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Methicillin-Resistant *Staphylococcus aureus* (MRSA) Detection: Comparison of Two Molecular Methods (IDI-MRSA PCR Assay and GenoType MRSA Direct PCR Assay) with Three Selective MRSA Agars (MRSA ID, MRSA*Select*, and CHROMagar MRSA) for Use with Infection-Control Swabs▽

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem. Rapid detection of MRSA-colonized patients has the potential to limit spread of the organism. We evaluated the sensitivities and specificities of MRSA detection by two molecular methods (IDI-MRSA PCR assay and GenoType MRSA Direct PCR assay) and three selective MRSA agars (MRSA ID, MRSA*Select*, and CHROMagar MRSA), using 205 (101 nasal, 52 groin, and 52 axillary samples) samples from consecutive known MRSA-infected and/or -colonized patients. All detection methods had higher MRSA detection rates for nasal swabs than for axillary and groin swabs. Detection of MRSA by IDI-MRSA was the most sensitive method, independent of the site (94% for nasal samples, 80% for nonnasal samples, and 90% overall). The sensitivities of the GenoType MRSA Direct assay and the MRSA ID, MRSA*Select*, and CHROMagar MRSA agars with nasal swabs were 70%, 72%, 68%, and 75%, respectively. All detection methods had high specificities (95 to 99%), independent of the swab site. Extended incubation for a further 24 h with selective MRSA agars increased the detection of MRSA, with a corresponding decline in specificity secondary to a significant increase in false-positive results. There was a noticeable difference in test performance of the GenoType MRSA Direct assay in detection of MRSA (28/38 samples [74%]) compared with detection of nonmultiresistant MRSA (17/31 samples [55%]) (susceptible to two or more non-β-lactam antibiotics). This was not observed with selective MRSA agar plates or IDI-MRSA. Although it is more expensive, in addition to rapid turnaround times of 2 to 4 h, IDI-MRSA offers greater detection of MRSA colonization, independent of the swab site, than do conventional selective agars and GenoType MRSA Direct.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem in health care facilities (6, 18). The burden of MRSA continues to rise, with a rate of 14% of all *Staphylococcus aureus* strains in 2004 documented from clinically significant samples in New South Wales, Australia (16). Rising colonization rates lead to increased infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient (12). Patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection (13).

Effective ways of reducing the MRSA disease burden in health care institutions include patient screening, dividing colonized patients into cohorts and/or isolating colonized patients, attempts at decolonization, and increased attention to staff hand hygiene (17). At our institution, infection control precautions are instituted immediately after a positive MRSA result becomes available from diagnostic and surveillance specimens. Since PCR-based methods are rapid, with turnaround times of 2 to 4 h, these tests are therefore able to improve the utilization of infection control resources. Furthermore, as colonized patients are divided into cohorts and/or isolated sooner, this step may prevent further pathogen transmission (10, 11). No study to date has compared different molecular methods or the use of these methods with a wide range of selective agar-based methods for the detection of MRSA colonization. The aim of this study was to determine whether molecular methods offer any other benefit in addition to rapid turnaround times to offset the additional expense of molecular testing.

We compared the relative sensitivities and specificities of the IDI-MRSA and GenoType MRSA Direct assays and three selective MRSA agars, MRSA ID, MRSA*Select*, and CHROMagar MRSA, with swabs from the three most commonly screened sites, i.e., the nose, groin, and axilla. Because multiple body sites are usually required for optimal detection of MRSA, we wanted to confirm the utility of the IDI-MRSA assay for use with these sites and to compare it to standard methods used in our laboratory (19).

MATERIALS AND METHODS

Two hundred five consecutive high-risk patients admitted to St. Vincent's Hospital, Sydney, Australia, were screened for MRSA colonization. The patients were known to previously be colonized and/or infected with MRSA.

Specimen collection and processing. Three concurrent specimens were obtained from each site swabbed. All swabs were collected by the same infection

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control nurse during the study. One hundred one patients had anterior nares swabs, 52 had axillary swabs, and the remaining 52 had groin swabs taken from the cutaneous area. Nasal swabs were composite swabs of the right and left nares. The three Copan Transystem Liquid Stuart swabs (Venturi Transystem; Copan Diagnostics, Corona, CA) were transported at room temperature and processed within 1 to 3 h of collection. The swabs were randomly assigned to a detection method.

