Original Article | Iran J Med Microbiol. 2024; 18(4): 214-222

10.30699/ijmm.18.4.214

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Prevalence of Chlorhexidine-Tolerant *Pseudomonas aeruginosa* and Correlation with Antibiotic Resistance

Amirhossein Farshchi Tabrizi[1], Abolfazl Rafati Zomorodi[1,2], Farshad Kakian[3], Aida Moazemy[1], Leila Kasraian[4], Sita Nakhaeitazeji[2], Mohammad Motamedifar[*5]

1. *Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Bacteriology and Virology, Larestan University of Medical Sciences, Larestan, Iran*
4. *Blood Transfusion Research Center, Higher Institute for Research and Education in Transfusion Medicine, Shiraz, Iran*
5. *HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran*

ABSTRACT

Background and Aim: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common environmental bacterium linked to the serious hospital-acquired infections. Chlorhexidine (CHX), a widely used antiseptic in hospitals, can create selection pressure that contributes to the cross-antibiotic resistance and the emergence of multidrug-resistant (MDR) strains. This study aimed to investigate the prevalence of tolerance to CHX and the presence of the *pslA*, *pelA*, *qacE,* and *qacΔE1* genes among *P. aeruginosa* isolates in Shiraz, Southwest Iran.

Materials and Methods: From October 2020 to July 2021, 120 *P. aeruginosa* isolates were collected from hospitalized patients at Nemazee Hospital in Shiraz, Southwest Iran. The Kerby-Bauer disk diffusion method was employed for the antimicrobial susceptibility testing. The susceptibility to CHX was evaluated using the microbroth dilution method. Finally, the prevalence of the *pelA*, *pslA, qacE,* and *qacΔE1* genes was assessed among all *P. aeruginosa* isolates.

Results: The highest resistance rate was observed against ceftriaxone and ceftazidime, with frequencies of 97.120 (80.8%) and 96.120 (80%), respectively. In contrast, the lowest resistance rates were noted for amikacin (5.120, or 4.2%), ofloxacin (7.120, or 5.8%), and meropenem (10.120, or 8.3%). Among the 120 *P. aeruginosa* isolates, 33 (27.5%) were CHX-tolerant, and 22 (18.3%) were MDR. There was a highly significant correlation between the rates of MDR and CHX-tolerant *P. aeruginosa* isolates (P<0.005).

Conclusion: A positive association between the percentages of MDR strains and CHX-tolerant *P. aeruginosa* isolates has reinforced the hypothesis that exposure to CHX may contribute to developing cross-resistance. Thus, concise monitoring of CHX susceptibility seems essential in the hospital and clinical settings.

Keywords: Biocide, Chlorhexidine, Multi-drug Resistance, *P. aeruginosa*

Received: 2024/06/27; **Accepted**: 2024/09/12; **Published Online:** 2024/09/29;

Corresponding Information: **Mohammad Motamedifar**, *HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran*
Email: motamedm@sums.ac.ir

Use a device to scan and read the article online

Farshchi Tabrizi A, Rafati Zomorodi A, Kakian F, Moazemy A, Kasraian L, Nakhaeitazeji S, et al. Prevalence of Chlorhexidine-Tolerant *Pseudomonas aeruginosa* and Correlation with Antibiotic Resistance. Iran J Med Microbiol. 2024;18(4):214-22.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks
Send citation to: Mendeley Zotero RefWorks

1. Introduction

The Centers for Disease Control and Prevention (CDC) defines germicides and microbicides interchangeably as agents to eliminate microorganisms **(1)**. Hospitals employ microbicides extensively to limit nosocomial infections **(2)**. However, their overuse causes selection pressure with the cross-antibiotic resistance, developing multi-drug resistant (MDR) strains **(3)**.

The cationic biguanide disinfectant chlorhexidine (CHX) is available as gluconate, hydrochloride, and acetate products. The CHX kills bacteria by damaging

the cell membrane when it reacts with negatively-charged phospholipid molecules on the membrane. This allows the cell contents to leak out, which is the goal of eliminating bacteria **(4, 5)**. In hospitals, CHX is a common topical antiseptic agent with broad-spectrum uses, including surface cleaning, hand sanitization, and skin preparation before invasive operations **(6)**.

The CHX is effective against several microorganisms, including Gram-positive and Gram-negative bacteria, enveloped viruses, and some fungi. It is also useable on some products such as hand rubs, body washes, and antiseptic mouthwashes **(7)**. Therefore, using CHX solutions as topical disinfectant is listed as a part of strategies for MDR bacteria control **(8)**. However, the increasing use of CHX is a severe concern regarding its possible role in developing MDR bacteria through resistance genes acquisition **(9)**.

Pseudomonas aeruginosa (*P. aeruginosa*) is a prevalent bacteria derived from the environment that is responsible for the various nosocomial infections, including wounds, urinary tract infections, and several others **(10)**. A review investigated by Reynold *et al*., determined that 7.1% -7.3% of all nosocomial infections were caused by *P. aeruginosa* **(11)**. Also, an international observational study estimated the prevalence of *P. aeruginosa* among the intensive care units (ICU)-acquired infections at 26% **(12)**. *Pseudomonas aeruginosa* may thrive in a wide range of environmental niches because of its large and dynamic genome, which confers interesting metabolic adaptability and genetic plasticity. Therefore, *P. aeruginosa* strains exhibit notable inherent resistance against antimicrobial agents **(13, 14)**. Bacterial biofilms are responsible for around 80% of the chronic human infections **(15)**. *Pseudomonas aeruginosa* biofilm formation contributes to the elevated morbidity and mortality rates by protecting the host immune system and limiting the antibiotic treatment effectiveness **(16)**.

