ORIGINAL RESEARCH

Distribution of Biocide Resistance Genes and Association with Clonal Complex Genotypes in *Staphylococcus aureus* Isolated from School-Age Children in Guangzhou

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Purpose: Chlorhexidine and mupirocin are often prescribed to children in affected communities to prevent colonization and transmission of *Staphylococcus aureus*, but this has led to an increasing rate of biocide resistance. In this study, we aimed to determine the distribution of biocide resistance genes among *S. aureus* isolates from school-age children in Guangzhou, investigate chlorhexidine gluconate and mupirocin susceptibility and clonal complex (CC) genotypes in strains carrying biocide-resistance genes, and further explore the role of biofilms in this resistance.

Patients and Methods: Antibiotic resistance and multilocus sequence genotyping were performed on 722 *S. aureus* isolates from previous study. The distribution of nine biocide genes (*qacA/B, mupA, mepA, sepA, norA, lmrS, smr, mupB, qacG*) was determined by PCR. Isolates carrying *qacA/B* or *mupA* genes were further tested for susceptibility to chlorhexidine gluconate (CHG) and mupirocin and biofilm formation abilities.

Results: The most prevalent of the nine biocide resistance genes were *mepA* (95.57%), followed by *norA* (78.81%), *lmrS* (77.01%), and *sepA* (58.17%). The *qacG* gene was not detected. Distribution of *sepA* was significantly decreased in CC30 and CC45 genotypes, and presence of *sepA* was associated with resistance to antibiotics such as CLI, ERY, TCY, SXT, CIP, and LVX. In addition, 64 (94.1%, n=68) *qacA/B+* isolates showed CHG resistance, 12 (100.0%, n=12) *mupA+* isolates were mupirocin resistant, and 4 (80%, n=5) and 5 (100%, n=5) *qacA/B+mupA+* isolates were CHG and mupirocin resistant, respectively. Of these 85 isolates, 98.8% (n=84) had different degrees of biofilm-forming abilities, which were positively associated with CHG and mupirocin resistance.

Conclusion: The distribution of biocide resistance genes was associated with special CCs. The *qacA/B* and *mupA* genes are highly associated with resistance to CHG and mupirocin, and biofilm formation was found to contribute to this biocide resistance.

Keywords: *Staphylococcus aureus*, biocide resistance genes, *qacA/B*, children, chlorhexidine, mupirocin, biofilm formation

Introduction

Staphylococcus aureus can cause a variety of infectious diseases, including moderate skin and soft tissue infections, fatal pneumonia, and sepsis. Additionally, the frequent occurrence of antibiotic resistance poses a great challenge for *S. aureus* prevention, management, and treatment.[1,2] Particularly, severe and invasive infections of community-associated methicillin-resistant *S. aureus* (CA-MRSA) pose a serious threat to one of the most vulnerable groups—children.[3–6]

S. aureus is reported to frequently colonize populations, and children in many regions have higher rates of *S. aureus* nasal colonization (up to 40%).[2,7–9] This is a significant issue as the colonization of *S. aureus* contributes to its transmission and increases the risk of infection. Decolonization agents, such as mupirocin and chlorhexidine, have

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been widely used in healthcare-associated MRSA infections, and have also been prescribed to children in community settings to prevent *S. aureus* infectious diseases. Mupirocin is a commonly used antiseptic that inhibits isoleucyl tRNA synthetase, thus preventing the protein synthesis of bacteria.[10] Chlorhexidine gluconate (CHG) can covalently bind to the *S. aureus* membrane and ultimately lead to cell depolarization and death. However, in recent decades, widespread biocide use has resulted in a general decrease in antibiotic susceptibility of *S. aureus*.[11–13]

With the deepening of our knowledge on the mechanism of MRSA drug resistance, an increasing number of antimicrobial agents, like pyrazole-based analogs, benzimidazole analogues, indole-based derivatives, and arylethene-sulfonyl fluorides, have recently proven efficient against MRSA.[14–17] At the same time, more factors associated with drug resistance of *S. aureus* also need to be discussed.

