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The Role of Efflux Pump Genes (*qacE, qacE-Δ1*,and *sug E1*) in Carbapenems and Biocides Resistance among *Pseudomonas aeruginosa* Isolated from Burn Infections

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Abstract: Burn injuries cause the skin's defenses against opportunistic infections to be compromised. One of the primary pathogens that colonize burn wounds and cause serious infections is *pseudomonas aeruginosa*. Appropriate treatment options and time are limited by antibiotic resistance, biofilm production, and other virulence factors. The study samples, which comprised 150 clinical specimens in the form of burn swabs from patients with burn infections, admitted to four hospitals in Baghdad, Iraq, were collected between November 2022 and the end of February 2023. Of all the bacterial cultures that were collected, 45 isolates (30%) were identified as *P. aeruginosa* based on the results of selective media, biochemical tests, and the VITEK2 system. The results of the disc diffusion method's antimicrobial susceptibility test for the isolates under investigation showed that *P. aeruginosa* clinical isolates had moderate resistance to most of the tested antibiotics. Most *P. aeruginosa* isolates exhibited high resistance to Tetracycline, Piperacillin, and Piperacillin-tazobactam, as well as to Ceftazidime (84.44%) and Cefepime (75.55%). Also, the highest sensitivity was recorded for Colistin, Tigecycline. All *P. aeruginosa* isolates that showed resistance to imipenem and meropenem antibiotics (n = 14) as well as ten isolates that were sensitive to these antibiotics were used for molecular study. Polymerase chain reaction (PCR) has been used for evaluate the presence of the identification gene (*16S rRNA*) and efflux pump genes (*qacE, qacE-Δ1,* and *sug E1*). The results of the efflux pump gene detection showed that all carbapenem-resistant isolates with high resistance to antibacterial agents (chlorohexidine, dettol, and EDTA) had the efflux pump genes, with the exception of the isolates P11, P13, and P14, where the isolates P11 and P14 did not have the gene *sug E1*, and isolate P12 showed the absence of the gene *qacE*. The findings showed that all of the resistant isolates had the gene *qacE-Δ1*. Also, it was found that the majority of the isolates which sensitive to carbapenems did not have the efflux pump genes. In conclusion, there is a significant correlation between the presence of efflux pump gene and the resistance of carbapenems and biocides (chlorohexidine, Dettol, and EDTA), where the high MICs values were related with isolates that have the efflux pump genes.

Keywords: Burns, Resistance, Carbapenems, Biocides, Efflux pump genes, *Pseudomonas aeruginosa*.

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Introduction

Burns are common, severe injuries that often result in significant long-term medical and psychological complications. Infectious complications continue to be the primary cause of death and additional morbidity in burn care, including skin replacement, creative wound dressings, fluid management, and renal replacement therapy. Severe burns are often associated with gram-negative bacteria such as *Acinetobacter baumanii, Proteus mirabilis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* (1). *Pseudomonas aeruginosa* is a common aerobic rod that is non-fermentative and Gram-negative in nature. One of the most important opportunistic pathogens, it has been connected to a number of hospital and community-acquired infections, burns, otitis media, nosocomial infections, and respiratory tract infections (2). *P. aeruginosa* isolates frequently exhibit extensive drug resistance (XDR), which occurs when the bacteria are sensitive to only one or two antimicrobial categories, as well as multidrug resistance (MDR), which occurs when the bacteria are resistant to at least one agent in three or more categories (3). The ability of *P. aeruginosa* to withstand antibiotics is largely due to its active multidrug efflux pumps. The resistance nodulation division family (RND), major facilitator superfamily, ATP-binding cassette superfamily, small multidrug resistance family, and multidrug and toxic compound extrusion family are the five families into which these pumps can be divided (4). The efflux pumps in *P. aeruginosa* direct extracellular substances (such as antibiotics, chlorhexidine, different medications, etc.) out of the bacteria and increase the pathogen's ability to survive, preventing the development of deadly concentrations of toxic compounds. MDR isolates are caused by increased expression of efflux pump genes (5).

The local isolates of *P. aeruginosa* exhibited high resistance to the most antibiotics used in the treatment (6). Efflux pump mechanism exists in *P. aeruginosa* to become resistant to antibacterial and disinfectant agents. Small multidrug resistance (SMR) proteins are among them; they are found in the cytoplasmic membrane's inner layer and confer resistance to several antibiotics as well as biocides. The SMP subgroup contains the genes *qacE* and *qacEΔ1*, which are found on integrons and plasmids of numerous drug-resistant gram-negative bacteria. The plasmid also contains *SUG* genes. There are proton-dependent efflux pumps in the SMR family. The *qac* genes cause resistance to quaternary ammonium compounds; in addition, these genes code for resistance to biguanides, diamidines, and intercalating dyes, among many other cationic substances (7)(8). Many local studies indicated to the impotence of efflux pumps of *P. aeruginosa* in antibiotics resistance (9)(10).

