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Determination of Disinfectant Resistance Genes in Clinical Bacterial Isolates

Eren BİÇER[1], Birgül ÖZCAN[2,*], Dilek GEZGİN[3]

[1]*Hatay Mustafa Kemal University, Faculty of Arts and Sciences, Department of Biology, 31060, Antakya-Hatay, Türkiye*
erenbicer1903@gmail.com, ORCID: 0000-0001-8735-6250
[2]*Hatay Mustafa Kemal University, Faculty of Arts and Sciences, Department of Biology, 31060, Antakya-Hatay, Türkiye*
birgulozcan@gmail.com, ORCID: 0000-0001-9003-8480
[3]*Hatay Mustafa Kemal University, Faculty of Arts and Sciences, Department of Biology, 31060, Antakya, Türkiye*
dilekuzundag@gmail.com, ORCID: 0000-0002-6256-574X

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Abstract

In healthcare settings such as hospitals, bacteria develop resistance to disinfectants, which play a critical role in infection control, through various mechanisms. The presence and expression of efflux pumps are considered one of the key mechanisms of disinfectant resistance. The main objective of this study is to detect the presence of *qacA/B, qacC/D, qacE, qacΔE1, qacG, qacH, qacJ,* and *acrA* genes in clinical bacterial isolates and to determine the minimum inhibitory concentrations (MICs) against two different disinfectants: a quaternary ammonium compound and a biguanide. The presence of the related genes was screened in 40 clinical isolates using conventional polymerase chain reaction. Disinfectant susceptibility was determined by the agar dilution method. Species-level identification of the isolates was confirmed by Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) technique. As a result of the screening, 6 isolates (15%), confirmed as *E. coli, K. pneumoniae, and P. aeruginosa*, were found to possess the *qacΔE1* gene. The MIC values for these isolates against benzalkonium chloride and chlorhexidine gluconate were found to be in the range of 32−256 mg/L and 2−16

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mg/L, respectively. The findings revealed that there was no direct correlation between observed MIC values and the presence or absence of biocide resistance genes in the clinical *E. coli, K. pneumoniae*, and *P. aeruginosa* isolates studied.

Keywords: Disinfectant; Resistance genes; Clinical isolates; *MICs*

Klinik Bakteri İzolatlarında Dezenfektan Direnç Genlerinin Belirlenmesi

Öz

Hastaneler gibi sağlık hizmeti sunulan birimlerde enfeksiyon kontrolünde kritik bir rol oynayan dezenfektanlara karşı bakteriler farklı mekanizmalarla direnç geliştirmektedir. Akış (efflux) pompalarının varlığı ve ifadesi, dezenfektan direncinin temel mekanizmalarından biri olarak kabul edilmektedir. Bu çalışmanın temel amacı, klinik kökenli bakteri izolatlarında *qacA/B, qacC/D, qacE, qacΔE1, qacG, qacH, qacJ* ve *acrA* genlerinin varlığını saptamak ve bir kuaterner amonyum bileşiği ve bir bisguanid olmak üzere iki farklı dezenfektana karşı minimum inhibisyon konsantrasyonlarını (MİK) belirlemektir. Toplam 40 adet klinik izolatta, konvansiyonel Polimeraz Zincir Reaksiyonu ile ilgili genlerin varlığı taranmıştır. Dezenfektanlara duyarlılık agar dilüsyon yöntemiyle belirlenmiştir. İzolatların tür düzeyinde tanımlanması, matriks destekli lazer desorpsiyon/iyonizasyon uçuş süresi kütle spektrometresi (MALDI-TOF MS) tekniği ile doğrulanmıştır. Yapılan tarama sonucunda, *E. coli, K. pneumoniae* ve *P. aeruginosa* olduğu teyid edilen 6 izolatın (%15) *qacΔE1* genine sahip olduğu tespit edilmiştir. Bu izolatların benzalkonyum klorür ve klorheksidin glukonat'a karşı MİK değerleri sırasıyla 32−256 mg/L ve 2−16 mg/L aralığında bulunmuştur. Elde edilen bulgular, çalışılan klinik *E. coli, K. pneumoniae* ve *P. aeruginosa* izolatlarında gözlenen MİK değerleri ile biyosit direnç genlerinin varlığı veya yokluğu arasında doğrudan bir korelasyon olmadığını ortaya koymuştur.

