Short Communication

Molecular detection of efflux pump genes in clinical isolates of MDR Pseudomonas aeruginosa

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ABSTRACT

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen and a leading cause of various infections, including community- acquired and healthcare-associated infections. This organism is highly resistant to different classes of antimicrobials through multiple mechanisms, presenting a major challenge for infection treatment in hospital settings. Therefore, this study aimed to detect RND-type efflux pump genes, including *mexAB, mexXY, mexC*, and *oprM*, in multi-drug resistant (MDR) *P. aeruginosa* clinical isolates from Diyala Province using PCR technology.

Methods: A total of 50 clinical isolates were recovered from patients with different infections between November 2022 and March 2023. Twenty MDR isolates were selected based on antimicrobial susceptibility testing and subsequently confirmed using the VITEK 2 Compact system. Demographically, the isolates were obtained from 23 males (46%) and 27 females (54%). Twenty MDR isolates were selected based on antimicrobial susceptibility testing against 10 distinct antimicrobials using the Kirby-Bauer disk diffusion method.

Results: Molecular screening revealed that 10/20 (50%) of the isolates harbored the *mexB* gene, while the *mexA* gene was not detected in any isolate (0/20, 0%). Notably, all isolates carried both the *mexC* and *mexY* genes (20/20, 100%). Furthermore, 2/20 (10%) of the isolates harbored the *mexX* gene, whereas only 1/20 (5%) was positive for the *oprM* gene.

Conclusion: The current study showed in conclusion a rise in the spread of Mex efflux pump genes between the MDR isolates of P. *aeruginosa bacteria* that we obtained from different clinical samples in Diyala province, and therefore the isolated positive efflux pumps were detected in this study and this indicates the extent of enhanced prevention and severe control of this infection.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a highly pathogenic bacterium whose cell surface structures, secreted compounds, and biofilm formation all contribute to its virulence. In immunocompromised patients, it causes complex, difficult- to- treat infections owing to this virulence combined with antibiotic resistance [1]. As a quintessential opportunistic gram-negative pathogen, *P. aeruginosa* has been responsible for life-threatening infections in patients with weakened immune systems [2]. Resistance to antimicrobial agents in *P. aeruginosa* arises through three fundamental mechanisms: intrinsic, acquired, and adaptive resistance. Its inherent resistance stems from efflux pump expression, restricted outer membrane permeability, and the production of antibiotic- inactivating enzymes, while acquired resistance can develop through horizontal transfer of variable resistance genes, including β- lactamases, metallo- β-lactamases, and aminoglycoside resistance genes [3,4]. This study therefore screened for efflux pump genes (*mexA*, *mexB*, *mexC*, *mexX*, and *mexY*) and the outer membrane protein gene *oprM*.

P. aeruginosa is a Gram-negative bacillus widely distributed across environments, particularly in soil and water subject to heavy human activity, such as wastewater-, hydrocarbon-, and pesticide- contaminated sites [5]. It ranks among the leading pathogens responsible for nosocomial infections — affecting the respiratory tract, urinary tract, and skin wounds — and frequently causes recurrent, prolonged chronic infections in patients with cystic fibrosis [6,7]. As the body's largest organ, the skin serves as a physical and immunological barrier against pathogenic microorganisms; cutaneous lesions therefore represent an entry point for microbial contamination that can progress to chronic wounds and other invasive infections. Given their substantial social, psychological, and economic burden, chronic wounds are regarded as a serious public health concern [8]. Infections caused by *P. aeruginosa* are particularly challenging because of both its intrinsic and acquired resistance to numerous otherwise effective antibiotic classes, with intrinsic multidrug resistance driven by limited outer membrane permeability, inducible β-lactamase production, and multidrug efflux systems [9]. This study aimed to detect the efflux pump genes *mexA*, *mexB*, *mexC*, *mexX*, and *mexY*, along with the outer membrane protein gene *oprM*, across a range of clinical isolates.

METHODS

Confirmation of bacterial isolates

A total of 50 *P. aeruginosa* isolates were obtained from various clinical samples collected from male and female patients of different ages admitted to Baquba General Hospital, the Burns Center, and private medical laboratories in Diyala Province. The 50 P. aeruginosa isolates were identified using macroscopic, microscopic, culture, and biochemical tests and confirmed by the VITEK 2 Compact system. Antimicrobial susceptibility testing was subsequently performed, from which 20 isolates demonstrated an MDR phenotype. The susceptibility of the *P. aeruginosa* isolates against 10 distinct antimicrobials was evaluated using the Kirby- Bauer disk diffusion method, confirming their multi- drug resistant (MDR) phenotype. Biofilm production was evaluated using the microtiter plate method, as previously described [10].