Evidence has shown that *S. aureus* strains exhibiting resistance to CHG and mupirocin are highly associated with chromosomally-encoded biocide resistance genes and plasmid-encoded multidrug resistance (MDR) efflux pumps. Plasmid-mediated genes such as *qacA/B* encode the major facilitator superfamily, whereas genes such as *smr* and *qacG* encode the small multidrug resistance family, which are the major MDR efflux pump proteins. Other plasmid-mediated genes such as *mupA* and *mupB* encode novel isoleucyl tRNA synthetases, thus conferring increased anti-microbial resistance of *S. aureus*, including to mupirocin.[10,18] In addition, chromosomally-encoded efflux genes such as *mepA, norA, lmrS, sepA* have been reported to provide bacteria with multidrug and biocide resistance or help them survive in severe conditions. The prevalence of biocide resistance genes is high among *S. aureus* strains worldwide.[19–21]

In addition, it is believed that biofilms, a type of dynamic and complex biological system, make *S. aureus* infections more challenging because they can increase the resistance of staphylococcal species to both the immune system and antibiotics.[22–24] Although some biocides targeting *S. aureus* biofilm mechanisms have been successfully developed and have showed promise,[25,26] it has also been reported that sub-inhibitory concentrations of CHG or mupirocin used in daily life may promote biofilm formation by *S. aureus*.[27,28] Therefore, the role of *S. aureus* biofilm formation in its resistance to biocides such as CHG and mupirocin needs to be further investigated.

Herein, we detected the distribution of nine biocide-resistant genes (*qacA/B, mupA, mepA, sepA, norA, lmrS, smr, mupB, qacG*) in 722 *S. aureus* isolates from nasal swabs of school-age children in Guangzhou, Southern China, and CHG and mupirocin resistance phenotypes, gene type characteristics, and biofilm formation ability in 85 *qacA/B* or *mupA* positive isolates were analyzed.

Materials and Methods

All methods were carried out in accordance with the relevant guidelines and regulations.

1. Bacterial isolates. A total of 722 *S. aureus* strains isolated from nasal swabs of 2375 school-age students (6–18 years) in Guangzhou were recovered between October and November 2018[29] (22 isolates were excluded due to quality problems caused by long-term storage). All the participants were recruited voluntarily and their guardians provided written informed consent. This study was approved by The Ethics Committee of Guangzhou Woman and Children's Medical Center (registration no. 2016081029). All *S. aureus* isolates were identified and detected for the presence of *femB* and *mecA*. Resistance to ten conventional antibiotics had been previously determined (PEN, penicillin; CLI, clindamycin; ERY, erythromycin; TCY, tetracycline; SXT, sulfamethoxazole-trimethoprim; RIF, rifampicin; CIP, ciprofloxacin; LVX, levofloxacin; MFX, moxifloxacin; GEN, gentamicin).[29]

2. Detection of biocide resistance genes by PCR. Total DNA was extracted and nine genes (*qacA/B, mupA, mepA, sepA, mupB, smr, norA, lmrS, qacG*) were amplified by polymerase chain reaction (PCR) as previously described.[30] The nucleotide sequences of the primers used in PCR are listed in Table 1. Positive amplicons were randomly selected and sent for sequencing at BGI (Shenzhen, China), and BLAST on the NCBI website (www.ncbi.nlm.nih.gov) was used to confirm sequence consistency. Finally, partial nucleotide sequences of eight genes (*qacA/B, mupA, mepA, sepA, mupB, smr, norA, lmrS*) were submitted to GenBank repository (https://www.ncbi.nlm.nih.gov/).

3. Chlorhexidine gluconate and mupirocin susceptibility test. Broth microdilution was used to test CHG and mupirocin susceptibility according to the Clinical and Laboratory Standards Institute (CLSI) M07.[31]

Table 1 Sequences of Primers for PCR of Biocide Resistance Genes

Gene	Annealing Temperature (°C)	Primer	Sequence (5'-3')	Size (bp)	References
qacA/B	53.725	F	ATGCCTTATATTTATTTAATAATAGCC	321	20
		R	ATGCGATGTTCCGAAAATGTTTAAC		
mupA	54.215	F	TATATTATGCGATGGAAGGTTGG	458	13
		R	AATAAAATCAGCTGGAAAGTGTTG		
mupB	53.985	F	CTAGAAGTCGATTTTGGAGTAG	674	53
		R	AGTGTCTAAAATGATAAGACGATC		
mepA	53.985	F	ATGTTGCTGCTGCTCTGTTC	718	20
		R	TCAACTGTCAAACGATCACG		
sepA	53.875	F	GCAGTCGAGCATTTAATGGA	103	20
		R	ACGTTGTTGCAACTGTGTAAGA		
smr	51.86	F	ATAAGTACTGAAGTTATTGGAAGT	285	20
		R	TTCCGAAAATGTTTAACGAAACTA		
norA	52.325	F	TTCACCAAGCCATCAAAAAG	620	20
		R	CTTGCCTTTCTCCAGCAATA		
qacG	49.625	F	CAACAGAAATAATCGGAACT	321	44
		R	TACATTTAAGAGCACTACA		
lmrS	59.43	F	GCAAGCTTATGGCTAAAGTTGAATTAACAAC	1441	20
		R	GCGGATCCTTAAAATTTCCTTCTATTACTTT		

