Evaluation of the BD GeneOhm StaphSR Assay for Detection of Methicillin-Resistant and Methicillin-Susceptible
Staphylococcus aureus Isolates from Spiked Positive Blood Culture Bottles

Sabine Gröbner, Mireille Dion, Mélanie Plante and Volkhard A. J. Kempf

Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany; BD Diagnostics GeneOhm, Québec, Canada; and Institute of Medical Microbiology and Infection Control, University of Frankfurt am Main, Frankfurt am Main, Germany

Received 13 November 2008/Returned for modification 30 January 2009/Accepted 3 April 2009

To improve the clinical outcome of Staphylococcus aureus septicemia, the early selection of appropriate antibiotic treatment is crucial. Molecular diagnostics represents an attractive approach for the rapid identification of S. aureus and the determination of its methicillin (meticillin) resistance. In direct comparison to other molecular assays (sa442 and mecA real-time PCRs) and standard laboratory procedures, we evaluated the BD GeneOhm StaphSR assay for its use in the detection of S. aureus and methicillin-resistant S. aureus (MRSA) from spiked blood culture bottles (n = 134). In the case of detecting S. aureus (n = 90; for methicillin-susceptible S. aureus, n = 45; for MRSA, n = 45), the BD GeneOhm StaphSR assay had a sensitivity and a specificity of 100% each (95% confidence intervals [CIs], 96.0 to 100% and 82.4 to 100%, respectively). For MRSA (n = 45), the test was 95.6% (95% CI, 84.9 to 99.5%) sensitive and 95.3% (95% CI, 86.9 to 99.0%) specific. Overall, five discrepant results arose with this assay due to the presence of methicillin-susceptible, revertant MRSA strains (3/45) and MRSA strains that were not detected by the BD GeneOhm StaphSR assay (2/45). Compared to other real-time PCR-based molecular approaches and to conventional standard laboratory methods, the BD GeneOhm StaphSR turned out to be an appropriate diagnostic tool for a rapid (~1.5 h), preliminary identification of S. aureus and MRSA from blood cultures.

Staphylococcus aureus septicemia is associated with high mortality rates, prolonged hospitalization, and increased costs (3, 5). The prevalence of S. aureus septicemia is increasing, primarily due to infections caused by methicillin (meticillin)-resistant S. aureus (MRSA) (20). Several studies have shown that mortality rates among patients suffering from MRSA septicemia is significantly higher than those of patients suffering from infections caused by methicillin-susceptible S. aureus (MSSA) (5, 18, 19).

The early selection of an appropriate antibiotic regime for the treatment of MSSA or MRSA is crucial for the patient's outcome (4, 14, 15). However, bacterial identification and preliminary antibiotic susceptibility testing by standard microbiological procedures still requires 24 to 48 h after growth detection by automated incubation systems. In contrast, new real-time PCR-based methods that use samples directly from positive blood culture bottles allows differentiation of MSSA, MRSA, and coagulase-negative staphylococci (CoNS) within 1.5 to 3 h (7, 12, 13, 16). Such tests promote an early appropriate antibiotic selection, thus avoiding the unnecessary use of vancomycin, and they reduce mortality, the length of hospitalization, and costs associated with bloodstream infections caused by these bacteria (3).

We described recently a real-time PCR method for the detection of MSSA, MRSA, and CoNS directly from positive blood cultures; it turned out to have 100% sensitivity and 100% specificity for the detection of MSSA and MRSA (7). In this study, the differentiation between MSSA and MRSA directly from signal-positive blood cultures was achieved by the separate detection of the S. aureus-specific chromosomal fragment sa442 and the mecA gene (encoding methicillin resistance). However, since this test is not a commercialized system and does not run on a common platform like, e.g., the SmartCycler (Cepheid, Sunnyvale, CA), its widespread application is limited. Moreover, in blood cultures containing a mixture of MSSA (sa442+ but mecA negative) and methicillin-resistant CoNS (MR-CoNS; sa442 negative but mecA+), the test is prone to lead to the incorrect identification of MRSA (sa442+ mecA+).

