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EPIDEMIOLOGY AND SURVEILLANCE

Impact of Health Care Exposure on Genotypic Antiseptic Tolerance in *Staphylococcus aureus* Infections in a Pediatric Population

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ABSTRACT *Staphylococcus aureus* possessing either the *smr* gene or the *qacA/B* genes is associated with decreased susceptibility to chlorhexidine gluconate (CHG) and other antiseptics. Previous studies of antiseptic-tolerant staphylococci have focused largely on high-risk populations, and the exact role of health care exposure in the acquisition of these organisms is unclear. We sought to describe the risk factors and features of infection caused by antiseptic-tolerant *S. aureus* in a general pediatric population. Isolates were selected from an ongoing *S. aureus* surveillance study. Every third sequential isolate in the year 2014 was selected for inclusion. All isolates underwent PCR for the genes *qacA/B* and *smr*. Medical records were reviewed. Five hundred six isolates were included in the study, with 377 (74.3%) being community acquired. One hundred (19.8%) isolates were *smr* positive and 79 (15.6%) *qacA/B* positive. In univariable analyses, the presence of either gene was associated with underlying medical conditions, nosocomial acquisition, recent hospitalization, central venous lines, and CHG exposure. In multivariable analyses, only differences between patients with chronic medical conditions (odds ratio [OR] = 1.72; 95% confidence interval [CI], 1.22 to 2.64) and nosocomial acquisition (OR = 2.48; 95% CI, 1.16 to 8.17) remained statistically significant. Among patients without risk factors, 27.9% had infection with an antiseptic-tolerant isolate. *smr*- or *qacA/B*-positive *S. aureus* isolates are common in children and are independently associated with nosocomial acquisition and underlying medical conditions. These findings imply a role for the health care environment in acquisition of these organisms. However, genotypic antiseptic tolerance was seen in >25% of healthy children with an *S. aureus* infection, indicating that these organism are prevalent in the community as well.

KEYWORDS chlorhexidine, *smr*, *qacA/B*, *Staphylococcus aureus*, children

Received 1 February 2017 **Returned for modification** 6 March 2017 **Accepted** 15 April 2017

Accepted manuscript posted online 24 April 2017

Citation McNeil JC, Hultén KG, Mason EO, Kaplan SL. 2017. Impact of health care exposure on genotypic antiseptic tolerance in *Staphylococcus aureus* infections in a pediatric population. Antimicrob Agents Chemother 61:e00223-17. https://doi.org/10.1128/AAC.00223-17.

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Health care-associated infections (HAIs) are associated with substantial morbidity and mortality for individual patients and also place increased resource burdens on the health care system (1, 2). A study published in 2013 revealed that the estimated costs of the five most common HAIs in the United States totaled $9.8 billion (3). One of the most commonly implemented strategies to minimize the incidence of HAIs includes the use of topical antimicrobials and antiseptics, among the most prevalent of which is chlorhexidine gluconate (CHG). CHG-based body washes, oral solutions, and central-line care bundles have been shown to decrease the incidence of HAIs in both adults and children (4–7).

Staphylococcus aureus remains one of the principal causes of HAI in children (8–11). In *S. aureus*, the *smr* gene and the *qacA/B* gene complex have been associated with elevated MICs and minimum bactericidal concentrations (MBCs) of CHG. A number of investigators have discovered an increase in the incidence of organisms bearing these genes following widespread use of CHG in hospital units (12–14) and, more rarely,

following exposure to CHG outside the hospital setting (15, 16). Importantly, the presence of these genes has been associated with resistance to other systemic antimicrobial agents, including clindamycin and ciprofloxacin (*smr*), as well as higher MICs of vancomycin (*qacA/B*) (17, 18).

The exact influence of health care exposure and, specifically, antiseptic exposure on the acquisition of these organisms is controversial. The impact of the use of these agents on the development of genotypic or *in vitro* CHG resistance has been minimal in large clinical trials of CHG-based decolonization regimens (15, 19). Previous studies of children have shown an association between antiseptic tolerance genes in *S. aureus* and the presence of central venous lines (CVLs), as well as a higher rate of invasive infection (18). Many previous pediatric studies, however, have been biased by including high-risk populations, such as neonates/infants and oncology and cardiac surgery patients (12, 18, 20, 21).