The aim of this study was to investigate the relationship between the efflux pump genes (*qacE, qacE-Δ1,* and *sug E1*) and the resistance of carbapenems and biocides (chlorohexidine, Dettol, and EDTA) among *P. aeruginosa* isolates of burns infections and comparison the results in resistant and sensitive isolates.

Materials and Methods

Isolation and identification of *P. aeruginosa*

Between November 2022 and February 2023, the burn swabs (150) were collected from hospitals in Baghdad, Iraq (Al-yarmuk teaching, Al-kindi teaching, and Al-imam Ali

teaching). Using MacConkey, Blood, and Cetramide agar, *P. aeruginosa* was isolated. As directed by the manufacturer, these isolates were identified by biochemical tests using the VITEK 2 system (bioMerieux, France).

Antibiotic Susceptibility Test

The antimicrobial susceptibility test was conducted using the disc diffusion method. To put it briefly, *P. aeruginosa* was grown overnight on Cetrimide agar and resuspended in sterile normal saline. Mueller-Hinton agar plates were inoculated with the suspension after its turbidity was adjusted to 0.5 McFarland. The following antibiotic discs were utilized in this investigation: Imipenem (IPM), Meropenem (MRP), Gentamicin (GEN), Ciprofloxacin (CIP), Piperacillin-tazobactum (PIT), Ceftazidim(CAZ), Cefepime (FEP), Azetronam (AT), Piperacillin (PI), Amikacin (AK), Levofloxacin (LE), and Tetracycline (TE), Colistin (CL) , Tigecycline (TGC). The inhibition zone was measured and interpreted using the percentage of susceptible, intermediate, or resistant isolates as determined by CLSI breakpoint interpretative criteria (CLSI, 2021) following a 24hour incubation period of the agar plates at 37 °C.

Susceptibility to Biocides

The biocidal agents were selected, chlorohexidine, dettol, and EDTA. Stock solutions were made by dissolving 1000 µg/mL of chlorohexidine, 4% of Dettol, and 102.4 of EDTA in sterile distilled water (as serial dilutions), in accordance with CLSI guidelines. After adding the inoculum (50 µL), stock solutions were further diluted in Mueller–Hinton broth (MHB) (HiMedia, India) to reach final well concentrations. Using Resazurin dye, the microdilution plates were visually read after a 24 hour incubation period at 37 °C. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the biocide that effectively stopped the isolate's growth (11).

DNA extraction and identification of efflux pumps genes by PCR

It was possible to extract genomic DNA from bacterial broth using a ready kit (Promega, USA). A Quantus Fluorometer (Promega, USA) was used to measure the extracted DNA concentration and purity in order to assess the sample quality. Within a 20 µL total volume, the *qacE, qacE delta1*, and *sugE1* genes were detected through PCR reactions. The reaction mixture comprised of 10 µL of Master Mix, 1 µL of forward and reverse primers, 2 µL of DNA template, and 6 µL of nuclease-free water. The primer sequences listed in Table 1 were utilized in this investigation to identify the genes encoding efflux pumps.

Table (1): Primer sequences for PCR detection of *Pseudomonas aeruginosa*

Primer name		sequence (5'—3')	Size	Reference
qacE	F	TGCGTTCCTGGATCTATCTG	206	(27)
	R	GACGATGCCAATGCCTTC		
qacE-Δ1	F	TTGTTATCGCAATAGTTG	202	
	R	AATGGCTGTAATTATGAC		
sugE1	F	CCGTTGGTCTGAAATACAC	196	
	R	ATGGATTCGCCGAACAGG		
16S rRNA	F	CCGTGTCTCAGTTCCAGT	104	(28)
	R	TGAGCCTAGGTCGGATTA		

Thermo Fisher Scientific, USA, used a thermocycler to amplify the efflux pump genes under PCR conditions based on the settings from earlier research, as shown in table 2. A 2% agarose gel was electrophoresed at 100V for 60 minutes. Following electrophoresis, fragments were visualized using ultraviolet light and stained with ethidium bromide.

