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Short Communication

Molecular study of resistance of *Staphylococcus aureus* to antiseptic quaternary ammonium compounds

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ABSTRACT

Objectives: This study determined the prevalence of *qac* and *smr* genes in clinical *Staphylococcus aureus* isolates from hospital-acquired infections and their susceptibility to quaternary ammonium compounds (QACs) and antibiotics, and correlated the presence of antiseptic resistance genes with antibiotic resistance.

Methods: Susceptibility of 150 non-duplicate clinical *S. aureus* isolates to antimicrobials and benzalkonium chloride (BAC) was determined by disk diffusion and MIC method, respectively. Resistant strains were analysed by multiplex PCR for the presence of *qac* and *smr* genes.

Results: Reduced susceptibility to BAC was detected in 30% of isolates (MIC cut-off >8 mg/L). QAC resistance genes were detected in 13 isolates with reduced BAC susceptibility. The most frequently detected genes were *qacA*/*B* (10 isolates; 22.2%), followed by *qacJ* (10; 22.2%), *smr* (8; 17.8%), *qacG* (8; 17.8%) and *qacH* (3; 6.7%). There was a strong positive correlation between presence of QAC resistance genes and higher BAC MIC associated with *qacA*/*B*, *qacJ* and *smr* genes. There was a statistically significant prevalence of antiseptic resistance genes among isolates resistant to cefoxitin, ciprofloxacin, clindamycin, oxacillin, tetracycline and erythromycin.

Conclusion: This study highlights the prevalence of *qac* and *smr* genes in clinical *S. aureus* isolates with resistance to QACs. There was an association between the presence of antiseptic resistance genes and resistance to different antibiotics, which may be attributed to the presence of both groups of genes on the same plasmid or to selection of resistant strains. More studies are needed on the clinical relevance of the presence of genes controlling resistance to antiseptics.

1. Introduction

Hospital-acquired infections (HAIs) are a major threat affecting hospitalised patients, especially with the emergence of antimicrobial resistance. Use of antiseptic solutions such as quaternary ammonium compounds (QACs) is among the infection control measures for HAIs. QACs can be used as antiseptic solutions for the hands and as disinfectants for inanimate surfaces [1]. QACs possess biological detergent activity owing to their cationic composition as well as antimicrobial activity by the *N*-alkyl chain that exhibits lipophilic activity towards bacteria and fungi [2]. QAC activity can also be mediated through disruption of enzymes and denaturation of proteins in microbial cells [3]. However, use of these compounds without subsequent washing with water leads to prolonged exposure of micro-organisms to low concentrations of QACs, with survival of resistant clones [4]. Reduced susceptibility to QACs may be mediated by the presence of *qac* genes that lead to over-expression of efflux pump activity resulting in the extrusion of antibiotics and biocides actively out of the bacterial cell [5]. Common genes linked to resistance to QACs are *qacA*, *qacB*, *qacC*, *qacD*, *ebr* and *smr*. Studies of the structures of these genes have led to their classification into two families, namely *qacA* and *smr* [6].

In *Staphylococcus* spp., *qac* genes do not confer resistance to benzalkonium chloride (BAC). This is a matter of terminology as resistance is defined relative to a clinical breakpoint, whereas for disinfectants and biocides there are no breakpoints but only epidemiological cut-offs (ECOFFs) [7].

The goals of the present study were (i) to determine the prevalence of *qac* and *smr* genes in clinical isolates of *Staphylococcus aureus* from HAIs, (ii) to determine the susceptibility of *S. aureus* to QACs and antibiotics and (iii) to correlate the presence of antiseptic resistance genes with antibiotic resistance.

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2. Materials and methods

2.1. Study design

This was a cross-sectional study of *S. aureus* isolates from Mansoura University Hospital (Mansoura, Egypt) from January 2017 to March 2018. The study was approved by Mansoura Faculty of Medicine Ethical Committee. The study included 150 non-duplicate *S. aureus* strains isolated from different types of HAIs (70 blood culture isolates, 30 wound culture isolates, 30 isolates from pneumonia and 20 urine culture isolates). Strains were identified using a VITEK®2 automated system (bioMérieux, Inc, Hazelwood, MO) according to the manufacturer's guidelines.

