Title. Chlorhexidine and octenidine use, *qac* genes carriage, and reduced antiseptic susceptibility in methicillin-resistant *Staphylococcus aureus* isolates from a healthcare network

Type. Original article

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Running title: Antiseptic susceptibility in a healthcare network

ABSTRACT

Objectives. With the widespread use of antiseptics in healthcare facilities for the prevention of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission, there are concerns for antiseptic tolerance and resistance. We sought to understand the use of chlorhexidine and octenidine, *qac* genes carriage and reduced antiseptic susceptibilities.

Methods. A serial cross-sectional study was conducted in an acute care hospital and three extended-care facilities of a healthcare network in June-July, 2014-2016. Two of the extended-care facilities were exposed to intranasal octenidine and universal daily chlorhexidine/octenidine bathing. The minimum inhibitory concentration (MIC) levels and *qac* genes were determined by broth microdilution tests and whole genome sequencing respectively. Multivariable logistic regression was used to assess for the independent associations between antiseptic exposures, *qac* genes and reduced antiseptic susceptibilities.

Results. A total of 878 MRSA isolates were obtained. There were associations between *qacA/B* carriage and chlorhexidine (adjusted odds ratio [aOR]: 7.80; 95% confidence interval [CI]: 3.25-18.71) and octenidine (aOR: 11.79; 95%CI: 5.14-27.04) exposures. Chlorhexidine exposure was associated with reduced chlorhexidine susceptibility (MIC≥4mg/L) (aOR: 3.15; 95%CI: 1.14-8.74). Carriage of *qacA/B* (aOR: 10.65: 95%CI: 4.14-27.40) or *qacC* (aOR: 2.55; 95%CI: 1.22-5.32) had an association with reduced chlorhexidine susceptibility; while MRSA sequence type modified the association. However, we found no direct association between (i) antiseptics use and *qacC* carriage, (ii) octenidine exposure and reduced susceptibility and (iii) reduced octenidine susceptibility and *qacA/B* or *qacC* carriage.

Conclusions. Antiseptic exposures were associated with *qac* genes carriage. Chlorhexidine exposure was associated with reduced chlorhexidine susceptibility, requiring continued surveillance for the emergence of resistance.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA), which predominantly resides in hospital environments and affects patients with serious underlying comorbidities, has been endemic in many parts of the world since the 1990s [1, 2]. MRSA remains a significant global threat for healthcare-associated infections since its discovery in the 1960s. Today, MRSA is responsible for 40-60% of all nosocomial *Staphylococcus aureus* infections [3]. MRSA-colonized individuals typically harbour the bacteria on mucocutaneous sites, most commonly in the nares, axillae, and groin. Carriage of MRSA can persist for years to decades, without any skin or wound infection [4]. Patient-to-patient direct and indirect transmission of MRSA within and between healthcare facilities have been well documented [5].

To control for the MRSA transmission in hospitals, on-admission active surveillance screening and isolation of MRSA-colonized patients have been frequently adopted. Furthermore, antiseptic agents have been widely used, with MRSA decolonization guidelines including whole-body bathing with antiseptics and topical nasal application of mupirocin [6]. With the emergence of mupirocin resistance, octenidine has been used as an alternative for nasal decolonization [6]. Octenidine, cationic biguanide, is structurally similar to chlorhexidine but has a broader antibacterial activity spectrum towards Gram-positive bacteria [7].

With the widespread use of antiseptics in healthcare settings, MRSA carrying proton motive force-dependent efflux pumps encoded by plasmid-borne *qacA/B* and *qacC* genes that confer resistance to cationic biocides such as chlorhexidine, have been reported [8]. However, reduced susceptibility to octenidine has yet to be reported.

Our study aims to assess for the association of the use of chlorhexidine and octenidine for the prevention of nosocomial MRSA transmission with the prevalence of (i) *qacA/B* and

67 *qacC* genes and (ii) susceptibility to chlorhexidine and octenidine in MRSA isolated in an
68 acute hospital and affiliated intermediate-care facilities in a healthcare network.

69 **METHODS**

70 **Study Design and Setting**

71 We conducted serial cross-sectional studies over three consecutive years from 2014 to
72 2016, every six-week periods in June-July, in Tan Tock Seng Hospital (TTSH) and its three
73 affiliated intermediate-term care facilities (ITCFs) in Singapore. TTSH is a 1600-bed adult
74 acute tertiary-care hospital. The three ITCFs were included: (i) a 100-bed rehabilitation
75 centre which specialized in managing patients with stroke, brain injury, spinal and
76 musculoskeletal disorders (ITCF-1), (ii) a 360-bed community hospital providing care for
77 patients with stroke and debilitating medical conditions (ITCF-2), and (iii) a 116-bed
78 community hospital focused on inpatient care for stroke and subacute medical conditions
79 (ITCF-3).