Table (2): PCR amplification conditions for efflux pumps genes in *pseudomonas aeruginosa* isolates

Steps	Temperature (°C)	Time/ sec	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	*qacE-Δ1* 51°C *sugE1* 53°C *qacE1* 53°C *16sRNA* 55°C	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Results and Discussion

Isolation and Identification of *p. aeruginosa*

The species *P. aeruginosa* was detected using 150 burn swabs from burn patients that were cultured. MacConkey agar and sterile cetrimide agar plates were streaked with each sample that was being studied. The MacConkey agar colonies were mucoid and showed no signs of lactose fermentation. Green-yellow colonies are produced by the colonies on cetrimide agar (figure 1). According to Quinn *et al.*, (12) and Carter *et al.*, (13), the greenish single colonies were taken up in sterile cetrimide agar and slants for further characterization by various biochemical tests, such as oxidase test, catalase test, nitrate reduction, indole test, methyl red test, Voges-Proskauer test, citrate utilization, and glucose fermentation test. To confirm the outcome, every positive sample underwent two tests. The VITEK 2 system results were used to confirm the identity of every isolate of *P. aeuginosa*. Forty-five *P. aeruginosa* isolates from burn infection patients were obtained from 150 burn swabs.

Figure (1): Colonies of *pseudomonas aeruginosa* on the selective media Cetrimide agar (light green).

Antibiotic Susceptibility of *P. aeruginosa*

Using the disc diffusion method, the antibiotic sensitivity and resistance of 45 *P. aeruginosa* isolates were assessed using 14 antibiotic discs. The results of the sensitivity test are displayed in (Table 3).

The results of this investigation showed that Colistin (84.44%), Levofloxacin and Tigecycline (66.66%), and Imipenem (64.44%) had the highest percentages of sensitivity. With regard to *P. aeruginosa*, ceftazidime (6.66%), tetracycline (15.55%), and piperacillin-tazobactam (22.22%) showed the lowest percentage of antibiotic sensitivity. The highest percentages of antibiotic resistance were shown for ceftazidime (84.44%), piperacillin (73.33%), and tetracycline and cefepime (75.55%). Furthermore, the isolates exhibited low levels of resistance to levofloxacin (31.11%), tigecycline (22.22%), and colistin (15.55%).

Table (3): Percentages of antimicrobial susceptibility rate of 45 *P. aeruginosa* isolates against 14 antimicrobial agents.

Antibiotics	Sensitive	Intermediate	Resistance	P-value
Piperacillin-tazobactam (PIT)	(10) 22.22%	(4) 8.88%	(31) 68.88%	0.0001 **
Ceftazidime (CAZ)	(3) 6.66%	(4) 8.88%	(38) 84.44%	0.0001 **
Cefepime (FEP)	(11) 24.44%	(0) 0.00%	(34) 75.55%	0.0001 **
Ciprofloxacin (CIP)	(26) 57.77%	(1) 2.22%	(18) 40.00%	0.0001 **
Levofloxacin (LE)	(30) 66.66%	(1) 2.22%	(14) 31.11%	0.0001 **
Imipenem (IPM)	(29) 64.44%	(0) 0.00%	(16) 35.55%	0.0001 **
Meropenem (MRP)	(26) 57.77%	(0) 0.00%	(19) 42.22%	0.0001 **
Gentamicin (GEN)	(20) 44.44%	(2) 4.44%	(23) 51.11%	0.0002 **
Tetracycline (TE)	(7) 15.55%	(4) 8.88%	(34) 75.55%	0.0001 **
Tigecycline (TGC)	(30) 66.66%	(5) 11.11%	(10) 22.22%	0.0001 **
Azetronam (AT)	(23) 51.11%	(7) 15.55%	(15) 33.33%	0.0134 *
Amikacin (AK)	(21) 46.66%	(4) 8.88%	(20) 44.44%	0.0001 **
Piperacillin (PI)	(12) 26.66%	(0) 0.00%	(33) 73.33%	0.0001 **
Colistin (CL)	(38) 84.44%	(0) 0.00%	(7) 15.55%	0.0001 **
P-value	0.0001 **	0.0378 *	0.0001 **	---
* (P≤0.05), ** (P≤0.01).				

One hundred and eleven specimens from burns and wounds from Baghdad hospitals were used in the local study; of these, 31 isolates contained *Pseudomonas aeruginosa*, 33 contained *Escherichia coli*, and 10 contained *Klebsiella* spp. The 31 *Pseudomonas aeruginosa* isolates were subjected to drug susceptibility tests against five antibiotics; the results indicated the percentage of resistance: 100% for carbenicillin, 61% for ticarcillin, 32% for pipracillin, 93% for cefprozil, and 84% for colonistin (14).

