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Dual Site Sampling Improved Detection Rates for MRSA Colonization in Patients with Cutaneous Abscesses

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Abstract

Extranasal sites are common reservoirs of *Staphylococcus aureus* colonization, and may be relevant for methicillin-resistant *S. aureus* (MRSA) screening and infection control strategies. The objective here was to determine whether inguinal specimens could also be screened using Xpert SA Nasal Complete assay for MRSA. Results were compared to broth enrichment culture. Among 162 consented adults seeking care in the Emergency Department for cutaneous abscesses, inguinal specimens were found positive for MRSA more often than nares specimens; 24% and 26% by PCR or culture, respectively compared to 19% each by PCR or culture. Overall, 6% of adults colonized with MRSA would have been missed by nares screening alone. Compared to culture, Xpert SA Nasal Complete assay demonstrated sensitivity and specificity of 89% and 97%, respectively for detecting nares and/or inguinal MRSA colonization. In conclusion, inguinal specimens were a more common reservoir for MRSA than nares specimens in this population of patients.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) transmission is a major public health problem both in the healthcare setting and in the community. While the anterior nares is the

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most commonly surveyed site for *S. aureus* colonization, extra-nasal sites have also been shown to be important for community acquired (CA)-MRSA transmission, with extra-nasal screening increasing MRSA carrier identification by one third compared to nasal screening alone.(1–4) Interestingly, CA-MRSA transmission has also been documented in hospitals worldwide (5–7), emphasizing the importance of building screening paradigms to identify MRSA carriers prior to hospital admission.

There are several high performance molecular-based tests that detect MRSA directly from clinical specimens in under 2 hours.(8) The Cepheid Xpert SA Nasal Complete is one such test; a rapid PCR-based assay designed to screen for nasal MRSA colonization with a reported sensitivity and specificity of 91.9% and 97.9%, respectively, compared to a direct culture method and a run-time of about one hour.(9) This assay boasts high specificity because it employs three molecular targets (*spa*, *mecA*, and *SCCmec*), all of which must be present to generate a positive MRSA result. Unfortunately, current FDA clearance for this and other rapid assays, including the BD GenOhm MRSA assay and Roche light cycler assay, is restricted to nares specimens.(10, 11)

MRSA screening is commonly practiced as an infection control measure for patients being admitted to hospitals to quickly identify colonized patients.(12) However, programs currently employ only nasal screening.(13) Because of the increased interest in identifying MRSA carriers rapidly to reduce hospital transmission of MRSA, we sought to validate the Xpert SA Nasal Complete for use with inguinal specimens to improve our chances of detecting MRSA colonization in an emergency department (ED) population presenting with cutaneous abscesses.(14) The ED is the ideal setting in which to determine MRSA carrier status prior to the patient's hospital admission to target isolation practices. Although the Xpert SA Nasal Complete Assay also detects methicillin-sensitive *S. aureus* we chose to focus solely on MRSA colonization due to its clinical importance for infection control efforts.

Materials and Methods

Enrollment

We conducted a prospective, observational study in an urban academic ED between May 2011 and October 2012. Eligibility criteria included adults ≥ 18 years who presented to the ED with ≥ 1 cutaneous abscesses, defined as a tender, swollen, fluctuant skin lesion necessitating incision and drainage. Patients with post-operative or post-procedure wound infections, those currently on antibiotics, or those treated for the same abscess within the past 14 days were excluded. All subjects were consented in English and completed a written survey to identify demographic and epidemiologic characteristics. The George Washington University Institutional Review Board approved this study protocol.

Specimen Collection and Transport

Bilateral nares and inguinal crease specimens were collected using 2 pairs of Copan swabs (Cat. #900-0370, Copan Italia, S.p.A., Brescia, Italy) either by healthcare providers using a standardized protocol, or by the patients themselves who were given the opportunity to self-

collect inguinal swabs after receiving verbal instruction. Of the 162 consented subjects, 2 declined nares swab collection and 20 declined inguinal swab collection. To ensure more equal distribution of specimen between the pairs of swabs collected from each individual body site, the pair of swabs were rubbed together before being placed in Copan Transystem Culture Swab Transport System. One of the pair was immediately analyzed for presence of MRSA using the Xpert SA Nasal Complete (Cepheid, Sunnyvale, CA), while the other was stored at 4°C for up to 1 week before being shipped overnight on cold packs to Cepheid where broth-enriched culturing and biochemical testing for presence of MRSA was conducted.

