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The Methicillin-resistant *Staphylococcus aureus* (MRSA) Nasal Real-time PCR: A Predictive Tool for Contamination of the Hospital Environment

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Abstract

Introduction—We sought to determine whether the bacterial burden in the nares, as determined by the cycle threshold (C_T) value from real-time MRSA PCR, is predictive of environmental contamination with MRSA.

Methods—Patients identified as MRSA nasal carriers per hospital protocol were enrolled within 72 hours of room admission. Patients were excluded if 1) nasal mupirocin or chlorhexidine body-wash was used within the past month or 2) an active MRSA infection was suspected. Four environmental sites, 6 body sites and a wound, if present, were cultured with pre-moistened swabs. All nasal swabs were submitted for both a quantitative culture and real-time PCR (Roche Lightcycler, Indianapolis, IN).

Results—82 patients had a positive MRSA-PCR at study enrollment. There was a negative correlation of moderate strength between the C_T value and the number of MRSA colonies in the nares ($r = -0.61$, $p<0.01$). Current antibiotic use was associated with lower levels of MRSA nasal colonization (C_T value: 30.2 vs. 27.7, $p<0.01$).

Patients who had concomitant environmental contamination had higher median log MRSA nares count (3.9 vs. 2.5, $p=0.01$) and lower C_T values (28.0 vs. 30.2, $p<0.01$). However, a ROC curve was unable to identify a threshold MRSA nares count that reliably excluded environmental contamination.

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Conclusions—Patients with a higher burden of MRSA in their nares, based on the C_T value, were more likely to contaminate their environment with MRSA. However, contamination of the environment cannot be predicted solely by the degree of MRSA nasal colonization.

Keywords

MRSA; environment; real-time PCR

The prevention of methicillin-resistant *Staphylococcus aureus* (MRSA) healthcare-associated infections has been a priority at many acute care hospitals. To this end, many hospitals routinely screen patients for nasal MRSA carriage and place identified carriers on contact precautions.[1-7] The rationale for these practices is that healthcare workers caring for MRSA-positive patients can contaminate their hands or clothing and thereby serve as vectors of MRSA transmission.[8,9]

Current screening and isolation techniques treat all MRSA nasal-carriers equally even though some MRSA-carriers may be more likely sources of transmission than others. Among all MRSA nasal carriers, 60% are colonized in both the nose and at least one other body site.[10-12] Patients colonized at multiple body sites with *S. aureus* are at higher risk of transmitting *S.aureus* than patients colonized solely in the nares.[13,14] Certain sites of MRSA colonization are associated with high rates of environmental contamination, particularly the urine, wounds, groin, and the gastrointestinal tract.[9,14,15] Environmental contamination is a predictor of bacterial transmission to healthcare-workers' attire.[16]

A few studies have suggested that the burden of nasal colonization is also an important determinant of transmission. In an older report, carriers with <100,000 colonies of *S.aureus* in the nares were at no greater risk of dissemination than non-carriers.[17] In a more recent study, a quantity of MRSA in the nares < 500 colony-forming units was associated with less skin colonization and less environmental contamination.[18] However, current techniques of MRSA nasal screening make no distinction between heavy and low bacterial burdens.

In this study, we examined whether using the real-time PCR in a quantitative manner and routinely assessing for extra-nasal colonization could help predict which MRSA nasal-carriers were more likely to contaminate their hospital surroundings.

Methods

The Richard Roudebush Indianapolis Veterans Affairs Medical Center is a 200-bed, tertiary-care facility. As part of the MRSA Prevention Initiative, swabs from the anterior nares are collected from each patient upon admission, transfer, and discharge.[19] This nasal sample is analyzed by using the Lightcycler 2.0 (Roche Diagnostics, Indianapolis, IN). According to the test's package insert, a positive result will be produced with 95% confidence for a swab containing 240 colony-forming units. The lab routinely uses manufacturer-supplied positive and negative controls when running this test.

Patients

Patients from any medical unit (except psychiatry) were eligible for inclusion within 72 hours of hospital admission or room transfer if the hospital's MRSA nasal swab was positive at the time of room assignment. Patients were excluded if, at the time of screening, they were likely to have an active MRSA infection, as defined by a clinical culture growing 1) gram-positive cocci in clusters, 2) *Staphylococcus aureus* with susceptibilities pending, or 3) MRSA. Patients were also excluded if they had been treated with nasal mupirocin or chlorhexidine body-wash within the past month.