80 **Antiseptic exposure**

81 In ITCF-1, all inpatients were universally bathed daily with chlorhexidine
82 (chlorhexidine gluconate 4%, Microshield*4, Johnson & Johnson, Australia) throughout the
83 study period. From March to July 2016, a 5-day regimen of intranasal octenidine gel
84 (octenidine hydrochloride 0.1%, Octenisan® md nasal gel, Schülke & Mayr GmbH,
85 Norderstedt, Germany) was administered for MRSA-colonized patients from the day of
86 admission to the ITCF. In ITCF-2, universal daily octenidine bathing (octenidine
87 hydrochloride 0.3%, Octenisan® wash lotion, Schülke & Mayr GmbH, Norderstedt,
88 Germany), with a 5-day application of intranasal octenidine (octenidine hydrochloride 0.1%,
89 Octenisan® md nasal gel, Schülke & Mayr GmbH, Norderstedt, Germany) from day of
90 admission for MRSA-colonized patients were implemented from March to July 2016. Prior to
91 March 2016, ITCF-2 had not used any antiseptic products for MRSA decolonization. No

antiseptic bathing or intranasal application was implemented in ITCF-3 and the acute care hospital (TTSH) throughout the study period. MRSA isolates were classified as being "exposed" or "unexposed" to chlorhexidine and octenidine respectively, depending on whether or not the isolates were obtained from patients who were exposed to chlorhexidine bathing and octenidine bathing/nasal gel.

Participants and MRSA screening

A randomly selected sample of 3,000 inpatients with ≥48-hour stay from the acute hospital who were systematically selected thrice over 15 days proportional to the bed census of the ward covering all wards, and all inpatients from ITCFs with ≥48-hour stay were included in the study. As the estimated mutation rate of one core single nucleotide polymorphism (SNPs) for MRSA is every six weeks [9], we completed the MRSA screening in all four institutions within six weeks each year. MRSA was screened with separate nasal, axillary and groin swabs taken by trained research nurses using a standardized protocol involving the use of swabs moistened with two sterile saline drops rolled five times in each nostril and ten times over the skin of the axillae and groin. The samples were inoculated onto selective chromogenic agar (Oxoid *Brilliance* MRSA2 Agar, Thermo Fisher Scientific, Basingstoke, United Kingdom) and incubated aerobically at 35-37°C for 18-24 hours at a common research laboratory. The results were read by the same medical technologist who was blinded to the origin of the samples, and hence the exposure to antiseptics. Growth of denim blue colonies were read as MRSA and referred to matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and cefoxitin disk diffusion test for confirmation of microbial identity and methicillin resistance.

Susceptibility testing

Susceptibility of isolates to chlorhexidine and octenidine were determined by the minimum inhibitory concentration (MIC) levels using modified broth microdilution method,

adhering to Clinical and Laboratory Standards Institute guidelines [10]. Fresh colonies were used and the range of susceptibility testing was from 0.125-8.0mg/L. Each isolate was tested in triplicates and incubated aerobically at 37^{o}C for 16-20 hours.

Whole genome sequencing

DNA from the MRSA isolates were extracted using a commercial kit (DNeasy kit; Qiagen, Hilden, Germany) for whole genome sequencing. The detailed method was described elsewhere [11]. Briefly, DNA libraries were created using a method adapted from the Illumina Indexing standard protocol. Illumina readings were mapped onto relevant reference sequences using Sequence Search and Alignment by Hashing Algorithm (SSAHA) (version2.2.1) [12] and candidate SNPs were identified using ssaha_pileup [9]. A resistome database comprised of previously described database of known resistance determinant gene sequences, both horizontally acquired and core [13, 14]. *Fastq* files generated from 878 isolates were mapped to the resistome database. Antimicrobial Resistance Identification By Assembly (ARIBA) (version2.12.1) [15] was run for resistance genes detection using the default settings. SNPs in chromosomal-encoded genes previously identified as being associated with antimicrobial resistance were then manually inspected to confirm the variation.

Statistical analysis

Frequencies and percentages for categorical variables, and medians and interquartile ranges (IQR) for continuous variables, were used for descriptive analyses. Pearson's χ^2 or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables were used for bivariable analyses. Univariable and multivariable logistic regression were used to assess for the association between exposure to antiseptics, carriage of *qac* genes, and reduced antiseptic susceptibilities, while adjusting potential confounding variables. In the absence of an established cut-off for antiseptic resistance [16, 17], we pragmatically defined

reduced susceptibility as an MIC level of ≥4mg/L for chlorhexidine and ≥2mg/L for octenidine for regression analyses. The odds ratio (OR) with 95% confidence interval (CI) from regression analyses were presented. All reported P values were two-tailed, with an α level of 0.05. Statistical analyses were conducted using Stata13.1 (CollegeStation, TX: StataCorp LP).