According to a study conducted in Brazil, *Pseudomonas aeruginosa* was the most common etiological agent among burn patients between the years 2015–2016 and 2019–2020. *Acinetobacter baumannii* resistant to carbapenems and *Staphylococcus aureus* were the next most common etiological agents in both periods. *P. aeruginosa* was the primary agent found responsible for the deaths. Between the two time periods, there was no difference in the predominant pathogens or bacterial antibiotic resistance (15). The epidemiological profile of *Pseudomonas aeruginosa* isolated from a burn unit in Tunisia was examined in a different study. In a span of three years, from July 1, 2008, to June 30, 2011, 544 *P. aeruginosa* strains were isolated from burn patients. Blood cultures and cutaneous infections were the most often isolated sites (83.4%). The following were the percentages of resistant isolates: 34% for ceftazidime, 37.1% for imipenem, 27.1% for ciprofloxacin, and 29.6% for amikacin. Except for colistin, all of these strains were resistant to every antibiotic that was tested (16).

The previous study in Romania, 202 adult patients who were admitted to the intensive care unit (ICU), between 1 October 2018 and 1 April 2022, were included in the study, which covered the first two years of the COVID-19 outbreak. Every patient had their urine, blood drawn for a blood culture, wound swabs, and endotracheal aspirates taken. *Pseudomonas aeruginosa* made up 39% of the bacteria that were isolated, followed by *Klebsiella* spp. and *Staphylococcus aureus* (12%). (11%), and *Acinetobacter baumannii* (9%). Regardless of the clinical specimen from which *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated, over 90% of them were MDR (17).

The pathogenic bacteria of *P. aeruginosa* strains isolated from burn patients at Tehran, Iran's Shahid Motahari Hospital are subjected to multilocus sequence typing (MLST) and analysis of antibiotic resistance genes in the study Abednezhad (18). This study characterized 63 isolates of *P. aeruginosa* in total. MLST analysis was used to genotype the isolates. The study's isolates were all MDR. All isolates were susceptible to colistin, but the highest resistance was seen against cefoxitin, tobramycin, and gentamicin (100%).

Molecular detection of genes by polymerase chain reaction

All *P. aeruginosa* isolates that showed resistance to imipenem and meropenem antibiotics (n = 14) as well as ten isolates that were chosen because they were sensitive to these antibiotics had their genomic DNA extracted. Extraction of the genomic DNA from 24 isolates, which gel electrophoresis verified as bands. The Quantus Fluorometer was used to measure the concentration and purity of DNA. The results showed that all isolates had DNA concentrations ranging from 50-100 ng/µl and 1.4-2 in terms of purity.

Polymerase chain reaction (PCR) has been used for each DNA extracted

sample to determine the presence of the identification gene (*16S rRNA*) and efflux pump genes (*qacE, qacE-Δ1,* and *sugE1*) as well as the prevalence of each gene among *P. aeruginosa* clinical isolates. By analyzing the bands on gel electrophoresis and comparing their molecular weight with a 100 bp DNA ladder, the PCR products have been verified.

Every sample that had been extracted using DNA was put through a PCR reaction using primer sets that included the efflux pump genes, *sug E1* (196 bp), *qacE-Δ1* (202 bp), *qacE* (206 bp) and *16S rRNA* (104 pb). Figures display the findings of the PCR used to identify these genes in each isolate, and Table 5 illustrates the distribution of efflux pump genes across the 24 *P. aeruginosa* isolates obtained from burns.

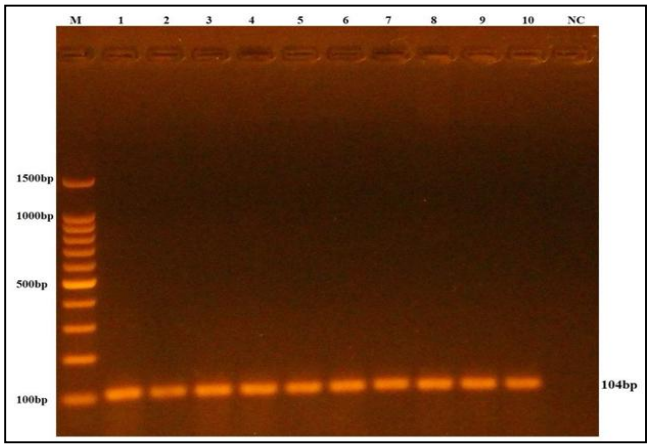

Figure (2): Agarose gel electrophoresis of PCR products for the Identification gene *16S rRNA*. Lane M: 100bp DNA ladder; lanes 1-10: P. aeruginosa isolates; lane 11: Negative control. (70V for 2hr).

