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Clinical Validation of the Molecular BD GeneOhm StaphSR Assay for Direct Detection of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* in Positive Blood Cultures▽

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The rapid detection of *Staphylococcus aureus* bacteremia and a swift determination of methicillin susceptibility has serious clinical implications affecting patient mortality. This study evaluated the StaphSR assay (BD GeneOhm, San Diego, CA), a real-time PCR assay, for the identification and differentiation of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) from 300 positive blood cultures. The BD GeneOhm StaphSR assay was performed and interpreted according to the manufacturer's recommendations. Positive blood cultures (containing predominantly gram-positive cocci in clusters) were subcultured on 5% sheep blood agar plates. After 18 to 24 h of incubation, isolates morphologically consistent with *S. aureus* were presumptively identified by latex agglutination (Staphaurex Plus; Remel, Lenexa, KS). Susceptibility testing was initially performed with the Phoenix automated microbiology system (BD Diagnostics, Sparks, MD). Additional susceptibility testing of samples with discrepant results was done using BBL oxacillin screen agar (BD Diagnostics, Sparks, MD), oxacillin and cefoxitin Etests (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar, an immunoassay for penicillin binding protein 2′ (Denka Seiken Co., Tokyo, Japan), and *mecA* PCR. The sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm StaphSR assay for MSSA detection were 98.9, 96.7, 93.6, and 99.5%, respectively. For the detection of MRSA, the BD GeneOhm StaphSR assay was 100% sensitive and 98.4% specific; positive and negative predictive values for MRSA detection were 92.6 and 100%, respectively. Inhibition was seen with only one sample, and the issue was resolved upon retesting. The BD GeneOhm StaphSR assay appears to be a valuable diagnostic tool for quickly differentiating bacteremia caused by MSSA and MRSA from that caused by other gram-positive cocci.

Staphylococcus aureus is a significant cause of community-acquired and nosocomial infections. Antimicrobial resistance to methicillin is on the rise (11, 40), complicating patient management (32, 39). *S. aureus* bacteremia increases one's risk of death (19, 39–41), and most studies have shown an increase in mortality among patients infected with methicillin-resistant *S. aureus* (MRSA) compared to those infected with methicillin-susceptible *S. aureus* (MSSA) (39, 40). In a meta-analysis published by Cosgrove et al., the authors reported a significant increase in mortality associated with MRSA bacteremia compared to that associated with bloodstream infections caused by MSSA (9). The increase in mortality remained after adjustment for confounding variables (9).

Traditionally, the detection of bloodstream infections caused by *S. aureus* requires at least a day for culture and another day for identification and susceptibility testing. Nucleic acid amplification methods can provide same-day results once the blood cultures are positive for microbial growth; however, the majority of presently described assays are not designed to differentiate between MSSA and MRSA. Additionally, those nucleic acid amplification procedures that can differentiate between MSSA and MRSA often require labor-intensive protocols, technologically advanced equipment, and a colony isolated from subculture, or such assays are not readily available (14, 17, 28, 30, 33, 37, 42). The detection of *mecA* or MRSA in positive blood culture bottles using molecular methods has previously been very successful (4, 36), but there are few reports on the rapid differentiation between MSSA and MRSA among other gram-positive organisms causing bacteremia (29, 34, 37).

A rapid, commercially available diagnostic test to detect bacteremia caused by *S. aureus* and MRSA has great potential to reduce mortality, the length of hospitalization, and costs associated with bloodstream infections (8). Additionally, from a laboratory work flow perspective, a highly sensitive and specific yet user-friendly assay with a common platform is desirable.

The StaphSR assay (BD GeneOhm, San Diego, CA) is a multiplex real-time PCR method that amplifies a specific target sequence of *S. aureus* and a specific target near the staphylococcal cassette chromosome (SCC) *mec* insertion site and the *orfX* junction in MRSA. This PCR assay provides distinctive results for each target and differentiates between MSSA and MRSA. We evaluated the performance of the StaphSR assay (BD GeneOhm, San Diego, CA) for the detection of MRSA and MSSA from positive blood cultures.

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MATERIALS AND METHODS

The study was performed as part of an industry-sponsored Food and Drug Administration clinical trial to evaluate the ability of the BD GeneOhm StaphSR molecular assay to distinguish *S. aureus* from other gram-positive cocci in positive blood cultures and to determine the susceptibility of the *S. aureus* to methicillin. The study was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Specimen selection. Blood culture bottles submitted to the Clinical Microbiology Laboratory of the Johns Hopkins Hospital, Baltimore, MD, for routine culture were eligible for inclusion in the study, and we attempted to maximize the recovery of staphylococci by including predominantly those bottles demonstrating the presence of gram-positive cocci in clusters. Cultures of 300 blood samples from 295 patients had been identified as positive by the BacT/ALERT 3D system (bioMerieux Inc., Hazelwood, MO) and were deidentified prior to testing. The majority of these cultures (99%) were positive for less than 36 h prior to enrollment. All personnel performing the conventional testing and all personnel performing the BD GeneOhm StaphSR molecular assay were blinded to the results of the group whose specimens they did not test.

