Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Diverse Clinical Specimens by the BD GeneOhm MRSA Assay and Comparison with Culture[▽]

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The efficacy of the BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) assay was assessed by analyzing nasal swabs and swabs from other body sites for the presence of MRSA in a low-prevalence area. From 681 patients with a high risk for MRSA carriage, 1,601 specimens were collected and transported in Amies agar. After discordant analysis, the sensitivity, specificity, positive predictive value, and negative predictive value of the BD GeneOhm MRSA assay were 84.3%, 99.2%, 88.4%, and 98.9%, respectively, compared to culture.

Rapid availability of laboratory results is paramount for early detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers, implementation of efficient control measures, and adequate therapy. To date, detection of MRSA is conventionally done by culture. Several chromogenic culture media have contributed to a more rapid detection of MRSA (24 versus 72 h with conventional culture [6]). In contrast to former PCR procedures, which required previous isolation of the organism via culture (21), the commercial BD GeneOhm MRSA assay (formerly IDI-MRSA; BD-GeneOhm, San Diego, CA) discriminates *mecA*-positive *Staphylococcus aureus* from coagulase-negative staphylococci (CoNS) and detects MRSA in clinical specimens within a few hours. This real-time PCR assay has been used particularly in high-prevalence areas (3, 6, 9). In Switzerland, MRSA prevalence ranges between 4 and 7% with the exception of Geneva (>25% [4, 14]). Surveillance strategies involve screening for MRSA from the nose and other body sites. The BD GeneOhm MRSA assay has been approved by the United States Food and Drug Administration (FDA) for the detection of MRSA from nasal swabs stored in liquid Stuart's medium. Analyzing specimens from other body sites by this test and transportation of the swab in media other than Stuart's medium are currently not FDA approved for use with this test.

In this study, we aimed at assessing (i) the performance of the BD GeneOhm MRSA assay compared to conventional culture in an extended spectrum of clinical specimens and (ii) the suitability of Amies agar as a transport medium for swabs in a low-prevalence setting.

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During 18 months, 1,601 single swabs from nose ($n = 676$), groin ($n = 643$), wound ($n = 153$), axilla ($n = 61$), throat ($n =$

37), rectum ($n = 9$), vagina ($n = 5$), and miscellaneous body sites ($n = 17$) were collected from 681 patients at high risk for MRSA carriage who were admitted to the Luzerner Kantonsspital, the major teaching hospital of central Switzerland, with approximately 1,300 beds, including a children's hospital. High-risk patients were individuals (i) admitted from out-bound hospitals/health care units of high-MRSA-prevalence areas in Switzerland or from hospitals/health care units of foreign countries; (ii) with skin lesions and intravenous drug abuse; or (iii) with a former positive MRSA test. Swabs were collected in Amies agar (Copanswab 108C; Copan, Brescia, Italy). If processing of the swabs was not possible on the same day, swabs were stored overnight at 4°C. Swabs were then broken off into the sample reagent buffer (blue-capped tubes supplied within the kit) and vortexed at high speed for 60 s. The full amount (approximately 1 ml) of buffer (without the swab) was transferred in another tube for cell lysis and DNA extraction according to the instructions of the manufacturer. PCR was performed with the Smart Cycler II instrument (Cepheid, Sunnyvale, CA). Positive and negative controls were included in each run. In the case of PCR inhibition, the sample was briefly frozen at –20°C to remove inhibitors and the run was repeated. Enrichment broth (1 ml; tryptic soy broth [Becton Dickinson, Allschwil, Switzerland] supplemented with 7.5% NaCl) was added to the sample buffer tube containing the swab and incubated at 35°C in ambient air for 24 h. The incubated enrichment broth (approximately 100 µl) was plated on chromogenic agar (Chrom ID MRSA agar; bioMérieux, Marcy l'Etoile, France) at 35°C in ambient air for 48 h. The chromogenic agar was screened for suspect colonies after 24 h and 48 h. Recovered blue colonies were then tested by the Staphaurex Plus coagulase test (Remel Europe Ltd., Dartford, Kent, United Kingdom) and confirmed as *S. aureus* by the Vitek 2 system (bioMérieux; GP colorimetric identification card; software version 04.03). Confirmation of methicillin resistance was done by disk diffusion testing with 30-µg cefoxitin (bioMérieux) according to the Clinical and Laboratory Standards Institute (7).

