

Prevalence of Resistance Genes to Biocides in Antibiotic-Resistant *Pseudomonas Aeruginosa* Clinical Isolates

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Research Article

Keywords: *Pseudomonas aeruginosa*, resistance, triclosan, benzalkonium chloride, chlorhexidine digluconate, formaldehyde

Posted Date: September 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-886208/v1>

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Abstract

Background: Biocides are frequently used as preservative, disinfectant and sterilizer against many microorganisms in hospitals, industry and home. However, the resistance rate of *Pseudomonas aeruginosa* (*P. aeruginosa*) strains to biocides is increasing. The aim of this study was to evaluate the antimicrobial activity of four frequently used biocides against *P. aeruginosa* and to determine the prevalence of genes involved in biocide resistance.

Methods: A total of 76 clinical isolates of *P. aeruginosa* strains were used in the present study. The minimum inhibitory concentrations (MICs) of four biocides, *i.e.* chlorhexidine digluconate, benzalkonium chloride, triclosan and formaldehyde, against *P. aeruginosa* strains were determined using agar dilution method. In addition, the prevalence of biocide resistance genes was determined using the polymerase chain reaction (PCR) method.

Results: In the present study, the highest MIC₉₀ value was observed for benzalkonium chloride (MIC₉₀=1024 µg/mL), followed by formaldehyde (MIC₉₀=512 µg/mL), triclosan (MIC₉₀=512 µg/mL) and chlorhexidine digluconate (MIC₉₀=64 µg/mL). Furthermore, the prevalence of *qacEΔ1*, *qacE*, *qacG*, *fabV*, *cepA* and *fabI* genes were 73.7% (n=56), 26.3% (n=20), 11.8% (n=9), 84.2% (n=64), 81.5% (n=62) and 0% (n=0), respectively. A significant association was observed between the presence of biocide resistance genes and MICs ($p<0.05$). Furthermore, there was no significant association between the presence of biocide resistance genes and antibiotic resistance ($p>0.05$), except for levofloxacin and norfloxacin antibiotics and *qacE* and *qacG* genes ($p<0.05$).

Conclusion: Our results revealed that chlorhexidine digluconate is the most effective biocide against *P. aeruginosa* isolates in Ardabil hospitals. However, we recommend continuous monitoring of the antimicrobial activity of biocides and the prevalence of biocide-associated resistance genes for a better prevention of microorganism dissemination and infection control in hospitals.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacillus, which is frequently isolated from hospital environments, especially from medical equipments used in intensive care units (ICUs) [1-3]. *P. aeruginosa* is an opportunistic human pathogen that is associated with various community-acquired and nosocomial infections such as ventilator-associated pulmonary infections, skin and soft-tissue infections, catheter-related urinary tract infections, eye and ear infections, bloodstream infections, endocarditis and surgical site/transplantation infections [1-3]. Therefore, it seems that hygiene control of surfaces and medical equipments in the hospital settings in terms of *P. aeruginosa* contamination is important because nosocomial infections are considered as a growing global threat in terms of economical and public health [3]. The most common antiseptic and disinfectant biocides used in clinical settings are chlorhexidine digluconate (a biguanide), benzalkonium chloride (a quaternary ammonium compound), triclosan (a bisphenol) and formaldehyde (an aldehyde) [4, 5]. However, several studies have reported bacterial resistance to various biocides due to the presence of resistance genes. On the other hand, the risk of *P. aeruginosa* infections in ICU-hospitalized patients is high (up to 30%) despite applying hygiene programs [5]. Such a high prevalence can be attributed to the resistance of *P. aeruginosa* strains against a variety of antiseptics and disinfectants over time [6]. Another problem is the emergence of multi-drug resistant (MDR) *P. aeruginosa* strains, which can lead to treatment failure [3]. Interestingly, *P. aeruginosa* strains have shown cross-resistance to biocides and antibiotics with probably similar mechanisms, thereby making bacterial elimination from hospital environments difficult [5, 7]. Therefore, obtaining information on the prevalence and mechanisms of bacterial resistance to antimicrobial agents and the selection of suitable biocides and antibiotics can be helpful for controlling hospital-acquired infections caused by *P. aeruginosa*. Several mechanisms of resistance to antibiotics and tolerance to biocides have been identified in *P. aeruginosa* strains including: 1) simple growth requirements and ability of biofilm formation, 2) enzymatic degradation, 3) target site modification, 4) outer membrane impermeability, and 5) presence of efflux pumps. These properties are involved in bacterial persistence in medical environments and development of nosocomial infections [2, 8-13].

