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Controlled Evaluation of the IDI-MRSA Assay for Detection of Colonization by Methicillin-Resistant *Staphylococcus aureus* in Diverse Mucocutaneous Specimens[▽]

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Rapid and reliable detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers is crucial for the effective control of MRSA transmission in healthcare facilities. The aim of this study was to verify the performance of the IDI-MRSA real-time PCR assay for direct MRSA detection in diverse mucocutaneous swabs from hospitalized patients. Swabs from nares ($n = 522$) and skin or other superficial sites ($n = 478$) were prospectively collected for MRSA screening from 466 patients admitted to an 858-bed teaching hospital. Swabs were inoculated onto selective chromogenic MRSA-ID agar, buffer extraction solution for IDI-MRSA assay, and enrichment broth. MRSA was detected by culture in 100 specimens from 47 patients. Compared to enrichment culture, the sensitivity and specificity of the PCR assay were 81.0 and 97.0%, respectively, and its positive and negative predictive values were 75.0 and 97.9%, respectively. The IDI-MRSA assay was more sensitive on swabs from nares (90.6%) than from other body sites (76.5%, $P < 0.01$). The PCR assay detected MRSA in 42 of 47 patients with culture positive study samples. Of 26 patients with culture-negative but PCR-positive study samples, 11 were probable true MRSA carriers based on patient history and/or positive culture on a new sample. The median turnaround time for PCR results was 19 h versus 3 days for agar culture results and 6 days for enrichment culture results. These data confirm the value of IDI-MRSA assay for rapid screening of MRSA mucocutaneous carriage among hospitalized patients. Cost-effectiveness studies are warranted to evaluate the impact of this assay on infection control procedures in healthcare settings.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens responsible for a wide spectrum of infections, including skin and soft tissue infections, pneumonia, bacteraemia, surgical site infections, and catheter-related infections. In Europe, up to 50% of nosocomial bloodstream *S. aureus* infections are due to MRSA. In Belgium, the proportion of MRSA isolates from blood cultures in hospitalized patients has risen from 22% in 1999 to 31.4% in 2005 (8). MRSA isolation from an inpatient is associated with increased risk of nosocomial infection and an excess of morbidity and hospitalization costs (4). The main mode of MRSA transmission is from MRSA-colonized or -infected patients to another one through indirect contact via the transiently colonized hands of healthcare workers. Therefore, the rapid identification of MRSA carriers is essential for implementation of targeted infection control measures to prevent dissemination. Active surveillance cultures for MRSA are now part of clinical practice recommendations both in Europe and the United States (16, 18, 22). The current Belgian recommendations for MRSA screening are to culture swabs from nares and other skin and mucosal sites with enrichment broths and selective media. However, the results of the conventional screening methods are generally not available before 48 h, in spite of shorter detection times with the latest-generation chromogenic selective agar media (3, 6). Several real-time PCR methods have been recently developed and evaluated for same-day MRSA detection directly from clinical samples (9, 13, 19).

The aim of the present study was to further evaluate the diagnostic performance of the IDI-MRSA assay (Infectio Diagnostic, Sainte-Foy, Canada, a subsidiary of BD Diagnostics GeneOhm, Erembodegem, Belgium) for MRSA detection from diverse mucocutaneous swabs in patients hospitalized in a tertiary-care hospital compared to current standard optimized selective culture methods using enrichment broth and chromogenic agar media.

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MATERIALS AND METHODS

Patients and clinical samples. The study was conducted prospectively in an 858-bed teaching hospital (Erasme Hospital) over a 4-month period. As defined by local policy for MRSA screening, samples from nares, throat, perineum, and skin wounds were prospectively collected on dry swabs from hospitalized patients and immediately inoculated into Stuart transport medium (Copan, Italy). The PCR assay was performed in parallel with processing by routine culture detection techniques. A sample size of 1,000 samples from 500 patients, with an expected rate of 10% prevalence of MRSA carriage, was planned to ensure sufficient statistical power. To this end, high-risk medicosurgical departments with retrospective 10% carriage rate prior to starting the study were selected for patient inclusion. The study protocol was approved by the ethical committee of our institution.