As there is scarcity of information regarding the CHX-tolerant *P. aeruginosa* and possible correlation with itsr antibiotic resistance, the current study investigated the prevalence of phenotypic tolerance to CHX and harboring *pslA*, *pelA*, *qacE, and qacΔE1* genes, and their correlation with *P. aeruginosa* isolated from the hospitalized patients in Shiraz, Southwest Iran.

2. Materials and Methods

Bacterial isolation

The *P. aeruginosa* isolates (120 samples) were collected from the hospitalized patients at Nemazee Hospital, a referral hospital in Shiraz, Iran, from October 2020 to July 2021. The laboratory isolates were obtained without limitations from various clinical sources, such as wounds, urine, blood, and sputum. All isolates were primarily identified as *P. aeruginosa* using the standard biochemical tests comprising Gram staining, colony morphology, catalase/oxidase, triple-sugar iron agar (TSI), and oxidation-fermentation (OF) test **(17)**.

Molecular confirmation of *P. aeruginosa* isolates

All *P. aeruginosa* isolates identified through the standard biochemical tests were further confirmed using the *16s rRNA* gene amplification by the polymerase chain reaction (PCR). The oligonucleotide sequences of the used primers were as follow: Forward: 5'-GGGGGATCTTCGGACCTCA-3' and Reverse: 5'-TCCTTAGAGTGCCCACCCG-3'. The PCR condition consisted of initial denaturation at 95°C for 2 min, 25 cycles of denaturation at 95°C for 20 sec, annealing at 58°C for 20 sec, and extension at 72°C for 40 sec; and eventually, a final extension at 72°C for 1 min **(17)**.

Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion method was used to determine the susceptibility of *P. aeruginosa* isolates to ten different antibiotic discs (HiMedia, India). They included ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), piperacillin-tazobactam (100.10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), and amikacin (30 µg). The test was conducted as recommended by the Clinical Laboratory and Standard Institute (CLSI, 2021); *P. aeruginosa* ATCC 27853 was tested as quality control **(18)**.

Determination of the minimum inhibitory concentration of CHX

The microbroth dilution method was accomplished to determine the minimum inhibitory concentration (MIC) of CHX for *P. aeruginosa* isolates. However, in susceptibility tests, the CLSI and related organizations do not have any established procedure for characterizing the bacterial resistance or susceptibility to the non-therapeutic antimicrobials. Therefore, susceptibility to the CHX was determined using the previously described *in vitro* MIC distributions **(19, 20)**. A stock solution of 1% CHX was made by mixing 1 gr of CHX (Sigma, USA) into 100 mL of Muller Hinton broth (MHB) medium (Merck, Germany) and passed through the sterile syringe filters (0.22 µm). Two-fold serial dilutions were prepared volumetrically in 50 µL MHB to achieve a range of 2500 to 5 µg/mL concentrations. The positive control well was filled with bacteria and MHB, while

MHB and CHX composed the negative control well. No bacterial growth was expected to be observed in this well, resulting in a clear appearance. As described previously, *P. aeruginosa* isolates with MIC>50 µg/mL were interpreted as CHX-tolerant **(21, 22)**.

Amplification of *qacE*, *qacΔE1* and *pelA*, *pslA* genes

The presence of biofilm-mediated genes (*pelA, pslA*) and antiseptic-associated resistance genes (*qacE*, *qacΔE1*) was detected using PCR. The genomic DNA of all isolates was extracted using the boiling method, as explained in a prior study **(23)**. The PCR amplifications were accomplished in 25 µL final volume comprising PCR 2X Master Mix (Amplicon, Denmark), 0.4 µM of each primer, 50 ng of template DNA, and nuclease-free water (Table 1).

Statistical analysis

SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis. Additionally, the evaluation was done using Chi-square and Fisher's exact test; the value was considered significant at $P<0.05$.

Table 1. The oligonucleotide sequences of primers

Primers' Name	5' – 3'	Annealing	Amplification size (bp)	References
16S rRNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	58 °C	965	(17)
pslA	TCCCTACCTCAGCAGCAAGC TGTTGTAGCCGTAGCGTTTCTG	55 °C	656	(24)
pelA	CATACCTTCAGCCATCCGTTCTTC CGCATTCGCCGCACTCAG	55 °C	786	
qacE	CCCGAATTCATGAAAGGCTGGCTT TAAGCTTTCACCATGGCGTCGG	55 °C	350	(20)
qacΔE1	TAGCGAGGGCTTTACTAAGC ATTCAGAATGCCGAACACCG	55 °C	300	

3. Results

A total of 120 *P. aeruginosa* isolates were recovered from the hospitalized patients in a proportion of 67 males (55.8%) and 53 females (44.2%), with an age median of 40 (ranged between 3 to 84 years). Generally, most *P. aeruginosa* isolates were recovered from blood 37.120 (30.8%), followed by sputum 20/120 (16.7%), wounds 18/120 (15%), and urine 13/120 (10.8%). Also, the majority of the isolates were obtained from ICU (35.120: 29.2%), internal (24.120: 20%), and acute (19.120: 15.8%) sections. The patinets' demographic information has been listed in Table 2.