Various compounds are used in hospitals and non-hospital environments in Ardabil city but there is no exact data on the rate of biocide effectiveness against local isolates of *P. aeruginosa*. Moreover, the mechanisms of biocide resistance in *P. aeruginosa* clinical isolates of Ardabil are still unclear. Therefore, the aim of the current study was to assess the distribution of biocide resistance genes, namely *qacEΔ1*, *qacE*, *qacG*, *fabV*, *cepA* and *fabI*, and to determine the minimum inhibitory concentrations (MICs) of various biocides against antibiotic-resistant *P. aeruginosa* strains isolated from clinical samples in Ardabil.

2. Methods

2.1 Data on *P. aeruginosa* isolates

A total of 76 confirmed clinical *P. aeruginosa* isolates were obtained from various specimens in Ardabil hospitals and then used to assess the distribution of biocide resistance genes as well as determination of the MICs of four biocides. Drug resistance characteristics of *P. aeruginosa* isolates was evaluated using the disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI, 2018) guideline [14]. Data on the prevalence of class I integron, harboring resistance genes to biocides and antibiotics, was used in this study in order to evaluate the association with resistance to biocides. It is noteworthy, the prevalence of antibiotic resistance along with class I integron rate were previously determined by authors [15, unpublished data].

2.2 Preparation of biocide solutions

Biocides used in this study were chlorhexidine digluconate (20%) (Sigma-Aldrich, USA), benzalkonium chloride (>95%) (Sigma-Aldrich, USA), triclosan (98%) (Bio Basic, Canada) and formaldehyde (37%) (Thermo Fisher Scientific, USA). Stock solutions of antimicrobial agents were prepared in distilled water or water-alcohol for water-insoluble antimicrobial agents. All antibacterial solutions were sterilized using sterile syringe filters (0.22 µm) before use.

2.3 Determination of the MICs of biocides

The MICs of antiseptics and disinfectants including chlorhexidine digluconate, benzalkonium chloride, triclosan and formaldehyde were determined by agar dilution technique and according to the CLSI guideline [14]. For this purpose, at first a range of biocide concentrations (0.125-1024 µg/mL) was prepared in Mueller-Hinton agar medium and then a 0.5 McFarland standard concentration of *P. aeruginosa* isolates (1.5×10^8 cfu/mL) was prepared in normal saline. Finally, diluted bacterial inoculum (1:10) (1.5×10^7 cfu/mL) was poured onto medium containing different biocides as a spot (10^4 cfu per spot). The plates were incubated at 37 °C overnight and then checked in terms of bacterial growth. In the current study, epidemiological cut-off value (ECOFF) for antibacterial susceptibility testing against four biocides was determined equal to MIC₉₅ (95% rule). Therefore, *P. aeruginosa* strains with higher MIC value compared with ECOFF value were considered as resistant organisms.

2.4 Detection of biocide resistance genes

Extraction of total genomic DNA was done using a simple boiling method. Extracted DNA was stored at -20 °C until use for detection of biocide resistance genes. The presence of *qacEΔ1*, *qacE*, *qacG*, *fabV*, *cepA* and *fabI* genes was detected by specific primers in polymerase chain reaction (PCR). According to the thermal cycling condition for amplification and the oligonucleotide primer sequences presented in Table 1, PCR was performed in a volume of 25 µL containing 20 µL of master mix (Ampliqon, Denmark), 1 µL of each primers (10 µmol/L) and 3 µL of extracted DNA. PCR products were detected by electrophoresis on 1% agarose gel in a TBE 0.5x buffer and then confirmed by sequencing technique.

