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Daniel J. Livorsi, Richard Roudebush VA Medical Center
Sana Arif, Indiana University
Patricia Garry, Richard Roudebush VA Medical Center
Madan G. Kundu, Indiana University
Sarah Satola, Emory University
Thomas H. Davis, Indiana University
Byron Batteiger, Richard Roudebush VA Medical Center
Amy B. Kressel, Indiana University

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

Daniel J. Livorsi, MD, MSc;1,2 Sana Arif MD;2 Patricia Garry, MT, ASCP;1 Madan G. Kundu, PhD;3 Sarah W. Satola, PhD;4,5 Thomas H. Davis, MD;6 Byron Batteiger, MD1,2 Amy B. Kressel, MD, MS2

**Objective.** We sought to determine whether the bacterial burden in the nares, as determined by the cycle threshold (CT) 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 premoistened swabs. All nasal swabs were submitted for both a quantitative culture and real-time PCR (Roche Lightcycler, Indianapolis, IN).

**Results.** At study enrollment, 82 patients had a positive MRSA-PCR. A negative correlation of moderate strength was observed between the CT 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 (CT value, 30.2 vs 27.7; \( P < 0.01 \)). Patients with concomitant environmental contamination had a higher median log MRSA nares count (3.9 vs 2.5, \( P = 0.01 \)) and lower CT 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.

**Conclusions.** Patients with a higher burden of MRSA in their nares, based on the CT 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.


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. 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 the attire of healthcare workers.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 noncarriers.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 MRSA nasal-screening techniques make no distinction between heavy and low bacterial burdens.

In this study, we sought to determine whether using the real-time polymerase chain reaction (PCR) in a quantitative...
manner and routinely assessing for extranasal colonization could help predict which MRSA nasal carriers were more likely to contaminate their hospital surroundings.

METHO DS
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. These nasal samples are analyzed using the Lightcycler 2.0 (Roche Diagnostics, Indianapolis, IN). According to the manufacturer’s protocol, a positive result is produced with 95% confidence for a swab containing 240 colony-forming units. The laboratory routinely uses positive and negative controls supplied by the manufacturer 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) S. 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 1 of 2 investigators (S.A. or D.L.) between July 13, 2012, and August 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. Each 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, premoistened rayon swabs (bioMérieux SA, Marcy l’Etoile, 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 (CT) was recorded. The CT 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 resuspended in 1 mL of sterile water; 1-µL and 10-µL calibrated loops 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 extranasal 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 second 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.

Statistical Analysis
The continuous variables (eg, quantitative nares culture and CT values) were summarized using median and interquartile range (IQR). The categorical variables (eg, environmental contamination and extranasal colonization) were summarized using frequency and percentage. The association of environmental contamination and extranasal 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 2-sided and were conducted using R statistical software, version 2.15.1 (http://cran.us.r-project.org) with a significance level of $P < 0.05$.

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

RESULTS
We enrolled 94 patients in this study. The median age of the 94 patients was 63 years (IQR, 57–71); 96% of the patients 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 patients had a positive MRSA nasal swab by PCR at the time of enrollment.

In the 82 cases with a positive PCR test for MRSA nasal colonization, the median CT 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 patients (13%), the nares quantitative culture yielded a colony count $>100,000$. In 14 patients (17%), the colony count was 0. A negative correlation of moderate strength was observed between the CT value and the number of MRSA colonies in the nares ($r = -0.61; P < 0.01$). A poor correlation was observed between CT values collected by the hospital nurse and the research team ($r = 0.34; P < 0.01$).

The CT value was not significantly different in patients with a prior history of MRSA infections (yes [30.2] vs no [29.0];
Environmental contamination was significantly higher in patients with MRSA nares counts $>1,000$ (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 cutoff 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 extranasal colonization\textsuperscript{21} and environmental contamination.\textsuperscript{17,18} Environmental contamination is a predictor of bacterial transmission to the attire of healthcare workers.\textsuperscript{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 CT value or by 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 areas are 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.\textsuperscript{14}

Higher MRSA nares count were not associated with more frequent extranasal colonization. In a study of 60 adults, there was a correlation between higher MRSA nares counts and the likelihood of extranasal colonization, but the use of systemic antibiotics was not reported, an important difference from the current project.\textsuperscript{21} In addition, because some MRSA carriers are primarily colonized at an extranasal site, such as the groin,\textsuperscript{22–24} a positive correlation between nares colonization and extranasal 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. 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 CT value and the number of MRSA colonies in the nares. We found a poor correlation between the CT 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 collected 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 nasal 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 patient. Even though the PFGE types matched, we cannot exclude the

