MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2014; 20: 544-550
DOI: 10.12659/MSM.890177

Received: 2013.12.12
Accepted: 2014.01.10
Published: 2014.02.02

Relationship between the resistance genes to quaternary ammonium compounds and antibiotic resistance in staphylococci isolated from surgical site infections

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Source of support: Departmental sources

Background: We aimed to investigate the prevalence of disinfectant resistance genes (*qacA/qacB,qacC*) and the aminoglycosides resistance genes [(*aac(6')aph(2'')*,*aph(3')-IIIa*,*ant(4')-Ia*)] in both *S. aureus* and coagulase-negative staphylococcal strains (*CoNS*) isolated from surgical site infections.

Material/Methods: Totally, 130 staphylococcal strains isolated from surgical site infections between January 2012 and February 2013 were included in the study. The PCR technique was employed to verify any presence of methicillin resistance gene (*mecA*), aminoglycoside resistance genes [(*aac(6')/aph (2''), aph(3)-III a ant (4')-1a*)], and disinfectant resistance genes (*qacA/qacB,qacC*) in staphylococci.

Results: *MecA* gene was determined in 58 (44.6%) of 130 staphylococcal isolates. A total of 28 (73.7%) of 38 *S. aureus* isolates were found to be positive for the *mecA* gene, and 4 (12.9%) of 31 isolates sensitive to amikacin were sensitive to methicillin. Eighteen (47.4%) of 38 amikacin-resistant *S. aureus* isolates were found to be positive for *qacA/qacB* genes and 11 (8.9%) of them were positive for *qacC* gene. Both *mecA* and *qacA/qacB* genes were found to be positive at the same time in 19 amikacin-resistant *S. aureus* strains. Seven (18.4%) *S. aureus* isolates were determined to be positive for *qacA/qacB* and *qacC* genes. Frequency of *qacA/B* genes was found to be 47.4% among amikacin-resistant *S. aureus* strains, while *qacC* gene was found to be 28.9% ($p<0.05$). The ratio of *qacA/B* and *qacC* genes in *CoNS* was found to be 37.9% and 20.7%, respectively ($p<0.05$).

Conclusions: Quaternary ammonium resistance genes were found to be positive at a remarkable ratio in the staphylococcal isolates from surgical wounds. Especially, the high rates of aminoglycosides and methicillin-resistance gene was remarkable in *S. aureus* isolates. Quaternary ammonium resistance genes were found to be positive.

MeSH Keywords: Surgical Site Infections • Staphylococcal Infections • Disinfectant Resistance • Drug Resistance, Microbial – drug effects • Staphylococcal Infections

Full-text PDF: http://www.medscimonit.com/download/index/idArt/890177

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Background

Mostly caused by staphylococci, surgical site infections are the most common complications occurring after surgery. Staphylococci can cause infections ranging from skin and soft-tissue infections to deep tissue infections such as osteomyelitis, bacteriemia, and endocarditis. Recently, methicillin and antibiotics resistance for various antibiotics have seemed to develop in both nosocomial *S. aureus* and coagulase-negative staphylococcal strains [1,2].

Very high resistance rates have been reported to develop against widely used antimicrobial agents and disinfectants in both *S. aureus* and coagulase-negative staphylococci. Combined antibiotics may often be required for the treatment of these kinds of infections caused by resistant bacterial strains. Due to their synergistic effects with many antibiotics, aminoglycosides are often administered in combination with other antibacterial agents in the treatment of staphylococcal infections [2].

As in all other antibiotic groups, resistance to antibiotics is a serious problem for aminoglycosides. The main mechanism of aminoglycoside resistance in staphylococci is based on the drug inactivation by aminoglycoside-modifying enzymes (AMEs). The most common AMEs among staphylococci is 6'-N-acetyltransferase-2''-O-phosphotransferase [*aac(6')-aph(2'')*]. It is encoded by *aac(6')-aph-ie (2'')* gene. This enzyme fulfills the inactivation of gentamicin, kanamycin, tobramycin, neomycin, and amikacin. Additionally, 4'-Oadenyltransferase I [*ant(4')-I*] is encoded by *ant(4')-Ia* gene, and it inactivates kanamycin, neomycin, tobramycin, and amikacin. Another enzyme is 3'-O-phosphotransferase *III[aph(3')-III]*,which inactivates kanamycin and amikacin and is encoded by *aph (3')-IIIa* gene [3–7].