Bacterial DNA extraction and PCR

Genomic DNA was extracted from the 20 MDR isolates using a commercial genomic DNA extraction kit according to the manufacturer's instructions (Favorgen Biotech Corp., Korea). The extracted DNA was stored at -20 °C in a deep freezer until further amplification. PCR assays were performed to screen for all the target genes listed in Table 1. The amplified PCR products were separated via gel electrophoresis on a 1% agarose gel (iNtRoN Biotech Inc., Korea) stained with 0.5 µg/mL ethidium bromide. Band migration and amplification profiles were subsequently visualized and photographed using a gel documentation system (Cleaver, United Kingdom).

PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min for all genes. Amplified PCR products were detected by agarose gel electrophoresis.

RESULTS

Molecular Detection of Efflux Pump Genes (m*exA*, *mexB*, *mexC*, *mexX*, *mexY* and *oprM*)

All 20 MDR isolates (100%) demonstrated biofilm production capabilities using the microtiter plate method. The molecular analysis of efflux pump genes summarized in Table 2 revealed that 0/20 (0%) and 10/20 (50%) of the *P. aeruginosa* isolates were positive for the *mexA* and *mexB* genes, respectively. Additionally, PCR data revealed that 20/20 (100%) of the isolates harbored the *mexC* and *mexY* genes. Furthermore, 2/20 (10%) of the isolates harbored the *mexX* gene. However, PCR data for outer membrane genes showed that 1/20 (5%) of the isolates were positive for the *oprM* gene.

In this study, all isolates were tested for the presence of the *mexA* and *mexB* genes, and the *mexA* gene was not amplified in any isolate as shown in Figure 1. The *mexA* gene was not amplified in any of the tested isolates.

While the *mexB* gene was detected 10/20 (50%) as in figure (2). The molecular results of efflux pump genes in figure (3) revealed that 20/ 20 (100%) of *P. aeruginosa* isolates were positioned for *mexC*. Uniplex results by PCR technique, showed 20/20 (100%) of isolates were positive for *mexY* in (Figure 4). PCR data revealed that 2/20 (10%) of the isolates contained the *mexX* gene (Figure 5). The results of the present study demonstrated that only 1/20 (5%) of the MDR *P. aeruginosa* isolates harbored the *oprM* gene (Figure 6).

Figures

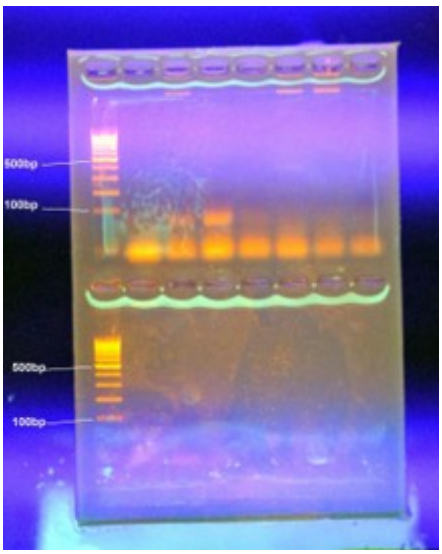

Figure 1: Agarose gel electrophoresis for the detection of mexA gene by PCR. Negative results for all samples are evident.

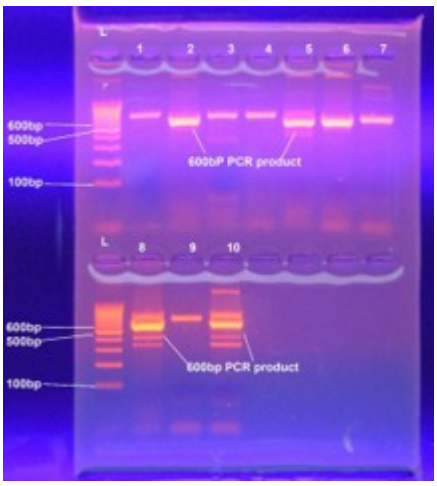

Figure 2: Agarose gel electrophoresis for the detection of mexB gene by PCR. Samples 2, 5, 6, 8 and 10 represent positive samples (600 bp amplicon). L: 100 bp DNA ladder.

