ORIGINAL ARTICLE

Importance of Bacterial Burden Among Methicillin-Resistant *Staphylococcus aureus* Carriers in a Long-Term Care Facility

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OBJECTIVE. To evaluate the prevalence and transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization, as well as risk factors associated with MRSA carriage, among residents of a long-term care facility (LTCF).

DESIGN. Prospective, longitudinal cohort study.

SETTING. A 100-bed Veterans Administration LTCF.

PARTICIPANTS. All current and newly admitted residents of the LTCF during an 8-week study period.

METHODS. Nasal swab samples were obtained weekly and cultured on MRSA-selective media, and the cultures were graded for growth on a semiquantitative scale from 0 (no growth) to 6 (heavy growth). Epidemiologic data for the periods before and during the study were collected to assess risk factors for MRSA carriage.

RESULTS. Of 83 LTCF residents, 49 (59%) had 1 or more nasal swab cultures that were positive for MRSA; 34 (41%) were consistently culture-negative (designated "noncarriers"). Of the 49 culture-positive residents, 30 (36% of the total of 83 residents) had all cultures positive for MRSA (designated "persistent carriers"), and 19 (23% of the 83 residents) had at least 1 culture, but not all cultures, positive for MRSA (designated "intermittent carriers"). Multivariate analysis showed that participants with at least 1 nasal swab culture positive for MRSA were likely to have had previous hospitalization (odds ratio, 3.9) or wounds (odds ratio, 8.2). Persistent carriers and intermittent carriers did not differ in epidemiologic characteristics but did differ in mean MRSA growth score (3.7 vs 0.7; $P < .001$).

CONCLUSIONS. Epidemiologic characteristics differed between noncarriers and subjects with at least 1 nasal swab culture positive for MRSA. However, in this LTCF population, only the degree of bacterial colonization (as reflected by mean MRSA growth score) distinguished persistent carriers from intermittent carriers. Understanding the burden of colonization may be important when determining future surveillance and control strategies.

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Longitudinal studies examining patients with nasal carriage of methicillin-susceptible *Staphylococcus aureus* have distinguished at least 3 patterns: persistent carriage, intermittent carriage, and noncarriage.[1] In certain populations, patients with persistent nasal carriage have higher bacterial loads than those with intermittent carriage and a higher risk of developing invasive infection.[1,2] Data suggest that the nasal carriage patterns for methicillin-resistant *S. aureus* (MRSA) may be similar to those of methicillin-susceptible *S. aureus,* even though, unlike methicillin-susceptible *S. aureus* colonization, MRSA nasal colonization has been associated with female sex and older age (ie, greater than 60 years).[1,3-5]

Long-term care facilities (LTCF) are an important reservoir for MRSA strains.[6] Prevalence of MRSA colonization among residents of LTCFs varies widely, ranging from 8% to 53% for nasal colonization and from 30% to 82% for wound colonization.[7] Risk factors for MRSA nasal carriage found in previous studies include decreased patient mobility, previous hospitalization, antibiotic exposure, invasive procedures, presence of wounds, and known history of MRSA infection.[4,8-10]

We performed a prospective, longitudinal pilot study to evaluate the prevalence and transmission of MRSA nasal colonization in residents of a Veterans Administration LTCF. We evaluated epidemiologic risk factors associated with MRSA carrier status and characterized MRSA carriage using a semiquantitative culture method to determine whether there was a correlation between carrier state and level of nasal colonization.

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METHODS

Study Population

The study took place in the Atlanta Veterans Administration nursing home care unit, a 100-bed facility physically connected to the Atlanta Veterans Administration Medical Center in Decatur, Georgia. The nursing home care unit had an average daily census of 80-85 residents and usually admitted 3-5 new residents each week during an 8-week period from November through December 2005. Approximately 50% of residents were long-stay residents (ie, with stays of more than 90 days), 30% were post–acute care residents (ie, those receiving skilled nursing care, wound management, or rehabilitation, with stays of 14-90 days), and 20% were short-stay respite care residents (ie, with stays of less than 14 days). The study was approved, with a waiver of the need for informed consent, by the Emory University institutional review board and the Atlanta Veterans Administration Research and Development Committee.