The BD GeneOhm StaphSR assay (BD Diagnostics GeneOhm, Québec, Canada) provides a rapid, simple, commercially available diagnostic test that runs on the SmartCycler for the detection of S. aureus and MRSA from nasal swabs, wounds, and blood cultures. This multiplex real-time PCR amplifies an S. aureus-specific target sequence and a specific target near the staphylococcal cassette chromosome mec (SCCmec) insertion site and the orfX junction in MRSA, thereby differentiating between MSSA and MRSA (9, 17).

Using the herein-described setting, we evaluated the BD GeneOhm StaphSR assay and the PCR that detects sa442 and mecA (designated sa442-mecA–PCR) for the detection of MSSA and MRSA directly from spiked blood cultures.
TABLE 1. Evaluation of the BD GeneOhm StaphSR assay and sa442-mecA real-time PCR for the detection of MSSA, MRSA, and CoNS in blood cultures spiked with defined blood culture isolates

<table>
<thead>
<tr>
<th>Isolate type</th>
<th>No. detected by BD GeneOhm StaphSR</th>
<th>No. detected by mecA-sa442-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (CoNS)</td>
<td>Reactive (S. aureus)</td>
</tr>
<tr>
<td>MSSA (45)</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>MRSA (45)</td>
<td>0</td>
<td>2*</td>
</tr>
<tr>
<td>CoNS (19)</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Total (109)</td>
<td>19</td>
<td>44</td>
</tr>
</tbody>
</table>

a Three MSSA isolates were incorrectly identified as MRSA by the BD GeneOhm StaphSR assay.
b Two MRSA isolates were not identified as MRSA by the BD GeneOhm StaphSR assay.

MATERIALS AND METHODS

Staphylococcus sp. blood culture isolates. In this study, randomly selected and non-duplicated Staphylococcus spp. (n = 109) were included that originally were isolated from signal-positive blood culture bottles that had been submitted for routine microbiological diagnostics. MSSA isolates (n = 45) were isolated between October 2006 to January 2008, MRSA isolates (n = 45) from December 2004 to February 2008, and CoNS isolates (n = 19) from June 2006 to January 2008. All isolates were subjected to our collection of blood culture isolates. Among the CoNS isolates tested were Staphylococcus epidermidis (n = 12), Staphylococcus capitis (n = 2), Staphylococcus haemolyticus (n = 2), Staphylococcus hominis (n = 2), and Staphylococcus warneri (n = 1).

All Staphylococcus sp. isolates were identified by standard laboratory methods (e.g., DNase testing, mannitol and glucose fermentation, and the latex slide agglutination test; Staphyctect Plus; Oxoid, Basingtouke, United Kingdom) and by the Vitek2 or API Staph system (bioMérieux, Nürtingen, Germany). Additionally and as described below, bacterial isolates were analyzed by the sa442-mecA–PCR (7).

Spiking of blood culture bottles with defined Staphylococcus sp. isolates. Staphylococcus sp. isolates were thawed and cultivated for 24 h on Columbia agar supplemented with 5% sheep blood (BD Diagnostics, Heidelberg, Germany) at 37°C under aerobic conditions. Blood was taken from volunteers (S. Gröbner and V. Kempf), and clotting was avoided by the addition of EDTA.

Aerobic blood culture bottles (BACTEC Plus Aerobic/F; BD Diagnostics) were spiked with 5 ml of blood and the respective bacterial isolates. For this purpose, the tip of an 18-gauge needle, attached to a blood-filled 5-ml syringe, was dipped into a corresponding bacterial colony prior to the inoculation of the blood culture bottle. After incubation in a BACTEC-9240 apparatus (BD Diagnostics), an aliquot from each positive blood culture was taken for (i) Gram staining, (ii) PCR analysis using the BD GeneOhm StaphSR assay (BD Diagnostics GeneOhm), (iii) PCR analysis using the sa442-mecA–PCR method (7), and (iv) subcultivation on Columbia agar plates for routine laboratory identification (as described above).

sa442-mecA real-time PCR. sa442-mecA real-time PCR from positive blood culture bottles was performed as recently described (7). Briefly, 8 ml of positive blood culture was transferred into a BD Vacutainer SST II Advance tube (BD Diagnostics). After centrifugation, DNA was extracted from the supernatant, and 2 μl of the extracted DNA was added directly into a LightCycler tube to 18 μl of the amplification mixture. Real-time PCRs were performed using a LightCycler (Roche, Mannheim, Germany).

