

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

4 **Type.** Original article

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17 **Running title:** Antiseptic susceptibility in a healthcare network

18 **ABSTRACT**

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

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

30 **Results.** A total of 878 MRSA isolates were obtained. There were associations between  
31 *qacA/B* carriage and chlorhexidine (adjusted odds ratio [aOR]: 7.80; 95% confidence interval  
32 [CI]: 3.25-18.71) and octenidine (aOR: 11.79; 95%CI: 5.14-27.04) exposures. Chlorhexidine  
33 exposure was associated with reduced chlorhexidine susceptibility (MIC $\geq$ 4mg/L) (aOR: 3.15;  
34 95%CI: 1.14-8.74). Carriage of *qacA/B* (aOR: 10.65; 95%CI: 4.14-27.40) or *qacC* (aOR:  
35 2.55; 95%CI: 1.22-5.32) had an association with reduced chlorhexidine susceptibility; while  
36 MRSA sequence type modified the association. However, we found no direct association  
37 between (i) antiseptics use and *qacC* carriage, (ii) octenidine exposure and reduced  
38 susceptibility and (iii) reduced octenidine susceptibility and *qacA/B* or *qacC* carriage.

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

## 42 INTRODUCTION

43 Methicillin-resistant *Staphylococcus aureus* (MRSA), which predominantly resides in  
44 hospital environments and affects patients with serious underlying comorbidities, has been  
45 endemic in many parts of the world since the 1990s [1, 2]. MRSA remains a significant  
46 global threat for healthcare-associated infections since its discovery in the 1960s. Today,  
47 MRSA is responsible for 40-60% of all nosocomial *Staphylococcus aureus* infections [3].  
48 MRSA-colonized individuals typically harbour the bacteria on mucocutaneous sites, most  
49 commonly in the nares, axillae, and groin. Carriage of MRSA can persist for years to  
50 decades, without any skin or wound infection [4]. Patient-to-patient direct and indirect  
51 transmission of MRSA within and between healthcare facilities have been well documented  
52 [5].

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

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

65 Our study aims to assess for the association of the use of chlorhexidine and octenidine  
66 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

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

### 97 **Participants and MRSA screening**

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

### 114 **Susceptibility testing**

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

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

## 120 **Whole genome sequencing**

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

## 134 **Statistical analysis**

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

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

## 147 **RESULTS**

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

### 156 **Carriage of *qac* genes**

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

164 Among *qacA/B* carrying MRSA, majority of *qacA/B* was found in ST45 (n=287/409;  
165 70.2%), followed by ST22 (n=71/409; 17.3%) and other STs (n=51/409; 12.5%) MRSA.  
166 However, *qacC* was more prevalent in ST22 (n=74/119; 62.2%) than ST45 (n=2/119; 1.7%)

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

### 170 **Minimum Inhibitory Concentration**

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

### 177 **Associations between antiseptic exposure, *qac* genes carriage and reduced antiseptic** 178 **susceptibility**

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

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



192 Finally, we compared the carriage of *qac* genes with the prevalence of reduced  
193 antiseptic susceptibility. The odds of reduced chlorhexidine susceptibility increased in  
194 *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,  
195 P=0.01) carrying MRSA, compared to those without. However, neither the presence of  
196 *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,  
197 P=0.99) were associated with reduced octenidine susceptibility (Table4).

198 In the secondary analysis, we further estimated the joint effects of *qac* genes and  
199 MRSA strains on chlorhexidine susceptibility (Table5). Using non-ST22/non-ST45/*qac*-  
200 absent isolates as the reference, the odds of reduced chlorhexidine susceptibility for ST22  
201 without *qacA/B* was 4.12 (95%CI: 2.30-7.35, P<0.001) which increased to 28.60 (95%CI:  
202 3.66-223.57, P<0.01) in the presence of *qacA/B*. Both ST22 without *qacC* carriage  
203 (aOR:2.87, 95%CI: 1.64-5.03, P<0.001) and with *qacC* carriage (aOR:5.99, 95%CI: 1.93-  
204 18.57, P<0.01) had increased odds of reduced chlorhexidine susceptibility. We found no  
205 association with reduced chlorhexidine susceptibility and ST45 with and without *qacA/B* or  
206 *qacC*.

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

## 214 DISCUSSION

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

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

224         The global distribution of *qac* genes is highly variable. One study reported that  
225 *qacA/B* can be found in 0.9-83.3% of clinical MRSA isolates worldwide [17]. Our study's  
226 finding of *qacA/B* period prevalence of 46.6% was comparable to the prevalence of *qacA/B*  
227 observed in other Asian countries ranging from 24-61%, and higher than in Canada, the  
228 United States, and Scotland (1-15%) but lower than Brazil (80%) [18]. We detected *qacC* in  
229 13.6% of MRSA isolates, similar to other Asian studies ranging from 1-20%, but higher than  
230 the prevalence of 7% in Canada and 6% in Europe [18].