RESULTS

We screened 5,456 patients who provided 878 MRSA isolates, of which 12% (n=106) and 14% (n=126) of isolates were respectively exposed to chlorhexidine and octenidine, for a median of 20 (IQR:6-49) and 28.5 (IQR:10-44) days. More MRSA were isolated from the ITCFs (n=528; 60.1%) than the acute care hospital (n = 350; 39.9%). Overall, about half (n=463; 52.7%) of the MRSA belonged to sequence type (ST)22, with the remaining being ST45 (n=290; 33.0%) and other STs (n=125; 14.3%). There were significant differences in sequence type of isolates between those exposed and unexposed to chlorhexidine (P<0.01) and octenidine (P<0.01) (Table1).

Carriage of *qac* genes

The overall period prevalence of *qacA/B* and *qacC* were 46.6% (n=409) and 13.6% (n=119) respectively. A significantly higher proportion of *qacA/B* was observed both in isolates exposed to (i) chlorhexidine (70.6% exposed *v.* 43.4% unexposed, P<0.001) and (ii) octenidine (65.1% exposed *v.* 43.5% unexposed, P<0.01). However, *qacC* was more frequently detected in unexposed isolates to (i) chlorhexidine (4.9% exposed *v.* 14.7% unexposed, P<0.01) and (ii) octenidine (10.3% exposed *v.* 14.1% unexposed, P=0.25) (Table1).

Among *qacA/B* carrying MRSA, majority of *qacA/B* was found in ST45 (n=287/409; 70.2%), followed by ST22 (n=71/409; 17.3%) and other STs (n=51/409; 12.5%) MRSA. However, *qacC* was more prevalent in ST22 (n=74/119; 62.2%) than ST45 (n=2/119; 1.7%)

and other STs (n=43/119; 36.1%). Stratifying the gene carriage by sequence types, a remarkably high proportion of ST45 carried *qacA/B* (n=287/290; 99.0%) compared to ST22 (n=71/463; 15.3%) (Figure1).

Minimum Inhibitory Concentration

The MIC ranged from 1-8mg/L for chlorhexidine and 0.5-2mg/L for octenidine. Chlorhexidine-exposed isolates had a higher proportion with reduced susceptibility (MIC≥4mg/L) to chlorhexidine than the unexposed ones (87.3% exposed *v.* 72.2% unexposed, P<0.01). However, there was no significant difference in the proportion with reduced susceptibility (MIC≥2mg/L) to octenidine between the octenidine-exposed and unexposed isolates (5.5% exposed *v.* 9.6% unexposed, P=0.14) (Table1).

Associations between antiseptic exposure, *qac* genes carriage and reduced antiseptic susceptibility

Firstly, we examined the association between antiseptic exposure and *qac* genes carriage. After adjusting for healthcare facilities, year of isolation, sequence types and duration of exposure; chlorhexidine (adjusted odds ratio [aOR]:7.80, 95%CI: 3.25-18.71, P<0.001) and octenidine (aOR:11.79, 95%CI: 5.14-27.04, P<0.001) exposures were strongly associated with *qacA/B.* Although *qacC* carriage was negatively associated with exposure to chlorhexidine (aOR:0.18, 95%CI: 0.04-0.94, P=0.04), it was not significantly associated with exposure to octenidine (aOR:0.55, 95%CI: 0.23-1.31, P=0.18) (Table2).

Next, we investigated the relationship between antiseptic exposure and susceptibility. A significant reduction in antiseptic susceptibility was observed in chlorhexidine-exposed isolates, with three times as many exposed isolates as unexposed ones to have MIC levels ≥4mg/L to chlorhexidine (aOR:3.15, 95%CI: 1.14-8.74, P=0.03). Interestingly, octenidine-exposed isolates were nearly four times less likely than unexposed ones to have MIC≥2mg/L to octenidine (aOR:0.27, 95%CI: 0.08-0.95, P<0.01) (Table3).

Finally, we compared the carriage of *qac* genes with the prevalence of reduced antiseptic susceptibility. The odds of reduced chlorhexidine susceptibility increased in *qacA/B* (aOR:10.65, 95%CI: 4.14-27.40, P<0.001) and *qacC* (aOR:2.55, 95%CI: 1.22-5.32, P=0.01) carrying MRSA, compared to those without. However, neither the presence of *qacA/B* (aOR:0.76, 95%CI: 0.33-1.73, P=0.51) nor *qacC* (aOR:0.99, 95%CI: 0.43-2.31, P=0.99) were associated with reduced octenidine susceptibility (Table4).