Antimicrobial susceptibility testing

The highest resistance rates were found against ceftriaxone and ceftazidime as the third-generation cephalosporins, with 97.120 (80.8%) and 96.120 (80%) frequencies, respectively. Also, the lowest resistance rates were against amikacin (5.120: 4.2%), ofloxacin (7.120: 5.8%), and meropenem (10.120: 8.3%) (Figure 1). Furthermore, out of 120 examined isolates, 22 (18.3%) were identified as MDR, exhibiting resistance to at least one antibiotic across three or more distinct antibiotic categories.

MIC of CHX

All isolates were grown in exposure to the CHX concentrations < 19 µg/mL. However, no growth was detected in exposure to the CHX concentrations > 313 µg/mL. The most prevalent MIC value was 19 µg/mL with frequency of 52/120 (43.3%). Out of 120 *P. aeruginosa* isolates 33 (27.5%) were CHX-tolerant. The MIC_{50} and MIC_{90} values were obtained at 39 µg/mL and 156 µg/mL, respectively. The prevalence of CHX-tolerant *P. aeruginosa* isolates among MDR and non-MDR isolates (90.9% vs. 30.9.4%) was significantly different ($P<0.001$). In addition, a significant relationship was found between the rates of CHX-tolerant *P. aeruginosa* isolates and resistance to the tested antibiotics, except for amikacin and ciprofloxacin (Table 3).

Prevalence of *pelA*, *pslA*, *qacE*, *qacΔE1* genes

The most predominant genes were *qacΔE1* and *pslA* with frequencies of 94.120 (78.3%) and 92.120 (76.7%), respectively. The rates for *pelA* and *qacE* genes came next, with frequencies of 79.120 (65.8%) and 76.120 (63.3%), respectively. The frequency of the isolates that

harbored all four genes was 35.120 (29.1%) that 18 (51.4%) samples were MDR. The presence of *pelA* and *qacE* genes was significant among MDR (90.9% vs. 95.6%) and non-MDR (60.2% vs. 56.1%) isolates (P=0.006 and P=0.001), respectively. Also, there was a positive correlation between the rates of CHX-tolerant *P. aeruginosa* isolates and harboring *pelA, qacE, and qacΔE1* genes (P<0.05).

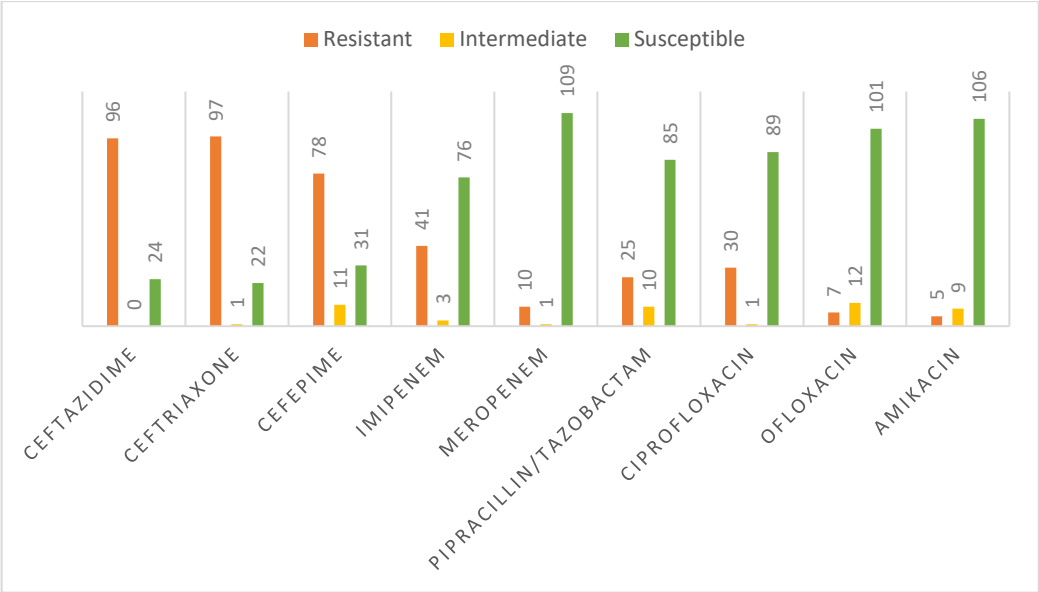

Figure 1. The antimicrobial resistance patterns of *P. aeruginosa* isolates (N=120).

