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Colonization Sites of USA300 Methicillin-Resistant *Staphylococcus aureus* in Residents of Extended Care Facilities

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Abstract

BACKGROUND—The anterior nares are the most sensitive single site for detecting methicillin-resistant *Staphylococcus aureus* (MRSA) colonization. Colonization patterns of USA300 MRSA colonization are unknown.

OBJECTIVES—To assess whether residents of extended care facilities who are colonized with USA300 MRSA have different nares or skin colonization findings, compared with residents who are colonized with non-USA300 MRSA strains.

METHODS—The study population included residents of 5 extended care units in 3 separate facilities who had a recent history of MRSA colonization. Specimens were obtained weekly for surveillance cultures from the anterior nares, perineum, axilla, and skin breakdown (if present) for 3 weeks. MRSA isolates were categorized as USA300 MRSA or non-USA300 MRSA.

RESULTS—Of the 193 residents who tested positive for MRSA, 165 were colonized in the anterior nares, and 119 were colonized on their skin. Eighty-four percent of USA300 MRSA–colonized residents had anterior nares colonization, compared with 86% of residents colonized with non-USA300 MRSA (P=.80). Sixty-six percent of USA300 MRSA–colonized residents were colonized on the skin, compared with 59% of residents colonized with non-USA300 MRSA (P=.30).

CONCLUSIONS—Colonization patterns of USA300 MRSA and non-USA300 MRSA are similar in residents of extended care facilities. Anterior nares cultures will detect most—but not all—people who are colonized with MRSA, regardless of whether it is USA300 or non-USA300 MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are increasing in the community. In the United States, community-associated MRSA infections predominantly involve skin and soft-tissue infections and are caused by the USA300 MRSA clone.[1–6] USA300 MRSA also causes healthcare-associated MRSA infections, such as sepsis among postpartum women, prosthetic joint infection in surgical patients, bloodstream infection in adults and neonates, and other types of infection in intensive care unit patients.[1,5–9] USA300

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MRSA isolates are distinguished from other MRSA isolates by the presence of the staphylococcal chromosome cassette *mec* type IV element, the Panton Valentin leukocidin (PVL) gene, and the arginine catabolic metabolic element (ACME) genes.[10,11] Sequence typing of USA300 isolates demonstrates that these isolates share a common *spa* type motif (MBQBLO).[11]

In recent guidelines, the Centers for Disease Control and Prevention (CDC) and Society for Healthcare Epidemiology of America (SHEA) recommend surveillance cultures as an infection-control method to control the transmission of multidrug-resistant organisms, such as MRSA, in the healthcare setting.[12,13] The anterior nares is the most common site to be screened in active surveillance programs, because this site has the highest sensitivity for the detection of MRSA colonization.[14,15] However, previous studies of screening sites for MRSA colonization have focused on MRSA colonization due to USA100 MRSA.

A number of investigators have suggested that nasal colonization with USA300 MRSA may be considerably less common than colonization with healthcare-associated MRSA.[16] If so, typical screening programs may fail to detect USA300 MRSA colonization. Only 34% of patients were colonized in the anterior nares in a study that assessed for USA300 MRSA colonization among patients who were admitted to an urban emergency department and who had USA300 MRSA infection. [17] In another study of an outbreak of USA300 MRSA infection among National Football League players, MRSA colonization was absent from the nares of players and trainers, despite the occurrence of multiple USA300 MRSA infections. [18] Little is known about the optimal screening site for USA300 MRSA colonization. We undertook this study to assess whether USA300 MRSA and non-USA300 MRSA have different distributions of anatomic sites for colonization among residents of extended care facilities.

METHODS

Study Setting and Population

The study population consisted of residents of extended care facilities at 2 healthcare systems located in Baltimore, Maryland. The Veterans Affairs Maryland Health Care System (VAMHCS) has 5 extended care units in 2 facilities. The Baltimore Rehabilitation and Extended Care Center has 2 units, with a total of 120 beds, providing postacute care, chronic rehabilitation, hospice, and residential care. The Perry Point Veterans Affairs Medical Center has 3 units, with a total of 150 beds, providing long-term rehabilitation and residential care. The University Specialty Hospital, which is part of the University of Maryland Medical System, is a 180-bed postacute care hospital that has an 88-bed rehabilitation extended care unit. The University of Maryland School of Medicine institutional review board and the VAMHCS research and development committee approved this study.