To control nosocomial infections, it is essential to secure hygienic conditions in hospitals. Of vital importance is the efficacy of disinfectants used for hand washing and surface disinfectants extensively used in hospitals against microorganisms. Nowadays, the most commonly used disinfectant agents in hospitals are quaternary ammonium compounds [8]. Recently, *qac* genes (*qacA/qacB and qacC or smr*), which are the resistance genes to quaternary ammonium compounds, have appeared more frequently in Staphylococcal isolates [9,10].

In this study, we aimed to investigate the prevalence of disinfectant resistance genes (*qacA/qacB, qacC*) and the aminoglycosides resistance genes [(*aac(6')aph(2'')*,*aph(3')-IIIa*,*ant(4')-Ia*)] in *S. aureus* and coagulase-negative staphylococcal strains isolated from surgical site infections. We also tried to determine, the relationship, if any, between the disinfectant resistance genes and the aminoglycosides resistance genes.

Material and Method

The study included a total of 130 staphylococcal strains isolated from surgical site infections between January 2012 and February 2013. Polymerase chain reaction (PCR) technique was employed to verify any presence of methicillin resistance gene (*mecA*), aminoglycoside resistance genes [(*aac(6')/aph (2''), aph(3)-III a ant (4')-1a*)], and disinfectant resistance genes (*qacA/qacB, qacC*) in staphylococci.

Bacterial isolates

The study involved a total of 130 consecutive isolates of staphylococci collected from Mustafa Kemal University Research Hospital, Department of General surgery. Bacterial isolates were obtained postoperatively from various infected wounds. To isolate staphylococci, samples were inoculated onto sheep blood agar plates and phenol-red mannitol salt agar plates. The plates were incubated at 37°C for 48 h. Identification of staphylococci was based upon colony morphology, biochemical activities, and coagulase test results [11]. If necessary, further confirmatory tests were done with the Vitek-2 automated microbiology system (bioMerieux, France).

Susceptibility testing

Disc diffusion method

Antimicrobial susceptibilities of the isolates to amikacin were tested by the agar disk diffusion method on Mueller-Hinton agar (Tiantan Biotechnology, PR China) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. All antibiotic discs [oxacillin (1 µg) and amikacin (10 µg)], were obtained from Oxoid. Mueller-Hinton broth was used as the growth medium. Plates were incubated aerobically at 35°C and 37°C for 24 h in oxacillin and amikacin susceptibilities tests, respectively. The diameter of each zone was measured in millimeters.

Quality control organisms

S. aureus ATCC 29213 and *S. aureus* ATCC (43300) were chosen as the negative and positive control strains, respectively.

Broth microdilution susceptibility tests

The minimum inhibitory concentrations (MICs) of chlorhexidine were determined by the reference broth microdilution procedure recommended by the CLSI. At the end of incubation, the MIC was defined as the lowest concentration of antibiotics that produced no growth [13].

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Table 1. Primer sequences and predicted sizes used in the multiplex PCRs for *fem-A*, *mecA*, *aac(6')/aph (2'')*, *aph(3)-III a*, *ant (4')-1a*, *qacA/qacB* and *qacC*.

Gene	Primers	Oligonucleotide sequence (5'-3')	Size of amplified product (bp)
mecA	*mecA-1*	ACTGCTATCCACCCTCAAAC	163
	mecA-2	CTGGTGAAGTTGTAATCTGG	
aac(6')/aph (2'')	*aac(6')/aph (2'')-1*	5'-GAAGTACGCAGAAGAGA-3'	491
	aac(6')/aph (2'')-2	5'-ACATGGCAAGCTCTAGGA-3'	
aph(3)-III a	*aph(3)-III a-1*	5'-AAATACCGCTGCGTA-3'	242
	aph(3)-III a-2	5'-CATACTCTTCCGAGCAA-3'	
ant (4')-1a	*ant (4')-1a-1*	5'-AATCGGTAGAAGCCCAA-3'	135
	ant (4')-1a-2	5'-GCACCTGCCATTGCTA-3'	
qacA/qacB	*qacA/qacB-1*	5'-TCCTTTTAATGCTGGCTTATACC-3'	220
	qacA/qacB-2	5'-AGCCKTACCTGCTCCAACTA-3'	
qacC	*luk PVL-1*	5'-GGCTTTTCAAAATTTATACCATCCT-3'	249
	luk PVL-2	5'-ATGCGATGTTCCGAAAATGT-3'	