Susceptibility to CHG was tested using a twofold dilution series from 32 to 0.0625 mg/L, starting with a 20% (w/v) CHG solution (Macklin, Shanghai, China).[32] Isolates were considered susceptible to CHG if the MIC was <4 mg/L and had a tolerance to CHG at MIC ≥4 mg/L.[33] Mupirocin susceptibility was determined by another twofold dilution series from 1024 mg/L to 1 mg/L (Macklin), which was classified into three categories, depending on the MIC to mupirocin: ≤4 mg/L as mupirocin-susceptible, 8–256 mg/L as low-level mupirocin resistance (LLMR), and ≥512 mg/L as high-level mupirocin resistance (HLMR).[34]

4. Biofilm formation using tissue culture plate (TCP) assay. The biofilm formation test in this study was based on Stepanovic's recommendations and minimally modified.[35] Briefly, *S. aureus* isolates from fresh Columbia blood agar plates were inoculated into 5 mL Trypticase Soy broth (TSB) and incubated on a 220-rpm shaker overnight at 37 °C. The cultures were then diluted to 1:50 with TSB containing 0.25% glucose, and 200 μL was added (in triplicate) to sterile 96-U-bottom polystyrene plates for 18 h at 37 °C. Uninoculated wells containing TSB and glucose served as negative controls. Wells were washed with PBS solution three times and dried at 60 °C for 1 h; then, 1% crystal violet was used to stain adherent bacteria for 10–15 minutes. After washing three times with distilled water to remove excess stain and allowing them to dry at room temperature, a microplate reader (Multiskan FC, Thermo Fisher, USA) was used to measure optical density (OD) at 570 nm. ODc = average OD of negative control + (3×SD of negative control); biofilm formation ability was divided into four degrees: non (OD≤ODc), weak (ODc<OD≤2ODc), moderate (2ODc<OD≤4ODc), and strong (4ODc<OD).

5. Multilocus sequence typing (MLST), clonal complex (CC) genotyping, and data analysis. Multilocus sequence typing numbers were determined previously,[29] and the eBURST v.3 program (https://www.mlst.net/eburst/) was used to conduct CCs. All data were entered and analyzed using GraphPad Prism 8.0.1 (GraphPad Software Inc,

San Diego, CA, USA) and categorical variables were compared using Fisher's exact test. The results were considered statistically significant at P<0.05.

Results

Prevalence and Distribution Characteristics of Biocide Resistance Genes

S. aureus isolates were collected from nasal swabs of school-age students in Guangzhou, where carriers were recruited from senior high school (n=192, 26.59%), junior middle school (n=182, 25.21%), and elementary school (n=348, 48.20%). The overall prevalence of *qacA/B, smr, norA, mepA, sepA, mupA, mupB*, and *lmrS* was 10.11% (n=73), 1.39% (n=10), 78.81% (n=569), 95.57% (n=690), 58.17% (n=420), 2.35% (n=17), 0.42% (n=3), 77.01% (n=556), respectively. Of note, five isolates harbored both *qacA/B* and *mupA* (*qacA/B+mupA+*) and four isolates harbored both *qacA/B* and *smr* (*qacA/B+smr+*). The *qacG* gene was not detected. The prevalence of all these genes in the MRSA and methicillin-susceptible S. aureus (MSSA) populations showed no significant difference (P>0.05) (Figure 1A).

Compared to the other educational age groups, elementary school children had lower *sepA* (53.7%) and *qacA/B* (7.18%) carrier rates, and senior school students had a higher prevalence of *sepA* (67.18%) and *norA* (92.18%) (P<0.05). Additionally, a significantly lower prevalence of *mepA* (91.76%), *norA* (69.78%), and *lmrS* (66.48%) and a higher prevalence of *qacA/B* (15.93%) and *mupA* (6.04%) were found in junior school children (P<0.05) compared to other populations (Figure 1B). Furthermore, among all CC genotype groups, the distribution of *sepA* and *norA* was significantly lower in CC30 groups and higher in CC45 groups (P<0.05), while the distribution of *qacA/B* was significantly higher in the CC30 genotype (P<0.05) (Figure 1C).