In the secondary analysis, we further estimated the joint effects of *qac* genes and MRSA strains on chlorhexidine susceptibility (Table5). Using non-ST22/non-ST45/*qac*-absent isolates as the reference, the odds of reduced chlorhexidine susceptibility for ST22 without *qacA/B* was 4.12 (95%CI: 2.30-7.35, P<0.001) which increased to 28.60 (95%CI: 3.66-223.57, P<0.01) in the presence of *qacA/B*. Both ST22 without *qacC* carriage (aOR:2.87, 95%CI: 1.64-5.03, P<0.001) and with *qacC* carriage (aOR:5.99, 95%CI: 1.93-18.57, P<0.01) had increased odds of reduced chlorhexidine susceptibility. We found no association with reduced chlorhexidine susceptibility and ST45 with and without *qacA/B* or *qacC*.

We further assessed for the co-occurrence of resistance to mupirocin, an antibiotic commonly used for the decolonization of nasal carriage of MRSA. The mupirocin resistance gene, iles-2, was found in 10% (n=89) of our study MRSA isolates. We observed a significantly higher proportion of isolates carrying iles-2 in isolates with reduced susceptibility to chlorhexidine (12.6% MIC≥4mg/L *v.* 3.1% MIC<4mg/L, P<0.001), but not in isolates with reduced susceptibility to octendine (3.8% MIC≥2mg/L *v.* 10.8% MIC<2mg/L, P=0.05) (data not presented).

DISCUSSION

In this study, we have demonstrated positive associations between (i) chlorhexidine/ octenidine exposures and *qacA/B* carriage (ii) chlorhexidine exposure and reduced

susceptibility to chlorhexidine, and (iii) *qacA/B* and *qacC* carriages and reduced chlorhexidine susceptibility, and the modifying effects of *qacA/B* and *qacC* on ST22's effects on reduced chlorhexidine susceptibility respectively. We further observed that neither octenidine exposure nor carriage of *qacA/B* or *qacC* genes was associated with reduced susceptibility to octenidine in our study's isolates. On the contrary, isolates exposed to octenidine were four times less likely than unexposed isolates to have reduced susceptibility to octenidine.

The global distribution of *qac* genes is highly variable. One study reported that *qacA/B* can be found in 0.9-83.3% of clinical MRSA isolates worldwide [17]. Our study's finding of *qacA/B* period prevalence of 46.6% was comparable to the prevalence of *qacA/B* observed in other Asian countries ranging from 24-61%, and higher than in Canada, the United States, and Scotland (1-15%) but lower than Brazil (80%) [18]. We detected *qacC* in 13.6% of MRSA isolates, similar to other Asian studies ranging from 1-20%, but higher than the prevalence of 7% in Canada and 6% in Europe [18].

Our findings on the association of chlorhexidine exposure and higher MIC levels to chlorhexidine, corroborated with observations by a study from the United Kingdom which described the correlation of chlorhexidine exposure with mean MIC levels of isolates including *Staphylococcus aureus* [19]. However, we did not find an association between octenidine exposure and higher MIC levels to octenidine. There have been limited published studies on octenidine exposure and susceptibility, although the effectiveness of octenidine as a decolonization regimen has been frequently reported [20-22].

As described in other studies [23, 24], our study also indicated the association between antiseptic exposure and *qacA/B* carriage, although not with *qacC* carriage. Whilst we observed that *qacA/B* and *qacC* carriages were associated with reduced chlorhexidine susceptibility, we did not find an association between *qac* genes and reduced octenidine

susceptibility. *qacA/B* is considered to be the most common gene encoding for resistance to biocides [25], and it significantly increases the risk of persistent MRSA carriage after decolonization therapy [26]. However, there have been suggestions that presence of *qacA/B* does not necessarily translate to the expression of reduced susceptibility to chlorhexidine [18]. Although almost all (99%) of our ST45 MRSA carried *qacA/B*, they were not positively associated with reduced susceptibility to chlorhexidine. Whilst the *qacA/B* carriage rate in ST22 (15%) was low, ST22 was positively associated with reduced susceptibility to chlorhexidine, consistent with findings from a recent study conducted in the United Kingdom [22]. An Australian study evaluating 123 MRSA isolates also noted the over-predominance of ST22 in the expression of reduced susceptibility to chlorhexidine [27]. The reason behind raised MIC levels in certain MRSA strains remains unclear. However, possible alternate mechanisms includes overexpression of mutant chromosomally encoded genes of efflux pump such as *norA*, *norB* and *mepA* [8, 18]. An *in vitro* study demonstrated the increased expression of the efflux pump genes in clinical isolates when exposed to low concentrations of antiseptics [28]. Future studies are required to elucidate the differences in resistance mechanisms between MRSA strains. For ST22 and STs other than ST45, we further observed that the presence of *qacA/B* and *qacC* genes enhanced the effects of the respective MRSA clones on reduced susceptibility to chlorhexidine. Whilst the observed clonal predominance of *qac* genes corroborated with other studies [23, 29], the modifying effects of *qac* genes on the effects of specific MRSA clones on antiseptic resistance have not been reported previously.

