JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2004, p. 5578–5581 Vol. 42, No. 12
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.12.5578–5581.2004
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Detection of Methicillin-Resistant *Staphylococcus aureus* Directly from Nasal Swab Specimens by a Real-Time PCR Assay

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Received 23 April 2004/Returned for modification 22 June 2004/Accepted 3 August 2004

Screening for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) is a key aspect of infection control to limit the nosocomial spread of this organism. Current methods for the detection of MRSA in clinical microbiology laboratories, including molecularly based techniques, require a culture step and the isolation of pure colonies that result in a minimum of 20 to 24 h until a result is known. We describe a qualitative in vitro diagnostic test for the rapid detection of MRSA directly from nasal swab specimens (IDI-MRSA; Infectio Diagnostic, Inc., Sainte-Foy, Québec, Canada), based upon a real-time PCR and direct detection of MRSA via amplicon hybridization with a fluorogenic target-specific molecular beacon probe. Samples from 288 patients were analyzed for the presence of MRSA with the IDI-MRSA assay, compared to detection by either direct plating or enrichment broth selective culture methods. The diagnostic values for this MRSA screening method were 91.7% sensitivity, 93.5% specificity, 82.5% positive predictive value, and 97.1% negative predictive value when compared to culture-based methods. The time from the start of processing of specimen to result was approximately 1.5 h. In our hands, the IDI-MRSA assay is a sensitive and specific test for detection of nasal colonization with MRSA and providing for same-day results, allowing more efficient and effective use of infection control resources to control MRSA in health care facilities.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been steadily increasing as a cause of infections among hospitalized patients in the United States since it was first reported in the 1960s. According to the National Nosocomial Infection Surveillance System of the Centers for Disease Control and Prevention, in 2002 MRSA accounted for 57.1% of all *S. aureus* isolates obtained from patients in more than 300 participating intensive care units throughout the United States (20). Large outbreaks of MRSA in other institutions, such as correctional facilities (4, 22), and among otherwise healthy individuals in the community (15) raise the concern that this organism is spreading outside of its traditional role as a health care-related pathogen. Infections caused by MRSA result in increased lengths of hospital stay, health care costs, morbidity, and mortality (10, 21, 24) compared to those caused by methicillin-sensitive strains.

Infection control measures, such as placing hospitalized patients colonized or infected with MRSA in contact precautions (i.e., the use of gowns and gloves), have been demonstrated to limit the spread of this pathogen (5, 13). The use of surveillance cultures (e.g., anterior nares, axillae, and perineum) greatly improves the detection of MRSA colonization compared to clinical cultures alone (7). The anterior nares is the most frequent site of MRSA colonization, with a single culture from this site having a sensitivity of approximately 85% (7, 26).

Methicillin resistance in *Staphylococcus* spp. is primarily mediated by the *mecA* gene, encoding penicillin-binding protein 2a (PBP2a). This protein has reduced affinity for β-lactam antibiotics. Because the *mecA* gene is heterogeneously expressed in vitro (6), selective media are necessary to facilitate recovery of MRSA in culture. The time from culture inoculation to identification of MRSA is typically 48 h, with some methods taking as long as 96 h (25). Furthermore, the sensitivity of any single selective medium method ranges between 65 and 100% (25). 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 (2, 17, 19, 30, 32), a colorimetric cycling probe assay to directly detect the *mecA* gene (1, 16, 31), and real-time PCR methods to detect the *mecA* gene (3, 8, 9, 14, 16, 23, 27) in conjunction with *S. aureus*-specific genome fragments, such as *nuc* (8, 9, 16) and *sa442* (23, 27). While these assays are sensitive in detecting MRSA, they are unable to distinguish MRSA from *mecA*-positive strains of coagulase-negative *Staphylococcus* spp. in mixed specimens, such as those obtained from the anterior nares, and therefore still require initial culture and identification steps.

IDI-MRSA (Infectio Diagnostic, Inc., Sainte-Foy, Québec, Canada) is a qualitative in vitro diagnostic test for the rapid detection of MRSA directly from nasal swabs. The test utilizes the real-time PCR method for the amplification of an MRSA-specific DNA sequence recovered from clinical samples and fluorogenic target-specific hybridization with a molecular beacon probe (29) for the detection of the amplified MRSA DNA. The sequences targeted in this assay are within *SCCmec*, the mobile genetic element which harbors the *mecA* gene (12), and *orfX*, a highly conserved open reading frame in *S. aureus* which is the site of *SCCmec* integration into the genome; the exact target sequences have been described previously (11). In the presence of these sequences, target-specific primers within the assay will bind and generate an MRSA-specific amplicon dur-

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ing the PCR, which is then detected by a complementary molecular beacon probe. We describe the validation of this assay for the detection of MRSA from swabs obtained from the anterior nares of hospitalized patients, compared to detection of MRSA by either of two culture-based methods.