The goals of the present study were (i) to define the relative prevalence of the *smr* and *qacA/B* genes among a random sample of clinical *S. aureus* isolates from a general pediatric population and (ii) to determine the clinical features and outcomes associated with *S. aureus* isolates positive for these genes compared to those of negative controls in children.

RESULTS

During the study period, 1,530 unique *S. aureus* isolates were catalogued in the Texas Children's Hospital (TCH) *S. aureus* surveillance database, with 506 viable nonduplicate isolates included in the present study and undergoing screening. The median age of patients was 2.4 years (interquartile range [IQR], 1.1 to 8.2 years), and the racial/ethnic makeup of patients was very similar to that of the greater Houston area (Table 1). The vast majority of infections were community acquired (377/506 [74.3%]), and the most common infectious disease diagnosis was skin and soft tissue infection (SSTI) (386/506 [76.3%]), followed by bacteremia/endocarditis (26/506 [5.1%]) and musculoskeletal infections (24/506 [4.7%]). Invasive infections occurred in 22.1% of patients. An underlying medical illness was present in 177 (35%) patients, of which the most common illnesses were eczema (36/506 [7.1%]), immunocompromising conditions (35/506 [6.9%]), allergic rhinitis (21/506 [4.2%]), and asthma (19/506 [3.8%]).

Antiseptic tolerance genes. Overall, 100 isolates (19.8%) were positive for the *smr* gene by PCR, 79 (15.6%) were positive for *qacA/B*, and 13 (2.5%) were positive for all three genes. In univariable analyses, *S. aureus* isolates positive for *smr* were less likely to be community acquired ($P = 0.03$) and more likely to be clindamycin resistant (21% versus 12.1%; $P = 0.02$) (Table 1) than isolates negative for *smr*. In addition, *smr*-positive isolates were more likely to be associated with prior antibiotic exposure (57% versus 45.3%; $P = 0.04$) and prior CHG use (15% versus 8.4%; $P = 0.04$). Furthermore, *smr*-positive organisms were more often associated with 30-day readmission (8% versus 2.9%; $P = 0.01$).

In contrast, *qacA/B*-positive infections were far less likely to be methicillin resistant (18.9% versus 58.3%; $P < 0.001$) and were associated with higher vancomycin MICs ($P = 0.09$) (Table 1) in univariable analyses. As with *smr*-positive organisms, *qacA/B*-positive organisms were less likely to be community acquired ($P = 0.02$) and were more likely to be seen in patients with underlying conditions, especially cardiac disease (10.1% versus 2.3%; $P = 0.003$). In addition, *qacA/B*-positive organisms were more often associated with invasive infections (31.6% versus 20.3%; $P = 0.04$), CVLs (11.4% versus 5.4%; $P = 0.05$), and intensive care unit (ICU) admission than *qacA/B*-negative organisms (13.9% versus 3.9%; $P = 0.002$).

Comparisons were made between patients infected with isolates bearing *smr* and/or *qacA/B* (see Table S1 in the supplemental material). Infections due to isolates bearing both *qacA/B* and *smr* were far less likely to be methicillin resistant ($P < 0.001$), more likely to be clindamycin resistant ($P = 0.02$), and more likely to be associated with CHG exposure ($P = 0.05$) and underlying medical conditions ($P < 0.001$) than isolates with only one tolerance gene or no tolerance genes. In addition, isolates bearing both genes

TABLE 1 Characteristics of the study group and univariable comparison of isolates with and without antiseptic tolerance genes[a]