Table 2. Demographic information of resistant *P. aeruginosa* isolates

Demographic	CZA N=96 (%)	CRO N=97 (%)	CFM N=78 (%)	IMP N=41 (%)	MER N=10 (%)	PIP/TZ N=25 (%)	CIP N=30 (%)	OFL N=7 (%)	AMK N=5 (%)
Gender									
Female	40 (41.7%)	38 (39.2%)	33 (42.3%)	16 (39%)	5 (50%)	12 (48%)	14 (46.7%)	5 (71.4%)	3 (60%)
Male	56 (58.3%)	59 (60.8%)	45 (57.7%)	25 (61%)	5 (50%)	13 (52%)	16 (53.3%)	2 (28.6%)	2 (40%)
Source									
Blood	26 (27.1%)	27 (27.8%)	19 (24.4%)	14 (34.1%)	3 (30%)	7 (28%)	8 (26.7%)	3 (42.9%)	2 (40%)
Sputum	17 (17.7%)	17 (17.5%)	16 (20.5%)	6 (14.6%)	3 (30%)	3 (12%)	7 (23.3%)	2 (28.6%)	1 (20%)
Wound	15 (15.6%)	16 (16.5%)	11 (14.1%)	8 (19.5%)	0	7 (28%)	6 (20%)	0	2 (40%)
Urine	12 (12.5%)	11 (11.3%)	11 (14.1%)	3 (7.3%)	3 (30%)	2 (8%)	4 (13.3%)	1 (14.3%)	0
ETT	7 (7.3%)	7 (7.2%)	5 (6.4%)	6 (14.6%)	1 (10%)	2 (8%)	2 (6.7%)	1 (14.3%)	0
Abscess	6 (6.3%)	6 (6.2%)	5 (6.4%)	2 (4.9%)	0	2 (8%)	1 (3.3%)	0	0
Nasal	5 (5.2%)	5 (5.2%)	5 (6.4%)	0	0	1 (4%)	0	0	0
Fluid	4 (4.2%)	4 (4.1%)	4 (5.1%)	1 (2.4%)	0	1 (4%)	0	0	0
Pleural	4 (4.2%)	4 (4.1%)	2 (2.6%)	1 (2.4%)	0	0	2 (6.7%)	0	0
Ward									

Demographic	CZA N=96 (%)	CRO N=97 (%)	CFM N=78 (%)	IMP N=41 (%)	MER N=10 (%)	PIP/TZ N=25 (%)	CIP N=30 (%)	OFL N=7 (%)	AMK N=5 (%)
ICU	29 (30.2%)	28 (28.9%)	19 (24.4%)	16 (39%)	2 (20%)	5 (20%)	7 (23.3%)	2 (28.6%)	2 (40%)
Internal	20 (20.8%)	19 (19.6%)	20 (25.6%)	3 (7.3%)	1 (10%)	3 (12%)	7 (23.3%)	1 (14.3%)	0
Pediatric	14 (14.6%)	12 (12.4%)	11 (14.1%)	6 (14.6%)	3 (30%)	3 (12%)	3 (10%)	1 (14.3%)	0
Acute	13 (13.5%)	16 (16.5%)	11 (14.1%)	4 (9.8%)	2 (20%)	6 (24%)	5 (16.7%)	2 (28.6%)	0
Emergency	11 (11.5%)	11 (11.3%)	10 (12.8%)	7 (17.1%)	2 (20%)	4 (16%)	5 (16.7%)	1 (14.3%)	2 (40%)
Surgical	9 (9.4%)	11 (11.3%)	7 (9%)	5 (12.2%)	0	4 (16%)	3 (10%)	0	1 (20%)

Abbreviation: CAZ, ceftazidime; CRO, ceftriaxone; CFM, cefepime; IMP, imipenem; MER, meropenem; PIP/TAZ, piperacillin/tazobactam; CIP, ciprofloxacin; OFL, ofloxacin; AMK, amikacin; ETT, endotracheal tube; ICU, intensive care unit.

Table 3. Distribution of CHX-tolerant and MDR *P. aeruginosa* isolates regarding resistance to the tested antibiotics

Antibiotics name	Tolerant N=33 (%)	Nontolerant N=87 (%)	*p* value	MDR N=22 (%)	Non-MDR N=98 (%)	P-value
CAZ (n=96)	33 (100%)	63 (72.4%)	0.001	22 (100%)	74 (75.5%)	0.009
CRO (n=97)	32 (97%)	65 (74.7%)	0.022	21 (95.5%)	76 (77.6%)	0.155
CFP (n=78)	27 (81.8%)	51 (58.6%)	0.01	19 (86.4%)	59 (60.2%)	0.066
IMP (n=41)	24 (72.7%)	17 (19.5%)	0.001	21 (95.5%)	20 (20.4%)	0.001
MER (n=10)	8 (24.2%)	2 (2.3%)	0.001	9 (40.9%)	1 (1%)	0.001
PAP/TAZ (n=25)	19 (57.6%)	6 (6.9%)	0.001	20 (90.9%)	5 (5.1%	0.001
OFLX (n=7)	6 (18.2%)	1 (1.1%)	0.002	7 (31.8%)	0	-*
CIP (n=30)	13 (39.4%)	17 (19.5%)	0.071	10 (45.5%)	20 (20.4%)	0.047
AMK (n=5)	3 (9.1%)	2 (2.3%)	0.289	4 (18.2%)	1 (1%)	0.008

Abbreviations: CAZ, ceftazidime; CRO, ceftriaxone; CFM, cefepime; IMP, imipenem; MER, meropenem; PIP/TAZ, piperacillin/tazobactam; CIP, ciprofloxacin; OFL, ofloxacin; AMK, amikacin. *It was not calculable.

