

Comparison of the Xpert Methicillin-Resistant *Staphylococcus aureus* (MRSA) Assay, BD GeneOhm MRSA Assay, and Culture for Detection of Nasal and Cutaneous Groin Colonization by MRSA[∇]

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Detection of methicillin (meticillin)-resistant *Staphylococcus aureus* colonization was assessed using combined nose and groin swabs in two commercial PCR assays (the Xpert MRSA assay and the BD GeneOhm MRSA assay). Compared to routine culture, both had similar sensitivities (87.0% versus 84.8%, respectively) and specificities (93.8% versus 92.7%, respectively). Combined PCR assays provide a rapid and more-complete assessment of colonization at a cost similar to that of single-site analysis.

The Xpert methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) assay (GXP-MRSA; Cepheid, Sunnyvale, CA), performed in the GeneXpert system (Cepheid), has the advantage that individual specimens can be rapidly processed without the need for batching of tests (4, 15). GXP-MRSA has FDA approval for direct detection of MRSA in nasal specimens only, and its performance on swabs from other sites has been assessed only on single swabs (11). However, processing combined nose and groin (CNG) swabs potentially provides a more complete assessment of MRSA colonization at an assay cost similar to that of assessing nasal colonization alone (3). We assessed the accuracy of GXP-MRSA for detecting MRSA colonization by the use of CNG swabs compared to that of processing both swabs separately and by culture assay (study A). Second, we prospectively compared the accuracy levels of GXP-MRSA, BD GeneOhm MRSA assay (BD-MRSA; Becton Dickinson Diagnostics, San Diego, CA), and culture for the detection of MRSA colonization by the use of CNG swabs (study B).

Both studies were conducted at the Austin Hospital, Melbourne, Australia, a 450-bed tertiary care university teaching hospital with known high (12 to 15%) rates of patient MRSA colonization (3, 9). The studies were approved by the hospital's human ethics committee. All participating patients gave informed consent, were ≥ 18 years old, and had their anterior nares (N) and the cutaneous area in their groin (G) swabbed with two sets of Copan Liquid Stuart double swabs (Venturi Transystem; Copan Diagnostics, Corona, CA). Both swabs from the first collected set (N1 and N2; G1 and G2) were used for PCR testing. A swab from the second collected set was used

for culture (N3 and G3), and the other (N4 and G4) was stored at 4°C for later use if required for repeat PCR.

All nose and groin swabs were cultured onto chromogenic MRSA agar (Oxoid, Basingstoke, England), and then each swab was placed into 1 ml of tryptone soya broth containing 6.5% sodium chloride. Chromogenic MRSA agar plates were incubated at 35°C and read after 24 and 48 h. Broth cultures were incubated for 48 h at 35°C and then subcultured onto chromogenic MRSA agar. All potential MRSA colonies were subcultured prior to routine identification and antibiotic susceptibility tests (1, 5). MRSA isolates were defined as nonmultiresistant (nmMRSA) if they were resistant to less than three non- β -lactam antibiotics (6). A patient was defined as "MRSA colonized" if, at the time of sample collection, the agar and/or broth cultures from the nose and/or groin were positive.

In study A, the GXP-MRSA assay was performed on specimens collected from patients who, based on our hospital's routine screening program, were suspected of being MRSA colonized or noncolonized. Each patient had separate nose (N1), groin (G1), and CNG swabs (N2 and G2 together) tested according to the manufacturer's instructions for handling a nose swab (4). If any of the GXP-MRSA results were invalid, the assay was repeated (when possible) using the spare nose (N4) and/or groin (G4) swab, which was frozen (-20°C for 30 min) then thawed (unpublished manufacturer's recommendations) prior to insertion in the elution reagent. We defined a specimen as "unresolved" if both the initial and repeat PCR assays were invalid or if the initial assay was invalid and no repeat swab was available for retesting. Unresolved specimens were excluded from final analysis. Crystalline material was noted when the elution reagent was removed from storage at 4°C during study A. Following discussions with the company representative, the elution reagent was routinely warmed for 15 to 30 min at 35°C after removal from storage at 4°C in study B. This aimed to dissolve any crystalline material and reduce the potential risk of an invalid result.