BD GeneOhm StaphSR assay. When a blood culture was flagged positive by the BACTEC-9240, indicating bacterial growth, a Gram stain was performed to confirm the presence of gram-positive cocci in clusters. BD GeneOhm StaphSR was performed according to the manufacturer’s instructions. Briefly, 2 μl of the culture broth was transferred to the sample buffer tube. After being vortexed for 15 s, 50 μl of the sample suspension was transferred into a lysis tube, vortexed for 5 min, spun quickly to centrifuge down solid contents, and heated at 95°C for 2 min. The lysed samples were kept on ice until further use. The master mix was reconstituted with 225 μl diluent and aliquoted (each 25 μl) into SmartCycler tubes (Cepheid, Sunnyvale, CA). Next, 3 μl of each of the lysed samples was added to the SmartCycler tubes. Positive controls provided by the test kit and negative controls (sample buffer) were included in each run. SmartCycler tubes were centrifuged with a quick spin at 4°C. Finally, the assay was run on the SmartCycler using the manufacturer’s amplification protocol. To work up potentially discrepant results, processed lysis tubes were frozen at −80°C.

The PCR results for the BD GeneOhm StaphSR assay were interpreted using the SmartCycler software (SmartCyclerDX software, version 1.7b), which delivered the following test results: negative (neither S. aureus nor MRSA DNA was detected), reactive (S. aureus DNA was detected), or positive (MRSA DNA was detected).

Additional analysis of isolates with discrepant results in the BD GeneOhm StaphSR assay. For the samples for which the BD GeneOhm StaphSR assay results did not agree with those of the sa442-mecA–PCR, the BD GeneOhm StaphSR assay was repeated with aliquots from the initial blood culture and, additionally, with the respective bacterial isolates grown on Columbia agar by inoculating a respective colony into the BD GeneOhm StaphSR sample buffer. Moreover, bacterial subcultivation on CHROMagar MRSA plates (Becton Dickinson, antibiotic testing (Vitek2) for methicillin resistance using a microbiolization method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (2), and spa typing (8) were performed. In addition, these isolates were tested for the presence of the penicillin binding protein 2 by using an immunosassay (PBP2′ test kit; Oxoid, Basingtouke, United Kingdom).

mec gene PCR analysis. The presence or absence of the mecA gene was determined by the analysis of Staphylococcus sp. DNA by an additional PCR assay specific to the mecA gene (11). Here, 1 μl of bacterial DNA was added to 19 μl of the PCR mixture. PCR amplification products were analyzed on 2% agarose gels. The size of the mecA-PCR product was 174 bp; positive (MRSA) and negative (MSSA) controls were included in each run (data not shown).

SCCmeC typing. The following methods were used for the SCCmeC typing of the discrepant S. aureus isolates. For the identification of the SCCmec type, the lysates first were analyzed with two multiplex PCR assays to determine the mec complex class, A, B, or C (1), and with a multiplex PCR to determine the crl complex based on a method described before (10). In both assays, 1 μl of each DNA sample was added to 49 μl of PCR mixture, and PCRs were performed using a PTC-200 thermal cycler (Bio-Rad, Hercules, CA). PCR amplification products were analyzed on 1% agarose gels (data not shown). Additionally, the presence of SCCmec types I to V was determined by a multiplex PCR assay based on a method described previously (21). Here, 2 μl of each DNA sample was added to 25 μl of PCR mixture. PCR amplification products were analyzed on a 1% agarose gel (data not shown).

