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

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The IDI-MRSA assay (Infectio Diagnostic, Inc., Sainte-Foy, Quebec, Canada) with the Smart Cycler II rapid DNA amplification system (Cepheid, Sunnyvale, CA) appears to be sensitive and specific for the rapid detection of nasal colonization by methicillin-resistant Staphylococcus aureus (MRSA). We assessed the sensitivity and specificity of this assay under conditions in which both the nose and cutaneous groin specimens were analyzed together and compared the accuracy of this PCR approach to that when these specimens were tested separately and by culture assays in an inpatient population with known high rates (12 to 15%) of MRSA colonization. Of 211 patients screened, 192 had results assessable by all three methods (agar-broth culture, separate nose and groin IDI-MRSA assay, and combined nose-groin IDI-MRSA assay), with MRSA carriage noted in 31/192 (16.1%), 41/192 (21.4%), and 36/192 (18.8%) patients by each method, respectively. Compared to agar culture results, the sensitivity and specificity of the combined nose-groin IDI-MRSA assay were 88.0% and 91.6%, respectively, whereas when each specimen was processed separately, the sensitivities were 90.0% (nose) and 83.3% (groin) and the specificities were 91.7% (nose) and 90.2% (groin). IDI-MRSA assay of a combined nose-groin specimen appears to have an accuracy similar to that of the current recommended PCR protocol, providing results in a clinically useful time frame, and may represent a more cost-effective approach to using this assay for screening for MRSA colonization.

Nasocomially acquired methicillin-resistant Staphylococcus aureus (MRSA) is a problem in health care facilities due to its associated morbidity, mortality, and health care costs (4, 5, 6–10, 18). Infection control measures, including patient screening for colonization, cohorting and isolation of infected and colonized patients, decolonization protocols, and increased emphasis on appropriate hand hygiene, have reduced the clinical MRSA disease burden (16, 20, 21). Early detection of MRSA-colonized patients may improve the timeliness of infection control measures and thereby reduce pathogen transmission (5, 13, 15). However, the utility of routine culture-based screening programs has been limited by the 2 to 3 days required to obtain accurate results. Such delays may have important infection control implications, especially among patients at high risk of colonization or where unnecessary isolation/cohorting is disruptive (19). Use of the PCR-based real-time IDI-MRSA assay (Infectio Diagnostic, Inc., Sainte-Foy, Quebec, Canada) with the Smart Cycler II rapid DNA amplification system (Cepheid, Sunnyvale, CA) has the potential to improve detection times for MRSA colonization and appears to be sensitive and specific (12, 23). The test is based on the detection of the right extremity sequence of the staphylococcal cassette chromosome mec and the chromosomal sequence of orfX, allowing discrimination between MRSA and methicillin-resistant coagulase-negative staphylococci (11, 12a). Currently, the test has FDA approval for the direct detection of MRSA from nasal specimens, but its use on specimens from alternative sites or pooled specimens has not been validated. Given that the accuracy of patient screening for MRSA carriage often involves sampling of both nasal and cutaneous sites (e.g., groin) (14), the utility of the IDI-MRSA assay could be greatly increased if proved to be sensitive and specific for detecting cutaneous MRSA colonization either in a separate assay or in a combined assay to assess nose and groin swabs concurrently.

We assessed the relative sensitivity and specificity of the IDI-MRSA assay to detect nose and cutaneous groin MRSA colonization when both specimens were processed separately (as currently recommended by the manufacturer for the nose swabs) and compared these results to those obtained when both specimens were tested together. The results obtained were compared to those obtained by routine culture analysis of these sites.

MATERIALS AND METHODS

The study was conducted at the Austin Hospital, Melbourne, Australia, a 450-bed tertiary care university teaching hospital, where previous screening programs suggested high (12 to 15%) rates of patient MRSA colonization (14). Recently, the hospital had undergone redevelopments such that all inpatients (except hematology-oncology patients) were scheduled for relocation to a new ward block in May to June 2005. To assess the rate of MRSA colonization and
assist with future infection control interventions in the new facility, all inpatients aged >18 years were to be screened for nose and cutaneous groin MRSA colonization during the 48- to 96-h period prior to relocation. The hospital’s Human Ethics Committee approved the study, and all participating patients gave consent.

Specimen collection and processing. Swabs were collected from the anterior nares and the cutaneous area in the groin using two separate dry Copan Tran-system Liquid Stuart swabs (Venturi Transystem; Copan Diagnostics, Corona, CA), transported at room temperature, and processed within 1 to 13 h of collection.

