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Evaluation of the IDI-MRSA Assay for Detection of Methicillin-Resistant *Staphylococcus aureus* from Nasal and Rectal Specimens Pooled in a Selective Broth

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Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) by PCR can be performed directly from nasal specimens with the IDI-MRSA assay. To improve the efficiency of screening, we evaluated the performance of the IDI-MRSA assay for the detection of MRSA from pooled and unpooled specimens cultured in a selective broth. Of the 287 specimens evaluated, 71 were culture and PCR positive, 203 were culture and PCR negative, 3 were culture positive and PCR negative, 8 were culture negative and PCR positive, and 2 remained inhibited. A methicillin-susceptible *Staphylococcus aureus* isolate was recovered from five of the eight specimens with false-positive PCR results. Compared to the results of culture, the sensitivity, specificity, and negative and positive predictive values of the IDI-MRSA assay for detection of MRSA from broth were 96%, 96%, 90%, and 98%, respectively. Following implementation of the IDI-MRSA assay, PCR-positive broths were subcultured for evaluation of assay performance. Of the 298 IDI-MRSA assay-positive broths, the results for 103 could not be confirmed by culture. A methicillin-susceptible *S. aureus* (MSSA) isolate was recovered from 77 of these 103 broths. Repeat testing by the IDI-MRSA assay directly with the MSSA isolates confirmed the original positive PCR result. The positive predictive value of the IDI-MRSA assay fell from 90% during the evaluation phase to 65% postimplementation. The IDI-MRSA assay performed well for the detection of MRSA from a selective broth compared to the performance of the detection of MRSA from culture. However, because of the burden associated with implementation of infection control precautions, cultures remain essential in confirming positive IDI-MRSA results.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen; and infections due to this organism have been associated with increased morbidity, mortality, and prolonged hospitalization (5, 23). According to the Canadian Nosocomial Infection Surveillance Program, MRSA rates across Canada increased from <1% in 1995 to 10% in 2003 (4). Although this rate is not as prevalent as that in the United States, a 10-fold increase in MRSA rates over this 8-year period is cause for concern.

Screening for MRSA colonization largely relies on the culture of specimens from appropriate sites, such as the nares, rectum, axilla, and wounds. Reliable detection of MRSA has traditionally depended on culture by using either selective broth or agar medium. However, depending on the culture method, a 2- to 4-day turnaround time for isolation, identification, and confirmation of MRSA can be expected (2, 3, 26).

Increasing MRSA rates place additional burden on hospital infection control programs, leading to increased costs and burdens to hospitals and patients (15). Detection of MRSA colonization within 24 h would facilitate identification of carriers and allow the early implementation of isolation precautions for colonized patients in hopes of preventing spread to other patients. Several nucleic acid amplification-based assays for detection of MRSA from clinical specimens or for culture confirmation have been described (6, 9, 10, 12, 24, 25, 31). The IDI-MRSA assay (GenOhm, San Diego, CA) is a multiplex qualitative real-time PCR assay for detection of MRSA from nasal swabs. Differentiation of *mecA*-positive *S. aureus* from coagulase-negative staphylococci (CoNS) is possible by targeting the right extremity sequences of the staphylococcal cassette chromosome *mec* (SCC*mec*) element and the 3′ end of the *S. aureus orfX* gene (12). Five primers targeting the currently recognized SCC*mec* right-junction sequences corresponding to SCC*mec* types I, II, III, IVa, IVb, and IVc are combined with one primer and three molecular beacons specific for the *orfX* gene. Currently, the IDI-MRSA assay is approved for use for the direct detection of MRSA from nasal swabs. The lack of approval for use with pooled specimens and specimens from sites other than the nares presents a logistical problem, since screening of multiple body sites is necessary to achieve optimal detection of MRSA carriers (27). Processing of individual specimens for PCR testing would be cost prohibitive and impractical. To facilitate processing and to reduce the workload, the performance of the IDI-MRSA assay for detection of MRSA from specimens pooled in a selective enrichment broth was evaluated. We also report on the postimplementation monitoring of this assay and the characterization of isolates with discrepant results.