Methicillin-resistant *S. aureus* (MRSA) was identified as resistant to cefoxitin and/or oxacillin by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. The reference strains *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively.

Minimum inhibitory concentrations (MICs) of the *S. aureus* isolates to QACs were determined by the microbroth dilution method. Detection of antiseptic resistant genes was performed by multiplex PCR.

2.2. Antimicrobial susceptibility testing by the disk diffusion method

Antimicrobial susceptibility testing was performed by the disk diffusion method using the following antibiotic disks (Oxoid Ltd., Basingstoke, UK): cefoxitin (30 μg); oxacillin (5 μg); penicillin (10 IU); ciprofloxacin (10 μg); trimethoprim/sulfamethoxazole (1.25/23.75 μg); gentamicin (10 μg); clindamycin (2 μg); tetracycline (30 μg); erythromycin (15 μg); and vancomycin (30 μg).

2.3. Minimum inhibitory concentration determination for benzalkonium chloride

S. aureus ATCC 43300 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively.

The concentration range used for BAC (Sigma-Aldrich, St Louis, MO) ranged from 0.5 mg/L to 64 mg/L. Two-fold serial dilutions of BAC in Mueller–Hinton broth were made in a microdilution plate and were inoculated with a 1/10 dilution of *S. aureus* suspension of 5×10^6 CFU/mL. The plate was incubated at 37 °C for 24 h and the MIC was reported for each strain as the lowest concentration with no visible growth. The ECOFF used in the present study was defined as the upper limit of the normal MIC of BAC for *S. aureus*, determined previously to be 6 mg/L [9]. Owing to differences in media and methods as well as differences between laboratories that possibly influence susceptibility testing, in the present study the ECOFF was determined to be ≥8 mg/L.

The presence of QAC resistance genes was determined by molecular method for *S. aureus* isolates with reduced susceptibility to BAC.

2.4. Molecular studies of Staphylococcus aureus quaternary ammonium compound resistance genes

S. aureus isolates with resistance to BAC were subjected to molecular studies for the presence of QAC resistance genes.

2.4.1. DNA extraction

DNA was extracted from pure *S. aureus* colonies using a QIAamp® Mini DNA Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.4.2. Multiplex PCR for antiseptic resistance genes

Sequences of the PCR primers used for multiplex PCR for antiseptic resistance genes are summarised in Table 1. The multiplex PCR included two protocols, one for the detection of *qacA/B* and *smr* genes and other for *qacG*, *qacH* and *qacJ* genes. The QIAGEN amplification mixture was used according to a previously described protocol [10]. Electrophoresis was performed using a 1% gel containing ethidium bromide and the bands were visualised under ultraviolet light.

Table 1
Primer sequences used for detection of antiseptic resistance genes.

Gene	Primer sequence	Amplicon size (bp)
qacA/B	5′-GCAGAAAGTGCAGAGTTCG-3′ 5′-CCAGTCCAATCATGCCTG-3′	361
smr	5′-GCC ATA AGT ACT GAA GTT ATT GGA-3′ 5′-GAC TAC GGT TGT TAA GAC TAA ACC T-3′	195
qacG	5′-CAA CAG AAA TAA TCG GAA CT-3′ 5′-TAC ATT TAA GAG CAC TACA-3′	275
qacH	5′-ATA GTC AGT GAA GTA ATA G-3′ 5′-AGT GTG ATG ATC CGA ATG T-3′	295
qacJ	5′-CTT ATA TTT AGT AAT AGC G-3′ 5′-GAT CCA AAA ACG TTA AGA-3′	301

2.5. Statistical analysis

Data were collected, revised, coded and entered into IBM SPSS Statistics v.24.0 (IBM Corp., Armonk, NY). Qualitative data are presented as the number and percentage. Comparison between the studied groups was done by the χ^2 test, and a *P*-value of <0.05 was considered statistically significant.

3. Results

This study included 150 non-duplicate clinical *S. aureus* isolates. Owing to differences in media and methods as well as differences between laboratories that possibly influence susceptibility testing, in the present study the ECOFF was determined to be ≥8 mg/L.

