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Chlorhexidine Gluconate Reduces Transmission of Methicillin-Resistant *Staphylococcus aureus* USA300 among Marine Recruits

Timothy J. Whitman, DO¹, Carey D. Schlett, MPH², Greg A. Grandits, MS³, Eugene V. Millar, PhD², Katrin Mende, PhD^{2,4}, Duane R. Hospenthal, MD, PhD⁴, Patrick R. Murray, PhD⁵, and David R. Tribble, MD, DrPH²

¹Infectious Diseases Service, Walter Reed National Military Medical Center, Bethesda, Maryland

²Infectious Disease Clinical Research Program, Uniformed Services University, Bethesda, Maryland ³Division of Biostatistics, University of Minnesota, Minneapolis, Minnesota ⁴San Antonio Military Medical Center, Fort Sam Houston, Texas ⁵National Institutes of Health, Bethesda, Maryland

Abstract

BACKGROUND—Methicillin-resistant *Staphylococcus aureus* (MRSA) pulsed-field type (PFT) USA300 causes skin and soft tissue infections in military recruits and invasive disease in hospitals. Chlorhexidine gluconate (CHG) is used to reduce MRSA colonization and infection. The impact of CHG on the molecular epidemiology of MRSA is not known.

OBJECTIVE—To evaluate the impact of 2% CHG-impregnated cloths on the molecular epidemiology of MRSA colonization.

DESIGN—Cluster-randomized, double-blind, controlled trial.

SETTING—Marine Officer Candidate School, Quantico, Virginia, in 2007.

PARTICIPANTS—Military recruits.

INTERVENTION—Thrice-weekly application of CHG-impregnated or control (Comfort Bath; Sage) cloths over the entire body.

MEASUREMENTS—Baseline and serial (every 2 weeks) nasal and/or axillary swab samples were assessed for MRSA colonization. Molecular analysis was performed with pulsed-field gel electrophoresis.

RESULTS—During training, 77 subjects (4.9%) acquired MRSA, 26 (3.3%) in the CHG group and 51 (6.5%) in the control group ($P = .004$). When analyzed for PFT, 24 subjects (3.1%) in the control group but only 6 subjects (0.8%) in the CHG group ($P = .001$) had USA300. Of the 167

Address correspondence to Timothy J. Whitman, DO, Department of Infectious Diseases, Walter Reed National Military Medical Center, 8901 Wisconsin Avenue, Bethesda MD 20889, (timothy.whitman@med.navy.mil).

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colonizing isolates recovered from 77 subjects, 99 were recovered from the control group, including USA300 (40.4%), USA800 (38.4%), USA1000 (12.1%), and USA100 (6.1%), and 68 were recovered from the CHG group, including USA800 (51.5%), USA100 (23.5%), and USA300 (13.2%).

CONCLUSIONS—CHG decreased the transmission of MRSA—more specifically, USA300—among military recruits. In addition, USA300 and USA800 outcompeted other MRSA PFTs at incident colonization. Future studies should evaluate the broad-based use of CHG to decrease transmission of USA300 in hospital settings.

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes skin and soft tissue infections (SSTIs) in community settings.^{1–3} MRSA SSTI control and prevention measures include hand hygiene, appropriate wound care, and environmental cleaning.⁴

Decolonizing agents such as chlorhexidine gluconate (CHG) have also been implemented in outbreak settings or with individuals who have recurrent SSTI, because colonization is believed to play a role in MRSA transmission and pathogenesis.^{1,4} However, the efficacy of decolonization in disease prevention has not been validated.⁴ In a cluster-randomized, controlled trial among high-risk military recruits, thrice-weekly applications of CHG wipes failed to prevent SSTI.⁵ The use of CHG wipes did reduce MRSA acquisition, which suggests that the antiseptic may provide at least some protective benefit in community settings.

Our current understanding of MRSA epidemiology—particularly, the characterization of clinical isolates—has been greatly enhanced by the incorporation of molecular methods such as pulsed-field gel electrophoresis (PFGE).⁶ To date, 8 major pulsed-field types (PFTs) of MRSA have been identified. The vast majority of clinical isolates collected in community-based studies were identified as USA300, and the colonization prevalence of this PFT appears to be increasing.^{7–11} Of further concern, the prevalence of USA300 in hospital settings has also increased.¹²

Studies are warranted to evaluate the impact of CHG on the molecular epidemiology of MRSA, because they may provide valuable insights into the dynamics of transmission, the pathogenesis of disease, and the optimization of decolonization strategies. Herein we describe the impact of CHG on MRSA clonal dynamics in a cohort of subjects at high risk for colonization and disease, and we suggest that the inhibitory effect of CHG on transmission of USA300 that was found in this community-based study may have relevance for infection control in the hospital setting.

