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Mupirocin and Chlorhexidine Resistance in *Staphylococcus aureus* in Patients with Community-Onset Skin and Soft Tissue Infections

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Decolonization measures, including mupirocin and chlorhexidine, are often prescribed to prevent *Staphylococcus aureus* skin and soft tissue infections (SSTI). The objective of this study was to determine the prevalence of high-level mupirocin and chlorhexidine resistance in *S. aureus* strains recovered from patients with SSTI before and after mupirocin and chlorhexidine administration and to determine whether carriage of a mupirocin- or chlorhexidine-resistant strain at baseline precluded *S. aureus* eradication. We recruited 1,089 patients with community-onset SSTI with or without *S. aureus* colonization. In addition to routine care, 483 patients were enrolled in a decolonization trial: 408 received intranasal mupirocin (with or without antimicrobial baths), and 258 performed chlorhexidine body washes. Patients were followed for up to 12 months with repeat colonization cultures. All *S. aureus* isolates were tested for high-level mupirocin and chlorhexidine resistance. At baseline, 23/1,089 (2.1%) patients carried a mupirocin-resistant *S. aureus* strain and 10/1,089 (0.9%) patients carried chlorhexidine-resistant *S. aureus*. Of 4 patients prescribed mupirocin, who carried a mupirocin-resistant *S. aureus* strain at baseline, 100% remained colonized at 1 month compared to 44% of the 324 patients without mupirocin resistance at baseline ($P = 0.041$). Of 2 patients prescribed chlorhexidine, who carried a chlorhexidine-resistant *S. aureus* strain at baseline, 50% remained colonized at 1 month compared to 48% of the 209 patients without chlorhexidine resistance at baseline ($P = 1.0$). The overall prevalence of mupirocin and chlorhexidine resistance is low in *S. aureus* isolates recovered from outpatients, but eradication efforts were less successful in patients carrying a mupirocin-resistant *S. aureus* strain at baseline.

Preventive measures for *Staphylococcus aureus* infections have been widely implemented in health care settings (1). Specifically, the topical antimicrobial agents mupirocin and chlorhexidine have been prescribed for decades for patients in intensive care units and those undergoing surgery and dialysis as a means to eradicate *S. aureus* carriage to reduce the risk of nosocomial infections (2–4). During the current epidemic of cutaneous abscesses associated with the emergence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains, these decolonization therapies have been extrapolated to outpatients in an effort to prevent recurrent skin infections (5–9).

S. aureus strains exhibiting resistance to mupirocin and chlorhexidine have been reported. While this phenomenon has been described in both inpatient and outpatient settings for mupirocin, chlorhexidine resistance outside the hospital setting has not been previously studied (10–16). At the population level, widespread use of mupirocin and chlorhexidine has been associated with dramatically increased prevalence of *S. aureus* strains resistant to mupirocin and chlorhexidine compared to periods of time or geographic regions with limited use of these agents, likely due to the selection of resistant strains (13, 17–20). Alarmingly, routine use of these measures in health care settings has contributed to outbreaks with resistant strains (18, 21). At the individual level, in patients undergoing peritoneal dialysis, repeated prophylaxis with mupirocin has been associated with the development of mupirocin resistance in *S. aureus* (22, 23).

Mupirocin inhibits bacterial isoleucyl-tRNA synthetase, resulting in protein synthesis inhibition (24). *S. aureus* strains may harbor low-level (MIC = 8 to 256 μg/ml) or high-level (MIC ≥ 512 μg/ml) resistance to mupirocin (25). Low-level mupirocin resistance is the result of an alteration in the isoleucyl-tRNA synthetase gene *ileS*, a mutation which is typically stable and nontransferrable. As mupirocin is frequently delivered directly to the site of infection as a topical agent, low level resistance is typically not clinically relevant. High-level mupirocin resistance is conferred by the *mupA* gene, which is carried on a plasmid, enabling the spread of this resistance mechanism. The *mupA* gene encodes a novel isoleucyl-RNA synthetase which is not inhibited by mupirocin (16, 17, 24, 26–28). The plasmid carrying the *mupA* gene may also carry resistance determinants to other systemic antimicrobial agents, raising concern that mupirocin use could select not only for mupirocin resistance, but also for increasing antimicrobial resistance overall (10, 15, 16).

Chlorhexidine is a biguanide cationic bactericidal agent which is rapidly taken up by *S. aureus* (29, 30). At low concentrations, chlorhexidine disrupts the integrity of the cell wall and membranes, resulting in leakage of the intracellular contents. At high concentrations, chlorhexidine causes coagulation of the intracellular contents. Chlorhexidine resistance is conferred by the plasmid-mediated *qacA/B* genes which encode proton-dependent multidrug efflux pumps (13, 30, 31). Some studies have suggested

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cross-resistance between the *qacA/B* genes and other systemic antimicrobial agents, as the plasmids may carry multiple determinants of antimicrobial resistance (30–32). Data regarding chlorhexidine resistance in *S. aureus* isolates recovered from patients in the United States are limited.

We recently performed two decolonization trials for patients with community-onset skin and soft tissue infections (SSTI) which prescribed brief regimens employing mupirocin application to the anterior nares and chlorhexidine body washes (7, 8). In the present study, we aimed to determine the epidemiology of mupirocin and chlorhexidine resistance in contemporary *S. aureus* isolates recovered from these patients. The primary objective was to measure the prevalence of high-level mupirocin resistance and chlorhexidine resistance in *S. aureus* isolates recovered from patients with *S. aureus* colonization and/or SSTI at baseline and after performing decolonization with mupirocin and chlorhexidine. Additionally, we aimed to identify patient-level epidemiologic risk factors and isolate-level features associated with mupirocin and chlorhexidine resistance and to determine whether carrying a mupirocin- or chlorhexidine-resistant strain at baseline resulted in failure of *S. aureus* eradication.