Culture methods. One swab was used to directly inoculate the MRSA selective agars MRSA ID (bioMerieux), MRSA*Select* (Bio-Rad Laboratories), and CHROMagar MRSA (CHROMagar, Paris, France; it should be noted that this is not the same formulation as CHROMagar MRSA available in the United States from Becton Dickinson Microbiology products). The order of plate inoculation was random. Plates were inoculated directly on the day of receipt of the swab, incubated at 35°C in O_2, and read after 24 and 48 h. In accordance with the manufacturers' instructions, a colony suggestive of MRSA was confirmed as *Staphylococcus aureus* by using a tube coagulase and DNase test, while methicillin resistance was confirmed with cefoxitin susceptibility testing according to the CLSI method (15). Nonmultiresistant MRSA (NORSA) was defined as MRSA susceptible to two or more non-β-lactam antibiotics (ciprofloxacin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole).

DNA amplification methods. The second swab was processed using the real-time PCR-based IDI-MRSA assay (Infecto Diagnostics, Inc., Sainte-Foy, Quebec, Canada) with a Smart Cycler II rapid DNA amplification system (Cepheid, Sunnyvale, CA). The third swab was processed using the GenoType MRSA Direct (Hain Lifescience, Nehren, Germany) method. In accordance with the manufacturers' recommendations, HotStarTaq (QIAGEN) DNA polymerase was utilized with the reagents and protocols supplied in the kit. Hybridization of the DNA · STRIP was performed using reagents in the kit and a TwinCubator machine per the manufacturer's instructions. All inhibitory results were recorded as such and repeated. All swabs, after molecular detection, were stored in tryptic soy broth supplemented with 6.5% NaCl (per the manufacturers' instructions). For all patients with selective agar-negative samples that were positive by a single molecular method, culture of MRSA was attempted by inoculation of nonselective blood agar plates with the original PCR-amplified stored swabs. Suspicious colonies were confirmed as MRSA as described above. Similarly, for all patients with selective agar-negative samples that were positive by both molecular methods, culture of MRSA was attempted to define the antibiotic susceptibility pattern.

Result definitions and discordant results. A true positive result was defined as giving identical results with two or more methods. A true negative result was defined as a negative MRSA result by all methods. With discordant results (single agar plate positive), MRSA was confirmed using a third molecular method, namely, conventional gel-based PCR for the presence of the *mecA* and *nuc* genes. A sample that was positive by a single DNA amplification method (the alternative discordant result) was defined as a false-positive result in the absence of MRSA isolation and a true positive result if MRSA was isolated upon culture as described above.

Quality control. Each new batch of selective agars and new molecular kits were tested using the known MRSA strain ATCC 43300 and the negative control methicillin-susceptible *S. aureus* (MSSA) strain ATCC 25923.

Data analysis. The results of the IDI-MRSA and GenoType MRSA Direct assays were compared to those with the MRSA selective agars. The sensitivity, specificity, and negative and positive predictive values for each test were calculated. Samples that demonstrated inhibitory results by molecular methods were excluded from the calculations.

RESULTS

Two hundred five patients underwent screening, with patients assessed for nose (n = 101), groin (n = 52) and axilla (n = 52) MRSA colonization. Seventy-eight true positive MRSA isolates were identified. This represents a colonization rate of 38% (78/205 isolates). Nasal swabs were positive in 52% of cases (53/101 isolates), while groin and axilla site colonization rates were 33% (17/52 isolates) and 15% (8/52 isolates), respectively. Detection of MRSA by at least two selective agars was 85% (66/78 isolates). One swab (axilla) was positive on one selective agar only (MRSA ID). This isolate was confirmed as MRSA by the presence of the *mecA* and *nuc* genes by conventional PCR. Two specimens were positive by the IDI-MRSA assay only and were confirmed as MRSA by culture on nonselective blood agar. For the remaining nine specimens positive for MRSA, further characterization was not possible because nonselective cultures from the retained swabs were negative. Since these isolates were positive in both molecular assays, they were truly positive according to our definition (Table 1).

TABLE 1. True positive, true negative, and discordant (positive by a single method) results obtained by IDI-MRSA assay, GenoType MRSA Direct, and selective MRSA agars, with final resolved allocation[a]

Parameter	No. of specimens		
	Nasal	Axilla	Groin
True positive results			
Positive by two or more methods	44	5	17
Positive by two molecular methods only	7	2	
Discordant results resolved to be truly positive			
IDI-MRSA positive only	2		
MRSA ID selective agar positive only		1	
True positive total (n = 78)	53	8	17
True negative results			
Negative by all methods	48	43	31
Discordant results resolved to be truly negative			
IDI-MRSA positive only		1	1
GenoType MRSA Direct positive only			3[b]
True negative total (n = 127)	48	44	35

[a] Resolution of the discordant results was done as described in Materials and Methods.

