BD GeneOhm-MRSA ASSAY FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* DIRECTLY IN NASAL AND NON-NASAL SWAB SPECIMENS FROM HAEMATOLOGIC PATIENTS

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Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are responsible for rising health care costs and have a high attribution to mortality. Reliable and rapid detection of MRSA carriage is essential. Real-time PCR allows an early detection of MRSA colonization within 2 h. By using the BD GeneOhm-MRSA assay we analysed directly swabs of different sampling sites and compared the assay with culture method. One thousand one hundred and sixty samples from 129 patients in Magdeburg were examined. Of the samples, 8 (0.69%) or 1117 (96.3%) were tested equally positive or negative by both methods whereas 16 (1.38%) specimens were MRSA positive only by the GeneOhm-MRSA assay and 6 (0.52%) were MRSA positive only by culture method. Thirteen samples (1.12%), which are culture negative, were unresolved by the GeneOhm-MRSA. With regard to the patients, seven were detected as MRSA carriers only by the GeneOhm-MRSA while one patient was tested positive for MRSA only by culture. Assuming 100% correct results by the culture method, sensitivity and specificity of GeneOhm-MRSA assay could be calculated as 84.4% and 96.1% for nasal swabs, 78.7% and 96.9% for all swabs under study, and 94.8% and 99.5% when focussed on patients. PPV and NPV were 70.3% and 98% for all specimens together, respectively. BD GeneOhm-MRSA assay is a sensitive test for the detection of MRSA colonization from swab specimens without the need for an initial culture, but should always be performed in parallel to the culture method for comparison reasons. Furthermore, our results indicate that in addition swabs taken from different body sites were successfully analysed by the BD GeneOhm-MRSA assay. However, we conclude that the PCR assay might not be a preferred tool for screening in haematologic patients with low MRSA rate; for screening haematologic patients, the culture method is sufficient enough.

Keywords: MRSA, screening, non-nasal specimens, haematology, real-time PCR

Introduction

Staphylococcus aureus is a major pathogen that causes a wide spectrum of infections in hospitalized patients and in the community setting [1–3]. Methicillin-resistant *S. aureus* (MRSA) is emerging steadily all over the world. MRSA spread occurs mainly in health-care settings [3]. Nasal colonization with MRSA provides an important and clinically unapparent reservoir of MRSA strains for subsequent nosocomial transmission and infection. Between 40% and 60% of people carrying MRSA will go on to develop related infections [4]. Propagation of MRSA is clonal and can only be controlled or reduced by preventive interventions such as detection and implementation of hygiene measures. In our institution, methicillin resistance in *S. aureus* is relatively low, approx. 10% of the isolates are resistant to methicillin; with the lowest occurrence at the department of haemato-oncology at the University Clinic in Magdeburg [5].

The time from culture inoculation to identification of MRSA is typically 48 h, with some methods taking as long as 96 h. The sensitivity of any single selective medium

method ranges between 65% and 100% [6]. Several techniques to shorten the time to identification of MRSA in the laboratory have been developed in the last decade, including slide latex agglutination assays to detect PBP2a [7, 8], a colorimetric cycling probe assay to directly detect the *mecA* gene [9] and real-time PCR methods to detect the *mecA* gene in conjunction with *S. aureus*-specific genome fragments [10, 11].

Warren et al. validated the IDI-MRSA assay for the detection of MRSA from nasal swabs of hospitalized patients and compared to culture-based methods [12]. The manufacturer does not recommend non-nasal specimens. However, besides the nasal area additional sites of MRSA colonization are the perineum, the anal area, the groins, the axillary areas and wounds.

We describe the routine use of the GeneOhm-MRSA PCR assay for the fast detection of MRSA in swabs obtained from different sampling sites of haemato-oncological patients including the anterior nares, the throat, the axillae, the peritoneum and different wound locations. So far, the patients from the department of haemato-oncology are

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interesting subjects due to the fact that they receive chemotherapeutics which might impact the PCR assay and on the other hand, the MRSA rate is low compared to the other departments from the same university clinic.

Materials and methods

This prospective study was performed in a German University Hospital that provides a comprehensive range of haematological patients ($n = 129$). One thousand one hundred and sixty different specimen swabs (nasal, pharyngeal, tracheal, wounds etc.) obtained from the respective patients were analysed from March to November 2007. COPAN (Brescia, Italy) swabs were used for sampling the different body sites.