All residents in the nursing home care unit underwent weekly surveillance for MRSA colonization: swab specimens were collected from the nasal passages and from the wound (if any) and cultured to detect MRSA. All residents newly admitted to the facility had swab specimens obtained within 48 hours after admission and as part of weekly surveillance. Data on demographic characteristics, epidemiologic risk factors known to be associated with MRSA colonization (ie, comorbid conditions, history of previous MRSA-positive clinical cultures, transfers to the hospital in the previous 12 months, and antibiotic exposure in the previous 3 months), and exposures during the study period (ie, medical device use, receipt of antibiotics, and wound management) were collected for all residents through the Veterans Administration electronic medical record system. Standard precautions have been used for patients infected or colonized with MRSA in the LTCF since 1993.[11]

MRSA Culture and Semiquantitative Growth Score

By means of a polyurethane-tipped swab (BBL CultureSwab EZ; Becton Dickinson), samples for culture were obtained from the anterior nares (1 for both nostrils) and wound surfaces. MRSA was isolated by plating swab specimens directly on MRSA Chromagar plates (BD Diagnostics), which were then incubated at 35°C. Cultures were not held for more than 48 hours, and confirmatory testing with a latex agglutination assay (Staphaurex; Remel) was performed on isolates that had grown by hour 48. Additionally, all isolates recovered in weeks 1, 5, and 8 underwent both catalase and latex agglutination testing; all were confirmed to be *S. aureus* (data not included). Microbiologists described growth at 24 hours on the culture plates qualitatively on the basis of the colony distribution in the 4 quadrants of the plate, similar to the way they describe growth in clinical cultures as "light growth," "moderate growth," and so forth. A semiquantitative assessment of the MRSA bacterial burden was determined by applying a numeric value to the descriptors, as follows: 0, no growth; 1, scant to few colonies; 2, light growth; 3, light-moderate growth; 4, moderate growth; 5, moderate-heavy growth; and 6, heavy growth. The assignment of the numeric score to each culture was done after all the cultures had been evaluated and described by the microbiologists. Mean bacterial growth scores were calculated as the sum of the growth scores for each week's cultures divided by the total number of cultures performed for each subject.

MRSA Carrier Status

Residents were categorized into 1 of 3 MRSA carrier cohorts on the basis of serial nares culture results as follows: "persistent carriers," subjects for whom every culture was positive for MRSA; "intermittent carriers," subjects for whom 1-7 cultures were positive and at least 1 culture was negative for MRSA; and "noncarriage," subjects for whom all cultures were negative for MRSA. Residents had to have at least 3 culture results during the study period to be assigned to 1 of the 3 cohorts. Those with 2 or fewer culture results were excluded from the analysis.

Molecular Typing

Initial molecular strain typing of all MRSA isolates was performed with a modified version of the multiple-locus variable-number tandem repeat analysis (MLVA) method described by Sabat et al.[12] Briefly, DNA was extracted using the GenElute Bacterial Genomic DNA kit (Sigma) and amplified by polymerase-chain reaction using primer sets specific for 5 staphylococcal loci with variable-number tandem repeats *(sdr, clfA, clfB, sSpa,* and *Spa)*. Relationships among isolates collected from any one individual were determined by visual analysis of digital images. We used pulsed-field gel electrophoresis (PFGE) to determine USA strain type for a representative isolate from every MRSA carrier. Additionally, any discrepant strain patterns determined by MLVA were confirmed by PFGE. Following digestion with *Sma*I (Promega), genomic DNA fragments were separated using the CHEF DR III system (Biorad). Digital images of all PFGE gels were analyzed using Gelcompar II (Applied Maths). Percentage similarities between strains were identified with a dendrogram based on Dice coefficients, as described elsewhere.[13]

Statistical Analysis

Risk factors and outcomes associated with a given MRSA carriage state were analyzed using exploratory univariate analysis of continuous and categorical variables. Clinically relevant and significant variables from univariate analysis were entered into a multivariable logistic regression model. Differences in the mean growth score among MRSA carriers were compared using the Wilcoxon rank sum test. All analyses were performed using SAS statistical software, version 9.1 (SAS Institute).

TABLE. Comparison of Risk Factors Predictive of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Colonization Between the Persistent Carrier and the Noncarrier Cohorts

Risk factor	Persistent carriers ($N = 49$)	Noncarriers ($N = 34$)	P[a]
Hospitalization in previous 12 months	29 (59)	11 (32)	.02
Receipt of antibiotics in previous 3 months	27 (55)	7 (21)	.002
Receipt of antibiotics during study period	29 (59)	9 (26)	.003
Presence of a wound	22 (45)	3 (9)	.0004
History of stroke	11 (22)	15 (44)	.04
History of MRSA isolation	14 (29)	4 (12)	.07
New infection during study period	22 (45)	7 (21)	.02
Presence of an indwelling device	21 (43)	7 (21)	.03
Transfer to hospital during study period	7 (14)	2 (6)	.30
Decreased activities of daily living score $\geqslant 6$	33 (67)	18 (53)	.18
Age, mean, years	73.6	72.3	.72[b]

NOTE. Data are no. (%) of residents, unless otherwise indicated.