PCR method for *ica A* and *ica D*

Primers specific for *mecA, femA, aac(6')/aph(2''), aph(3')-IIIa,* and *ant(4')-Ia* were selected from the studies of Strommenger et al. [14], Martineau et al. [15], and Duran et al. [16] (Table 1). The oligonucleotide primers for the *qacA/qacB* and *qacC* genes were selected based on the study by Zmantar et al. (Table 1) [17].

Multiplex PCR assay was carried out for the detection of *mecA, femA, aac(6')/aph(2''), aph(3')-IIIa, ant(4')-Ia, qacA/qacB,* and *qacC* genes in all staphylococcal strains. PCR amplification was carried out in a total volume of 25 µl. PCR amplification was achieved as follows: 5 µl of genomic DNA (approximately 50 ng) sample was added to 20 µl of PCR mixture (20 mmol/L Tris-HCl, pH 8.4; 50 mmol/L KCl, 10 mmol/L $MgCl_2$) and 200 µmol/L each of deoxynucleoside triphosphates (dNTPs), 0.6 µmol/L each primers and 1 U *Taq* DNA polymerase. The amplification process for *mecA, femA, aac(6')/aph(2''), aph(3')-IIIa* and *ant(4')-Ia* was started with an initial denaturation step (94°C, 4 min). Each cycle consists of 3 steps: denaturation, annealing, and extension. Each PCR reaction consisted of 30 cycles of amplification. Amplification consisted of denaturation at 94°C for 45 s, annealing at 52°C for 30 s, and DNA chain extension at 72°C for 1 min.

The amplification process for *qacA/qacB* and *qacC* was started with an initial denaturation step (94°C, 5 min). Each cycle consists of 3 steps: denaturation, annealing, and extension. Each PCR reaction consisted of 30 cycles of amplification. Amplification consisted of denaturation at 94°C for 1.5 min, annealing at 56°C for 30 s, and DNA chain extension at 72°C for 1.5 min. A final extension cycle was performed at 72°C for 7 min.

After the amplification of the slime and adhesin genes, 10 µL volumes of PCR samples were mixed with 3 µL of loading buffer (10%, w/v, Ficoll 400; 10 mmol/L Tris-HCl, pH 7.5; 50 mmol/L EDTA; 0.25% bromophenol blue). The PCR products were analyzed in a 2% (w/v) agarose gel in 1xTAE buffer (40 mmol/L Tris-acetate, 1 mmol/L EDTA). Ethidium bromide (0.5 µg/mL TAE)-stained DNA amplicons were visualized using a gel imaging system (Wealtec, Dolphin-View, USA).

Statistical analysis

Statistical analysis was performed using the chi-square test and *p* values less than 0.05 were considered statistically significant. The statistical analyses were performed using Statistical Package for Social Sciences (*SPSS*, ver. 17.5, Chicago, USA) software.

Results

We found that 69 of the isolates (53.1%) were coagulase positive. *MecA* gene was determined in 58 (44.6%) of 130 staphylococcal isolates. A total of 28 (73.7%) of 38 *S. aureus* isolates were positive for the *mecA* gene, and 4 (12.9%) of 31 isolates sensitive to amikacin were sensitive to methicillin. Eighteen (47.4%) of 38 amikacin-resistant *S. aureus* isolates were positive for *qacA/qacB* genes and 11 (%8.9%) were positive for *qacC* gene. *mecA* and *qacA/qacB* genes were positive at the same time in 19 amikacin-resistant *S. aureus* strains, and 7 (18.4%) *S. aureus* isolates were positive for *qacA/qacB* and *qacC* genes (Table 2).