Patients were enrolled by one of 2 investigators (S.A. or D.L.) between 7/13/2012 and 8/19/2013. Enrollment involved collection of culture samples, including a repeat nasal swab, and the completion of a short survey on hygiene habits at home. The patient's medical record was reviewed to extract pertinent information, including relevant comorbidities: diabetes mellitus, intravenous drug use, organ transplantation, hemodialysis, liver cirrhosis, skin disease, malignancy, or human immunodeficiency virus (HIV) infection. For the duration of this study, Environmental Services was not cleaning high-touch surfaces on a daily basis. Terminal cleaning of all rooms was performed with a standard disinfectant, Wexcide (Wexford Labs, Kirkwood, MO).

Sample collection and processing

Sterile, pre-moistened rayon swabs (bioMérieux SA, Marcy l'Etaile, France) were used to sample all body and environmental sites in a standardized fashion.

The nasal culture, which included 2 swabs, was processed as follows: the 2 swabs were rubbed against each other using a circular motion to ensure equal distribution of bacteria. One of these swabs was analyzed using the Roche Lightcycler (Indianapolis, IN), and the cycle threshold value (C_T) was recorded. The C_T value is the cycle number at which MRSA DNA is first detected; it is inversely proportional to the amount of MRSA on the sample.

The second nasal swab was re-suspended in 1 mL of sterile water; a 1 µL and a 10 µL calibrated loop were used to inoculate separate ChromeID MRSA agar plates (bioMérieux SA). The second nasal swab was also suspended in 6.5% sodium chloride. Likewise, the swabs from all extra-nasal and environmental sites were cultured on ChromeID MRSA agar. All plates were incubated at 35°C for 48 hours. At 24 and 48 hours, plates were examined for the presence of green-colored colonies. An accurate colony count was only performed on the plates from the 2nd nasal swab; a semi-quantitative count was performed for all other cultures. For each case, the nares isolate and one randomly chosen isolate from a body site and the environment, if positive, were typed by pulsed-field gel electrophoresis (PFGE) as previously described.[20]

Statistical analysis

The continuous variables (e.g. quantitative nares culture and C_T values) were summarized using median and inter-quartile range (IQR). The categorical variables (e.g. environmental contamination and extra-nasal colonization) were summarized using frequency and percentage. The association of environmental contamination and extra-nasal colonization with categorical and continuous covariates was evaluated through Fisher's exact test and Wilcoxon rank sum test, respectively. Logistic regression was used to model environmental contamination in terms of log MRSA count, and threshold values were determined using a receiver operating characteristic (ROC) curve. All the statistical tests were carried out at two-sided 5% level of significance in R statistical software, version 2.15.1 (http://cran.us.r-project.org).

The protocol and conduct of this study were reviewed and approved by the Indiana University Institutional Review Board.

Results

Ninety-four subjects were enrolled in the study. The median age of the 94 subjects was 63 years (IQR 57-71). Ninety-six percent of subjects were men, and 89% were white.

In 12 patients, the study nasal swab did not yield a positive PCR result for the following reasons: negative PCR test (8), no PCR result provided due to the presence of inhibitors on the swab (3), and loss of specimen (1). Therefore, 82 subjects had a positive MRSA-nasal swab by PCR at the time of enrollment.

In these 82 cases with a positive PCR test for MRSA nasal colonization, the median C_T value was 29.8 (IQR 26.7–32.2). The median colony count on nares quantitative culture was 200 (IQR 10-40,000). In 11 (13%) patients, the nares quantitative culture yielded a colony count of >100,000. In 14 patients (17%), the colony count was 0. There was a negative correlation of moderate strength between the C_T value and the number of MRSA colonies in the nares ($r= -0.61$, $p<0.01$). There was a poor correlation between C_T values collected by the hospital nurse and the research team ($r=0.34$, $p<0.01$).

The C_T value was not significantly different in patients with a prior history of MRSA infections (yes 30.2 vs. no 29.0, $p=0.23$) or in patients with or without comorbidities (29.8 vs. 28.5, $p=0.10$).

Fifty-three subjects (65%) were on antibiotics at the time of study enrollment. The degree of nasal colonization, as measured by the C_T value, was lower in patients on antibiotics compared to those who were not (C_T value: 30.5 vs. 27.7, $p<0.01$). Of note, only 3 patients were on antibiotics traditionally thought to affect nasal carriage: doxycycline (2) and trimethoprim/sulfamethoxazole (1). Twenty-six patients were on antibiotics with anti-MRSA activity: vancomycin (23), clindamycin (2) and trimethoprim/sulfamethoxazole (1). Two of these patients on anti-MRSA antibiotics were also receiving doxycycline. Patients on an anti-MRSA antibiotics had higher C_T values, or less nasal colonization, than those not receiving anti-MRSA antibiotics (C_T value: 30.8 vs. 28.6, $p<0.01$).

Extra-nasal MRSA colonization

At least one body site was colonized in 58 (70.7%) patients. The most common body sites colonized were the groin 53.7%, chest wall 40.2%, abdominal wall 29.3%, axilla 28.0%, and forearm 20.7%. The urine culture was MRSA-positive in 4.2% of the 70 patients tested. In 14 patients with open wounds, 50% had wound colonization with MRSA.