Table 1. Used primers and PCR programs in the present study

Gene	Primer sequence	Thermal cycling condition					Amplicon size (bp)	Reference
		1	2	3	4	Cycle		
<i>qacEΔ1</i>	F: AATCCATCCCTGTCGGTGT	94	94	53	72	30	190	16
	R: CGCAGCGACTTCCACGATGGGGAT	4 min	1 min	50 sec	1 min			
<i>qacE</i>	F: TTAGGATGGAGACGAAATTTTCA	94	94	59	72	30	240	This study
	R: CGCTTAACACCTAGTATTATTACCGT	4 min	1 min	1 min	1 min			
<i>fabI</i>	F: ATGCTGAAAATTGTTTTGAGTGAGA	94	94	59	72	30	830	This study
	R: TTCATCATCCTTCATAGATTGGCTC	4 min	1 min	1 min	1 min			
<i>qacG</i>	F: TTGAATAATTGGTTATTTCTGGCT	94	94	59	72	30	333	This study
	R: TTAGTGAACACTTGCCTTAGATAG	4 min	1 min	1 min	1 min			
<i>cepA</i>	F: GCTCGCTGATGTCGGTAGG	94	94	59	72	30	481	This study
	R: CTGCTGGCAGTGCCTATTC	4 min	1 min	1 min	1 min			
<i>fabV</i>	F: TCGACCTGGTGGTCTACAGC	94	94	59	72	30	530	This study
	R: GACCTGCTCGATGCAACC	4 min	1 min	1 min	1 min			

Abbreviations: 1-Initial denaturation, 2-Denaturation, 3-Annealing, 4-Extension.

2.5 Data analysis

All data on the MICs of biocides against *P. aeruginosa* strains, the presence of resistance genes to biocides and *P. aeruginosa* resistance to various antibiotics were collected and their correlation analyzed by the SPSS software version 16. The Chi-square test was used to analyses and a *p* value of <0.05 was considered statistically significant.

3. Results

In the present study, among 76 *P. aeruginosa* clinical isolates, the prevalence of *qacEΔ1*, *qacE*, *qacG*, *fabV*, *cepA* and *fabI* genes were 73.7% (n=56), 26.3% (n=20), 11.8% (n=9), 84.2% (n=64), 81.5% (n=62) and 0%, respectively. As shown in Table 2, the MIC range for various biocides was as follows: benzalkonium chloride 256-1024 µg/mL, formaldehyde 32-512 µg/mL, triclosan 32-512 µg/mL and chlorhexidine digluconate 4-64 µg/mL. In total, the highest MIC₉₀ was observed for benzalkonium chloride (MIC₉₀=1024 µg/mL), followed by formaldehyde (MIC₉₀=512 µg/mL), triclosan (MIC₉₀=512 µg/mL) and chlorhexidine digluconate (MIC₉₀=64 µg/mL). Therefore, it seems that chlorhexidine digluconate and benzalkonium chloride had the highest and the lowest effects, respectively, in terms of growth inhibition of *P. aeruginosa* isolates in this study. As shown in Table 3, a significant association was observed between the presence of biocide resistance genes and MICs (*p*<0.05). Additionally, biocide resistance gene profiles revealed that isolates simultaneously harboring *qacEΔ1*, *cepA* and *fabV* genes are more prevalent (n=35, 46%), while 8 isolates (10.5%) did not harbor any biocide resistance genes (Table 4). Moreover, strains containing biocide resistance genes had higher MIC₅₀ and MIC₉₀ values compared with isolates without these genes. In this study, ECOFF for resistance to benzalkonium chloride, triclosan, formaldehyde and chlorhexidine digluconate were determined as 1024, 512, 512 and 64 µg/mL, respectively.

Isolates with and without biocide resistance genes were compared in terms of resistance rate to the following antibiotics: piperacillin, piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, cefepime, aztreonam, doripenem, imipenem, meropenem, gentamicin, tobramycin, amikacin, netilmicin, ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin and ofloxacin. There was no significant association between the presence of biocide resistance genes and antibiotic resistance (*p*>0.05), except for levofloxacin and norfloxacin antibiotics and *qacE* and *qacG* genes (*p*<0.05). The prevalence of integron I positive *P. aeruginosa* strains harboring *qacEΔ1* gene was 32 out of 76 (42.1%). No significant association was observed between the presence of class I integron and biocide resistance genes (*qacEΔ1*, *qacE*, *cepA* and *fabV*), except for *qacG* gene (*p* = 0.00).