Selective cultures for MRSA screening. Swabs were first plated onto selective MRSA agar (MRSA-ID medium; bioMérieux) and then suspended by vortex agitation in the sample buffer for PCR assay. Thereafter, swabs were inoculated

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TABLE 1. IDI-MRSA diagnostic performance compared to enrichment culture for MRSA detection in hospitalized patients[a]

Sample type (no. of specimens) and IDI-MRSA result	No. of samples tested by using culture and enrichment broth		%		PPV	NPV
	Positive	Negative	Sensitivity	Specificity		
Nasal swabs (522)						
Positive	29	14	90.6	97.1	67.4	99.4
Negative	3	476				
Other samples (475)						
Positive	52	13	76.5	96.8	80.0	96.1
Negative	16	394				
All specimens (997)						
Positive	81	27	81.0	97.0	75.0	97.9
Negative	19	870				
Patients (466)						
Positive	42	20	89.4	95.2	67.7	98.8
Negative	5	399				

[a] PPV, positive predictive value; NPV, negative predictive value.

into enrichment broth composed of brain heart infusion supplemented with 7% NaCl.

After 24 h of incubation at 35°C, broths were subcultured onto MRSA-ID agar. Selective agar plates were incubated for 48 h at 35°C and inspected daily. Suspected MRSA (green-pigmented) colonies were identified by coagulase testing and the oxacillin resistance test by cefoxitin disk diffusion method according to CLSI recommendations. MRSA isolates were conserved at −20°C.

IDI-MRSA assay. (i) Sample preparation and DNA extraction. The IDI-MRSA assay was performed according to the manufacturer's recommendations. Briefly, the sample buffer was vortexed at high speed, and the cell suspension was transferred into a lysis tube and centrifuged at a minimum of 14,000 rpm at room temperature. The supernatant was discarded, and 50 μl of sample buffer was added to the lysis tube. After being vortexed, the lysis tube was briefly centrifuged, warmed up to inactivate inhibitors, and placed on a cooling block until processing. A total of 25 μl of reconstituted master mix and 2.8 μl of the lysate solution were added to the reaction tube. Tubes were centrifuged briefly and placed on cooling block until ready to load. Positive and negative controls were included in each run.

(ii) Real-time PCR analysis. Each reaction tube was inserted into a SmartCycler II (Cepheid, Sunnyvale, CA) instrument for PCR amplification using SmartCycler Dx software version 1.7b. The results were obtained in approximately 1 h. Specimens received in the laboratory from 8 a.m. to 2 p.m. were processed within 2 h by the IDI-PCR assay; those received after 2 p.m. were refrigerated and analyzed the following day.

(iii) Data analysis. IDI-MRSA results were compared to those obtained using enrichment cultures as the gold standard, broken down by specimen and by patient. For discrepant results, PCR and culture were repeated when possible on a new sample from the same body site, which was collected with the informed consent of the patient. In addition, medical charts of patients with a negative culture but a positive PCR result were reviewed to collect information on (i) MRSA colonization previously documented during hospital stay, (ii) concurrent or recent patient treatment with mupirocin and/or antiseptics for MRSA decolonization, and (iii) glycopeptide treatment.

RESULTS

Included patients were hospitalized in the intensive care, cardiology, orthopedic surgery, geriatrics, neurology, revalidation, vascular surgery, cardiac surgery, endocrinology, gastroenterology, dermatology, and thoracic surgery departments. A total of 1,000 swabs were collected from nares ($n = 522$), throat ($n = 212$), perineum ($n = 206$), and skin wound samples ($n = 60$) in 466 patients (1 to 18 samples per patient, with a mean of 2.1 samples per patient). Three samples (0.3%) were excluded from the study analysis because of the presence of PCR inhibitors.

TABLE 2. Discrepancy analysis for patients with PCR-positive, culture-negative discordant results based on patient history and testing of control swab specimens

No. of patients	History of MRSA	Control swab result		Decolonization therapy[a]	Conclusion
		PCR	Culture		
4	Yes	ND[b]	ND	Yes	Probable MRSA carrier
3	Yes	+	+	Yes	Probable MRSA carrier
4	Yes	+	+	No	Probable MRSA carrier
9	No	ND	ND	No	Unresolved
3	No	ND	−	No	False positive
2	No	−	−	No	False positive
1	No	+	−	No	False positive

[a] Decolonization therapy consisted of daily bathing with chlorhexidine-containing soap and nasal mupirocin.
[b] ND, not done.