The median nares C_T value in patients with extra-nasal colonization was comparable to that of patients without extra-nasal colonization (28.9 vs. 29.8, p=0.60). The median log MRSA nares count in the individuals who had extra-nasal colonization was also comparable with that of those who did not (3.48 vs. 2.30, p=0.06). We did not observe any association (Table 2, p=0.08) in the proportion of patients with extra-nasal colonization and different degrees of MRSA nares colonization.

Environmental contamination with MRSA

At least one environmental surface was contaminated in 34 (41.5%) patients. The frequency of contamination was as follows: call button 23.2%, bedside table 22.0%, telephone 17.1%, and bedrail 14.6%. In 30/30 (100%) patients with environmental contamination whose isolates underwent PFGE typing, the environmental isolate had the same PFGE type to the nares and/or body-site isolate.

Table 1 shows the association between measured covariates and environmental contamination. Environmental contamination was significantly associated with extra-nasal colonization at certain body sites, including the abdominal wall (p<0.01), chest wall (p<0.01), forearm (p=0.01), and groin (p<=0.01). The median nares C_T value was lower in patients with environmental contamination compared to patients without environmental contamination (27.5 vs. 30.3, p<0.01). The median log MRSA nares count was also significantly higher in the individuals who had environmental contamination than those who did not (3.90 vs. 2.45, p=0.01). The proportions of environmental contamination based on different levels of MRSA nares colonization is displayed in Table 2. The incidence of environmental contamination was significantly higher in patients with MRSA nares counts greater than 1,000 (57.1% vs 25%, p<0.01).

Using logistic regression, environmental contamination was modeled with log MRSA nares count as the only predictor. The associated ROC curve was not helpful in identifying environmental contamination. For example, using a cut-point of 13, the nares count can correctly identify 76% of the cases with environmental contamination, but its ability to identify patients without any environmental contamination is very poor (50%).

Discussion

Prior studies have demonstrated that the burden of *S. aureus* nasal colonization is predictive of extra-nasal colonization [21] and environmental contamination.[17,18] Environmental contamination is a predictor of bacterial transmission to healthcare-workers' attire.[16] However, current methods for MRSA nasal screening make no distinction between heavy and low bacterial burdens.

In this study, patients who had higher nasal burdens of MRSA were more likely to contaminate the hospital environment with MRSA. This was true whether the nasal burden was measured by the C_T value or a quantitative culture. However, this study was unable to identify a threshold level of MRSA nasal colonization that would reliably exclude any MRSA contamination of the hospital surroundings. It is tempting to speculate that low-level

MRSA carriers may have only produced low-level environmental contamination, but this study was not designed to assess environmental contamination in a quantitative fashion.

Certain body sites were more likely to be associated with environmental contamination: abdominal wall, chest wall, forearm, and groin. These are areas in frequent contact with healthcare workers' hands and other equipment, so the association with environmental contamination is biologically plausible. A prior report also found that groin colonization was a significant predictor of environmental contamination.[14]

Higher MRSA nares count were not associated with more frequent extra-nasal colonization. In a study of 60 adults, there was a correlation between higher MRSA nares counts and the likelihood of extra-nasal colonization, but the use of systemic antibiotics was not reported, an important difference from the current project.[21] In addition, since some MRSA carriers are primarily colonized at an extra-nasal site, such as the groin,[22-24] a positive correlation between nares colonization and extra-nasal carriage may not be expected.

In this study, antibiotic use was associated with less MRSA colonization of the nares. This association was seen even though very few patients were receiving systemic antibiotics used for decolonization of the nares. Furthermore, patients who were on antibiotics with anti-MRSA activity—primarily parenteral vancomycin—had lower colony counts than those who were not receiving anti-MRSA therapy. Although parenteral vancomycin is not thought to affect MRSA nasal colonization, prior studies have not assessed vancomycin's effect on nasal colonization in a quantitative manner.[25,26] Based on our findings, the effect of parenteral vancomycin on MRSA nasal colonization may be more complicated than previously described.

A standardized method for swabbing the nares is important to accurately assess the degree of MRSA nasal colonization. In agreement with a prior report, we found a negative correlation between the C_T value and the number of MRSA colonies in the nares.[27] We found a poor correlation between the C_T value from the study's nasal swabs and the nasal swabs collected by the hospital nurses. We believe this poor correlation reflects a wide variability in collection techniques. While all study swabs were collected by 1 of 2 investigators using a standardized approach, hundreds of different hospital nurses were collecting nasal swabs on behalf of the hospital's MRSA program.