METHODS

Study Population and Setting

A cluster-randomized, double-blind, controlled trial to evaluate the effectiveness of the use of CHG-impregnated body wipes in preventing SSTI was conducted with military recruits attending Officer Candidate School (OCS) at Marine Corps Base Quantico (MCBQ), Virginia, during the summer and fall of 2007 (ClinicalTrials identifier: NCT00475930; <http://clinicaltrials.gov>). Details of the study have been described elsewhere.⁵ Briefly,

military recruits were assigned to platoons of 40–70 individuals upon arrival at OCS. Platoon members resided together in open bays and had minimal contact with recruits from other platoons. Each platoon was randomly assigned to the CHG group or the control group. Written, informed consent was obtained from the recruits who agreed to participate in the study. Participants in each platoon received the same intervention, either 2% CHG-impregnated cloths (Sage; 500 mg CHG per cloth; hereafter referred to as the CHG group) or control cloths (Comfort Bath, Sage; hereafter referred to as the CB group), in similar packaging. One packet (containing 2 cloths) was dispensed to each subject thrice weekly; subjects were instructed to use the cloths to scrub the entire body, with the exception of genitalia, the face, and any areas with large, open wounds. The duration of the trial spanned 4 training classes, 3 of 6 weeks' duration and 1 of 10 weeks' duration.

Study procedures included completion of a questionnaire and collection of nares and axillary swab samples at enrollment and at biweekly follow-up visits. A self-administered questionnaire at enrollment assessed recent antibiotic use (in the prior 3 months). Throughout the study, antibiotic use was recorded, including information about antibiotic name, indication for use, and start/stop dates. Recruits with SSTIs were referred to the OCS clinic for evaluation and treatment by healthcare providers.

Specimen Collection and Identification

Specimens collected from the external nares and axillae were collected, using BD BBL CultureSwabs with Liquid Amies Medium (BD Diagnostic Systems, Sparks, MD). For clinical infections, a specimen was obtained from the base of a cutaneous lesion by OCS healthcare providers, using a sterile Dacron swab after the wound surface was cleaned. Specimens were transported to the National Naval Medical Center clinical laboratory, where they were processed and cultured according to standard methods.¹³

Laboratory Methods

After identification and isolation procedures were performed, isolates were assessed by PFGE as described elsewhere.⁶ PFGE patterns were analyzed, using BioNumerics software (Applied Maths, Austin, TX), and grouped according to PFT, using established criteria.^{6,14} Polymerase chain reaction (PCR) was conducted to detect the following virulence and resistance markers: Pantón-Valentin leukocidin genes (PVL), arginine catabolic mobile element (ACME), and staphylococcal cassette chromosome type *mec* (SCC*mec*).^{11,15} All isolates were assessed for PFT and the presence of each of these markers.

In addition, as a part of assessing the safety of CHG use, a subset of isolates were tested for CHG resistance, using the agar dilution method.¹⁶ Isolates from both treatment groups were included. No Clinical and Laboratory Standards Institute minimum inhibitory concentration (MIC) breakpoints exist for CHG; however, several studies suggest that CHG MICs can be used as reliable indicators of trends toward decreasing biocide susceptibility.¹⁷ We also performed PCR for *qacA/B* and *smr* genes, because the presence of these genes has been correlated with elevated CHG MICs (>4 µg/mL).¹⁸

Statistical Analysis

Subjects were classified as being colonized if either swab sample (from nares or axillae) had positive test results for MRSA. Of the 199 MRSA episodes, 41 (21%) were identified solely from axilla samples. If MRSA-positive results were obtained for both samples ($n = 35$), the result from the nares sample was used for PFT classification.

Incidence and prevalence was computed for the study groups for overall and PFT-specific MRSA. Incidence was defined as either a new MRSA colonization or a new PFT isolated (among subjects who had MRSA colonization at baseline). Analysis was performed at the subject level and at the platoon level. At the subject level, simple percentages were computed for each group and compared using standard χ^2 tests. At the platoon level, the prevalence of overall MRSA colonization and of PFT-specific colonization were calculated for each platoon, as was the percentage of subjects in each platoon who acquired MRSA during the follow-up period. From these platoon-level values, mean platoon prevalence and cumulative incidence of overall MRSA colonization and colonization with types USA300 and USA800 (the 2 most common types) were computed and compared among the CHG- and CB-randomized platoons, using a 2-way weighted ANOVA with factors for OCS class (3 df) and treatment (1 df); analyses were weighted with the inverses of the estimated platoon rate variances, to account for different platoon sizes.¹⁹

The PFT distribution among subjects with MRSA colonization was compared between groups. This was done at baseline, for incident MRSA acquisition, and using all follow-up visits in which subjects had positive test results for MRSA. Distributions between treatment groups were compared, using χ^2 tests (with 2 df; classifying types as USA300, USA800, and all other types combined). We used SAS software, version 9.2 (SAS Institute), for all analyses.