Our study has several strengths. To our knowledge, this is the first clinical study reporting octenidine susceptibility in MRSA from acute- and intermediate-care settings in a healthcare network. Secondly, we demonstrated the joint effects of MRSA strains and *qac* genes on reduced chlorhexidine susceptibility, providing new observations that can advance

the understanding of antiseptic resistance in MRSA with further studies. Thirdly, samples were collected by trained research nurses who followed standardized procedures, tested in a single laboratory by the same medical technologist, and were confirmed with MALDI-TOF minimizing any potential measurement error and misclassification. Fourthly, blinded microbiologic assessment of samples reduced any potential detection bias. Finally, our MRSA clones were consistent with the epidemiology of MRSA in Singapore, rendering any potential selection bias unlikely [11, 30].

There are several limitations. We acknowledge that the MIC cut-off we used to define reduced susceptibility might not be internationally adopted. Nonetheless, studies have defined chlorhexidine MIC≥4mg/L to represent reduced susceptibility [18, 22]. To date, no study has determined the MIC cut-off for octenidine. Hence, we selected the most plausible cut-off of MIC≥2mg/L to define reduced octenidine susceptibility for our study. Likewise, there have not been any standard definition nor standardized methods to determine antiseptic resistance. Whilst the majority of published literature have adopted MIC-based methods for antiseptic susceptibility testing, minimum bactericidal concentration (MBC) has been suggested by some papers to better reflect clinical outcomes. We have chosen to determine MIC levels in this study for comparability with other studies. Furthermore, we did not test for other mechanisms of antiseptic resistance including *norA/B* and there could be residual confounding due to unknown confounders despite adjusting for key confounders defined *a prior* in the multivariable regression analyses.

In summary, chlorhexidine and octenidine are essential antiseptics used in the prevention and control of MRSA in healthcare settings worldwide. This study provided evidence of reduced susceptibility to chlorhexidine with exposure, although we did not find a reduction with octenidine. This finding has important clinical implications, as more

healthcare institutions implement universal chlorhexidine and octenidine bathing programs to prevent nosocomial MRSA transmission.

Transparency declarations. None to declare.

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Ethics approval

The study was approved by Domain Specific Review Board of National Healthcare Group Singapore (DSRB – 2015/00369). Informed consent was provided by all cognitively intact participants or the legally authorized representatives (LARs) of cognitively impaired participants. A waiver of informed consent was granted for cognitively impaired participants from the ITCFs who had no LARs.

