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Chlorhexidine and Mupirocin Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Isolates in the REDUCE-MRSA Trial

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Whether targeted or universal decolonization strategies for the control of methicillin-resistant *Staphylococcus aureus* (MRSA) select for resistance to decolonizing agents is unresolved. The REDUCE-MRSA trial (ClinicalTrials registration no. NCT00980980) provided an opportunity to investigate this question. REDUCE-MRSA was a 3-arm, cluster-randomized trial of either screening and isolation without decolonization, targeted decolonization with chlorhexidine and mupirocin, or universal decolonization without screening to prevent MRSA infection in intensive-care unit (ICU) patients. Isolates from the baseline and intervention periods were collected and tested for susceptibility to chlorhexidine gluconate (CHG) by microtiter dilution; mupirocin susceptibility was tested by Etest. The presence of the *qacA* or *qacB* gene was determined by PCR and DNA sequence analysis. A total of 3,173 isolates were analyzed; 2 were nonsusceptible to CHG (MICs, 8 μg/ml), and 5/814 (0.6%) carried *qacA* or *qacB*. At baseline, 7.1% of MRSA isolates expressed low-level mupirocin resistance, and 7.5% expressed high-level mupirocin resistance. In a mixed-effects generalized logistic regression model, the odds of mupirocin resistance among clinical MRSA isolates or MRSA isolates acquired in an ICU in intervention versus baseline periods did not differ across arms, although estimates were imprecise due to small numbers. Reduced susceptibility to chlorhexidine and carriage of *qacA* or *qacB* were rare among MRSA isolates in the REDUCE-MRSA trial. The odds of mupirocin resistance were no different in the intervention versus baseline periods across arms, but the confidence limits were broad, and the results should be interpreted with caution.

Health care-associated infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with high attributable mortality, increased length of stay, and excess cost (1). Colonization with MRSA typically precedes infection and plays a major role in its dissemination in hospitals (2). Both targeted decolonization (i.e., decolonization of patients who are identified as carrying MRSA) and universal decolonization (i.e., decolonization of populations of hospital patients regardless of MRSA colonization status) have been demonstrated to decrease cross-transmission and infection (3, 4).

The anterior nares are the primary reservoir for MRSA in humans, and the application of topical nasal mupirocin is a common decolonization strategy (5). Mupirocin interferes with bacterial protein synthesis by competitive inhibition of bacterial isoleucyl-tRNA-synthetase (6). High-level mupirocin resistance (HLMR) is conferred by the *mupA* or *mupB* gene, both of which encode novel isoleucyl-tRNA-synthetases (6). These genes are carried on plasmids, enabling their spread. Low-level mupirocin resistance (LLMR) results from mutations in the native chromosomal isoleucyl-tRNA-synthetase gene; these mutations are typically stable and nontransferable (7). HLMR has been associated with decolonization failure (8), while LLMR may predispose to early recolonization (9). Prolonged and widespread use of mupirocin for decolonization has been associated frequently, but not universally, with the development of mupirocin resistance (10, 12).

Antiseptic bathing of patients, most commonly with chlorhexidine gluconate (CHG), is another evidence-based approach to MRSA decolonization; antiseptic baths are often employed together with nasal mupirocin (4, 13, 14). CHG kills by binding covalently to the bacterial cell membrane, resulting in depolarization and cell death. CHG susceptibility testing methods and breakpoints have not been standardized. Broth microdilution, which was developed to predict the activity of systemic antibiotics,

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is the susceptibility testing method reported most frequently, despite questions about its relevance for predicting the activity of a topical biocide such as CHG (15). Reduced susceptibility to CHG in MRSA occurs via efflux, and identification of plasmid-mediated genes, such as *qacA* and *qacB*, that encode multidrug efflux pumps has been considered genotypic evidence of CHG nonsusceptibility by some (16, 17). However, the relationship between the carriage of multidrug efflux pump genes and decreased susceptibility to CHG is inconsistent; phenotypic susceptibility has been demonstrated in MRSA strains that carry *qacA* or *qacB*, and reduced susceptibility has been reported in strains that lack these or other multidrug efflux pump genes (15, 18–20). The inconsistency may be due in part to the ability of the pump encoded by *qacA*, but not that encoded by *qacB*, to utilize CHG as a substrate. Despite this important functional difference, *qacA* and *qacB* are closely related genetically and are difficult to differentiate without DNA sequence analysis; most publications do not distinguish between the two genes.