4. Discussion

Pseudomonas aeruginosa is regarded as a significant cause of nosocomial infections that are associated with high morbidity and mortality. This is due to the emergence of severe antimicrobial resistance and an increase in MDR and extensively-drug resistant (XDR) strains, which have limited treatment options. This study also revealed alarming resistance to the most commonly used antibiotics in our clinical wards. Among the antibiotics studied, the frequency of resistance rates against the third- and the fourth-generation cephalosporins in our study were as follows: ceftriaxone 80.8%, ceftazidime 80%, and cefepime 60%, respectively. Previous investigations in Iran have revealed lower resistance rates to ceftazidime (46.5%–59.8%) and cefepime (50%–37.9%) (25, 26). However, studies conducted in Qatar (cefepime, 97.5%), Pakistan (ceftazidime, 100%), and Nigeria (ceftazidime, 98%) have reported higher resistance rates against ceftazidime and cefepime (27-29).

On the other hand, our findings determined resistance frequencies against ofloxacin, meropenem, and amikacin with less than 10% rates. Similarly, previous surveys indicated low prevalence of resistance to meropenem in Egypt (5%), China (11.5%), and Spain (9.6%) (30-32). However, an earlier

narrative review study by Rafaella Rosito *et al*., suggested that a new generation of β-lactamase inhibitors (e.g., avibactam, relebactam) in combination with β-lactams are suitable options for the treatment of infections caused by MDR *P. aeruginosa* **(33)**. Also, piperacillin-tazobactam, a familiar antipseudomonal antibiotic, has demonstrated 93% treatment efficacy among the patients with *P. aeruginosa* infections **(34)**. Our results revealed a 25% resistance rate against piperacillin-tazobactam among *P. aeruginosa* isolates.

Compared to the antibiotic resistance, resistance to the antiseptics has been widely neglected in recent scientific literature. However, CHX, one of the most frequently used antiseptic agents in the hospital environments, has become attractive to the researchers because of the recent increase in the CHX-tolerant *P. aeruginosa*. Remarkably, in this study 27.5% of the investigated *P. aeruginosa* were CHX-tolerant; this contrasts with previous observations that indicated lower frequencies of CHX-tolerant *P. aeruginosa* in their experiments **(4,7)**. This discrepancy may be attributed to the various factors, such as the sample size or differences in geography of the studies. However, Buxser *et al*., found that the numbers of CHX-tolerant *P. aeruginosa*, *Acinetobacter baumannii,* and *Klebsiella pneumonia* strains have risen for 70 years **(35)**.

Significantly, MDR and CHX-tolerance have shown a positive correlation among *P. aeruginosa* isolates. Therefore, hospitals need to monitor and control the CHX-tolerance among *P. aeruginosa* isolates. However, the standard guidelines need to be established for the laboratory identification of the tolerant bacteria or the surveillance of their prevalence. Indeed, evaluating the susceptibility of bacteria, including *P. aeruginosa*, to CHX is commonly performed using the epidemiological cut-off values. These determinations were made following various bacteria investigations, irrespective of the antiseptic treatments outcomes. In addition, lacking the standard CHX susceptibility test creates restrictions in monitoring the frequency of CHX-tolerant *P. aeruginosa*, deficient of appropriate clinical data, and challenges in comparing data across the studies and drawing meaningful conclusions **(7)**. Several researchers, who repeatedly call for the assessment of the benefits and safety of using CHX-based decolonization, further confirm this urgent need **(36, 37)**.

Producing biofilm and efflux pump systems are two of the most critical antimicrobial resistance mechanisms among *P. aeruginosa* isolates. Various compounds comprising alginate, Psl, Pel, and lipopolysaccharide (LPS) are involved in the biofilm production **(38)**.

The prevalence of *pslA* and *pelA* genes among the tested *P. aeruginosa* isolates were 76.7% and 65.8%, respectively. Also, harboring the *pslA* gene was significant among MDR and CHX-tolerant *P. aeruginosa* isolates (P<0.005). These results were aligned with earlier studies that reported the frequency of *pslA* and *pelA* genes in a range of 89%–94% and 69%–87%, respectively **(39-41)**.

Furthermore, cross-resistance to antibiotics and CHX was investigated by harboring the *qacE* and *qacE1* genes. Notably, harboring these genes demonstrated significant differences among the MDR and CHX-tolerant *P. aeruginosa* isolates compared to not resistant and not CHX-tolerant isolates (P<0.05). Other investigations have also found these correlations **(42, 43)**. Therefore, there is a positive possibility that long-term exposure to the CHX may contribute to developing MDR or cross-resistance among *P. aeruginosa* isolates. Remarkably, cross resistance happens for the related antibiotics. For instance, colistin and CHX are positive-charge molecules attached to the bacterial cell membrane with a negative charge, which cleared the reason for finding cross-resistance for these antibiotics **(44)**. Colistin is recommended as the last resort treatment of the infections caused by carbapenem-resistant *P. aeruginosa*, thus, increasing CHX-tolerant *P. aeruginosa* complicated the treatment of such infections **(45)**.

Of course, the present research has some limitations including inability to determine the biofilm production or assess the expression level of the tested genes. Thus, further investigations are suggested to study the molecular mechanisms affecting different bacteria through the exposure to CHX or other biocides.