Anahtar Kelimeler: Dezenfektan; Direnç geni; Klinik izolat; MİK

1. Introduction

Used to control infections in healthcare facilities, especially hospitals, disinfectants are one of the most important tools in modern medicine. However, the extensive and sometimes incorrect use of these chemicals leads to the development of resistance in bacteria. In particular, due to improper dilution, insufficient contact time, or incorrect application, disinfectants can affect bacteria at sub-inhibitory levels, facilitating the acquisition of resistance [1]. The development of resistance not only complicates infection control but can also lead to bacteria becoming more

resistant to antibiotics [2]. For this reason, understanding the mechanisms of disinfectant resistance is critical for both infection control strategies and public health.

Disinfectants are generally divided into two main groups: oxidizing and non-oxidizing. Oxidizing disinfectants (e.g., chlorine compounds and hydrogen peroxide) kill microorganisms by oxidizing intracellular proteins and nucleic acids. Non-oxidizing disinfectants include substances such as quaternary ammonium compounds (QAMs), biguanides, phenols, and glutaraldehyde. These compounds generally act by disrupting cell membrane integrity or inhibiting enzymatic functions [3]. Quaternary ammonium compounds and chlorhexidine, in particular, are among the most commonly used agents in hospitals.

Bacteria employ a variety of mechanisms to develop resistance to these agents. Chief among these are "efflux pumps," which expel toxic substances that enter the cell. Genes encoding these pumps, such as *qacA/B*, *qacC/D*, *qacE*, *qacΔE1*, *qacG*, *qacH*, *qacJ*, and *acrA*, have been widely identified in both Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa)* and Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis)* bacteria [4]. The presence of these genes is generally associated with higher minimum inhibitory concentration (MIC) values and may reduce the effectiveness of disinfectants. Recent studies reveal clinically important findings. The frequent detection of qac genes in *E. coli* and *P. aeruginosa* isolates suggests that disinfectant resistance has become a significant clinical problem [5]. It has been reported that the *qacEΔ1* gene is present in 62% of multidrug-resistant *K. pneumoniae* isolates and that this gene is significantly associated with cephalosporin resistance [6]. For this reason, it has been reported that these genes may reside on the same plasmids or integrons as antibiotic resistance genes, thus co-transmitting both disinfectant and antibiotic resistance. It has also been reported that the use of low concentrations of disinfectants can lead to cross-resistance in bacteria not only to biocides but also to antibiotics [2, 7]. Recently, the emergence of multidrug-resistant bacteria (MDR) and antibiotic resistance has become a significant global problem [8]. For this reason, the most effective protection against unwanted bacterial growth in medical and industrial settings today is provided by disinfectants and advanced biosecurity measures [9]. However, the lack of scientific management and proper planning, as well as the excessive and improper use of disinfectants, leads to an increase in antibiotic resistance genes (ARGs) [10]. For example, it has been reported that quaternary ammonium compounds detected in environmental wastewater may be effective in selecting for antibiotic resistance genes. This suggests that resistance development and spread may also occur outside of hospital settings.

Despite increasing research on disinfectant resistance, there are still significant standardization gaps in this area. Although international MIC breakpoints exist for antibiotics, the lack of a similar reference framework for disinfectants makes it difficult to compare the results of studies. Also, the methodologies used, different terminologies, and heterogeneous experimental designs have led to limited epidemiological data on disinfectant resistance. This further complicates the problem of disinfectant resistance and poses a serious threat to global health. For this reason, a detailed understanding of the mechanisms of disinfectant resistance and the factors affecting this resistance is vital [11].

Findings indicate that disinfectant resistance is encountered in daily hospital practice and may be a risk factor for infection control. The potential contribution of this resistance to the spread of resistance genes in bacterial populations highlights the need for further investigation. For this reason, our study aimed to determine the presence of several disinfectant resistance-associated genes (*qacA/B, qacC/D, qacE, qacΔE1, qacG, qacH, qacJ,* and *acrA*) in hospital-acquired clinical isolates from the Molecular Biology Laboratory of Hatay Mustafa Kemal University using conventional PCR. We also aimed to identify isolates harboring these genes and determine their minimum inhibitory concentration (MIC) values against benzalkonium chloride and chlorhexidine gluconate.