The results of study A are shown in Table 1. Among the

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TABLE 1. Comparison of results for the GXP-MRSA assay versus culture using individual and CNG swabs for detection of MRSA colonization

GXP-MRSA assay result (<i>n</i> = 43)	MRSA culture result ^a							
	Agar alone			Agar and/or broth				
	No. of specimens		Sensitivity (%)	Specificity (%)	No. of specimens		Sensitivity (%)	Specificity (%)
Culture positive	Culture negative	Culture positive			Culture negative			
Nose swabs								
Positive	16	1	94.1	96.0	17	0	89.5	100
Negative	1	24			2	23		
Unresolved ^b	1 ^{c,f}	0			1 ^{c,f}	0		
Groin swabs								
Positive	10	3	90.9	90.0	11	2	78.6	92.6
Negative	1	27			3	25		
Unresolved ^b	1 ^c	1			1 ^c	1		
CNG swabs								
Positive	17 ^d	1	89.5	95.0	17 ^d	1	85.0	94.7
Negative	2	19 ^e			3	18 ^e		
Unresolved ^b	2 ^f	2			2 ^f	2		

^a Sensitivity and specificity of PCR assays compared to culture result.

^b See text for definition; excluded from further analysis.

^c Initial PCR assay invalid and no repeat specimen available.

^d Four specimens were invalid on initial PCR assay and resolved on repeat testing.

^e One specimen was invalid on initial PCR assay and resolved on repeat testing.

^f One unresolved CNG specimen and nose specimen were from the same patient.

selected 43 patients, 22 were MRSA colonized and 21 noncolonized. Initially, 10 patients (12 specimens) had invalid results with GXP-MRSA assays of nose specimens, groin specimens, or CNG swabs, resulting in “invalid/inhibited” rates of 1/43 (2.3%), 2/43 (4.7%), and 9/43 (20.9%), respectively. After repeat testing, 37/43 patients (86%) had valid PCR results for all three assays (N1, G1, and CNG [N2 and G2]), and the unresolved rates for the respective specimen assays were 1/43 (2.3%), 2/43 (4.7%), and 4/43 (9.3%) (Table 1). The explanation for the CNG specimens having higher initial “invalid/inhibited” and subsequent unresolved rates than the individual specimens is unclear, but we believe the need to dissolve the crystalline material (see above) may have been a contributing factor. In study B (see below), in which the crystalline material was dissolved, the “invalid/inhibited” rates for CNG specimens were comparable to those reported by the manufacturer for assessment of nasal specimens alone.

GXP-MRSA performed well using CNG swabs, demonstrating 100% concordance with results from separately processed nose (N1) and groin (G1) swabs and similar levels of sensitivity and specificity as those of culture. In addition, the sensitivity and specificity of GXP-MRSA for detecting nasal MRSA colonization compared to culture (agar and broth) were very similar to those reported by the manufacturer (89.5% versus 86.3% and 100.0% versus 94.9%, respectively) (4) and others (11, 15).

In study B, we prospectively assessed MRSA colonization in a randomly selected group of inpatients from wards known to have a high background rate of MRSA colonization (intensive care, renal, spinal, and liver transplant) in which we compared GXP-MRSA and BD-MRSA assays against each other and against culture for the detection of MRSA in CNG swabs. Nose and groin swabs were combined for processing by GXP-

MRSA as described above. Similarly, CNG swabs were processed using the BD-MRSA assay on the SmartCycler II rapid DNA amplification system (Cepheid, Sunnyvale, CA) as previously described, except that all BD-MRSA DNA lysates were frozen at -20°C until tested (2, 3).

A total of 210 patients were assessed for MRSA colonization in study B, of whom 46/210 (21.9%) were colonized with MRSA (agar culture) in either the nose alone ($n = 24$ [11.4%]), the groin alone ($n = 4$ [1.9%]), or both sites ($n = 18$ [8.6%]) (Table 2). An additional 10 patients were identified as MRSA positive by broth culture (seven in the nose alone, one in the groin alone, two in both sites). Concordance between the two PCR assays was observed in 94% (195/208) of patients. Compared to direct agar culture, the sensitivity and specificity levels of GXP-MRSA and BD-MRSA were very similar: 87.0% versus 84.8% and 93.8% versus 92.7%, respectively (Table 2). These findings are similar to those of Wolk et al. (15), who also found the accuracies of these two assays to be comparable, although these authors assessed only nasal swabs. In our study, groin swabs identified additional patients (5/56 [9%]) who would otherwise have been considered noncolonized. Similarly, other studies have also shown that screening nonnasal sites in addition to nasal swabs increases the sensitivity of detection of MRSA carriers (3, 7, 10).

