AAC Journals.ASM.org

Prevalence of Chlorhexidine-Resistant Methicillin-Resistant *Staphylococcus aureus* following Prolonged Exposure

Carey D. Schlett,[a] Eugene V. Millar,[a] Katrina B. Crawford,[a] Tianyuan Cui,[a] Jeffrey B. Lanier,[b] David R. Tribble,[a] Michael W. Ellis[c]

Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biometrics, Uniformed Services University, Bethesda, Maryland, USA[a]; Martin Army Community Hospital, Fort Benning, Georgia, USA[b]; Department of Medicine, Uniformed Services University, Bethesda, Maryland, USA[c]

Chlorhexidine has been increasingly utilized in outpatient settings to control methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks and as a component of programs for MRSA decolonization and prevention of skin and soft-tissue infections (SSTIs). The objective of this study was to determine the prevalence of chlorhexidine resistance in clinical and colonizing MRSA isolates obtained in the context of a community-based cluster-randomized controlled trial for SSTI prevention, during which 10,030 soldiers were issued chlorhexidine for body washing. We obtained epidemiological data on study participants and performed molecular analysis of MRSA isolates, including PCR assays for determinants of chlorhexidine resistance and high-level mupirocin resistance and pulsed-field gel electrophoresis (PFGE). During the study period, May 2010 to January 2012, we identified 720 MRSA isolates, of which 615 (85.4%) were available for molecular analysis, i.e., 341 clinical and 274 colonizing isolates. Overall, only 10 (1.6%) of 615 isolates were chlorhexidine resistant, including three from the chlorhexidine group and seven from nonchlorhexidine groups ($P > 0.99$). Five (1.5%) of the 341 clinical isolates and five (1.8%) of the 274 colonizing isolates harbored chlorhexidine resistance genes, and four (40%) of the 10 possessed genetic determinants for mupirocin resistance. All chlorhexidine-resistant isolates were USA300. The overall prevalence of chlorhexidine resistance in MRSA isolates obtained from our study participants was low. We found no association between extended chlorhexidine use and the prevalence of chlorhexidine-resistant MRSA isolates; however, continued surveillance is warranted, as this agent continues to be utilized for infection control and prevention efforts.

Skin and soft-tissue infections (SSTIs), particularly those attributed to methicillin-resistant *Staphylococcus aureus* (MRSA), remain a persistent cause of morbidity in community settings. Over the past decade, ambulatory care and emergency department visits for SSTIs have nearly doubled (1, 2). The emergence of MRSA, especially strain USA300 (3), as a community pathogen is recognized as underlying this surge in SSTI rates (1, 4). Individuals in congregate settings, such as children in day care centers, athletes, inmates, and military personnel, are at increased risk for MRSA SSTIs (5–8).

Chlorhexidine, a topical antiseptic, has had a longstanding role in infection prevention in health care settings (9) and has been increasingly utilized in outpatient settings (9). It has been an integral component of prevention and control measures during MRSA outbreaks (5, 10, 11). Additionally, chlorhexidine has been demonstrated to be effective against recurrent MRSA SSTIs (12) and in limiting the household spread of SSTIs (13) and is recommended when MRSA decolonization of individuals is a goal (14). In the absence of an effective *S. aureus* vaccine (15), chlorhexidine has also been employed as an SSTI prevention strategy among military trainees, a group known to be at increased risk for MRSA colonization and disease (16, 17).

The increased use of chlorhexidine in SSTI prevention has raised concerns about the possible emergence of chlorhexidine-resistant strains (18, 19). The epidemiology of chlorhexidine-resistant MRSA in health care settings has been described (20, 21); however, there are limited data with regard to its prevalence in community-based settings (22, 23). Moreover, few studies have evaluated the association between concurrent chlorhexidine use and the prevalence of chlorhexidine-resistant *S. aureus* (24, 25). We recently performed a large-scale, field-based, cluster-randomized trial evaluating the effectiveness of chlorhexidine against MRSA SSTIs among high-risk U.S. Army recruits in basic training at Fort Benning, Georgia (26). The objective of the current study was to determine the prevalence of chlorhexidine resistance in clinical and colonizing MRSA isolates obtained in the context of this cluster-randomized controlled trial.

MATERIALS AND METHODS

Study design and population. The methods of the cluster-randomized trial have been reported previously; in brief, a 20-month investigation, from May 2010 to January 2012, was conducted to evaluate hygiene-based intervention strategies to reduce SSTI incidence among infantry trainees at Fort Benning, Georgia (26). The trial involved randomization, by training company, to three study groups with various intervention components. Recruits in the standard group received an educational briefing on MRSA SSTI recognition and prevention. Recruits in the enhanced standard group received, in addition to the briefing, supplemental educational material and a first aid kit and were instructed to take a weekly 10-min shower in addition to routine showering. Recruits in the chlorhexidine group received an 8-oz bottle of 4% chlorhexidine (Hibiclens; Mölnlycke Heath Care, Norcross, GA) to use during the weekly 10-min shower.