Subculture. One milliliter from each positive blood culture bottle was transferred to a 1.5-ml microtube in a biological safety cabinet, and 1 drop (100 to 200 μl) was inoculated onto a 5% sheep blood agar (SBA) plate (BD Diagnostics, Sparks, MD) for primary isolation. The 5% SBA plate was incubated aerobically for 24 h at 35°C and reviewed again at 48 h if necessary. Gram-positive cocci in clusters that were catalase positive and classified as *S. aureus* by Staphaurex Plus (Remel, Lenexa, KS) were identified as *S. aureus*. Oxacillin susceptibility was determined using the BD Phoenix automated microbiology system (BD Diagnostics, Sparks, MD) as previously reported (3). The BD Phoenix instrument was also used to confirm the species identification of any isolate that yielded questionable Gram staining, catalase, or Staphaurex Plus results. When all testing was completed, isolates were frozen at −70°C in Trypticase soy broth with 10% glycerol after identification.

BD GeneOhm StaphSR assay. The BD GeneOhm StaphSR assay uses the same primers and probes for MRSA detection as the MRSA assay (BD GeneOhm, San Diego, CA), and these primers and probes have been described in detail previously by Huletsky et al. (20). The MRSA assay (formerly known as the IDI-MRSA assay; BD GeneOhm, San Diego, CA) uses primers specific for various SCC *mec* right-extremity sequences and a primer and probes specific for the *S. aureus* chromosomal *orfX* gene located to the right of the SCC *mec* insertion site (20, 38). The *S. aureus* species-specific gene targets are proprietary and are not available. The analytical sensitivity (limit of detection) is 15 DNA copies per reaction mixture or 10 CFU per reaction mixture for both MRSA and *S. aureus* (S. Paradis, BD GeneOhm, personal communication). The assay was performed according to the manufacturer's instructions. In brief, 2 μl of a 1.0-ml aliquot removed from the positive blood culture bottle was inoculated into 1 ml of sample buffer in a tube. To the remaining aliquot of blood, approximately 100 μl of glycerol was added, and the aliquot from the positive blood culture bottle was frozen at −70°C. The sample buffer tube was subjected to a vortex at high speed for 5 to 10 s, and 50 μl of the sample buffer was transferred into a dry lysis tube containing glass beads. Sample processing according to the BD GeneOhm StaphSR assay protocol continued. The lysis tube was subjected to a vortex for 5 min at high speed, spun briefly, and incubated in a 95°C dry block for 2 min to inactivate possible PCR inhibitors. The samples were placed on ice for immediate testing or refrigerated at 3 to 5°C until the assay was performed. The master mix (25 μl) was reconstituted and aliquoted into labeled SmartCycler tubes (Cepheid, Sunny Vale, CA) for each sample tested. Three microliters of the sample from the lysis tube was added to the corresponding labeled SmartCycler tube. Positive and negative PCR controls were prepared according to the package insert and were included with each run. Additionally, a positive *S. aureus* control (≈600 CFU per reaction mixture) provided by the manufacturer was run each day as an external extraction control. All reagents and samples were kept at 2 to 8°C on tube cooler blocks. Once the samples and master mix were pipetted, the SmartCycler tubes were centrifuged with a quick spin to remove air bubbles prior to being loaded into the SmartCycler CORE module. The BD GeneOhm StaphSR assay includes a non-*S. aureus* sequence from a linearized plasmid which serves as an internal control to detect inhibition of the PCR. The assay was run on the SmartCycler using the manufacturer's amplification protocol. Sample buffer and processed lysis tubes were frozen at −70°C after testing was completed.

The PCR results for the BD GeneOhm StaphSR assay were interpreted using the SmartCycler software (version 1.7b) which uses a decisional algorithm to interpret the assay result as negative, i.e., no *S. aureus* or MRSA DNA detected, or positive, i.e., *S. aureus* and/or MRSA DNA detected. This interpretation is accomplished by determining the slope of the fluorescent output curve, which then determines the cycle threshold. This threshold is the first cycle in which there is a significant increase in fluorescence above a predetermined level. The PCR results were considered positive according to the manufacturer's specifications when any *S. aureus* or MRSA DNA signal was detected during amplification (5).

Data analysis. Blood culture outcomes were defined by laboratory confirmation of isolates to be MSSA, MRSA, or other organisms (not MSSA or MRSA) from initial culture results. Descriptive statistics with 95% confidence intervals (95% CI) were calculated using standard methods (35) on Stata 7 (Stata Corporation, TX).

Discordant sample results. Testing of samples for which the culture result and the PCR result from the BD GeneOhm StaphSR assay did not agree was repeated by PCR in duplicate with aliquots from the initial lysis tubes. The corresponding culture work was reviewed for accuracy by a staff member not involved in the original culture workup. Isolates whose susceptibility patterns did not match the results of the molecular assay were tested again with the Phoenix system and were also tested using BBL oxacillin screen agar (BD Diagnostics, Sparks, MD), oxacillin and cefoxitin Etests (AB Biodisk, Solna, Sweden) on BBL Mueller-Hinton II agar (BD Diagnostics, Sparks, MD) according to the Clinical and Laboratory Standards Institute (CLSI) standards (7), and an immunoassay for penicillin binding protein 2′ (Denka Seiken Co., Tokyo, Japan) (23).