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

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

242 susceptibility. *qacA/B* is considered to be the most common gene encoding for resistance to  
243 biocides [25], and it significantly increases the risk of persistent MRSA carriage after  
244 decolonization therapy [26]. However, there have been suggestions that presence of *qacA/B*  
245 does not necessarily translate to the expression of reduced susceptibility to chlorhexidine  
246 [18]. Although almost all (99%) of our ST45 MRSA carried *qacA/B*, they were not positively  
247 associated with reduced susceptibility to chlorhexidine. Whilst the *qacA/B* carriage rate in  
248 ST22 (15%) was low, ST22 was positively associated with reduced susceptibility to  
249 chlorhexidine, consistent with findings from a recent study conducted in the United Kingdom  
250 [22]. An Australian study evaluating 123 MRSA isolates also noted the over-predominance  
251 of ST22 in the expression of reduced susceptibility to chlorhexidine [27]. The reason behind  
252 raised MIC levels in certain MRSA strains remains unclear. However, possible alternate  
253 mechanisms includes overexpression of mutant chromosomally encoded genes of efflux  
254 pump such as *norA*, *norB* and *mepA* [8, 18]. An *in vitro* study demonstrated the increased  
255 expression of the efflux pump genes in clinical isolates when exposed to low concentrations  
256 of antiseptics [28]. Future studies are required to elucidate the differences in resistance  
257 mechanisms between MRSA strains. For ST22 and STs other than ST45, we further observed  
258 that the presence of *qacA/B* and *qacC* genes enhanced the effects of the respective MRSA  
259 clones on reduced susceptibility to chlorhexidine. Whilst the observed clonal predominance  
260 of *qac* genes corroborated with other studies [23, 29], the modifying effects of *qac* genes on  
261 the effects of specific MRSA clones on antiseptic resistance have not been reported  
262 previously.

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

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

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

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

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

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297 **Ethics approval**

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

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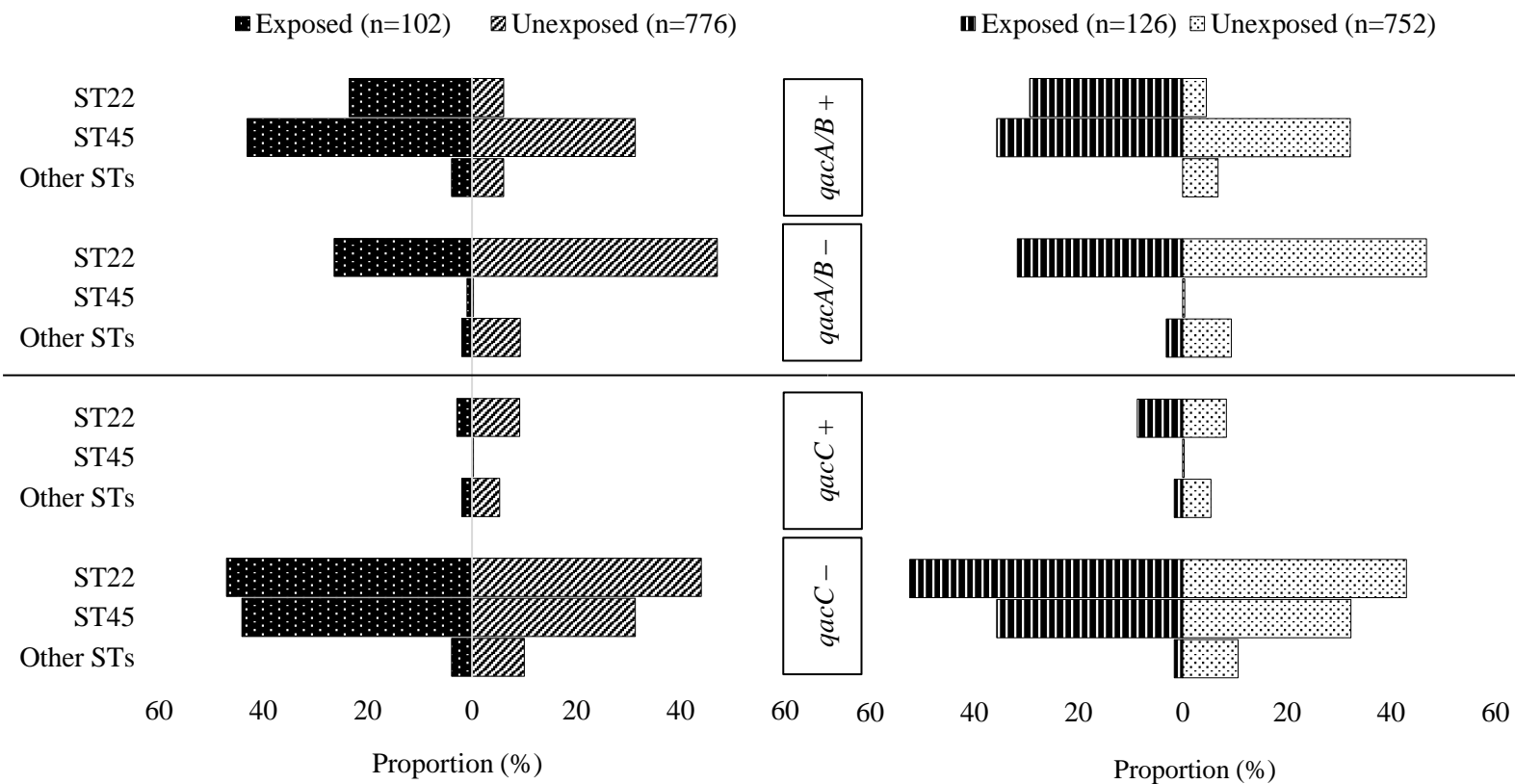
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397 **Figure 1.** Distribution of different methicillin-resistant *Staphylococcus aureus* sequence types carrying *qacA/B* & *qacC* genes based on **A.**