### Table 1. Factors Associated with MRSA Contamination of the Hospital Environment in 82 Patients with Known MRSA Nasal Colonization

<table>
<thead>
<tr>
<th></th>
<th>Environmental Contamination (n = 34)</th>
<th>No Environmental Contamination (n = 48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>34 (100)</td>
<td>46 (95.8)</td>
<td>0.5086</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>32 (94.1)</td>
<td>40 (83.3)</td>
<td>0.1830</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>63 (51, 68)</td>
<td>64 (59, 72)</td>
<td>0.1919c</td>
</tr>
<tr>
<td>Any comorbidity, n (%)</td>
<td>20 (58.8)</td>
<td>32 (66.7)</td>
<td>0.5195</td>
</tr>
<tr>
<td>Central line, n (%)</td>
<td>3 (8.8)</td>
<td>9 (18.8)</td>
<td>0.6928</td>
</tr>
<tr>
<td>Urinary catheter, n (%)</td>
<td>5 (14.7)</td>
<td>11 (22.9)</td>
<td>0.1513</td>
</tr>
<tr>
<td>Current antibiotic use, n (%)</td>
<td>22 (64.7)</td>
<td>31 (64.6)</td>
<td>0.9969</td>
</tr>
<tr>
<td>Prior MRSA infections, n (%)</td>
<td>9 (26.5)</td>
<td>12 (25.0)</td>
<td>0.1179</td>
</tr>
<tr>
<td>Time since admission in current room (h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>40.4 (36.4, 43.7)</td>
<td>42.5 (40.0, 60.2)</td>
<td>0.1497c</td>
</tr>
<tr>
<td>Prior history of a boil, rising, or abscess</td>
<td>15/32 (46.9)</td>
<td>16/47 (34.0)</td>
<td>0.3481</td>
</tr>
<tr>
<td>Hand washing &gt;6 times per day</td>
<td>14/32 (43.8)</td>
<td>14/46 (30.4)</td>
<td>0.2419</td>
</tr>
<tr>
<td>Bathing &gt;4 times/week</td>
<td>17/32 (53.1)</td>
<td>22/47 (46.8)</td>
<td>0.6500</td>
</tr>
<tr>
<td>Nose picking</td>
<td>25/31 (80.6)</td>
<td>41/47 (87.2)</td>
<td>0.5261</td>
</tr>
<tr>
<td>Log MRSA nares count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>3.90 (2.77, 4.84)</td>
<td>2.45 (1.00, 4.11)</td>
<td>0.0148c</td>
</tr>
</tbody>
</table>

### Table 2. Prevalence of Environmental Contamination and Extranasal Colonization Based on the MRSA Nares Bacterial Count

<table>
<thead>
<tr>
<th>MRSA Nares Count</th>
<th>Total</th>
<th>Environmental Contamination n (%)</th>
<th>Extranasal Colonization n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>4 (28.6)</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>0–10</td>
<td>15</td>
<td>4 (26.7)</td>
<td>12 (80.0)</td>
</tr>
<tr>
<td>10–100</td>
<td>2</td>
<td>0 (0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>100–1,000</td>
<td>9</td>
<td>2 (22.2)</td>
<td>5 (55.6)</td>
</tr>
<tr>
<td>1,000–10,000</td>
<td>14</td>
<td>9 (64.3)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>10,000–100,000</td>
<td>28</td>
<td>15 (53.6)</td>
<td>22 (78.6)</td>
</tr>
</tbody>
</table>

**NOTE.** MRSA, methicillin-resistant *Staphylococcus aureus*; IQR, interquartile range.

*Urine cultures were examined in 28 and 42 patients, respectively.

*bWound cultures were examined in 4 and 10 patients, respectively.

^P value based on Wilcoxon rank sum test. Otherwise, P is based on Fisher’s exact test.

Based on our findings, the effect of parenteral vancomycin on MRSA nasal colonization may be more complicated than previously described.
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 (ie, swabbing) was used to test for environmental contamination. Because we used the same sampling technique for all patients’ rooms, it is 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 Cq 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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Conflicts of interest. All authors report no conflicts of interest relevant to this article.

Address correspondence to Daniel Livorsi, MD, MSc, Assistant Professor, Division of Infectious Diseases, Indiana University School of Medicine, 545 Barnhill Drive, EH 421 Indianapolis, IN 46202 (dlivorsi@iu.edu).

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