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MATERIALS AND METHODS

Specimen collection. Screening of high-risk patients for MRSA colonization was performed on admission according to established Ottawa Hospital Infection Control policies. The patients included those who were direct transfers from another health care facility, those with a previous admission to any health care facility in the previous 6 months, and those with a previous history of infection with an antibiotic-resistant organism (MRSA, vancomycin-resistant *Enterococcus*, and extended-spectrum beta-lactamase-producing organisms). Nasal and rectal specimens and up to two wound swabs were obtained from at-risk patients and submitted to the laboratory in Amies transport medium (Starplex Scientific, Etobicoke, Ontario, Canada).

MRSA screening by broth culture. Swabs collected at the same time from each individual patient were cultured in a single tube of selective broth consisting of brain heart infusion broth (Becton Dickinson, Sparks, MD) supplemented with 5 μg/ml of aztreonam (ICN, Costa Mesa, CA) and 75 μg/ml of ceftizoxime (GlaxoSmithKline, Inc.) (B. Slinger, A. Lee, C. Martel, I. Gaboury, P. Jessamine, K. Ramotar, and B. Toye, 70th Conjoint Meeting, Canadian Association of Clinical Microbiology and Infectious Diseases, abstr. D4, 2002). Because of the low MRSA prevalence in our patient population, specimens for MRSA screening were selected to provide an adequate number of MRSA-positive samples in order to properly evaluate the IDI-MRSA assay. Two swabs were pooled in a single tube of broth, incubated overnight at 35°C in ambient air, and subcultured to 5% sheep blood agar (Trypticase soy agar; Oxoid Inc., Ottawa, Ontario, Canada). The *S. aureus* isolates recovered from the broths were screened for methicillin resistance by using oxacillin (6 μg/ml) salt agar screen plates (MH-Ox; Becton Dickinson). Confirmation of methicillin resistance was performed by disk diffusion testing with 30-μg cefoxitin disks (Oxoid, Ottawa, Ontario, Canada), according to Clinical and Laboratory Standards Institute (formerly NCCLS) recommendations (20, 21, 22), and detection of the modified penicillin binding protein (PBP 2′) by latex agglutination (Oxoid Inc.).

MRSA PCR testing from broth. Screening for MRSA by the IDI-MRSA assay was performed by transferring a 50-μl aliquot of the overnight broth into the sample reagent buffer provided with the assay kit (GenOhm). The PCR was then performed according to the manufacturer's instructions by using a SmartCycler II device (Cepheid, Sunnyvale, CA). Lysates found to be inhibited on initial PCR testing were frozen and thawed at −20°C for 1 h before they were retested. Repeatedly inhibited specimens were reported as unresolved. The sensitivity, specificity, and positive and negative predictive values for the IDI-MRSA assay were calculated by comparison to the results of broth culture of the specimens, which was considered the "gold standard."

Characterization of discrepant *S. aureus* isolates. Methicillin-susceptible *S. aureus* (MSSA) isolates that were recovered from PCR-positive broths but that failed to grow on MH-Ox were further characterized for oxacillin susceptibility by cefoxitin (30-μg) disk diffusion testing and MIC determination by agar dilution according to the Clinical and Laboratory Standards Institute recommendations (21), repeat testing on MH-Ox screen plates, and determination of PBP 2′ expression by latex agglutination. Testing of the MSSA isolates was also performed by the IDI-MRSA assay directly with the pure colonies.

Postimplementation evaluation of the IDI-MRSA assay. Following implementation of the IDI-MRSA assay as our routine method for MRSA detection, positive broths were subcultured onto blood agar (BA) and incubated overnight at 35°C. *S. aureus* isolates recovered on subculture were characterized for oxacillin susceptibility by cefoxitin disk diffusion, growth on MH-Ox screen plates, and determination of PBP 2′ expression by latex agglutination. Detection of the *mecA* gene and confirmation of *S. aureus* identification were performed by a multiplex PCR assay by using a previously described method with *mecA* and *nuc* gene-specific primers and 16S rRNA-specific primers as internal controls (17). The expected *nuc* (624-bp), *mecA* (1,235-bp), and 16S rRNA (228-bp) products were separated on a 2% agarose minigel.

PFGE of *Staphylococcus aureus*. Genotyping by pulsed-field gel electrophoresis (PFGE) of MSSA isolates that were positive by the IDI-MRSA assay was performed as described previously (29). SmaI digests of genomic DNA were electrophoresed in a 1% pulse-field-certified agarose gel by using a CHEF DR III apparatus (Bio-Rad, Hercules, CA). The gels were stained in ethidium bromide and photographed after UV transillumination. Restriction fragment profiles were compared visually and interpreted based on the guidelines of Tenover and colleagues (30). Isolates were classified according to the Canadian nomenclature (C-MRSA), as described previously (28).

