV), and other or nontypeable isolates ($n = 0$ and 6). Important numbers of false-positive strains were recorded by this assay ($n = 42$ and 38 by protocols Hain 1 and Hain 2, respectively).

A significant proportion of the discrepant results could be related to the design of the study that was performed with isolated strains instead of swab specimens. However, our determinations clearly indicate that these assays failed to detect isolates harboring nontypeable or recently described SCC*mec* cassettes (variants of IV and V [20]) as well as specific SCC*mec* variants resulting from several recombination events (17). This observation confirms that the emergence of new SCC*mec* elements warrants iterative modifications of the assay design and revalidation as new molecular variants continuously emerge (2).

The high rate of false-positive results could be explained by the homology between the *orfX* moieties in *S. aureus* and other coagulase-negative species, such as *S. haemolyticus* (GenBank accession number AY751823.1) or *S. epidermidis* (GenBank accession number AY751823.1), or with the capsular polysaccharide cluster (GenBank accession number CP000029.1), as observed previously (6). Donnio described that the partial excision of SCC*mec* occurs not infrequently and might explain some of the false-positive results (9). Freezing-thawing has been suggested as a potential cause of *mecA* excision (23). This type of event, previously reported in vivo in epidemic clones with or without antibiotic pressure (7), probably contributed to the large number of samples in our collection for which false-positive results were recorded. In our study, MSSA strains were obtained directly from the routine laboratory and were tested immediately after identification. Thus, our results are in accordance with those of a recent report by Desjardins et al. showing the similar performance (positive predictive value, 64%) of the BD assay with nasal and rectal swab specimens (8) or swab specimens from patients admitted to an intensive care unit (22).

The IDI-MRSA assay has been evaluated by others, and its sensitivity and specificity with nasal swab samples appeared to be appreciable (10); but its performance was not superior to the performance of the standard PCR approach (3). In our study, despite efficiencies of >90% for the identification of MRSA strains for the two commercial assays, the implementation of infection control measures would have been considerably affected in terms of costs by the rate of false-positive results, yielding unnecessary isolation (16). Our study shows that, despite the strain diversity, the *mecA* gene is a robust target for the detection of MRSA isolates. On the other hand, tests that target the *orfX* region failed to detect insertions into several backgrounds, which is an important observation, considering the diverse and rapidly evolving epidemiology of MRSA strains.

In summary, our study clearly indicates that the local epidemiology could have a major impact on the diagnostic accuracies of rapid molecular assays targeting evolving or mobile regions of the bacterial chromosome. The cost-effectiveness of screening for MRSA requires rapid and robust identification strategies, which are prerequisites to avoiding the biases related to erroneous identification.

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