The relationship between reduced CHG susceptibility and clinical resistance in MRSA is even more tenuous. CHG has been used widely in health care for more than 50 years, and reduced susceptibility as measured by *in vitro* methods has been reported across the globe (18, 20–24, 46), yet decolonization failure related to nonsusceptibility has been described only rarely (16, 25, 26). Of note, topical concentrations of CHG used for decolonization remain >200-fold higher than the highest CHG MICs and minimum bactericidal concentrations (MBCs) recorded for staphylococci (15, 25).

The Randomized Evaluation of Decolonization versus Universal Clearance to Eradicate MRSA (REDUCE-MRSA) trial (ClinicalTrials registration no. NCT00980980) was a cluster-randomized, multicenter study designed to compare three MRSA control strategies: (i) screening and isolation, (ii) screening, isolation, and targeted decolonization with mupirocin and CHG, and (iii) universal decolonization with CHG and mupirocin without screening (27). Universal decolonization with CHG and mupirocin was found to be superior to both alternative strategies in reducing MRSA infections. In order to evaluate the effect of decolonization on the susceptibility of MRSA to CHG and mupirocin, we conducted a secondary analysis of isolates collected during the baseline and intervention periods from all three study arms and subjected them to phenotypic and genotypic susceptibility testing.

(This research was presented in part at the 20th Annual Meeting of the Society for Healthcare Epidemiology of America 2011, Dallas, TX, 1 to 4 April 2011, and at IDWeek 2014, Philadelphia, PA, 8 to 12 October 2014 [28].)

MATERIALS AND METHODS

Selection of MRSA isolates. Isolates were collected over a 7-month baseline period (1 August 2009 to 28 February 2010) and an 18-month intervention period (8 April 2010 to 30 September 2011) from patients in intensive-care units (ICUs) that were participating in REDUCE-MRSA (27). In this pragmatic clinical trial, 43 Hospital Corporation of America (HCA)-affiliated hospitals in 16 states were assigned at random to one of three MRSA prevention strategies, with all adult ICUs in a given hospital assigned to the same strategy. Arm 1 employed admission screening for MRSA and isolation of patients who were found to be colonized or infected; arm 2, targeted decolonization (i.e., admission screening, isolation, and decolonization of patients who were colonized or infected with MRSA with a 5-day regimen of twice-daily intranasal mupirocin ointment and daily CHG baths); and arm 3, universal decolonization (i.e., no screening; decolonization of all patients with 5 days of twice-daily intranasal mupirocin ointment and daily CHG baths for the entire ICU stay).

During the trial, microbiology laboratories at each participating hospital were instructed to collect the first clinical or anterior-nares screening isolate of MRSA per ICU patient. If both a clinical isolate and a surveillance isolate were available, the clinical isolate was preferred. MRSA isolates were shipped to a central laboratory at Rush University Medical Center (RUMC) for confirmation of MRSA status and further testing. Isolates were excluded if they were not MRSA or if they could not be verified to have been collected from a patient during or after an ICU stay during a study period. Additionally, in arm 3, because screening for MRSA was to have been discontinued during the intervention period, isolates collected from screening cultures were excluded. Clinical isolates were considered to have been acquired during an ICU stay (to be "ICU attributable") if they were collected between 3 days after ICU admission and 2 days after ICU discharge.

All isolates were tested for susceptibility to CHG and mupirocin. A sample of 15 isolates per hospital per study period, as well as any isolate with a CHG MIC of >4 μg/ml, was tested further for the presence of *qacA* and *qacB*. Baseline period isolates were selected at random. During the intervention period, the isolates with the latest culture dates were preferred, in order to enrich for isolates with the greatest potential exposure to the intervention. If fewer than 15 isolates were available, all isolates were tested.