2. Materials and Methods

Hospital-acquired bacterial isolates from the Molecular Biology Laboratory of the Faculty of Arts and Sciences at Hatay Mustafa Kemal University were used in the study. Genomic DNA purification kit, Safe-T-stain, 100-1500 bp DNA marker (100 bp ladder), Müller Hinton agar/broth, Brain Heart Infusion (BHI) agar/broth, 10X TBE buffer, NaCl, Lysozyme, 10X PCR buffer, dNTP, $MgCl_2$, Taq polymerase, agarose, Safe-T-stain, chlorhexidine gluconate, and benzalkonium chloride were supplied by Thermofisher, Bmlabosis, Biomatik, Sigma, Merck, and Ataman Kimya.

Isolates preserved as glycerol stocks were activated in BHI medium, and purity controls were performed on agar medium. The species of clinical bacterial isolates prepared from fresh cultures (18-24 hours at 37°C) were confirmed using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). The identification of bacteria was performed by comparing the spectra obtained with the instrument's flex control software program (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) with the Maldi Biotyper Real-Time Classification (RTC) software [12].

Genomic DNA was obtained from the isolates using a commercial DNA purification kit. Additionally, a freshly prepared 20 mg/ml lysozyme solution was used for DNA isolation from Gram-positive isolates, as recommended in the kit. The obtained DNA samples were run on a 0.8% agarose gel and stored at -20°C until PCR studies. Polymerase chain reaction (PCR) was performed using specific primers (Table 1) for disinfectant resistance genes (*qacA/B*, *qacC/D*, *qacΔE1*, *qacE*, *qacG*, *qacH*, *qacJ*, and *acrA* in the strain). The products were separated by gel electrophoresis and visualized under UV light.

Table 1: Primers used in the study.

Gene	Primer pairs	Amplicon size (bp)
qacA/B	5' -GCTGCATTTATGACAATGTTTG-3' 5' -AATCCCACCTACTAAAGCAG-3'	629 [13]
qacC/D	5'GGCTTTTCAAAATTTATACCATCCT-3' 5'-ATGCGATGTTCCGAAAATGT-3'	249 [14]
qacΔE	5'- TAGCGAGGGCTTTACTAAGC -3' 5'-ATTCGAAATGCCGAACACCG-3'	335 [15]
qacE	5'-GCCCTACACAAATTGGGAGA-3' 5'- TTAGTGGGCACTTGCTTTGG -3'	350 [13]
qacG	5'- CAACAGAAATAATCGGAACT -3' 5'- TACATTTAAGAGCACTACA -3'	275 [16]
qacH	5'- ATAGTCAGTGAAGTAATAG -3' 5'- AGTGTGATGATCCGAATGT -3'	295 [16]
qacJ	5'- CTTATATTTAGTAATAGCG -3' 5'- GATCCAAAAACGTTAAGA -3'	306 [16]
acrA	5'- CCTCAAGTTAGCGGGATTAT -3' 5'- ACCGTCCTGCGGGAACTTAA -3'	567 [13]

Disinfectant resistance was determined by the agar dilution method, as recommended by the Clinical and Laboratory Standards Institute [17]. Benzalkonium chloride and chlorhexidine gluconate were diluted two-fold and added to sterile Mueller-Hinton agar medium at final temperatures ranging from 0.125 to 1024 mg/L, and bacterial temperatures were spot-inoculated. After incubation (37°C, 24 hours), the MIC was determined as the low disinfectant range where growth was completely inhibited [18].

3. Results and Discussion

3.1. Detection of Disinfectant Resistance Genes

In the present study, the presence of *qacA/B*, *qacC/D*, *qacΔE1*, *qacE*, *qacG*, *qacH*, *qacJ*, and *acrA* genes was investigated using PCR in a total of 40 clinical bacterial strains (Table 2) isolated from different hospitals and units (intensive care, urology, internal medicine, general surgery, gynecology, chest diseases, and palliative care) in the culture collection of the Microbiology Laboratory of the Faculty of Arts and Sciences at Mustafa Kemal University.

Table 2: Source and type of clinical isolates.