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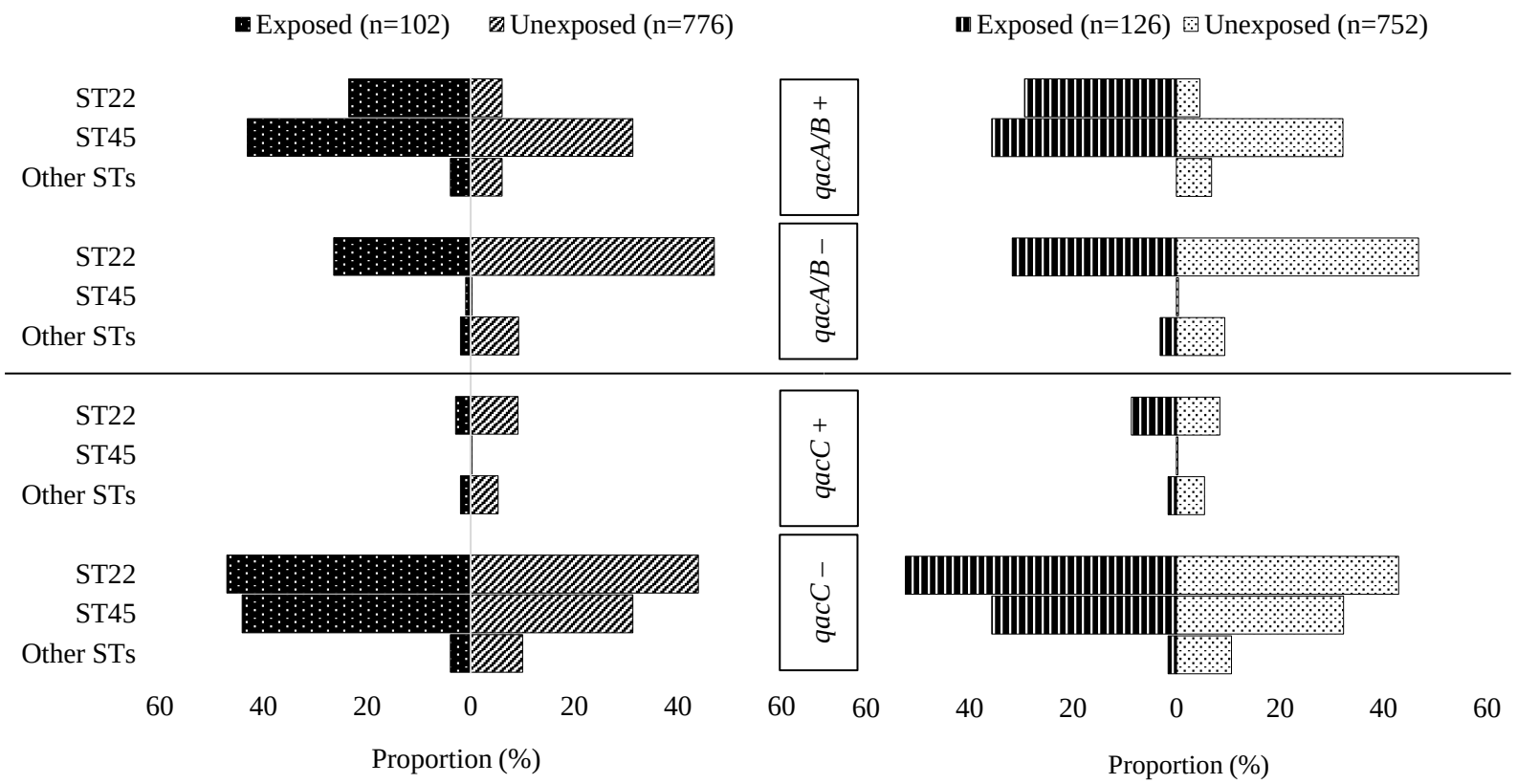

A. Chlorhexidine exposure **B.** Octenidine exposure

Figure 1. Distribution of different methicillin-resistant *Staphylococcus aureus* sequence types carrying *qacA/B* & *qacC* genes based on **A.** chlorhexidine exposure, and **B.** octenidine exposure

399 **Table 1**. Characteristics of methicillin-resistant *Staphylococcus aureus* isolates from the acute- and intermediate-term care facilities

Characteristics	Total isolates (n = 878)	Chlorhexidine			Octenidine		
		Exposed isolates* (n = 102)	Unexposed isolates* (n = 776)	P_1	Exposed isolates* (n = 126)	Unexposed isolates* (n = 752)	P_2
Healthcare institutions				<0.001			<0.001
ACH	350 (39.9)	0	350 (45.1)		0	350 (46.5)	
ITCF-1	102 (11.6)	102 (100.0)	0		17 (13.5)	85 (11.3)	
ITCF-2	330 (37.6)	0	330 (42.5)		109 (86.5)	221 (29.4)	
ITCF-3	96 (10.9)	0	96 (12.4)		0	96 (12.8)	
Healthcare facilities				<0.001			<0.001
ACH	350 (39.9)	0	350 (45.1)		0	350 (46.5)	
ITCFs	528 (60.1)	102 (100.0)	426 (54.9)		126 (100.0)	402 (53.5)	
Year of MRSA isolation				<0.001			<0.001
2014	43 (4.9)	43 (42.1)	0		0	43 (5.7)	
2015	497 (56.6)	42 (41.2)	455 (58.6)		0	497 (66.1)	

2016	338 (38.5)	17 (16.7)	321 (41.4)		126 (100.0)	212 (28.2)	
Duration of antiseptic exposure, days							
median (IQR)	–	20 (6 - 49)	–	–	28.5 (10 - 44)	–	–
Sequence type				<0.01			<0.01
ST22	463 (52.7)	51 (50.0)	412 (53.1)		77 (61.1)	386 (51.3)	
ST45	290 (33.0)	45 (44.1)	245 (31.6)		45 (35.7)	245 (32.6)	
Other STs[#]	125 (14.3)	6 (5.9)	119 (15.3)		4 (3.2)	121 (16.1)	
Carriage of *qacA/B* genes	409 (46.6)	72 (70.6)	337 (43.4)	<0.001	82 (65.1)	327 (43.5)	<0.001
Carriage of *qacC* genes	119 (13.6)	5 (4.9)	114 (14.7)	<0.01	13 (10.3)	106 (14.1)	0.25
MIC level to chlorhexidine				<0.01[†]			–
1 mg/L	11 (1.3)	0	11 (1.4)		–	–	
2 mg/L	218 (24.8)	13 (12.7)	205 (26.4)		–	–	
4 mg/L	647 (73.7)	88 (86.3)	559 (72.1)		–	–	
8 mg/L	2 (0.2)	1 (1.0)	1 (0.1)		–	–	
MIC level to chlorhexidine ≥ 4mg/L	649 (73.9)	89 (87.3)	560 (72.2)	<0.01	–	–	
MIC level to octenidine				–			0.15[†]