[a] $P \leqslant .05$ was considered statistically significant. P values were obtained with the χ^2 test, unless otherwise indicated.

[b] Student t test.

RESULTS

Study Population

During the 8-week study period, 104 residents were enrolled in the study: 80 residents at study inception and 24 newly admitted residents after study inception. Demographic characteristics of the subjects enrolled were consistent with those of the Veterans Administration population: 94% of study subjects were male, and approximately 50% were between the ages of 70 and 89. Of these 104 enrolled subjects, 83 (80%) had at least 3 weekly nares swab culture results available for inclusion in the risk factor analysis. Of the 21 enrolled subjects excluded because of an insufficient number of nares culture results, 5 were from the inception group and 16 were from the new admission group.

MRSA Colonization

The prevalence of nasal MRSA colonization was 48% (38 of 80 residents) among residents tested in the first week of surveillance. The overall prevalence of MRSA colonization remained similar from week to week. In contrast, the prevalence among residents newly admitted to the facility was 29% (7 of 24 residents); this proportion was not statistically different from the intrafacility prevalence ($P = .16$, Fisher exact test). Longitudinal surveillance results obtained over the study period for the 83 residents (75 present at the inception of the study and 8 residents admitted during the study) revealed 3 distinct cohorts of MRSA nasal colonization: 34 residents (41%) were noncarriers, 30 (36%) were persistent carriers, and 19 (23%) were intermittent carriers. Thirty-one (91%) of the 34 noncarriers completed all 8 weeks of surveillance. Of note, in the intermittent carriage cohort, there were no patterns in the distribution of positive nasal culture results that suggested initial noncarriage followed by acquisition of persistent carriage. Results of cultures of wound samples did not change the carrier cohort assignment for any resident in the noncarriage or the intermittent carriage groups. Only 5 (24%) of the 21 subjects excluded because of insufficient culture results had nasal carriage of MRSA.

Risk Factor Analysis

The distribution of comorbid conditions (such as diabetes, heart disease, or pulmonary disease) was similar among the subjects with at least 1 nares culture positive for MRSA and among the noncarriers. However, the previous occurrence of a stroke was more frequent among noncarriers than MRSA carriers (44% vs 22% of subjects; $P = .04$, χ^2 test]. The distribution of risk factors associated with MRSA carriage (such as receipt of antibiotics and previous hospitalization) was significantly different between the residents with at least 1 culture positive for MRSA and the noncarriers (Table). In multivariate analysis, only previous hospitalization (odds ratio, 3.9 [95% confidence interval, 1.2-13.3]) and presence of a pressure wound (odds ratio, 8.2 [95% confidence interval, 1.3-51.2]) remained significantly associated with MRSA nasal colonization. There were no significant differences in the prevalence of these risk factors between the persistent carriers and the intermittent carriers (data not shown).

Bacterial Burden

When calculated for the individuals in the persistent carriage and intermittent carriage cohorts, the mean bacterial growth score over the 8-week surveillance period was significantly higher for the persistent carriage cohort than for the intermittent carriage cohort (3.7 vs 0.7; $P < .001$, Wilcoxon rank sum test) (Figure 1). The mean bacterial growth score for

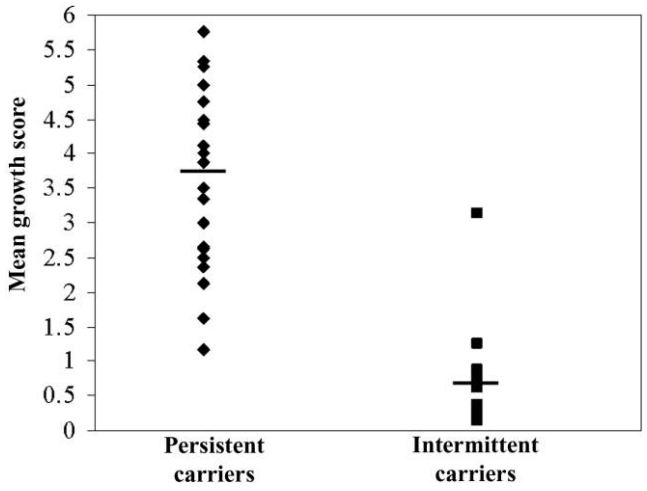

FIGURE 1. Comparison of mean growth scores for the persistent carriage and intermittent carriage cohorts. *Horizontal line,* mean value for each group (3.7 vs 0.7; $P < .001$).