[b] All three samples were Genotype MRSA Direct positive and selective and nonselective agar negative. Two samples were IDI-MRSA negative, and one sample was IDI-MRSA inhibitory.

There were 12 specimens that demonstrated inhibitory results upon molecular testing by the IDI-MRSA assay (3 specimens) and GenoType MRSA Direct (9 specimens). Two swabs each from the nose (n = 2) and axilla (n = 2) were inhibitory in both molecular tests. Seven swabs (from the nose [n = 2] and axilla [n = 5]) were GenoType MRSA Direct assay inhibitory and IDI-MRSA assay negative. One swab (from the groin) was IDI-MRSA assay inhibitory and GenoType MRSA Direct assay positive. All 12 specimens were negative on selective agar culture and remained unresolved upon repeat molecular testing.

There were two IDI-MRSA-positive (from the axilla [n = 1] and groin [n = 1]) and culture- and GenoType MRSA Direct-negative samples and two GenoType MRSA Direct-positive (from the groin [n = 2]) and culture- and IDI-MRSA-negative samples. The remaining discordant result was GenoType MRSA Direct positive (from the groin [n = 1]), culture negative, and IDI-MRSA assay inhibitory (Table 1).

The sensitivities, specificities, and positive and negative predictive values for MRSA detection with all swabs (nasal, axilla, and groin) can be found in Table 2. The IDI-MRSA assay was the most sensitive test, at 90%, compared to selective agars (63

TABLE 2. Comparison of IDI-MRSA assay, GenoType MRSA Direct, and selective MRSA agars in the detection of MRSA with all swabs (nasal, groin, and axilla)[a]

Test	No. of positive samples[b]	No. of negative samples[b]	No. of inhibited samples	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Molecular detection tests							
IDI-MRSA	75	127	3	90	96	93	94
GenoType MRSA Direct	59	137	9	69	96	92	82
Selective agars at 24 h							
MRSA ID	58	147		71	98	95	84
MRSA*Select*	50	155		64	95	89	81
CHROMagar MRSA	56	149		63	99	98	81
Selective agars at 48 h							
MRSA ID	123	82		82	53	52	83
MRSA*Select*	98	107		69	74	62	79
CHROMagar MRSA	87	118		71	67	56	79

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] True positive ($n = 78$) and true negative ($n = 127$) samples, defined as described in the text.

to 71%) and GenoType MRSA Direct (69%). The sensitivities of MRSA ID, MRSA*Select*, and CHROMagar MRSA increased 11%, 5%, and 8%, respectively, with extended incubation to 48 h. Specificities were similar (>95%) for all detection methods at 24 h. However, the specificities of the selective agars declined with a further 24-h incubation, secondary to a significant increase in the number of false-positive isolates. False-positive isolates increased by 56, 41, and 27 isolates for MRSA ID, MRSA*Select*, and CHROMagar MRSA, respectively, with coagulase-negative *Staphylococcus* species present in 93% of samples (124/134 isolates). The remaining 7% of isolates (10/134 isolates) were MSSA all occurred in nasal swabs isolated on CHROMagar MRSA. None of these 10 isolates was positive by either molecular method.

The sensitivities, specificities, and positive and negative predictive values for MRSA detection with nasal swabs can be found in Table 3. The test performances for MRSA detection with nasal swabs were similar to those for MRSA detection with swabs from all sites, except in the case of CHROMagar MRSA, with a lower specificity of 88% at 24 h. This was a result of high MSSA detection in nasal swabs. Similar trends were seen with extended incubation on selective agars, with a small increase in sensitivity and a marked reduction in specificity secondary to a significant rise in false-positive results ($P < 0.001$).

Table 4 summarizes the sensitivities, specificities, and positive and negative predictive values for MRSA detection with swabs from nonnasal sites (axilla and groin). The IDI-MRSA assay's sensitivity declined from 94% with nasal swabs to 80% with swabs from other sites. Groin swabs accounted for 75% (4/5 samples) of the missed samples. However, the sensitivities of all other detection methods for MRSA in nonnasal swabs were lower than those with nasal swabs, with sensitivities of 68%, 68%, 50%, and 40% for the GenoType MRSA Direct assay, MRSA ID, MRSA*Select*, and CHROMagar MRSA, respectively. Extended incubation of the selective agars caused false-positive results as described above.