No	Bacteria/Gram reaction	Source	No	Bacteria/Gram reaction	Source
1	*Acinetobacter baumannii* (Gr-)	Wound	21	*Klebsiella oxytoca*	Urine
2	*Escherichia coli* (Gr-)	Urine	22	*Pseudomonas aeruginosa*	Wound
3	*Escherichia coli*	Wound	23	*Acinetobacter Baumann*	Blood
4	*Klebsiella oxytoca*	Urine	24	*Escherichia coli*	Urine
5	*Escherichia coli*	Urine	25	*Escherichia coli*	Urine
6	*Escherichia coli*	Urine	26	*Acinetobacter baumannii*	Sputum
7	*Pseudomonas aeruginosa* (Gr-)	Urine	27	*Pseudomonas aeruginosa*	Aspirate
8	*Enterococcus faecium* (Gr+)	Wound	28	*Acinetobacter baumannii*	Urine
9	*Enterococcus faecalis* (Gr+)	Urine	29	*Acinetobacter baumannii*	Blood
10	*Acinetobacter baumannii*	Wound	30	*Klebsiella ozoenae*	Blood
11	*Escherichia coli*	Urine	31	*Klebsiella pneumoniae*	Urine
12	*Pseudomonas aeruginosa*	Wound	32	*Pseudomonas aeruginosa*	Urine
13	*Acinetobacter baumannii*	Blood	33	*Escherichia coli*	Urine
14	*Escherichia coli*	Urine	34	*Staphylococcus aureus* (Gr+)	Blood
15	*Klebsiella pneumoniae* (Gr-)	Blood	35	*Escherichia coli*	Urine
16	*Pseudomonas aeruginosa*	Aspirate	36	*Acinetobacter baumannii*	Aspirate
17	*Klebsiella pneumoniae*	Urine	37	*Staphylococcus aureus*	Blood
18	*Pseudomonas aeruginosa*	Wound	38	*Streptococcus agalactiae* (Gr+)	Vagina
19	*Pseudomonas aeruginosa*	Urine	39	*Pseudomonas aeruginosa*	Tissue
20	*Escherichia coli*	Wound	40	*Serratia marcescens* (Gr-)	Blood

The *qacΔE1* gene, measuring 335 bp, was detected in 6 of 40 hospital-acquired bacterial isolates. Two of these were found in urine-acquired *E. coli* strains (strains 2 and 24), two in blood-acquired *K. pneumoniae* strains (strains 15 and 17), and the other two in aspirate- and urine-acquired *P. aeruginosa* strains (strains 27 and 34) (Table 2 and Figure 1). In our study, the percentages of the relevant gene were 18.2% among *E. coli* isolates, 22.2% among *P. aeruginosa* isolates, and 66.7% among *K. pneumoniae* isolates, and in 15% of a total of 40 bacteria. Other biocide resistance genes (*qacA/B*, *qacC/D*, *qacE*, *qacG*, *qacH*, *qacJ*, and *acrA*) were not detected in the bacterial species within the scope of the study.

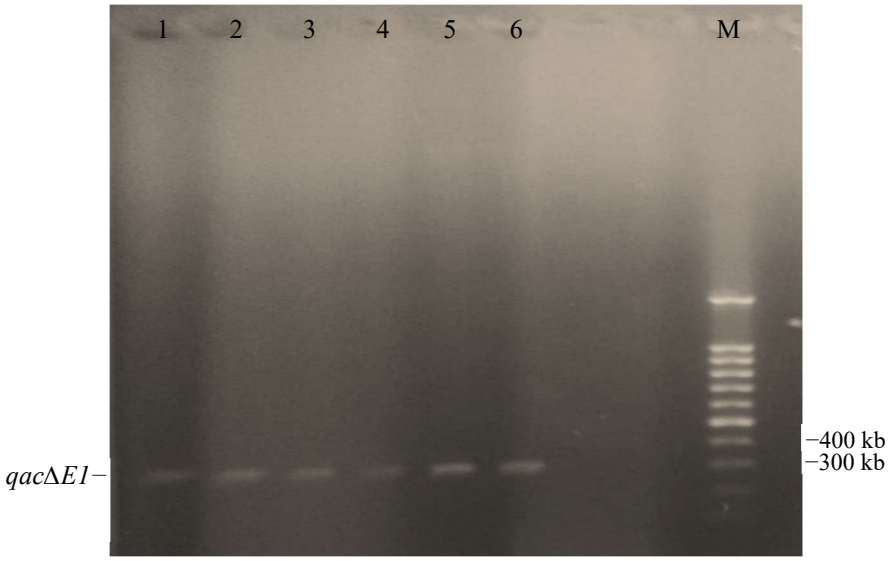

Figure 1: Gel electrophoresis image of PCR results for the *qacΔE1* gene in *E. coli* (1-strain 2 and 2-strain 24), *P. aeruginasa* (3-strain 27 and 4-strain 34), and *K. pneumoniae* (5-strain 5 and 6-strain 17). M: marker