Clinical and demographic data were extracted from HCA electronic corporate data warehouses. This study was reviewed and approved by the Harvard Pilgrim Health Care Institutional Review Board (IRB), the central institutional review board for the trial (reference number 367981), and by the IRB of RUMC. Written informed consent was waived (27).

Laboratory procedures. (i) Confirmation of MRSA. Local clinical laboratories identified MRSA isolates according to their standard practices. At the RUMC laboratory, *S. aureus* identification was confirmed by examination of colony morphology and the results of a rapid latex agglutination test (BactiStaph kit; Remel, Lenexa, KS). Isolates with atypical results underwent Gram staining, tube coagulase testing, and automated identification by the MicroScan WalkAway system (Siemens, Washington, DC). Methicillin resistance was confirmed by Kirby-Bauer disk diffusion testing on Mueller-Hinton agar (Becton, Dickinson [BBL], Sparks, MD) using a 30 μg cefoxitin disk (Oxoid, Lenexa, KS) (29). Isolates that tested susceptible to methicillin underwent *mecA* PCR (30); *mecA*-positive isolates were classified as MRSA.

(ii) CHG and mupirocin susceptibility testing. CHG susceptibility testing was performed using broth microdilution and a complete inhibition endpoint (47). Starting with a 20% (wt/vol) CHG solution (Sigma-Aldrich, St. Louis, MO), a 2-fold dilution series (from 32 to 0.0625 μg/ml) was prepared daily. An isolate was classified as nonsusceptible to CHG if the MIC was >4 μg/ml, which is outside the wild-type distribution of CHG MICs for *S. aureus* (epidemiologic cutoff) (31, 48). Susceptibility to mupirocin was determined by the Etest method (bioMérieux, Durham, NC) according to the manufacturer's instructions. LLMR was defined as a MIC of 8 to 256 μg/ml and HLMR as a MIC of ≥512 μg/ml (6). When MICs were compared between groups, a 4-fold difference was considered significant (32). MBCs of CHG were determined in triplicate for all *qacA*- or *qacB*-positive isolates and for isolates with CHG MICs of >4 μg/ml (33).

(iii) PCR for *qacA* and *qacB*. A real-time PCR assay was developed and was used to identify *qacA* and *qacB* (see the Supplemental Data and Table S1 in the supplemental material) (34). Isolates were tested after overnight growth on tryptic soy agar with 25 μg/ml ethidium bromide (Sigma-Aldrich), which was added to provide selective pressure for strains that harbored efflux pumps such as those encoded by *qacA* and *qacB* (35). *S. aureus* SK2355 (*qacA*), *S. aureus* SK2725 (*qacB*) (gifts from Arnold Bayer