Processing of swabs and preparation of DNA lysates. A flow diagram of the processing protocol for swabs is depicted in Fig. 1. As part of the study design, we elected to use a single swab from each site (nose and groin) to validate and compare the performance of the IDI-MRSA assay using both swabs tested separately and a sample representing a combined or pooled site (both nose and groin together). This approach was chosen to prevent potential sampling variation that may have otherwise occurred if we had used one set of swabs for separate-site testing and another for the combined-specimen assay. Crude DNA lysates were prepared from each swab using the reagents supplied with the IDI-MRSA assay kit (Infectio Diagnostic, Inc.). In order to prepare the combined nose-groin DNA lysate specimen, additional sample buffer tubes were generously provided by Infectio Diagnostic, Inc. For each patient, nose and groin swabs were inoculated directly onto mannitol salt agar (Oxoid, Basingstoke, England) containing 4 μg/ml oscarilin (MSSO) and then placed into separate sample buffer tubes containing 1 ml sample buffer (as recommended by the manufacturer). After vortexing for 1 min, 0.5 ml of sample buffer from each nose and groin sample buffer tube was transferred into separate lysis tubes, and 0.25 ml of sample buffer from each of the nose and groin sample buffer tubes was combined into a third lysis tube (total volume, 0.5 ml). Thus, there were three DNA lysis tubes for each patient containing equivalent volumes of the specimen (as advised by the manufacturer [personal communication]): nose specimen alone (0.5 ml), groin specimen alone (0.5 ml), and combined nose and groin specimen (0.25 + 0.25 = 0.5 ml). The two sample buffer tubes, each containing a swab and residual sample buffer (~0.25 ml), were sent for MRSA culture (Fig. 1). The DNA lysate preparation was completed for the three lysis tubes as described by the manufacturer’s protocol, except that (as noted above) 0.5 ml of the specimen was processed instead of the recommended 1.0 ml.

Briefly, the lysis tubes were centrifuged at 21,000 × g for 5 min at room temperature. The supernatant was removed and discarded, and 50 μl fresh sample buffer was then added to each lysis tube. Lysis tubes were vortexed for 5 min and pulse centrifuged for 30 s at 21,000 × g, followed by heating at 95°C for 2 min. DNA lysates were then stored on ice prior to immediate testing by the IDI-MRSA assay procedure. Long-term storage of DNA lysates was at −30°C. For the purposes of this study, all DNA lysates are henceforth referred to as “specimens”; thus, each patient had three specimens.

IDI-MRSA assay. The IDI-MRSA assay was performed on all three DNA specimens (nose, groin, and combined nose and groin) from each patient as recommended by the manufacturer. Briefly, 2.8 μl of DNA lysate specimen was added to 25 μl of reconstituted Mastermix, and the sample was loaded into a Smart Cycler tube. Each tube was inserted into the I-CORE module of the Smart Cycler, and the machine was run using the preprogrammed IDI-MRSA run protocol. Positive control DNA (supplied with the kit) and a negative control (sample buffer) were included with each run. When specimens demonstrated inhibition in the assay, the test was repeated using the stored frozen DNA lysate sample. Specimens were excluded from analysis if inhibition was noted upon retesting.

Culture methods. All sample buffer tubes containing the patient swab and residual sample buffer (~0.25 ml) had 1 ml of tryptone soya broth containing...
The sensitivity and specificity of each IDI-MRSA assay approach were compared to each other and to MRSA results obtained using culture obtained from the nose and groin (nose, groin, or combined nose-groin specimen). The sensitivity and specificity of each IDI-MRSA assay approach were comparable to those obtained when the IDI-MRSA assay was performed on agar and/or broth culture (48 h at 35°C and then subcultured onto MSO prior to storage at 4°C. All MSO cultures (both those directly inoculated with swabs and those subcultured from TSBNaC1) (Fig. 1) were incubated at 35°C for 48 h, after which potential colonies of S. aureus were further subcultured onto horse blood agar and DNase agar (Oxoid, Basingstoke, England). MRSA isolates were confirmed by using routine identification methods (1), and antibiotic susceptibility was assessed by agar dilution using CLSI methods (6).