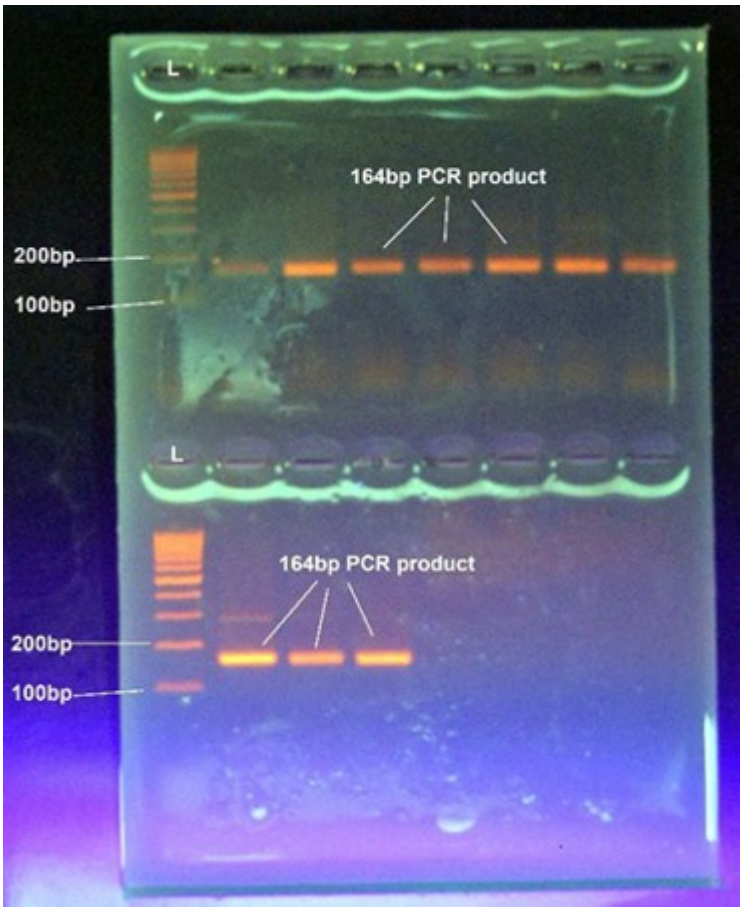

Figure 3: Agarose gel electrophoresis for the detection of mexC gene by PCR. All samples tested positive and produced 164bp amplicon (each)..

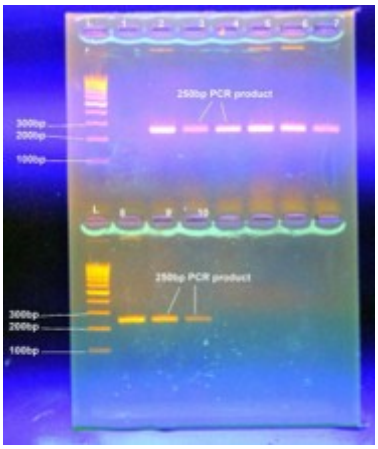

Figure 4: Representative agarose gel electrophoresis for the detection of the mexY gene by PCR. Samples 2–10 shown in the gel are positive and produced 250-bp amplicon (each).

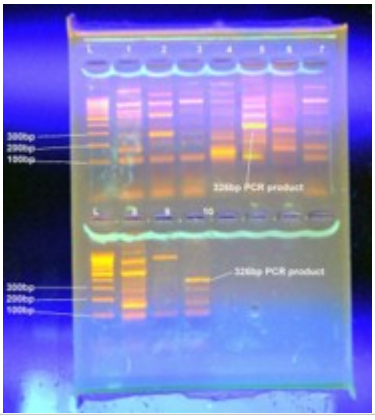

Figure 5: Agarose gel electrophoresis for the detection of the mexX gene by PCR. Samples 5 and 10 are positive, producing a 326 bp amplicon (each). L: 100 bp DNA ladder.

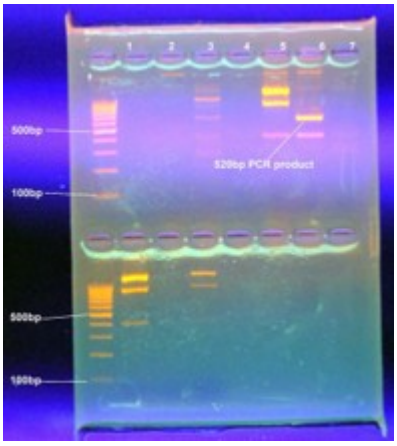

Figure 6: Agarose gel electrophoresis for the detection of oprM gene by PCR, only one sample is positive (sample 6) that produced 520bp amplicon (each).