MRSA was recovered by culture in 100 specimens (10.0%) from 47 patients (10.1%) (Table 1). Among these positive specimens, 81 (81.0%) were detected by agar culture, and 19 (19.0%) were detected by enrichment broth only. The turnaround time ranged from 1 to 9 days, with a median of 3 days for the final reporting of agar culture results and from 2 to 9 days with a median of 6 days for the enrichment culture results.

A total of 108 specimens (10.8%) collected from 62 patients were determined to be positive by the IDI-MRSA assay (Table 1). A total of 81 swabs from 42 patients were concordantly positive for MRSA both by culture and by PCR. Compared to culture, the sensitivity and specificity of PCR with all samples were 81.0 and 97.0%, respectively (Table 1). The sensitivity was significantly higher with nasal swabs (90.6%) than with other samples (76.5%) ($P < 0.01$). The specificity was similar with nasal and extranasal swabs (97.1 and 96.8%, respectively). The overall positive and negative predictive values were 75.0 and 97.9%, respectively. The median turnaround time for PCR results was 19 h, with a range from 4 to 27 h.

By analysis at the patient level, the sensitivity and specificity of the PCR assay for detecting MRSA colonization by multiple site sampling were 89.4 and 95.2%, respectively. The positive and negative predictive values in the study population were 67.7 and 98.8%, respectively.

A total of 19 samples from 17 patients yielding MRSA by culture showed false-negative PCR results. These specimens were sampled from the nose ($n = 3$), the perineum ($n = 6$), the throat ($n = 8$), and other skin sites ($n = 2$). DNA extracted from MRSA isolates from these specimens all tested positive by PCR.

Of 26 patients who had culture-negative but PCR-positive discordant results (Table 2), 11 patients were considered to be probable MRSA carriers because they had a recent history of MRSA carriage. In addition, seven of these patients were re-

ceiving topical decolonization treatment at the time of sampling (Table 2). For 7 of the 11 patients, a second sample obtained for control testing was MRSA positive by culture. No patient was under glycopeptide therapy. The remaining 15 patients were considered to have false-positive IDI-MRSA results because they had no history of MRSA carriage and repeat swabs were culture negative for 6 patients and not available for 9 patients (Table 2). After reassessment by a revised gold standard combining culture on control samples and MRSA carrier history to resolve discrepant cases, the performance of PCR would increase to a sensitivity of 90.2% and a specificity of 96.1%.

DISCUSSION

There is a need for a reliable, rapid, and efficient technique to identify MRSA carriers (3). The current recommendations for MRSA screening in many European countries are to perform surveillance culture on swabs from nares and other skin and mucosal sites with selective enrichment broths and solid media (3). Although some improvement in reducing time to detection has been achieved by using chromogenic media, culture-based techniques are time-consuming and can fail to detect low-level heteroresistant MRSA strains (20, 23). More recently, multiplex PCR have been developed to simultaneously detect the methicillin resistance determinant, the *mecA* gene, and the *S. aureus* species-specific gene (*femA*, *nuc*, etc.) for detection of MRSA in screening swabs (9, 15, 19, 21). These tests showed a good performance in endemic settings but required an overnight incubation of the swabs in broth culture (15). Moreover, this PCR design is subject to producing false-positive results with samples containing both methicillin-susceptible *S. aureus* and methicillin-resistant coagulase-negative staphylococci. Such cocolonization is detected in ca. 3 to 5% of nasal swab samples from hospitalized patients (1).

To overcome this limitation, a commercial real-time PCR test, the IDI-MRSA assay, was developed to target a highly conserved sequence in *S. aureus* that bridges the SCC*mec*-chromosomal junction (*orfX*) and the major variant SCC*mec* types I to IV (11, 12, 14). This design prevents the occurrence of false-positive signal from specimens containing methicillin-susceptible *S. aureus* and methicillin-resistant coagulase-negative staphylococci mixed flora (12). On the other hand, it is liable to produce false-negative results with MRSA strains harboring novel SCC*mec* types. The assay allows same-day results with batching of specimens (24). Previous evaluations performed on nasal swab specimens reported a sensitivity ranging from 89 to 100% and a specificity greater than 93.5% (2, 13, 24), whereas two recent studies evaluating the assay performance on non-nasal specimens reported a sensitivity of 82 to 96% and a specificity of 92 to 96% (2, 7). The present study, which examined the largest series of positive samples to date, showed a somewhat lower sensitivity of 81.0% and a similar specificity of 97.0% for this assay compared to optimized selective culture method on both nasal and non-nasal mucocutaneous swabs. It is likely that the lower sensitivity found here was related to the optimized gold standard that we used by combining enrichment cultures and chromogenic MRSA selective medium. Although some community-acquired MRSA strains grow slowly in medium containing 2.5% NaCl, the medium used here has been previously validated and demonstrated 100% sensitivity with a collection of representative Belgian HA and CA-MRSA strains (17). In the majority of previous evaluations, investigators used less-sensitive culture methods based on direct swab plating on selective, nonchromogenic media. Moreover, in many of these studies, enrichment cultures were added for post hoc analysis of samples with discrepant results. This diagnostic performance evaluation strategy, however, is inherently biased toward inflated estimates of sensitivity and specificity (10).