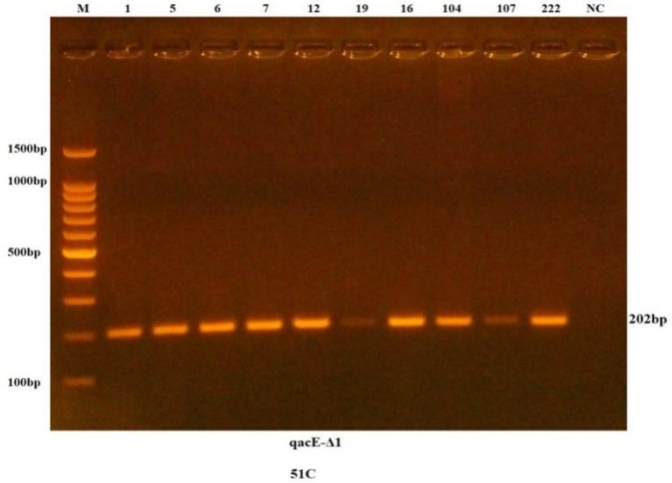

Figure (3): Agarose gel electrophoresis of PCR products for the resistance gene *qacEdelta1*Lane M: 100bp DNA ladder; lanes 2-14: P. aeruginosa K60 –K72 isolates; lane 1: Negative control. (70V for 2hr).

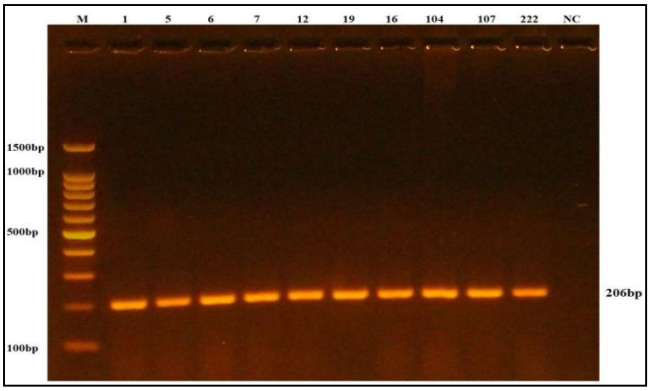

Figure (4): Agarose gel electrophoresis of PCR products for the resistance gene *qacE* Lane M: 100bp DNA ladder; lanes 2-14: P. aeruginosa K60 –K72 isolates; lane 1: Negative control. (70V for 2hr).

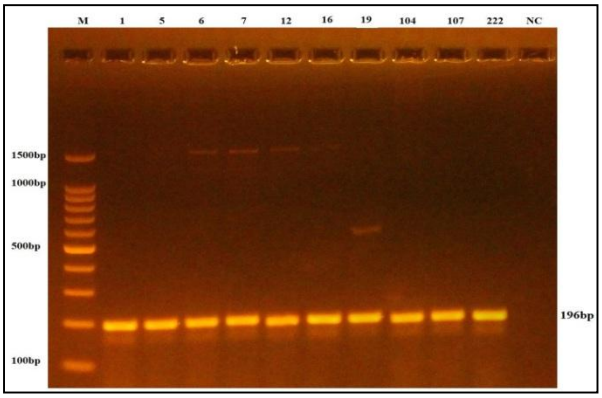

Figure (5): Agarose gel electrophoresis of PCR products for the resistance gene *Sug* E1. Lane M: 100bp DNA ladder; lanes 2-14: P. aeruginosa K60 –K72 isolates; lane 1: Negative control. (70V for 2hr).

The current results showed that all isolates examined by PCR assay for *P. aeruginosa* species identification contained the identification gene *16S rRNA*. The gene *16S rRNA* was used for the molecular identification of *P. aeruginosa* species in several previous studies (19)(20).