Reduced susceptibility to BAC at MIC > 8 mg/L was detected in 30% of isolates (45/150). MRSA represented 58% of isolates. Fig. 1 summarises the distribution of BAC MICs among the 150 *S. aureus* isolates. Reduced susceptibility to BAC was most frequent with an MIC concentration of ≥8 mg/L (53.3%).

QAC resistance genes were detected in 13 of the 45 *S. aureus* isolates with reduced susceptibility to BAC determined by BAC MIC > 8 mg/L. The most frequently detected genes were *qacA/B* in 10 isolates (22.2%), *qacJ* in 10 isolates (22.2%), *smr* in 8 isolates (17.8%) and *qacG* in 8 isolates (17.8%), with a low prevalence of *qacH* (3 isolates; 6.7%). A combination of two genes was present in 10 isolates (22.2%), including *qacA/B*+ *smr* in 8 isolates (17.8%) and *qacA/B*+ *qacJ* in 10 isolates (22.2%); moreover, three isolates (6.7%) had three genes (*qacA/B* + *qacG* + *qacJ*) (Table 2).

There was a strong positive correlation between the presence of QAC genes and higher BAC MIC associated with *qacA/B*, *qacJ* and *smr* genes (Table 3).

There was a statistically significant prevalence of antiseptic resistant genes among isolates resistant to cefoxitin, ciprofloxacin, clindamycin, oxacillin, tetracycline ($P = 0.0001$) and erythromycin ($P = 0.002$).

4. Discussion

qac genes are named after their principal resistance activity mainly towards QACs. These genes are reported to be distributed widely, especially in staphylococcal species [11].

In the present study, the most prevalent antiseptic resistance genes were *qacA/B* (22.2%), *qacJ* (22.2%), *smr* (17.8%) and *qac*G (17.8%), with a low prevalence of *qacH* (6.7%). The prevalence of