In addition, 20 (69.0%) of 29 amikacin-resistant coagulase-negative staphylococci were found to carry *mecA* gene, while

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Table 2. Correlation between antibiotics resistance and quaternary ammonium compounds resistance genes (*qacA/qacB* and *qacC*).

Isolates	Amikacin resistance	Methicillin resistance gene		Quaternary ammonium resistance genes			
		mecA		*qacA/qacB*		*qacC*	
		n	%	n	%	n	%
I – *S. aureus*	38 (resistant)	28	73.7	18	47.4	11	28.9
II – *S. aureus*	31 (sensitive)	4	12.9	6	19.4	2	6.5
III – *CoNS*	29 (resistant)	20	69.0	11	37.9	6	20.7
IV – *CoNS*	32 (sensitive)	6	18.8	3	9.4	0	0.0
Total	130	58	44.6	38	29.2	19	14.6

p values for *mecA*: I–II: 0.000, I–III: NS, I–IV: 0.000, II–IV: <0.05; p values for *qacA/qacB*: I–II: 0.000, I–III: <0.05, I–IV: 0.000, II–IV: 0.01; p values for *qacc*: I–II: 0.000, I–III: <0.05, I–IV: 0.000, II–IV: 0.00; NS – non-significant.

Table 3. The relation between amikacin resistance and amikacin resistance genes [*aac(6')/aph(2'')*,*aph(3')-IIIa, ant(4')-Ia*].

Isolates	Amikacin resistance	Amikacin resistance genes						Number of PCR negative isolates	
		aac(6')/aph(2''		*aph(3')-IIIa*		*Ant(4')-Ia*			
		n	%	n	%	n	%	n	%
I – *S. aureus*	38 (resistant)	17	48.6	11	28.9	10	26.3	0	0.0
II – *S. aureus*	31 (sensitive)	3	9.7	1	3.2	0	0.0	27	87.1
III – *CoNS*	29 (resistant)	13	44.8	11	37.9	7	24.1	0	0.0
IV – *CoNS*	32 (sensitive)	2	6.3	3	9.4	1	3.1	26	81.3
Total	130	35	100.0	26	100.0	18	100.0	53	100.0

p values for *aac(6')/aph(2'')*: I–II: 0.000, I–III: NS, I–IV: 0.000, II–IV: <0.05; p values for *aph(3')-IIIa*: I–II: 0.000, I–III: NS, I–IV: 0.00, II–IV: 0.01; p values for *Ant(4')-Ia*: I–II: 0.000, I–III: NS, I–IV: 0.000, II–IV: NS; NS – non-significant.

6 (18.8) of 32 amikacin-sensitive isolates carried the methicillin resistance gene. Eleven (37.9%) and 6 (20.7%) of 29 amikacin-resistant coagulase-negative staphylococci were positive for *qacA/qacB* genes and *qacC* genes, respectively. In addition, 4 (13.8%) and 2 (6.9%) of these strains were positive for *mecA* and *qacA/qacB* genes, and *mecA* and *qacC* genes, respectively (Table 2). Aminoglycoside resistance genes were detected by PCR method in all of the 38 *S. aureus* strains, which were phenotypically determined to have amikacin resistance, while 3 (9.7%) of the 31 *S. aureus* strains sensitive to amikacin were found to carry *aac(6')/aph(2'')* genes. The *aph (3')-IIIa* gene was identified in 1 strain (3.2%). Similarly, all 29 amikacin-resistant coagulase-negative strains had at least 1 aminoglycoside-resistant gene, while *aac(6')/aph(2'', aph(3')-IIIa,* and *ant(4')-Ia* genes were 6.3%, (2/32), 9.4% (3/32) and 3.1% (1/32) positive, respectively, in the 32 strains found to be phenotypically susceptible to amikacin (Table 2).

The occurrence of *mecA* gene among amikacin-resistant *S. aureus* strains was determined as 73.7%, and this ratio was 12.9% among amikacin-sensitive *S. aureus* strains. There was a statistically significant difference among the methicillin-sensitive and -resistant strains in amikacin resistance ($p<0.001$). This difference was also recognized among coagulase-negative strains. Occurrence of methicillin resistance genes among amikacin-resistant *S. aureus* strains was 69.0%, and the same ratio was 18.8% among coagulase-negative strains (Table 2).