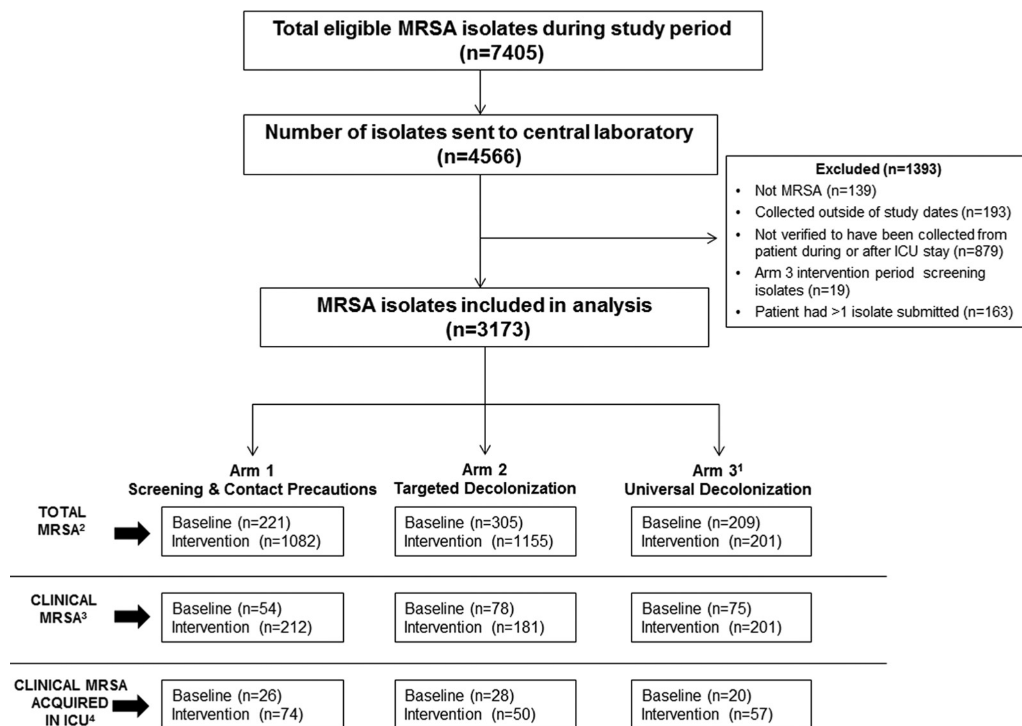

FIG 1 Eligible, collected, and analyzed methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. [1], Surveillance cultures were discontinued in arm 3 during the intervention period; only clinical cultures were collected. [2], All qualifying surveillance and clinical isolates. [3], MRSA isolates identified in clinical cultures (surveillance isolates excluded). [4], Clinical isolates of MRSA were attributed to an intensive-care unit (ICU) stay if the specimen was collected during the period from the third day after ICU admission through the second day after ICU discharge.

[36]), and *S. aureus* NRS123 (*qac* negative [37]) were included as control strains in every run.

(iv) DNA sequence analysis of *qacA* and *qacB* amplicons. To distinguish between *qacA* and *qacB*, a conventional PCR primer pair was designed to amplify a 1,423-bp region internal to both genes (Supplemental Data and Table S1). pCR-BluntII-TOPO vector (Invitrogen, Carlsbad, CA)-cloned products were sequenced bidirectionally (ACGT Inc., Wheeling, IL). Sequences were compared to canonical *qacA* and *qacB* reference sequences (GenBank accession no. GU565967.1 and AF053772.1).

(v) MLST. All isolates positive for *qacA* or *qacB* underwent multilocus sequence typing (MLST). The protocol, including allelic number and sequence type (ST) assignment methods, is available at the *S. aureus* MLST database (http://saureus.mlst.net/).

Statistical analysis. Because this was a secondary analysis, the sample size was not calculated. Demographic and clinical characteristics were compared between arms with generalized linear mixed models that accounted for within-hospital clustering. A generalized logistic mixed-effects regression model was used to estimate the effects of the trial on mupirocin resistance. Generalized logistic regression resembles logistic regression except that it simultaneously estimates the odds ratios (OR) for both low- and high-level resistance relative to no resistance. Mixed effects were used to account for randomization by cluster (38). Because hospitals did not submit every MRSA isolate they collected, we used multiple imputation to generate estimated effects that incorporated the isolates with unknown susceptibility patterns (39–41). Trial effects were assessed by a 4-degree-of-freedom difference-in-differences test of the interaction, comparing the difference in the odds ratio for each level of resistance, for the baseline versus the intervention period, across arms. Analyses were performed using SAS proc glimmix software, version 9.3 (SAS Institute).

RESULTS

Characteristics of MRSA isolates and patients. A total of 7,405 eligible MRSA isolates were identified by local laboratories during the baseline and intervention periods; 4,566 isolates were sent to the central laboratory. Of these, 1,393 were excluded, leaving 3,173 (43%) isolates for analysis (Fig. 1).

Most isolates were collected from the anterior nares of patients on the first day of hospitalization (Table 1). On average, patients from whom MRSA was isolated were elderly and had multiple comorbid conditions. Isolate or population descriptors were similar among the three arms during both the baseline and the intervention periods. Collection site and hospital day of specimen collection differed in arm 3 during the intervention period, when surveillance cultures for MRSA were discontinued (Table 1).