RESULTS

MRSA Prevalence and PFT Distribution at Baseline

Of the 2,572 recruits in OCS, 1,562 (60.7%) from 44 platoons were enrolled in our study, 781 (from 23 platoons) in the CHG group and 781 (from 21 platoons) in the CB group. At enrollment, 32 subjects (2.0%; 16 from the CHG group and 16 from the CB group) had MRSA colonization (Figure 1). The most common PFT was USA800 (31.3%), followed by USA100 (25.0%), USA1000 (15.6%), USA300 (12.5%), non-USA types (9.4%), and USA700 (6.3%; Table 1). PFT distribution at baseline did not differ between the groups ($P = .50$; Figure 2).

MRSA Acquisition and Prevalence and PFT Distribution at Follow-Up

Of the 1,562 subjects who were enrolled in our study, we collected additional swab samples from 1,358 (86.9%) at 2 weeks, 1,193 (76.4%) at 4 weeks, and 1,040 (66.6%) at 6 weeks. Of the 445 subjects in the 10-week training class, we collected additional swab samples from 234 (52.6%) and 227 (51.0%) at 8 and 10 weeks, respectively. A total of 4,048 swab samples were collected from 1,382 individuals during the follow-up period.

A total of 77 subjects (4.9%) acquired MRSA, 26 (3.3%) in the CHG group and 51 (6.5%) in the CB group ($P = .004$; Figures 1, 2). In addition, 2 subjects in the CB group experienced a change in MRSA PFT from baseline (1 from USA100 to USA300 and 1 from USA1000 to USA 300); their cases are included as incident cases. Differences in acquisition (CB vs CHG) were greatest for USA300 (24 [3.1%] vs 6 [0.8%]; $P < .001$) and USA800 (23 [2.9%] vs 14 [1.8%]; $P = .13$) and there was no difference for all other PFTs combined (6 [0.8%] vs 6 [0.8%]; Figure 2).

During the follow-up period, 101 subjects (77 with incident cases and 24 with prevalent cases) had MRSA colonization at 1 or more biweekly assessments, representing a total of 167 MRSA isolates. Of these 167 MRSA isolates, 68 were isolated from the CHG group and 99 were from the CB group (Figure 1; Table 1). The proportions of isolates that were USA800 or USA100 were higher in the CHG group than in the CB group (USA800: 35 [51.5%] vs 38 [38.4%], respectively; USA100: 16 [23.5%] vs 6 [6.1%], respectively). By contrast, the proportion of isolates that were USA300 was higher in the CB group than in the CHG group (40 [40.4%] vs 9 [13.2%], respectively). USA1000 accounted for 12 (12.1%) isolates in the CB group but only 2 (2.9%) in the CHG group.

Average platoon rates of USA300 and USA800 prevalence and acquisition are displayed in Figure 3. The results of these analyses paralleled those of the individual analyses. The prevalence of USA300 increased in both groups, but it was consistently lower in the platoons randomized to the CHG group. The mean cumulative incidence of USA300 acquisition was lower in the CHG group than in the CB group (0.9% vs 3.6%; $P = .058$). By contrast, the mean cumulative incidence of USA800 acquisition did not differ significantly between study groups (2.3% vs 3.4%; $P = .54$). Prevalence between the CHG and CB groups followed the same pattern as the incidence rates (ie, higher rates of colonization with type USA300 in the CB group and similar rates of colonization with type USA800 between groups).

Genotypic Characteristics of MRSA Isolates

Of the 199 MRSA isolates obtained from samples collected from 109 recruits, 64 (32.2%) were positive for PVL, 54 (27.1%) were positive for ACME, and 165 (82.9%) had *SCCmec* type IV (Table 2). Genotypic characteristics varied widely by PFT ($P < .001$). All 53 USA300 isolates had *SCCmec* type IV and tested positive for PVL and ACME, whereas none of the USA100 isolates had these markers. Only 1 other isolate (of type USA800) had the ACME marker. Of the 199 isolates, 4 (2.0%) carried the *qacA/B* gene (1 USA700 and 3 USA800 isolates) and 5 (2.5%) had the *smr* gene (all were USA300).