the intermittent carriage cohort remained significantly lower than that of the persistent carriage cohort even if the mean score for the intermittent carriage cohort was calculated only for the positive culture results (3.7 vs 1.6; $P < .001$, Wilcoxon rank sum test).

Molecular Typing

Overall, only 2 (4%) of 49 carriers had more than 1 MRSA strain type present in their nares, as determined by the combination of MLVA and PFGE. Of the 30 persistent carriers, 25 were colonized with only 1 strain type, according to the results of MLVA (Figure 2). Although MLVA patterns suggested that there were 2 strain types present in the isolates recovered from weekly surveillance cultures for each of 5 residents, PFGE confirmed the presence of 2 different strain types for only 1 of the 5. Among the 19 intermittent carriers, MLVA typing showed only 1 person with 2 strain types, which was confirmed by PFGE. The rate of carriage of multiple MRSA strain types was not significantly different between the persistent carriers and the intermittent carriers ($P = .99$, Fisher exact test).

PFGE typing was performed on a representative isolate (the isolate recovered from the first MRSA-positive culture) from 49 MRSA carriers and revealed 20 unique strain types. Thirty-seven (76%) of 49 MRSA carriers were colonized with strains that usually are hospital associated, USA100 and USA500. Five isolates (10%) belonged to USA300, a strain that typically is community associated.[13]

Intrafacility Transmission

Of the 83 subjects who had enough surveillance culture results to be included in the analysis, 43 who had negative results for the first nasal swab culture were designated as having a risk of intrafacility acquisition. Of those 43 subjects, 34 had negative culture results throughout the study, and 9 (21%) had a subsequent culture positive for MRSA. Only 2 of those 9 subjects had at least 2 consecutive cultures positive for MRSA without any subsequent negative culture results, a pattern we conservatively defined as suggestive of acquisition within the facility. Neither of these individuals had a history of MRSA colonization prior to study entry. Most of the subjects in the study (64 [77%] of 83) were either persistent carriers or noncarriers.

DISCUSSION

The prevalence of 48% MRSA carriage in the LTCF we studied was similar to the higher prevalence estimates reported in the literature (range, 8%-53%).[7] Only the presence of a wound and prior hospitalization remained predictive of MRSA carriage in multivariate analysis. However, no risk factors could distinguish persistent carriers from intermittent carriers; only measures of bacterial burden could distinguish these 2 MRSA cohorts.

Most studies of MRSA carriage in LTCF residents have been point prevalence surveys [9,14,15]; only a few studies have studied LTCF residents longitudinally.[4,5] Bradley et al.[4] performed active surveillance for MRSA in nares and wound specimens obtained on admission and monthly thereafter for 1 year. They categorized subjects as persistent and probable persistent carriers, intermittent carriers, transient carriers, or noncarriers on the basis of the number and distribution of positive culture results. They reported that 65% of residents never carried MRSA, 25% were carriers when the first culture was performed, and the remaining 10% acquired MRSA in the facility. Muder et al.[5] also did active surveillance for *S. aureus* in the Pittsburgh Veterans Administration LTCF, first monthly, then bimonthly, for 3 years, and they classified residents as either persistent carriers, transient carriers, or noncarriers but did not comment on carriage acquired in the facility. Neither of these studies described the quantitative burden of bacterial colonization among the MRSA carriers.

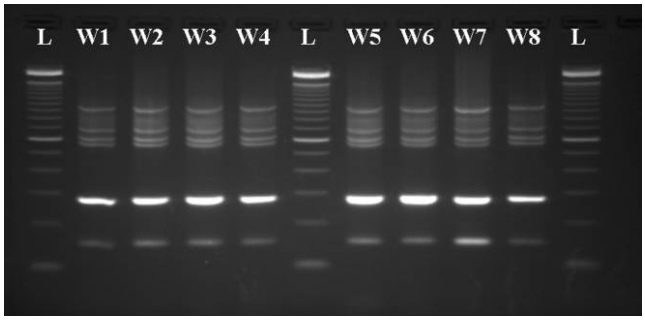

FIGURE 2. Example of a multiple-locus variable-number tandem repeat analysis gel of a methicillin-resistant *Staphylococcus aureus* isolate recovered from a persistent carrier. *Lanes W1-W8,* isolates from 8 weekly surveillance cultures; *lanes L,* reference 100–base pair ladder.