Table 2. Minimal inhibitory concentration of biocides against clinical isolates of *P. aeruginosa*

Biocides	MIC ($\mu\text{g}/\text{mL}$)										ECOFF* ($\mu\text{g}/\text{mL}$)
	4	16	32	64	128	256	512	1024	MIC ₅₀	MIC ₉₀	
Formaldehyde	-	-	2 (2.6%)	47 (61.9%)	15 (19.7%)	-	12 (15.8%)	-	64	512	512
Benzalkonium chloride	-	-	-	-	-	12 (15.8%)	53 (69.7%)	11 (14.5%)	512	1024	1024
Triclosan	-	-	11 (14.5%)	3 (3.9%)	5 (6.6%)	-	57 (75%)	-	512	512	512
Chlorhexidine digluconate	4 (5.3%)	10 (13.2%)	22 (28.9%)	40 (52.6%)	-	-	-	-	64	64	64

*ECOFF-epidemiological cut-off value

Table 3. Association between biocide resistance genes and MIC

Gene	Biocide (MIC)	Formaldehyde		Benzalkonium chloride		Triclosan		Chlorhexidine	
		≤ 128	$256 \leq$	≤ 256	$512 \leq$	≤ 128	$256 \leq$	≤ 16	$32 \leq$
<i>qacEΔ1+</i>		46	10	3	53	5	51	6	50
<i>qacEΔ1-</i>		18	2	9	11	13	7	8	12
<i>p value</i>		0.019		0.00		0.00		0.02	
<i>qacE+</i>		15	6	2	19	2	19	2	19
<i>qacE-</i>		49	6	10	45	16	39	12	43
<i>p value</i>		0.24		0.00		0.426		0.491	
<i>qacG+</i>		7	2	0	9	0	9	0	9
<i>qacG-</i>		57	10	12	55	18	49	14	53
<i>p value</i>		0.88		0.00		0.53		0.32	
<i>cepA+</i>		50	12	7	55	8	54	9	53
<i>cepA-</i>		14	0	5	9	10	4	5	9
<i>p value</i>		0.00		0.03		0.00		0.00	
<i>fabV+</i>		52	12	7	57	8	56	10	54
<i>fabV-</i>		12	0	5	7	10	2	4	8
<i>p value</i>		0.00		0.01		0.00		0.00	

Table 4. Gene profile and minimal inhibitory concentration of biocides

Gene	Isolate (n)	Chlorhexidine digluconate		Triclosan		Benzalkonium chloride		Formaldehyde	
		MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
<i>qacEΔ1</i>	2	32	32	0.5	64	256	512	64	128
<i>qacE</i>	1	32	32	512	512	512	512	64	64
<i>qacEΔ1+cepA</i>	1	64	64	512	512	512	512	64	64
<i>qacEΔ1+fabV</i>	2	32	64	512	512	512	512	128	128
<i>cepA+fabV</i>	5	32	64	128	512	256	512	64	64
<i>qacE+cepA+fabV</i>	6	32	64	512	512	512	1024	128	512
<i>qacEΔ1+cepA+fabV</i>	35	64	64	512	512	512	512	64	512
<i>qacEΔ1+qacE+cepA+fabV</i>	7	64	64	512	512	512	1024	64	512
<i>qacEΔ1+qacG+cepA+fabV</i>	2	64	64	512	512	512	1024	64	64
<i>qacEΔ1+qacE+qacG+cepA+fabV</i>	7	64	64	512	512	1024	1024	128	512
No gene	8	4	64	0.5	64	256	512	64	64

4. Discussion

Biocides are basic compounds to control microorganism dissemination and ensuing infections and are frequently used as preservative, disinfectant and sterilizer against various microorganisms, in particular *P. aeruginosa* [17]. One of the most useful biocides against microbes, especially Gram-positive bacteria, is triclosan, which is widely used in toothpastes, soaps and other daily products [17]. Triclosan is an anionic and lipophilic compound, which its anti-bacterial function stems from inhibition of enoyl-acyl-carrier protein reductase (ENR), an enzyme involved in fatty acid synthesis [19, 21]. However, *P. aeruginosa* strains are inherently resistant to this biocide (MIC>2,000 µg/mL) [21]. ENR enzymes show diversity among different bacteria in terms of sequence and structure, and contain four isozymes including FabI (triclosan-sensitive ENR), FabL, FabV and FabK (triclosan-resistant ENRs) [19, 21]. The FabV isozyme is involved in swimming motility, energy metabolism, protein secretion and adherence, and is responsible for *P. aeruginosa* resistance to triclosan biocide [21]. Genes encoding FabI and FabV enzymes are found in most bacterial chromosomes such as *P. aeruginosa* [19, 21]. Bacterial resistance to triclosan is associated with mutation in the active site of *fabI* gene and the presence of *fabV* gene [17, 21]. In the current study, the prevalence *fabI* resistance gene among *P. aeruginosa* isolates was 0%. Unlike *fabI* gene, the frequency of *fabV* gene was high in the present study (84.2%). Zhu *et al.* showed that deletion of *fabV* gene confers extremely high susceptibility to triclosan (>2,000 folds) in *P. aeruginosa* isolates [19]. Similar result was reported by Huang *et al.* [21]. In this study, the MIC₅₀ and MIC₉₀ values of triclosan for *fabV* resistance gene- harboring *P. aeruginosa* strains was higher than *fabV* gene-negative strains (Table 4).