Trainees who presented to the Troop Medical Clinic (TMC) with an SSTI during training were approached and consented to allow investigators to abstract SSTI-related information from their clinical records and to obtain clinical specimens collected during routine care. Subjects were also asked to complete a questionnaire assessing personal hygiene prac-

Received 30 January 2014 Returned for modification 25 April 2014
Accepted 12 May 2014
Published ahead of print 19 May 2014
Address correspondence to Carey D. Schlett, cschlett@idcrp.org.

doi:10.1128/AAC.02419-14

tices, compliance with the hygiene-based intervention, and knowledge of SSTIs, particularly MRSA SSTIs, and to provide a nasal swab. Some trainees presenting to the TMC for noninfectious conditions, such as musculoskeletal injuries or physical therapy, were also approached for enrollment in the study, as controls. They completed the same questionnaire as subjects with SSTIs and also provided nasal swabs for evaluation. This study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University (protocol IDCRP-055).

Specimen collection, laboratory methods, and antimicrobial susceptibility testing. Clinical culture specimens were obtained from purulent SSTIs as part of routine care and were processed at the Martin Army Community Hospital microbiology laboratory, according to standard techniques. Study personnel obtained bilateral anterior nares swabs from trainees at the time of enrollment, using BBL CultureSwabs (BD Diagnostic Systems, Sparks, MD). These specimens were placed in 5 ml of Trypticase soy broth (TSB) supplemented with 6.5% NaCl (BBL; BD Diagnostic Systems, Sparks, MD) and were incubated for 18 to 24 h at 35°C. After incubation, a 75-μl aliquot of broth was plated on mannitol salt agar (MSA). Mannitol-fermenting colonies were isolated, plated on Trypticase soy agar with 5% sheep's blood, and incubated overnight. *S. aureus* isolates were identified based on colony morphology, Gram staining, latex agglutination (Staphaurex; Remel, Lenexa, KS), and slide catalase testing results. *S. aureus* isolates were tested with oxacillin screening agar (Mueller-Hinton agar with oxacillin at 6 μg/ml; Becton, Dickinson). Using MicroScan WalkAway-96 (DadeBehring, Inc., Deerfield, IL), all MRSA isolates underwent susceptibility testing with ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, rifampin, tetracycline, and trimethoprim-sulfamethoxazole. MIC breakpoints and quality control protocols were used according to standards established by the Clinical and Laboratory Standards Institute (CLSI) (27). Inducible clindamycin resistance was assessed with a double-disk diffusion test (28).

Detection of chlorhexidine resistance and high-level mupirocin resistance. DNA was extracted using a QIAmp DNA minikit (Qiagen, Valencia, CA), according to the manufacturer's instructions. A previously described real-time quadruplex PCR assay was used in duplicate, to confirm methicillin resistance in *S. aureus* (*mecA* and *femA*) and to detect high-level mupirocin resistance (*mupA*) and chlorhexidine resistance (*qacA/B*) simultaneously (23). We defined chlorhexidine resistance on the basis of MRSA isolates possessing *qacA/B*. There are no CLSI methods for testing susceptibility to chlorhexidine; however, we used standard broth dilution techniques to test all MRSA isolates (29). We used 4% chlorhexidine gluconate (Hibiclens) as the starting material and ATCC 700699 as our control strain (22). Isolates that were positive for *mupA* underwent MIC determinations by Etest (bioMérieux, St. Louis, MO). We used the following MIC breakpoints for definitions: susceptible, ≤4 μg/ml; low-level resistance, 8 to 64 μg/ml; high-level resistance, ≥512 μg/ml (30).

Pulsed-field gel electrophoresis. We performed pulsed-field gel electrophoresis (PFGE), using SmaI (Roche Molecular Biochemicals) as a restriction endonuclease, for all available MRSA isolates (31). PFGE findings were resolved and analyzed using BioNumerics (Applied Math, Austin, TX), and isolates were grouped into PFGE types using Dice coefficients and 80% similarity, as described previously (32). We obtained control strains of known PFGE types from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA).

Statistical analysis. Differences in proportions were evaluated by the chi-square test or Fisher's exact test, as appropriate. Median ages were compared using the Kruskal-Wallis *H* test. Analysis of variance (ANOVA) was used to analyze other continuous variables. All tests of significance were two tailed. *P* values of ≤0.05 were considered significant. Statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Study population. A total of 30,209 trainees (all male) constituted the overall study population, and 10,030 (33.2%) were issued chlorhexidine during the study period. Trainees were assigned to chlorhexidine-randomized battalions over the duration of the study period, and the distribution did not vary by study month (data not shown). During the investigation, 1,203 (4.0%) trainees with SSTIs were enrolled in the study. Of the 1,203 SSTIs, 650 (54.0%) had a drainable focus and were cultured; 550 (84.6%) of those were culture positive for *S. aureus* (57.5% MRSA). Subjects with MRSA SSTIs were young (median age, 19 years [range, 17 to 37 years]) and predominately white (80.5%) (Table 1). Among subjects with SSTIs, no demographic differences were observed between study groups. Control subjects were similar in age to subjects with SSTIs (median age, 20 years [range, 17 to 42 years]).