MATERIALS AND METHODS

Setting and study population. This study was approved by the Washington University Institutional Review Board and informed consent was obtained for all study participants. From April 2007 to November 2009, 1,089 patients, 6 months of age and older with community-onset SSTI, were recruited from nine community pediatric practices in metropolitan St. Louis affiliated with a practice-based research network, the St. Louis Children's Hospital (SLCH) Emergency Department (ED) and ambulatory wound center, and the Barnes-Jewish Hospital (BJH) ED. Colonization cultures to detect *S. aureus* carriage were obtained from the anterior nares, axillae, and inguinal folds. Isolates associated with clinical infection were obtained from the SLCH or BJH clinical microbiology laboratories. A questionnaire was administered to assess health, hygiene, and household factors. Patients with traditional risk factors for health care-associated MRSA infections (e.g., indwelling catheter, percutaneous medical device, receiving dialysis, or residing in a long-term-care facility) were excluded. Each participant had at least one *S. aureus* isolate available for the analysis of mupirocin and chlorhexidine resistance.

Of the 1,089 patients with community-onset SSTI, 483 with *S. aureus* colonization were subsequently enrolled into 1 of 2 decolonization trials, described in detail elsewhere (7, 8). Briefly, in the first trial, participants were randomized to 1 of 4 decolonization regimens: (i) hygiene education alone; (ii) hygiene education plus 2% mupirocin ointment applied to the anterior nares twice daily for 5 days; (iii) hygiene education, twice daily application of intranasal mupirocin, and daily body washes with 4% chlorhexidine solution for 5 days; and (iv) hygiene education, twice daily application of intranasal mupirocin, and daily baths in dilute bleach water for 5 days. Longitudinal colonization cultures were collected from the anterior nares, axillae, and inguinal folds of the study participants 1 month and 4 months following decolonization (7). In the second trial, the 5-day decolonization regimen consisted of hygiene education, 2% mupirocin ointment applied to the anterior nares twice daily, and daily body washes with 4% chlorhexidine solution. Patients and their households were randomized to either the index decolonization group, in which only the patient presenting with the skin infection performed the decolonization regimen, or the household decolonization group, in which all household members were asked to perform the decolonization measures. Longitudinal colonization cultures were collected from the anterior nares, axillae, and inguinal folds of the index patients 1, 3, 6, and 12 months following decolonization (8). For both trials, *S. aureus* eradication was defined as the absence of *S. aureus* at the 3 sampled body sites.

TABLE 1 Primers used in this study

Primer name	Primer sequence	Source or reference
Mup-F	TAATGGGAAATGTCTCGAGTAGA	34
Mup R	AATAAAATCAGCTGGAAAGTGTTC	
qacA/B-F	CTATGGCAATAGGAGATATGGTGT	31
qacA/B-R	CCACTACAGATTCTTCAGCTACATG	
qacA/B-RT-F	AGTGAAGCCATACCAGCTCCAACT	This study
qacA/B-RT-R	TTGCACCAATTGCACCCGGATTAG	
Mup1	CCCATGGCTTACCAGTTGA	38
Mup2	CCATGCAGCACTATCCGAA	
MupA-1f	ACTTTACTTTATCCAATATATCTTTC	38
MupA-1r	AATGTAGATAATATATTCCATACAC	
MupA-2f	TACTGGGTTGACATGGACTCCC	38
MupA-2r	TCTTTGTTATAACATTTAAGAAATCC	
MupA-3f	TGGTATTGTTCATATAGCACCA	38
MupA-3r	AACCAAACATCGATTACTTCTTC	
MupA-4f	ATATTGAGTTGCATAGACCTTATG	38
MupA-4r	ATCGTAATTATTTACATAAATATTAC	
MupA-5f	AATACATTAGATAATTGGGCTCTT	38
MupA-5r	TTTAAGCTCATAGGTAATATAGTG	
MupA-6f	GGTGATTAAACCTAATAGTCAATTAAAC	38
MupA-6r	TTTATTTGGTAATTTAGAATAATC	

Laboratory methods. Colonization swabs were placed in tryptic soy broth with 6.5% sodium chloride (BBL; Becton Dickinson, Sparks, MD) and incubated at 35°C overnight. The broth was subsequently plated onto Trypticase soy agar with 5% sheep blood (BBL; Becton Dickinson) for overnight incubation. *S. aureus* isolates were identified based on colony morphology, Gram stain, results of a rapid latex agglutination test for *S. aureus* identification (Staphaurex; Remel, Lenexa, KS), and catalase activity. Disk diffusion testing on Mueller-Hinton agar (BBL; Becton Dickinson) was performed to detect resistance to cefoxitin (as an indicator of methicillin resistance), clindamycin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, rifampin, and tetracycline according to Clinical and Laboratory Standards Institute guidelines (25). Inducible clindamycin resistance was detected by the double-disk approximation "D test" (25, 33); for the final analysis, strains which possessed inducible clindamycin resistance were considered resistant to clindamycin. All *S. aureus* isolates were frozen in glycerol for future analyses.

Genomic DNA extraction. Total DNA extraction was performed using either the BiOstic bacteremia DNA kit (MoBio Laboratories, Carlsbad, CA) or the GeneOhm lysis kit (Becton Dickinson) according to the manufacturers' directions. An analysis of the two DNA extraction methods for downstream PCRs found the methods to be comparable (not shown).

Detection of high-level mupirocin resistance. A real-time PCR assay detecting a 124-bp portion of *mupA* was used to detect high-level mupirocin resistance using established primers (34). Briefly, 50 to 100 ng of DNA was added to a reaction mix containing 1× iQ SYBR green Supermix (Bio-Rad, Hercules, CA), 200 nM (each) primers Mup-F and Mup-R (Table 1) (34), and molecular-grade water to a final reaction volume of 25 μl. PCR was performed using the Cepheid SmartCycler, with an initial cycle of 95°C for 120 s, followed by 35 cycles of 95°C for 10 s (optics off), 60°C for 15 s (optics on; FCTC25 dye set), and 72°C for 15 s (optics off). A melt curve analysis was then performed from 60 to 95°C, at a rate of 0.2 degrees per second. Samples were considered positive if they had a threshold cycle (C_T) of less than 31 cycles and a melting temperature within ±0.5°C of the positive control, *S. aureus* strain BAA-1708 (80.5°C ± 0.5°C).