Association Between Biocide Resistance Genes and Antibiotic Resistance

Table 2 shows the association between ten biocide resistance genes and conventional antibiotic resistance among 722 *Staphylococcus aureus* isolates. The *qacA/B* and *mupA* genes were associated with *S. aureus* resistance to clindamycin (P = 0.0004, P = 0.0253) and erythromycin (P = 0.006, P = 0.0216), the *mepA* gene was associated with penicillin (P = 0.0163) and clindamycin (P = 0.0001) resistance, and the *norA* gene was correlated with resistance to penicillin (P = 0.0011), clindamycin (P = 0.0002), and erythromycin (P = 0.0012). In addition, *sepA+* isolates were more frequently resistant to clindamycin, erythromycin, tetracycline, sulfamethoxazole-trimethoprim, ciprofloxacin, and levofloxacin (P<0.05). No additional antibiotic associations were found for *smr, mupB* and *lmrS* genes.

CHG and Mupirocin Resistance and Its Distribution by CC Genotype

In 722 *S. aureus* isolates, 85 isolates carrying *qacA/B* or *mupA* were screened to test the susceptibility to CHG and mupirocin (68 isolates carried *qacA/B* but no *mupA* gene, 12 isolates carried only the latter, and 5 isolates carried both). Among 68 isolates carrying *qacA/B* only, 64 (94.1%) were resistant to CHG and 4 (5.9%) were susceptible to CHG. All 12 isolates carrying only *mupA* were resistant to mupirocin, including seven (58.3%) LLMR and five (41.7%) HLMR isolates. Among the five isolates carrying both genes, four (80%) were resistant to CHG, and five (100%) were HLMR (Table 3).
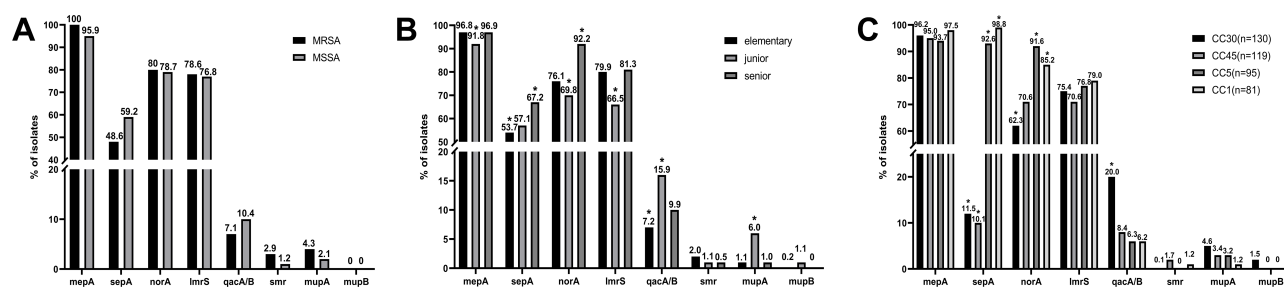

Figure 1 The distribution of biocide resistance genes in different groups. (**A**) MRSA and MSSA, (**B**) educational ages, and (**C**) four main clonal complex (CC) genotypes among 722 *S. aureus* isolates from school-age students in Guangzhou. The data shown above the column are percentages in that population. "*" indicates statistics which were significantly different (P<0.05).

Table 2 Association Between ten Biocide Resistance Genes and Conventional Antibiotic Resistance Among 722 *Staphylococcus Aureus* Isolates