398 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		P <sub>1</sub>	Octenidine		P <sub>2</sub>
		Exposed	Unexposed		Exposed	Unexposed	
		isolates* (n = 102)	isolates* (n = 776)		isolates* (n = 126)	isolates* (n = 752)	
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 <sup>#</sup>	125 (14.3)	6 (5.9)	119 (15.3)		4 (3.2)	121 (16.1)	
Carriage of <i>qacA/B</i> genes	409 (46.6)	72 (70.6)	337 (43.4)	<0.001	82 (65.1)	327 (43.5)	<0.001
Carriage of <i>qacC</i> 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 <sup>†</sup>			–
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 $\geq$ 4mg/L	649 (73.9)	89 (87.3)	560 (72.2)	<0.01	–	–	
MIC level to octenidine				–			0.15 <sup>†</sup>

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 $\geq$ 2mg/L	79 (9.0)	–	–	–	7 (5.6)	72 (9.6)	0.14

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

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

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

405 P<sub>1</sub>; statistical test between chlorhexidine exposed and unexposed isolates

406 P<sub>2</sub>; statistical test between octenidine exposed and unexposed isolates

407 #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),

408 ST672 (n=1), ST1178 (n=5), ST1218 (n=2), ST1232 (n=1), NF (n=9)

409 †; Fisher's exact test

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

Variables	Total isolates (n = 878)	Isolates carrying <i>qac</i> genes [no./total no. (%)]	Crude odds ratio (95% CI)	P	Adjusted odds ratio <sup>a</sup> (95% CI)	P
<b><i>qacA/B</i> genes</b>						
<b>Chlorhexidine</b>	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)
	<b><i>qacC</i> genes</b>					
	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
<b><i>qacA/B</i> genes</b>						
<b>Octenidine</b>	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)
	<b><i>qacC</i> genes</b>					
	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

412 <sup>a</sup>adjusted for year, facility of MRSA isolate detection, duration of exposure and sequence type (categorized as ST22, ST45 and other STs)

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

414 *Staphylococcus aureus* isolates

Variables	Total isolates (n = 878)	Isolates with reduced antiseptic susceptibility <sup>a</sup> [no./total no. (%)]	Crude odds ratio (95% CI)	P	Adjusted odds ratio <sup>b</sup> (95% CI)	P
<b>Chlorhexidine exposure</b>						
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
<b>Octenidine exposure</b>						
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

415 <sup>a</sup>reduced antiseptic susceptibility is defined as MIC  $\geq$ 4mg/L for chlorhexidine, and MIC  $\geq$ 2mg/L for octenidine

416 <sup>b</sup>adjusted for year, facility of MRSA isolate detection, duration of exposure, sequence type (categorized as ST22, ST45 and other STs), and

417 presence of *qacA/B* and *qacC* genes

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

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



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

420 <sup>a</sup>reduced antiseptic susceptibility is defined as MIC  $\geq$ 4mg/L for chlorhexidine, and MIC  $\geq$ 2mg/L for octenidine.

421 <sup>b</sup>adjusted for year, facility of MRSA isolate detection, sequence types (categorized as ST22, ST45 and other STs), antiseptic exposure and

422 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

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

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<i>qacC</i> + & ST22	4 (1.8)	70 (10.8)	10.64 (3.53 - 32.02)	<0.001	5.99 (1.93 - 18.57)	<0.01
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425 <sup>a</sup>adjusted for year, facility of MRSA isolate detection, chlorhexidine exposure and duration of exposure