Direct Culture for Wound Specimens

Clinician-ordered wound cultures were performed onsite at The George Washington University hospital clinical microbiology laboratory according to the following protocol. Specimens were inoculated directly onto 5% sheep blood agar (Thermo Scientific), chocolate agar (Thermo Scientific), MacConkey agar (Thermo Scientific) and Colistin Nalidixic Acid agar (Thermo Scientific) agar plates and incubated at 35°C for 24–48 h. *S. aureus* was identified using standard methods based on colony morphology, Gram stain, catalase test, mannitol fermentation, and coagulase results. Methicillin resistance was assessed for all *S. aureus* isolates using MRSASelect chromogenic agar (Bio-Rad, Redmond, WA) incubated at 35°C for 24 h before visual inspection for pink colony growth by a trained clinical laboratory technician. All specimens were plated within 2 h of receipt if received between 8 AM and midnight; otherwise, swabs were refrigerated at 4°C and plated the following morning on the above combination of agar plates.

Broth-Enrichment Culture for Colonization Specimens

Upon arrival at Cepheid, each swab was incubated overnight in 2.2 ml Tryptic Soy Broth with 6.5% NaCl (Cat. #R065032, Remel, Lenexa, KS) at 35°C. Enrichment broth was plated onto HardyCHROM MRSA agar (Cat. #G307 Hardy Diagnostics, Santa Monica, CA) and incubated for 20–24 hours at 35°C. Presence of MRSA was determined visually for pink/magenta colonies. Colonies with ambiguous results were tested for catalase and coagulase production; susceptibility to cefoxitin and oxacillin was confirmed via disk diffusion as described by the Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton agar (Cat. # R01620, Remel, Lenexa, KS) (15).

Xpert SA Nasal Complete Assay

Individual inguinal and nares swabs were tested directly for MRSA using the Xpert SA Nasal Complete assay (Cat. # GXSACOMP-10, Cepheid) according to the manufacturer's recommendation; inguinal specimens were processed in the same manner as nares swabs. Testing was performed by non-laboratory, non-clinical research personnel trained on proper use of the Xpert SA Nasal Complete assay by Cepheid staff in the hospital clinical laboratory. Specimens with Xpert SA Nasal Complete and enriched culture discordant results were further analyzed by testing 10 µl of enrichment broth using the Xpert SA Nasal Complete assay. The resulting pre and post-enrichment cycle threshold (Ct) values were compared.

Statistical Analysis

Data analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Demographic data were calculated with descriptive frequencies. Contingency tables were set up to evaluate the analytical performance of the Xpert SA Nasal Complete compared to broth-enrichment culture for detecting MRSA colonization directly from nares and inguinal specimens.

Results

Patient Demographic and Clinical Characteristics

Of the 200 ED patients approached for this study, a total of 162 participants (84 women, 78 men) were enrolled, with 160 nares specimens and 142 inguinal specimens collected. Mean age of participants was 37 years of age, 80% classified themselves as African-American, 26% reported at least one co-morbid medical condition, and 23% reported hospitalization within the past twelve months (Table 1). The footnote in Table 2 provides an explanation for the missing data on 22 of the 162 participants.

Wound Culture Results

Of the 162 patients, 88% had available wound culture results. Of the wound cultures completed by the hospital microbiology laboratory, 31% were MRSA, 18% were methicillin-susceptible *Staphylococcus aureus* (MSSA), 14% were coagulase-negative *Staphylococcus spp.*, and 6% were gram-negative bacilli. Seventeen percent of wound cultures contained polymicrobial growth, while 2% percent of cultures were sterile

Broth-Enrichment Culture Results for MRSA Colonization—Nineteen percent (30/160) of nares samples and 26% (37/142) of inguinal samples were positive for MRSA when tested using the broth-enrichment culture method (Table 2). Combining culture results from both body sites when available resulted in 31% (44/142) of individuals being positive for MRSA colonization.