The study was cross-sectional in design and examined data from the VAMHCS and UMMS extended care units from March 2005 through December 2007. Residents with a history of MRSA colonization provided a minimum of 3 weekly culture sets and had at least 1 culture positive for MRSA during the study period. We collected culture specimens from the anterior nares, perineum, axilla, and the largest area of skin breakdown, if present. All surveillance culture specimens were collected by a research nurse by means of a rayon-tipped swab with Aimes charcoal transport medium (BactiSwab; Remel). The swab was premoistened with transport gel before the nares culture specimen was obtained.

Microbiological Methods

All swabs were streaked for isolation onto tryptic soy agar containing 5% sheep blood agar (Remel). In addition, perineum swabs were plated on phenylethyl alcohol agar plates (Remel).

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Plates were incubated at 37°C for 48 hours. Isolates were characterized as *S. aureus* on the basis of catalase and coagulase production (Pastorex; Bio-Rad Laboratories). *S. aureus* isolates were plated on oxacillin (6 μg/mL) agar screen plates and incubated at 37°C. Growth on the oxacillin agar screen was classified as oxacillin resistant (ie, MRSA) and was plated on a fresh 5% blood agar plate for DNA extraction.

Molecular Methods

Chromosomal DNA was extracted from cells after growth in an overnight culture of tryptic soy broth at 37°C. The cells were lysed using a 1:5 ratio of lysostaphin to cell suspension that was incubated for 2–3 hours at 37°C. DNA isolation was performed using the Prepman Ultra kit (Applied Biosystems) in accordance with manufacturer's guidelines. All MRSA isolates recovered from swabs taken from the anterior nares, axilla, perineum, and wound over the 3-week period were molecularly typed. All isolates were characterized using polymerase chain reaction assays to amplify PVL and ACME genes, as described elsewhere.[19] The typing of the polymorphic region of protein A *(spa)* was performed as described previously.[20] For validation purposes, a sample of each of the resident's first isolates underwent pulsed-field gel electrophoresis (PFGE) strain typing performed with *Sma*I digestion. The resulting PFGE band patterns were analyzed by visual inspection, followed by analysis with the Fingerprinting software (BioRad Laboratories) for determination of isolate relatedness. PFGE polymorphisms were identified by generating dendograms with the Dice coefficient method, and patterns were clustered by the unweighted pair group method by means of arithmetic averages, with an optimization of 0.25% and a tolerance of 1.0%.

Variable Definitions

MRSA colonization was categorized as USA300 or non-USA300. We used the established genetic algorithm of having the *spa* type motif MBQBLO and the presence of PVL and ACME genes as a marker of USA300.[10,11,20,21] We validated this algorithm in our population by assessing a sample of residents' first MRSA isolate by performing PFGE on one-third of the t008 MBQBLO *spa* types and on all of the other MBQBLO *spa* types. Isolates were classified as closely related to USA300 MRSA if the tested MRSA isolates demonstrated more than 80% similarity to the USA300 MRSA PFGE pattern. We also tested 8% of the isolates that did not have the MBQBLO *spa* type motif, that were PVL negative, or that were ACME negative. Isolates were classified as non-USA300 MRSA if they demonstrated less than 80% similarly to the USA300 MRSA PFGE pattern. Thus, residents were classified as "USA300 MRSA colonized" if they had at least 1 MRSA isolate in a swab set that had the MBQBLO *spa* type motif and that was PVL and ACME positive; all others were categorized as non-USA300 MRSA.

Nares colonization was defined as at least 1 culture positive for MRSA of 3 anterior nares cultures. Skin colonization was defined as at least 1 positive culture result for specimens of the axilla, perineum, or the largest area of skin breakdown, if present.

During the study, a research nurse collected and entered into a relational database (Access; Microsoft) the following variables from the medical staff and the electronic medical records: demographic characteristics (ie, age, race, and sex), presence of skin breakdown (eg, pressure ulcer, surgical wound, or placement of a percutaneous foreign device), level of care (eg, residential, intermediate, or acute care), level of mobility (as measured by the ability to attend means therapy and other activities with little assistance and by the requirement to have meals in the room and to require assistance with most activities), level of dependency (as measured by activities of daily living), and the following underlying diseases: diabetes mellitus, renal insufficiency, and HIV infection. Two of the authors (S.M.S. and M.-C.R.) validated the data

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by performing logic checks on the database and independently checking source documentation for 5% of residents enrolled.