One of the fundamental mechanisms enabling bacteria to develop resistance to biocides is the expression of efflux pumps. Qac (quaternary ammonium compounds) genes, one of which is widely distributed in both Gram-positive and Gram-negative bacteria, are generally carried on class I integrons [19, 20]. The *qacEΔ1* efflux pump, which belongs to the small multidrug resistance (SMR) superfamily and is one of six families of efflux pump systems, can extrude quaternary am5monium compounds and chlorhexidine. Overexpression of this gene, particularly in *E. coli* biofilms, has been reported to be associated with increased chlorhexidine MIC values [21]. Similar to our study findings, a high prevalence of *qacEΔ1* has been reported in multidrug-resistant clinical *Klebsiella* spp., carbapenem-resistant *P. aeruginosa* isolates, and nosocomial *E. coli* isolates [6, 21, 22]. The higher occurrence of *qacEΔ1* among all resistance genes is related to the longer history of use of quaternary ammonium compound disinfectants than antibiotics. The high prevalence of these genes facilitates their rapid spread among populations through horizontal gene transfer [23]. Furthermore, the fact that these genes share genetic structures with antibiotic resistance genes suggests that disinfectant and antibiotic resistance may be co-transmitted [21]. The frequent presence of the *qacΔE* gene in hospital isolates, as well as in strains from industrial environments where quaternary ammonium compounds are used extensively, suggests that disinfectant resistance is a widespread problem outside hospital settings [24].

3.2. Minimum Inhibitory Concentrations of Benzalkonium Chloride and Chlorhexidine Gluconate

The MIC values of benzalkonium chloride on strains using agar dilution were found to be 32 mg/L for *E. coli* and *K. pneumoniae* strains, including those containing the *qacΔE1* gene, and

256 mg/L for *P. aeruginosa* strains (Table 3). The MIC values of chlorhexidine gluconate were found to be 2 mg/L for the gene-deficient strain and 2 and 16 mg/L for the gene-containing strains of *E. coli*. For *K. pneumoniae* and *P. aeruginosa* strains, the MIC values were 64 mg/L for the gene-deficient strains and 16 mg/L for the gene-containing strains (Table 4).

Table 3: Minimum inhibitory concentration of benzalkonium chloride.

BK (mg/L)	**5** ***E. coli****	**33** ***K. pneu.****	**7** ***P. aer.****	**24** ***E. coli***	**2** ***E. coli***	**15** ***K. pneu.***	**17** ***K. pneu.***	**27** ***P. aer.***	**34** ***P. aer.***
0.125	+	+	+	+	+	+	+	+	+
0.250	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+
32	-	-	+	-	-	-	-	+	+
64	-	-	+	-	-	-	-	+	+
128	-	-	+	-	-	-	-	+	+
256	-	-	-	-	-	-	-	-	-
512	-	-	-	-	-	-	-	-	-
1024	-	-	-	-	-	-	-	-	-
Control	+	+	+	+	+	+	+	+	+

**qacΔE1* negative

Table 4: Minimum inhibitory concentration of chlorhexidine gluconate.

BK (mg/L)	**5** ***E. coli****	**33** ***K. pneu.****	**7** ***P. aer.****	**24** ***E. coli***	**2** ***E. coli***	**15** ***K. pneu.***	**17** ***K. pneu.***	**27** ***P. aer.***	**34** ***P. aer.***
0.125	+	+	+	+	+	+	+	+	+
0.250	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	+
2	-	+	+	-	+	+	+	+	+
4	-	+	+	-	+	+	+	+	+
8	-	+	+	-	+	+	+	+	+
16	-	+	+	-	-	-	-	-	-
32	-	+	+	-	-	-	-	-	-
64	-	-	-	-	-	-	-	-	-
128	-	-	-	-	-	-	-	-	-
256	-	-	-	-	-	-	-	-	-
512	-	-	-	-	-	-	-	-	-
1024	-	-	-	-	-	-	-	-	-
Control	+	+	+	+	+	+	+	+	+

**qacΔE1* negative

Studies have shown that benzalkonium chloride and chlorhexidine MIC values can be higher in strains containing the *qacΔE1* gene than in those lacking it [13, 25]. This suggests that disinfectant resistance genes may be more prevalent in drug-resistant strains. However, some

studies have found no significant correlation between the presence of this gene and increased susceptibility to disinfectants [26].