5. Conclusion

In summary, the frequencies of CHX-tolerance and MDR among *P. aeruginosa* isolates from Nemazee Hospital in Shiraz, Iran were found 27.5% and 18.3%, respectively. A positive correlation was observed between the rates of MDR and CHX-tolerance in *P. aeruginosa* isolates. Furthermore, the presence of CHX-tolerant isolates was significantly different among the isolates that were resistant to the tested antibiotics, except for amikacin and ciprofloxacin. These findings strengthen the hypothesis that biocide exposure, like CHX, contributes to the cross-resistance development. Thus, monitoring and evaluating the susceptibilities to CHX and such biocides seems essential in the hospital and clinical settings.

Acknowledgment

The authors would like to thank the staff of Nemazee Hospital for their cooperation.

Ethical Considerations

This study underwent a rigorous ethical approval process by the Ethics Committee of Shiraz University of Medical Sciences, ensuring the highest standards of research ethics (Approval No. IR.SUMS.MED.REC.1399.203). The samples were taken as part of the regular procedure and isolated anonymously.

Authors' Contributions

Amirhossein F. and Ayda M.: conceptualized and conducted the experiments. Farshad K. and Sita N.: collected, analyzed, and interpreted data. Leila K.: developed the study concept and design, edited, and approved the final manuscript. Abolfazl R.Z.: collaborated in the preparation of the manuscript. Mohammad M.: supervised, edited, and reviewed the manuscript. All authors have read the final manuscript and approved the submission.

Funding

As Ayda Moazemi's and Amirhossein Farshchitabrizi's MD theses, the work was supported by the Vice-Chancellor for Research of Shiraz University of Medical Sciences (code number: IR.SUMS.REC.98-01-43-20128).

Conflict of Interest

The authors declare no conflict of interest in this study.

References

1. Glossary Disinfection and Sterilization Guidelines Library Infection Control CDC. Accessed on 01 October 2024. Available online: [https://www.cdc.gov/infection-control/hcp/disinfection-and-sterilization/index.html]
2. Betchen M, Giovinco HM, Curry M, Luu J, Fraimow H, Carabetta VJ, et al. Evaluating the effectiveness of hospital antiseptics on multidrug-resistant Acinetobacter baumannii: Understanding the relationship between microbicide and antibiotic resistance. Antibiotics. 2022;11(5):614. [DOI:10.3390/antibiotics11050614]
3. Namaki M, Habibzadeh S, Vaez H, Arzanlou M, Safarirad S, Bazghandi SA, et al. Prevalence of resistance genes to biocides in antibiotic-resistant Pseudomonas aeruginosa clinical isolates. Mol Biol Rep. 2022;49(3):2149-55. [DOI:10.1007/s11033-021-07032-2]
4. Zheng X, Zhang X, Zhou B, Liu S, Chen W, Chen L, et al. Clinical characteristics, tolerance mechanisms, and molecular epidemiology of reduced susceptibility to chlorhexidine among Pseudomonas aeruginosa isolated from a teaching hospital in China. Int J Antimicrob Agents. 2022;60(1):106605. [DOI:10.1016/j.ijantimicag.2022.106605]
5. Bock LJ, Wand ME, Sutton JM. Varying activity of chlorhexidine-based disinfectants against Klebsiella pneumoniae clinical isolates and adapted strains. J Hosp Infect. 2016;93(1):42-8. [DOI:10.1016/j.jhin.2015.12.019]
6. Rania K, Mohamed M, Waheed H, Nader N. Efflux pump genes and chlorhexidine resistance: Clue for Klebsiella pneumoniae infections in intensive care units, Egypt. Afr J Microbiol Res. 2014;8(21): 2162-7. [DOI:10.5897/AJMR2014.6656]
7. Leshem T, Gilron S, Azrad M, Peretz A. Characterization of reduced susceptibility to chlorhexidine among Gram-negative bacteria. Microbes Infect. 2022;24(2):104891. [DOI:10.1016/j.micinf.2021.104891]
8. Gall E, Long A, Hall KK. Chlorhexidine bathing strategies for multidrug-resistant organisms: a summary of recent evidence. Journal of Patient Safety. 2020;16(3):S16-S22. [DOI:10.1097/PTS.0000000000000743]
9. Williamson DA, Carter GP, Howden BP. Current and emerging topical antibacterials and antiseptics: agents, action, and resistance patterns. Clin Microbiol Rev. 2017;30(3):827-60. [DOI:10.1128/CMR.00112-16]
10. Ahmadi N, Salimizand H, Zomorodi AR, Abbas JE, Ramazanzadeh R, Haghi F, et al. Genomic diversity of β-lactamase producing Pseudomonas aeruginosa in Iran; the impact of global high-risk clones. Ann Clin Microbiol Antimicrob. 2024;23(1):1-8. [DOI:10.1186/s12941-024-00668-5]
11. Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of Pseudomonas

aeruginosa infections: an update. Drugs. 2021; 81(18):2117-31. [DOI:10.1007/s40265-021-01635-6]