Statistical Analysis

The proportion of residents who had nasal or skin colonization with USA300 MRSA was compared with the proportion who had nasal or skin colonization with non-USA300 MRSA and was assessed using the χ^2 test or Fisher exact test, as appropriate. Prevalence ratios and 95% confidence intervals (CIs) were calculated. The presence of skin breakdown was assessed as a potential confounder by evaluating a change in the adjusted association of more than 10%, compared with the unadjusted association. We estimated the sensitivity (the number of MRSA-positive residents by specimen site divided by the total number of MRSA-positive residents) of the anterior nares and skin surveillance cultures for MRSA colonization, with estimates stratified on the basis of USA300 and non-USA300 MRSA colonization. All statistical tests were 2-sided, and *P* values less than .05 were considered to be statistically significant. Data were analyzed using the SAS Statistical Software, version 9.1 (SAS Institute).

RESULTS

During the study period from March 2005 through December 2007, a total of 388 patients with a recent history of MRSA colonization or infection were enrolled and had culture specimens obtained over a 3-week period. One hundred eighty-six residents (48%) were excluded because they were no longer colonized with MRSA, and 9 residents (2%) were excluded because they were concurrently colonized with USA300 and non-USA300 MRSA strains. The remaining 193 residents were culture positive for at least 1 of 3 culture sets and constituted our final study population (Table 1). The mean age of patients was 64 years, 164 (85%) were men, and 112 (58%) were white. Approximately one-half (102 of 193) of the population was in residential long-term care. A history of diabetes (77 patients [40%]) was common, and most of the population (138 patients [72%]) had skin breakdown, with foreign devices (97 patients [50%]) being the predominant type, followed by pressure ulcers (85 patients [44%]) and surgical incisions (49 patients [25%]).

Overall, seventy-one residents (38%) were classified as having USA300 MRSA colonization on the basis of the MBQBLO *spa* type, PVL positivity, and ACME positivity algorithm. A total of 122 residents were classified as being colonized with non-USA300 MRSA. Table 1 compares characteristics of residents who were classified as USA300 and non-USA300 MRSA colonized at the extended care facilities. Compared with non-USA300 MRSA–colonized residents, those who were colonized with USA300 MRSA were more likely to be fully mobile and to be female.

One hundred sixty-five residents (85%) had a positive anterior nares culture result, and 119 (62%) had a positive skin culture result. Of those with skin colonization, 56% were colonized in the axilla and perineum alone, and 30% had an area of skin breakdown. There was no difference in the prevalence of nares colonization between residents who were colonized with USA300 MRSA and those who were colonized with non-USA300 MRSA (prevalence ratio, 1.0 [95% CI, 0.9– 1.1]; *P* = .77). Among 71 residents who were colonized with USA300 MRSA, 60 (84%) were colonized in the anterior nares, whereas 105 (86%) of the 122 residents who were colonized with non-USA300 MRSA were colonized in the anterior nares. There was no difference in the prevalence of skin colonization between residents who were colonized with USA300 MRSA and those who were colonized with non-USA300 MRSA (prevalence ratio, 1.1 [95% CI, 0.9–1.4]; *P* = .32). Among residents who were colonized with USA300 MRSA, 47 (66%) were colonized on the skin, compared with 72 (59%) of 122 residents who were colonized with non-USA300. Stratification of residents with skin colonization on the basis of the presence of skin breakdown (ie, presence of foreign device, pressure ulcers, or surgical

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incision) demonstrated no difference in skin colonization between residents who were colonized with USA300 MRSA and those who were colonized with non-USA300 MRSA in both groups.

The anterior nares were the most sensitive single site for detection of MRSA colonization in residents who were colonized with either USA300 or non-USA300 MRSA. Comparisons of the sensitivity at different anatomic sites are shown in Table 2. Among residents with no skin breakdown, a nares culture alone had the greatest overall sensitivity (93%). However, among residents with skin breakdown, we found that the overall pair combination of nares or skin breakdown culture improved the sensitivity to approximately 95% for detection of any MRSA type, with decreasing detection found using nares or perineum (92%) and nares or axilla (87%).

DISCUSSION

USA300 MRSA is a common cause of infection in community settings; however, it is now endemic in the healthcare system, including in extended care facilities. A number of investigators have postulated that USA300 MRSA may colonize other anatomic sites, such as the skin, more readily than the anterior nares.[16] We found that, in our extended care facilities, the frequency of anterior nares and skin colonization in residents who were colonized with USA300 MRSA was similar to that in residents who were colonized with non-USA300 MRSA. The anterior nares cultures will detect most (but not all) people who are colonized with MRSA, regardless of whether it is USA300 or non-USA300 MRSA.