TABLE 1. Evaluation of IDI-MRSA PCR assay for detection of MRSA isolates from 287 nasal and rectal swabs pooled in a selective broth compared to culture

IDI MRSA PCR result[b]	No. of swabs with the following selective broth culture result:	
	Positive	Negative
Positive	71	8[a]
Negative	3	203

[a] Among the eight specimens with false-positive results, two were from patients whose other specimens were PCR and culture positive. For one patient, no additional specimen was available to resolve the PCR assay result. For five patients, an MSSA isolate that was IDI-MRSA PCR was recovered.

[b] The sensitivity, specificity, and positive and negative predictive values for the IDI-MRSA PCR assay were 96%, 96%, 90%, and 98%, respectively. Two specimens remained inhibited, and both were culture negative (data not included in table).

RESULTS

MRSA detection by the IDI-MRSA assay. A total of 174 of 287 broths were inoculated with pooled nasal and rectal swabs. The other 113 were inoculated with individual swabs: 53 nasal swabs, 28 rectal swabs, and 32 other swabs (of catheter and tracheal sites and wounds). Of 287 broths, 74 were MRSA culture positive and 213 were MRSA culture negative. For two broths, the PCR assay remained unresolved. Among the MRSA culture-positive broths, 71 were positive and 3 were negative by the PCR assay (Table 1). For the three PCR false-negative broths (culture positive, PCR negative), one was a rectal swab from a patient found to be MRSA PCR and culture positive from a nasal specimen collected on the same day. The second false-negative result was for a patient who was found to be PCR positive on subsequent screening. For the remaining patient, the false-negative IDI-MRSA assay result from pooled nasal and rectal swabs could not be resolved, as additional specimens from the same patient were not available for further testing.

For the 213 culture-negative broths, 203 were negative, 8 were positive, and 2 remained unresolved by the IDI-MRSA assay (Table 1). Of the eight false-positive PCRs (culture negative, PCR positive), three were for previously MRSA culture-positive patients; and for the remaining five, an *S. aureus* isolate was recovered on culture of the broth. Oxacillin susceptibility was confirmed for all five of these isolates by cefoxitin disk diffusion testing, the absence of growth on the MH-Ox agar, and the lack of PBP 2′ expression (data not shown). False-positive results were found for all types of specimens and regardless of whether the swabs were pooled or not pooled.

Compared to the results of culture, the sensitivity, specificity, and negative and positive predictive values of the IDI-MRSA assay for detection of MRSA from the selective broth were 96%, 96%, 90%, and 98%, respectively.

Postimplementation evaluation of the IDI-MRSA assay. Following implementation of the IDI-MRSA assay as routine practice at our hospital for MRSA surveillance swabs, all PCR-positive broths were subcultured to BA. From November 2004 to February 2005, 4,201 broths were processed, with 298 (7.1%) being positive by the PCR assay. Of these, MRSA was

TABLE 2. Postimplementation culture outcomes of IDI-MRSA PCR-positive broths

Culture outcome	No. (%) of broths	No. (%) of patients
MRSA	195 (65)	93 (62)
MSSA[a]	77 (26)	38 (25)
No *S. aureus*	26 (9)	19 (12)
Total	298	150

[a] The positive IDI-MRSA PCR result was confirmed by repeat testing of pure cultures of the isolated MSSA isolate.

TABLE 3. Characterization of the 38 IDI-MRSA PCR-positive MSSA isolates

mecA PCR result	No. of isolates					Oxacillin MIC_{90}[a] (μg/ml)
	Total	*nuc* positive	With growth on MH-Ox	Cefoxitin susceptible	Positive for PBP 2′ expression	
Negative	36	36	0	36	0	0.5
Positive	2	2	0	2	0	0.5

[a] MIC_{90}, MIC at which 90% of isolates are inhibited.

cultured from 195 broths (65%) and the result was confirmed (Table 2). Cultures were negative for *S. aureus* for 26 (9%) of the PCR-positive broths, and an MSSA isolate was recovered from 77 (26%) broths of specimens from 38 patients (Table 2).