Antimicrobial Agents	Susceptibility	qacA/B		smr		mupA		mupB		mepA		sepA		lmrS		norA	
		Positive (%)	P value	Positive (%)	P value	Positive (%)	P value	Positive (%)	P value	Positive (%)	P value	Positive (%)	P value	Positive (%)	P value	Positive (%)	P value
PEN	R (n=618)	64(10.4)	0.7257	9(1.5)	>0.9999	17(2.8)	0.1523	3(0.5)	>0.9999	596(96.4)	**0.0163***	353(57.1)	0.1971	470(76.1)	0.1655	475(76.9)	**0.0011***
	S (n=104)	9(8.7)		1(1.0)		0(0)		0(0)		94(90.4)		67(64.4)		86(82.7)		94(90.4)	
CLI	R (n=295)	44(15.0)	**0.0006***	5(1.7)	0.7478	12(4.1)	**0.0216***	3(1.0)	0.0678	292(99.0)	**0.0001***	105(35.6)	**<0.0001***	227(77.0)	>0.9999	212(71.9)	**0.0002***
	S (n=427)	29(6.8)		5(1.2)		5(1.2)		0(0)		398(93.2)		315(73.8)		329(77.0)		357(83.6)	
ERY	R (n=311)	46(14.7)	**0.0004***	5(1.6)	0.7524	12(3.9)	**0.0253***	3(1.0)	0.0795	301(96.8)	0.2023	121(38.9)	**<0.0001***	241(77.5)	0.8583	227(73.0)	**0.0012***
	S (n=411)	27(6.6)		5(1.2)		5(1.2)		0(0)		389(94.6)		299(72.7)		315(76.6)		342(83.2)	
TCY	R (n=60)	6(10)	>0.9999	2(3.3)	0.1992	3(5)	0.1616	0(0)	>0.9999	55(91.7)	0.17	51(85.0)	**<0.0001***	42(70.0)	0.1982	47(78.3)	0.8698
	S (n=662)	67(10.1)		8(1.2)		14(2.1)		3(0.1)		635(95.9)		369(55.7)		514(77.6)		522(78.9)	
SXT	R (n=24)	1(4.2)	0.499	0(0)	>0.9999	1(4.2)	0.4413	0(0)	>0.9999	24(100)	0.6191	21(87.5)	**0.0026***	18(75)	0.8064	20(83.3)	0.7999
	S (n=698)	72(10.3)		10(1.4)		16(2.3)		3(0)		666(95.4)		399(57.2)		538(77.1)		549(78.7)	
RIF	R (n=12)	0(0)	0.6219	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	11(91.7)	0.4221	4(33.3)	0.1367	9(75)	0.743	10(83.3)	>0.9999
	S (n=710)	73(10.3)		10(1.4)		17(2.4)		3(0)		679(96.6)		416(58.6)		547(77.0)		559(78.7)	
CIP	R (n=7)	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	7(100)	>0.9999	7(100)	**0.0455***	6(85.7)	>0.9999	5(71.4)	0.6366
	S (n=715)	73(10.2)		10(1.4)		17(2.4)		3(0)		690(96.5)		420(58.7)		556(77.8)		564(78.9)	
LVX	R (n=7)	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	7(100)	>0.9999	7(100)	**0.0455***	6(85.7)	>0.9999	5(71.4)	0.6366
	S (n=715)	73(10.2)		10(1.4)		17(2.4)		3(0)		690(96.5)		420(58.7)		556(77.8)		564(78.9)	
MFX	R (n=5)	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	0(0)	>0.9999	5(100)	>0.9999	5(100)	0.0785	5(100)	0.5945	4(80)	>0.9999
	S (n=717)	73(10.2)		10(1.4)		17(2.4)		3(0)		685(95.5)		415(57.9)		551(76.8)		555(77.4)	
GEN	R (n=4)	1(25)	0.3477	0()	>0.9999	1(25)	0.0911	0(0)	>0.9999	4(100)	>0.9999	4(100)	0.1441	3(75)	>0.9999	3(75)	>0.9999
	S (n=718)	72(10.0)		10(1.4)		16(2.2)		3(0)		686(95.5)		416(57.9)		566(78.8)		566(78.8)	

Note: *In bold indicates statistics were significantly different (P<0.05).
Abbreviations: R, resistant; S, susceptible.

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Table 3 The Susceptibility to CHG & Mupirocin and Biofilm Formation Ability of *qacA/B* and *mupA* Genes Positive Isolates

Categories	Degree	*qacA/B+* (n=68) (%)	*mupA+* (n=12) (%)	*qacA/B+mupA+*(n=5) (%)
CHG susceptibility	S	5(5.9)	–	1(20)
	R	68(94.1)		4(80)
Mupirocin susceptibility	S	–	0(0)	0(0)
	L		7(58.3)	0(0)
	H		5(41.7)	5(100)

Abbreviations: *qacA/B+*, *S. aureus* isolates carried *qacA/B* gene but no *mupA* gene; *mupA+*, *S. aureus* isolates carried *mupA* gene but no *qacA/B* gene; *qacA/B+mupA+*, *S. aureus* isolates carried *qacA/B* and *mupA* genes simultaneously; S, susceptible; R, resistant; L, low-level resistance; H, high-level resistance.

Next, 68 and 17 isolates resistant to CHG and mupirocin, respectively, were divided to determine their distribution by CC genotype. Among the isolates resistant to CHG, the top nine CCs were CC30 (36.76%), CC45 (14.72%), CC5 (8.82%), CC1 (7.35%), CC398 (7.35%), CC22 (4.41%), CC59 (4.41%), CC15 (4.41%), and CC7 (4.41%), whereas the other CCs included only one isolate (Figure 2A). Among the 17 isolates resistant to mupirocin, the main CCs were CC30 (29.41%), CC45 (23.53%), and CC5 (17.65%), and the others included only one isolate (Figure 2B).