Analysis of clinical MRSA isolates. The clinical care of the 316 subjects with MRSA SSTIs yielded 382 MRSA isolates. Of these clinical isolates, 341 isolates (89.3%) from 283 subjects were available for molecular analysis, including 93 from the standard group, 143 from the enhanced standard group, and 105 from the chlorhexidine group. No differences in demographic or clinical characteristics were observed when subjects who contributed isolates for molecular analysis were compared with those who did not (data not shown). Furthermore, the proportions of unavailable isolates were evenly distributed across study groups.

Among clinical MRSA isolates, there was no difference in the prevalence of genetic determinants of chlorhexidine resistance between study groups. Of the 341 clinical MRSA isolates tested, only five (1.5%) were positive for *qacA/B*, including three from the standard group and two from the chlorhexidine group (3.2% and 1.9%, respectively; $P = 0.64$). In broth dilution testing of these five isolates, two of the isolates, both from the standard group, had a chlorhexidine MIC of 4 μg/ml. The remaining three isolates had a chlorhexidine MIC of 2 μg/ml. All five isolates were USA300 (Table 2). Three (60%) of the five isolates possessed *mupA* (Table 2). The three *mupA*-positive isolates, all from the standard group, also demonstrated high-level resistance (>1,024 μg/ml) by Etest. Of note, two of the five clinical isolates were from subjects in the same training company.

Among the 283 SSTI cases, abscess (210 cases [74.2%]) and cellulitis (138 cases [48.8%]) were the most common clinical diagnoses, and many subjects developed more than one infection (104 cases [36.7%]). The most common site of infection was the lower extremities (126 cases [44.5%]). The time from training start to clinical presentation for MRSA SSTIs did not differ among the groups, and clinical presentation occurred around week 8 to 9 of training.

Analysis of colonizing MRSA isolates. Anterior nares samples were obtained from 1,200 of the 1,203 SSTI subjects; of these, 265 samples (22.1%) yielded MRSA, i.e., 63 from the standard group, 97 from the enhanced standard group, and 105 from the chlorhexidine group. We also assessed nasal colonization in 1,712 control subjects; among these, 73 samples (4.3%) yielded MRSA, including 28 from the standard group, 19 from the enhanced standard group, and 26 from the chlorhexidine group. The total number of colonizing nasal isolates was 338, of which 274 (81.1%) were available for molecular analysis. No difference across study groups in the distribution of isolates available for testing was observed, and isolates were recovered throughout the study period.

Among colonizing MRSA isolates, there were no differences in the prevalence of *qacA/B* in the chlorhexidine group versus the standard and enhanced standard groups combined (0.9% versus 2.4%; $P = 0.65$). Analysis of the 274 colonizing MRSA isolates revealed five (1.8%) that were positive for *qacA/B*, i.e., three

TABLE 1 Characteristics of MRSA cases by study group

Characteristic	Standard ($n = 78$)	Enhanced standard ($n = 121$)	Chlorhexidine ($n = 84$)	P
Race/ethnicity, no. (%)[a]				
White, non-Hispanic	21 (80.8)	40 (74.1)	30 (90.9)	0.61
Hispanic	2 (7.7)	8 (14.8)	2 (6.1)	
Black, non-Hispanic	2 (7.7)	3 (5.6)	1 (3.0)	
Other, non-Hispanic	1 (3.8)	3 (5.6)	0 (0.0)	
Age, median (range)	20 (17–37)	19 (17–34)	19 (17–28)	
Clinical infection, no. (%)				0.03
More than one	32 (41.0)	35 (28.9)	37 (44.1)	
Abscess	22 (28.2)	58 (47.9)	37 (44.1)	
Cellulitis	21 (26.9)	22 (18.2)	8 (9.5)	
Folliculitis	3 (3.9)	2 (1.7)	1 (1.2)	
Infected blister	0 (0.0)	3 (2.5)	1 (1.2)	
Impetigo	0 (0.0)	1 (0.8)	0 (0.0)	
Site of infection, no. (%)				0.56
Lower extremity	33 (42.3)	56 (46.3)	37 (44.1)	
Upper extremity	20 (25.6)	38 (31.4)	21 (25.0)	
More than one	18 (23.1)	17 (14.1)	17 (20.2)	
Thorax	4 (5.1)	5 (4.1)	1 (1.2)	
Head	2 (2.6)	3 (2.5)	4 (4.8)	
Groin/inguinal/perineal area	1 (1.3)	2 (1.7)	4 (4.8)	
Time from training start to first MRSA clinical isolate (days)				
Mean ± SD	66.6 ± 25.1	61.7 ± 26.6	66.3 ± 25.6	0.32
Range	23–118	20–118	20–124	

[a] Race/ethnicity information was available for 26 subjects in the standard group, 54 in the enhanced standard group, and 33 in the chlorhexidine group.