Phenotypic MIC testing was performed on a subset of the isolates to validate the molecular assay. Seventy-three *mupA* negative isolates were phenotypically mupirocin susceptible (MIC ≤ 0.50 μg/ml) by Etest (bio-

Mérieux, St. Louis, MO), suggesting that mechanisms other than *mupA* were not contributing to phenotypic mupirocin resistance in our population. Ninety-two of the 94 *mupA*-positive isolates had a mupirocin MIC of ≥1,024 μg/ml. The remaining 2 *mupA*-positive isolates (recovered from the same participant) had a mupirocin MIC of ≤0.50 (i.e., were phenotypically mupirocin susceptible). For all analyses, these two isolates were considered mupirocin susceptible.

Detection of chlorhexidine resistance. Amplification of the *qacA/B* genes, the genetic determinants for chlorhexidine resistance, was performed. All strains possessing these genetic determinants were classified as chlorhexidine resistant. PCR of *qacA/B* was performed using a modification of a previously published assay (31). Briefly, approximately 100 ng of DNA was added to a Ready-To-Go PCR bead (GE Life Sciences), with 2.5 μl of a 2 μM solution of each primer (qacA/B-F and qacA/B-R) (Table 1) and molecular-grade water (added to a final volume of 25 μl) (31). Following an initial denaturation step at 94°C for 10 min, 30 cycles of PCR were performed under the following conditions: 94°C for 30 s, 52°C for 30 s, and 74°C for 30 s. A final elongation step was performed for 10 min at 72°C for an expected amplicon of 321 bp. A positive control (*S. aureus* strain NB01264) (21) and a negative control (*S. aureus* ATCC 29213) were included with each PCR run. A second PCR for *qacA/B* with an alternate genetic target was performed on all isolates that were positive in the first *qacA/B* PCR, as well as on all isolates that were both *mupA* positive and *qacA/B* negative in the first PCR. The PCR and cycling conditions were identical to the initial *qacA/B* PCR except for the primer sequences (qacA/B-RT-F and qacA/B-RT-R) (Table 1) and an expected amplicon size of 172 bp. All PCR products were resolved and visualized on a 2% agarose gel stained with ethidium bromide.

Molecular typing by rep-PCR. To determine the clonality of the mupirocin- and chlorhexidine-resistant *S. aureus* strains, molecular typing was performed by repetitive-sequence-based PCR (rep-PCR). DNA amplification was performed using random amplified polymorphic DNA Ready-To-Go RAPD analysis beads (GE Life Sciences, Piscataway, NJ) in a final volume of 25 μl, including ~100 ng of total genomic DNA and 75 pmol of the primer RW3A (35, 36). Rep-PCR products were resolved using Diversilab DNA chips for the Agilent 2100 (Agilent Technologies, Santa Clara, CA). Diversilab Bacterial Barcodes software was used to compare banding patterns and to determine similarity among isolates (bioMérieux Clinical Diagnostics, Marcy I'Etoile, France) (37). Isolates with a similarity index of ≥95% were considered to represent the same strain type.

Genetic analysis of *mupA* genotype-phenotype discordant strains. Both of the isolates that were *mupA* positive but phenotypically mupirocin susceptible were from the same patient; these were determined to be identical using rep-PCR. The *mupA* gene of one of these isolates and the reference isolate *S. aureus* BAA-1708 were bidirectionally sequenced (Sanger sequencing) using a series of overlapping primers (Table 1) (38). The patient isolate was aligned with the reference isolate using BioEdit software (Ibis Biosciences, Carlsbad, CA) for analysis.

Statistical methods. Statistical analyses were performed with SPSS for Windows 20 (IBM SPSS, Chicago, IL) and SAS version 9.2 (SAS Institute, Cary, NC). Chi-square analysis was performed to compare patient-level and isolate-level factors associated with mupirocin or chlorhexidine resistance. As age was not normally distributed, we calculated median age and compared groups with the Mann-Whitney U test. To evaluate the association between mupirocin or chlorhexidine resistance and strain resistance to other systemic antibiotics, only the baseline wound isolate was included in the analysis. Fisher's exact tests were used to determine whether the proportion of participants who remained colonized at the same sites (or additional sites) at the 1-month follow-up differed between participants colonized at baseline with a mupirocin-resistant strain versus a mupirocin-susceptible strain. Student's *t* test was used to analyze continuous variables. All tests of significance were two tailed. Odds ratios (OR) were considered significant if the 95% confidence interval (CI) did not include 1. A *P* value of ≤0.05 was considered significant.

RESULTS

Study population. We enrolled 1,089 patients with community-onset SSTI. Of these patients, 761 (69.9%) had a confirmed *S. aureus* SSTI plus *S. aureus* colonization, 191 (17.5%) had *S. aureus* SSTI without *S. aureus* colonization, and 137 (12.6%) were colonized with *S. aureus*, but *S. aureus* was not recovered from the infecting site. The median age of the study population was 7.3 years (range, 6 months to 70 years). Females comprised 58% of the study population. A large proportion of the participants was African-American (64%) and had government-issued health insurance or no health insurance (70%) (Table 2).

At baseline, 2,425 *S. aureus* isolates were recovered from the 1,089 participants; 901 (37.2%) isolates were recovered from SSTI and 1,524 (62.8%) were colonizing isolates (Table 2). The majority of isolates (74.5%) were identified as MRSA, while 25.5% were identified as methicillin-susceptible *S. aureus* (MSSA).

Baseline prevalence of and risk factors for mupirocin resistance. At baseline, 23 (2.1%) of the 1,089 participants were colonized and/or infected with a mupirocin-resistant *S. aureus* strain, yielding 50 mupirocin-resistant isolates among the 2,425 *S. aureus* isolates tested (2.1%). Epidemiologic risk factors associated with carriage of a mupirocin-resistant strain at baseline included younger age ($P = 0.05$), systemic antibiotic use in the prior year ($P = 0.006$), an emergency department or urgent care visit in the prior year ($P = 0.04$), hospitalization in the prior year ($P = 0.02$), and having a household contact with an SSTI in the prior year (0.006). Participants with a pet in the household were less likely to carry a mupirocin-resistant *S. aureus* strain ($P = 0.03$) (Table 2).