Frequency of *qacA/B* genes was 47.4% among amikacin-resistant *S. aureus* strains, while *qacC* gene was 28.9% ($p<0.05$). The ratio of *qacA/B* and *qacC* genes in *CoNS* was 37.9% and 20.7%, respectively ($p<0.05$).

At least 1 of the amikacin resistance genes existed in 38 amikacin-resistant *S. aureus* strains. The *aac(6')/aph(2'')* resistance gene positivity (48.6%) was highest, followed by *aph(3')-IIIa* (28.9%) and *ant (4')-Ia* (26.3%) genes. As in *S. aureus* isolates, at least 1 or more resistance genes existed in all of the amikacin-resistant *CoNS*. Two amikacin-sensitive *CoNS* had *aac(6') aph(2'')* gene, while 3 *CoNS* had *aph(3)-IIIa* gene (Table 3). It was observed that 2 amikacin-sensitive *CoNS* had *aac(6')aph(2'')* gene, while 3 *CoNS* had *aph(3)-IIIa* gene (Table 3).

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Table 4. The relation between antibiotics resistance and quaternary ammonium resistance genes (*qacA/qacB, qacC*).

Isolates	Amikacin resistance	*mecA* gene in combination with a quaternary ammonium resistance genes					
		mecA and *qacA/qacB* positivity		*mecA* and *qacC* positivity			
		n	%	n	%	Total	%
I – *S. aureus*	38 (resistant)	19	50	7	18.4	26	68.4
II – *S.aureus*	31 (sensitive)	3	9.7	0	0.0	3	9.7
III – *CoNS*	29 (resistant)	4	13.8	2	6.9	6	20.7
IV – *CoNS*	32 (sensitive)	1	3.1	1	3.1	2	6.3
Total	130	27	20.8	10	7.7	37	28.5

p values for *mecA* and *qacA/qacB* positivity: I–II: 0.000, I–III: 0.00, I–IV: 0.000, II–IV: NS;
p values for *mecA* and *qacC* positivity: I–II: 0.000, I–III: 0.00, I–IV: 0.00, II–IV: <0.05; NS – non-significant.

Table 5. The relation between quaternary ammonium resistance genes (*qacA/qacB, qacC*) and chlorhexidine MIC values.

Isolates	n	Chlorhexidine resistance and quaternary ammonium resistance genes					
		Both chlorhexidine resistant and *qacA/qacB* positivity		Both chlorhexidine resistant and *qacC* positivity			
		n	%	n	%	Total (n)	%
I – *S. aureus*	69	24/24	100.0	11/13	84.6	35	50.7
II – *CoNS*	61	13/14	92.9	5/6	83.3	18	29.9
Total	130	37/130	285.0	16	12.3	53/130	40.8

p values for chlorhexidine and *qacA/qacB* positivity: I–II: NS; p values for chlorhexidine and *qacC* positivity: I–II: NS;
NS – non-significant.

We found that *mecA* gene and *qacA/B* genes were both positive in 27 (20.8%) staphylococcal isolates, while *mecA* and *qacC* occurred in 10 (7.7%) of the isolates. Methicillin and *qacA/B* genes positivity were determined in 50% of 38 amikacin-resistant *S. aureus* isolates, while *qacC* gene was detected in 18.4% of them. Of a total of 38 *S. aureus* isolates, 68.4% carried both methicillin and disinfectant resistance genes. Similarly, *qacA/B* genes were positive in 14.8% of 29 amikacin-resistant *CoNS*, while *qacC* gene was observed in 2 (6.9%) isolates. There was a significant difference between *S. aureus* isolates carrying *mecA* genes and *CoNS* carrying *mecA* genes containing the disinfectant resistance genes. Methicillin and disinfectant resistance genes were detected in 20.7% of amikacin-resistant *CoNS*. *mecA* plus *qacA* positivity was detected as 9.7% of the 31 *S. aureus* strains that were resistant to amikacin. No common positivity for *mecA* and *qacA* was found ($p<0.01$) among the amikacin-sensitive *S. aureus* strains. One isolate was positive for *mecA* and *qacA/B* genes among amikacin-sensitive *CoNS*, and 1 isolate was positive for *mecA* plus *qacC* genes (Table 4).