CHG susceptibility. CHG MICs for all MRSA isolates collected during the baseline period displayed a narrow, unimodal distribution that did not change significantly during the intervention period (Fig. 2). The CHG MIC_{50} and MIC_{90} were 2 μg/ml and 4 μg/ml, respectively, for both the baseline and intervention periods. There were no significant differences in MIC distributions or in the MIC_{50} or MIC_{90} when isolates were stratified by arm or when only the clinical isolates acquired in an ICU were analyzed (data not shown). Two clinical MRSA isolates (both identified in arm 1, intervention period) were nonsusceptible to CHG (MIC, 8 μg/ml) and carried *qacA* (see Table 3). For the subset of non-CHG-susceptible or *qacA*- or *qacB*-positive isolates tested, MBCs were never more than

TABLE 1 Characteristics of MRSA isolates ($n = 3173$) and patient population according to study period and intervention arm

Variable	Baseline (7 mo)			Intervention (18 mo)			
	Arm 1	Arm 2	Arm 3	Arm 1	Arm 2	Arm 3	P value[a]
Isolate descriptors							
No. of isolates	221	305	209	1,082	1,155	201	
Median (IQR) month of collection	3 (2)	3 (2)	3 (2)	11 (9)	10 (9)	10 (9)	
Median (IQR) hospital day of collection	1 (1)	1 (1)	1 (2)	1 (1)	1 (1)	2 (4)	0.0087
No. (%) collected from:							
Anterior nares	168 (76.0)	230 (75.4)	134 (64.1)	869 (80.3)	974 (84.3)	0[b]	
Blood	7 (3.2)	11 (3.6)	16 (7.7)	44 (4.1)	43 (3.7)	42 (20.9)	
Sputum or tracheal aspirate	30 (13.6)	32 (10.5)	29 (13.9)	98 (9.1)	70 (6.1)	79 (39.3)	
Other site	16 (7.2)	32 (10.5)	30 (14.4)	71 (6.6)	68 (5.9)	80 (39.8)	
Population descriptors							
Median (IQR) patient age (yr)	69 (22)	68 (21)	65 (25)	69 (24)	70 (23)	68 (25)	
No. (%) female	104 (47.1)	134 (43.9)	100 (47.9)	513 (47.4)	570 (49.4)	91 (45.3)	
No. (%) with the following comorbid condition:							
Chronic obstructive pulmonary disease	103 (46.6)	120 (39.3)	87 (41.6)	424 (39.2)	463 (40.1)	67 (33.3)	
Diabetes	88 (39.8)	123 (40.3)	78 (37.3)	412 (38.1)	431 (37.3)	75 (37.3)	
Congestive heart failure or myocardial infarction	104 (47.1)	131 (43.0)	91 (43.5)	438 (40.5)	495 (42.9)	81 (40.3)	
Renal failure	66 (29.9)	99 (32.5)	71 (34.0)	341 (31.5)	349 (30.2)	62 (30.9)	
Cerebrovascular disease	23 (10.4)	50 (16.4)	30 (14.4)	154 (14.2)	148 (12.8)	32 (15.9)	
Peripheral vascular disease	38 (17.2)	37 (12.1)	33 (15.8)	123 (11.4)	142 (12.3)	26 (12.9)	
Cancer	17 (7.7)	26 (8.5)	24 (11.5)	96 (8.9)	108 (9.4)	19 (9.5)	
No. (%) with a history of MRSA[c]	80 (36.2)	100 (32.8)	70 (33.5)	333 (30.8)	251 (21.7)	57 (28.4)	0.0496

[a] For difference among intervention arms during the baseline or intervention period. Comparisons were conducted with generalized linear mixed models that accounted for within-hospital clustering. Only significant P values (<0.05) are shown. P values are not shown for baseline period comparisons, because there were no significant differences among intervention arms during the baseline period.

[b] During the intervention period, collection of surveillance cultures was discontinued in arm 3.

[c] MRSA isolated from a surveillance or clinical culture within 1 year prior to the date of collection of the MRSA isolate included in this study.

4-fold higher than MICs, indicating that the isolates were not tolerant to CHG (see Table 3) (33).