At baseline, there was not a statistically significant difference in mupirocin resistance between strains recovered from infecting sites and strains from colonizing sites or between colonizing strains recovered from the nose, axilla, or inguinal fold (Table 3). Overall, a higher proportion of isolates recovered at longitudinal samplings (31 of 696, 4.5%) were mupirocin resistant compared to those obtained at baseline (50 of 2,425, 2.1%; OR, 2.21; 95% CI, 1.40 to 3.49; $P = 0.001$).

To evaluate the association between high-level mupirocin resistance and resistance to commonly prescribed systemic antibiotics, we analyzed the baseline infecting isolates recovered from the site of SSTI ($n = 901$). Isolates resistant to clindamycin were more likely to be resistant to mupirocin (6 of 96, 6.2%) compared to clindamycin-susceptible isolates (10 of 805, 1.2%; $P = 0.004$) (Table 4).

Baseline prevalence of and risk factor for chlorhexidine resistance. At baseline, 10 (0.9%) of the 1,089 patients carried a chlorhexidine-resistant *S. aureus* strain, yielding 17 chlorhexidine-resistant isolates of 2,425 total *S. aureus* isolates (0.7%). Chlorhexidine resistance was not associated with the participant-level epidemiologic risk factors (Table 2). There was not a statistically significant difference in chlorhexidine resistance at baseline between strains recovered from infecting sites and strains from colonizing sites or between sites of colonization from which the strains were obtained (Table 3). Again, overall, a high proportion of isolates recovered at longitudinal samplings (11 of 696, 1.6%) were chlorhexidine resistant compared to those obtained at baseline (17 of 2,425, 0.7%; OR, 2.28; 95% CI, 1.06 to 4.88; $P = 0.043$). In evaluating the baseline infecting isolate, chlorhexidine resistance was not associated with resistance to other systemic antibiotics (Table 4).

TABLE 2 Carriage of a mupirocin- or chlorhexidine-resistant *S. aureus* strain at baseline by participant factors

Participant factor	No. of participants (n = 1,089) (%)	No. of participants with mupirocin-resistant strain (n = 23) (%)	No. of participants with mupirocin-susceptible strain (n = 1,066) (%)	OR (95% CI)	P value	No. of participants with chlorhexidine-resistant strain (n = 10) (%)	No. of participants with chlorhexidine-susceptible strain (n = 1,079) (%)	OR (95% CI)	P value
Female	633/1,089 (58)	10/23 (43)	623/1,066 (58)	0.55 (0.24–1.26)	0.20	7/10 (70)	626/1,079 (58)	1.69 (0.43–6.58)	0.53
African-American[a]	739/1,083 (68)	18/23 (78)	721/1,060 (68)	1.69 (0.62–4.60)	0.37	4/10 (40)	735/1,073 (69)	0.31 (0.09–1.09)	0.08
Median age (yr) (range)	7.3 (0.5–70)	2.5 (0.6–45.8)	7.6 (0.5–70)	NA[d]	0.05	2.5 (1.6–48.8)	7.4 (0.5–70)	NA	0.56
Health insurance: government issued or self-pay	557/791 (70)	15/17 (88)	542/774 (70)	3.21 (0.73–14.15)	0.18	2/6 (33)	555/785 (71)	0.21 (0.04–1.14)	0.07
Site of skin infection									
Head and neck	91 (8)	3/23 (13)	88/1,064 (8)	1.66 (0.49–5.71)	0.43	0/10 (0)	91/1,077 (8)	NA	1.00
Trunk	211 (19)	8/23 (35)	203/1,064 (19)	2.26 (0.95–5.41)	0.10	2/10 (20)	209/1,077 (19)	1.04 (0.22–4.93)	1.00
Groin or buttock	442 (41)	6/23 (26)	436/1,064 (41)	0.51 (0.20–1.30)	0.20	5/10 (50)	437/1,077 (41)	1.47 (0.42–5.09)	0.54
Upper extremity	111 (10)	3/23 (13)	108/1,064 (10)	1.33 (0.39–4.54)	0.72	1/10 (10)	110/1,077 (10)	0.98 (0.12–7.78)	1.00
Lower extremity	301 (28)	6/23 (26)	295/1,064 (28)	0.92 (0.36–2.36)	1.00	2/10 (20)	299/1,077 (28)	0.65 (0.14–3.08)	0.74
Comorbid health condition[b]	260/483 (54)	6/9 (67)	254/474 (54)	1.73 (0.43–7.01)	0.52	4/7 (57)	256/476 (54)	1.15 (0.25–5.18)	1.00
Skin disorder[c]	280/818 (34)	7/15 (47)	273/803 (34)	1.70 (0.61–4.73)	0.41	3/8 (38)	277/810 (34)	1.16 (0.27–4.87)	1.00
Systemic antibiotic use in prior year	466/806 (58)	14/15 (93)	452/791 (57)	10.50 (1.37–80.24)	0.006	6/8 (75)	460/798 (58)	2.20 (0.44–10.99)	0.48
Emergency Department or urgent care visit in prior year	198/483 (41)	7/9 (78)	191/474 (40)	5.19 (1.07–25.23)	0.04	4/7 (57)	194/476 (41)	1.94 (0.43–8.76)	0.45
Hospitalization in prior year	133/815 (16)	6/15 (40)	127/800 (16)	3.53 (1.24–10.10)	0.02	0/8 (0)	133/807 (17)	NA	0.37
Surgery in prior year	92/483 (19)	1/9 (11)	91/474 (19)	0.53 (0.07–4.26)	1.00	3/7 (43)	89/476 (19)	3.26 (0.72–14.83)	0.13
SSTI in prior year	355/813 (44)	9/15 (60)	346/798 (43)	1.96 (0.69–5.56)	0.29	5/8 (63)	350/805 (44)	2.17 (0.51–9.13)	0.31
Household contact SSTI in prior year	348/813 (43)	12/15 (80)	336/798 (42)	5.50 (1.54–19.64)	0.006	3/8 (38)	345/805 (43)	0.80 (0.19–3.37)	1.00
Health care worker in household	216/817 (26)	6/15 (40)	210/802 (26)	1.88 (0.66–5.34)	0.24	3/8 (38)	213/809 (26)	1.68 (0.40–7.09)	0.44
Pet in household	351/818 (43)	2/15 (13)	349/803 (44)	0.20 (0.05–0.89)	0.03	4/8 (50)	347/810 (43)	1.33 (0.33–5.37)	0.73

[a] Number of African-American and biracial participants (739) compared to the number of participants in all other racial groups: Caucasian (338), Asian (4), American Indian (2).