Parameter	Values for all patients (n = 506)[b]	Values for patients infected with isolates that were		P value (*smr*-positive isolates vs *smr*-negative isolates)	Values for patients infected with isolates that were		P value (*qacA/B*-positive isolates vs *qacA/B*-negative isolates)
		smr positive (n = 100)	*smr* negative (n = 406)		*qacA/B*-positive (n = 79)	*qacA/B* negative (n = 427)	
Median age (yr)	2.4 (1.1–8.2)	2.1 (0.8–5.5)	2.5 (1.2–8.3)	0.2	3.1 (0.8–10.3)	2.2 (1.1–8.2)	0.6
Female gender	259 (51.2)	48 (48)	211 (52)	0.5	34 (43)	225 (52.3)	0.1
African American race	130 (25.5)	25 (25)	105 (25.9)	0.9	22 (27.9)	108 (25.9)	0.6
Hispanic ethnicity	236 (46.6)	43 (43)	213 (52.4)	0.4	33 (41.8)	203 (47.5)	0.4
Site of acquisition of infection				0.03			0.02
Community acquired	377 (74.5)	64 (64)	313 (77.1)		53 (67)	324 (75.9)	
Community-onset health care associated	104 (20.6)	29 (29)	75 (18.5)		17 (21.5)	87 (20.4)	
Nosocomial	25 (4.9)	7 (7)	18 (4.4)		9 (11.3)	16 (3.7)	
Any underlying medical condition	177 (35)	43 (43)	134 (33)	0.06	39 (49.4)	138 (32.3)	0.005
Immunocompromising conditions	35 (6.9)	11 (11)	24 (5.9)	0.08	7 (8.9)	28 (6.6)	0.46
Cardiac disease	18 (3.6)	5 (5)	13 (3.2)	0.37	8 (10.1)	10 (2.3)	0.003
Hospitalization in the prior 3 mo	90 (17.8)	23 (23)	67 (16.5)	0.14	20 (25.3)	70 (16.4)	0.07
Surgery in the prior 3 mo	56 (11.1)	16 (16)	40 (9.9)	0.37	10 (12.7)	46 (10.7)	0.55
Receipt of antibiotics in the prior 3 mo	241 (47.8)	57 (57)	184 (45.3)	0.045	35 (44.3)	206 (48.2)	0.54
Central venous catheter *in situ*	32 (6.3)	9 (9)	23 (5.7)	0.12	9 (11.4)	23 (5.4)	0.07
Any receipt of CHG in the prior 3 mo	49 (9.7)	15 (15)	34 (8.3)	0.04	11 (13.9)	38 (8.9)	0.21
Infection leading to hospital admission	270 (53.4)	59 (59)	211 (51.9)	0.2	37 (46.8)	233 (54.6)	0.7
Admission for ≥24 h	190 (37.5)	55 (55)	145 (35.7)	0.001	30 (37.9)	160 (37.4)	1
Infection leading to ICU admission	28 (5.5)	6 (6)	22 (5.4)	0.8	11 (13.9)	17 (3.9)	0.002
Median length of hospital stay (days)	5 (2–10)	3 (2–9)	3 (2–7)	0.3	8 (4–20)	3 (2–6)	<0.001
30-day readmission	18 (3.6)	8 (8)	10 (2.4)	0.01	3 (3.8)	15 (3.3)	1
All-cause mortality	3 (0.6)	1 (1)	2 (0.5)	0.48	1 (1.3)	2 (0.4)	0.4
Invasive infections[c]	112 (22.1)	29 (29)	83 (20.4)	0.08	25 (31.6)	87 (20.3)	0.04
Methicillin resistance	264 (52.2)	59 (59)	205 (50.4)	0.1	15 (18.9)	249 (58.3)	<0.001
Clindamycin resistance	70 (13.8)	21 (21)	49 (12.1)	0.02	16 (20.3)	54 (12.6)	0.07
Vancomycin MIC of ≥2 μg/ml	12 (2.4)	5 (5)	7 (1.7)	0.06	4 (5.1)	8 (1.9)	0.09

[a]Continuous variables are expressed as medians with interquartile ranges (IQRs). Categorical variables expressed as numbers (percentages) of patients.

[b]The most common diagnoses overall were skin and soft tissue infections (386 [76.3%]), bacteremia/endocarditis (26 [5.1%]), and musculoskeletal infections (24 [4.7%]).

[c]Invasive infections included bacteremia, central-line-associated bloodstream infections, endocarditis, musculoskeletal infection, pneumonia/empyema, peritonitis, central nervous system infection, deep abscesses (such as deep neck abscesses), and deep surgical-site infections.