We found that bacterial burden distinguished persistent carriage from the low-level carriage seen in our intermittent carriage cohort. The low burden of carriage in the latter group may result in initially negative culture results, followed later by positive results, a pattern that could be misinterpreted as intrafacility transmission. Therefore, the presence of intermittent carriers in a population may complicate the way intrafacility acquisition is defined if the burden of colonization is not considered. When we interpreted these findings in light of the bacterial burden data, we did not find significant evidence of transmission within our facility: of the 43 subjects whose first culture was negative for MRSA, only 2 had carriage patterns suggestive of MRSA acquisition. However, 1 of these 2 individuals had culture data censored at 4 weeks because of discharge from the facility.

Unlike some studies, which have shown intermittent carriers to have multiple *S. aureus* strains colonizing the nares,[1] this study found that the majority of intermittent carriers in this study were colonized with a single strain. MLVA, a polymerase chain reaction–based strain typing method, was used to distinguish between strains because it was technically easier and less costly than PFGE. However, banding patterns that showed discrepancies by MLVA were often found to be a single indistinguishable pattern by PFGE. This suggests that MLVA and PFGE cannot be considered equivalent typing methods.

This pilot study has several limitations. Female patients were underrepresented because our study population was from a Veterans Administration facility, so the MRSA prevalence findings may not generalize to non–Veterans Administration LTCFs. Although the method for collection of swab specimens was consistent among the 3 researchers who performed serial swab sampling, the consistency of culture results for the samples from the different collectors was not evaluated, nor was patient cooperativeness with serial swabbing, either of which could influence the culture results for some of the intermittent carriers. The assessment of bacterial growth by means of a semiquantitative scoring system needs to be further validated to show whether results are reproducible with different plate readers and whether results for a given individual are reproducible with repeat swab sampling. The inability to distinguish persistent carriers from intermittent carriers on the basis of epidemiologic factors may have been due to the relatively small sample size for each group. However, bacterial burden did distinguish between these 2 cohorts, suggesting that either the host immune response or the bacteria's capacity to adhere to tissue in colonized regions may play a role in persistent MRSA colonization.[16,17] Eight weeks may be too short a period for accurate identification of intermittent MRSA carriage, resulting in misclassification of people as noncarriers, especially if they have only 3 or 4 surveillance culture results available. However, longitudinal surveillance provides a better opportunity to detect intermittent carriage than a one-time surveillance, and 91% of individuals in the noncarrier cohort completed all 8 weeks of surveillance. Although there was little evidence of intrafacility transmission, it is possible that the number of subjects and the duration of the study period were not enough to detect new acquisition of MRSA and subsequent development of high-burden, persistent carriage. We are initiating a larger study at 3 LTCFs with follow-up for 6 months, to examine these limitations. Finally, the use of the MLVA technique for molecular typing may have identified isolates as belonging to the same strain that would have been identified as belonging to different strains if PFGE had been used to type all isolates. However, this seems unlikely, because for those isolates that were typed by both methods, PFGE more often determined that the isolates belonged to the same clone than did MLVA. That finding suggests that band patterns detected by MLVA change more rapidly than do band patterns detected by PFGE and that MLVA is a more sensitive indicator of strain differences.[18]

Because the prevalence of MRSA colonization is high in LTCFs, the financial and human cost of active surveillance and control strategies for this organism may be substantial. Understanding the biology that accounts for different levels of MRSA carriage may lead to other approaches to addressing MRSA colonization, especially if underlying host factors are playing a role. Further work may also be needed to determine whether bacterial burden plays a role in patients' responses to decolonization strategies. Finally, if patients with low-level colonization are misclassified as having acquiring MRSA in the healthcare facility, intrafacility transmission rates could be overestimated. The presence of intermittent carriers in the population could call into question the validity of using a single positive or negative culture result to define carrier status. Thus, further understanding of the distribution of MRSA carriage and the burden of colonization may have an impact on future strategies both for surveillance of and for prevention of transmission of MRSA among LTCF residents.

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