Among the subset of 29 MRSA isolates (9 USA100, 13 USA800, 1 USA300, 1 USA1000, and 5 non-USA types) in which CHG resistance was tested, 6 (20.7%) were resistant (MIC 4 $\mu\text{g/mL}$) to CHG; all 6 were of type USA100. All 13 USA800 isolates had an MIC value of 2.

Antibiotic Use during the Study

Rates of antibiotic use did not differ between study groups. A total of 152 subjects (19.5%) in the CHG group received anti-MRSA antibiotics (eg, trimethoprim-sulfamethoxazole,

tetracyclines, clindamycin, rifampin, and fluoroquinolones), compared with 159 (20.4%) in the CB group.

Molecular Characteristics of Disease Isolates

Of the 98 subjects who developed an SSTI, swab samples from 30 were collected at the infection site; of these, 5 had culture results that were positive for MRSA. USA300 was the PFT of 4 (80%) of these isolates, and the other isolate was a non-USA type. Three of the 4 USA300 isolates had positive test results for PVL and ACME.

DISCUSSION

To our knowledge, this is the first longitudinal cohort study to evaluate the impact of CHG on the molecular epidemiology of MRSA colonization. Our community-based study had 2 important findings. First, USA300 and USA800 have a competitive fitness advantage in colonization over other PFTs. Second, and more importantly, from an infection control perspective, CHG can prevent transmission of the most pathogenic strain of MRSA (USA300).

Type-specific competition among MRSA isolates has been observed in hospital-based studies, where PFTs that were once associated only with community-acquired infections (ie, USA300) have now become a significant cause of bloodstream infections.^{20,21} Mathematical models suggest that the enhanced fitness of USA300 will eventually lead to its predominance among hospital-associated strains of MRSA.²²

It is likely that the colonization fitness advantage of specific MRSA PFTs is dependent on a highly complex interplay between host, environment, and pathogen.^{23–25} Our cohort consisted of a homogeneous group of individuals who were young, healthy, and engaged in rigorous physical training in a hot, humid environment, factors that may have been advantageous for the transmission and acquisition of USA300 and USA800. In addition, it is possible that certain characteristics unique to certain PFTs may provide a selective advantage, including genes that encode for clumping factor B, surface adhesions, *agrB* and *clfA*, and the mobile genetic element ACME.^{23–25} Of note, ACME was present in all USA300 isolates but only 1% of USA800 isolates; this may highlight type-specific differences in factors that affect colonization mechanisms.

Very few clinical isolates were collected in our study. We suspect that this was because SSTIs were identified early, before the development of an abscess, and antibiotics were immediately prescribed for treatment of these cases.⁵ As expected, most of the clinical isolates were USA300 and positive for PVL and ACME. The predominance of USA300 among cases of SSTI is consistent with previous studies in military¹¹ and nonmilitary settings.^{10,26} By contrast, USA800 was commonly found among colonizing isolates but not among clinical isolates.

CHG is an antistaphylococcal topical antiseptic commonly used in hospital settings to reduce catheter-related blood-stream and surgical site infections.^{27–29} In these settings, the

use of CHG has also been associated with decreased acquisition of MRSA and the partial decolonization of patients.^{30,31}

This study demonstrates that thrice-weekly applications of CHG decreased the incidence of acquisition of USA300 among military recruits, suggesting that if it is applied more frequently (ie, daily) and over longer periods of time, CHG has the potential to prevent SSTI. Further studies evaluating a daily application regimen are required to better elucidate the effects of this antiseptic on the colonization dynamics of MRSA and its ability to prevent SSTI.

Of interest, CHG appeared to have less of an effect on the acquisition of USA800, which may imply that CHG resistance is intrinsic to this specific type. In hospital settings, CHG resistance (isolates with MICs $\geq 4 \mu\text{g/mL}$) and expression of associated genetic markers (eg, *qacA/B* or *smr* genes) have been noted following long-term use of this antiseptic.³² Noguchi et al identified 30 hospital-acquired MRSA isolates in Asia, belonging to 21 different PFTs, that had the *qacA/B* gene.³³ However, data are lacking that identify CHG resistance genes in the USA pulsed-field pattern of MRSA, and there are no studies that link PFT patterns with in vivo CHG resistance. On the basis of our findings, further studies are needed to investigate in vivo or in vitro CHG resistance of MRSA genotypes in the United States.