(4.1%) from the standard group, one (1.0%) from the enhanced standard group, and one (0.9%) from the chlorhexidine group. In broth dilution testing of these five colonizing isolates, three of the isolates, i.e., two from the standard group and one from the chlorhexidine group, had a chlorhexidine MIC of 2 μg/ml. The remaining two, one each from the standard and enhanced standard groups, had a chlorhexidine MIC of 1 μg/ml. All five isolates were USA300. Among these five isolates, *mupA* was detected in only one isolate, from a subject who also contributed a clinical chlorhexidine-resistant isolate (Table 2); the isolate demonstrated high-level resistance (>1,024 μg/ml) by Etest.

Antimicrobial susceptibilities. We evaluated the association between *qacA/B*-positive MRSA isolates and resistance to commonly prescribed antimicrobials (Table 3). Most clinical and colonizing *qacA/B*-positive MRSA isolates were susceptible to most agents. Among the chlorhexidine-resistant MRSA isolates, resistance to ciprofloxacin and erythromycin was observed for both clinical and colonizing isolates; however, the number of isolates was small.

DISCUSSION

In the context of a community-based, cluster-randomized, controlled trial, during which more than 10,000 soldiers were issued chlorhexidine, we found no association between extended chlo-

TABLE 2 Genotypic chlorhexidine resistance for individual subjects

Subject[a]	Study group	Specimen type	Time to isolate collection (days)	Isolate collection date	PFGE type	*mupA*[b]	Chlorhexidine MIC (μg/ml)
A	Chlorhexidine	Clinical	49	October 2010	USA300	No	2
B	Chlorhexidine	Clinical	96	September 2011	USA300	No	2
C	Standard	Clinical	42	March 2011	USA300	Yes	4
D	Standard	Clinical	42	March 2011	USA300	Yes	4
E	Standard	Clinical	6	September 2011	USA300	Yes	2
D	Standard	Colonizing	42	March 2011	USA300	Yes	2
F	Standard	Colonizing	38	July 2010	USA300	No	1
G	Standard	Colonizing	17	July 2010	USA300	No	2
H	Enhanced standard	Colonizing	38	July 2010	USA300	No	1
I	Chlorhexidine	Colonizing	82	September 2011	USA300	No	2

[a] Subjects C and D were from the same training company, and subjects B and I were from the same training company.
[b] All isolates with *mupA* demonstrated high-level resistance (>1,024 μg/ml) by Etest.

TABLE 3 Genotypic chlorhexidine resistance by antimicrobial resistance

Antibiotic and phenotype	No. (%)			
	Clinical isolates ($n = 341$)		Colonizing isolates ($n = 274$)	
	qacA/B negative ($n = 336$)	*qacA/B* positive ($n = 5$)	*qacA/B* negative ($n = 269$)	*qacA/B* positive ($n = 5$)
Ciprofloxacin[a]				
Susceptible	216 (100.0)	0 (0)	179 (100.0)	0 (0)
Resistant	120 (96.0)	5 (4.0)	90 (94.7)	5 (5.3)
Clindamycin[b]				
Susceptible	302 (98.4)	5 (1.6)	225 (97.9)	5 (2.1)
Resistant	34 (100.0)	0 (0)	44 (100.0)	0 (0)
Daptomycin				
Susceptible	335 (98.5)	5 (1.5)	269 (98.2)	5 (1.8)
Resistant	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin				
Susceptible	35 (100.0)	0 (0)	32 (100.0)	0 (0)
Resistant	301 (98.4)	5 (1.6)	237 (97.9)	5 (2.0)
Gentamicin				
Susceptible	334 (98.5)	5 (1.5)	268 (98.2)	5 (1.8)
Resistant	1 (100.0)	0 (0)	1 (100.0)	0 (0)
Linezolid				
Susceptible	335 (98.5)	5 (1.5)	269 (98.2)	5 (1.8)
Resistant	0 (0)	0 (0)	0 (0)	0 (0)
Rifampin				
Susceptible	335 (98.5)	5 (1.5)	268 (98.2)	5 (1.8)
Resistant	1 (100.0)	0 (0)	0 (0)	0 (0)
Tetracycline				
Susceptible	324 (98.5)	5 (1.5)	246 (98.0)	5 (2.0)
Resistant	12 (100.0)	0 (0)	23 (100.0)	0 (0)
Trimethoprim-sulfamethoxazole				
Susceptible	336 (98.5)	5 (1.5)	268 (98.2)	5 (1.8)
Resistant	0 (0)	0 (0)	1 (100.0)	0 (0)

[a] $P < 0.01$ by Fisher's exact test for both clinical and colonizing isolates.
[b] Twenty-nine (10.0%) of the 290 clinical isolates tested and 28 (12.4%) of the 225 colonizing isolates tested showed inducible clindamycin resistance.

rhexidine use and the prevalence of chlorhexidine-resistant MRSA isolates. Overall, the prevalence of chlorhexidine resistance during the 2-year period was low (1.6%), and isolation of resistant isolates did not seem to increase over time. Furthermore, 70% of chlorhexidine-resistant isolates were from subjects in groups that did not receive chlorhexidine.