Chlorhexidine MIC values were determined as 0.5–8 mg/l. Chlorhexidine resistance was 50.7% among *S. aureus* isolates carrying *qacA/B* genes, while the same ratio was 29.5% among *CoNS* isolates ($p<0.01$). Chlorhexidine resistance was detected in all of the 24 *S. aureus* strains whose *qacA/B* genes were positive, while chlorhexidine resistance was positive in 11 of 13 *qacC* genes (84.6%) ($p<0.05$). Chlorhexidine resistance was determined in 13 (92.3%) of 14 isolates among *CoNS* strains that were detected with *qacA/B* gene, while chlorhexidine resistance was observed in 5 of 6 *CoNS* strains (83.3%) with *qacC* gene positivity (Table 5).

Discussion

A number of disinfectant agents are used for both hand hygiene and the prevention of nosocomial infections. It has been reported that *qacA/B* and *qacC* (or *smr*) genes transmitted by plasmids are responsible for the development of resistance in staphylococci against cationic antiseptic agents. Medically,

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S. aureus and *CoNS* are remarkable microorganisms, which may cause diseases of skin and soft-tissue, as well as bacteremia and endocarditis. Staphylococci can readily develop resistance to most antimicrobial agents, including antiseptic and disinfectants [18,19].

Quaternary ammonium compounds are disinfectants frequently used for controlling nosocomial infections. Recently, resistance to quaternary ammonium compounds has frequently appeared among clinical staphylococcal strains. In a study exploring the relation between the antiseptic resistance genes (*qacA/B, qacC*) and antibiotic resistance in clinical *S. aureus* isolates, it was observed that *qacC* genes occurred at a higher ratio than did *qacA/B* genes. Presence of *qacC* gene was positive in 36% of MRSA isolates. No *qacC* resistance gene could be detected in MSSA isolates, but *qacA/B* gene was positive in them at the rate of 4%. In that study, *smr* resistance gene was detected only in MRSA isolates, while *qacA/B* genes were determined in MRSA isolates at lower rates [20]. Unlike this study, *qacA/B* ratio in MRSA isolates was found to be statistically significantly higher than *qacC* ratio ($p<0.01$) in our study. In contrast, in our research *qacC* resistance gene was detected both in MRSA and methicillin-sensitive *CoNS* isolates. In addition, *qacA/B* and *qacC* resistance genes were found positive in 8.2% (5/61) and 4.9% (3/61) *CoNS*, respectively.

Previous studies have reported that the frequency of occurrence of disinfectant resistance genes varied from country to country and even from hospital to hospital. Noguchi et al. studied the frequency of disinfectant genes in MRSA strains. For this purpose, they used clinical MRSA isolates from different countries. *QacC* gene frequency among MRSA isolates from Indian isolates was 31.6%, while the same values were 1.9% in Asian samples. *QacA/B* frequency in Asian isolates was reported to be 41.6% and these isolates were found resistant to acriflavine at the rate of 57.7%. In the study, it was pointed out that *qacA/B* genes were widespread in MRSA isolates in Asia and that *qacA/B* play role in resistance to disinfectants [21]. In our study, the frequency of *qacA/B* genes with Asian roots was rather high. Chlorhexidine resistance was found in 28.5% of the isolates carrying *qacA/B* genes.

In a study by Zhanq et al., [22], the relation between efflux proteins was encoded by *qac* genes and biocide resistance. They investigated the prevalence of antiseptic resistance genes (*qacA/qacB qacC*) in staphylococci colonized in medical staff and nurses and found that *qacA/B* and *qacC* genes were related to the presence of *mecA* genes. In addition, rather high antibiotic resistance was detected in *S. aureus* strains where presence of *qac* genes was verified and in all *qac* gene-positive isolates, and quite high MIC and MBC values were found against antiseptics [22].

In our study, *qacA/B* ratio was 20.8% in 27 isolates in which presence of *mecA* gene was verified, while *qacC* genes existed at the rate of 7.7%. We also found a high chlorhexidine resistance value in isolates in which *qacA/qaB* and *qacC* resistance genes were verified. Our findings are in line with the results of Zhang and et al. [22].