In addition, personnel at BD GeneOhm Canada examined the 10 samples with discordant results from culture and the BD GeneOhm StaphSR assay. Frozen aliquots of the original positive blood culture were thawed and recultured. Isolates were identified as MSSA, MRSA, or not MSSA or MRSA, and standard susceptibility testing and *mecA* PCR (25) were done when necessary. The BD GeneOhm StaphSR assay of 9 of the 10 samples with discordant culture and StaphSR assay results was repeated according to the clinical trial protocol. The contents of the SmartCycler tubes from the PCR of the two samples which were initially identified as MRSA positive by the BD GeneOhm StaphSR assay but were culture negative were tested by gel analysis to detect *S. aureus* and MRSA DNA. Three microliters of electrophoresis loading buffer was added to each SmartCycler tube, and 15.0 μl of the solution was loaded into a 2% agarose gel in Tris-borate-EDTA 1× buffer with 0.25 mg of ethidium bromide/ml. A 100-bp molecular size ladder (Amersham Biosciences, Baie d'Urfé) was used to compare the sizes of the bands present after samples were run at 120 V for 90 min.

RESULTS

Culture results. Positive cultures (identified by the BacT/ALERT system) of 300 blood samples from 295 patients were evaluated. Five patients (1.7%) contributed two samples each, but the blood samples were drawn at different times. All but four blood samples were processed for culture and PCR within 36 h of the determination of a positive result. Ninety samples (30%) were cultured in BacT/ALERT FA bottles, 104 (35%) were cultured in BacT/ALERT aerobic bottles, 105 (35%) were cultured in BacT/ALERT anaerobic bottles, and 1 (0.33%) was cultured in a BacT/ALERT pediatric FA bottle. Of the 89 samples (29.7%) that grew *S. aureus*, 56.2% (50 of 89) grew MRSA (Table 1). Two of the MSSA-positive blood cultures grew multiple organisms; one grew *S. aureus* and coagulase-negative staphylococci (CoNS), and the other grew *S. aureus* and *Streptococcus* sp. One MRSA-positive blood culture also grew multiple organisms, including MRSA, *Streptococcus* sp., and a gram-negative rod. Species other than *S. aureus* or MRSA grew in 211 of the BacT/ALERT bottles (Table 1). CoNS alone were recovered from 190 cultures. The remaining 21 bottles grew a mixture of CoNS, gram-positive rods, *Streptococcus* sp., *Micrococcus* sp., *Candida parapsilosis*, and gram-negative rods. One remaining bottle demonstrated the presence of gram-positive cocci in clusters upon Gram staining; however, upon subculture, there was no bacterial growth, and this sample was negative by the multiplex PCR assay.

TABLE 1. Isolates recovered from 300 positive blood cultures

Microorganism(s) recovered	No. of isolates	Frequency[a] (%)
S. aureus, methicillin resistant[b]	50	16.7
S. aureus, methicillin susceptible[c]	39	13.0
Coagulase-negative staphylococci[d]	190	63.3
Coagulase-negative staphylococci and streptococci	3	1.0
Coagulase-negative staphylococci and gram-positive rods	5	1.7
Coagulase-negative staphylococci and gram-negative rods	1	0.3
Micrococcus species	2	0.7
Streptococci	4	1.3
Gram-positive rods	3	1.0
Gram-negative rods	1	0.3
Yeast	1	0.3
None[e]	1	0.3
Total	300	

[a] Percentages do not add up to 100 due to rounding.
[b] One culture contained MRSA colonies with two types of morphology, and one culture was mixed, containing streptococci and gram-negative rods.
[c] One culture contained MSSA colonies with two types of morphology, one culture showed MSSA mixed with CoNS, and one mixed culture included streptococci.
[d] Eleven cultures contained CoNS colonies with two types of morphology; one culture contained colonies with three types of morphology.
[e] Organisms were seen upon Gram staining but failed to grow upon subculture.

BD GeneOhm StaphSR assay performance. Ninety-five samples (31.7%) were positive by the BD GeneOhm StaphSR assay: 94 samples (31.3%) tested positive for *S. aureus*, and 54 samples (18.0%) tested positive for MRSA (Table 2). The multiplex PCR assay gave negative results for 205 samples. All but one MRSA-positive sample also tested positive for *S. aureus* by the BD GeneOhm StaphSR assay. Only one sample, negative by culture, resulted in an internal control failure during the initial PCR, but upon retesting, the issue was resolved; this sample was negative for both *S. aureus* and MRSA.