These findings are similar to those observed by Fritz et al. (25). They found no significant increase in *qacA/B* acquisition in their investigation, which involved randomly assigning 258 subjects to receive 5 days of 4% chlorhexidine antiseptic wash (25). Similarly, during a study that involved the use of chlorhexidine-impregnated cloths thrice weekly over a 6-week period, Johnson et al. failed to detect any incident infections with chlorhexidine-resistant strains (24). Additionally, low prevalence rates of chlorhexidine-resistant isolates have been reported for MRSA isolates obtained from the global U.S. military network (0.9%) (23) and U.S. nursing home residents (0.6%) (21). As with other community-based interventions that have assessed the impact of chlorhexidine use on the development of resistance, the periods of use and/or observation might have been too brief to capture the emergence of chlorhexidine-resistant MRSA strains.

These findings are in contrast to those observed in the hospital setting, where the prevalence of *qacA/B* among clinical MRSA isolates may be as high as 80% (33–35). In Taiwan, the prevalence of chlorhexidine-resistant MRSA strains increased from 1.7% in 1990 to 47% in 2005 (36). Moreover, the prevalence of *qacA/B* in strains of MRSA colonizing health care workers appears to be rising (37). Additional studies to monitor chlorhexidine resistance in the hospital setting, where chlorhexidine is increasingly being used for daily bathing of patients, are needed.

The emergence of chlorhexidine resistance among MRSA isolates, whether clinical or colonizing, has implications for prevention and control efforts. In the hospital setting, long-term chlorhexidine use and the presence of *qacA/B* genes have been associated with decolonization failure and the spread of resistant strains. In a case-control study, Lee et al. found that the presence of *qacA/B* genes in combination with mupirocin resistance independently predicted failure of MRSA decolonization (20). In an

investigation employing chlorhexidine to prevent MRSA transmission, Batra et al. found that the chlorhexidine intervention was effective only for patients who lacked chlorhexidine resistance genes (*qacA/B*) (18). Future MRSA prevention strategies that involve widespread use of chlorhexidine over extended periods should incorporate long-term surveillance for the emergence of chlorhexidine-resistant strains.

Genotypic resistance to chlorhexidine is indicated by the presence of *qacA/B* and *smr* genes, which encode drug efflux proteins that confer high-level and low-level *S. aureus* determinants (38, 39). However, chlorhexidine MICs have also been reported as indicators of decreasing susceptibility (36, 37). The challenges with this method are the absence of clear CLSI breakpoints, variable assays, and the fact that, while correlation of elevated MICs (>4 μg/ml) with the presence of *qacA/B* genes has been demonstrated (22), sometimes they are poorly correlated (21, 23). In our study, two of the *qacA/B*-positive isolates (20%) had an MIC of 4 μg/ml, both isolates from subjects in the standard group. Six of the isolates had an MIC of 2 μg/ml, and two had an MIC of 1 μg/ml. McGann et al. found all of the *qacA/B*-positive isolates in their study to have an MIC of 2 μg/ml (23).

We observed that the presence of *qacA/B* was associated with resistance to ciprofloxacin. *qacA/B* may share mobile genetic elements with other antibiotic resistance genes (40). Indeed, chlorhexidine resistance is more common in MRSA than in methicillin-sensitive *S. aureus* (MSSA) (41). Our finding is similar to other reports of antibiotic resistance associated with *qacA/B* (34, 37, 42). This observation may hint at the presence of another efflux-mediated resistance gene present in our isolates (perhaps *norA*) (42). Interestingly, all of our isolates harboring *qacA/B* were of the same lineage (USA300). Specific MRSA strains with associations with *qacA/B* have been reported, but no such associations have been noted in the United States (18, 35, 43).

Our study has several strengths. First, our large-scale, field-based, cluster-randomized trial among trainees at high risk for MRSA colonization and infection presented a unique opportunity to evaluate the emergence of chlorhexidine-resistant MRSA. Over the course of the 2-year study, nearly 625 gallons of chlorhexidine were distributed to over 10,000 trainees, creating an opportunity for evolutionary pressure and for the development and detection of resistant isolates. Second, our closed study population and unified health care network allowed us to obtain and to evaluate almost all MRSA clinical isolates collected from study subjects. Third, we collected nasal specimens from participants with and without clinical infections. Through this broad sampling distribution, we still observed low rates of chlorhexidine resistance among MRSA isolates not associated with clinical disease.