Characterization of IDI-MRSA assay-positive MSSA. We selected 38 MSSA isolates (one from each patient) for further study. None of these isolates grew on MH-Ox screening agar, all were susceptible to cefoxitin by disk diffusion, and PBP 2′ was not detected by latex agglutination (Table 3). The oxacillin MIC at which 90% of isolates are inhibited for all 38 isolates was 0.5 μg/ml. Repeat testing by the IDI-MRSA assay directly with these MSSA isolates confirmed the original positive PCR result for the pooled specimens. By multiplex PCR, *nuc* was detected in all 38 isolates and the *mecA* gene was identified in 2 of the 38 isolates (Table 3).

Genetic profile of IDI-MRSA assay-positive MSSA. Seventeen different PFGE genotypes were identified among the 38 IDI-MRSA assay-positive MSSA isolates. We found evidence of patient-to-patient transmission for two of these strains in two unrelated events involving four patients (data not shown). For 21 of the isolates, the PFGE patterns were consistent with common the MRSA genotypes found in this region. For 11 of these isolates the genotypes were consistent with variants of C-MRSA type 2 (U.S. MRSA clone USA 500), for 2 isolates the genotypes were consistent with variants of C-MRSA type 1 (U.S. MRSA clone USA 100), and for 8 isolates the profiles were consistent with uncommon MRSA clones previously identified at The Ottawa Hospital. The profiles for 17 isolates were not consistent with those for currently recognized MRSA clones from this region.

DISCUSSION

Rapid detection of MRSA-colonized patients has the potential of improving patient care and positively affecting hospital infection control practices. The development of simple single-step nucleic acid amplification assays for MRSA detection has been complicated by difficulties with the reliable determination of the origin of *mecA* in mixed cultures containing both CoNS and *S. aureus*. Since optimal screening for MRSA colonization is not limited to the testing of nasal swab specimens, alternative approaches that allow pooling of multiple specimens and testing of the pooled specimens in a single PCR are desirable. Use of a broth-PCR method for detection of MRSA has been described previously and has been implemented for routine screening for MRSA colonization (24). By this approach, a quantitative real-time PCR assay for detection of *nuc* is required to differentiate the growth of MRSA from that of MSSA from clinical specimens pooled in a selective broth. The IDI-MRSA assay, a commercial PCR method, is designed to be able to differentiate both CoNS and MSSA from MRSA in clinical specimens in a rapid single-step procedure.

We did not find that testing of pooled specimens from broth interfered with the performance of the IDI-MRSA assay, but rather, it was reliable and performed well compared to the performance of culture of nasal and rectal swabs pooled in a selective broth. Although rectal swabs should also be considered for use for screening for MRSA colonization, testing of specimens from rectal sites by amplification methods may be problematic because of the expected higher rates of inhibition from these complex specimens (1, 16). By pooling the rectal and nasal swabs in a broth, potential inhibitors are diluted and are less likely to interfere with the PCR. In fact, we found that the inhibition rate for the IDI assay was less than 1% when we tested samples from broth cultures. One drawback of combining the PCR assay with the broth culture is the delay in the reporting of the results compared to the time to the reporting of the results of direct testing. Overnight incubation of specimens in the selective broth was used in the laboratory, as it was convenient for work-flow purposes. Due to the high sensitivity of PCR, overnight incubation may not be necessary and a shorter incubation may be as effective. However, we did not evaluate this assay under these conditions. After incubation of the broth, routine cultures required an additional 40 to 72 h to confirm the presence of MRSA and an additional 48 h if no *S. aureus* isolate was recovered, whereas less than 4 h of additional time was required for a negative result by the IDI-MRSA assay. However, culture confirmation of a positive IDI-MRSA assay result required an additional 40 to 72 h. Despite this, pooling of specimens in the selective broth with the overnight incubation prior to PCR testing still provides an improvement over the time required for routine culture.

Although the IDI-MRSA assay performed well compared to the performance of culture, we continued to culture PCR-positive broths to recover MRSA isolates for genotyping and epidemiologic purposes. This also provided us with an opportunity to continue monitoring the performance of the assay outside of a research laboratory setting. In the 4 months postimplementation, we found that the positive predictive value of the assay fell from 90% during the evaluation phase to 65%. This significant reduction in performance can be explained in part by the fact that 38 IDI-MRSA assay-positive MSSA isolates were recovered. It is unlikely that the selective broth interfered with the recovery of MRSA by favoring the growth of the MSSA isolates, since for seven of these patients,

the same MSSA isolates were recovered when nasal swabs were directly cultured onto BA (data not shown). We failed to recover *S. aureus* from 9% of the PCR-positive broths. Some of these were from patients who were previously positive for MRSA and may reflect low-level colonization that may not be detectable by culture. For the seven patients described above in whom MSSA isolates were recovered by direct culture of a nasal specimen onto BA, initial screening cultures failed to recover any *S. aureus* isolate from the PCR-positive broth. Therefore, the selective broth may suppress the growth of some MSSA isolates and may explain some of the false-positive PCR outcomes in which no *S. aureus* isolate was recovered.