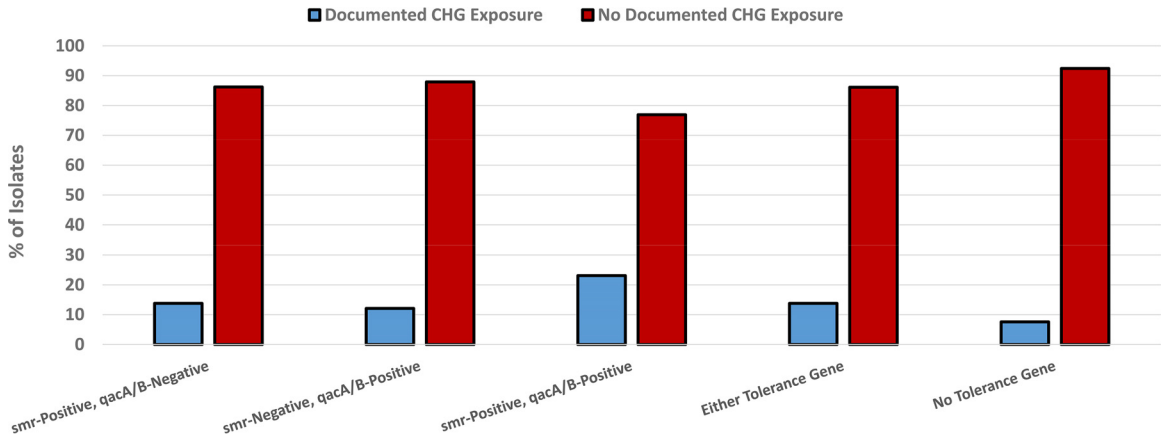

FIG 1 Impact of CHG exposure on antiseptic tolerance in *S. aureus* isolates with and without *smr* and *qacA/B*. Comparisons of genotypes were done in terms of documented CHG exposure. *P* was equal to 0.08 in comparisons across all categories; *P* was equal to 0.03 in a comparison of isolates with either tolerance gene and no tolerance gene.

were more likely to be invasive in nature ($P = 0.03$) and associated with ICU admission ($P = 0.007$).

Chlorhexidine exposure. Only 49 patients (9.7%) had documented receipt of at least one application of CHG in the 3 months prior to presentation. Among these, 26 (53.1%) had CHG exposure associated with the presence of a CVL and 33 (67.3%) had CHG exposure associated with surgical procedures; 30 (61.2%) cases were community-onset health care associated (CO-HCA), and 17 (34.7%) were nosocomial. Among isolates obtained from those patients with documented CHG exposure, the presence of either tolerance gene (46.9% versus 31.3%; $P = 0.04$), specifically *smr* (30.6% versus 18.6%; $P = 0.06$), was more common than among those isolates obtained from patients without a history of CHG exposure (Fig. 1). Six patients (1.2%) had CHG exposure without a history of CVLs or surgery. Among these, four had been prescribed topical CHG preparations for recurrent skin infections, and two received CHG-based mouthwashes for gingivitis; four out of these six patients (66.6%) had infection with an *smr*-positive organism ($P = 0.009$).

Risk factors for any antiseptic tolerance gene. Comparisons were made between infections due to organisms harboring at least one of the antiseptic tolerance genes ($n = 166$) and isolates without these genes ($n = 340$) (Table 2). Underlying chronic medical conditions, nosocomial acquisition of infection, previous hospital admission, the presence of central venous lines, and previous exposure to CHG were associated with the presence of either antiseptic tolerance gene in univariable analyses. When these clinical features were included in a multivariable logistic regression model for antiseptic tolerance, only underlying conditions ($P = 0.004$; odds ratio [OR], 1.72; 95% confidence interval [CI], 1.22 to 2.64) and nosocomial acquisition ($P = 0.04$; OR, 2.48; 95% CI, 1.16 to 8.17) remained statistically significant. The presence of a combination of these two clinical factors in any given patient with an *S. aureus* infection was examined in terms of the likelihood of an antiseptic-tolerant isolate. There was an additive effect of the number of risk factors present on the proportion of isolates with genotypic antiseptic tolerance ($P < 0.001$), ranging from 27.2% (89/328) with neither of these factors present to 40.9% (63/154) with one and 58.3% (14/24) with 2 risk factors present. Among nosocomial *S. aureus* isolates, 15/25 (60%) were positive for either *smr* or *qacA/B*; among isolates obtained from patients with underlying conditions, 76/177 (42.9%) carried at least one of these genes.