Overall, there was 96.7% agreement between the results of the BD GeneOhm StaphSR assay and culture (results were the same for 290 of 300 samples). The agreement between the methods was 97.7% (293 of 300 samples) for the detection of all types of *S. aureus* and 98.7% (296 of 300 samples) for the detection of MRSA. Compared to bacterial culture for *S. aureus*, the *S. aureus* PCR component of the BD GeneOhm StaphSR assay was 98.9% sensitive (95% CI, 95.9 to 100%) and 96.7% specific (95% CI, 94.3 to 99.1%) with a positive predictive value of 93.6% (95% CI, 88.7 to 98.6%) and a negative predictive value of 99.5% (95% CI, 98.2 to 100%). In the evaluation of the BD GeneOhm StaphSR assay compared to standard culture and susceptibility testing, the sensitivity for MRSA detection was 100% (95% CI, 92.9 to 100%) and the specificity was 98.4% (95% CI, 96.8 to 100%) with a positive predictive value of 92.6% (95% CI, 85.6 to 99.6%) and a negative predictive value of 100% (95% CI, 98.5 to 100%).

Discrepant results. For a total of 10 samples, the results of culture and the BD GeneOhm StaphSR assay, as interpreted using the manufacturer's recommendations, were discrepant (Table 3). One culture was mixed, comprising *Streptococcus* species and a light growth of *S. aureus*; however, the BD GeneOhm StaphSR assay result was negative even after the retesting in duplicate of aliquots from the lysis tube. The isolate from culture was tested with the BD GeneOhm StaphSR assay and was identified as *S. aureus*.

For two samples that tested positive for *S. aureus* and MRSA by the BD GeneOhm StaphSR assay, the blood cultures yielded only MSSA. The original lysates and the isolates also were retested by the BD GeneOhm StaphSR assay. The retested lysates were positive for MRSA and the isolates themselves were identified as MRSA, suggesting that the organisms were genotypically consistent with MRSA. Both isolates were tested again on the Phoenix instrument, and they were both identified as MSSA. Additional testing of these two isolates showed susceptibility to oxacillin (MICs of 1.0 and 0.5 μg/ml) and cefoxitin (MIC of 8.0 μg/ml) as demonstrated by Etest results and their failure to grow on oxacillin screening agar plates. Both isolates tested negative for the product of the *mecA* gene, namely, penicillin binding protein 2′, by a slide latex agglutination test (Denka Seiken Co., Tokyo Japan) and were negative for the *mecA* gene.

Seven samples grew only CoNS but tested positive for *S. aureus* by the BD GeneOhm StaphSR assay. Five of these were positive by PCR for *S. aureus* alone. Aliquots from the lysis tubes corresponding to these samples were tested again in duplicate by the PCR assay. Three of the five samples gave repeat positive results in at least one of the two duplicate PCR tests. The remaining two samples which grew CoNS initially tested positive for MRSA. One of these two samples tested

TABLE 2. Direct comparison of culture results to the StaphSR results interpreted using the manufacturer's protocol[a]

Culture identification (no. of samples)	StaphSR result			% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
	No. of negative samples	No. of samples positive for *S. aureus*	No. of samples positive for MRSA				
Not MSSA or MRSA (211)	204	5	2[b]				
MSSA only (39)	1	36	2[c]	98.9 (95.9–100)	96.7 (94.3–99.1)	93.6 (88.7–98.6)	99.5 (98.2–100)
MRSA only (50)	0	0	50	100 (92.9–100)	98.4 (96.8–100)	92.6 (85.6–99.6)	100 (98.5–100)
Total (300)	205	41	54				

[a] PPV, positive predictive value; NPV, negative predictive value. Calculations used to compute sensitivity, specificity, and positive and negative predictive values were as follows: for *S. aureus*, sensitivity, 88/89 = 98.9%; specificity, 204/211 = 96.7%; positive predictive value, 88/94 = 93.6%; negative predictive value, 205/206 = 99.5%; for MRSA, sensitivity, 50/50 = 100%; specificity, 246/250 = 98.4%; positive predictive value, 50/54 = 92.6%; negative predictive value, 246/246 = 100%.
[b] One sample tested positive for MRSA only.
[c] Organisms in two samples were phenotypically MSSA, but the assay and culture results identified the isolates genotypically as MRSA.

TABLE 3. Analysis of samples with discrepant results[a]