Two of the 38 MSSA isolates were found to be *mecA* positive but repeatedly oxacillin susceptible. Although the exact nature and significance of these two isolates is not known, they may represent pre-MRSA clones requiring inactivation of the *mecI* repressor gene before methicillin resistance can be expressed (11). Further characterization of these isolates is ongoing.

For the remaining 36 PCR-positive MSSA isolates, all were found to be negative for *mecA*. Persistent colonization with these strains was demonstrated in seven patients by repeat screening. Furthermore, we found evidence of patient-to-patient transmission in at least two unrelated cases, suggesting that these strains can easily establish themselves and persist in hospital settings. The origins and significance of these isolates are not known. Our findings are consistent with reports of both *S. aureus* and CoNS isolates with non-*mecA*-containing SCC elements (8, 14, 19). Huletsky et al. also reported that 4.6% of MSSA strains tested were positive in a similar PCR assay for MRSA targeting the right-junction element (12). Because SCC*mec* is a mobile genetic element, complete or partial loss of the cassette may result in isolates harboring a portion of the right-junction sequence while having lost the *mecA* gene complex. In fact, Donnio et al. recently reported on the characterization of nine MSSA isolates which likely originated from MRSA epidemic clones but underwent partial excision of SCC*mec* (8). These isolates lost the *mecA* gene complex but retained the right extremity of the SCC element integrated in the 5′ end of the *orfX* gene (8). Interestingly, 21 of our MSSA isolates did have PFGE profiles consistent with those of commonly circulating MRSA clones. However, the remaining isolates had profiles that were not consistent with those of any MRSA clones from this region, suggesting that the origin of these *mecA*-negative SCC elements is manifold. These isolates require further characterization. For a more comprehensive review of the mechanisms of resistance in MRSA, readers are referred to the review article by Hiramatsu (11).

It is possible that other types of SCC elements with similarities to *SCCmec* may integrate in the same insertion sites. In fact, an SCC element carrying capsular genes (SCCcap1) and with similarities to SCC*mec* type II but lacking *mecA* has been described (18). A PCR assay similar to the IDI-MRSA assay also targeting the right-junction sequence of SCC*mec* was positive for an *S. aureus* isolate carrying SCCcap1 (7). There is evidence that other types of insertion elements do share similarities with SCC*mec* and could be a problem for the IDI-MRSA assay. MSSA isolate ATCC 25923, which is commonly used for quality control of in vitro susceptibility testing, has been shown to contain an element that shows similarities to SCC*mec* and that is inserted in the same integration site as SCC*mec* (13). Based on these similarities, we tested this strain with the IDI-MRSA assay and found that it provided a false-positive result (data not shown).

Although *mecA*-negative SCC elements are not unique, the extent to which these *mecA*-negative SCC elements were found in our patient population was surprising. Regardless of their origins, these common and diverse genetic elements will affect the performance of assays targeting the right-junction sequences as surrogate markers for *mecA*-mediated resistance. The extent to which these strains will affect the assay will depend on how widespread they are. In settings of high MRSA prevalence, these MSSA strains may be displaced and may not be as relevant as they are in settings of lower MRSA incidence, such as Canada. At our institution, because of the rapid turnaround time and the excellent negative predictive value, the IDI-MRSA assay was implemented for routine screening for MRSA. However, because of the significant burden associated with the implementation of infection control precautions, cultures remain essential for confirmation of positive IDI-MRSA assay results. Until broth cultures are finalized, PCR-positive results are considered preliminary and require confirmation. However, pending this confirmation, newly identified patients are placed in isolation because of the potential presence of MRSA. Therefore, it is important that both the microbiology laboratory and infection control evaluate the usefulness of this assay for their local settings. This includes determination of whether IDI-MRSA assay-positive results require culture confirmation and how these results will be used for determination of when to implement appropriate prevention measures.

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