MRSA screening by culture methods

In-house produced mannitol salt agar (MSA) plates were inoculated directly with specimen swabs prior to testing of the same swab with BD GeneOhm-MRSA assay. The agar permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and 6.0 µg of oxacillin per ml. MRSA colonies appeared yellowish on the chromogenic agar plate, whereas other colonies were inhibited. The plates were incubated at 37 °C for 18–48 h. Culture was observed for growth signs after 18 and 48 h.

Confirmation of the identification of the staphylococcal strains was done by use of the VITEK 2® (bioMérieux). The susceptibility results were interpreted according to the guidelines of the CLSI (Clinical and Laboratory Standards Institute) [13].

Processing swabs with the BD GeneOhm-MRSA assay

BD GeneOhm-MRSA utilizes PCR for the amplification of MRSA DNA and fluorogenic target-specific hybridization probes for the detection of the amplicons. The different specimen swabs were placed directly in sample preparation buffer, and after additional processing, an aliquot of the specimen is added to PCR reagents containing MRSA specific primers. PCR and detection of MRSA amplicons are performed on the SmartCycler instrument. Processing to detection requires about 1.5 h. The BD GeneOhm-MRSA assay includes an internal control DNA sequence with complementary primers that is incorporated into each sample to make sure that the PCR takes place. The internal control used is based upon a previously described linearized plasmid [14, 15].

Clinical samples

A total of 1160 swabs referred to our department for MRSA screening was used in this study. These swabs were ob-

tained from the following sites: the nose ($n = 465$), the genital ($n = 224$), the throat ($n = 242$), axillae ($n = 180$), the perineum ($n = 220$) and wounds plus ulcers ($n = 6$).

Data analysis

Sensitivity and specificity were determined by comparing the results of BD GeneOhm-MRSA™ assay with results of the culture method. Ninety-five percent confidence intervals (CI) for sensitivity and specificity were calculated by standard methods [16]. In the case of discordant results between culture methods and the BD GeneOhm-MRSA assay, the assay was repeated and the results were noted; however, for purposes of calculating sensitivity and specificity, only the initial result was considered.

Results

A comparison of the BD GeneOhm-MRSA assay to direct plating on MSA plates for the detection of MRSA is shown in Table 1. From 1160 specimens, BD GeneOhm-MRSA assay revealed 24 positive (2.07%), 1123 negative (96.81%) and 13 unresolved (0.96%) results, whereas culture detected 14 positive MRSA (1.21%) and 1146 negative results (98.8%). In detail, as is apparent from Table 1, among the total of 1160 specimens tested, 16 (1.38%) specimens were positive for MRSA only by the BD GeneOhm-MRSA assay, whereas vice versa 6 (0.52%) were positive for MRSA only by direct plating. Thus, 8 (0.69%) or 1117 (96.3%) specimens were positive or negative by the methods BD GeneOhm-MRSA and the direct plating, respectively. The six samples positive for MRSA only by culture were from nasal swabs ($n = 2$), pharyngeal/tracheal swabs ($n = 2$), genital swab ($n = 1$) and from anal swab ($n = 1$). The 16 specimens positive for MRSA only by BD GeneOhm-MRSA were mainly from nasal swabs ($n = 7$), pharyngeal/tracheal swabs ($n = 5$) and from genital/anal swabs ($n = 4$).

In three cases from a total of 16 BD GeneOhm-MRSA positive results methicillin-resistant but coagulase-negative staphylococci (*S. haemolyticus*, *S. epidermidis*, *S. simulans*) were cultured from the MSA plates. The respective pure cultures were tested by the BD GeneOhm-MRSA. All results were negative for MRSA. Thus, a misidentification of methicillin-resistant coagulase-negative staphylococci by

Table 1. Direct comparison of the results obtained by BD GeneOhm-MRSA assay and culture method showing the discrepancies in general ($n = 1160$)

Culture	PCR			Total
	Negative	Positive	Unresolved	
Negative	1117	16	13	1146
Positive	6	8	–	14
Total	1123	24	13	1160

the BD GeneOhm-MRSA assay could be excluded. Vice versa all culture-positive, BD GeneOhm-MRSA negative specimens were available for additional testing from stored culture plates. All isolates were identified as MRSA by the BD GeneOhm-MRSA assay. Therefore, failure of amplification by the respective primers may be excluded as well. This is true from the culture plate but not necessarily from the initial specimens.