We found a lower sensitivity of the IDI-MRSA PCR assay with swabs from the perineum, throat, and skin compared to nasal specimens, as observed in groin versus nasal specimens in the previous evaluation, which also used enrichment culture methods (2). This difference did not come from the presence of inhibitors to the PCR, since the internal control in each of these tubes was positive. Positive PCR results with the MRSA isolates from false-negative samples showed that the negative PCR results were not caused by a divergent sequence variant in the SCC*mec* or *orfX* sequences. This is in agreement with validation studies of this assay on international *S. aureus* strain collections and clinical samples, which indicate that these atypical strains are uncommon (12). A low inoculum of MRSA in the specimen below the limit of detection for the PCR assay or DNA degradation by mucosal enzymes could explain these discordant results. The limitation in PCR sensitivity can be mitigated by multiple site sampling (3). Pooling diverse specimens in the same assay would limit the extra cost (2, 7), but further evaluation of potential interference from degrading enzymes in some specimens is advisable before this practice can be recommended for all specimen types.

In one-third of the cases, the IDI-MRSA assay gave a positive result, whereas culture failed to grow MRSA from the same sample. The interpretation of false-positive PCR results is unclear. In a third of our patients, these specimens were collected from known MRSA carriers under topical decolonization therapy. In this case, the positivity of the PCR assay can be explained by the presence of DNA from noncultivable MRSA in the specimen. Although one may argue that these patients may be still potentially colonized with transmissible MRSA, we do not believe that the revised evaluation based on an "expanded gold standard" combining culture and MRSA history would provide a more accurate estimate of PCR diagnostic performance because of bias inherent to discrepancy analysis. Because of the use of a selective medium, we were unable in the present study to detect another potential cause of false-positive PCR result due to *S. aureus* strains with a deletion in the methicillin resistance *mec* region (12), which have been observed in our patient population (5).

The predictive values reported here are biased by the sampling strategy, since only patients admitted to wards with an expected pretest probability of MRSA carriage of 10% were included. In our patient population under routine screening for carriage, the average MRSA carriage rate is ca. 5%. This would represent a predictive value of positive PCR of 47% and of negative PCR of 99.4%. Based on this performance in our setting, it would appear prudent to confirm at least PCR-positive samples by culture to avoid placing patients under contact isolation without confirmation of MRSA colonization,

until the epidemiological relevance of MRSA DNA detection alone is better understood.

In summary, we found that with batch processing during working hours, the IDI-MRSA PCR assay was rapid (median reporting time of 19 h), sensitive (89%), and specific (95%) for the detection of MRSA colonization when performed on multiple mucocutaneous swab specimens compared to 1 to 9 days for detection by optimized MRSA selective culture using enrichment broth and chromogenic agar. The assay was, however, found for the first time to be more sensitive in testing nasal swabs than throat and skin swabs. Our findings support further evaluation of the cost-effectiveness of rapid PCR screening for MRSA control in healthcare settings.