Even with the rigorous study design, our study has several limitations. First, the MRSA isolates evaluated for chlorhexidine resistance were obtained from trainees presenting to the TMC with SSTIs or noninfectious conditions. The prevalence of chlorhexidine resistance in the overall recruit population is not known. Second, due to the nature of the military training, we were unable to obtain baseline or terminal (at the end of training) colonization cultures from individual study participants. Such data might have helped us estimate the rate of *qacA/B* acquisition longitudinally and determine whether *qacA/B* was associated with chlorhexidine exposure or a chlorhexidine-resistant strain was introduced by a recruit upon entry. Third, due to limitations imposed by military training requirements, we were unable to capture data on individual levels of adherence to the once-weekly chlorhexidine shower, and we were able to assess only nasal colonization. However, a subset of subjects enrolled in the study completed a questionnaire assessing adherence to the prevention strategies, including chlorhexidine use. The majority (85.7%) of subjects assigned to the chlorhexidine group reported receiving the soap for use and 71.2% reported using it at least weekly, with an additional 11.3% reporting biweekly use. Although this represents fairly good adherence to the study intervention and is similar to findings from other studies that employed chlorhexidine (16, 25), we recognize that these are self-reported rates from a sample of the study population. Although a correlation between the presence of *qacA/B* and elevated MICs has been demonstrated, this correlation is not fully understood and the clinical implications of elevated MICs need to be further elucidated.

In summary, we found that extensive use of chlorhexidine in a community-based study conducted in a high-risk population was not associated with the emergence of chlorhexidine-resistant MRSA. Nevertheless, as MRSA SSTIs continue to cause morbidity in the community setting and chlorhexidine continues to be an integral component of prevention strategies, surveillance for acquired resistance is needed, particularly in high-risk settings.

ACKNOWLEDGMENTS

We thank the study team of clinical research coordinators, laboratory personnel, and data management staff members whose efforts made this project possible.

This work was supported by the Infectious Disease Clinical Research Program, a Department of Defense (DoD) program executed through the Uniformed Services University. This project has been funded with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), through an interagency agreement (grant Y1-AI-5072). Additional funding was provided by the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Healthcare Quality Promotion, through an interagency agreement (grant 09FED914272 to M.W.E.), and by the DoD Global Emerging Infections Surveillance program (grant C0366-11-HS to M.W.E.).

We have no financial, consultant, institutional, or other relationships that would constitute a conflict of interest.

The content of this publication is the sole responsibility of the authors and does not necessarily reflect the views or policies of the NIH, the Department of Health and Human Services, the DoD, or the Departments of the Army, Navy, or Air Force. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. Government.

REFERENCES

1. **Hersh AL, Chambers HF, Maselli JH, Gonzales R.** 2008. National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. Arch. Intern. Med. **168:**1585–1591. http://dx.doi.org/10.1001/archinte.168.14.1585.
2. **Pallin DJ, Egan DJ, Pelletier AJ, Espinola JA, Hooper DC, Camargo CA, Jr.** 2008. Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin-resistant *Staphylococcus aureus*. Ann. Emerg. Med. **51:**291–298. http://dx.doi.org/10.1016/j.annemergmed.2007.12.004.
3. **Talan DA, Krishnadasan A, Gorwitz RJ, Fosheim GE, Limbago B, Albrecht V, Moran GJ, EMERGEncy ID Net Study Group.** 2011. Comparison of *Staphylococcus aureus* from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clin. Infect. Dis. **53:**144–149. http://dx.doi.org/10.1093/cid/cir308.
4. **Klein E, Smith DL, Laxminarayan R.** 2009. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States,

1999–2006. Emerg. Infect. Dis. **15:**1925–1930. http://dx.doi.org/10.3201/eid1512.081341.