The results of the efflux pump gene detection showed that all carbapenem-resistant isolates with high resistance to antibacterial agents (chlorohexidine, Dettol, and EDTA) had these genes (*qacE-Δ1, qacE*, and *sug E1*), with the exception of the isolates p11, p13, and p14, where the isolates p11 and p14 did not have the gene *sug E1*, and isolate p12 showed the absence of the gene *qacE*. The findings showed that all of the resistant isolates had the gene *qacE-Δ1*. (Table 5) shows that of the isolates sensitive to carbapenems, the majority did not have the efflux pump genes identified. Of these, one isolate (p18) had the gene *qacE-Δ1*,two isolates (p16 and p22) had the gene *qacE*, and three isolates (p19, p21, and p22) had the gene *sug E1*.

Table (5): Association of Efflux pump genes with the phenotypic resistance patterns of *Pseudomonas aeruginosa* isolates against Carbapenems and antiseptics.

The code of isolate	Genes detection			Carbapenems susceptibility		MICs of Antimicrobial agent		
	qacE	*qacE-Δ1*	*sug-E1*	IMP	MEM	Dettol (%)	EDTA (µg/ml)	CLX (µg/ml)
P1	+	+	+	R	R	2	500	51.2
P2	+	+	+	R	R	2	500	25.6
P3	+	+	+	R	R	0.25	250	6.4
P4	+	+	+	R	R	0.5	500	25.6
P5	+	+	+	R	R	2	1000	102.4
P6	+	+	+	R	R	0.5	250	25.6
P7	+	+	+	R	R	0.25	125	12.8
P8	+	+	+	R	R	2	125	51.2
P9	+	+	+	R	R	2	1000	51.2
P10	+	+	+	R	R	2	250	51.2
P11	+	+	-	R	R	1	250	25.6
P12	+	+	+	R	R	2	500	51.2
P13	-	+	+	R	R	1	250	12.8
P14	+	+	-	R	R	0.25	125	6.4
P15	-	-	-	S	S	0.01	7.81	1.6
P16	+	-	-	S	S	0.25	62.5	3.2
P17	-	-	-	S	S	0.06	3.90	1.6
P18	-	+	-	S	S	0.125	31.25	3.2
P19	-	-	+	S	S	0.125	31.25	3.2
P20	-	-	-	S	S	0.03	3.90	0.8
P21	-	-	+	S	S	0.06	7.81	1.6
P22	+	-	+	S	S	0.25	62.5	6.4
P23	-	-	-	S	S	0.03	15.62	0.8
P24	-	-	-	S	S	0.125	31.25	3.2

MIC: Minimum inhibitory concentration; CLX: Chlorohexidine; IMP: imipenem; MEM: Meropenem.

The findings of (Table 5) demonstrated the clear correlation between *P. aeruginosa's* high level of resistance to carbapenems and antiseptics and the presence of efflux pump genes.

According to the findings of Goodarzi (21), 30 (32.6 %) of the *P. aeruginosa* isolates lacked any genes, while 62 (67.4%) of the isolates carried at least one of the investigated biocides genes. Sixty-nine strains (60.9%) possessed the *cepA* gene. One isolate (1.1%) and thirty-four isolates (36.7%) had their *qacE* and *qacEΔ1* genes amplified, respectively. One (1.1%) isolate and thirty-two (34.8%) isolates both possessed both the *cepA* and *qacEΔ1* genes. Furthermore, a significant correlation was observed between the existence of biocide resistance genes (*cepA* and *qacEΔ1*) and resistance to chlorohexidine (22).

According to a different study, the MIC of digluconate chlorhexidine (64 μg/mL). In addition, the corresponding prevalence rates for *qacEΔ1, qacE,* and *qacG* were 73.7% (n = 56), 26.3% (n = 20), and 11.8% (n = 9). A noteworthy correlation was noted between the existence of genes resistant to biocide and MICs. Additionally, the presence of biocide resistance genes did not significantly correlate with antibiotic resistance ($p > 0.05$), with the exception of the antibiotics levofloxacin and norfloxacin as well as the *qacE* and *qacG* genes ($p < 0.05$). Also, the best biocide for *P. aeruginosa* isolates is chlorhexidine digluconate.

An earlier study illustrated the significant impact of the PaβN inhibitor efflux pump, with MIC values for didecyldimethylammonium chloride decreasing more than those for carbapenems. Furthermore suggested that the overexpression of the RND efflux pump system's intrinsic mechanism could account for this decreased susceptibility. There has been a correlation found between the tolerance to biocides and the well-known drug efflux system constitutively expressed in *P. aeruginosa*, which adds to intrinsic antibiotic resistance (23).