The anterior nares yielded a similar frequency for detection of any MRSA genotype, regardless of whether it was USA300 MRSA (84%) or non-USA300 MRSA (86%). The frequency of anterior nares colonization among our USA300 MRSA– colonized residents was considerably higher than the reported 34% incidence among adult emergency department patients with skin and soft-tissue infections due to USA300 MRSA.[17] The differences in our results are likely associated with the different study populations, with culture specimens obtained in different clinical scenarios. Our population consisted of extended care residents who were known to be positive for MRSA on the basis of prior surveillance or clinical culture results, whereas the emergency department patients consisted of community-dwelling adults with skin and soft-tissue infections. Investigators have suggested that the pathogenesis of USA300 MRSA skin and soft-tissue infections differs from that of other *S. aureus* skin and soft-tissue infections, in which people are usually colonized before infection.[16] Although it may be true that the pathogenesis of USA300 MRSA is different, our results demonstrate that USA300 MRSA and non-USA300 MRSA colonize the anterior nares with equal frequency in the extended care setting.

The sensitivity of anterior nares culture alone in our study among the non-USA300 MRSA–colonized patients (86%) is consistent with results from most previous studies. Sanford et al.[14] and Sewell et al.22 reported sensitivities of 93% and 83%, respectively, among residents in the extended care setting; Girou et al.23 reported sensitivity of 84% in an acute care setting; and Lucet et al.24 reported sensitivity of 78% among intensive care patients. Several investigators have suggested that the prevalence of nasal colonization with USA300 MRSA may be considerably lower than that of non-USA300 MRSA and that, therefore, typical surveillance programs that rely on nares cultures may fail to detect USA300 MRSA colonization. [16] In our study, we demonstrated that the anterior nares cultures are as useful for detecting USA300 MRSA as they are for detecting non-USA300 MRSA. The sensitivity of the anterior nares did not significantly differ for extended care residents who were colonized with USA300 MRSA (84%) versus those who were colonized with non-USA300 MRSA (86%). This lack of a difference in anterior nares colonization rates suggests that screening programs that rely on nares cultures will detect USA300 MRSA–colonized individuals.

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Although the anterior nares were equally sensitive for detection of USA300 MRSA and non-USA300 MRSA colonization, culture of specimens from the anterior nares alone did not identify all persons who were colonized with MRSA. We found that the combination of cultures of specimens from the nares and from areas of skin breakdown improved the overall sensitivity to approximately 95% for detection of any MRSA. Other studies from extended care settings similar to ours have reported that sampling from different sites— especially the skin—increases the sensitivity for detecting MRSA colonization.[14] Thus, the measurement of MRSA colonization should involve screening cultures from multiple anatomic sites performed more than once, to increase the likelihood of detection of MRSA in colonized patients.

Our study has a number of limitations. Our study population consisted of extended care residents and not community-dwelling people or patients who are hospitalized from the community, thus limiting our ability to draw conclusions about USA300 MRSA colonization patterns in those latter populations. There may be substantial differences in the general healthy community-dwelling population, and as such, USA300 MRSA colonization findings may be different in the community. Another limitation is our sample size. We were powered to detect a 22% decrease in nares colonization or 30% increase in skin colonization of USA300 MRSA–colonized residents, compared with residents who were colonized with non-USA300 MRSA. The possibility exists that the difference between USA300 and non-USA300 MRSA colonization may be smaller than this; however, this small difference may not be clinically meaningful. In addition, we did not perform PFGE of all of our MRSA isolates to determine USA300 status; however, we did validate our algorithm for determining USA300 with PFGE and found it to be accurate. Other investigators have also validated the use of the presence of MBQBLO *spa* type, PVL gene, and ACME gene as biomarkers of USA300 MRSA.[10,11,21]

Despite these limitations, our study methods are strong. This was a study that sampled multiple body sites over a 3-week period, with all cultures performed by a single research nurse (and not multiple clinical personnel) who used a standard technique. In addition, we prospectively collected clinical information, thus limiting missing data and improving the accuracy of the data collected, compared with use of a retrospective chart review. Furthermore, all of our MRSA isolates were available for molecular characterization.