While the MIC values obtained in our study are consistent with similar results in the literature, no clear relationship was found between the presence of the gene and decreased susceptibility. As stated in the literature, efflux pumps alone may not be sufficient to explain biocide resistance, and that bacterial species, test methods, or other resistance mechanisms may also be influential [27].

3.3. Identification

Since the clinical isolates used in this study were identified using an automated system used in routine bacterial identification based on phenotypic characteristics, a total of nine isolates, including control groups containing and lacking the *qacΔE1* gene, were confirmed at the species level using a MALDI-TOF MS device. The protein spectra obtained from the analysis were compared with the reference database. Data between 2,000 and 3,000, highlighted in green, as suggested by the system's program, were considered reliable (Figure 2).

Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
27	Pseudomonas aeruginosa	2.456	Pseudomonas aeruginosa	2.439
34	Pseudomonas aeruginosa	2.364	Pseudomonas aeruginosa	2.275
7	Pseudomonas aeruginosa	2.389	Pseudomonas aeruginosa	2.298
33	Klebsiella pneumoniae	2.479	Klebsiella pneumoniae	2.393
17	Klebsiella pneumoniae	2.392	Klebsiella pneumoniae	2.38
15	Klebsiella pneumoniae	2.513	Klebsiella pneumoniae	2.398
24	Escherichia coli	2.508	Escherichia coli	2.427
5	Escherichia coli	2.459	Escherichia coli	2.449
2	Escherichia coli	2.167	Escherichia coli	2.131

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Figure 2: MALDI-TOF MS analysis score values

A total of 9 isolates selected for the study were confirmed at the species level using MALDI-TOF MS. As a result of this analysis, 8 isolates were identified with high confidence and 1 isolate with probable confidence. These findings further demonstrate that MALDI-TOF MS is

a powerful method for rapid, cost-effective, and accurate identification of clinical bacteria [12, 28].

4. Conclusion

In the present study, the presence of biocide resistance-associated genes and disinfectant susceptibility levels were examined in 40 clinical bacterial isolates obtained from hospital environments. PCR analysis revealed the presence of the qacΔE1 gene in 15% of the isolates. The detection of this gene, particularly in *E. coli, K. pneumoniae*, and *P. aeruginosa* strains, is consistent with the literature, which reports that this gene is widespread among the *Enterobacteriaceae* and *Pseudomonadaceae* families [23, 29]. When MIC (Minimum Inhibitory Concentration) values determined for disinfectants were examined, it was determined that the *P. aeruginosa* isolate exhibited a high tolerance to benzalkonium chloride at 256 mg/L. This result is consistent with previous reports suggesting that increased tolerance may be observed in *qacΔE1* gene-positive isolates [30]. This situation indicates that, as stated in the literature, additional factors such as membrane permeability, enzymatic inactivation, and biofilm formation may also be effective in biocide resistance [3, 31].

The literature indicates that misuse of disinfectants, especially at low concentrations, can create pressure for the selection of resistance genes and pave the way for the development of cross-resistance to antibiotics [32]. In this context, rapid identification methods such as MALDI-TOF MS have been reported to be an important tool for the early detection of resistant strains and the effective implementation of infection control policies [28].

In conclusion, this study aimed to evaluate the relationship between genetic disinfectant resistance and phenotypic susceptibility in clinical isolates. The findings suggest that not only disinfectant selection but also application doses and environmental factors should be considered when determining disinfection policies in hospitals.

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References

[1] Golfiroozi, S., Fashayi, F., Rajabi, A., Shahryar, A., *Disinfectants efficacy in reducing pathogens related to health-care infection associated in universities hospitals of Gorgan, North of Iran*, BMC Infectious Diseases, 24(1), 1113, 2024.

[2] Staats, G.J., Mc Carlie, S.J., Van der Walt, B., Bragg, R.R., *The linkage between antibiotic and disinfectant resistance*, In Antimicrobial Research and One Health in Africa, Cham: Springer International Publishing, 241-274, 2023.