5. **Kazakova SV, Hageman JC, Matava M, Srinivasan A, Phelan L, Garfinkel B, Boo T, McAllister S, Anderson J, Jensen B, Dodson D, Lonsway D, McDougal LK, Arduino M, Fraser VJ, Killgore G, Tenover FC, Cody S, Jernigan DB.** 2005. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N. Engl. J. Med. **352:**468–475. http://dx.doi.org/10.1056/NEJMoa042859.
6. **Aiello AE, Lowy FD, Wright LN, Larson EL.** 2006. Methicillin-resistant *Staphylococcus aureus* among US prisoners and military personnel: review and recommendations for future studies. Lancet Infect. Dis. **6:**335–341. http://dx.doi.org/10.1016/S1473-3099(06)70491-1.
7. **Army Medical Surveillance Activity.** 2011. Ambulatory clinic visits among members of active components, U.S. Armed Forces, 2010. MSMR **18:**16–21.
8. **Salgado CD, Farr BM, Calfee DP.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin. Infect. Dis. **36:**131–139. http://dx.doi.org/10.1086/345436.
9. **Milstone AM, Passaretti CL, Perl TM.** 2008. Chlorhexidine: expanding the armamentarium for infection control and prevention. Clin. Infect. Dis. **46:**274–281. http://dx.doi.org/10.1086/524736.
10. **Campbell KM, Vaughn AF, Russell KL, Smith B, Jimenez DL, Barrozo CP, Minarcik JR, Crum NF, Ryan MA.** 2004. Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infections in an outbreak of disease among military trainees in San Diego, California, in 2002. J. Clin. Microbiol. **42:**4050–4053. http://dx.doi.org/10.1128/JCM.42.9.4050-4053.2004.
11. **Wootton SH, Arnold K, Hill HA, McAllister S, Ray M, Kellum M, LaMarre M, Lane ME, Chaitram J, Lance-Parker S, Kuehnert MJ.** 2004. Intervention to reduce the incidence of methicillin-resistant *Staphylococcus aureus* skin infections in a correctional facility in Georgia. Infect. Control Hosp. Epidemiol. **25:**402–407. http://dx.doi.org/10.1086/502413.
12. **Miller LG, Tan J, Eells SJ, Benitez E, Radner AB.** 2012. Prospective investigation of nasal mupirocin, hexachlorophene body wash, and systemic antibiotics for prevention of recurrent community-associated methicillin-resistant *Staphylococcus aureus* infections. Antimicrob. Agents Chemother. **56:**1084–1086. http://dx.doi.org/10.1128/AAC.01608-10.
13. **Fritz SA, Hogan PG, Hayek G, Eisenstein KA, Rodriguez M, Epplin EK, Garbutt J, Fraser VJ.** 2012. Household versus individual approaches to eradication of community-associated *Staphylococcus aureus* in children: a randomized trial. Clin. Infect. Dis. **54:**743–751. http://dx.doi.org/10.1093/cid/cir919.
14. **Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, Rybak MJ, Talan DA, Chambers HF.** 2011. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin. Infect. Dis. **52:**e18–e55. http://dx.doi.org/10.1093/cid/ciq146.
15. **Daum RS, Spellberg B.** 2012. Progress toward a *Staphylococcus aureus* vaccine. Clin. Infect. Dis. **54:**560–567. http://dx.doi.org/10.1093/cid/cir828.
16. **Whitman TJ, Herlihy RK, Schlett CD, Murray PR, Grandits GA, Ganesan A, Brown M, Mancuso JD, Adams WB, Tribble DR.** 2010. Chlorhexidine-impregnated cloths to prevent skin and soft-tissue infection in Marine recruits: a cluster-randomized, double-blind, controlled effectiveness trial. Infect. Control Hosp. Epidemiol. **31:**1207–1215. http://dx.doi.org/10.1086/657136.
17. **Morrison SM, Blaesing CR, Millar EV, Chukwuma U, Schlett CD, Wilkins KJ, Tribble DR, Ellis MW.** 2013. Evaluation of methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infection prevention strategies at a military training center. Infect. Control Hosp. Epidemiol. **34:**841–843. http://dx.doi.org/10.1086/671278.
18. **Batra R, Cooper BS, Whiteley C, Patel AK, Wyncoll D, Edgeworth JD.** 2010. Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant *Staphylococcus aureus* in an intensive care unit. Clin. Infect. Dis. **50:**210–217. http://dx.doi.org/10.1086/648717.
19. **Skovgaard S, Larsen MH, Nielsen LN, Skov RL, Wong C, Westh H, Ingmer H.** 2013. Recently introduced *qacA/B* genes in *Staphylococcus epidermidis* do not increase chlorhexidine MIC/MBC. J. Antimicrob. Chemother. **68:**2226–2233. http://dx.doi.org/10.1093/jac/dkt182.
20. **Lee AS, Macedo-Vinas M, Francois P, Renzi G, Schrenzel J, Vernaz N, Pittet D, Harbarth S.** 2011. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant *Staphylococcus aureus* carriage after decolonization therapy: a case-control study. Clin. Infect. Dis. **52:**1422–1430. http://dx.doi.org/10.1093/cid/cir233.
21. **McDanel JS, Murphy CR, Diekema DJ, Quan V, Kim DS, Peterson EM, Evans KD, Tan GL, Hayden MK, Huang SS.** 2013. Chlorhexidine and mupirocin susceptibilities of methicillin-resistant *Staphylococcus aureus* from colonized nursing home residents. Antimicrob. Agents Chemother. **57:**552–558. http://dx.doi.org/10.1128/AAC.01623-12.
22. **McGann P, Kwak YI, Summers A, Cummings JF, Waterman PE, Lesho EP.** 2011. Detection of *qacA/B* in clinical isolates of methicillin-resistant *Staphylococcus aureus* from a regional healthcare network in the eastern United States. Infect. Control Hosp. Epidemiol. **32:**1116–1119. http://dx.doi.org/10.1086/662380.
23. **McGann P, Milillo M, Kwak YI, Quintero R, Waterman PE, Lesho E.** 2013. Rapid and simultaneous detection of the chlorhexidine and mupirocin resistance genes *qacA/B* and *mupA* in clinical isolates of methicillin-resistant *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **77:**270–272. http://dx.doi.org/10.1016/j.diagmicrobio.2013.06.006.
24. **Johnson MD, Schlett CD, Grandits GA, Mende K, Whitman TJ, Tribble DR, Hospenthal DR, Murray PR.** 2012. Chlorhexidine does not select for resistance in *Staphylococcus aureus* isolates in a community setting. Infect. Control Hosp. Epidemiol. **33:**1061–1063. http://dx.doi.org/10.1086/667744.
25. **Fritz SA, Hogan PG, Camins BC, Ainsworth AJ, Patrick C, Martin MS, Krauss MJ, Rodriguez M, Burnham CA.** 2013. Mupirocin and chlorhexidine resistance in *Staphylococcus aureus* in patients with community-onset skin and soft tissue infections. Antimicrob. Agents Chemother. **57:**559–568. http://dx.doi.org/10.1128/AAC.01633-12.
26. **Ellis MW, Schlett CD, Millar EV, Wilkins KJ, Crawford KB, Morrison-Rodriguez SM, Pacha LA, Gorwitz RJ, Lanier JB, Tribble DR.** 2014. Hygiene strategies to prevent methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections: a cluster-randomized controlled trial among high-risk military trainees. Clin. Infect. Dis. **58:**1540–1548. http://dx.doi.org/10.1093/cid/ciu166.
27. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
28. **Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH.** 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J. Clin. Microbiol. **41:**4740–4744. http://dx.doi.org/10.1128/JCM.41.10.4740-4744.2003.
29. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
30. **Patel JB, Gorwitz RJ, Jernigan JA.** 2009. Mupirocin resistance. Clin. Infect. Dis. **49:**935–941. http://dx.doi.org/10.1086/605495.
31. **Bannerman TL, Hancock GA, Tenover FC, Miller JM.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
32. **McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. **41:**5113–5120. http://dx.doi.org/10.1128/JCM.41.11.5113-5120.2003.
33. **Miyazaki NH, Abreu AO, Marin VA, Rezende CA, Moraes MT, Villas Boas MH.** 2007. The presence of *qacA/B* gene in Brazilian methicillin-resistant *Staphylococcus aureus*. Mem. Inst. Oswaldo Cruz **102:**539–540. http://dx.doi.org/10.1590/S0074-02762007000400018.
34. **Vali L, Davies SE, Lai LL, Dave J, Amyes SG.** 2008. Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant *Staphylococcus aureus* isolates. J. Antimicrob. Chemother. **61:**524–532. http://dx.doi.org/10.1093/jac/dkm520.
35. **Ho J, Branley J.** 2012. Prevalence of antiseptic resistance genes *qacA/B* and specific sequence types of methicillin-resistant *Staphylococcus aureus* in the era of hand hygiene. J. Antimicrob. Chemother. **67:**1549–1550. http://dx.doi.org/10.1093/jac/dks035.
36. **Wang JT, Sheng WH, Wang JL, Chen D, Chen ML, Chen YC, Chang SC.** 2008. Longitudinal analysis of chlorhexidine susceptibilities of nosocomial methicillin-resistant *Staphylococcus aureus* isolates at a teaching