[b] Comorbid health conditions surveyed included asthma, allergies, seizures, heart disease, high blood pressure, diabetes, cancer, sickle cell disease, cystic fibrosis, dialysis, HIV/AIDS, gastroesophageal reflux disease (GERD), depression/bipolar disorder, and attention deficit disorder (ADD).

[c] Skin disorders surveyed included eczema, acne, psoriasis, and folliculitis.

[d] NA, not applicable.

TABLE 3 Mupirocin and chlorhexidine resistance at baseline by isolate factors ($n = 2,425$ isolates)[b]

Isolate factor	Mupirocin-resistant isolate ($n = 50$) (%)	Mupirocin-susceptible isolate ($n = 2,375$) (%)	OR (95% CI)	*P* value	Chlorhexidine-resistant isolate ($n = 17$) (%)	Chlorhexidine-susceptible isolate ($n = 2,408$) (%)	OR (95% CI)	*P* value
Infecting isolate ($n = 901$)	16 (1.8)	885 (98.2)	0.79 (0.44–1.44)	0.55	4 (0.4)	897 (99.6)	0.52 (0.17–1.59)	0.32
Colonizing isolate ($n = 1,524$)	34 (2.2)	1,490 (97.8)			13 (0.9)	1511 (99.1)		
Site of colonization[a]								
Anterior nares ($n = 601$)	10 (1.7)	591 (98.3)	Reference	0.16	4 (0.7)	597 (99.3)	Reference	0.59
Axilla ($n = 302$)	11 (3.6)	291 (96.4)	2.23 (0.94–5.32)		4 (1.3)	298 (98.7)	2.00 (0.50–8.06)	
Inguinal fold ($n = 621$)	13 (2.1)	608 (97.9)	1.26 (0.55–2.90)		5 (0.8)	616 (99.2)	1.21 (0.32–4.53)	

[a] OR calculated separately for the axilla and inguinal fold sites of colonization in comparison to the anterior nares site of colonization.
[b] The percentages listed represent the percentages for the row (not the column).

Nine isolates (from 3 participants) were resistant to both mupirocin and chlorhexidine. No predictive factors for this cross-resistance could be identified; these isolates were recovered from a variety of body sites (nasal, axilla, groin, and wound) and exhibited varying susceptibly profiles to systemic antimicrobial agents. Eight of these isolates were identified as MRSA, and one isolate was identified as MSSA.

Longitudinal sampling. Of the 1,089 participants sampled at baseline, 483 were enrolled into one of two decolonization trials. Of these, 408 were assigned mupirocin nasal applications and 258 were assigned chlorhexidine body washes. Of the 483 participants enrolled in a decolonization trial, 404 (83.6%) provided at least 1 follow-up culture.

Seven of 404 (1.7%) patients with culture data carried mupirocin-resistant *S. aureus* at a longitudinal sampling. Of 75 patients not prescribed mupirocin, 3 (4%) carried mupirocin-resistant *S. aureus* at baseline; 2 of these patients had follow-up data and 1 patient carried mupirocin-resistant *S. aureus* during the longitudinal study period (Fig. 1A and Table 5). Of 408 patients receiving mupirocin, 6 (1.5%) carried mupirocin-resistant *S. aureus* at baseline; of these, 4 of 4 patients with follow-up data carried mupirocin-resistant *S. aureus* during follow-up. Of patients not carrying mupirocin-resistant *S. aureus* at baseline, carriage of mupirocin-resistant *S. aureus* during follow-up occurred in 0 of 64 patients not receiving mupirocin compared to 2 of 334 (0.6%) patients receiving mupirocin ($P = 1.0$) (Fig. 1A and Table 5).

During the longitudinal study period, 4 of 404 (1.0%) patients

TABLE 4 Baseline wound isolate mupirocin and chlorhexidine resistance by systemic antibiotic susceptibility ($n = 901$ isolates)[b]

Antibiotic resistance[a]	Mupirocin-resistant isolate ($n = 16$) (%)[b]	Mupirocin-susceptible isolate ($n = 885$) (%)	OR (95% CI)	*P* value	Chlorhexidine-resistant isolate ($n = 4$) (%)	Chlorhexidine-susceptible isolate ($n = 897$) (%)	OR (95% CI)	*P* value
Methicillin								
R ($n = 755$)	14 (1.9)	741 (98.1)	1.36 (0.31–6.05)	1.00	4 (0.5)	751 (99.5)	NA	1.00
S ($n = 146$)	2 (1.4)	144 (98.6)			0 (0)	146 (100)		
Clindamycin								
R ($n = 96$)	6 (6.2)	90 (93.8)	5.30 (1.88–14.93)	0.004	1 (1.0)	95 (99.0)	2.81 (0.29–27.32)	0.36
S ($n = 805$)	10 (1.2)	795 (98.8)			3 (0.4)	802 (99.6)		
Erythromycin								
R ($n = 804$)	13 (1.6)	791 (98.4)	0.52 (0.14–1.84)	0.40	4 (0.5)	800 (99.5)	NA	1.00
S ($n = 97$)	3 (3.1)	94 (96.9)			0 (0)	97 (100)		
TMP-SMX								
R ($n = 2$)	0 (0)	2 (100)	NA	1.00	0 (0)	2 (100)	NA	1.00
S ($n = 897$)	16 (1.8)	881 (98.2)			4 (0.4)	893 (99.6)		
Ciprofloxacin								
R ($n = 86$)	0 (0)	86 (100)	NA	0.24	0 (0)	86 (100)	NA	NA
S ($n = 84$)	2 (2.4)	82 (97.6)			0 (0)	84 (100)		
Rifampin								
R ($n = 3$)	0 (0)	3 (100)	NA	1.00	0 (0)	3 (100)	NA	1.00
S ($n = 891$)	16 (1.8)	875 (98.2)			4 (0.4)	887 (99.6)		
Tetracycline								
R ($n = 21$)	1 (4.8)	20 (95.2)	2.61 (0.33–20.80)	0.34	0 (0)	21 (100)	NA	1.00
S ($n = 744$)	14 (1.9)	730 (98.1)			4 (0.5)	740 (99.5)		

[a] R, resistant; S, susceptible.
[b] The percentages listed represent the percentages for the row (not the column). NA, not applicable.