Biofilm Formation Among *qacA/B* and *mupA* Positive Isolates

A tissue culture plate (TCP) assay was used to assess the biofilm-forming ability of 85 isolates carrying *qacA/B* or *mupA*. Overall, 84 (98.8%) isolates produced biofilms, and one (1.2%) isolate did not. Among the biofilm-producing isolates, 40 (47.1%) produced weak biofilms, 37 (43.5%) produced moderate biofilms, and seven (8.2%) isolates produced strong biofilms.

Among the 73 *qacA/B* positive isolates, 7 (9.6%) were firmly adherent, 34 (47.2%) were moderately adherent, 31 (42.5%) were weakly adherent, and 1 (1.4%) was non-adherent; among the 17 *mupA* positive isolates, 8 (47.1%) were moderate biofilm producers, and 9 (52.9%) isolates produced weak biofilms. To meet the condition of all expected values being greater than 1.0 and at least 20% of the expected values being greater than 5, we combined non and weak biofilm producers into a "+" group and moderate and strong biofilm producers into a "++" group (Table 4). The relationship between biofilm formation ability and the resistance of S. *aureus* to CHG and mupirocin was discovered. The results

A CC genotype distribution of CHG resistance isolates(n=68)
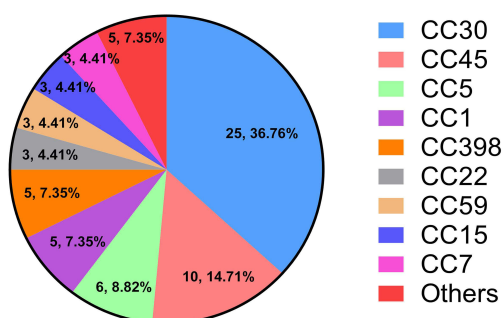
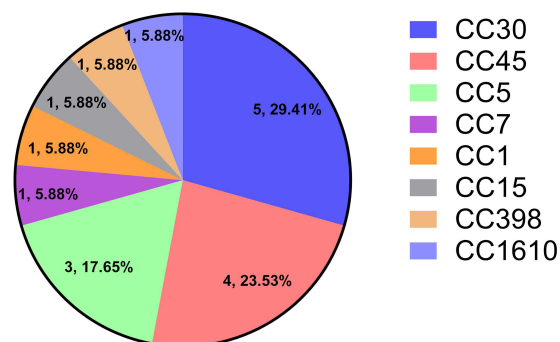**B CC genotype distribution of mupirocin resistance isolates (n=17)**

Figure 2 CC genotype distribution of (**A**) CHG and (**B**) mupirocin resistance isolates. "Others" represent CC8, CC25, CC121, CC944 and CC1610 genotypes; each includes only one isolate.

Table 4 Relationship Between CHG & Mupirocin Susceptibility and Biofilm Formation Ability Among *qacA/B* & *mupA* Positive Isolates

Isolates	Antimicrobial Agents	Susceptibility	Biofilm Formation Ability				P value
			+ (%)		++ (%)		
			Weak	Non	Moderate	Strong	
qacA/B gene positive (n=73)	CHG	S (n=5)	1(20)	4(80)	0(0)	0(0)	**0.0134***
		R (n=68)	0(0)	27(39.7)	34(50)	7(10.3)	
mupA gene positive (n=17)	Mupirocin	L (n=7)	0(0)	6(85.7)	1(14.3)	0(0)	**0.0498***
		H (n=10)	0(0)	3(30)	7(70)	0(0)	

Note: *In bold indicates statistics were significantly different (P<0.05).
Abbreviations: S, susceptible; R, resistant; L, low-level resistance; H, high-level resistance; +, non and weak biofilm producers; ++, moderate and strong biofilm producers.

showed that among *qacA/B*- and *mupA*- positive isolates, biofilm formation ability was positively associated with resistance to CHG and mupirocin (P<0.05).

Discussion

Over the past decade, the susceptibility of *S. aureus* to CHG and mupirocin has been decreasing, mainly owing to the prevalence of a variety of biocide resistance genes.[34,36,37] It has been reported that these genes on plasmids or chromosomes are distributed worldwide and differ significantly throughout the different regions infected with *S. aureus*.[38] From the data available, the plasmid-mediated *qacA/B* and *smr* genes prevailed at 35.4–41.6%, with 0–31% of *S. aureus* in the southern Asian region.[38–40] In our study, only 10.11% and 1.39% of isolates carried *qacA/B* and *smr* genes, respectively, and 0.55% (n=5) of isolates carried both genes simultaneously, which was lower than that reported by McNeil et al for the Texas children's hospital at 22.7% and 33.1% of isolates harboring *qacA/B* and *smr* genes, respectively, and 10.9% carrying both simultaneously.[41] This could likely be owing to the lower prevalence of these genes in community-associated isolates compared to hospital-associated isolates. In addition, sporadic harboring of the *smr* gene is thought to confer less advantageous resistance compared to *qacA/*B.[40] Plasmid-mediated genes, *mupA* and *mupB*, were detected in 2.35% and 0.42% of the isolates, respectively, and this prevalence was similar to that of clinical *S. aureus* isolates in children from Iran and France.[42,43] The *qacG* gene has been rarely reported, except in strains isolated from food sources and animals,[44,45] and likewise, no *qacG* gene was found in any of the 722 S. aureus isolates analyzed in this study.