Antibiotic resistance and biocide tolerance are correlated, which may lead to surface disinfection failures and the spread of pathogens resistant to disinfectants and antibiotics used in healthcare settings. Actually, this issue may arise from cross-resistance, which happens when the same mechanism underlies both biocide tolerance and antibiotic resistance, as seen, for instance, from the expression of efflux pumps and modifications in the permeability of the outer membrane, or co-resistance, which is observed when genes encoding biocide tolerance and antibiotic resistance are found on the same mobile genetic element (24).The isolates of *P. aeruginosa* have previously been reported to possess resistance to biocides, particularly quaternary ammonium compounds, which is often linked to the *qac* genes, particularly *qacE* and *qacEΔ1*, which are encoded in integrons and plasmids (25). According to the local study conducted by Khudair and Mahmood (26), 63.88% of the *P. aeruginosa* isolates had the *qacEdelta1* gene prevalence.

Conclusion

The efflux pump genes (*qacE, qacE-Δ1*, and *sugE1*) were present in most *P. aeruginosa* isolates that are resistant to biocides and carbapenmes, suggesting that efflux pumps play a crucial role in the development of resistance against these antibiotics and biocides.

References

1. Salerian, A. J. (2020). Burn wound infections and Pseudomonas aeruginosa. *Burns: journal of the International Society for Burn Injuries*, *46*(1): 257-258.
2. Pachori, P.; Gothalwal, R. and Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. *Genes & diseases*, 6(2): 109-119.
3. Recio, R.; Mancheño, M.; Viedma, E.; Villa, J.; Orellana, M. Á. and Lora-Tamayo, J. (2020). Predictors of mortality in bloodstream infections caused by Pseudomonas aeruginosa and impact of antimicrobial resistance and bacterial virulence. *Antimicrobial agents and chemotherapy*, 64(2): 10-1128.
4. Pang, Z.; Raudonis, R.; Glick, B. R.; Lin, T. J. and Cheng, Z. (2019). Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnology advances*, 37(1): 177-192.
5. Kampf, G. (2019). Adaptive bacterial response to low level chlorhexidine exposure and its implications for hand hygiene. *Microbial Cell*, 6(7): 307.
6. AL-Sabagh, F. S. H. and Ghaima, K. K. (2023). Sh. AL-Dabbagh A H. The antibacterial activity of LL-37 peptide against multidrug-resistant Pseudomonas aeruginosa isolated from burn infections. *Revista Bionatura*, 8(1): 69.
7. Bakht, M.; Alizadeh, S. A.; Rahimi, S.; Kazemzadeh Anari, R.; Rostamani, M. and Javadi, A.(2022). Phenotype and genetic determination of resistance to common disinfectants among biofilm-producing and non-producing Pseudomonas aeruginosa strains from clinical specimens in Iran. *BMC microbiology*, *22*(1): 124.
8. Quddus, S.; Liaqat, Z.; Azam, S.; Haq, M. U.; Ahmad, S. and Alharbi, M.(2023). Identification of Efflux Pump Mutations in *Pseudomonas aeruginosa* from Clinical Samples. *Antibiotics*, 12(3): 486.
9. Yaseen, N. N. and Ahmed, D. A. (2023). Detection of *mexB* multidrug efflux gene in some local isolates of *Pseudomonas aeruginosa*. *Iraqi Journal of Science*, 111-118.
10. Mahdi, L.F. and Ahmed H. AL-Azawi, A.H. (2022). Synergistic Effect of Conocarpus erectus Extract and some Antibiotics against Multi-Drug Resistant *Pseudomonas aeruginosa. Iraqi Journal of Biotechnology*, 21(2): 308-325.
11. Taj, Z.; Rasool, M. H.; Khurshid, M.; Aslam, B. and Qamar, M. U. (2023). Insights into the Intersection of Biocide Resistance, Efflux Pumps, and Sequence Types in Carbapenem-Resistant Acinetobacter baumannii: A Multicenter Study. *Pathogens*, *12*(7): 899.
12. Quinn, P. J.; Markey, B. K.; Leonard, F. C.; Hartigan, P.; Fanning, S. and Fitzpatrick, E. (2011). *Veterinary microbiology and microbial disease*. John Wiley and Sons.
13. Carter, G. R.; Wise, D. J. and Flores, E. F. (2004). A concise review of veterinary virology.
14. AL-Shamaa, N.F.K.; Abu-Risha, R.A. and AL-Faham, M.A. (2016). Virulence Genes Profile of Pseudomonas aeruginosa Local Isolates from Burns and Wounds. *Iraqi Journal of Biotechnology*, 15(3): 31-39.
15. Rissetto, N. G.; de Oliveira, J. W. and Nisihara, R. (2024). Main pathogens causing infections and antibiotic resistance profile in major burns in Brazil comparing two periods: 2015/2016 and 2019/2020. *Journal of Burn Care & Research*, *45*(1): 145-150.
16. Zoghlami, A. Kanzari, L.; Boukadida, J.; Messadi, AA. and Ghanem, A. (2012). Profil épidémiologique et résistance aux antibiotiques des Souches de *Pseudomonas* aeruginosa isolées au centre de Traumatologie et Grands Brûlés en Tunisie durant trois ans [Epidemiological profile and antibiotic resistance of *Pseudomonas aeruginosa* isolates in burn and traumatology center in Tunisia over a three-year period]. *Tunis Med*. 90(11):803-6.
17. Nițescu, B.; Pițigoi, D.; Tălăpan, D.; Nițescu, M.; Aramă, S. Ș. and Pavel, B. (2023). Etiology and Multi-Drug Resistant Profile of Bacterial Infections in Severe Burn Patients, Romania 2018–2022. *Medicina*, *59*(6):1143.
18. Abednezhad, A.; Bakhshi, B.; Moghadam, N. A.; Faraji, N.; Derakhshan-Nezhad, E. and Mohammadi Barzelighi, H. (2023). Characteristics of multiresistant Pseudomonas aeruginosa isolates from burn patients in Iran. *Acta Microbiologica et Immunologica Hungarica*, *70*(1): 29-37.
19. Altaai, M.E.; Aziz, S.H. and Marhoon, A.A. (2014). Identification *Pseudomonas aeruginosa* by *16s rRNA* gene for Differentiation from Other *Pseudomonas*