Antimicrobial susceptibility determination was possible for 69 (88%) samples, with 38 MRSA (55%) and 31 NORSA (45%) isolates. The IDI-MRSA assay and selective MRSA agar plates were able to detect MRSA and NORSA equally well, with similar sensitivities, specificities, and positive and

TABLE 3. Comparison of IDI-MRSA assay, GenoType MRSA Direct, and selective MRSA agars in the detection of MRSA with nasal swabs[a]

Test	No. of positive samples[b]	No. of negative samples[b]	No. of inhibited samples	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Molecular detection tests							
IDI-MRSA	53	47	1	94	94	94	94
GenoType MRSA Direct	39	59	3	70	96	95	73
Selective agars at 24 h							
MRSA ID	40	61		72	95	95	75
MRSA*Select*	37	64		68	98	97	73
CHROMagar MRSA	46	55		75	88	86	76
Selective agars at 48 h							
MRSA ID	60	41		81	65	72	75
MRSA*Select*	56	45		77	69	73	73
CHROMagar MRSA	52	49		79	79	80	78

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] True positive ($n = 53$) and true negative ($n = 48$) samples, defined as described in the text.

TABLE 4. Comparison of IDI-MRSA assay, GenoType MRSA Direct, and selective MRSA agars in the detection of MRSA with groin and axilla swabs[a]

Test	No. of positive samples[b]	No. of negative samples[b]	No. of inhibited samples	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Molecular detection tests							
IDI-MRSA	22	80	2	80	97	90	94
GenoType MRSA Direct	20	78	6	68	96	85	90
Selective agars at 24 h							
MRSA ID	18	86		68	98	94	91
MRSA*Select*	14	90		52	98	93	87
CHROMagar MRSA	10	94		40	100	100	84
Selective agars at 48 h							
MRSA ID	63	41		84	47	33	90
MRSA*Select*	42	62		60	66	36	84
CHROMagar MRSA	35	69		48	70	34	81

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] True positive ($n = 25$) and true negative ($n = 79$) samples, defined as described in the text.

negative predictive values for all sites, whether nasal or nonnasal. However, the sensitivities of MRSA detection were 74% (28/38 isolates) and 55% (17/31 isolates) for MRSA and NORSA detection, respectively, by the GenoType MRSA Direct assay.

DISCUSSION

Detection of MRSA is important for patient care and appropriate utilization of infection control resources. In this evaluation, we compared the performances of two molecular detection assays and three commonly used selective agar plates for MRSA detection with nasal, groin, and axilla swabs in Australia.

IDI-MRSA was the most sensitive method for the detection of MRSA with nasal swabs, with 90% sensitivity, similar to previously reported rates (8, 20). Although not validated for nonnasal sites, the IDI-MRSA assay remained the most sensitive, at 80%, compared to alternative methods. Notably, this is similar to the reported sensitivity of 83.3% by Bishop et al. (1).

Previous studies have reported IDI-MRSA assay-positive MSSA samples secondary to possible complete or partial loss of the SCCmec genetic element (3). These organisms retain a portion of right-junction sequence (the IDI-MRSA target), having lost the *mecA* gene (9). Nine samples were positive by both molecular methods, but we were unable to isolate *Staphylococcus aureus* from the nonselective cultures of the retained swabs. Since the IDI-MRSA assay and the GenoType MRSA Direct assay target different components of the SCCmec gene, the likelihood of these being falsely positive is small (7, 9). However, whether these positive results represent viable and/or transmissible bacteria is unknown.

The alternative molecular test, GenoType MRSA Direct, had a sensitivity of 69%. independent of the swab site. This result was lower than the published rate of 95% (7). The overall reduced sensitivity may be due to the lower rate of NORSA (55%; 17/31 isolates) as opposed to MRSA (74%; 28/38 isolates) detection. The GenoType MRSA Direct assay amplifies SCCmec I to IV as its target and therefore should detect most of the NORSA strains in Australia (2, 16). A possible reason for the lower sensitivity of GenoType MRSA Direct than that of IDI-MRSA may be related to the difference in the detection limits of the assays (IDI-MRSA detection limit, 25 CFU/nasal swab; GenoType MRSA Direct detection limit, 30 CFU/5 μl). This could be secondary to the differences in DNA extraction, with both methods using a heating step but only the IDI-MRSA assay using a bead agitation step. However, attempts to amplify IDI-MRSA-positive, GenoType MRSA Direct-negative samples by using the IDI-MRSA lysate with the GenoType MRSA Direct assay were unsuccessful.

Rapid detection of MRSA-colonized patients has the potential to limit the spread of MRSA. Each IDI-MRSA run could be completed in 2 to 3 h, and the GenoType MRSA Direct assay could be completed in 6 to 7 h, in contrast to selective MRSA agars, which take 48 to 72 h.