Specimen no.	Initial culture identification	Repeat culture identification at:		Initial StaphSR PCR result for:		Repeat StaphSR PCR result for:		Isolate analysis					
		JHH	GeneOhm	*S. aureus*	MRSA	*S. aureus*	MRSA	StaphSR PCR result for:		Phoenix OX MIC (μg/ml)[b]	Result of PBP2′ assay	Result of *mecA* PCR	Final assignment
								S. aureus	MRSA				
7903	CoNS	No *S. aureus*	No *S. aureus*	Pos	Neg	Pos[c]	Neg[c]	Neg	Neg	ND	ND	ND	False-pos for *S. aureus*
8409	CoNS	No *S. aureus*	No *S. aureus*	Pos	Neg	Pos[c]	Neg[c]	Neg	Neg	ND	ND	ND	False-pos for *S. aureus*
8433	CoNS	No *S. aureus*	No *S. aureus*	Pos	Neg	Pos	Neg[c]	Neg	Neg	ND	ND	ND	False-pos for *S. aureus*
8452	CoNS	No *S. aureus*	No *S. aureus*	Pos	Neg	Neg[c]	Neg[c]	ND	ND	ND	ND	ND	False-pos for *S. aureus*
8455	CoNS	No *S. aureus*	No *S. aureus*	Pos	Neg	Neg[c]	Neg[c]	ND	ND	ND	ND	ND	False-pos for *S. aureus*
7052	CoNS	No *S. aureus*	No *S. aureus*	Neg	Pos	Neg[c]	Neg[c]	ND	ND	ND	ND	ND	False-pos for MRSA
8445	CoNS	No *S. aureus*	No *S. aureus*	Pos	Pos	Pos[c]	Neg[c]	Neg	Neg	ND	ND	ND	False-pos for MRSA
7054	*S. aureus*	ND	*S. aureus*	Neg	Neg	Neg[c]	Neg[c]	Pos	Neg	0.5	Neg	ND	False-neg
7083	*S. aureus*	*S. aureus*	*S. aureus*	Pos	Pos	Pos[c]	Pos[c]	Pos	Pos	0.5	Neg	Neg	False-pos for MRSA
7987	*S. aureus*	*S. aureus*	*S. aureus*	Pos	Pos	Pos[c]	Pos[c]	Pos	Pos	0.5	Neg	Neg	False-pos for MRSA

[a] JHH, Johns Hopkins Hospital; GeneOhm, BD GeneOhm Canada; Pos, positive; Neg, negative; OX, oxacillin; PBP2′, penicillin binding protein 2′; ND, not done.
[b] Phoenix MICs were corroborated by Etest MICs of oxacillin and cefoxitin and by failure to grow on oxacillin screening agar plates. See the text for values.
[c] Testing was repeated at BD GeneOhm Canada with concordant results.

positive for MRSA but was negative for *S. aureus* by the BD GeneOhm StaphSR assay, and when the sample was retested in duplicate, the results were negative. The remaining sample which grew only CoNS was positive for *S. aureus* and MRSA by the BD GeneOhm StaphSR assay, but when the sample was retested in duplicate, the initial result of positive for *S. aureus* only was confirmed by both repeats.

Culture of the 10 samples with discordant results at BD GeneOhm Canada confirmed the original culture results for 8 of the 10 samples (Table 3). Aliquots from nine of the original lysis tubes tested by the BD GeneOhm StaphSR assay yielded the same results in Canada as in Baltimore. The sample that initially tested positive for MRSA alone was tested at BD GeneOhm and was negative for the presence of *S. aureus* and MRSA. The contents of the initial SmartCycler PCR tube corresponding to the sample that was culture negative and PCR positive for *S. aureus* and MRSA by the BD GeneOhm StaphSR assay were analyzed on a gel (see Materials and Methods) and gave the result of positive for *S. aureus* and MRSA.

Within the confines of our Food and Drug Administration evaluation with anonymous testing, we were unable to determine the clinical relevance of the 10 samples with discrepant results from culture and the BD GeneOhm StaphSR assay.

DISCUSSION

To our knowledge, this is the first published report of the performance of the BD GeneOhm StaphSR assay for detecting *S. aureus* and simultaneously differentiating MRSA in positive blood cultures. The performance characteristics of the BD GeneOhm StaphSR assay compared favorably to standards from routine culture and susceptibility testing methods: 99% sensitivity for *S. aureus* detection and 100% sensitivity for MRSA detection. The BD GeneOhm StaphSR assay was 97 and 98% specific for *S. aureus* and MRSA detection, respectively. Our results are similar to those reported by Fuller et al. for a prototype of the assay. In that study, the authors reported 100% sensitivity and specificity (15).

During the 20 weeks of testing, the assay performed well, with consistently high levels of sensitivity and specificity. The only culture-positive, PCR-negative specimen contained a *Streptococcus* species with a small quantity of *S. aureus* growth when subcultured onto 5% SBA, and testing of the *S. aureus* isolate by the StaphSR assay gave positive results. Quantification of the *S. aureus* organisms was not performed. However, it is possible that the quantity of *S. aureus* organisms in the blood culture bottle was below the limit of detection of the BD GeneOhm StaphSR assay (20, 24). The analytical sensitivity of the assay has been reported by the manufacturer to be 15 DNA copies per reaction mixture or 10 CFU per reaction mixture for *S. aureus* and MRSA (S. Paradis, BD GeneOhm, personal communication). The drop (≈150 μl) from the blood culture bottle was applied directly onto the SBA, whereas for the PCR assay, the specimen was diluted in the sample buffer (2:1,000) and again diluted in the PCR tube (3:25). The *Streptococcus* species was likely the microorganism that signaled the BacT/ALERT instrument. High concentrations of *Streptococcus* species may have inhibited *S. aureus* growth in the bottle and/or possibly inhibited the PCR. However, this inhibition has not been observed with other heavily mixed cultures in our laboratory. Contamination of the blood culture bottle or SBA subculture plate during manipulation is another possible, but unlikely, explanation.