RESULTS

Analysis of blood cultures spiked with one Staphylococcus species per bottle. To evaluate the BD GeneOhm StaphSR assay in comparison to the sa442-mecA–real-time PCR assay, 109 signal-positive blood cultures, each spiked with one particular Staphylococcus sp. blood culture isolate, were analyzed in parallel in both assays. At the time of sample preparation, all signal-positive blood cultures showed the growth of gram-positive cocci in clusters when analyzed by Gram staining and, accordingly, all bacterial isolates exhibited the typical phenotypic characteristics when undergoing standard laboratory identification (e.g., DNase, the expression of clumping factor, etc.; data not shown). All S. aureus isolates (45 MSSA, 45 MRSA) were correctly identified by the sa442-mecA–PCR method (Table 1). However, the BD GeneOhm StaphSR assay repeatedly identified three of the MSSA isolates as MRSA and two MRSA isolates as S. aureus (but not as MRSA) when
tested directly from blood cultures. None of the 19 CoNS was misidentified in either assay, excluding the presence of *S. aureus* in these samples (Table 1). In summary, the sensitivity and specificity of the BD GeneOhm StaphSR assay was 100% each (95% confidence interval [CI], 96.0 to 100% and 82.4 to 100%, respectively) for the detection of *S. aureus* (*n* = 90), including MSSA (*n* = 45) and MRSA (*n* = 45). For the detection of MRSA (*n* = 45), the test was 95.6% (43/45) sensitive (95% CI, 84.9 to 99.5%) and 95.3% (61/64) specific (95% CI, 86.9 to 99.0%).

**Analysis of blood culture bottles spiked with mixtures of staphylococcal isolates.** To evaluate the BD GeneOhm StaphSR assay for its use in positive blood cultures in greater detail, blood culture bottles were spiked with two different staphylococcal isolates (Table 2). This setting mimics a polymicrobial septicemia prone to deliver incorrect results in rapid molecular diagnostics.

All mixtures of MSSA and MRSA (*n* = 5) were identified correctly as MRSA-containing samples by both assays. Since the BD GeneOhm StaphSR assay targets a specific sequence for MRSA and a specific sequence for *S. aureus* in the same reaction, the assay will report MRSA in the case of mixed MSSA and MRSA cultures; therefore, the appraisal of the result MRSA gets priority over the appraisal of *S. aureus*. Similarly, the *sa442-mecA*-PCR assay (detecting *sa442* as an *S. aureus*-specific sequence and *mecA* coding for methicillin resistance) leads to the identification as MRSA and neglects the presence of MSSA in the same sample.

The *sa442-mecA*-PCR assay suffers from potentially incorrect MRSA detection, as this assay detects the *S. aureus*-specific *sa442* gene and the *mecA* gene encoding methicillin resistance in two single PCRs. Therefore, in blood cultures containing both MSSA and *mecA*-positive methicillin-resistant CoNS (MRCoNS), this approach might lead to the incorrect diagnosis of MRSA, and this is a known limitation of the method. Indeed, due to the simultaneous detection of the *sa442* and *mecA* genes from two independent staphylococcal genomes, the *sa442-mecA*-PCR setting leads to the incorrect diagnosis of MRSA (*sa442*<sup>+</sup> *mecA*<sup>+</sup>) in such mixtures (*n* = 5) (Table 2).

In contrast, the BD GeneOhm StaphSR assay correctly identified four of the MSSA isolates from mixtures of MSSA and MRCoNS. One mixture of MSSA and MRCoNS was identified as MRSA by the BD GeneOhm StaphSR assay. However, this particular MSSA-MRCoNS mixture contained one of those three MSSA isolates that were misidentified as MRSA (isolate 114; see below). Therefore, the diagnosis of MRSA resulted from the MSSA falsely detected as MRSA and not from the MRCoNS.

Mixtures containing (i) MRSA and methicillin-susceptible CoNS (MSCoNS) (*n* = 5), (ii) MRSA and MRCoNS (*n* = 5), and (iii) MSCoNS and MRCoNS (*n* = 5) all were correctly identified in both assays (Table 2).

**Analysis of *S. aureus* isolates with discrepant results with the BD GeneOhm StaphSR assay.** Isolates 13, 30, and 114 (all MSSA; evaluated by conventional laboratory methods and by using the *sa442-mecA*-PCR from a singular bacterial colony) were repeatedly identified as MRSA by the BD GeneOhm StaphSR assay when performed directly from positive blood cultures. Furthermore, isolates 35 and 126 (both MRSA) were identified as *S. aureus* and not MRSA by the BD GeneOhm StaphSR assay when repeatedly tested directly from blood culture (Table 3). In contrast, when tested from singular bacterial colonies grown on Columbia agar plates (not recommended by the manufacturer for routine diagnostics; performed only for internal purposes during our investigations), isolate 126 was correctly identified as MRSA, whereas isolate 35 still was identified as *S. aureus* and not as MRSA.