0.5 mg/L	3 (0.3)	−	−		1 (0.8)	2 (0.3)	
1 mg/L	796 (90.7)	−	−		118 (93.6)	678 (90.1)	
2 mg/L	79 (9.0)	−	−		7 (5.6)	72 (9.6)	
MIC level to octenidine ≥ 2mg/L	79 (9.0)	−	−	−	7 (5.6)	72 (9.6)	0.14

Abbreviations: ACH, Acute care hospital; IQR, Interquartile range; ITCFs, Intermediate-term care facilities; MIC, Minimum inhibitory concentration; ST, sequence type.

*MRSA isolates were classified as being “exposed” or “unexposed” to chlorhexidine and octenidine respectively, depending on whether or not the isolates were obtained from patients who were exposed to chlorhexidine bathing and octenidine bathing/nasal gel.

Values are expressed in no. (%) unless stated otherwise

P_1; statistical test between chlorhexidine exposed and unexposed isolates

P_2; statistical test between octenidine exposed and unexposed isolates

[#]Other STs include ST5 (n=1), ST6 (n=3), ST59 (n=1), ST80 (n=1), ST88 (n=1), ST188 (n=1), ST239 (n=45), ST573 (n=17), ST622 (n=37), ST672 (n=1), ST1178 (n=5), ST1218 (n=2), ST1232 (n=1), NF (n=9)

†; Fisher's exact test

Table 2. Associations between chlorhexidine and octenidine exposures and carriage of *qacA/B* and *qacC* genes among methicillin-resistant *Staphylococcus aureus* isolates

	Variables	Total isolates (n = 878)	Isolates carrying *qac* genes [no./total no. (%)]	Crude odds ratio (95% CI)	P	Adjusted odds ratio[a] (95% CI)	P
Chlorhexidine	***qacA/B* genes**						
	Unexposed	776 (88.4)	337/776 (43.4)	Reference		Reference	
	Exposed	102 (11.6)	72/102 (70.6)	3.13 (1.99 - 4.90)	<0.001	7.80 (3.25 - 18.71)	<0.001
	***qacC* genes**						
	Unexposed	776 (88.4)	114/776 (14.7)	Reference		Reference	
	Exposed	102 (11.6)	5/102 (4.9)	0.30 (0.12 - 0.75)	0.01	0.18 (0.04 - 0.94)	0.04
Octenidine	***qacA/B* genes**						
	Unexposed	752 (85.6)	327/752 (43.5)	Reference		Reference	
	Exposed	126 (14.4)	82/126 (65.1)	2.42 (1.63 - 3.59)	<0.001	11.79 (5.14 - 27.04)	<0.001
	***qacC* genes**						
	Unexposed	752 (85.6)	106/752 (14.1)	Reference		Reference	
	Exposed	126 (14.4)	13/126 (10.3)	0.70 (0.38 - 1.29)	0.25	0.55 (0.23 - 1.31)	0.18

[a]adjusted for year, facility of MRSA isolate detection, duration of exposure and sequence type (categorized as ST22, ST45 and other STs)

Table 3. Associations between chlorhexidine and octenidine exposures and reduced antiseptic susceptibility among methicillin-resistant *Staphylococcus aureus* isolates

Variables	Total isolates (n = 878)	Isolates with reduced antiseptic susceptibility[a] [no./total no. (%)]	Crude odds ratio (95% CI)	P	Adjusted odds ratio[b] (95% CI)	P
Chlorhexidine exposure						
Unexposed	776 (88.4)	560/776 (72.2)	Reference		Reference	
Exposed	102 (11.6)	89/102 (87.3)	2.64 (1.45 - 4.82)	<0.01	3.15 (1.14 - 8.74)	0.03
Octenidine exposure						
Unexposed	752 (85.6)	72/752 (9.6)	Reference		Reference	
Exposed	126 (14.4)	7/126 (5.6)	0.56 (0.25 - 1.24)	0.15	0.27 (0.08 - 0.95)	<0.01