Two *S. aureus* isolates were identified as MRSA by the BD GeneOhm StaphSR assay but as MSSA by the Phoenix instrument. The isolates were confirmed as methicillin susceptible by using an Etest, oxacillin screening agar, and the penicillin binding protein 2′ assay. Further PCR testing at BD GeneOhm

Canada found the isolates to be lacking an amplifiable *mecA* gene. This phenomenon in the context of the BD GeneOhm MRSA assay (formerly the IDI-MRSA assay) has been described previously by several investigators (10, 25). Both Desjardins et al. and Oberdorfer et al. confirmed the findings of Huletsky et al. that approximately 5% of specimens identified as MRSA by the BD GeneOhm MRSA assay are phenotypically MSSA (10, 20, 26). Desjardins et al., using a previous version of the assay with similar primer arrangements to detect MRSA, found that of the 38 *S. aureus* isolates that were classified as MRSA by the BD GeneOhm MRSA assay, only 2 contained the *mecA* determinant (10). The authors reasoned that the two isolates containing *mecA* may require the inactivation of the *mecI* repressor gene before expression (25); the 36 *mecA*-negative isolates were felt to be similar to those described in reports of *S. aureus* and CoNS strains with non-*mecA*-containing SCC elements (10, 12, 21). In addition, as has been previously suggested (10, 26), these strains may represent some MSSA lineages that carry SCC *mec* without the functioning region containing the *mecA* (12). The genetic differences found in the strains that are phenotypically MSSA but genotypically MRSA are worthy of further investigation.

Seven samples which grew only CoNS yielded false-positive PCR results. None of the CoNS isolates recovered from these seven samples gave positive *S. aureus* results when tested directly by the PCR assay. Two of these samples were also positive for MRSA by the BD GeneOhm StaphSR assay. Both samples failed to yield repeated MRSA results, although one of the samples was repeatedly identified as *S. aureus*. Perhaps the CoNS obscured or inhibited the *S. aureus*. Five samples were positive by the BD GeneOhm StaphSR assay for *S. aureus* only, but CoNS were isolated from the blood culture bottles. Aliquots from three of the five initial lysis tubes tested positive for *S. aureus* again upon duplicate testing. Samples from four of these five blood culture bottles were subcultured onto BBL CHROMagar SA (BD Diagnostics, Sparks, MD) to look for the *S. aureus*, but all showed no growth. It is possible that there was enough genetic material present for amplification in the absence of viable organisms, as may be the case with patients who are receiving therapy. Contamination at some point during initial culture collection or subsequently during laboratory work flow is another possible explanation. We believe that contamination in the laboratory was not the source, as there was no amplification from any negative controls and a unidirectional work flow with other work practice controls was implemented.

Almost no inhibition was seen with the blood culture extraction method, unlike instances described in previous reports of nucleic acid amplification testing of blood (1, 31) and other sample types using the BD GeneOhm MRSA assay (2, 13, 26). The sample extraction method is an easy, one-step procedure that requires minimal processing and is much quicker than most manual and robotic extraction methods, greatly minimizing the time required to perform the assay (1.5 to 2.5 h for five samples and the extraction control).

As mentioned above, standard culture methods require at least 24 to 48 h for the recovery and identification of *S. aureus* and additional confirmatory tests (23) or susceptibility testing methods to determine methicillin resistance. Other nonamplification methods that have been developed to streamline MRSA identification include inoculating specimens from positive blood culture bottles onto chromogenic medium that detects MRSA (29) and using peptide nucleic acid probes for the identification of gram-positive cocci in clusters as *S. aureus* (6, 16, 18, 22, 27). The former instance requires at least overnight growth on the selective medium, and the latter requires susceptibility testing or another method to distinguish MSSA from MRSA (22).

A variety of test methods that rapidly detect or amplify nucleic acid from *S. aureus* and/or *mecA* from positive blood cultures have been described previously (14, 22, 30, 37). Some of the newer assays, although sensitive and specific, require complicated procedures for processing or detection (14) or are not commercially available (37). Those that are available commercially do not discriminate between MSSA and MRSA (16, 22) or require a subcultured isolate (18, 33), which is the aforementioned time constraint with culture-based methods.

In summary, the BD GeneOhm StaphSR assay has excellent sensitivity for distinguishing MSSA and MRSA from other gram-positive organisms causing bacteremia. The negative predictive value is also excellent. While the specificity is acceptable and similar to those of other reported platforms and prototypes of this assay, the explanation for the false-positive results needs to be explored. Likewise, the lower positive predictive values need to be considered in the context of the prevalence of oxacillin resistance among *S. aureus* in a particular institution. A major advantage of this assay is the much shorter time to report a result, 2 h after the detection of a positive blood culture, compared to the 18 to 48 h required for conventional culture and susceptibility testing methods. In addition, the extraction method is easy and the SmartCycler is user friendly, allowing for flexibility in testing.