Furthermore, there may be the possibility that the number of MRSA organisms present in the specimens was below the limit of detection for the BD GeneOhm-MRSA assay or vice versa for culture by the direct plating method. Therefore we reanalysed our data with regard to the individual patients. The 1160 specimens studied belonged to 129 different patients. From each patient, we obtained swabs from up to six different sampling sites. One hundred and twenty patients were excluded and only nine patients were identified as MRSA carriers by the BD GeneOhm-MRSA assay and the direct plating method, respectively. A patient was defined to be a MRSA carrier when at least one sample was tested positive for MRSA by the respective method. Nonetheless, seven patients were detected as MRSA carrier only by the BD GeneOhm-MRSA while one patient was tested positive for MRSA only by the culture method.

In case of unresolved PCR assays, we repeated testing with frozen lysates of the specimens. In total, 13 specimens (0.96%) were unresolved even after repeating the BD GeneOhm-MRSA assay. Details of information are given in Table 2. Among the 13 unresolved PCR results, four samples belonged to nasal swabs, four to pharyngeal swabs and five to genital/anal swabs. All respective specimens tested negative for MRSA by the direct plating method.

Assuming that direct plating detects all MRSA-positive specimens, the sensitivity and specificity of the BD GeneOhm-MRSA could be calculated as 57.14% and 98.6% for all sampling sites. When patients, and not specimen, were considered the sensitivity and specificity of the BD GeneOhm-MRSA were calculated as 94.8% and 99.5%, respectively.

Discussion

Standard culture methods for the identification of *S. aureus* and the determination of oxacillin susceptibility are time-consuming, usually requiring 2–4 days. For these reasons, it has become important to develop rapid diagnostic tests for the detection of MRSA [12]. To our knowledge, this is the first report on prospective data obtained due to the use of the BD GeneOhm-MRSA assay as a routine method for nasal and non-nasal specimens considering the department of haematology at university clinic with rather low MRSA prevalence rate as compared to other departments within the same clinic [5].

A preliminary evaluation of the IDI-MRSA™ assay with MRSA-spiked nasal samples indicated 100% sensitivity and 97.9% specificity compared with primary isolation and broth-enrichment techniques for the detection of MRSA [14, 15]. A subsequent retrospective evaluation with 1657 culture isolates of MRSA found a sensitivity of 98.7% and a specificity of 98.4% [14]. The IDI-MRSA™ has also been tested against nasal swabs from 288 patients processed with combined direct plating on MSA and another swab enriched in TSB with salt followed by screening of all presumptive *S. aureus* on 6% oxacillin screening agar. Values of 98.5% sensitivity and 92.8% specificity were obtained. Accordingly our results support and extend the previous findings with regard to nasal swabs. For direct comparison, we first picked up only the results of the nasal swabs ($n = 465$). One has to consider that the evaluation of our data is based on the assumption that 100% MRSA are isolates using the MSA plates. In nasal swabs, sensitivity and specificity values were 84.4% and 96.1% for the BD GeneOhm-MRSA assay, respectively. Among the 10 PCR-positive nasal MRSA isolates five were negative by culture method and in consequence culture failed to detect 50% positive nasal specimens as compared to the MRSA PCR assay. This may be due to the known limitations in sensitivity of selective culture methods in detecting MRSA from nasal swabs [6]. As shown by Warren et al. [12], neither the direct plating method alone nor the culture en-

Table 2. Comparative results for MRSA detection by both methods (BD GeneOhm-MRSA [BDGO] assay and culture) according to the sampling sites ($n = 1160$)

Sampling sites (n)	Culture negative			Culture negative total	Culture positive		Culture positive total
	BDGO negative	BDGO positive	BDGO unresolved		BDGO negative	BDGO positive	
Anal (220)	209	3	4	216	1	3	4
Genital (224)	221	1	1	223	1		1
Nasal (465)	449	7	4	460	2	3	5
Pharyngeal (236)	227	4	4	235	1		1
Sputum (3)	3			3			
Tracheal (6)	2	1		3	1	2	3
Wound (6)	6			6			
Total (1160)	1117	16	13	1146	6	8	14

BDGO, BD GeneOhm-MRSA assay

richment broth method alone detected 100% of the patients with MRSA-positive nasal colonization. MSA or variations of this medium have been extensively used as a primary screening medium for MRSA. The reported sensitivity of these media with clinical specimen has varied widely [17].

Although nasal carriage of *S. aureus* is accepted as one of the most important risk factors for nosocomial infections, doubtless other sites of a patient are important as well to be screened for MRSA. Studies have shown that in comparison to MRSA nasal carriers, the non-nasal-carriers who acquire exogenous *S. aureus* bacteraemia have fourfold increased mortality rate [18, 19]. Therefore, nasal swabs as well as swabs from different sampling sites were included in this study. A total of 1160 samples were analysed by the BD GeneOhm-MRSA and by direct plating under study. Sensitivity and specificity values of 78.7% and 96.9% were obtained for all the sampling sites.