Species that isolated from Patients and environment. *Baghdad Sci.J.* 11(2):1028-34.

20. Gawad, M.A. and Gharbi, W.A. (2022). Molecular Detection of oprI and oprL Virulence Genes of *Pseudomonas aeruginosa* Isolated from Burns and Wounds. *Iraqi Journal of Biotechnology*, 21(2):215-224.
21. Goodarzi, R.; Yousefimashouf, R.; Taheri, M.; Nouri, F. and Asghari, B. (2021). Susceptibility to biocides and the prevalence of biocides resistance genes in clinical multidrug-resistant *Pseudomonas aeruginosa* isolates from Hamadan, Iran. *Molecular Biology Reports*, *48*, 5275-5281.
22. Namaki, M.; Habibzadeh, S.; Vaez, H.; Arzanlou, M.; Safarirad, S. and Bazghandi, S. A. (2022). Prevalence of resistance genes to biocides in antibiotic-resistant *Pseudomonas aeruginosa* clinical isolates. *Molecular Biology Reports*, *49*(3): 2149-2155.
23. Pottier, M.; Gravey, F.; Castagnet, S.; Auzou, M.; Langlois, B. and Guérin, F. (2023). A 10-year microbiological study of *Pseudomonas aeruginosa* strains revealed the circulation of populations resistant to both carbapenems and quaternary ammonium compounds. *Scientific Reports*, *13*(1): 2639.
24. Elekhnawy, E.; Sonbol, F., Abdelaziz, A., and Elbanna, T. (2020). Potential impact of biocide adaptation on selection of antibiotic resistance in bacterial isolates. *Future Journal of Pharmaceutical Sciences*, *6*, 1-10.
25. Verdial, C.; Serrano, I.; Tavares, L.; Gil, S. and Oliveira, M. (2023). Mechanisms of antibiotic and biocide resistance that contribute to *Pseudomonas aeruginosa* persistence in the hospital environment. *Biomedicines*, *11*(4): 1221.
26. Khudair, A. N. A. and Mahmood, S. S. (2021). Detection of the antiseptic resistance gene among Pseudomonas aeruginosa isolates. *Iraqi Journal of Science*, *62*(1): 75-82.
27. Bakht, M.; Alizadeh, S. A.; Rahimi, S.; Kazemzadeh Anari, R.,; Rostamani, M. and Javadi, A. *et al.* (2022). Phenotype and genetic determination of resistance to common disinfectants among biofilm-producing and non-producing Pseudomonas aeruginosa strains from clinical specimens in Iran. BMC microbiology, 22(1): 124.
28. Joshi, H.; Dave, R. and Venugopalan, V. P. (2014). Pumping iron to keep fit: modulation of siderophore secretion helps efficient aromatic utilization in Pseudomonas putida KT2440. Microbiology, 160(7): 1393-1400.