**Misidentified MSSA isolates.** Isolates 13, 30, and 114 were correctly identified as MSSA by the *sa442-mecA*-PCR setting (*sa442*<sup>−</sup> and *mecA* negative) but were identified to be MRSA with the BD GeneOhm StaphSR assay. Moreover, all three isolates were susceptible for oxacillin tested by microdilution test, no growth was observed on oxacillin-containing Mueller-Hinton agar plates and CHROMagar MRSA plates, and the PBP2<sup>−</sup> test was negative (Table 3; not all data are shown). Accordingly, *mecA*-specific PCRs (11) were negative for all three isolates, confirming the absence of a functional *mecA* gene (data not shown). Those isolates turned out to represent revertant strains in which a part of the SCC (including the *mecA* gene) was deleted. All three strains were not typeable by SCCmec multiplex PCRs (data not shown) and belonged to different *spa* types (Table 3), indicating that there was no epidemiological relationship among those isolates.

**Misidentified MRSA isolates.** Strains 35 and 126 (both MRSA; identified by the *sa442-mecA*-PCR setting [*sa442*<sup>−</sup> *mecA*<sup>+</sup>]) and by the described conventional identification tech-
### Table 3. Analysis of the isolates with discrepant results in BD GeneOhm StaphSR, –PCR, and phenotypic testing

<table>
<thead>
<tr>
<th>Isolate no. (type)</th>
<th>Real-time PCR result directly from blood culture</th>
<th>Isolate analysis (subcultivation of positive blood culture on agar plates)</th>
<th>Final assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OX:MIC (µg/ml)</td>
<td>Growth in CHROMagar PBP2</td>
</tr>
<tr>
<td>13 (MSSA)</td>
<td>Positive (MSSA)</td>
<td>≤0.25</td>
<td>No</td>
</tr>
<tr>
<td>30 (MSSA)</td>
<td>Positive (MSSA)</td>
<td>≤0.25</td>
<td>No</td>
</tr>
<tr>
<td>114 (MSSA)</td>
<td>Positive (MSSA)</td>
<td>≥4</td>
<td>Yes</td>
</tr>
<tr>
<td>35 (MRSA)</td>
<td>Positive (MRSA)</td>
<td>≥4</td>
<td>Yes</td>
</tr>
<tr>
<td>126 (MRSA)</td>
<td>Reactive (S. aureus)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

This report describes, for the first time, the evaluation of the BD GeneOhm StaphSR assay for detecting MSSA (based on the detection of *S. aureus* not being MRSA) and MRSA from spiked positive blood cultures in direct comparison with another rapid molecular approach, the *sa442-mecA*–PCR assay, which recently was analyzed by us for its potential use in the rapid molecular identification of staphylococci from blood cultures (7). Since the number of MRSA blood culture isolates at our hospital is relatively low (~20 per year), we performed the herein-described study with spiked blood cultures from our collection of blood culture isolates. In total, we analyzed 134 blood cultures, of which 45 were spiked with MSSA isolates, 45 with MRSA isolates, 19 with CoNS, and 25 with mixtures of staphylococci. When blood cultures were spiked with CoNS, none of these assays led to incorrect results. Using the *sa442-mecA*–PCR assay, all blood cultures spiked with singular MSSA or MRSA isolates were correctly identified. In contrast, five discrepant results arose by using the BD GeneOhm StaphSR assay. When analyzing mixtures of staphylococci, both assays revealed a good reliability influenced by the specific limitations of each assay.