Mupirocin susceptibility. Among all MRSA isolates collected during the baseline period ($n = 735$), 7.1% expressed LLMR (range, 5.9% in arm 2 to 8.1% in arm 1) and 7.5% expressed HLMR (range, 5.0% in arm 1 to 11% in arm 3) with no significant differences in prevalence among arms. Because arm 3 did not collect surveillance cultures during the intervention period, we restricted further analyses to clinical isolates. Using imputed values for the number of mupirocin-resistant isolates, we did not find a significant difference-in-differences in the proportion of LLMR or HLMR isolates between baseline and intervention periods across arms for all clinical isolates or for clinical isolates attributable to an ICU stay (Table 2.) The small number of clinical isolates attributable to an ICU stay (Fig. 1) resulted in extremely wide confidence intervals (CI) for these comparisons (Table 2).

***qacA*, *qacB*, and MLST.** We tested 411 baseline isolates and 403 intervention isolates for *qacA* and *qacB* by PCR. Five MRSA isolates (0.6%) were found to carry *qac* genes: 4 carried *qacA*, and 1 carried *qacB* (Table 3). One isolate was collected during the baseline period (arm 1), and 4 isolates were collected during the intervention period (3 in arm 1 and one in arm 3). Isolates were submitted from 5 different hospitals; 4 isolates were submitted from hospitals located in Florida.

We identified three different *S. aureus* sequence types by MLST, including a novel sequence type (ST2484) characterized by a *tpi* allele that had not been described previously (Table 3). The novel sequence type was submitted to the MLST website

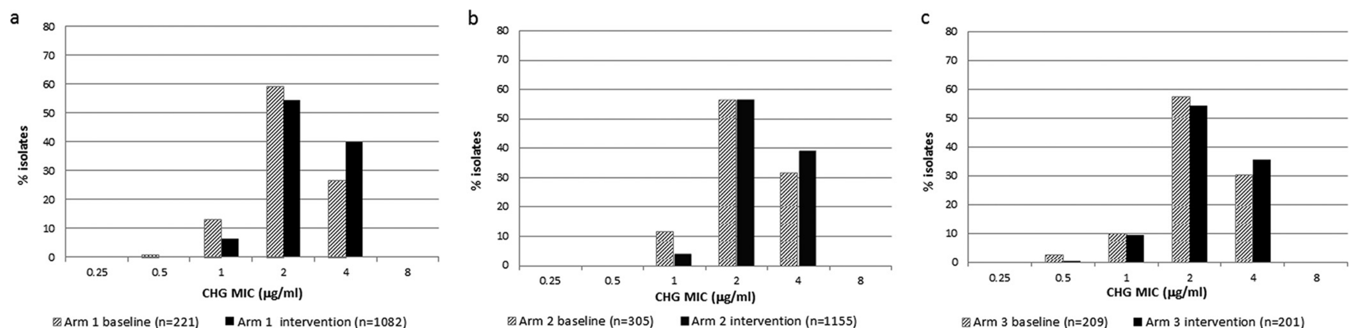

FIG 2 Distributions of chlorhexidine gluconate (CHG) MICs for all evaluable methicillin-resistant *Staphylococcus aureus* (MRSA) isolates ($n = 3,173$) collected during the baseline (hatched bars) and intervention (black bars) periods for arm 1 (a), arm 2 (b), and arm 3 (c). Two isolates for which the CHG MIC was 8 µg/ml (nonsusceptible) were identified in arm 1 during the intervention period.

TABLE 2 Odds of ICU MRSA isolates expressing low-level or high-level mupirocin resistance in the intervention versus the baseline period, by intervention arm[a]

MRSA isolate category	Mupirocin resistance category	Arm 1: No. of resistant isolates[b]/1,000 trial participants[c] Baseline	Arm 1: Intervention	Arm 1: OR (95% CI)	Arm 2: No. of resistant isolates[b]/1,000 ICU trial participants[c] Baseline	Arm 2: Intervention	Arm 2: OR (95% CI)	Arm 3: No. of resistant isolates[b]/1,000 trial participants[c] Baseline	Arm 3: Intervention	Arm 3: OR (95% CI)
Clinical MRSA isolates	Low-level	8.6	8.0	1.0 (0.22, 4.85)	4.2	6.4	1.4 (0.34, 6.14)	6.6	3.0	0.5 (0.1, 2.14)
	High-level	6.5	5.1	0.8 (0.11, 5.68)	5.4	5.5	0.9 (0.15, 5.44)	5.2	3.5	0.7 (0.16, 3.01)
ICU-attributable MRSA isolates	Low-level	2.1	3.8	1.7 (0.37, 7.55)	3.5	3.1	0.4 (<0.01, >99)	2.5	1.6	0.7 (0.10, 4.64)
	High-level	2.5	3.4	1.5 (0.25, 9.02)	3.8	2.8	0.7 (0.09, 5.06)	1.6	2.0	1.4 (0.13, 15.63)