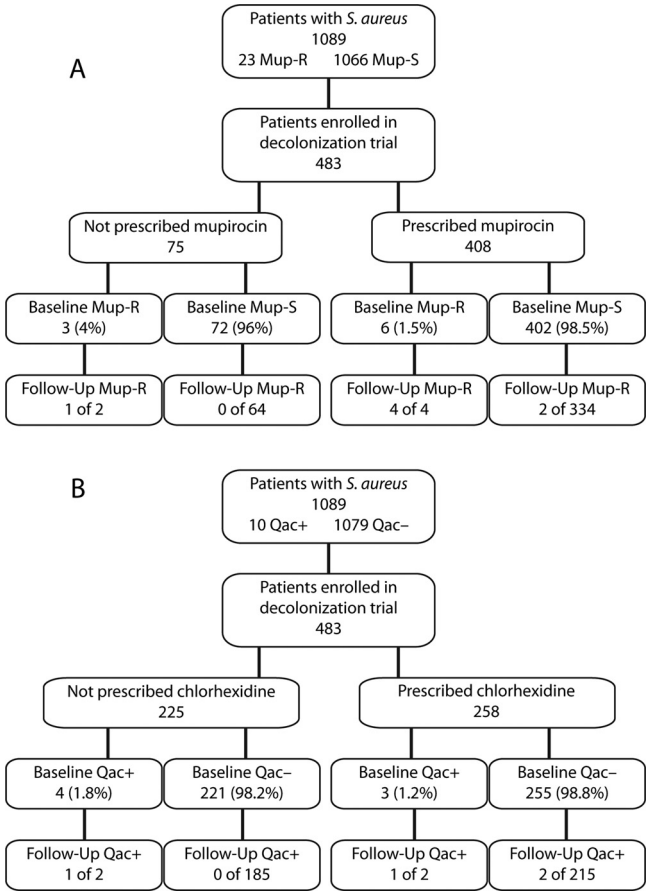

FIG 1 (A) Baseline and longitudinal carriage of mupirocin-resistant *S. aureus* strains stratified by randomization to perform mupirocin decolonization. Mup-R, mupirocin-resistant strain; Mup-S, mupirocin-susceptible strain. (B) Baseline and longitudinal carriage of chlorhexidine-resistant *S. aureus* strains stratified by randomization to perform chlorhexidine decolonization. Qac^+, strain carrying the *qacA/B* genes and therefore classified as chlorhexidine resistant; Qac^-, strain not carrying the *qacA/B* genes and classified as chlorhexidine susceptible.

with culture data carried chlorhexidine-resistant *S. aureus*. Of 225 patients not prescribed chlorhexidine, 4 (1.8%) carried chlorhexidine-resistant *S. aureus* at baseline; 2 of these patients had follow-up data, and 1 patient carried chlorhexidine-resistant *S. aureus* during the longitudinal study period (Fig. 1B and Table 6). Of 258 patients receiving chlorhexidine, 3 (1.2%) carried chlorhexidine-resistant *S. aureus* at baseline; of these, 1 of 2 patients with follow-up data carried chlorhexidine-resistant *S. aureus*. Of patients not carrying chlorhexidine-resistant *S. aureus* at baseline, carriage of chlorhexidine-resistant *S. aureus* during follow-up occurred in 0 of 185 patients not receiving chlorhexidine compared to 2 of 215 (0.9%) patients receiving chlorhexidine ($P = 0.5$) (Fig. 1B and Table 6).

Mupirocin and chlorhexidine resistance and prediction of *S. aureus* eradication. At the 1-month sampling, of 4 patients prescribed mupirocin, who carried a mupirocin-resistant *S. aureus* strain at baseline and provided follow-up cultures, 100% remained colonized compared to 44% of the 324 patients without mupirocin resistance at baseline ($P = 0.04$) (Fig. 2A). In evaluating sites of colonization, 5 of 6 (83%) participants colonized at baseline at one or more sites with a mupirocin-resistant strain remained colonized at the same or additional sites at the 1-month follow-up sampling, while only 83 of 384 (22%) participants colonized at baseline with only mupirocin-susceptible strains remained colonized at the same site(s) at 1 month ($P = 0.003$). Of the 2 patients prescribed chlorhexidine, who carried a chlorhexidine-resistant *S. aureus* strain at baseline and provided follow-up cultures, 50% remained colonized at 1 month compared to 48% of the 209 patients without chlorhexidine resistance at baseline ($P = 1.0$) (Fig. 2B).

Diversity of resistant strains. To determine whether all of the mupirocin-resistant or chlorhexidine-resistant strains represented a single *S. aureus* clone, rep-PCR was performed on all mupirocin- and chlorhexidine-resistant isolates. These isolates were not clonal; among 113 isolates, 16 distinct strain types were detected.

Sequence of strains possessing *mupA* demonstrating phenotypic susceptibility. Two *S. aureus* isolates recovered from the same participant were genotypically mupirocin resistant (i.e., possessed the *mupA* gene) but were phenotypically mupirocin susceptible as determined by Etest, with a mupirocin MIC of ≤0.50 μg/ml. The *mupA* gene was sequenced from one of the strains recovered from the participant concomitantly with a mupirocin-resistant reference strain possessing the *mupA* gene (*S. aureus* ATCC BAA-1708). When the sequences from the two isolates were aligned, no variants were detected within the *mupA*-coding region of the phenotype-genotype discordant isolate.