In a study conducted in Malaysia in 2012 to investigate the frequency of antiseptic resistance genes in MRSA strains, it was determined that antiseptic resistance genes *qacA/B* and *qacC* occurred in 83.3% and 1.6% of the isolates, respectively. It was established in the study that *qacA/B* genes carriage leads to decline in chlorhexidine digluconate and benzethonium sensitivity. It was reported that MIC values were 0.5 to 2 mg/l against 3 disinfectant agents (benzalkonium chloride, benzethonium chloride, and chlorhexidine digluconate) in MRSA strains. The presence of antiseptic resistance genes was reported to be related to rising resistance values against disinfectant agents [23]. In our study, chlorhexidine MIC value was determined as 0.5–8 mg/l in isolates in which *qacA/B* and *qacC* genes were detected, and MIC value was <1 mg/l in strains where no resistance genes were detected.

Chlorhexidine is among the most widely used disinfectants for controlling nosocomial infections. Although chlorhexidine-impregnated catheter practice is reported to reduce catheter-related infections, chlorhexidine resistant *S. aureus* isolates have been found in recent years. In a study investigating MRSA isolates, the efficiency of chlorhexidine resistance genes (*qacA* and *qacA/B*), and chlorhexidine-impregnated catheters, MIC values against chlorhexidine in *qacA/B* genes carrying *S. aureus* isolates were >2 mg/l, while the MIC values in isolates not carrying *qacA/B* genes were >1 mg/l. It was also found out that MIC values were 1 mg/l in MSSA isolates and 2 mg/l in MRSA isolates, and *qacA/B* prevalence in MRSA and MSSA isolates was 43.8% and 3.3%, respectively. The prevalence of *qacC* gene in MRSA and MSSA isolates was 5% and 25%, respectively [24]. As in this study, MIC values against chlorhexidine were found to be high (>2–8) in our study.

In a study of 522 *S. aureus* isolates from a hospital in Japan, *qacA* and *qacB* genes were investigated; the presence of *qacA/B* in MRSA isolates was 32.6% and that of *qacA/B* genes was 7.5% among MSSA isolates. The presence of *qacC* in MRSA and MSSA isolates was 3.3% and 5.9%, respectively. Among *S. aureus* isolates, *qacA/B* gene frequency was widespread. In *qacA/B*-positive isolates, *qacB* and *qacA* genes were positive at the rate of 59.3% and 40.7%, respectively [25]. As in our study, *qacA/B* genes were more frequent among MRSA isolates.

In a study carried out with 98 methicillin-resistant clinical isolates, distribution and sensitiveness to antiseptics of

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antiseptic resistance genes (*qacA* and *qacC*) were investigated. Frequencies of *qacA* and *qacC* were 20.2% and 20.4%, respectively [26].

In our study, *qacC* gene prevalence was at a lower level than in the study by Nogachi et al. [26]. The disinfectant resistance genes (*qacA/B, qacC*) frequency was determined as a remarkably high ratio in this first study conducted in our region. In our hospital, the prevalence of *qacA/B* genes was higher than that of *qacC* gene. There was a statistically significant difference between the *qacA/B* gene-positive and *qacA/B* gene-negative groups in chlorhexidine resistance. Similarly, resistance to chlorhexidine was significantly higher in presence of *qacC* gene.

In our study, 68.7% of the isolates in which antiseptic resistance genes were detected had amikacin resistance, while in the isolates lacking disinfectant resistance genes, amikacin resistance was 31.3%.

Conclusions

Rather high resistance to chlorhexidine was determined in the isolates found to carry *qacA/B* and *qacC* antiseptic resistance genes. Our findings indicate a significant relation between the presence of *qacA/B* and *qacC* resistance genes and amikacin and methicillin resistance.

Quaternary ammonium resistance genes were found to be positive at a remarkable ratio in the staphylococcal isolates from the surgical wounds. The rates of aminoglycoside- and methicillin-resistance genes were remarkably high in *S. aureus* isolates, especially when quaternary ammonium resistance genes were positive.

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