12. Vincent JL, Sakr Y, Singer M, Martin-Loeches I, Machado FR, Marshall JC, et al. Prevalence and outcomes of infection among patients in intensive care units in 2017. JAMA. 2020; 323(15):1478-87. [DOI:10.1001/jama.2020.2717]
13. Botelho J, Grosso F, Peixe L. Antibiotic resistance in Pseudomonas aeruginosa–Mechanisms, epidemiology and evolution. Drug Resist Updates. 2019;44:100640. [DOI:10.1016/j.drup.2019.07.002]
14. Simanek KA, Paczkowski JE. Resistance is not futile: the role of quorum sensing plasticity in Pseudomonas aeruginosa infections and its link to intrinsic mechanisms of antibiotic resistance. Microorganisms. 2022;10(6):1247. [DOI:10.3390/microorganisms10061247]
15. Hemati S, Kouhsari E, Sadeghifard N, Maleki A, Omidi N, Mahdavi Z, Pakzad I. Sub-minimum inhibitory concentrations of biocides induced biofilm formation in Pseudomonas aeruginosa. New Microbes New Infect. 2020;38:100794. [DOI:10.1016/j.nmni.2020.100794]
16. El-Banna T, Abd El-Aziz A, Sonbol F, El-Ekhnawy E. Adaptation of Pseudomonas aeruginosa clinical isolates to benzalkonium chloride retards its growth and enhances biofilm production. Mol Biol Rep. 2019;46:3437-43. [DOI:10.1007/s11033-019-04806-7]
17. Zomorodi AR, Mohseni N, Hafiz M, Nikoueian H, Hashemitabar G, Salimizand H, et al. Investigation of mobile colistin resistance (mcr) genes among carbapenem resistance Pseudomonas aeruginosa isolates from bovine mastitis in Mashhad, Iran. Gene Rep. 2022;29: 101695. [DOI:10.1016/j.genrep.2022.101695]
18. Wayne PA. CLSI Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document Clinical Laboratory Standards Institute (CLSI): Wayne, PA, USA. 2017.
19. Tag ElDein MA, Yassin AS, El-Tayeb O, Kashef MT. Chlorhexidine leads to the evolution of antibiotic-resistant Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis. 2021;40(11):2349-61. [DOI:10.1007/s10096-021-04292-5]
20. Gomaa FA, Helal ZH, Khan MI. High prevalence of Bla NDM-1, Bla VIM, qacE, and qacEΔ1 genes and their association with decreased susceptibility to antibiotics and common hospital biocides in clinical isolates of Acinetobacter baumannii. Microorganisms. 2017;5(2):18. [DOI:10.3390/microorganisms5020018]
21. Kampf G. Acquired resistance to chlorhexidine–is it time to establish an 'antiseptic stewardship' initiative?. J Hosp Infect. 2016;94(3):213-27. [DOI:10.1016/j.jhin.2016.08.018]
22. Nakahara H, Kozukue H. Isolation of chlorhexidine-resistant Pseudomonas aeruginosa from clinical lesions. J Clin Microbiol. 1982;15(1):166-8. [DOI:10.1128/jcm.15.1.166-168.1982]
23. Rafati Zomorodi A, Rad M, Hashemitabar GR, Salimizand H. Molecular typing of cephalosporin resistant serovars of Salmonella enterica from poultry and farm animals. Bulg J Vet Med. 2020; 23(2):178-86. [DOI:10.15547/bjvm.2196]
24. Ghadaksaz A, Fooladi AA, Hosseini HM, Amin M. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. J Appl Biomed. 2015;13(1):61-8. [DOI:10.1016/j.jab.2014.05.002]
25. Bazghandi SA, Arzanlou M, Peeridogaheh H, Vaez H, Sahebkar A, Khademi F. Prevalence of virulence genes and drug resistance profiles of Pseudomonas aeruginosa isolated from clinical specimens. Jundishapur J Microbiol. 2021;14(8): e118452 [DOI:10.5812/jjm.118452]
26. Heidari R, Farajzadeh Sheikh A, Hashemzadeh M, Farshadzadeh Z, Salmanzadeh S, Saki M. Antibiotic resistance, biofilm production ability and genetic diversity of carbapenem-resistant Pseudomonas aeruginosa strains isolated from nosocomial infections in southwestern Iran. Mol Biol Rep. 2022;49(5):3811-22. [DOI:10.1007/s11033-022-07225-3]
27. Ugwuanyi FC, Ajayi A, Ojo DA, Adeleye AI, Smith SI. Evaluation of efflux pump activity and biofilm formation in multidrug resistant clinical isolates of Pseudomonas aeruginosa isolated from a Federal Medical Center in Nigeria. Ann Clin Microbiol Antimicrob. 2021;20:1-7. [DOI:10.1186/s12941-021-00417-y]
28. Saleem S, Bokhari H. Resistance profile of genetically distinct clinical Pseudomonas aeruginosa isolates from public hospitals in central Pakistan. J Infect Public Health. 2020; 13(4):598-605. [DOI:10.1016/j.jiph.2019.08.019]
29. Sid Ahmed MA, Abdel Hadi H, Abu Jarir S, Al Khal AL, Al-Maslamani MA, Jass J, et al. Impact of an antimicrobial stewardship programme on antimicrobial utilization and the prevalence of MDR Pseudomonas aeruginosa in an acute care

hospital in Qatar. JAC-Antimicrob Resist. 2020; 2(3):dlaa050. [DOI:10.1093/jacamr/dlaa050]