In parallel, a high distribution of chromosomally-encoded efflux genes, *mepA* (95.57%) and *norA* (78.81%), was determined in the present study, which was similar to those found in Europe, Africa, and Asia.[19–21] However, compared to some European and African studies,[19,20] a lower prevalence of *lmrS* (77.01%) and *sepA* (58.17%) was found in *S. aureus* isolates from children's nasal swabs in Guangzhou, suggesting that the distribution of these genes varied by region. Overall, we provided updated data on the prevalence of biocide resistance genes among *S. aureus* colonies in the nasal cavity of school-aged children in Guangzhou, which showed large regional differences.

In addition, we found that the distribution of biocide resistance genes among *S. aureus* was related to the educational age and CC groups in this study. Although *qacA/B* or *smr* genes were reported to be more common in MRSA populations,[46,47] our results showed that MRSA and MSSA populations have a similar distribution of these genes (P>0.05).

To the best of our knowledge, the relationship between biocide resistance gene distribution among *S. aureus* and children's educational age has scarcely been investigated. We found that among school-age students from elementary school through to senior school, junior school students had a significantly higher prevalence of *qacA/B* and *mupA* genes and senior school students had a higher prevalence of *norA* and *sepA* genes (P<0.05), suggesting that there may be a wide distribution of biocide resistance genes in children of specific age groups.

MDR efflux pumps encoded by genes such as *qacA/B, mepA, sepA, norA, lmrS, smr, qacG* may lead to high-level resistance of *S. aureus* to multiple antibiotics.[38] We found that *qacA/B* was related to resistance to erythromycin and clindamycin, which was consistent with the findings of Teresa et al[19] However, unlike the known roles of *mepA* and *norA* in *S. aureus* to confer fluoroquinolone resistance (norfloxacin and ciprofloxacin),[38,48] the present study discovered that the *mepA* gene in *S. aureus* was positively associated with penicillin and clindamycin resistance, whereas the *norA* gene was inversely associated with resistance to penicillin, clindamycin, and erythromycin. However, these findings need to be further verified using a larger sample size. The *smr* gene has been reported to be related to ciprofloxacin resistance in other studies,[41] but it was not detected in our study, probably owing to its low prevalence.

Previously, Isa et al revealed that higher MICs of chlorhexidine were exhibited among *S. aureus* isolates from CC5 and CC22 groups, which originated from humans and animals in Germany,[49] suggesting that the phenotype or genotype of biocide resistance may also be related to CC groups.

It is worth noting that among all CC genotypes of the 722 *S. aureus* isolates, the distribution of *sepA* was significantly low in CC30 (11.5%) and CC45 (10.1%) groups and high in CC5 (92.6%) and CC1 (98.8%) groups (P<0.05), while the distribution of *qacA/B* (20.0%) and *mupA* (4.6%) genes had the highest proportion in the CC30 group. In addition, in this study we found that *sepA* was inversely associated with resistance to CLI and ERY, while *qacA/B* and *mupA* were positively associated with resistance to these antibiotics. These results explain our previous findings that CC30 genotypes had the highest proportion of ERY-and CLI-resistant *S. aureus* isolates, while CC1 possessed the lowest proportion thereof.[29] Moreover, we also revealed that the reason for the lower prevalence of *sepA* (58.17%) among *S. aureus* isolates found in Guangzhou compared to other regions (often as high as 90%)[19,20] was owing to varying distributions of this gene by region. The *sepA* gene was prevalent in CC5 (92.6%) and CC1 (98.8%) groups, but sporadically distributed in CC30 (11.5%) and CC45 (10.1%) groups (P<0.05), while CC30 and CC45 were the main *S. aureus* CC genotypes carried by children in Guangzhou.