[3] Maillard, J.Y., Pascoe, M., *Disinfectants and antiseptics: mechanisms of action and resistance*, Nature Reviews Microbiology, 22(1), 4-17, 2024.

[4] Dulanto Chiang, A., Dekker, J.P., *Efflux pump-mediated resistance to new beta lactam antibiotics in multidrug-resistant gram-negative bacteria*, Communications Medicine, 4(1), 170, 2024.

[5] Fernandes, Â.R., Rodrigues, A.G., Cobrado, L., *Effect of prolonged exposure to disinfectants in the antimicrobial resistance profile of relevant micro-organisms: a systematic review*, Journal of Hospital Infection, 151, 45-59, 2024.

[6] Kosyakova, K.G., Esaulenko, N B., Kameneva, O.A., Kazakov, S.P., Dubinina, A., Mezina, E., et. al., *Prevalence of carbapenemase genes, qacE, qacEΔ1 and cepA in multidrug-resistant gram-negative bacteria with different susceptibility to chlorhexidine*. Epidemiology and Vaccinal Prevention, 19(5), 49-60, 2020.

[7] Mc Carlie, S., Boucher, C.E., Bragg, R.R., *Molecular basis of bacterial disinfectant resistance*, Drug Resistance Updates, 48, 100672, 2020.

[8] Bassetti, M., Righi, E., *Multidrug-resistant bacteria: what is the threat?*, Hematology, the American Society of Hematology Education Program Book, 2013(1), 428-432, 2013.

[9] Davies, R., Wales, A., *Antimicrobial resistance on farms: a review including biosecurity and the potential role of disinfectants in resistance selection*, Comprehensive Reviews in Food Science and Food Safety, 18(3), 753-774, 2019.

[10] Han, Y., Zhou, Z. C., Zhu, L., Wei, Y.Y., Feng, W. Q., Xu, L., et. al., *The impact and mechanism of quaternary ammonium compounds on the transmission of antibiotic resistance genes*, Environmental Science and Pollution Research, 26(27), 28352-28360, 2019.

[11] Gerba, C.P., Boone, S., Nims, R.W., Maillard, J.Y., Sattar, S.A., Rubino, J.R., et. al., *Mechanisms of action of microbicides commonly used in infection prevention and control*, Microbiology and Molecular Biology Reviews, 88(3), e00205-22, 2024.

[12] Uysul, A., Kurt, Ş., Soylu, S., Soylu, E.M., Kara, M., *Yaprağı yenen sebzelerdeki mikroorganizma türlerinin MALDI-TOF MS (matris destekli lazer desorpsiyon/iyonizasyon uçuş Süresi Kütle Spektrometresi) Tekniği Kullanılarak Tanılanması*, Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 29(4), 595-603, 2019.

[13] Wei, X.Y., Luo, T., Jia, B., Chang, L.J., Li, X.Q., Huang, W.X., *Prevalence and molecular typing of the antiseptic resistance genes qacA/B among Staphylococcus aureus strains isolated in a teaching hospital*, African Journal of Biotechnology, 11(47), 10785-10791, 2012.

[14] Liu, W. J., Fu, L., Huang, M., Zhang, J.P., Wu, Y., Zhou, Y.S., et. al., *Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant Acinetobacter baumannii*, Journal of Medical Microbiology, 66(1), 13-17, 2017.

[15] Bischoff, M., Bauer, J., Preikschat, P., Schwaiger, K., Mölle, G., Hölzel, C., *First detection of the antiseptic resistance gene qac A/B in Enterococcus faecalis*, Microbial Drug Resistance, 18(1), 7-12, 2012.

[16] Correa, J.E., De Paulis, A., Predari, S., Sordelli, D.O., Jeric, P. E., *First report of qacG, qacH and qacJ genes in Staphylococcus haemolyticus human clinical isolates*, Journal of Antimicrobial Chemotherapy, 62(5), 956-960, 2008.

[17] CLSI., *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S15*. CLSI, Wayne, PA, USA, 2010.

[18] Kim, J.M., Ahn, Y., Li Puma, J.J., Hussong, D., Cerniglia, C.E., *Survival and susceptibility of Burkholderia cepacia complex in chlorhexidine gluconate and benzalkonium chloride*, Journal of Industrial Microbiology and Biotechnology, 42(6), 905-913, 2015.