DISCUSSION

Community-onset *S. aureus* SSTI are a significant public health burden. The incidence of recurrent SSTI has been reported to be as high as 50% over 1 year (8). Thus, many health care practitioners prescribe decolonization measures, especially mupirocin and

TABLE 5 Longitudinal carriage of mupirocin-resistant isolates[a]

Participant	Randomized to use mupirocin	Baseline	1-month follow-up	3- to 4-month follow-up	6-month follow-up	12-month follow-up
A	Yes	Mup-S	NC	NC	NC	Mup-R
B	Yes	Mup-S	Mup-R	NC	–	–
C	Yes	Mup-R	Mup-R	Mup-R	Mup-R	Mup-R
D	Yes	Mup-R	Mup-R	Mup-R	–	–
E	Yes	Mup-R	Mup-R	NC	–	–
F	Yes	Mup-R	Mup-R	Mup-R	–	–
G	No	Mup-R	Mup-R	Mup-R	–	–
H	No	Mup-R	Mup-S	NC	–	–

[a] Mup-S, mupirocin susceptible; Mup-R, mupirocin resistant; NC, not colonized at this time point; -, colonization data not collected.

TABLE 6 Longitudinal carriage of chlorhexidine-resistant isolates[a]

Participant	Randomized to use chlorhexidine	Baseline	1-month follow-up	3- to 4-month follow-up	6-month follow-up	12-month follow-up
A	Yes	Qac-neg	Qac-neg	Qac-pos	Qac-neg	Qac-neg
B	Yes	Qac-neg	Qac-pos	NC	Qac-neg	NC
C	Yes	Qac-pos	Qac-pos	Qac-pos	Qac-pos	Qac-pos
D	Yes	Qac-pos	NC	NC	NC	NC
E	Yes	Qac-pos	Qac-neg	Qac-neg	-	-
F	Yes	Qac-pos	Qac-pos	Qac-pos	-	-

[a] Qac-neg, chlorhexidine susceptible; Qac-pos, chlorhexidine resistant; NC, not colonized at this time point; -, colonization data not collected.

chlorhexidine, in an effort to prevent recurrent staphylococcal disease (5). Of concern is the potential for increasing prevalence of *S. aureus* strains resistant to these antimicrobial agents with widespread use, which has been reported in countries outside the United States (19, 21). Thus, it is important to be aware of the prevalence of *S. aureus* strains encoding resistance to these measures, risk factors associated with resistance, and the clinical significance of resistant *S. aureus* strains.

In our study population of healthy adults and children presenting to Emergency Departments and ambulatory centers, the prevalence of infection and/or colonization with *S. aureus* strains exhibiting high-level mupirocin resistance at baseline was very low (2.1%) and, over the course of the whole study, remained low (2.3%). Interestingly, in a prior prevalence survey of mupirocin resistance in MRSA colonization strains recovered from patients in an adult surgical intensive care unit at our medical center (Barnes-Jewish Hospital), 8.6% (26 of 302) of isolates exhibited high-level resistance to mupirocin despite relatively infrequent

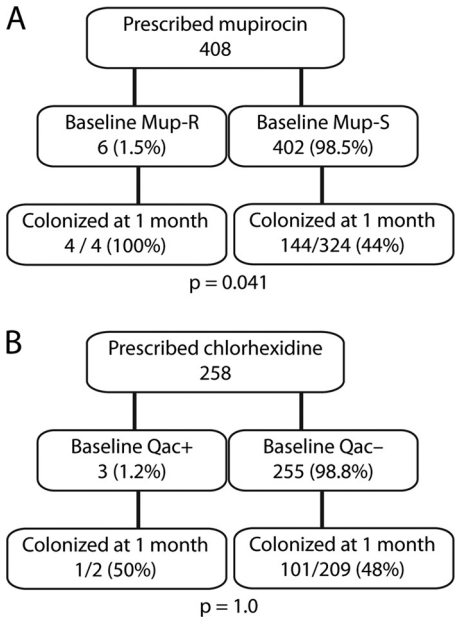

FIG 2 (A) *S. aureus* colonization at the 1-month longitudinal sampling of patients prescribed mupirocin stratified by a mupirocin-resistant or -susceptible isolate at baseline. Mup-R, mupirocin-resistant strain; Mup-S, mupirocin-susceptible strain. (B) *S. aureus* colonization at the 1-month longitudinal sampling of patients prescribed chlorhexidine stratified by a chlorhexidine-resistant or -susceptible isolate at baseline. Qac^+, strain carrying the *qacA/B* genes and therefore classified as chlorhexidine resistant; Qac^-, strain not carrying the *qacA/B* genes and classified as chlorhexidine susceptible.

in-hospital mupirocin use (6.08 treatment days per 1,000 patient days) (12). A nationwide study of hospitalized patients in the United States found that less than 5% of MRSA isolates recovered from the nares and blood exhibited high-level mupirocin resistance (39). Two pediatric studies have previously been conducted evaluating mupirocin resistance in *S. aureus* isolates. A study by Hogue et al., conducted on a military base, evaluated first-time MRSA isolates recovered from children in both inpatient and outpatient settings. Similar to our study, the investigators detected a low prevalence (1.8% [3 of 167]) of high-level mupirocin-resistant *S. aureus* isolates from both infecting and colonizing sites (34). A study of pediatric patients with recurrent *S. aureus* SSTI conducted in Houston, TX, by McNeil et al. determined a relatively high prevalence of mupirocin resistance. Of 68 patients, 12 (18%) were infected with an isolate possessing *mupA*. Resistance to mupirocin occurred more commonly among *S. aureus* isolates causing recurrent SSTI (19%) than primary infecting isolates (10%) (15). Prior exposure to mupirocin was unknown in this study.