Three MSSA strains (13, 30, and 114) were misidentified as MRSA by the BD GeneOhm StaphSR assay. None of these strains harbored a functional *mecA* gene as analyzed by PCR techniques, and no SCC*mec* type could be determined. Interestingly, the analysis of these results (MSSA strains that appear to be *mecA* negative but BD GeneOhm StaphSR positive) revealed that these three isolates represent MRSA revertant strains, and this explains on a molecular basis why these MSSA isolates were reactive in the BD GeneOhm StaphSR assay, leading to the misidentification as MRSA (data not shown). Knowing that the *mecA* gene is carried by the SCC*mec* and that the MRSA target of the BD GeneOhm StaphSR assay is located in the 3’ sequence downstream of SCC*mec*, these results suggest that the revertant isolates (13, 30, and 114) harbor residual fragments of the *mec* right-extremity junction belonging to the SCC*mec* cassette. SCCs are mobile elements that are able to insert into and be excised from the chromosome. Such excision of the SCC*mec* can be complete or partial; in the latter case, some fragments can be left at the integration site of the SCC*mec* (6). Therefore, residual SCC*mec* extremity fragments containing such MRSA target sequences most likely are the molecular reason for the misidentification of these three MSSA isolates.

Two MRSA strains (35 and 126) were identified as *S. aureus*...
but not as MRSA by the BD GeneOhm StaphSR assay from blood cultures. When tested from colonies grown on agar plates (not recommended by the manufacturer; performed only for internal purposes during this investigation), strain 35 still was identified as *S. aureus* (and not as MRSA), whereas for strain 126 the result changed to MRSA. The molecular explanation for this phenomenon remains unclear. Most probably, the identification as MRSA from culture has to be judged as an incidental correct identification of this particular isolate based on an unspecified amplification of the MRSA-specific sequences (intellectual property of BD GeneOhm, details not shown). Misidentifications occurring with the BD GeneOhm StaphSR assay can be explained by the fact that the assay was designed to detect the most common MRSA strains. However, a minor population of MRSA strains cannot be identified, because the specific DNA sequence is not targeted by the BD GeneOhm StaphSR assay; in fact, isolates 35 and 126 were such rare strains. Considering that these strains represent only a minor population of all MRSA strains (data not shown), the detection of MRSA in blood cultures by the BD GeneOhm StaphSR technology represents a reasonable trade off between practicability and correct MRSA identification. However, these results also imply that the rapid detection of MRSA or MSSA by the BD GeneOhm StaphSR assay nevertheless should be followed up by conventional culture-based laboratory methods, which still are necessary for antimicrobial susceptibility testing.

When the two methods are compared to each other, the advantage of the *sa442-mecA* real-time PCR method is obviously its higher specificity for the detection of MRSA. Although this assay is predisposed to deliver incorrect results in the detection of MRSA in mixtures of MSSA and MRCoNS, we did not observe such incorrect identification results until now in our routine laboratory. The reason for this fact most likely is that mixed staphylococcal blood cultures are rare in our hospital: such mixtures did not appear in a former study with 475 routine blood cultures (7). Nevertheless, this limitation of the *sa442-mecA*–PCR assay is overcome by the BD GeneOhm StaphSR assay. The latter delivers the correct identification of MSSA and MRSA even in mixed-blood cultures containing *S. aureus* and MRCoNS, since the precondition for the diagnosis of MRSA by this assay is the simultaneous amplification of a target near the SCCmec insertion site and the *sa442-aureus* opfX junction. Moreover, this assay is very rapid (~1.5 h) and easy to perform in laboratories where the SmartCycler apparatus has already been implemented for other applications.

In summary, both herein-described real-time PCR methods (BD GeneOhm StaphSR assay and *sa442-mecA*–PCR assay) are reliable, useful tools for the detection of MSSA and MRSA directly from positive blood cultures. Even though studies have shown that revertant strains and MRSA strains undetected by the BD GeneOhm StaphSR assay are rare (data collected during the BD GeneOhm StaphSR clinical study showed that the prevalence of revertant strains represents less than 1% of the specimens tested), their occurrence currently is being monitored and will be taken into consideration when improving the BD GeneOhm StaphSR assay in the future.

ACKNOWLEDGMENTS

We thank Mariola Gwodz, Kerstin Kromer, Cornelia Lüth, and Stefanie Richt (Tübingen, Germany) for excellent technical assistance.

REFERENCES