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REFERENCES

1. **Al-Soud, W. A., and P. Radstrom.** 2001. Purification and characterization of PCR-inhibitory components in blood cells. J. Clin. Microbiol. **39:**485–493.
2. **Bishop, E. J., E. A. Grabsch, S. A. Ballard, B. Mayall, S. Xie, R. Martin, and M. L. Grayson.** 2006. Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual-specimen PCR and routine culture assays for detection of colonization by methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**2904–2908.
3. **Carroll, K. C., A. P. Borek, C. Burger, B. Glanz, H. Bhally, S. Henciak, and D. C. Flayhart.** 2006. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of staphylococci and enterococci. J. Clin. Microbiol. **44:**2072–2077.
4. **Carroll, K. C., R. B. Leonard, P. L. Newcomb-Gayman, and D. R. Hillyard.** 1996. Rapid detection of the staphylococcal *mecA* gene from BACTEC blood culture bottles by the polymerase chain reaction. Am. J. Clin. Pathol. **106:**600–605.
5. **Cepheid.** 2003. SmartCycler II operator's manual. Cepheid, Sunny vale, CA.
6. **Chapin, K., and M. Musgnug.** 2003. Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. J. Clin. Microbiol. **41:**4324–4327.
7. **CLSI.** 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement, 16th ed. M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Cosgrove, S. E.** 2006. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin. Infect. Dis. **42**(Suppl. 2)**:**S82–S89.
9. **Cosgrove, S. E., Y. Qi, K. S. Kaye, S. Harbarth, A. W. Karchmer, and Y. Carmeli.** 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect. Control Hosp. Epidemiol. **26:**166–174.