[a]reduced antiseptic susceptibility is defined as MIC ≥4mg/L for chlorhexidine, and MIC ≥2mg/L for octenidine

[b]adjusted for year, facility of MRSA isolate detection, duration of exposure, sequence type (categorized as ST22, ST45 and other STs), and presence of *qacA/B* and *qacC* genes

Table 4. Associations between carriage of *qacA/B* and *qacC* genes and reduced chlorhexidine and octenidine susceptibility among methicillin-resistant *Staphylococcus aureus* isolates

	Variables	Isolates susceptible to antiseptic [no./total no. (%)]	Isolates with reduced antiseptic susceptibility[a] [no./total no. (%)]	Crude odds ratio (95% CI)	P	Adjusted odds ratio[b] (95% CI)	P
Chlorhexidine	***qacA/B* genes**						
	qacA/B –	92/229 (40.2)	377/649 (58.1)	Reference		Reference	
	qacA/B +	137/229 (59.8)	272/649 (41.9)	0.48 (0.36 - 0.66)	<0.001	10.65 (4.14 - 27.40)	<0.001
	***qacC* genes**						
	qacC –	219/229 (95.6)	540/649 (83.2)	Reference		Reference	
	qacC +	10/229 (4.4)	109/649 (16.8)	4.42 (2.27 - 8.61)	<0.001	2.55 (1.22 - 5.32)	0.01
Octenidine	***qacA/B* genes**						
	qacA/B –	430/799 (53.8)	39/79 (49.4)	Reference		Reference	
	qacA/B +	369/799 (46.2)	40/79 (50.6)	1.19 (0.75 - 1.90)	0.45	0.76 (0.33 - 1.73)	0.51
	***qacC* genes**						

qacC –	688/799 (86.1)	71/79 (89.9)	Reference		Reference	
qacC +	111/799 (13.9)	8/79 (10.1)	0.70 (0.33 - 1.49)	0.35	0.99 (0.43 - 2.31)	0.99

[a]reduced antiseptic susceptibility is defined as MIC ≥4mg/L for chlorhexidine, and MIC ≥2mg/L for octenidine.

[b]adjusted for year, facility of MRSA isolate detection, sequence types (categorized as ST22, ST45 and other STs), antiseptic exposure and duration of exposure

423 **Table 5**. Joint association of *qacA/B* or *qacC* carriage and sequence types (ST), and reduced **chlorhexidine** susceptibility among methicillin-

424 resistant *Staphylococcus aureus* isolates

	qac genes & ST	Isolates susceptible to chlorhexidine (MIC <4 mg/L) (n = 229)	Isolates with reduced chlorhexidine susceptibility (MIC ≥4 mg/L) (n = 649)	Crude odds ratio (95% CI)	P	Adjusted odds ratio[a] (95% CI)	P
***qacA/B* genes**	*qacA/B* – & other ST	30 (13.1)	44 (6.8)	Reference		Reference	
	qacA/B – & ST45	3 (1.3)	0 (0.0)	–		–	
	qacA/B – & ST22	59 (25.8)	333 (51.3)	3.85 (2.24 - 6.61)	<0.001	4.12 (2.30 - 7.35)	<0.001
	qacA/B + & other ST	5 (2.2)	46 (7.1)	6.27 (2.23 - 17.62)	<0.001	10.37 (3.53 - 30.46)	<0.001
	qacA/B + & ST45	131 (57.2)	156 (24.0)	0.81 (0.48 - 1.36)	0.43	0.62 (0.35 - 1.10)	0.11
	qacA/B + & ST22	1 (0.4)	70 (10.8)	47.73 (6.28 - 362.57)	<0.001	28.60 (3.66 - 223.57)	<0.01
***qacC* genes**	*qacC* – & other ST	31 (13.5)	51 (7.9)	Reference		Reference	
	qacC – & ST45	132 (57.6)	156 (24.0)	0.72 (0.43 - 1.19)	0.20	0.43 (0.25 - 0.74)	<0.01
	qacC – & ST22	56 (24.5)	333 (51.3)	3.61 (2.13 - 6.13)	<0.001	2.87 (1.64 - 5.03)	<0.001
	qacC + & other ST	4 (1.7)	39 (6.0)	5.93 (1.93 - 18.19)	<0.01	4.93 (1.55 - 15.69)	<0.01
	qacC + & ST45	2 (0.9)	0 (0.0)	–		–	

qacC + & ST22	4 (1.8)	70 (10.8)	10.64 (3.53 - 32.02)	<0.001	5.99 (1.93 - 18.57)	<0.01

[a]adjusted for year, facility of MRSA isolate detection, chlorhexidine exposure and duration of exposure