Staphylococcal strains with discrepant results for the BD

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TABLE 1. Resolution of discrepancies from PCR-positive and culture-negative cases

No. of specimens	History of MRSA	Comment	No. of amplicons analyzed by gel electrophoresis/no. of specimens	Conclusion
7	Yes	After decolonization	1/7	Probably true positive
3	Yes	Known MRSA carrier	1/3	Probably true positive
2		No information available	2/2	Probably true positive
2	No	Positive PCR and positive culture from other body sites	Not done	Probably true positive
1	No	Antibacterial wound dressing	1/1	Probably true positive
8	No	Culture with MSSA	Not done	False positive
1	No	Culture with CoNS	Not done	False positive
2	No	Positive PCR result not reproducible upon repeat	Not done	False positive
1	No	Superficial wound, no growth in culture	Not done	Unresolved

GeneOhm MRSA assay and culture were further studied. At first the BD GeneOhm MRSA assay was repeated from the DNA extract of the same specimen to exclude a confusion of samples. MRSA isolates from PCR-negative but culture-positive specimens were retested from subculture by the BD GeneOhm MRSA assay. For specimens with PCR-positive but culture-negative results, the patients' medical history was studied (antibiotic therapy and previous carriage of MRSA). For these cases gel electrophoresis was performed to determine the size of amplicons in a 2% agarose gel (AgaroseUltraPured; Invitrogen Corporation, Carlsbad, CA). Discrepancies between the BD GeneOhm MRSA assay and culture were resolved according to at least one of the following criteria: PCR-positive but culture-negative results were considered true positive if (i) patients had been decolonized, (ii) the PCR product had the expected molecular size (as described previously by Huletsky et al. [15]), or (iii) there were PCR-positive and culture-positive results from other body sites of the same patient at the same time (Table 1). Sensitivity and specificity for the BD GeneOhm MRSA assay as well as the positive predictive value (PPV) and negative predictive value (NPV) including confidence intervals (CI; according to Wilson's method [1]) were calculated and compared to culture (Table 2).

Six of 1,601 specimens (0.4%; three from wound, one from rectum, one from groin, and one from axilla) were excluded due to persisting PCR inhibition. Seventeen initially inhibited specimens (1.0%) were eventually included in the data analysis because results were available after freezing and repeating the PCR. Our initial inhibition rate (1.4%) is well in line with that

observed by others using Stuart's liquid medium (8, 26). Some authors (5, 16, 17) who used an agar-based medium reported higher inhibition rates and, therefore, pretreated the swabs before performing PCR. In our hands, Amies agar medium did not significantly interfere with the PCR, and therefore, additional processing of the swabs prior to PCR was considered unnecessary. Of the 1,595 specimens, 103 specimens were PCR positive (6.5%) and 1,492 were PCR negative. By culture (broth/chromogenic agar medium) MRSA was recovered from 93 (5.8%) of 1,595 specimens. Of the 93 culture-positive specimens 17 PCR assays were negative initially as well as upon repeat testing of the lysate and, thus, considered false negative. When colonies were directly tested following subculture from broth medium by the BD GeneOhm MRSA assay, all isolates yielded a positive result. Conversely, for 27 of 103 PCR-positive specimens culture remained negative. Compared to culture, overall sensitivity, specificity, PPV, and NPV of the BD GeneOhm MRSA assay were 81.7%, 98.2%, 73.8%, and 98.9%, respectively, before resolution of discrepant results.

Retrospective analysis of the 27 presumably false-positive PCR results is shown in Table 1. PCR products of 5 specimens with discrepant results were further analyzed by gel electrophoresis. The obtained amplicons of 278 bp and 176 bp represent parts of the *SCCmec* and *orfX* region, respectively (15). Of the 27 presumably false-positive PCR results, 15 specimens were considered true positive, while for the remaining 12 specimens discrepancies could not be resolved with certainty. PCR was, thus, considered false positive. Eventually, the BD GeneOhm MRSA assay resulted in a sensitivity of 84.3%, a specificity of 99.2%, and a PPV of 88.4% (Table 2). Sensitivity,

TABLE 2. Performance characteristics of the BD GeneOhm MRSA assay versus culture after resolution of discrepant results

Origin of swab (no. of specimens)	No. of results by PCR compared with culture ^a				% (95% CI)			
	TP	TN	FN	FP	Sensitivity	Specificity	PPV ^b	NPV ^b
Nose (672)	34	629	6	3	85.0 (70.9–93.0)	99.5 (98.6–99.9)	91.9 (78.6–97.3)	99.1 (97.9–99.6)
Groin (642)	30	603	5	4	85.7 (70.6–93.8)	99.3 (98.3–99.8)	88.2 (73.3–95.4)	99.2 (98.0–99.7)
Wound (150)	20	125	1	4	95.2 (77.3–99.2)	96.9 (92.2–98.8)	83.3 (64.1–93.4)	99.2 (95.6–99.9)
Other ^c (131)	7	118	5	1	78.3 (31.9–80.7)	99.2 (95.3–99.9)	87.5 (52.9–97.8)	95.9 (90.8–98.3)
Total (1,595)	91	1,475	17	12	84.3 (76.7–90.0)	99.2 (98.5–99.6)	88.4 (80.7–93.3)	98.9 (98.1–99.3)

^a TP, true positive; TN, true negative; FN, false negative; FP, false positive.