This study has several strengths. We used a consistent, standardized approach to assessing the degree of MRSA colonization and environmental contamination in a defined cohort of hospitalized patients. Other factors predictive of environmental contamination were assessed through a survey and chart review. PFGE was performed on nearly all environmental isolates to determine whether they originated from the case patient. Even though the PFGE types matched, we cannot exclude the possibility that these isolates actually reflected contamination from prior room occupants.

Our study had some limitations. First, our cohort largely included elderly white men, so our findings may not be generalizable to other populations. Second, an insensitive technique (i.e. swabbing) was used to test for environmental contamination. Since we used the same sampling technique for all patients' rooms, it's doubtful that this would have introduced bias into our results. Third, although we assumed that rubbing the 2 nasal swabs together would equally distribute the bacteria, we did not validate this assumption. Fourth, the amount of time patients spent in their rooms prior to enrollment was not standardized, which may have introduced variability into our results. In our analysis, the amount of environmental contamination did not differ by time spent in the room. Fifth, the effect of prior nasal swabs on the current degree of MRSA colonization is unknown, and our study did not assess the day-to-day variability in MRSA burden. Eight patients found to be MRSA-positive on admission were no longer MRSA-positive at the time of enrollment, which suggests that there may have been intermittent nasal carriage of MRSA.[28] Finally, behavioral factors were based on patient self-reporting, which may have been prone to bias. In addition, hygiene habits at home were assessed, but these may not reflect hygiene habits in the hospital.

In conclusion, patients with a higher burden of MRSA in their nares, as determined by the C_T value, are more likely to have environmental contamination with MRSA. However, contamination of the environment cannot be predicted solely by the degree of MRSA nasal colonization.

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Table 1

Factors associated with MRSA contamination of the hospital environment in 82 patients with known MRSA nasal colonization

	Environmental contamination (n=34)	No environmental contamination (n=48)	p-value
Male, n(%)	34 (100%)	46 (95.8%)	0.5086
White, n (%)	32 (94.1%)	40 (83.3%)	0.1830
Age			
Median (IQR)	63 (51, 68)	64 (59, 72)	0.1919[c]
Any comorbidity, n (%)	20 (58.8%)	32 (66.7%)	0.5195
Central line, n (%)	3 (8.8%)	9 (18.8%)	0.6928
Urinary catheter, n (%)	5 (14.7%)	11 (22.9%)	0.1513
Current antibiotic use, n (%)	22 (64.7%)	31 (64.6%)	0.9969
Prior MRSA infections, n (%)	9 (26.5%)	12 (25.0%)	0.1179
Time since admission in current room (hours)			
Median (IQR)	40.4 (36.4, 43.7)	42.5 (40.0, 60.2)	0.1497[c]
Prior history of a boil, rising, or abscess	15/32 (46.9%)	16/47 (34.0%)	0.3481
Handwashing > 6 times per day	14/32 (43.8%)	14/46 (30.4%)	0.2419
Bathing > 4 times/week	17/32 (53.1%)	22/47 (46.8%)	0.6500
Nose-picking	25/31 (80.6%)	41/47 (87.2%)	0.5261
Log MRSA nares count			
Median(IQR)	3.90 (2.77, 4.84)	2.45 (1.00, 4.11)	0.0148[c]
Cycle threshold (C_T)			
Median (IQR)	27.5 (26.1, 30.4)	30.3 (27.7, 33.2)	0.0053[c]
Extra-nasal colonization, n (%)			
Abdominal wall	16 (47.1%)	8 (16.7%)	0.0060
Axilla	13 (38.2%)	10 (20.8%)	0.1334
Chest wall	23 (67.7%)	10 (20.8%)	<0.0001
Forearm	12 (35.3%)	5 (10.4%)	0.0114
Groin	9 (26.5%)	19 (39.6%)	0.0034
Urine [a]	2 (7.1%)	1 (2.4%)	0.5507
Wound [b]	2 (50%)	5 (50%)	>0.999

[a]Urine cultures were examined in 28 and 42 patients, respectively.

[b]Wound cultures were examined in 4 and 10 patients, respectively.

[c]p-value based on Wilcoxon rank sum test. Otherwise, p-value is based on Fisher's exact test

Table 2

Prevalence of environmental contamination and extra-nasal colonization based on the MRSA nares bacterial count

MRSA nares count	Total	Environmental contamination n (%)	Extra-nasal colonization n (%)
0	14	4 (28.6%)	6 (42.9%)
0-10	15	4 (26.7%)	12 (80.0%)
10-100	2	0 (0%)	1 (50.0%)
100 -1,000	9	2 (22.2%)	5 (55.6%)
1,000 – 10,000	14	9 (64.3%)	12 (85.7%)
10,000 – 100,000	28	15 (53.6%)	22 (78.6%)