**Discordant specimen results.** When the results of the IDI-MRSA assay on any specimen (nose, groin, or combined nose-groin specimen) were discordant with routine culture findings, the following additional investigations were undertaken. For PCR-positive but culture-negative specimens, stored broth cultures were subcultured onto antibiotic-free mannitol salt agar and nonselective media (horse blood agar and MacConkey agar [Oxoid, Basingstoke, England]) to further test for the presence of MRSA. Although these results were noted, they were not used for the analysis of sensitivity and specificity of the IDI-MRSA assay since they represented a more detailed culture methodology than what would be routinely applied to such specimens. In addition, patients who had PCR-positive but culture-negative specimens had their laboratory records reviewed 1 month before and 5 days after the screening cultures were collected to assess for evidence of recent MRSA infection at any site. For specimens in which the IDI-MRSA PCR assay was negative but the culture assay was positive, the isolates obtained were tested using conventional gel-based PCR for the presence of mecA and nuc genes (2, 3).

**Data analysis.** Results of the IDI-MRSA assay of DNA lysate specimens obtained from the nose and groin (nose, groin, or combined nose-groin specimen) were compared to each other and to MRSA results obtained using culture methods. The sensitivity and specificity of each IDI-MRSA assay approach were calculated and compared to those of routine culture assay.

### RESULTS

In total, 211 patients were screened for nose and cutaneous groin MRSA colonization (422 swabs and 633 DNA lysate specimens) and assessed by IDI-MRSA assay and culture. The results from 19 patients were excluded from further analysis due to either the failure of the IDI-MRSA assay external (negative) control (10 patients; 30 specimens) or persistent PCR inhibition in one or more specimens (nine patients; 10 of 27 specimens [eight nose, one groin, and one combined nose-groin specimen]). A further 18 patients (23 specimens) had one or more specimens that were inhibited on the initial PCR assay but that were not inhibited on the assay of the stored frozen lysate specimens. These 18 patients were included in the overall analysis. Thus, 192 of 211 (91%) patients had valid results by all assessment methods (576 specimens) (Table 1).

Overall, 31/192 (16.1%) patients were colonized with MRSA based on culture assay. Based on routine agar culture assay, 25/192 (13%) patients were colonized with MRSA in either the nose alone (n = 7; 3.6%), groin alone (n = 5; 2.6%), or both sites (n = 13; 6.8%), while a further six patients were positive by broth culture assays alone (four nose alone, one groin alone, and one nose and groin). Thus, compared to nose culture alone, groin culture identified an additional five patients who would otherwise have been missed using agar culture assays or six patients if both agar and broth cultures were performed.

Results from the IDI-MRSA assay and a comparison of those results with routine culture findings are shown in Table 1. Compared to direct agar culture assay, the sensitivity and specificity of the IDI-MRSA assay on nose and cutaneous groin specimens processed separately were comparable to those obtained when the IDI-MRSA assay was performed on the combined nose-groin specimen (sensitivity, 90.0% for the nose, 83.3% for the groin, and 88.0% for the combined nose-groin specimen; specificity, 91.7%, 90.2%, and 91.6%, respectively) (Table 1). Inclusion of broth culture results affected the sensitivity and specificity of the PCR assay only slightly (Table 1). Forty-one patients (41/192; 21.4%) tested positive by PCR when each nose and groin swab was assessed separately (9 nose alone, 9 groin alone, and 23 both sites). Of these, 36/41 patients were PCR positive by the combined nose-groin assay.

Twenty patients had a total of 32 specimens (9 nose, 14 groin, and 9 combined nose-groin specimens) for which the IDI-MRSA assay was positive but the corresponding cultures (agar and broth) were negative. However, repeated subculture of stored TSBNaC1 cultures identified MRSA in five of these patients, and a further four patients had MRSA cultured from a noncorresponding site. Of the remaining 11 PCR-positive, culture-negative patients, 5 had MRSA cultured from clinical specimens during the month before or within 5 days after the study. Thus, the likelihood that the IDI-MRSA assay incor-

### TABLE 1. Comparison of the IDI-MRSA assay using individual and combined nose-groin DNA specimens with culture of swabs for detection of MRSA

<table>
<thead>
<tr>
<th>IDI-MRSA assay sample (n = 192)</th>
<th>No. of positive samples</th>
<th>No. of negative samples</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>No. of positive samples</th>
<th>No. of negative samples</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
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<tbody>
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<td><strong>Nose</strong></td>
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<td>Positive</td>
<td>18</td>
<td>14</td>
<td>90.0</td>
<td>91.7</td>
<td>56.3</td>
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<td><strong>Groin</strong></td>
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<tr>
<td>Positive</td>
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<td><strong>Combined nose-groin</strong></td>
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<tr>
<td>Positive</td>
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<td>88.0</td>
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<td>4</td>
<td>152</td>
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</table>

a Culture results obtained following direct plating of swabs on MSO (see the text).
b Culture results obtained by direct plating onto MSO or by enrichment broth culture in TSBNaC1 (see the text).
c PPV, positive predictive value.
d NPV, negative predictive value.
directly identified a patient as being colonized by MRSA, compared to culture assays, occurred in ≤ 6 patients.