hospital in Taiwan. J. Antimicrob. Chemother. **62:**514–517. http://dx.doi.org/10.1093/jac/dkn208.

37. **Zhang M, O'Donoghue MM, Ito T, Hiramatsu K, Boost MV.** 2011. Prevalence of antiseptic-resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonising nurses and the general population in Hong Kong. J. Hosp. Infect. **78:**113–117. http://dx.doi.org/10.1016/j.jhin.2011.02.018.
38. **Paulsen IT, Brown MH, Littlejohn TG, Mitchell BA, Skurray RA.** 1996. Multidrug resistance proteins QacA and QacB from *Staphylococcus aureus*: membrane topology and identification of residues involved in substrate specificity. Proc. Natl. Acad. Sci. U. S. A. **93:**3630–3635. http://dx.doi.org/10.1073/pnas.93.8.3630.
39. **Paulsen IT, Skurray RA, Tam R, Saier MH, Jr, Turner RJ, Weiner JH, Goldberg EB, Grinius LL.** 1996. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol. Microbiol. **19:**1167–1175. http://dx.doi.org/10.1111/j.1365-2958.1996.tb02462.x.
40. **Horner C, Mawer D, Wilcox M.** 2012. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J. Antimicrob. Chemother. **67:**2547–2559. http://dx.doi.org/10.1093/jac/dks284.
41. **Mayer S, Boos M, Beyer A, Fluit AC, Schmitz FJ.** 2001. Distribution of the antiseptic resistance genes *qacA*, *qacB* and *qacC* in 497 methicillin-resistant and -susceptible European isolates of *Staphylococcus aureus*. J. Antimicrob. Chemother. **47:**896–897. http://dx.doi.org/10.1093/jac/47.6.896.
42. **DeMarco CE, Cushing LA, Frempong-Manso E, Seo SM, Jaravaza TA, Kaatz GW.** 2007. Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **51:**3235–3239. http://dx.doi.org/10.1128/AAC.00430-07.
43. **Alam MM, Kobayashi N, Uehara N, Watanabe N.** 2003. Analysis on distribution and genomic diversity of high-level antiseptic resistance genes *qacA* and *qacB* in human clinical isolates of *Staphylococcus aureus*. Microb. Drug Resist. **9:**109–121. http://dx.doi.org/10.1089/107662903765826697.