Chlorhexidine digluconate, an antiseptic, disinfectant and preservative, is a bactericidal biocide, which has higher antibacterial activity against Gram-positive compared with Gram-negative bacteria [22]. This biocide is used in oral health antiseptics, hand washes and other hygienic solutions. The antibacterial mechanism of chlorhexidine digluconate is *via* the bacterial cell membrane [17]. However, *P. aeruginosa* is intrinsically resistant to this biocide due to the presence of an outer membrane [22]. Adaptive resistance to chlorhexidine biocide is mediated by a membrane protein encoded by *Acinetobacter* chlorhexidine efflux gene (*aceI*). The *AceI* protein identified in *Acinetobacter baumannii* is involved in chlorhexidine efflux *via* an energy-dependent mechanism [23]. However, genes encoding this protein were not identified in *P. aeruginosa* strains in the current study (data not shown). The antiseptic resistance gene *cepA*, an efflux pump gene, is associated with chlorhexidine resistance in Gram-negative bacteria causing high chlorhexidine MICs [24, 25]. In our study, 62 (81.5%) *cepA*-positive strains were found, which is higher than those reported by Mendes *et al.* (44.5%) and Vijayakumar *et al.* (63.6%) [24, 25]. According to MIC results, chlorhexidine digluconate is more effective than other biocides against *P. aeruginosa* isolates (MIC range=4-64 µg/mL) (Table 2). In this study, the presence of *cepA* gene had variable effects on the MIC₅₀ and MIC₉₀ values of chlorhexidine (Table 4).

A major biocide resistance mechanism in Gram-negative bacteria including *P. aeruginosa* is the action of efflux pumps such as the small multidrug resistance family (SMR) [13, 18]. Biocide resistance genes *qacEΔ1*, *qacE* and *qacG* encode multidrug efflux pumps, which confer resistance to quaternary ammonium compounds like benzalkonium chloride [13, 18]. In our study, the *qacEΔ1* gene was observed in 73.7% of clinical isolates of *P. aeruginosa*, while in studies conducted by Subedi *et al.*, Romão *et al.*, Kücken *et al.*, Helal *et al.* and Mahzounieh *et al.* the *qacEΔ1* gene was detected in 46.1%, 48%, 10%, 48% and 91.5% of the isolates, respectively [13, 18, 26-28]. According to the reports of Subedi *et al.*, Kücken *et al.*, Helal *et al.* and Mahzounieh *et al.*, 100%, 2.7%, 13.5% and 50% of *P. aeruginosa* strains, respectively, had the *qacE* gene [13, 26-28], while we detected this gene in 26.3% of isolates. The frequency of *qacG* gene in the present study was 11.8%, which is higher compared to the frequency reported by Subedi *et al.* (0%) [13]. The MIC₅₀ and MIC₉₀ values of benzalkonium chloride were significantly high for *qacEΔ1*-, *qacE*- and *qacG*-positive *P. aeruginosa* strains compared with the negative strains (Table 4).

Class I integron carries *qacEΔ1* and antibiotic resistance genes in clinical isolates of *P. aeruginosa* [13]. Therefore, *P. aeruginosa* strains harboring class I integron are resistant to benzalkonium chloride and various antibiotics [26]. Comparison of our current and previous study (unpublished data) showed that the frequency of integron I-positive *P. aeruginosa* strains harboring *qacEΔ1* gene was 32 out of 76 (42.1%). No significant association was observed between the presence of class I integron and biocide resistance genes (*qacEΔ1*, *qacE*, *cepA* and *fabV*), except for *qacG* gene ($p = 0.00$).