[a] A mixed-effects generalized logistic regression model was used to estimate the effects of the trial on mupirocin resistance.
[b] Numerators are based on imputed values.
[c] The numbers of study participants in each trial phase were as follows: in arm 1, 5,680 during the baseline period and 23,963 during the intervention period; in arm 2, 5,775 during the baseline period and 22,522 during the intervention period; in arm 3, 6,101 during the baseline period and 26,500 during the intervention period.

curator and was approved. All other isolates belonged to clonal complex 8, a lineage of MRSA identified commonly in the United States (42).

DISCUSSION

In a large sample of MRSA isolates collected during the REDUCE-MRSA trial, decreased susceptibility to CHG, as measured by CHG MICs and carriage of *qacA* or *qacB*, was rare and was similar in frequency among MRSA isolates identified in decolonization arms and in the screening and isolation arm. The prevalence of mupirocin resistance at baseline was moderate (7.1% LLMR and 7.5% HLMR), and the odds of mupirocin resistance during the intervention versus the baseline period did not differ between the targeted or universal decolonization arms, on the one hand, and the screening and isolation arm, on the other. One theoretical explanation for our findings is that the success of decolonization applied diligently in all ICUs of a hospital reduced the prevalence of MRSA sufficiently to prevent the selection of resistance. The generalizability of our findings is strengthened by the large number of isolates tested and their broad geographic distribution across 16 states.

The low prevalence of nonsusceptibility to CHG in the population is striking. Two of 3,173 isolates tested nonsusceptible to CHG (MIC, 8 μg/ml), and 5 of 814 isolates were found to carry *qacA* or *qacB*. Only one of these isolates was identified in a decolonization arm, suggesting that CHG nonsusceptibility and *qacA* or *qacB* carriage were independent of exposure to decolonization.

The optimum susceptibility testing method and breakpoints for CHG and *S. aureus* are not defined. We used protocols endorsed by the Clinical and Laboratory Standards Institute that are widely accepted as reference methods for categorizing bacterial susceptibility to antibiotics and that have been used often in other studies of staphylococcal susceptibility to CHG, allowing our results to be compared to those in published reports. The susceptibility breakpoint for CHG applied in this study was derived from the MIC epidemiologic cutoff, a standard approach used when validated breakpoints are not available (31, 48). As in other reports (15, 19, 20), *qacA* was not a specific predictor of CHG resistance: three of five *qac*A-positive isolates were susceptible to CHG, and MBCs were always within 2 doubling dilutions of MICs. Of note are two isolates that carried *qacA* and expressed LLMR; the combination of LLMR and *qacA* carriage was a risk factor for decolonization failure in one case-control study (16).

Our results are consistent with several other published surveys that infrequently identified phenotypic or genotypic evidence of reduced susceptibility to CHG in MRSA isolates from U.S. health care facilities (22, 23, 35). More recently, reports have emerged of

TABLE 3 Characteristics of MRSA isolates that carried the *qacA* or *qacB* gene

Isolate ID	Hospital location[a]	Intervention arm	Study period	Culture type	*qac* identity[b]	ST	CHG MIC[c] (μg/ml)	CHG MBC[c] (μg/ml)	Mupirocin susceptibility profile[d]
B4607	Florida	1	Baseline	Surveillance	*qacA*	8	4	4	S
I06127	Florida	1	Intervention	Surveillance	*qacA*	2484	8	8	S
I44233	Florida	1	Intervention	Surveillance	*qacB*	8	4	8	S
I01137	Texas	1	Intervention	Clinical	*qacA*	8	8	8	LL
I1939[e]	Florida	3	Intervention	Clinical	*qacA*	450[f]	4	16	HL