MATERIALS AND METHODS

Study enrollment and collection of clinical specimens. Study subjects were selected from inpatients at Barnes-Jewish Hospital, a 1,442-bed academic, tertiary care hospital in St. Louis, Mo. Subjects were selected from both new and currently admitted patients. Patients were considered for inclusion in the study if they met any of the following criteria: (i) prior MRSA infection or colonization, (ii) current hospital stay exceeding 3 days, or (iii) known colonization or infection with a hospital-acquired pathogen (e.g., vancomycin-resistant *Enterococcus* spp.). These criteria were chosen to select a patient population at high risk for MRSA nasal colonization. Patients were excluded from the study if they had received treatment with intranasal mupirocin or polysporin in the previous 14 days, treatment with oral antimicrobials for the purpose of eradicating MRSA colonization within the past 14 days (including oral rifampin therapy for any reason), or had contraindications to nasal sampling. After written informed consent was obtained, each patient had a specimen collected from the nares with a dry, unmoistened swab (Venturi Transystem; Copan Diagnostics, Corona, Calif.). The tip of the collection swab was inserted approximately 1 in. (2.56 cm) into the nares and rolled five times in each nostril. Collected specimens were transported and stored at room temperature. Cultures were inoculated and specimens were processed for PCR analysis within 24 h of being collected (time to culture inoculation: median, 1.7 h; range, 0.3 to 17.9 h; time to processing for PCR, median, 14.4 h; range, 2.7 to 19.0 h). Each collection swab was initially inoculated into *S. aureus* selective agar and then into a buffer solution for the IDI-MRSA assay and finally into *S. aureus* selective broth medium (see below). This study was approved by the Washington University Human Studies Committee.

Selective media and culture conditions. Mannitol salt agar plates (Becton Dickinson, Cockeysville, Md.) were inoculated directly with specimen swabs prior to testing of the same swab with the IDI-MRSA assay. The mannitol salt agar plates were incubated for 24 to 48 h at 35°C and examined for growth. Strains that produced yellow colonies on mannitol salt agar screen were confirmed as *S. aureus* with Gram stain, 3% catalase testing, and coagulase testing with the Staph Latex agglutination assay (LifeSign, Somerset, N.J.). After the specimen swabs were processed for testing with the IDI-MRSA assay, the swabs were used to inoculate an enrichment broth medium containing tryptic soy broth and 6.5% sodium chloride (Remel, Lenexa, Kans.). The enrichment broth was incubated overnight at 35°C and then subcultured to 5% sheep blood agar (Becton Dickinson) for 18 to 24 h at 35°C. Colonies on the 5% sheep blood agar plates consistent with those for *S. aureus* were confirmed as previously described.

Detection of MRSA by culture methods. Confirmed *S. aureus* isolates were subcultured from the 5% sheep's blood agar plates onto oxacillin screen agar containing 6.0 μg of oxacillin per ml (Becton Dickinson). Plates were incubated at 35°C for 18 to 24 h and examined for evidence of growth. Strains showing distinct growth were considered to be methicillin resistant.

Processing specimen swabs with the IDI-MRSA assay. Specimens were processed for PCR analysis per the manufacturer's instructions. Cell lysis and DNA preparation were achieved with a set of two tubes per patient sample provided with the IDI-MRSA kit. Nasal swabs were initially vortexed in a tube containing 1 ml of sample buffer, and 850 μl of this was transferred to a lysis tube. Following centrifugation at 21,000 × *g* for 5 min and removal of the supernatant, 50 μl of sample preparation buffer was then added to each lysis tube and bacterial cell lysis was achieved by vortexing for 5 min. Lysis tubes were then centrifuged, heated for 2 min at 95°C, and stored at 4°C prior to analysis by real-time PCR.