There were 6 samples which were positive for MRSA by culture and negative by BD GeneOhm-MRSA assay. The reason for the false-negative BD GeneOhm-MRSA results may be the variability in either the *SCCmec* or *orfX* sequences that did not allow amplification with the panel of primers in the assay [20]. However, pure colonies from all MRSA detected by culture were directly tested by the BD GeneOhm-MRSA assay and all were identified as MRSA. Thus, amplification failure due to changed primer binding sites can be excluded. Another reason might be the fact that the specimens were directly plated on the culture agar prior to proceeding for PCR assay so that the sensitivity was influenced in a negative manner for the amplification assay. Indeed, we assume that the number of MRSA organisms present in the specimens was below the limit of detection (LoD) for the assay (~25 CFU per nasal swab) according to Huletsky et al. [14], though the manufacturer states that the kit has an LoD of 325 CFU per swab. The six PCR-negative samples belonged to three patients. Among these, only one culture-based MRSA positive patient was negative for all the specimens by BD GeneOhm-MRSA assay.

In total, 16 specimens were positive for MRSA by BD GeneOhm-MRSA assay only. The respective specimens were obtained from nine patients. However, among the nine patients, seven were negative for MRSA by culture in all specimens. The *mec* region can undergo deletion *in vivo* [21] and there has been a recent report in which the deletion of the *SCCmec* region has not been complete and some elements have been found to remain within the genome [22]. On the other site, PCR tests targeted at *mecA* detection in an *S. aureus* background can detect markedly heteroresistant strains, which may be difficult to detect in phenotypic tests, as recognized in the Netherlands [23]. Of 47 patients with culture-negative but PCR-positive study samples, 21 were probably true MRSA carriers based on patient history and/or positive culture on a new sample. The prospective study confirms the value of MRSA PCR assay for rapid screening of MRSA mucocutaneous carriage among in- and out-patients. Recently, de San et al.

[24] demonstrated similar discrepancies in various mucocutaneous specimens.

The majority of the unresolved BD GeneOhm-MRSA results ($n = 13$), which may be due to inhibition of the PCR, were obtained from patient's specimens of the haemato-oncology department and the medical ICU. A possible reason for the unresolved results might be due to the treatment which the patients received at these departments. Thus, PCR inhibition through medication might be a plausible explanation for unresolved results by the BD GeneOhm-MRSA assay. This is supported by the fact that all specimens from the individual patients (up to four swabs) gave unresolved results. Additionally, the patients from the haemato-oncology department were not detected as MRSA positive elsewhere in any previous investigation and this department is among the low-rate departments for MRSA [5]. Further investigations are in progress in order to resolve the problem of inhibition.

According to the sensitivities of the BD GeneOhm-MRSA assay under study, 78.7% for all the body sites and 84.4% for the nasal specimens, it is not acceptable to perform this screening method for the detection of MRSA colonization directly from swabs without direct culturing method, especially if sampling was done from a single body site of the patient. The same day-result accounts as an advantage of the BD GeneOhm-MRSA assay. But caution is needed in cases of discrepant results as compared to culture methods. Rapid screening on admission of high-risk patients for MRSA is one of the control measures to limit the spread of MRSA strains. Though culture-based methods are performed with less costs, these are indeed time-consuming and misinterpretation occurs when methicillin resistance is simulated by hyperproduction of beta-lactamase [3] or coagulase activity is not convincing enough [25]. Based on our results, BD GeneOhm-MRSA assay needs rather more amendments in terms of primer sequences and cycling processes to improve the efficiency and effectiveness of measures to control the spread of this resistant organism in health-care facilities. The false-positive MRSA results revealed by the BD GeneOhm-MRSA assay may be resolved by comparing the results of the patient's different sampling sites or on the basis of the patient's MRSA history as also applied in the recent study of Struelens [25]. BD GeneOhm-MRSA-positive/culture-negative isolates require DNA sequencing to look for "*mecA* drop-outs" as the assays target the right junction of *SCCmec* and not *mecA* itself. However, culture methods should be done in parallel and culture method still cannot be replaced totally by BD GeneOhm-MRSA assay. Based on the presented data, we do not recommend the PCR assay for MRSA screening in patients at the department of haematology/oncology, although both the sensitivity and NPV were exceptionally high for the BD GeneOhm-MRSA assay, as also shown elsewhere [26, 27, 28]. However, culture method is the gold standard because the lower PPV for BD GeneOhm-MRSA assay, besides the susceptibility testing, can be performed for epidemiological issues.

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