The current CDC and SHEA guidelines recommend the use of the anterior nares as the single most sensitive site for detection of MRSA colonization.[12,13] Our study provides evidence that these guidelines can be extended to USA300 MRSA–colonized individuals from the healthcare setting. We also have demonstrated that obtaining both nares and skin surveillance culture specimens from persons who are experiencing skin breakdown improves MRSA detection rates. Finally, the similar frequency of nares colonization in this study population for USA300 MRSA and non-USA300 MRSA suggests that the pathogenesis of USA300 MRSA infections may be similar to that of non-USA300 MRSA infections in extended care residents. Similar studies should be performed in community-dwelling people with and without skin and soft-tissue infections.

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TABLE 1

Description of Study Population and Characteristics Associated with USA300 Methicillin-Resistant *Staphyloco ccus aureus*(MRSA) Colonization among MRSA-Colonized Residents in Extended Care Setting Units of Veterans Affairs Maryland Health Care System and University Specialty Hospital, 2004–2006

Variable	All patients (*n* = 193)	Patients colonized with USA300 MRSA (*n* = 71)	Patients colonized with non-USA300 MRSA (*n* = 122)	*P*
Age, mean ± SD, years	64 ± 18	62 ± 17	66 ± 18	.12
Male sex	164 (85)	55 (77)	109 (89)	.03
Race				.50
White	112 (58)	39 (55)	73 (60)	
Black	81 (42)	32 (45)	49 (40)	
Level of care				.11
Residential care, hospice, or respite	102 (53)	31 (44)	71 (58)	
Long-term rehabilitation (>90 days)	45 (23)	18 (25)	27 (22)	
Short-stay rehabilitation or postacute care (<90 days expected)	46 (24)	22 (31)	24 (20)	
Level of dependency				.16
Minimal assistance required	50 (26)	23 (32)	27 (22)	
Moderate assistance required	68 (35)	26 (37)	42 (34)	
Requires assistance with most ADLs	75 (39)	22 (31)	53 (43)	
Level of mobility				.03
Able to attend meals, therapies, and other activities	88 (46)	41 (58)	47 (38)	
Able to attend meals and required therapies only	54 (28)	14 (20)	40 (33)	
Eats meals in room and unable to attend therapies	51 (26)	16 (22)	35 (29)	
History of diabetes	77 (40)	31 (44)	46 (38)	.41
History of HIV infection	4 (2)	3 (4)	1 (1)	.11
Renal replacement therapy	5 (3)	2 (3)	3 (2)	.88
Current *S. aureus* infection	13 (7)	7 (10)	6 (5)	.19
Skin breakdown				
Any type	138 (72)	50 (70)	88 (72)	.79
Pressure ulcers	85 (44)	32 (45)	53 (43)	.83
Foreign devices	97 (50)	34 (48)	63 (52)	.61
Surgical incisions	49 (25)	23 (32)	26 (21)	.09
Source of first MRSA isolate				.39
Surveillance culture	92 (48)	31 (44)	61 (51)	
Clinical culture	101 (52)	40 (56)	61 (50)	
Screening site				
Nares colonization	165 (85)	60 (84)	105 (86)	.77
Skin colonization (any type)	119 (62)	47 (66)	72 (59)	.32
Axilla colonization	57 (30)	23 (32)	34 (28)	.51
Perineum colonization	75 (39)	32 (45)	43 (35)	.18
Skin breakdown colonization	57 (30)	24 (34)	33 (27)	.32

NOTE. Data are no. (%) of patients, unless otherwise indicated. ADLs, activities of daily living.

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TABLE 2

Sensitivities of Anatomic Sites in Detecting Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 193 Residents in Extended Care Units of Veterans Affairs Maryland Health Care System and University Specialty Hospital, 2004–2006

Culture rule	Overall sensitivity, no. (%) of patients
Overall	
Nares only	165 (85)
Nares or perineum	178 (92)
Nares or axilla	171 (87)
Nares or skin breakdown	184 (95)
Nares, skin breakdown, or perineum	192 (99)
Nares, skin breakdown, or axilla	185 (95)
Without skin breakdown (*n* = 55)	
Nares only	51 (93)
Nares or perineum	55 (100)
Nares or axilla	51 (93)
With skin breakdown (*n* = 138)	
Nares only	114 (82)
Nares or skin breakdown	133 (96)
Nares, skin breakdown, or perineum	137 (99)
Nares, skin breakdown, or axilla	134 (97)