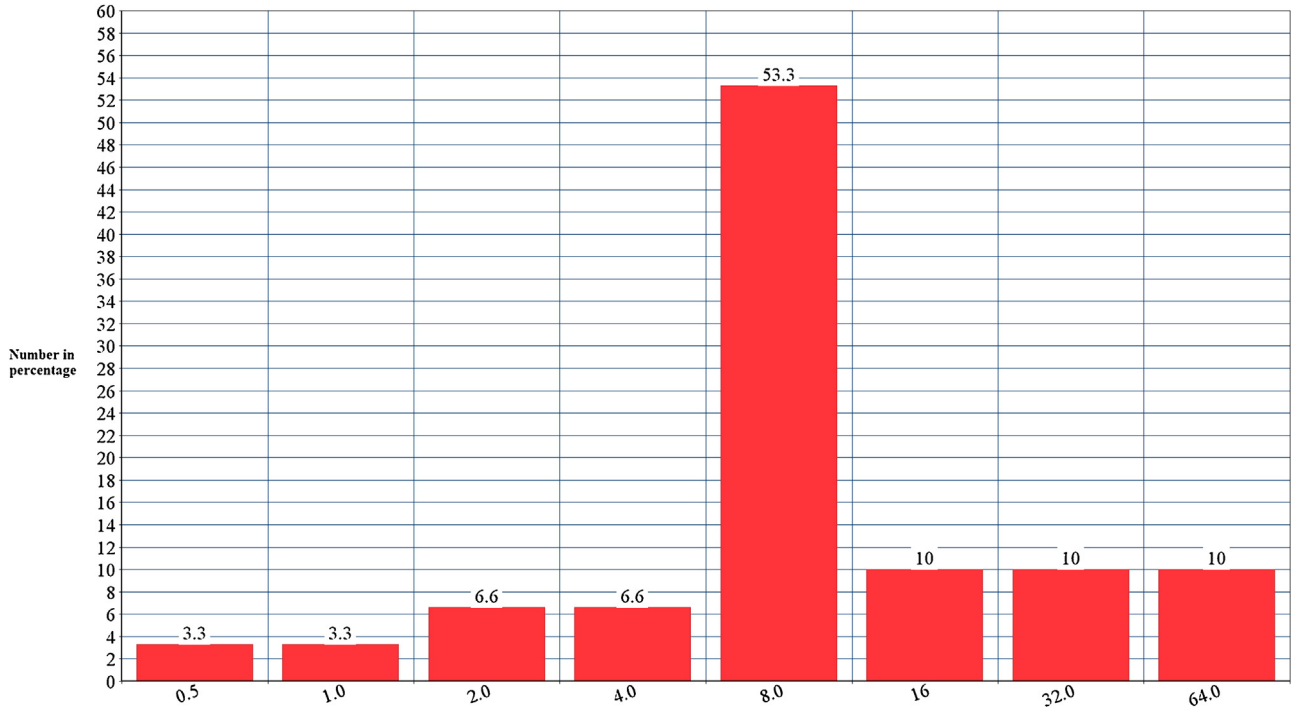

Fig. 1. Distribution of *Staphylococcus aureus* isolates according to minimum inhibitory concentration (MIC) of benzalkonium chloride.

Table 2
Frequency of quaternary ammonium compound (QAC) resistance genes determined by PCR among *Staphylococcus aureus* strains with MIC > 8 mg/L ($n = 45$).

Gene	No. (%)
qacA/B	10 (22.2)
qacJ	10 (22.2)
smr	8 (17.8)
qacG	8 (17.8)
qacH	3 (6.7)
Two genes combined	10 (22.2)
qacA/B + smr	8 (17.8)
qacA/B + qacJ	10 (22.2)
Three genes combined	
qacA/B+ qacG + qacJ	3 (6.7)

Table 3
Relationship between quaternary ammonium compound (QAC) resistance genes and minimum inhibitory concentrations (MICs) of benzalkonium chloride (BAC).

Gene	No. (%) at MIC (mg/L) of:			
	8	16	32	64
qacA/B ($n = 10$)	1 (10)	1 (10)	3 (30)	5 (50)
R^*	0.98			
R^2	0.97			
qacJ ($n = 10$)	1 (10)	2 (20)	2 (20)	5 (50)
R^*	0.96			
R^2	0.92			
smr ($n = 8$)	1 (12.5)	2 (25)	3 (37.5)	2 (25)
R^{**}	0.59			
R^2	0.34			
qacG ($n = 8$)	2 (25)	3 (37.5)	2 (25)	1 (12.5)
R	−0.06			
R^2	0.003			
qacH ($n = 3$)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)
R	−0.34			
R^2	0.12			

R^*, strong positive correlation; R^{**}, moderate positive correlation.

antiseptic genes differs by different geographic location, with a wide range varying from 10% up to 80% [11–14] for the predominant gene *qacA/B*. However, another study determined a low prevalence of *qacA/B* in *S. aureus* isolates, with a total absence of the *qacH* gene [15]. Differences in prevalence rates may be attributed to the location of QAC resistance genes studied in different clones of *S. aureus*, as these genes are commonly carried on plasmids leading to rapid transfer, whereas in some *S. aureus* clones different genes controlling resistance to QACs are carried on the chromosome [15,16].

Resistance to BAC was detected in 30% of the isolates by MIC > 8 mg/L. The method is easy to use and is applicable for many strains. However, the phenotype of reduced susceptibility to BAC tested on hundreds of strains is very small and in microbroth dilution it is of only one dilution [17] and such a weak change in susceptibility cannot be termed resistance.

There was a significant association between antibiotic resistance and the presence of antiseptic resistance genes, in line with a previous report [18]. This may be attributed to selective pressure of the use of antibiotics and antiseptic solutions with the predominance of resistant strains. However, in another study of more than 1600 strains, the presence of *qac* genes has been shown to be associated with resistance to antibiotics but the correlation coefficients were low [19].

The study highlights the prevalence of *qac* and *smr* genes in clinical *S. aureus* isolates with resistance to QACs. There was an association between the presence of antiseptic resistance genes and resistance to different antibiotics, which may be attributed to presence of both groups of genes on the same plasmid or to selection of resistant strains. There is a need for more studies on the clinical relevance of the presence of genes controlling resistance to antiseptic solutions.

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Competing interests

None declared.

Ethical approval

This study was approved by the Ethical Committee of Mansoura Faculty of Medicine (Mansoura, Egypt) [R/17.03.92].

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