Overall, the presence of either gene was associated with a higher rate of invasive infection (29.5% versus 18.6%; $P = 0.006$), intensive care unit admission (9% versus 3.8%; $P = 0.03$), and a longer median length of stay (5 days [IQR, 2 to 10 days] versus 2 days [IQR, 1 to 6 days]; $P = 0.001$) than found with isolates lacking these genes.

TABLE 2 Characteristics of infections secondary to antiseptic-tolerant versus -susceptible organisms[a]

Parameter	Values for patients infected with isolates with:		Univariable *P* value	Adjusted multivariable *P* value	Odds ratio	95% CI
	Either tolerance gene ($n = 166$)	No tolerance gene ($n = 340$)				
Age (yr)	2.4 (0.8–8.2)	2.4 (1.2–8.2)	0.8			
Female gender	75 (45.2)	184 (54.1)	0.08			
African American race	45 (27.1)	85 (25)	0.6			
Hispanic ethnicity	71 (42.8)	165 (48.5)	0.3			
Underlying conditions	76 (45.8)	101 (29.7)	0.001	0.004	1.72	1.22–2.64
Immunocompromised	17 (10.2)	19 (5.6)	0.05			
Cardiac disease	11 (6.6)	7 (2.1)	0.02			
Nosocomial acquisition	15 (9)	10 (2.9)	0.004	0.04	2.48	1.16–8.17
Hospitalization in prior 3 mo	40 (24.1)	50 (14.7)	0.013	0.35	1.33	0.74–2.44
Antibiotic use in prior 3 mo	87 (52.4)	154 (45.3)	0.2			
Surgery in prior 3 mo	24 (14.5)	32 (9.4)	0.11			
CVL *in situ*	16 (9.6)	16 (4.7)	0.05	0.58	1.43	0.54–2.88
Any CHG use in prior 3 mo	23 (13.9)	26 (7.6)	0.04	0.68	1.1	0.44–2.36
Clinical outcomes						
Methicillin-resistant isolate	72 (43.6)	192 (56.5)	0.006			
Clindamycin-resistant isolate	32 (19.3)	38 (11.2)	0.02			
Vancomycin MIC of ≥2 μg/ml	7 (6.6)	5 (1.5)	0.01			
Invasive infection	49 (29.5)	63 (18.6)	0.006			
Infection leading to hospital admission	87 (52.4)	183 (53.8)	0.7			
ICU admission	15 (9)	13 (3.8)	0.02			
Length of stay (days)	5 (2–10)	2 (1–6)	0.001			
30-day readmission	7 (4.2)	6 (1.8)	0.1			
All-cause mortality	2 (1.2)	1 (0.3)	0.2			

[a]Continuous variables are expressed as medians with interquartile ranges. Categorical variables are expressed as numbers (percentages) of patients.

Genotypic antiseptic tolerance in the absence of risk factors. Two hundred ninety-five patients did not have any of the above-identified clinical features associated with *smr*- and/or *qacA/B*-positive *S. aureus* isolates in univariable analyses (nosocomial acquisition of infection, admission in the prior 3 months, CVL *in situ*, CHG use, or underlying chronic medical conditions); of these 295 patients, 79 (26.7%) had infection with an isolate with genotypic antiseptic tolerance. Among these healthy children, 50 patients had isolates that were *smr* positive (16.9%), and 36 had isolates that were *qacA/B* positive (12.2%). In patients without risk factors, *S. aureus* isolates with genotypic antiseptic tolerance were more often associated with a longer length of hospital stay (4 days versus 2 days; $P = 0.04$) and readmission within 30 days (5.2% versus 0.2%; $P = 0.04$) than susceptible isolates.