10. **Desjardins, M., C. Guibord, B. Lalonde, B. Toye, and K. Ramotar.** 2006. Evaluation of the IDI-MRSA assay for detection of methicillin-resistant *Staphylococcus aureus* from nasal and rectal specimens pooled in a selective broth. J. Clin. Microbiol. **44:**1219–1223.
11. **Diekema, D. J., B. J. BootsMiller, T. E. Vaughn, R. F. Woolson, J. W. Yankey, E. J. Ernst, S. D. Flach, M. M. Ward, C. L. Franciscus, M. A. Pfaller, and B. N. Doebbeling.** 2004. Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clin. Infect. Dis. **38:**78–85.
12. **Donnio, P. Y., D. C. Oliveira, N. A. Faria, N. Wilhelm, A. Le Coustumier, and H. de Lencastre.** 2005. Partial excision of the chromosomal cassette containing the methicillin resistance determinant results in methicillin-susceptible *Staphylococcus aureus*. J. Clin. Microbiol. **43:**4191–4193.
13. **Drews, S. J., B. M. Willey, N. Kreiswirth, M. Wang, T. Ianes, J. Mitchell, M. Latchford, A. J. McGeer, and K. C. Katz.** 2006. Verification of the IDI-MRSA assay for detecting methicillin-resistant *Staphylococcus aureus* in diverse specimen types in a core clinical laboratory setting. J. Clin. Microbiol. **44:**3794–3796.
14. **Fujita, S., Y. Senda, T. Iwagami, and T. Hashimoto.** 2005. Rapid identification of staphylococcal strains from positive-testing blood culture bottles by internal transcribed spacer PCR followed by microchip gel electrophoresis. J. Clin. Microbiol. **43:**1149–1157.
15. **Fuller, D., T. Davis, M. Parker, R. Buckner, and J. Talbott.** 2006. Clinical evaluation of a molecular-based assay for detection of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* in positive blood cultures, abstr. O-175. Abstr. 16th Eur. Congr. Clin. Microbiol. Infect. Dis.
16. **Gonzalez, V., E. Padilla, M. Gimenez, C. Vilaplana, A. Perez, G. Fernandez, M. D. Quesada, M. A. Pallares, and V. Ausina.** 2004. Rapid diagnosis of *Staphylococcus aureus* bacteremia using *S. aureus* PNA FISH. Eur. J. Clin. Microbiol. Infect. Dis. **23:**396–398.
17. **Grisold, A. J., and H. H. Kessler.** 2006. Use of hybridization probes in a real-time PCR assay on the LightCycler for the detection of methicillin-resistant *Staphylococcus aureus*. Methods Mol. Biol. **345:**79–89.
18. **Grisold, A. J., E. Leitner, G. Muhlbauer, E. Marth, and H. H. Kessler.** 2002. Detection of methicillin-resistant *Staphylococcus aureus* and simultaneous confirmation by automated nucleic acid extraction and real-time PCR. J. Clin. Microbiol. **40:**2392–2397.
19. **Harbarth, S., O. Rutschmann, P. Sudre, and D. Pittet.** 1998. Impact of methicillin resistance on the outcome of patients with bacteremia caused by *Staphylococcus aureus*. Arch. Intern. Med. **158:**182–189.
20. **Huletsky, A., R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. **42:**1875–1884.
21. **Katayama, Y., F. Takeuchi, T. Ito, X. X. Ma, Y. Ui-Mizutani, I. Kobayashi, and K. Hiramatsu.** 2003. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome *mec* of methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. **185:**2711–2722.
22. **Levi, K., and K. J. Towner.** 2003. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in blood with the EVIGENE MRSA detection kit. J. Clin. Microbiol. **41:**3890–3892.
23. **Louie, L., S. O. Matsumura, E. Choi, M. Louie, and A. E. Simor.** 2000. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **38:**2170–2173.
24. **Martineau, F., F. J. Picard, D. Ke, S. Paradis, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 2001. Development of a PCR assay for identification of staphylococci at genus and species levels. J. Clin. Microbiol. **39:**2541–2547.
25. **Martineau, F., F. J. Picard, N. Lansac, C. Ménard, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 2000. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **44:**231–238.
26. **Oberdorfer, K., S. Pohl, M. Frey, K. Heeg, and C. Wendt.** 2006. Evaluation of a single-locus real-time polymerase chain reaction as a screening test for specific detection of methicillin-resistant *Staphylococcus aureus* in ICU patients. Eur. J. Clin. Microbiol. Infect. Dis. **25:**657–663.
27. **Oliveira, K., S. M. Brecher, A. Durbin, D. S. Shapiro, D. R. Schwartz, P. C. De Girolami, J. Dakos, G. W. Procop, D. Wilson, C. S. Hanna, G. Haase, H. Peltroche-Llacsahuanga, K. C. Chapin, M. C. Musgnug, M. H. Levi, C. Shoemaker, and H. Stender.** 2003. Direct identification of *Staphylococcus aureus* from positive blood culture bottles. J. Clin. Microbiol. **41:**889–891.
28. **Palka-Santini, M., S. Putzfeld, B. E. Cleven, M. Kronke, and O. Krut.** 2007. Rapid identification, virulence analysis and resistance profiling of *Staphylococcus aureus* by gene segment-based DNA microarrays: application to blood culture post-processing. J. Microbiol. Methods **68:**468–477.
29. **Pape, J., J. Wadlin, and I. Nachamkin.** 2006. Use of BBL CHROMagar MRSA medium for identification of methicillin-resistant *Staphylococcus aureus* directly from blood cultures. J. Clin. Microbiol. **44:**2575–2576.
30. **Paule, S. M., A. C. Pasquariello, R. B. Thomson, Jr., K. L. Kaul, and L. R. Peterson.** 2005. Real-time PCR can rapidly detect methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* directly from positive blood culture bottles. Am. J. Clin. Pathol. **124:**404–407.
31. **Radstrom, P., R. Knutsson, P. Wolffs, M. Lovenklev, and C. Lofstrom.** 2004. Pre-PCR processing: strategies to generate PCR-compatible samples. Mol. Biotechnol. **26:**133–146.
32. **Rehm, S. J., and J. G. Bartlett.** 2006. Challenges in the management of serious infections. Clin. Infect. Dis. **42**(Suppl. 2)**:**S63–S64.
33. **Sabet, N. S., G. Subramaniam, P. Navaratnam, and S. D. Sekaran.** 2006. Simultaneous species identification and detection of methicillin resistance in staphylococci using triplex real-time PCR assay. Diagn. Microbiol. Infect. Dis. **56:**13–18.
34. **Shrestha, N. K., M. J. Tuohy, G. S. Hall, C. M. Isada, and G. W. Procop.** 2002. Rapid identification of *Staphylococcus aureus* and the *mecA* gene from BacT/ALERT blood culture bottles by using the LightCycler system. J. Clin. Microbiol. **40:**2659–2661.
35. **Szklo, M., and F. J. Nieto.** 2000. Epidemiology: beyond the basics. Aspen, Gaithersburg, MD.
36. **Tan, T. Y., S. Corden, R. Barnes, and B. Cookson.** 2001. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. J. Clin. Microbiol. **39:**4529–4531.
37. **Thomas, L. C., H. F. Gidding, A. N. Ginn, T. Olma, and J. Iredell.** 2007. Development of a real-time *Staphylococcus aureus* and MRSA (SAM−) PCR for routine blood culture. J. Microbiol. Methods **68:**296–302.
38. **Warren, D. K., R. S. Liao, L. R. Merz, M. Eveland, and W. M. Dunne, Jr.** 2004. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. J. Clin. Microbiol. **42:**5578–5581.
39. **Weinstein, M. P., M. L. Towns, S. M. Quartey, S. Mirrett, L. G. Reimer, G. Parmigiani, and L. B. Reller.** 1997. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin. Infect. Dis. **24:**584–602.
40. **Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond.** 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. **39:**309–317.
41. **Wisplinghoff, H., H. Seifert, S. M. Tallent, T. Bischoff, R. P. Wenzel, and M. B. Edmond.** 2003. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. Pediatr. Infect. Dis. J. **22:**686–691.
42. **Zhang, K., J. Sparling, B. L. Chow, S. Elsayed, Z. Hussain, D. L. Church, D. B. Gregson, T. Louie, and J. M. Conly.** 2004. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J. Clin. Microbiol. **42:**4947–4955.