In our outpatient study population, the prevalence of *S. aureus* strains possessing the genetic determinants of chlorhexidine resistance was extremely low at 0.9% (1.1% over the entire study period). This phenomenon has not been studied extensively in the United States, and this is the first investigation measuring chlorhexidine resistance in an outpatient population in the United States. In a similar population, a study conducted in the United Kingdom detected no *qacA/B* genes in CA-MRSA isolates (40). In a study of Canadian intensive care units, only 2% of the MRSA strains possessed the *qacA/B* genes (29). However, in health care settings in some countries where chlorhexidine use is standard practice, including Brazil, Taiwan, and European countries, the prevalence of the *qacA/B* genes in *S. aureus* isolates ranges from 10 to 80% (13, 21, 31, 41–43). As chlorhexidine is a topical antiseptic agent, applied directly to the site of *S. aureus* colonization and therefore achieving high local concentrations, the clinical significance of *in vitro* resistance is unclear (3, 21). We do not know the MIC or minimal bactericidal concentration (MBC) of the *qacA/B*-positive strains in this study. However, Smith et al. (40) correlated higher chlorhexidine MBCs for *qacA/B*-positive strains compared to negative controls. In addition, several reports raise concern for the presence of *S. aureus* strains possessing the *qacA/B* genes in the clinical setting. For example, in one hospital in the United Kingdom, although implementation of a chlorhexidine-based antiseptic protocol in intensive care units led to a reduction in acquisition of strains not carrying the *qacA/B* genes, this protocol led to increased transmission of an outbreak MRSA strain exhibiting chlorhexidine resistance (21). Therefore, increased prevalence of *qacA/B*-containing isolates is a potential public

health concern. Lee et al. conducted a nested case-control study in Switzerland to investigate the significance of low-level mupirocin and chlorhexidine resistance. This hospital routinely decolonizes patients with MRSA carriage with intranasal mupirocin and chlorhexidine bathing. Culturing of the nares, groin, and other clinically relevant sites revealed a strong association between carriage of MRSA strains encoding low-level mupirocin plus genotypic chlorhexidine resistance and persistent colonization following decolonization measures (13).

In the present study, chlorhexidine resistance at baseline was not associated with persistent *S. aureus* carriage, possibly due to the low prevalence of these strains in our population. Although the prevalence of mupirocin resistance was low in our population, carriage of a high-level mupirocin-resistant *S. aureus* strain at baseline did predict decolonization failure. This has similarly been reported in hospital-based studies. In an investigation conducted in Veterans Affairs facilities, patients colonized with high-level mupirocin-resistant MRSA strains were significantly more likely to remain colonized over 4 weeks compared to patients colonized with mupirocin-susceptible MRSA strains (28). Thus, although testing for mupirocin resistance is not routine clinical practice in the United States, the possibility of *S. aureus* infection and/or colonization with a mupirocin-resistant strain should be considered in individuals in whom decolonization efforts are unsuccessful or in those who develop recurrent *S. aureus* SSTI following the performance of decolonization with mupirocin.

Another concern surrounding routine topical decolonization is the development of resistance in recolonizing strains (44). Simor et al. conducted a trial that employed a 7-day decolonization regimen with intranasal mupirocin, chlorhexidine body washes, and systemic rifampin and doxycycline for hospitalized patients in Canada with MRSA colonization. The study determined that 5% of patients carrying a mupirocin-susceptible MRSA strain at baseline were colonized with a mupirocin-resistant strain at follow-up (45). In the present study of patients in ambulatory settings, a short-course of decolonization with mupirocin was not associated with the emergence of resistant *S. aureus* strains. Similar findings were reported from a military study comparing the application of intranasal mupirocin to placebo for 5 days. Of 199 CA-MRSA isolates tested, no mupirocin resistance was detected, suggesting that resistance is not selected when a limited approach is employed (6).

Determinants of resistance to systemic antibiotics have been associated with mupirocin and chlorhexidine resistance in prior studies, leading to the postulate that factors driving resistance to agents used for decolonization (e.g., increased use) may increase staphylococcal resistance to other antibiotics. In Europe, the prevalence of both mupirocin and chlorhexidine resistance has been reported to be significantly higher in MRSA strains than MSSA strains in hospitalized patients (31, 46). In the United States, a survey of multidrug-resistant MRSA isolates, defined as resistance to ≥3 classes of non-beta-lactam antibiotics, found that 6.8% of 191 such isolates carried the *mupA* gene compared with none of the 130 non-multidrug-resistant isolates (10). In the study of pediatric patients by McNeil et al., one-third of the *S. aureus* isolates possessing the *mupA* gene were also clindamycin resistant (15). This association was also observed in our study population, in which clindamycin-resistant *S. aureus* strains were more likely to also be resistant to mupirocin.

High-level mupirocin resistance is encoded by the *mupA* gene. In our population, two *S. aureus* isolates, recovered from the same patient, possessed the *mupA* gene but were phenotypically mupirocin susceptible. This discordance in genotypic and phenotypic resistance has been reported by others as well (38). In a survey of mupirocin susceptibility by Driscoll et al., one strain testing positive for the *mupA* gene by PCR was demonstrated to be mupirocin susceptible by broth microdilution. Sequencing of *mupA* revealed a single-base-pair deletion, resulting in a frameshift mutation and, ultimately, a truncated protein which did not confer mupirocin resistance (38). In the present study, *mupA* in our discordant strain was also sequenced, but when aligned with the control strain, no mutations were detected within the *mupA* coding sequence. This suggests the possibility that an alternative mutation exists within the promoter region or other regulatory element, resulting in disparate results between genotypic and phenotypic testing. Evaluation of other genetic factors contributing to this scenario could be the focus of future investigations. This finding does raise a point of caution that studies relying solely on genotypic detection of high-level mupirocin resistance may overestimate prevalence.

This study has several limitations. Although this study tested an enormous number of isolates, the number of *S. aureus* isolates that were resistant to mupirocin or chlorhexidine was small, and thus we may not have been able to detect associations between resistance to these agents and other patient- and isolate-level risk factors. In addition, use of mupirocin and/or chlorhexidine by study participants prior to the baseline sampling is unknown. Although the low prevalence of mupirocin- and chlorhexidine-resistant *S. aureus* strains is clinically relevant to practitioners, these findings may not be applicable to geographic areas outside metropolitan St. Louis.

The genes conferring high-level mupirocin resistance and resistance to chlorhexidine are plasmid borne, permitting facile transmission among *S. aureus* strains. Although the prevalence of both mupirocin and chlorhexidine resistance in *S. aureus* isolates was low in our outpatient population, carriage of a mupirocin-resistant strain at baseline precluded eradication efforts, consistent with the findings of other investigators (13, 28). This may be germane for patients experiencing recurrent infections despite performing decolonization measures.

As the contemporary MRSA epidemic persists in the community, and as this clone has recently entered the health care environment (47, 48), the prevalence of strains resistant to these frequently prescribed decolonization measures will likely continue to increase. Thus, ongoing monitoring is needed and practitioners should consider this when prescribing these interventions.

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