Xpert SA Nasal Complete Results for MRSA Colonization—Nineteen percent (31/160) of nares samples and 24% (34/142) of inguinal samples tested positive for MRSA directly from swabs using Xpert SA Nasal Complete. Combining the test results from both body sites, when available, resulted in 30% (42/142) of patients colonized with MRSA (Table 2). Fifty-five percent (17/31) of MRSA positive nares specimens occurred in patients with abscesses of the axilla, trunk or face; 23% (7/31) in those with abscesses of the perineum and buttock; and 23% (7/31) for extremity abscesses compared to 44% (15/34), 35% (12/34), and 21% (7/34) for inguinal specimens, respectively.

Analytical Performance Characteristics of the Xpert SA Nasal Complete Assay—Compared to broth-enrichment culturing, the Xpert SA Nasal Complete demonstrated 83% sensitivity and 95% specificity for detecting MRSA colonization in the nares, and 84% sensitivity and 97% specificity for detecting MRSA colonization from inguinal samples. Combining nares and inguinal colonization data, the Xpert SA Nasal Complete assay demonstrated 89% sensitivity and 97% specificity for detecting MRSA directly from swab-

based specimens compared to broth enrichment culture (Table 2). Molecular-based testing of nares specimens yielded a positivity rate of 81% compared to 83% by culture. Molecular-based testing of inguinal specimens yielded a positivity rate of 91% compared to 84% by culture. There were 20 discordant samples, 11 nares and 9 inguinal samples (Table 2), the most common (11/20; 55%) being false negative results generated with Xpert SA Nasal Complete. We hypothesize that most of these represent specimens containing MRSA at levels below the limit of detection for the Xpert SA Nasal Complete assay. The other discrepancy seen was false positive results with Xpert SA Nasal Complete (9/20; 45%); two isolates contained *SCCmec* empty cassettes, while a third isolated displayed *mecA* resistant genotype not expressed phenotypically.

Discussion

To our knowledge, this was the first study that evaluated the performance of the Xpert SA Nasal Complete assay for both inguinal and nares specimens using trained non-laboratory personnel; prior assay performances were conducted in traditional laboratory settings (16). This study employed a convenience sample population of high-risk ED patients with cutaneous abscesses and an expected high rate of MRSA infection; 31% of wound specimens isolated MRSA in culture. MRSA was found more often in inguinal specimens than in nares specimens regardless of the testing method used. Most notably, 6% of MRSA carriers in this high-risk population would have been missed had nares screening alone been performed. Importantly, only 57% of those colonized with MRSA would have been identified through a MRSA positive wound culture.

Analytical sensitivity of the Xpert SA Nasal Complete assay improved with dual screening from 83% with nares alone, to 89% for combined testing, while specificity and PPV improved from 95% to 97%, and 81% to 93% respectively. Furthermore, there was no significant association between colonization site and abscess location, suggesting that extra-nasal colonization is important in patients at risk of MRSA regardless of the location of the wound infection. This data highlights the value of including non-nasal specimens for improved MRSA screening.

Patients at high risk for MRSA colonization or infection who are admitted to the hospital are typically placed in contact precautions and isolation due to concerns for healthcare transmission. There can be unintended consequences for these patients including less patient interaction and care, increase in adverse events, possibly anxiety and depression and patient dissatisfaction. (17–21) Therefore using the Xpert Nasal Complete assay in the ED setting to screen this type of patient has the potential to reduce adverse effects associated with inappropriate isolation for patients not colonized with MRSA, as well as facilitate appropriate isolation of those patients who do screen positive for MRSA in the ED. Use of a rapid molecular test could also potentially decrease boarding times in the ED by avoiding delays in transfer to the inpatient ward, as hospitals typically suffer from overcrowding with limited isolation beds. (22)

Another key finding from this study was the ease of use and excellent performance characteristics demonstrated in the hands of minimally trained non-laboratory staff. These

results suggest an important opportunity for supporting near patient testing in the ED by the clinical laboratory as well as use of rapid molecular testing platforms under CLIA waiver. While personnel performing the testing were not clinical providers, most had very little laboratory experience. The results of this study illustrated that clinical staff could perform this testing satisfactorily in episodic care settings. Although current regulations in the US and elsewhere do not support using the Xpert assays by non-laboratory personnel, from our experience in the ED, we believe that these moderately complex assays might be considered for CLIA waiver.