Considering *qacA/B* and *mupA* are well known to be highly associated with resistance to CHG and mupirocin,[30,50] 85 isolates carrying *qacA/B* or *mupA* were selected to further test their phenotypes. The three major CC genotypes for CHG and mupirocin resistance were CC30, CC45, and CC5; this trend was consistent with the distribution of the whole population.[29] In this study, 64 *qacA/B* genes were associated with CHG resistance in 94.1% (n=68) of *S. aureus* isolates and the *mupA* gene was associated with resistance to mupirocin in 100% (n=12) of the isolates (including 7 LLMR and 5 HLMR), which verified that the *qacA/B* and *mupA* genes were highly resistant to chlorhexidine and mupirocin. Notably, we found five *S. aureus* isolates harboring both *qacA/B* and *mupA* genes, four (80%) resistant to CHG, and five (100%) highly resistant to mupirocin, suggesting that the *qacA/B* gene may also increase high-level mupirocin resistance among *mupA+ S. aureus* isolates. Furthermore, we discovered whether the co-occurrences of *qacA/B* and *mupA* may also promote resistance to multiple antibiotics; among the five *qacA/B+mupA+* isolates, three (60%) isolates were resistant to ERY and CLI, one (20%) isolate was resistant to OXA, SXT, and TCY, and two (40%) were intermediately resistant to RIF; this resistance rate was higher than when the two genes were present alone, but the underlying mechanism needs to be further explored.

Biofilms have been receiving much attention because of their association with resistance to antibiotic agents in *S. aureus*; strains within biofilms are thought to be hundreds of times more resistant to antibiotics than planktonic strains.[23,51] It was previously reported that a larger proportion (up to 90%) of *S. aureus* produced biofilms from nasal swabs compared to clinical isolates;[52] in this study, we also found a high proportion of biofilm producers (98.8%) among *qacA/B*-positive and *mupA*-positive *S. aureus* isolates from nasal swabs of school-age children. Similar to the study by Barakat et al,[53] we suggested that the biofilm formation ability of *S. aureus* was positively correlated with its resistance to CHG and mupirocin (P<0.05). This finding raises additional concerns about the potential risks of *S. aureus* nasal colonization in school-aged children.

This study has several limitations. We only performed susceptibility to CHG and mupirocin and biofilm formation tests among *S. aureus* isolates carrying *qacA/B* and *mupA*. Thus, comparative analysis or a larger sample size will enhance the reliability of our results. Subsequently, we plan to further determine the distribution characteristics of *S. aureus* resistance to mupirocin and chlorhexidine in school-age children in Guangzhou and investigate the underlying mechanisms of mupirocin and CHG resistance in *S. aureus*. Otherwise, in the face of the complex mechanism of *S. aureus'* antibiotic resistance, the development of drugs targeting its structure-activity relationship is expected in future.

Conclusion

This study revealed the distribution characteristics of biocide resistance genes among *S. aureus* isolates colonizing the nasal cavity of school-aged children in Guangzhou. A significant association was observed between the distribution of these genes, educational age, and specific CCs. Furthermore, we verified that *qacA/B* and *mupA* could be highly resistant to chlorhexidine and mupirocin, and preliminarily discovered that biofilms formed by *S. aureus* contribute to this resistance. Our results suggest that chlorhexidine and mupirocin are still effective against the decolonization of *S. aureus* in children in Guangzhou, but it should be noted that some biocide resistance genes are widely carried and closely related to multidrug resistance. Moreover, this study indicated that the biofilm-forming ability of *S. aureus* should receive more attention for the prevention and management of community and nosocomial infections.

Abbreviations

S. aureus, Staphylococcus aureus; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; CHG, chlorhexidine gluconate; MDR, multidrug resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; TCP, tissue culture plate; TSB, trypticase soy broth; PBS, phosphate-buffered saline; PEN, penicillin; CLI, clindamycin; ERY, erythromycin; TCY, tetracycline; SXT, sulfamethoxazole-trimethoprim; RIF, rifampicin; CIP, ciprofloxacin; LVX, levofloxacin; MFX, moxifloxacin; GEN, gentamicin; PCR, polymerase chain reaction; OD, optical density; LLMR, low-level mupirocin resistance; HLMR, high-level mupirocin resistance.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available in the GenBank repository (https://www.ncbi.nlm.nih.gov/), the GenBank accession numbers for the eight partial nucleotide sequences in this study were below: ON448386 (*sepA*), ON448387 (*lmrS*), ON448388 (*mupA*), ON448389 (*qacA/B*), ON448390 (*mepA*), ON448391 (*norA*), ON448393 (*smr*), ON448392 (*mupB*).

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (registration no. 2016081029). All the participants were recruited voluntarily and their guardians have provided written informed consent. We declare that our study complies with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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