Because infection control is usually poorly reimbursed and molecular methods cost approximately five to six times more per test than agar-based methods, cost remains an issue (Table 5). However, the most sensitive agar, MRSA ID, is able to

TABLE 5. Cost per test in Australian dollars

Test	Cost per test	Additional costs[a]	Processing time (min) at $34/h[b]	Time to final result (h)	Total cost[c]
IDI-MRSA	$45	$0.20	15	2	$53.60
GenoType MRSA Direct	$56	$4.60	2.5 h[d]	6	$144.80
MRSA ID	$1.80	$2.40	10	48–72	$9.80
MRSA*Select*	$1.50	$2.40	10	48–72	$9.50
CHROMagar MRSA	$2.05	$2.40	10	48–72	$10.05

[a] Additional costs include plastic wear, filter tips, consumables, sensitivity plates and disks, master mix, etc.
[b] Scientist level (year 8) = $33.68 per hour. Processing time reflects the time for a single agar sample or the time for 16 samples (14 samples, one positive control, and one negative control) for IDI-MRSA or 8 samples for GenoType MRSA Direct. Both molecular tests are limited by the final step, specifically by the number of spaces on the Smartcycler (IDI-MRSA) or Twincubator (GenoType MRSA Direct) machine.
[c] Total cost = cost per test + additional consumable/reagent cost + (processing time × 33.68).
[d] Processing time consists of extraction, setting up PCRs, and 12 hybridization steps, with 2 steps of 30 min each and 10 steps ranging from 1 to 15 min each, therefore restricting laboratory staff from multitasking.

detect only 71% and 82% of isolates at 24 and 48 h, respectively. Therefore, approximately 18% of MRSA-colonized patients may remain undetected at 48 h. Not identifying these patients may cost the institution more in the long term and prevent effective eradication of MRSA from our hospitals.

Selective MRSA agars (MRSA ID, MRSA*Select*, and CHROM agar MRSA) performed best in detecting MRSA from nasal sites, with sensitivities of 72%, 68%, and 75%, respectively, with a fall in sensitivity for detection from nonnasal sites. There was an increase in MRSA detection at 48 h of incubation, independent of the swab site. However, all selective agar plates had poorer detection performances than the previously published rates of around 95% (4, 5, 14). An inoculum effect could partly explain the differences in the selective agar sensitivities, as one swab was used to inoculate all three selective agars. However, this should have affected all methods equally, as swabs were randomly assigned to each detection method and, similarly, the order of selective agar inoculation was random. In addition, if we excluded all positive specimens that were not detected by all three agars (inoculum effect), the overall sensitivity for all sites did not improve significantly and was 69% and 82% at 24 and 48 h, respectively.

In a busy laboratory, processing and reading screening swabs are time-consuming. Selective agar-based detection methods attempt to reduce this time consumption. However, in our laboratory, all isolates are confirmed with tube coagulase and susceptibility testing. Therefore, keeping agar plates for an extended period (48 h) increases the workup of suspicious colonies significantly, with a small increase in MRSA detection. Furthermore, as the specificities of the selective agars at 24 h were similar to the published rates of 95%, 98%, and 99% for MRSA ID, MRSA*Select*, and CHROMagar MRSA, respectively, the need for confirmation may be eliminated if plates are read at 24 h (4, 5, 14).

Our study has some limitations and caveats. Our study population consisted entirely of a known adult MRSA-colonized and/or -infected population (38% positive rate), and therefore the application and performance of these tests are not known for a low-risk population (our institution's overall MRSA colonization rate of approximately 1%). With a reducing prevalence of MRSA colonization, the number of false-positive results would increase (i.e., the positive predictive value would decrease). However, the positive and negative likelihood ratios (markers independent of disease prevalence) revealed that our results would have a substantial bearing on our general hospital population by increasing the pretest odds of MRSA colonization by a factor of 22 with a positive result and reducing the pretest odds of not carrying MRSA by a factor of 0.11 with a negative result by IDI-MRSA (data not shown). Since the MRSA strains were not typed, application of these results to alternative populations (i.e., non-Australian populations) is uncertain.

In conclusion, molecular detection of MRSA by IDI-MRSA remains the most sensitive test in detecting MRSA, independent of site. In addition to greater detection rates, IDI-MRSA has the shortest turnaround time, i.e., 2 to 3 h. Although molecular testing remains expensive relative to conventional agar-based detection, there is an overall cost savings, especially if molecular testing is directed at high-risk populations.

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