Four patients had negative IDI-MRSA assay results for all three specimens despite a positive MRSA culture from at least one site (one nose, one groin, and two both nose and groin). When the IDI-MRSA assay was repeated using the stored DNA lyse specimens for these patients, a positive result was obtained in one of the four patients. Assessment for meca and nuc in the MRSA isolates obtained from the three remaining patients revealed the presence of both these genes.

Assessment of swab and specimen processing times revealed that each batch of 14 samples took approximately 2.5 h to be appropriately processed using the IDI-MRSA assay and Smart Cycler II apparatus.

**DISCUSSION**

In this study, we validated the performance of the IDI-MRSA assay for detection of nasal and cutaneous (groin) MRSA colonization using both separate swabs and a combined sample using both the nose and groin swabs together. Using our modified protocol, we found the IDI-MRSA assay to be rapid, sensitive (90%), and specific (91.7%) for identifying MRSA colonization of the nose compared to routine and detailed culture analysis. This is similar to the findings reported previously by Huletsky et al., who reported 100% sensitivity and 96.5% specificity, and Warren et al., who noted 91.7% sensitivity and 93.5% specificity, respectively (12, 23). We also found the IDI-MRSA assay to be sensitive (83.3%) and specific (90.2%) for detecting cutaneous MRSA colonization of the groin. Notably, we have found for the first time that IDI-MRSA assay of a single DNA lysate specimen prepared from both nose and groin swabs together was equally sensitive (88.0%) and specific (91.6%) compared to when these same specimens are processed separately (Table 1). In our patient population, both nose and groin cultures appeared to be necessary to accurately identify MRSA colonization since nose cultures alone identified only 25/31 (80.6%) colonized patients. Given this finding, our data suggest that rather than performing two IDI-MRSA assays (nose and groin) for each patient, a single combined assay could be performed without a significant loss of accuracy in detecting MRSA colonization in these sites. This approach is likely to result in cost savings, given the reductions in the numbers of IDI-MRSA assays used and scientist processing time.

From a practical, laboratory perspective, it was inconvenient that 33 of the 603 assessable specimens (5.5%) were initially inhibited when the IDI-MRSA assay was used on fresh specimens. However, this overall rate of inhibition is consistent with the 4.5% inhibition reported by the manufacturer for assessment of nose specimens (12a). Interestingly, we found that the inhibition rate using the combined nose-groin specimen was less than that found for nose specimens processed separately (8/201 [4.0%] versus 15/201 [7.5%], respectively; \( P = 0.133 \), chi-square test). Repeat testing on stored frozen DNA lysates, as recommended by the manufacturer, resolved inhibition in 23 of the 33 specimens. It is unclear whether our observed rates of inhibition for combined nose-groin specimens relate to the volumes we used in the preparation of the DNA lysate for the IDI-MRSA assay, given that they are different from those routinely recommended by the manufacturer.

We found that each assay run of 14 clinical specimens could be processed within 2.5 h. This suggests that by testing combined nose-groin specimens in the manner that we propose, results for a number of patients can be obtained relatively rapidly compared to conventional culture assays of all these sites.

Our study has some limitations and caveats. Since our study population consisted entirely of adults, we cannot be sure that the IDI-MRSA assay of combined nose-groin specimens will perform similarly in other patient populations such as children and neonates. Secondly, our protocol required half the recommended volume of sample buffer to prepare the DNA lysates (Fig. 1). This may have affected the results obtained compared to swabs processed using the recommended sample buffer volumes. However, the fact that the sensitivity and specificity of the IDI-MRSA assay using our approach were so similar to those reported previously by others using the recommended approach suggests this protocol modification had little effect on test performance (12, 12a, 23).

Rapid detection of MRSA colonization allows for the early implementation of appropriate infection control interventions to limit the spread of MRSA (22). Our data suggest that the use of the IDI-MRSA assay on combined nose-groin specimens is similar to routine culture techniques in accuracy but provides results in a more clinically useful time frame without the additional cost of processing each specimen separately.

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**REFERENCES**


17. Reference deleted.