Real-time PCR using the IDI-MRSA assay. PCR tubes containing the lyophilized master mix were reconstituted with diluent, and specimen DNA was added. The PCR was performed with a Smart Cycler II device (Cepheid, Sunnyvale, Calif.). The run time for the PCR component of the assay was 45 min. To ensure that the PCR takes place, the IDI-MRSA assay includes an internal control DNA sequence with complementary primers that is incorporated into each sample. The internal control used is based upon a previously described linearized plasmid (11) and consists of a 335-bp DNA fragment composed of a 277-bp non-MRSA sequence flanked by the sequences of the meciv511 and mecv492 primers. This fragment was cloned into the pCR2.1 vector (Invitrogen, Burlington, Ontario, Canada) and linearized for use in the assay. External positive and negative controls were included with each PCR run. PCRs of individual specimens were repeated if the internal control was invalid due to the presence of inhibitors or if the positive or negative controls were invalid.

Data analysis. Statistical analysis was performed with SPSS for Windows, version 11.0 (SPSS, Inc., Chicago, Ill.). Ninety-five percent confidence intervals (CI) for sensitivity and specificity were calculated by standard methods (28). In the case of discordant results between culture methods and the IDI-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

Nasal swab specimens were obtained from 290 patients between June and September 2003. Two patients were subsequently found to be ineligible for the study due to concurrent rifampin-containing therapy for mycobacterial infections. Of the samples from the remaining 288 patients, 2 (0.7%) with negative internal controls due to the presence of PCR inhibitors were examined again. Both samples were resolved upon repeat testing. Thus, 288 specimens were valid for determining sensitivity and specificity. Sixty-four (22.2%) of the source patients had a past history of MRSA infection of colonization, and 203 (70.5%) had received oral or intravenous antimicrobial therapy within the 2 weeks prior to testing.

Of the 288 specimens tested, 72 (25%) were positive for MRSA by direct plating or broth enrichment methods. Fifty-nine (20.5%) specimens were positive for MRSA by both culture methods, while 7 (2.4%) specimens were MRSA positive only by broth enrichment, and 6 (2.1%) were positive by the direct plating method alone.

A comparison of the IDI-MRSA assay to culture methods for the detection of methicillin-resistant *S. aureus* is shown in Table 1. Compared to the direct plating method alone, the assay had a sensitivity of 98.5% (95% CI, 95.5 to 100%) and a specificity of 92.8% (95% CI, 89.4 to 96.2%). However, the direct plating method alone detected only 65 of 72 (90.3%) patients with nasal colonization with MRSA. Likewise, the enrichment broth method detected 66 (91.7%) of MRSA nasal carriers. When compared to either culture method, the IDI-MRSA assay had a sensitivity of 91.7% (95% CI, 85.3 to 98.1%) and a specificity of 93.5% (95% CI, 90.2 to 96.8%), with a positive predictive value of 82.5% and negative predictive value of 97.1%. To examine the effects of delayed processing on assay sensitivity, when specimens that were processed for PCR analysis at greater than the median time after collection (14.4 h; $n = 145$) were considered, the sensitivity and specificity of the assay were 90.0 and 96.5%, respectively. Typically, 8 to 10 specimens were batched for processing and analyzed with external positive and negative control samples per each run. The time from specimen centrifugation to generation of results for this number of specimens was approximately 1.5 h.

DISCUSSION

Rapid identification of MRSA colonization is critical to the effectiveness of infection control, with delays in detection resulting in either late institution of infection control measures and resultant occult transmission of MRSA between patients or unnecessary contact precautions being applied to high-risk patients, resulting in increased hospital cost. In our hands, the IDI-MRSA assay demonstrated a sensitivity and specificity

TABLE 1. Sensitivity and specificity of the IDI-MRSA real-time PCR assay compared to culture methods for detection of MRSA from nasal swabs

Culture method result[a]	No. of samples with IDI-MRSA assay result:		% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (%)[b]	NPV (%)[c]
	Positive	Negative				
Direct plating			98.5 (95.5–100)	92.8 (89.4–96.2)	80	99.5
Positive	64	1				
Negative	16	207				
Enrichment broth			92.4 (86.0–98.8)	91.4 (87.8–95.1)	76.2	97.6
Positive	61	5				
Negative	19	203				
Either culture method			91.7 (85.3–98.1)	93.5 (90.2–96.8)	82.5	97.1
Positive	66	6				
Negative	14	202				

[a] Direct plating involved culture on mannitol salt agar followed by subculture on Mueller-Hinton agar supplemented with 6 μg of oxacillin/ml. The enrichment broth method involved culture in tryptic soy broth with 6.5% NaCl followed by subculture on Mueller-Hinton agar supplemented with 6 μg of oxacillin/ml.
[b] NPV, negative predictive value.
[c] PPV, positive predictive value.

above 90% for the detection of MRSA nasal colonization compared to two selective culturing methods. The assay can be performed by a single technologist, after receiving appropriate training, and allows same-day results with batching of specimens. The Smart Cycler system used in this study consisted of a single unit that can run 16 samples at once; however, up to six units per controller can be used, allowing 96 samples to be tested simultaneously.