The initial rate of “invalid/inhibited” results for GXP-MRSA in study B was 5.2% (11/210), with only two of these specimens (2/210 [1%]) unresolved on repeat testing. This rate was lower than the rate observed in study A for CNG specimens (4/43 [9.3%]) and is comparable to that reported by the manufacturer and others for GXP-MRSA assessment of nasal/single-site swabs (4, 11). We believe this difference relates to the off-label step of warming the GXP-MRSA elution reagent prior to use. It is notable that in the new kit format, the elution

TABLE 2. Comparison of GXP-MRSA and BD-MRSA assay results versus culture results, using CNG swabs for the detection of MRSA colonization

PCR assay result (n = 210)	MRSA culture result ^a											
	Agar alone						Agar and/or broth					
	No. of specimens		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No. of specimens		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture positive	Culture negative	Culture positive					Culture negative					
GXP-MRSA												
Positive	40 ^e	10	87.0	93.8	80.0	96.2	42 ^g	8	75.0	94.7	84.0	91.1
Negative	6 ^{d,f}	152 ^c					14 ^{d,h}	144 ^c				
Unresolved ^b	0	2					0	2				
BD-MRSA												
Positive	39 ⁱ	12	84.8	92.7	76.5	95.6	41 ^k	10	73.2	93.5	80.4	90.6
Negative	7 ^j	152					15 ^l	144				
Unresolved	0	0					0	0				

^a Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PCR assays compared to culture result.

^b See text for definition; excluded from further analysis.

^c Eight specimens were invalid on initial PCR assay and resolved on repeat testing.

^d One specimen was invalid on initial PCR assay and resolved on repeat testing.

^e Consists of 19 nose specimens, three groin specimens, and 18 CNG specimens.

^f Consists of five nose specimens and one groin specimen.

^g Consists of 13 nose, two groin, and 27 CNG specimens.

^h Consists of 12 nose specimens, one groin specimen, and one CNG specimen.

ⁱ Consists of 18 nose, three groin, and 18 CNG specimens.

^j Consists of six nose specimens and one groin specimen.

^k Consists of 12 nose, three groin, and 26 CNG specimens.

^l Consists of 13 nose and two CNG specimens.

reagent may now be stored at room temperature. In study B, there were no inhibited specimens for BD-MRSA. This may be related to the freeze-thawing step involved, as previously published (inhibition rates of 4.0% and 0.5% for CNG specimens before and after freeze-thawing, respectively) (3).

Results were discordant (positive for one or both PCR assays, but negative for cultures) for 14/208 (6.7%) patients (four GXP-MRSA and BD-MRSA, six BD-MRSA only, four GXP-MRSA only). Five of these patients had MRSA cultured from recent clinical specimens and/or were receiving vancomycin therapy at the time of specimen collection. The remaining 9/208 (4.3%) discordant results may represent "false-positive" PCR results, possibly due to non-*mecA*-containing *Staphylococcus* chromosome cassette (SCC) elements (8, 11, 13–16).

In 16 patients (16/208 [7.6%]), MRSA was cultured (agar and/or broth) (Table 2) but was not detected by either GXP-MRSA or BD-MRSA (13 were negative by both assays, two were BD-MRSA negative only, and one was GXP-MRSA negative only). Although five of these 16 patients were colonized with nmMRSA, the rate of nmMRSA was no different from that observed in the group that tested PCR positive ($P = 0.96$; chi-square). Primers included in both molecular assays may have been unable to detect these isolates—possibly because of variability in the SCC_{mec} or *orfX* sequence regions (14). Another explanation for the discrepancy between PCR and culture results may be differences in the lower limits of detection of the PCR assays. Rossney et al. (11) have previously reported that the limit of detection for agar culture is 171 CFU/swab, compared to 9 CFU/swab for broth culture and 58 and 190 CFU, respectively, for GXP-MRSA and BD-MRSA. Similar to others, we noted an increased MRSA isolation rate with broth enrichment compared to that with agar culture alone (3, 12, 15); hence, by this benchmark, the sensitivity levels of both

PCR assays were reduced. However, the use of broth enrichment increases both workload and result turnaround times.

In conclusion, our study demonstrates that the GXP-MRSA assay of CNG swabs has sensitivity and specificity similar to those for when the assay is used on individual nose and groin swabs, for the detection of MRSA colonization. Furthermore, GXP-MRSA and BD-MRSA have similar levels of accuracy using a CNG approach. CNG assessment by either PCR assay appears to enhance MRSA colonization detection and allows for a more complete and cost-effective approach to rapid molecular testing without the potential additional cost of processing each specimen separately, as has previously been the case (11, 15).

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