There are limitations to this study. First, specimens were collected from study participants biweekly, for a maximum duration of 10 weeks. It is not known whether colonization episodes of less than 2 weeks' duration occurred between study visits or whether changes in MRSA colonization occurred beyond the period of observation, as has been reported elsewhere.³⁴ Second, colonization was determined by collecting samples from the nares and the axilla. It is possible that MRSA and/or particular PFTs may be more adept at colonizing sites other than the nares or the axilla, and thus it is possible that we underestimated the actual prevalence.³⁵ Third, there was significant attrition in the cohort over time due to individuals withdrawing from the trial or from OCS altogether. Finally, our study was nested in a single-site evaluation of CHG effectiveness, utilizing a relatively closed cohort consisting of young, otherwise healthy individuals engaged in military training. These data might not be representative of MRSA colonization dynamics or PFT distributions among other populations known to be at high risk for MRSA (eg, people with diabetes, day care participants, intravenous drug abusers) or in physical environments that differ from those of military training facilities (eg, less crowding, lower frequency of skin abrasions).

Our study revealed variation in PFT among MRSA isolates over time in a single cohort of military recruits. This observation may have relevance for disease outcomes as well as prevention and screening strategies in other groups at increased risk for MRSA infection. Further studies are needed to determine why certain MRSA PFTs are more adept at colonizing than causing disease (and vice versa), why CHG has differential effects on specific PFTs, and, most importantly, whether this impact can result in the prevention of infection.

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Figure 1. Flow diagram comparing incident cases of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization by treatment group.

Figure 2.

Distribution of pulsed-field type (PFT) at baseline and during follow-up, by treatment group (CHG, chlorhexidine; CB, Comfort Bath). Included in the incidence episodes are 2 subjects, both in the CB group, who experienced a change in PFT between baseline and follow-up. One subject had USA100 at baseline and USA300 during follow-up, and the other had USA1000 at baseline and USA300 during follow-up.

Figure 3.

Mean platoon prevalence and cumulative incidence of colonization with the USA300 and USA800 types of methicillin-resistant *Staphylococcus aureus* (MRSA) in the axilla or nares. The dotted-dashed lines represent week 8 and week 10 data from class 2 (June 4) only. MRSA was isolated from both the nares and the axilla in the same subject during the same visit, results were combined into 1 result (and the nares result was used).

Table 1

Pulsed-Field Types (PFTs) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates Collected at Baseline and during Training

PFT	Enrollment	Training	Total
USA100	8 (25.0)	22 (13.2)	30 (15.1)
USA300	4 (12.5)	49 (29.3)	53 (26.6)
USA700	2 (6.3)	3 (1.8)	5 (2.5)
USA800	10 (31.3)	73 (43.7)	83 (41.7)
USA1000	5 (15.6)	14 (8.4)	19 (9.5)
Non-USA PFT ^a	3 (9.4)	6 (3.6)	9 (4.5)
Total	32 (100.0)	167 (100.0)	199 (100.0)

NOTE. Data are no. (%) of isolates. “Enrollment” indicates the measurement at week 0 (baseline) and “training” indicates measurements taken during weeks 2–10. When MRSA was isolated from both the nares and the axilla of the same subject during the same visit, results were combined into 1 result (and the nares result was used).

^aIncludes Quantico type 1 ($n = 6$), other Quantico type ($n = 1$), and eMRSA15 ($n = 2$).

TABLE 2

Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates (by Pulsed-Field Type [PFT]) Colonizing Recruits

	USA100	USA300	USA700	USA800	USA 1000	Non-USA PFT ^a	Total
Total number of isolates	30	53	5	83	19	9	199
Genotypic characteristics							
PVL	0 (0)	53 (100)	1 (20.0)	1 (1.2)	9 (47.4)	0 (0)	64 (32.2)
ACME	0 (0)	53 (100)	0 (0)	1 (1.2)	0 (0)	0 (0)	54 (27.1)
SCCmec type IV	0 (0)	53 (100)	5 (100)	83 (100)	18 (94.7)	6 (66.7)	165 (82.9)
<i>qacA/B</i>	0 (0)	0 (0)	1 (20.0)	3 (3.6)	0 (0)	0 (0)	4 (2.0)
<i>smr</i>	0 (0)	5 (9.4)	0 (0)	0 (0)	0 (0)	0 (0)	5 (2.5)

NOTE. Data are no. (%) of isolates. When MRSA was isolated from both the nares and the axilla of the same subject during the same visit, results were combined into 1 result (and the nares result was used). This resulted in the following selections for the few discordant axilla versus nares sample results: 1 discordant PFT result (USA800 and USA900; USA800 result used), 2 discordant SCCmec results (both type IV vs no SCCmec present; type IV result used), 1 discordant ACME result (present vs absent; present result used), and 1 discordant PVL result (present vs absent; present result used).

^aIncludes Quantico type 1 ($n = 6$), other Quantico type ($n = 1$), and eMRSA15 ($n = 2$).