^b Estimated from patients at high risk for MRSA.

^c Includes specimens from axilla, throat, rectum, and vagina, as well as miscellaneous specimens.

specificity, PPV, and NPV of the assay were also separately calculated for nasal, inguinal, and wound swabs. The numbers of specimens from axilla, throat, rectum, and vagina were too low to draw any reliable conclusions (results under "Other," Table 2).

Independent of the type of specimen, the specificity of the PCR was high. Specificity of wound specimens (96.9%) was slightly lower than that of nasal (99.5%) and inguinal (99.3%) specimens. Eight of the 27 PCR-positive/culture-negative wound specimens contained methicillin-sensitive *S. aureus* (MSSA), a fact which has been observed also by others. For instance, Farley et al. (12) reported that 55% of the false-positive PCR specimens contained MSSA, some of the MSSA lysates being, again, positive upon repeated PCR testing. Likewise, Desjardins et al. (9) reported a false-positive PCR result with an MSSA strain (ATCC 25923) carrying an element similar to *SCCmec* which was inserted in the same integration site as *SCCmec*. In our clinical laboratory, MSSA strains were not tested for the presence of an *SCCmec* element with a *mecA* deletion.

The use of antiseptic wound dressings may lead to nonviability of the bacteria. We showed by gel electrophoresis that the analyzed PCR product from a wound specimen had the expected molecular size (15), but MRSA could not be cultured. For PCR-positive and culture-negative results observed in patients after/under decolonization by DNA (ranging from 1 year ago to present), noncultivable MRSA strains were most likely present in their specimens. This demonstrates that for such patients culture should be preferred, i.e., PCR should not be used to monitor the effectiveness of decolonization. However, it remains unclear how long PCR will be positive after a successful decolonization procedure.

Analyzing a low number of specimens collected in Amies agar, Drews et al. (11) used direct plating of single swabs on solid medium prior to PCR and reported a sensitivity of 96%. In contrast, we performed the PCR assay first followed by culture, which resulted in a sensitivity of merely 84.3%. This may be explained by the use of enrichment broth as the reference method, which yields conceivably a higher sensitivity than does direct plating (17, 20, 23). Overall sensitivity of PCR for all swabs ($n = 1,595$) in Amies agar corresponds well to the results reported previously using Stuart's medium (sensitivity ranging from 81% to 92.3%) (3, 8, 19, 22, 26). However, a direct comparison of Amies agar with Stuart's medium for the same clinical specimens has not been undertaken yet. In our hands, the sensitivity of the PCR from nasal specimens (85%) was similar to the sensitivity observed for inguinal specimens (85.7%), suggesting that inguinal swabs are suitable specimens for PCR screening as well. Sensitivity was highest for wound specimens (92.9%), which might reflect a higher number of MRSA strains present in this type of specimen.

The false-negative PCR results ($n = 17$) may largely be explained by low numbers of MRSA strains, i.e., below the detection limit of the assay (22). In agreement with others (10, 18, 25), the chromogenic medium used for subcultures from enrichment broth proved to be highly sensitive. In three cases, 1 to 2 CFU could be detected on the agar plate, while PCR was negative. When PCR was repeated from subcultures, all 17 initially false-negative results were positive. With this, a failure of the PCR assay due to possible variabilities in the *SCCmec* or

the *orfX* region that may have prevented amplification can be excluded (2, 13, 24). Conversely, false-negative culture results cannot be excluded, since culture never attains a sensitivity of 100% (23).

To a certain extent, the PPVs obtained in this study are biased by the swab-collecting strategy, inasmuch as only patients with a high risk of carrying MRSA were screened. Due to the low prevalence of MRSA in central Switzerland, we have obtained an expectedly low PPV (88.4%). A focused indication for PCR analysis by the clinician will undoubtedly increase the PPV.

As a whole, the true strength of the BD GeneOhm MRSA assay is its exceptionally high NPV (98.9%), making the test an ideal tool for rapid exclusion of MRSA carriers in hospitals. As a consequence, this would dramatically shorten the patients' isolation time in areas with low prevalence where high-risk patients are precautionarily isolated until the test result is negative. However, the overall sensitivity of 84.3% found in this study is a disadvantage for MRSA screening on admission in a setting of high prevalence since undetected MRSA carriers would not be appropriately isolated.

In conclusion, the BD GeneOhm MRSA assay represents a reliable screening test when applied to nasal, inguinal, and wound specimens. We have also demonstrated that swabs transported in Amies agar can reliably be used. With its excellent NPV, MRSA colonization can safely be ruled out by PCR, questioning the necessity of culture in low-prevalence settings. However, the BD GeneOhm MRSA assay should not be applied when patients have undergone decolonization, since the length of persistence of MRSA DNA is unknown and permanent carriage of MRSA cannot be excluded either.

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