[a] Five different hospitals.
[b] Determined by DNA sequence analysis of a 1,453-bp coding region of *qacA* and *qacB* that included 6 codons (codons 26, 152, 167, 291, 323, and 380) that predicted amino acid differences between *qacA* and *qacB*. The sequences obtained were compared with those of the canonical *qacA* (GenBank accession no. GU565967.1) and *qacB* (GenBank accession no. AF053772.1) genes. Three isolates (I06127, I01137, and I1939) matched the canonical *qacA* gene at all 6 codons. B4607 matched *qacA* at 3/6 codons, including codon 323, coding for aspartic acid, which predicts a multidrug efflux pump with the ability to use CHG as a substrate. I44233 matched the canonical *qacA* gene at 5/6 loci but coded for alanine at codon 323, a pattern consistent with *qacB*, which codes for an efflux pump that is not active against CHG.
[c] Testing was done in triplicate. Results represent consensus values.
[d] S, susceptible; LL, low-level resistance; HL, high-level resistance.
[e] ICU-attributable isolate.
[f] ST450 is a single-locus variant of ST8 (differs at *aroE*).

a higher prevalence of CHG nonsusceptibility or of an increase in the frequency of acquired multidrug efflux genes among MRSA isolates from wards where CHG bathing had been ongoing for years. McNeil et al. reported that 22.7% of MRSA surveillance isolates collected over a 7-year period at a children's hospital in Texas carried *qacA* or *qacB* (18). Warren and colleagues identified a statistically significant increase in the annual prevalence of *qacA* and *qacB* among MRSA surveillance isolates cultured from patients in a surgical ICU between 2005 and 2012, although the increase was not linear (49). These reports, together with the few descriptions of decolonization failure associated with reduced CHG susceptibility (16, 25, 26), justify surveillance for CHG resistance during sustained decolonization programs that employ routine CHG bathing.

While our inability to detect increases in CHG or mupirocin resistance in the decolonization arms of this trial is encouraging, our study has limitations. First, most of the MRSA isolates available for investigation were identified in cultures collected from patients on the first day of hospital admission; these patients would have had little exposure to CHG or mupirocin during targeted or universal decolonization interventions. While some patients may have undergone decolonization during an earlier ICU stay, readmissions to study ICUs were not tallied, so information about prior exposure to decolonization is unknown. Second, isolates were not selected randomly but instead comprised a convenience sample. We may have reduced the risk of sampling bias by testing isolates in a central laboratory and delaying the release of results to participating hospitals until after the trial closed. Third, the intervention period was relatively short (18 months), and results may not be generalizable to longer periods of routine decolonization. Fourth, we looked for only two efflux pump genes; other efflux pumps that can use CHG as a substrate have been described in *S. aureus* (18, 35).

Some limitations of our study apply specifically to the detection of mupirocin resistance. Most notably, few ICU-attributable isolates were available for analysis, resulting in imprecise estimates of odds ratios for a difference-in-differences in the prevalence of mupirocin resistance during the intervention versus the baseline period, across arms. Other investigators have reported increases in both LLMR and HLMR associated with increases in mupirocin use in hospitals (43, 44); exposure to mupirocin was an independent risk factor for colonization with mupirocin-resistant MRSA in a case-control study of general inpatients in one hospital (45). The risk of the development of mupirocin resistance appears to be greatest when mupirocin use is widespread and sustained over long periods, and when it is applied to extranasal anatomic sites, such as vascular catheter exit sites and wounds (4, 6). Whether the application of mupirocin to the anterior nares of all ICU patients will eventually select for mupirocin resistance and reduce the effectiveness of the universal decolonization strategy could not be determined by our analysis, but the issue remains a concern and should be monitored.

In summary, CHG nonsusceptibility and carriage of *qacA* or *qacB* were rare in a large sample of MRSA isolates from the REDUCE-MRSA trial. We did not detect an increase in the odds of mupirocin resistance in the intervention versus the baseline period, but confidence limits were broad, and our results should be interpreted with caution. Health care facilities that use CHG and mupirocin for targeted or universal decolonization should monitor *S. aureus* isolates for resistance to these agents.

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