30. Hassuna NA, Darwish MK, Sayed M, Ibrahem RA. Molecular epidemiology and mechanisms of high-level resistance to meropenem and imipenem in Pseudomonas aeruginosa. Infect Drug Resist. 2020:285-93. [DOI:10.2147/IDR.S233808]
31. Feng W, Huang Q, Wang Y, Yuan Q, Li X, Xia P, et al. Changes in the resistance and epidemiological characteristics of Pseudomonas aeruginosa during a ten-year period. J Microbiol Immunol Infect. 2021;54(2):261-6. [DOI:10.1016/j.jmii.2019.08.017]
32. Cabrera R, Fernández-Barat L, Vázquez N, Alcaraz-Serrano V, Bueno-Freire L, Amaro R, et al. Resistance mechanisms and molecular epidemiology of Pseudomonas aeruginosa strains from patients with bronchiectasis. J Antimicrob Chemother. 2022;77(6):1600-10. [DOI:10.1093/jac/dkac084]
33. Losito AR, Raffaelli F, Del Giacomo P, Tumbarello M. New drugs for the treatment of Pseudomonas aeruginosa infections with limited treatment options: a narrative review. Antibiotics. 2022; 11(5):579. [DOI:10.3390/antibiotics11050579]
34. Ferreiro JL, Otero JÁ, Rivo AS, González LG, Conde IR, Soneira MF, et al. Outpatient therapy with piperacillin/tazobactam using elastomeric pumps in patients with Pseudomonas aeruginosa infection. Sci Rep. 2021;11(1):8610. [DOI:10.1038/s41598-021-88179-7]
35. Buxser S. Has resistance to chlorhexidine increased among clinically-relevant bacteria? A systematic review of time course and subpopulation data. PLoS One. 2021;16(8): e0256336. [DOI:10.1371/journal.pone.0256336]
36. Huang SS. Chlorhexidine-based decolonization to reduce healthcare-associated infections and multidrug-resistant organisms (MDROs): who, what, where, when, and why?. J Hosp Infect. 2019;103(3):235-43. [DOI:10.1016/j.jhin.2019.08.025]
37. Huang SS, Septimus E, Kleinman K, Moody J, Hickok J, Heim L, et al. Chlorhexidine versus routine bathing to prevent multidrug-resistant organisms and all-cause bloodstream infections in general medical and surgical units (ABATE Infection trial): a cluster-randomised trial. The Lancet. 2019;393(10177):1205-15. [DOI:10.1016/S0140-6736(18)32593-5]
38. Ma LZ, Wang D, Liu Y, Zhang Z, Wozniak DJ. Regulation of biofilm exopolysaccharide biosynthesis and degradation in Pseudomonas aeruginosa. Annu Rev Microbiol. 2022;76(1):413-33. [DOI:10.1146/annurev-micro-041320-111355]
39. Farhan RE, Solyman SM, Hanora AM, Azab MM. Molecular detection of different virulence factors genes harbor pslA, pelA, exoS, toxA and algD among biofilm-forming clinical isolates of Pseudomonas aeruginosa. Mol Cell Biol. 2023; 69(5):32-9. [DOI:10.14715/cmb/2023.69.5.6]
40. Motevasel M, Haghkhah M, Azimzadeh N. Phylogenetic Aspects of Antibiotic Resistance and Biofilm Formation of P. aeruginosa Isolated from Clinical Samples. Can J Infect Dis Med Microbiol. 2024;2024(1):6213873. [DOI:10.1155/2024/6213873]
41. Elmaraghy N, Abbadi S, Elhadidi G, Hashem A, Yousef A. Virulence genes in Pseudomonas aeruginosa strains isolated at Suez Canal University Hospitals with respect to the site of infection and antimicrobial resistance. Int J Clin Microbiol Biochem Technol. 2019;2(1):008-19. [DOI:10.29328/journal.ijcmbt.1001006]
42. Bakht M, Alizadeh SA, Rahimi S, Kazemzadeh Anari R, Rostamani M, Javadi A, et al. Phenotype and genetic determination of resistance to common disinfectants among biofilm-producing and non-producing Pseudomonas aeruginosa strains from clinical specimens in Iran. BMC Microbiol. 2022;22(1):124. [DOI:10.1186/s12866-022-02524-y]
43. Chowdhury CS, Khan JA, Khanam J, Nila SS, Ahmed S, Haque N, et al. Detection of Biocide Resistance Genes (qacE and qacΔE1) in Pseudomonas spp Isolated from Patients with CSOM at Mymensingh Medical College Hospital, Bangladesh. Mymensingh Med J. 2021;30(4): 954-9.
44. Hashemi MM, Holden BS, Coburn J, Taylor MF, Weber S, Hilton B, et al. Proteomic analysis of resistance of Gram-negative bacteria to chlorhexidine and impacts on susceptibility to colistin, antimicrobial peptides, and ceragenins. Front Microbiol. 2019;10:210. [DOI:10.3389/fmicb.2019.00210]
45. Garcia RC, Rodrigues RD, Garcia EC, Rigatto MH. Comparison between Colistin and Polymyxin B in the Treatment of Bloodstream Infections Caused by Carbapenem-resistant Pseudomonas aeruginosa and Acinetobacter baumannii-calcoaceticus Complex. Antibiotics. 2023;12(8): 1317. [DOI:10.3390/antibiotics12081317]