Formaldehyde is an organic electrophilic biocide, which its mechanism of action involves cross-linking of macromolecules (proteins, RNA and DNA) [17, 29]. Our results indicated that the MIC₅₀ and MIC₉₀ values of formaldehyde were high for biocide resistance genes-positive *P. aeruginosa* strains compared with the negative strains (Table 4).

A study by Chuanchuen *et al.* showed a cross-resistance between biocide and antibiotic resistance. They demonstrated a link between *P. aeruginosa* exposure to triclosan biocide and efflux-mediated resistance to ciprofloxacin [12]. In the present study, there was no significant association between biocide resistance genes and antibiotic resistance, except for levofloxacin and norfloxacin antibiotics and *qacE* and *qacG* genes. However, more studies are needed to substantiate the existence of biocide-antibiotic cross-resistance.

5. Conclusion

Our results revealed that the frequency of resistance genes to benzalkonium chloride, chlorhexidine digluconate, triclosan and formaldehyde was high in clinical isolates of *P. aeruginosa*. Furthermore, *P. aeruginosa* isolates harboring resistance genes had higher MIC values compared with those lacking these genes. On the other hand, chlorhexidine digluconate was the most effective biocide against *P. aeruginosa* isolates in Ardabil hospitals. We recommend continuous monitoring of the antimicrobial activity of biocides and biocide-associated resistance genes in order to prevent microorganism dissemination and infection control in hospitals.

Abbreviations

MIC: Minimum inhibitory concentration; PCR: Polymerase chain reaction; *Pseudomonas aeruginosa*: *P. aeruginosa*; ICU: Intensive care unit; MDR: Multi-drug resistant; CLSI: Clinical and Laboratory Standards Institute; ECOFF: Epidemiological cut-off value; ENR: Enoyl-acyl-carrier protein reductase.

Declarations

Ethics approval and consent to participate

This research was approved by the Research Ethics Committee of Ardabil University of Medical Sciences (approval ID: IR.ARUMS.REC.1398.591). Informed written consent was given to subjects from whom the samples were obtained for this study.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

Authors' contributions

MN, SS and SAB collected the data. FK and HV analyzed the data and led the writing of the manuscript. SH and MA revised the manuscript.

Funding

This research was supported by Ardabil University of Medical Sciences, Iran (grant number: 1005761).

Acknowledgements

The authors would like to acknowledge the Vice Chancellor for Research and Technology, Ardabil University of Medical Sciences, Ardabil, Iran, due to financial support.

References

1. Driscoll JA, Brody SL, Kollef MH (2007) The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 67(3):351–368
2. Vaez H, Salehi-Abargouei A, Khademi F (2017) Systematic review and meta-analysis of imipenem-resistant *Pseudomonas aeruginosa* prevalence in Iran. *Germes* 7(2):86–97
3. Moradali MF, Ghods S, Rehm BH (2017) *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol* 7:39
4. Ignak S, Nakipoglu Y, Gurler B (2017) Frequency of antiseptic resistance genes in clinical staphylococci and enterococci isolates in Turkey. *Antimicrob Resist Infect Control* 6(1):1–7
5. Vásquez-Giraldo DF, Libreros-Zúñiga GA, Crespo-Ortiz MD (2017) Effects of biocide exposure on *P. aeruginosa*, *E. coli* and *A. baumannii* complex isolates from hospital and household environments. *Infectio* 21(4): 243 – 50
6. Wesgate R, Grasha P, Maillard J-Y (2016) Use of a predictive protocol to measure the antimicrobial resistance risks associated with biocidal product usage. *Am J Infect Control* 44(4):458–464
7. Ortega-Morente E, Fernández-Fuentes MA, Grande-Burgos MJ, Abriouel H, Pérez-Pulido R, Gálvez A (2013) Biocide tolerance in bacteria. *Int J Food Microbiol* 162(1):13–25
8. Murray PR, Rosenthal KS, Pfaller MA (2015) *Medical microbiology*, 8th edn. Elsevier Health Sciences, UK, pp 272–277
9. Bridier A, Dubois-Brissonnet F, Greub G, Thomas V, Briandet R (2011) Dynamics of the action of biocides in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* 55(6):2648–2654
10. Mima T, Joshi S, Gomez-Escalada M, Schweizer HP (2007) Identification and characterization of TriABC-OpmH, a triclosan efflux pump of *Pseudomonas aeruginosa* requiring two membrane fusion proteins. *J Bacteriol* 189(21):7600–7609
11. Vikram A, Bomberger JM, Bibby KJ (2015) Efflux as a glutaraldehyde resistance mechanism in *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* 59(6):3433–3440
12. Chuanchuen R, Beinlich K, Hoang TT, Becher A, Karkhoff-Schweizer RR, Schweizer HP (2001) Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects nfxB mutants overexpressing MexCD-OprJ. *Antimicrob Agents Chemother* 45(2):428–432
13. Subedi D, Vijay AK, Willcox M (2018) Study of disinfectant resistance genes in ocular isolates of *Pseudomonas aeruginosa*. *Antibiotics* 7(4):88
14. CLSI (2018) *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement*. CLSI Document M100. Clinical and Laboratory Standards Institute, Wayne
15. Bazghandi SA, Safarirad S, Arzanlou M, Peeri-Dogaheh H, AliMohammadi H, Khademi F (2021) Prevalence of multidrug-resistant *Pseudomonas aeruginosa* strains in Ardabil. *J Ardabil Univ Med Sci* 20(2):280–286
16. Rizzotti L, Rossi F, Torriani S (2016) Biocide and antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolated from the swine meat chain. *Food Microbiol* 60:160–164