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REFERENCES

1. **Becker, K., I. Pagnier, B. Schuhen, F. Wenzelburger, A. W. Friedrich, F. Kipp, G. Peters, and C. von Eiff.** 2006. Does nasal co-colonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-positive methicillin-resistant *S. aureus* determinations by molecular methods? J. Clin. Microbiol. **44:**229–231.
2. **Bishop, E. J., E. A. Grabsch, S. A. Ballard, B. Mayall, S. Xie, R. Martin, and M. L. Grayson.** 2006. 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*. J. Clin. Microbiol. **44:**2904–2908.
3. **Brown, D. F., D. I. Edwards, P. M. Hawkey, D. Morrison, G. L. Ridgway, K. J. Towner, and M. W. Wren.** 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother. **56:**1000–1018.
4. **Cosgrove, S. E., Y. Qi, K. S. Kaye, S. Harbarth, A. W. Karchmer, and Y. Carmeli.** 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect. Control Hosp. Epidemiol. **26:**166–174.
5. **Deplano, A., P. T. Tassios, Y. Glupczynski, E. Godfroid, and M. J. Struelens.** 2000. In vivo deletion of the methicillin resistance *mec* region from the chromosome of *Staphylococcus aureus* strains. J. Antimicrob. Chemother. **46:**617–620.
6. **Diederen B. M., M. L. van Leest, I. van Duijn, P. Willemse, P. H. van Keule, and J. A. Kluytmans.** 2006. Performance of MRSA ID, a new chromogenic medium for detection of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**586–588.
7. **Drews, S. J., B. M. Willey, N. Kreiswirth, M. Wang, T. Ianes, J. Mitchell, M. Latchford, A. J. McGeer, and K. C. Katz.** 2006. Verification of the IDI-MRSA assay for detecting methicillin-resistant *Staphylococcus aureus* in diverse specimen types in a core clinical laboratory setting. J. Clin. Microbiol. **44:**3794–3796.
8. **EARSS Management Team.** 2005 EARSS annual report 2005. EARSS Management Team, Bilthoven, The Netherlands. [Online.] http://www.rivm.nl/earss/database/.
9. **Francois, P., D. Pittet, M. Bento, B. Pepey, P. Vaudaux, D. Lew, and J. Schrenzel.** 2003. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. J. Clin. Microbiol. **41:**254–260.
10. **Hadgu, A.** 1999. Discrepant analysis: a biased and an unscientific method for estimating test sensitivity and specificity. J. Clin. Epidemiol. **52:**1231–1237.
11. **Hanssen, A. M., and J. U. Ericson-Sollid.** 2006. SCC*mec* in staphylococci: genes on the move. FEMS Immunol. Med. Microbiol. **46:**8–20.
12. **Huletsky, A., R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. **42:**1875–1884.
13. **Huletsky, A., P. Lebel, F. J. Picard, M. Bernier, M. Gagnon, N. Boucher, and M. G. Bergeron.** 2005. Identification of methicillin-resistant *Staphylococcus aureus* carriage in less than one hour during a hospital surveillance program. Clin. Infect. Dis. **40:**976–981.
14. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* in the chromosome of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:**1323–1336.
15. **Jonas, D., M. Speck, F. D. Daschner, and H. Grundmann.** 2002. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. J. Clin. Microbiol. **40:**1821–1823.
16. **Muto, C. A., J. A. Jernigan, B. E. Ostrowsky, H. M. Richet, W. R. Jarvis, J. M. Boyce, and B. M. Farr.** 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. Infect. Control Hosp. Epidemiol. **24:**362–386.
17. **Nonhoff, C., O. Denis, and M. J. Struelens.** 2006. Evaluation of three chromogenic media for the detection of MRSA from Belgian hospitalized patients. Abstr. 46th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-822.
18. **Nijssen, S., M. J. Bonten, and R. A. Weinstein.** 2005. Are active microbiological surveillance and subsequent isolation needed to prevent the spread of methicillin-resistant *Staphylococcus aureus*? Clin. Infect. Dis. **40:**405–409.
19. **Paule, S. M., A. C. Pasquariello, D. M. Hacek, A. G. Fisher, R. B. Thomson, K. L. Kaul, and L. R. Peterson.** 2004. Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens using real-time polymerase chain reaction. J. Mol. Diagn. **6:**191–196.
20. **Safdar, N., L. Narans, B. Gordon, and D. G. Maki.** 2003. Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*: a prospective study comparing 32 methods. J. Clin. Microbiol. **41:**3163–3166.
21. **Towner, K. J., D. C. Talbot, R. Curran, C. A. Webster, and H. Humphreys.** 1998. Development and evaluation of a PCR-based immunoassay for the rapid detection of methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **47:**607–613.
22. **Verbrugh, H. A.** 2005. Value of screening and isolation for control of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **41:**268–269.
23. **Wannet, W.** 2002. Spread of an MRSA clone with heteroresistance to oxacillin in The Netherlands. Eur. Surveill. **7:**73–74.
24. **Warren, D. K., R. S. Liao, L. R. Merz, M. Eveland, and W. M. Dunne.** 2004. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. J. Clin. Microbiol. **42:**5578–5581.