Invasive infections. One hundred twelve patients in the study had invasive infections. Comparisons were made between invasive and noninvasive infections (Table S2). Several clinical variables were associated with invasive infections in univariable analyses; however, in multivariable analyses, only nosocomial acquisition (OR = 10.8; 95% CI, 1.78 to 66.2), underlying medical conditions (OR = 2.02; 95% CI, 1.15 to 3.53), and previous admission (OR = 2.6; 95% CI, 1.2 to 5.63) remained significantly associated with invasive infection.

DISCUSSION

Numerous studies have illustrated the clear benefit of the use of CHG and other antiseptic preparations in the prevention of HAIs. There is concern, however, regarding the potential development of decreased susceptibility to these agents over time. We have performed a cross-sectional study with a large random sample of pediatric *S. aureus* isolates at a tertiary care children's hospital and found that 32.8% of isolates harbored *smr* and/or *qacA/B*. This is higher than the rate of genotypic antiseptic tolerance of 18.5% among a random sample of pediatric *S. aureus* isolates described in a study performed at Vanderbilt from 2004 to 2009 (22). This discrepancy may reflect

both geographic variation and a temporal trend for an increased prevalence of these genes among staphylococci.

There are numerous reports of a temporal relationship between CHG use and the detection of genotypically antiseptic-tolerant staphylococci in hospital units (13, 14). The presence of *smr*- and/or *qacA/B* in *S. aureus* is associated with health care exposure, as illustrated by an association in multivariable analyses with nosocomial acquisition and underlying medical conditions. Notably, the presence of these risk factors in a given patient had an additive effect, such that in patients with both of these factors, the proportion of *S. aureus* isolates positive for *smr* and/or *qacA/B* was 58.3%. Interestingly, while any CHG exposure was associated with *qacA/B* or *smr* in univariable analyses, it lost statistical significance in our multivariable analysis; this may potentially be a consequence of the high degree of collinearity between any CHG exposure (as defined in this study) and CO-HCA/nosocomial acquisition and CVLs.

Only a small proportion of our study population (9.7%) had documented CHG exposure in the 3 months preceding their sampling. However, among isolates taken from patients with previous CHG exposure, the proportion of cases bearing either a tolerance gene or specifically *smr* was higher than among those without CHG exposure. This is consistent with reports of increasing prevalence of *qacA/B* among staphylococci in hospital settings following the initiation of daily CHG bathing (14). While this finding was statistically significant, the overall effect size is small, with an absolute difference of only 15.6%. This small effect size, which was detectable with our overall large data set, in part may explain the lack of emergence of CHG nonsusceptibility in some clinical trials of this agent for decolonization, particularly with declines in HAI rates after CHG use (19, 23). Fritz et al., in a trial of a mupirocin- and CHG-based regimen to prevent recurrent skin and soft tissue infection in the community, found the emergence of *qacA/B*-positive *S. aureus* in only 2/215 (0.9%) patients receiving CHG (15). Interestingly, among six patients with a history of CHG exposure independent of prior surgery or CVL placement, 4/6 (66.7%) had infection secondary to infection with an *smr*-positive staphylococcus, suggesting that CHG regimens used by practitioners in the community may select for genotypic antiseptic tolerance.

It is notable that 26.7% of patients without any of the above-described risk factors had infection caused by an isolate with either *smr* or *qacA/B*. While it is apparent from our data that health care exposure is associated with infection with staphylococci bearing genotypic antiseptic tolerance, it also appears that these organisms exist at a high baseline level in our community. Given the retrospective nature of this study, it is likely that the prevalence of true community-acquired antiseptic-tolerant staphylococci may have been overrepresented; any CHG use and/or other health care exposures not documented in the medical record would not have been captured by our study design. However, the classification of any patient receiving surgery or CVL at our center being considered as having CHG exposure by our study definition helps to minimize this limitation.