Previous real-time PCR assays (8, 14, 16, 23, 27) have demonstrated the capability of rapidly detecting MRSA from culture. Reischl et al. (23) reported a duplex assay for *mecA* and the *S. aureus*-specific *sa442* genome fragments using paired FRET probes with a Light Cycler (Roche Diagnostics Corp., Indianapolis, Ind.) real-time PCR instrument. They reported 100% sensitivity and specificity for detecting MRSA from pure colonies. Elsayed et al. (8) used a duplex molecular beacon-based real-time PCR assay containing primers and molecular beacon probes to sequences within the *mecA* gene and an *S. aureus*-specific *nuc* gene. The authors tested 181 strains, including methicillin-sensitive and -resistant *S. aureus*, methicillin-sensitive and resistant coagulase-negative *Staphylococcus* spp., and nonstaphylococcus bacteria, and reported 100% sensitivity and specificity for this assay in detecting and differentiating *Staphylococcus* spp. from pure strain isolates. However, neither of the described assays was able to differentiate methicillin-sensitive *S. aureus* from methicillin-resistant coagulase-negative *Staphylococcus* spp. in primary specimens where both of these organisms could coexist, such as the anterior nares. By utilizing primer sequences for *SCCmec* and *orfX* regions, an MRSA-specific amplicon is generated, which is then detected by a complementary molecular beacon probe. Theoretically, other specimens (i.e., wound, blood, and perineum) could be directly tested for the presence of MRSA using this assay, but this would require further validation.

The assay in our hands was not 100% sensitive or specific, compared to prior real-time PCR assays performed on pure culture isolates (8, 23). Six of the samples that were positive by at least one of the culture methods were negative by the IDI-MRSA assay. This was not due to the presence of inhibitors to the PCR, as the internal control in each of these tubes was positive. Four of these culture-positive, PCR assay-negative specimens were available for additional testing from saved broth cultures. *S. aureus* was isolated from all four specimens by inoculation of 5% sheep blood agar plates and identification methods described above. Three of these specimens were found to grow on subculture to oxacillin screen agar and therefore were felt to represent phenotypic MRSA. The presence of the *mecA* gene in these three isolates was examined with a *mecA* gene-specific PCR assay described by Martineau et al. (18); all three isolates were negative for the *mecA* gene, suggesting that methicillin resistance in these isolates was mediated by methods other than PBP2a, such as hyperproduction of β-lactamase or modified PBP genes (6). Other potential explanations for why the assay failed to detect MRSA in false-negative PCR assay samples include possible variability in either the *SCCmec* or *orfX* sequences that did not allow amplification with the panel of primers in the assay or the possibility that the number of MRSA organisms present in the specimens was below the limit of detection for the assay. Conversely, 14 specimens were positive for MRSA by PCR assay and negative by either culture method. This may be due to the known limitations in sensitivity of selective culture methods in detecting MRSA from nasal swabs (25). Cross-contamination of specimens during preparation is also a possibility—3 of the 14 samples were PCR negative upon repeated testing with saved lysate solution. We also noted that the broth enrichment culture method in this study was as sensitive as the direct plating method used to detect MRSA, in contrast to previous studies (25). This might have been due to the order of inoculation in our study (i.e., the broth was inoculated after the direct plating and processing for the PCR assay).

In summary, the IDI-MRSA assay is a sensitive and specific test for the detection of MRSA nasal colonization directly from a swab specimen, without the need for an initial culture. This assay can be performed by a microbiologist technician with minimal additional training and allows same-day results, even with batching of specimens. This promises to improve the

efficiency and effectiveness of measures to control the spread of this resistant organism throughout health care facilities.

ACKNOWLEDGMENTS

This project was funded in part by a research grant from Infectio Diagnostic, Inc., who had no part in the recruitment of patients or analysis of data for this study.

We thank Godson Onwubiko for enrolling patients and obtaining specimens. We also acknowledge Cherie Hill and Stacy Leimbach for the management of research data for this study.

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