17. Chapman JS (2003) Biocide resistance mechanisms. *Int Biodeterior Biodegradation* 51(2):133–138
18. Romão C, Miranda CA, Silva J, Clementino MM, de Filippis I, Asensi M (2011) Presence of *qacEΔ1* gene and susceptibility to a hospital biocide in clinical isolates of *Pseudomonas aeruginosa* resistant to antibiotics. *Curr Microbiol* 63(1):16–21
19. Zhu L, Lin J, Ma J, Cronan JE, Wang H (2010) Triclosan resistance of *Pseudomonas aeruginosa* PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. *Antimicrob Agents Chemother* 54(2):689–698
20. Heath RJ, Rock CO (2000) A triclosan-resistant bacterial enzyme. *Nature* 406(6792):145–146
21. Huang YH, Lin JS, Ma JC, Wang HH (2016) Functional characterization of triclosan-resistant enoyl-acyl-carrier protein reductase (FabV) in *Pseudomonas aeruginosa*. *Front Microbiol* 7: 1903
22. Thomas L, Maillard JY, Lambert RJ, Russell AD (2000) Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a 'residual' concentration. *J Hosp Infect* 46(4):297–303
23. Hassan KA, Liu Q, Henderson PJ, Paulsen IT (2015) Homologs of the *Acinetobacter baumannii* Acel transporter represent a new family of bacterial multidrug efflux systems. *MBio* 6(1):e01982–e01914
24. Mendes ET, Ranzani OT, Marchi AP, da Silva MT, Amigo Filho JU, Alves T, Guimarães T, Levin AS, Costa SF (2016) Chlorhexidine bathing for the prevention of colonization and infection with multidrug-resistant microorganisms in a hematopoietic stem cell transplantation unit over a 9-year period: Impact on chlorhexidine susceptibility. *Medicine* 95(46):1–8
25. Vijayakumar R, Sandle T, Al-Aboody MS, Alfonaisan MK, Alturaiki W, Mickymaray S, Premanathan M, Alsagaby SA (2018) Distribution of biocide resistant genes and biocides susceptibility in multidrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*—A first report from the Kingdom of Saudi Arabia. *J Infect Public Health* 11(6):812–816
26. Kücken D, Feucht HH, Kaulfers PM (2000) Association of *qacE* and *qacEΔ1* with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS Microbiol Lett* 183(1):95–98
27. Helal ZH, Khan MI (2015) *QacE* and *QacEΔ1* Genes and Their Correlation to Antibiotics and Biocides Resistance *Pseudomonas aeruginosa*. *Am J Biomed Sci* 7(2): 52–62
28. Mahzounieh M, Khoshnood S, Ebrahimi A, Habibian S, Yaghoubian M (2014) Detection of antiseptic-resistance genes in *Pseudomonas* and *Acinetobacter* spp. isolated from burn patients. *Jundishapur J Nat Pharm Prod* 9(2):e15402
29. McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12(1):147–179