There were several limitations to our investigation. First, we compared the Xpert Nasal Complete assay to broth enrichment culture rather than the commonly used, more rapid, cheaper, but less sensitive direct culture method. Not surprisingly, previous studies show higher sensitivities for direct PCR-based tests compared to direct culture. (16) Secondly, our study was designed such that specimens were held at 4°C for weekly shipment to an off-site laboratory for *S. aureus* culturing rather than be cultured on-site, while wound cultures were processed within 24 hours onsite. This delay in shipping samples for culture could have negatively impacted organism viability. While the vast majority of the specimens were shipped within four days of collection, we believe this was an issue in 9 specimens with culture-negative, PCR-positive results. Finally, although all clinicians were provided with a video on how to collect the nares specimens, and handouts and instruction were given to both providers and patients regarding inguinal specimen collection, we did not monitor the quality of specimen collection on an ongoing basis. While PCR testing by non-laboratory personnel also may have impacted study results, all personnel were tested for competency, prior to performing study testing, and performance did not vary substantially from other published results.(23) That being said, we believe this scenario was an accurate representation of how molecular testing would occur in an ED setting.

In summary, controversy exists regarding current MRSA screening recommendations, particularly with regards to universal nasal screening. (24) In addition, extra-nasal screening is not routinely performed.(25) Hospitals are overcrowded, and have limited numbers of isolation beds (22) so implementing rapid MRSA screening of both nares and inguinal specimens would not only improve chances of identifying colonized patients but also the confidence in negative results to eliminate the need for isolation. This approach in the ED for high-risk patients could help to reduce the need for hospital resources and improve patient care. Future studies should be undertaken to determine the cost-effectiveness of near patient MRSA screening. Consideration should also be given for co-testing nares and inguinal swabs in the same cartridge to improve the sensitivity of detecting MRSA colonization without the added cost and time of analyzing a second specimen.

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Table 1

Patient demographics and clinical characteristics

Patient Characteristic (n = 162)	Breakdown (n, %)
Gender	
Female	84 (52)
Male	78 (48)
Age	
Mean (years)	36.8
Range (years)	18–84
Race	
Black	129 (80)
White	26 (16)
Hispanic	2 (1)
Other	5 (3)
Comorbidities	
Diabetes	23 (14)
HIV	14 (9)
Other (IDU, immunosuppressed)	5 (3)
None	120 (74)
Prior Hospitalization (within last 12 months)	37 (23)
Abscess Location	
Axilla	39 (24)
Trunk	31 (19)
Extremities	31 (19)
Buttock	29 (18)
Perineum	16 (10)
Face	16 (10)
Clinical abscess(es) presentation	
Single	139 (86)
Multiple	23 (14)
Prescribed Antibiotics provided during this ED visit	122 (75)

HIV, human immunodeficiency virus; IDU, injection drug use, ED; emergency department.

Table 2

Analytical performance characteristics of the Xpert SA Nasal Complete Assay in which swabs were tested directly compared to broth enrichment culture for detecting MRSA colonization in nares and/or inguinal samples.

Direct Xpert SA Nasal Complete Assay Results	Broth Culture Results		% (95% CI)			
	MRSA +	MRSA -	Sensitivity	Specificity	PPV	NPV
Nares ^a						
MRSA +	25	6	83 (66–93)	95 (90–98)	81 (63–91)	96 (91–99)
MRSA -	5	124				
Inguinal ^b						
MRSA +	31	3	84 (64–88)	97 (93–99)	91 (76–98)	94 (88–98)
MRSA -	6	102				
Combined Nares/Inguinal ^c						
MRSA +	39	3	89 (76–96)	97 (90–99)	93 (80–98)	95 (89–99)
MRSA -	5	95				

^a n = 160.

^b n = 142.

^c n = 142

If either nares or inguinal specimens were positive, then the combined result was considered positive. MRSA, Methicillin-resistant *Staphylococcus aureus*; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

There was missing data for 22 of the 162 enrolled participants; 20/22 were from inguinal samples and 2/22 from nasal samples. Reasons for these included: 12/162 patients declined inguinal swab collection, 6/162 samples generated errors on the Xpert instrument (5 increased syringe pressure, all from inguinal samples and 1 probe check failure from a nasal sample), and 4/162 samples with invalid results (3 inguinal, 1 nares).