The actual impact that the *smr* and *qacA/B* genes in *S. aureus* have on the efficacy of CHG-based antisepsis is controversial. Most studies show that the CHG MICs for these organisms are in the range of 2 to 4 μg/ml, and while this range is higher than for staphylococci lacking these genes (0.5 to 1 μg/ml) (18), it is much lower than the concentration of most CHG preparations in clinical use. What is clear, however, is that these organisms are associated with a more severe clinical phenotype, as manifested by more frequent invasive infections, more ICU admissions, and longer lengths of stay. Importantly, the finding of a longer length of stay was consistently shown in the group of healthy children analyzed. The reasons for the increased severity of illness are unclear but may be related to an unrecognized *S. aureus* virulence factor that should be further investigated. These findings must be interpreted with caution, as the study lacked a control group with CHG exposure but no infection. Furthermore, the study design precludes determining a causal relationship between genotypic antiseptic tolerance and severity of illness.

The findings in this study have several limitations. The restriction of a 3-month

period in our definition of antibiotic and antiseptic exposure may have underrepresented the impact of these particular factors on genotypic antiseptic tolerance. In addition, given the prevalence of *smr*- and *qacA/B*-positive staphylococci in otherwise-healthy children, it is possible that this is a consequence of the spread of an advantageous *S. aureus* clone or clones in our community. Previous work has illustrated that antiseptic-tolerant *S. aureus* isolates are of highly diverse genetic backgrounds (18), which minimizes, however, the impact of this limitation. In addition, isolates did not undergo CHG MIC determinations for this study, and the presence of *smr* and/or *qacA/B* might not directly equate to CHG resistance/tolerance. The large number of isolates in our study makes traditional broth dilution studies very labor-intensive and impractical. In addition, previous work in our center has demonstrated that the presence of these genes is associated with elevated MICs/MBCs of CHG even for community-acquired isolates (24). Finally, given that patients are not routinely screened for *S. aureus* colonization at our center, we are unable to evaluate the impact that *qacA/B* or *smr* may have on the efficacy of CHG-based decolonization regimens in hospitalized patients.

In conclusion, health care exposure, specifically nosocomial acquisition of infection and underlying medical conditions, is associated with genotypic antiseptic tolerance in *S. aureus*. Furthermore, these infections are associated with a more severe clinical phenotype, including more invasive infections, more ICU admissions, and reduced susceptibility to other systemic antibiotics. *smr*- and *qacA/B*-positive *S. aureus* strains are also common in our community even in the apparent absence of health care exposure. Further study and surveillance are necessary to better understand the consequences of these organisms.

MATERIALS AND METHODS

Patient and isolate selection. Isolates were selected from an *S. aureus* surveillance study at TCH ongoing since 2001. All *S. aureus* isolates identified by the clinical microbiology laboratory at TCH are subcultured and stored in horse blood at −80°C in the Infectious Diseases Research Laboratory. The surveillance study captures only isolates from infectious sources and does not capture colonization cultures. Every third sequential isolate in the calendar year 2014 was selected for inclusion in this study. TCH and the affiliated Texas Children's Pediatric Associates clinics (a network of 52 primary care clinics in the greater Houston area) have an integrated electronic medical record system. For all isolates, a retrospective review of the corresponding inpatient and outpatient electronic medical and pharmacy records was undertaken during a 3-month window prior to their presentation with the infection under study. This study was approved by the institutional review board of Baylor College of Medicine.

Infection control practices. The routine use of CHG for infection control purposes at our institution has been described elsewhere (18). Briefly, at our institution, CHG is the skin cleanser of choice prior to insertion of central venous lines (CVLs) and their maintenance. Daily CHG bathing is employed at TCH for all hospitalized patients with a CVL *in situ*. All patients undergoing elective surgery at TCH are encouraged to take a CHG bath the night prior to the operation, and this agent is the skin cleanser of choice in our operating rooms immediately prior to surgery. Daily CHG mouthwashes are routinely prescribed at TCH for hematopoietic stem cell transplant (HSCT) recipients and those with acute myeloid leukemia (AML). Colonization screening for methicillin-resistant *S. aureus* (MRSA), or for *S. aureus* in general, is not performed routinely at TCH.