[19] Chen, S., Fu, J., Zhao, K., Yang, S., Li, C., Penttinen, P., et. al., *Class 1 integron carrying qacEΔ1 gene confers resistance to disinfectant and antibiotics in Salmonella*, International Journal of Food Microbiology, 404, 110319, 2023.

[20] Zheng, W., Huyan, J., Tian, Z., Zhang, Y., Wen, X., *Clinical class 1 integron-integrase gene–A promising indicator to monitor the abundance and elimination of antibiotic resistance genes in an urban wastewater treatment plant*, Environment International, 135, 105372, 2020.

[21] Kuznetsova, M.V., Nesterova, L.Y., Mihailovskaya, V.S., Selivanova, P.A., Kochergina, D. A., Karipova, M.O., et. al., *Nosocomial Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus: Sensitivity to chlorhexidine-based biocides and prevalence of efflux pump genes*, International Journal of Molecular Sciences, 26(1), 355, 2025.

[22] Gülbudak, H., Emiroğlu, E., Görgülü, Y., Tezcan Ülger, S., Delialioğlu, N., Aslan, G., *Karbapenem dirençli Pseudomonas aeruginosa izolatlarında qacE, qacEΔ1 ve cepA biyosit direnç genlerinin araştırılması*, Türk Hijyen ve Deneysel Biyoloji Dergisi, 80(3), 277-284, 2023.

[23] Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., *Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution*, The ISME Journal, 9(6), 1269-1279, 2015.

[24] Han, Y., Zhou, Z.C., Zhu, L., Wei, Y.Y., Feng, W.Q., Xu, L., et. al., *The impact and mechanism of quaternary ammonium compounds on the transmission of antibiotic resistance genes*, Environmental Science and Pollution Research, 26(27), 28352-28360, 2019.

[25] Ahmed, S.S., Hassan, J.S., Mahdi, Q. A., *Detection of the quaternary ammonium Compound antiseptic resistance gene (qacA/B, qacC and qacE genes) among Staphylococcus aureus recovered from wound infection*, Iraqi Journal of Medical Sciences, 23(1), 2025.

[26] Zhao, K., Wang, L., Deng, J., Zuo, Q., Adila, M., Wang, X., et. al., *Determining the disinfectants resistance genes and the susceptibility to common disinfectants of extensively drug-resistant carbapenem-resistant Klebsiella pneumoniae strains at a tertiary hospital in China*, Microbial Drug Resistance, 30(10), 407-414, 2024.

[27] Blanco, P., Hernando-Amado, S., Reales-Calderon, J.A., Corona, F., Lira, F., Alcalde-Rico, M., et. al., *Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants*, Microorganisms, 4(1), 14, 2016.

[28] Abdulla, A.A., Abed, T.A., Almuttairi, A.A.H., *Molecular analysis of the antiseptic resistance genes among multidrug-resistant Pseudomonas aeruginosaiIsolated from burn patients*, Medical Journal of Babylon, 21(2), 456-461, 2024.

[29] Makled, A.F., Labeeb, A.Z., Moaz, H.M., Sleem, A.S., *Chlorhexidine and benzalkonium chloride: promising adjuncts in combating multidrug resistant Klebsiella pneumoniae in healthcare settings,* BMC Infectious Diseases, 25(1), 670, 2025.

[30] Farahani, H.E., Asgharzadeh, S., Golmoradizadeh, R., Akrami, S., Ghaznavi-Rad, E., Mirkalantari, S., et. al., *Resistance to chlorhexidine and benzalkonium chloride in E. Coli ST131, A. Baumannii, and P. Aeruginosa isolates*, Clinical Laboratory, 70(1), 2024.

[31] Tag ElDein, M.A., Yassin, A.S., El-Tayeb, O., Kashef, M.T., *Chlorhexidine leads to the evolution of antibiotic-resistant Pseudomonas aeruginosa*, European Journal of Clinical Microbiology & Infectious Diseases, 40(11), 2349-2361, 2021.

[32] Liu, X., Gong, L., Liu, E., Li, C., Wang, Y., Liang, J. *Characterization of the disinfectant resistance genes qacEΔ1 and cepA in carbapenem-resistant Klebsiella pneumoniae isolates*, The American Journal of Tropical Medicine and Hygiene, 110(1), 136, 2023.