Definitions. The following types of acquisition of infection were considered: community acquired, community-onset health care associated (CO-HCA), and nosocomial. Community-acquired infections were those occurring in otherwise-healthy children who exhibited the onset of signs and symptoms of infection as outpatients. CO-HCA infections were considered those in which signs and symptoms of infection developed in the outpatient setting in children with underlying medical conditions (25), excluding well-controlled asthma, eczema, and allergic rhinitis. Nosocomial infections were those in which the patient developed signs/symptoms of infection ≥72 h after hospital admission (26). For purposes of this study, patients with underlying medical conditions included all patients with a documented chronic medical illness (including even mild conditions, such as well-controlled asthma). Patients were considered to have CHG exposure in the prior 3 months if they had documented use of any CHG preparation, surgery, or CVL placement at TCH or diagnosis of AML or HSCT; all patients with surgery or CVL placement at TCH were assumed to have CHG exposure even if not clearly documented in the medical record. Invasive *S. aureus* infections included bacteremia/endocarditis, musculoskeletal infection (osteomyelitis, septic arthritis, and pyomyositis), pneumonia/empyema, peritonitis, central nervous system infection, deep abscesses (such as deep neck abscesses), and deep surgical-site infections (27). Noninvasive infections included skin and soft tissue infections (SSTIs) (such as cellulitis, impetigo, cutaneous abscesses, pustulosis, folliculitis, paronychia, etc.), otitis media, sinusitis, lymphadenitis, and superficial surgical-site infections. Immunocompromising conditions were considered to be malignancy/HSCT, HIV infection, primary immunodeficiency, end-stage renal disease, solid organ transplant, and rheumatologic conditions if the patient was receiving corticosteroids or immunomodulatory

agents. For purposes of this study, patients with underlying cardiac disease included patients with congenital heart disease, cardiomyopathy, and medically/surgically managed arrhythmia. Mortality, for purposes of this study, refers to all-cause mortality during hospital admission.

Microbiology and molecular studies. Testing for susceptibility to oxacillin and clindamycin was performed by the clinical microbiology laboratory in the routine course of care. Vancomycin MICs were determined with the Etest micromethod in the Infectious Diseases Research Laboratory. Whole DNA was prepared from all isolates with the assistance of QIAcube (Qiagen, Valencia, CA). All isolates underwent PCR to detect the presence of *smr* and *qacA/B* using previously published primers (18).

Statistical analyses and sample size considerations. Based on previous studies of health care-associated as well as community-acquired infections at our institution, the proportion of isolates carrying *qacA/B* or *smr* was estimated to be between 15 and 25% (18, 20, 24). Given that 1,530 isolates were known to be catalogued in the *S. aureus* database in 2014, selecting every third sequential isolate allowed for >500 isolates to be included in the study. In the event that the sequential selection of isolates resulted in any given patient having >1 isolate, only the first isolate was included in the study. A 20% prevalence of any given antiseptic tolerance gene was estimated *a priori*, with allowance for 4:1 matching of susceptible and tolerant isolates. Such a design allowed >80% power to detect a 15% absolute difference in the presence of any given risk factor with an α of 0.05 using a continuity-corrected χ^2 value.

Continuous variables were analyzed with either the Wilcoxon rank sum or Kruskal-Wallis test. Categorical variables were compared using Fisher's exact test. In analyses of length of hospital stay, patients admitted for 23-h observation were excluded from these calculations but not excluded from the study as a whole. In analyses of clinical features associated with the presence of either or both antiseptic tolerance genes, variables with a *P* value of ≤0.1 were included in a multivariable logistic regression model; *a priori*, it was decided to not include gender or race/ethnicity in the multivariable model. In specific analyses regarding the impact of the mode of infection acquisition (i.e., community-acquired, CO-HCA, or nosocomial), a comparison of nosocomial versus nonnosocomial infections (a combined group of community-acquired and CO-HCA infections) was used in the logistic regression model due to collinearity between CO-HCA acquisition and the presence of underlying medical conditions. The presence of any underlying chronic medical condition rather than individual conditions was included in the regression model. Variables found to be statistically significant in a multivariable analysis were included in a scheme to determine the additive effect of multiple risk factors on the likelihood of antiseptic tolerance. Additional analyses included comparisons of invasive and noninvasive infections (as defined above). All analyses were performed with the assistance of Stata v.13 (StataCorp, College Station, TX).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00223-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

ACKNOWLEDGMENT

This study was funded by NIAID grant K23AI099159 to J. Chase McNeil.

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