Tables

Gene name	Oligo Sequence (5'-3')	Product size bp	Reference
mexA	F- GACGGTGACCCTGAATACCG	620	[11]
	R- CGACGGAAACCTCGGAGAAT		
mexB	F- GTCTACCCGTACGACACCAC	600	
	R- GGTGGAAAGGAACATCCGGT		
oprM	F- GGTAGCCCAGGACCAGAATG	520	
	R- GAGCTGGTAGTACTCGTCGC		
mexC	F- GTACCGGCGTCATGCAGGGTTC	164	
	R- TTACTGTTGCGGCGCAGGTGACT		
mexX	F- TGA AGG CGG CCC TGG ACA TCA GC	326	
	R- GAT CTG CTC GAC GCG GGT CAG CG		
mexY	F - CCGCTACAACGGCTATCCCT	250	
	R- AGCGGGATCGACCAGCTTTC		
Housekeeping gene *rpsL*	F- GCAAGCGCATGGTCGACAAGA	201	
	R- CGCTGTGCTCTTGCAGGTTGTGA		

Table 1: The primers for efflux pumps genes with their nucleotide sequence and product size.

Genes Monoplex	NO. of Positive isolates/ Total isolate (%)
mexA	0/20 (0%)
mexB	10/20 (50%)
mexC	20/20 (100%)
mexX	2/20 (10%)
mexY	20/20 (100%)
oprM	1/20 (5%)

Table 2: Detection of efflux pump genes in MDR pseudomonas aeruginosa isolates.

DISCUSSION

The prevalence of efflux pump genes in the present study was generally lower than that reported

in previous literature.

For *mexA*, Abbas *et al.,* [12] reported a 100% detection rate among *Pseudomonas aeruginosa* isolates in Egypt, while Murugan *et al.,* [13] detected it in 51% (or 54.2%) of isolates which are both markedly higher than the present study, in which the gene was not detected. Similarly, Al-Zowaid *et al.,* [16] and Kishk *et al.,* [17] reported higher *mexA* detection frequencies of 83.5% and 88.2%, respectively.

Regarding *mexB*, the current findings contrast with a Baghdad-based study where 100% of isolates carried the gene [15], as well as reports by Al-Zowaid *et al.,* (63.29%) [16], Kishk *et al.,* (70.5%) [17], and Murugan *et al.,* (46.5%) [13]. Kishk *et al.,* [17] also noted that 58.8% (80 strains) of isolates co-harbored both *mexA* and *mexB*. In contrast, our *mexB* findings are consistent with a study conducted in Iran, which observed the gene in 53.3% of isolates [14]. For the outer membrane gene *oprM*, the detection rate in this study was considerably lower than the 40.5% reported by Murugan *et al.,* [13] and the 37.5% (12/32) reported by Al-Zowaid *et al.,* [16].

Discrepancies were also evident across other efflux pump systems. The mexC gene was detected in 78.12% of isolates in a study conducted in Najaf City, Iraq [18], and a similarly high prevalence (89.47%) was reported by Al Saadi [19] in Diyala, both considerably higher than the rate observed in the current study. Conversely, Al-Jubori [20] identified *mexY* in 28.5% (8/28) of isolates in Iraq, representing a lower prevalence than that found in the present study.

The variation in efflux pump gene distribution across these studies likely reflects regional differences in multidrug-resistance patterns and the selective pressure driven by local antibiotic usage.

CONFLICT OF INTEREST

There is no conflict of interest to be declared by authors.

AUTHOR CONTRIBUTIONS

Muslim M. Kadhim and Aseel R. Mardan contributed equally to conceptualization, execution and reporting of this study.

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AI language tools were used in the introduction section to [only] improve readability of the text.

REFERENCES

1. Killough M, Rodgers AM, Ingram RJ. Pseudomonas aeruginosa: Recent Advances in Vaccine Development. Vaccines, (2022); 10(7): 1100.
2. Wang BX, Wheeler KM, Cady KC, Lehoux S, Cummings RD,
3. Mahmoud SF, Fayez M, Swelum AA, Alswat AS, Alkafafy M,
4. Azam MW, Khan AU. Updates on the pathogenicity status of Pseudomonas aeruginosa. Drug discovery today, (2019); 24(1): 350-9.
5. Crone S, Vives-Flórez M, Kvich L, Saunders AM, Malone M,
6. Angeletti S, Cella E, Prosperi M, Spoto S, Fogolari M,
7. Brao KJ, Wille BP, Lieberman J, Ernst RK, Shirtliff ME, Harro JM. Scnn1b-Transgenic BALB/c Mice as a Model of P. aeruginosa Infections of the Cystic Fibrosis Lung. Infection and Immunity, (2020); 88: e00237-20.
8. Vale De Macedo GH, Costa GD, Oliveira ER, Damasceno GV, Mendonça JS,
9. Mohamad SM, Rostami S, Zamanzad B, Gholipour A, Drees F. Detection of exotoxins and antimicrobial susceptibility pattern in clinical Pseudomonas aeruginosa isolates. Avicenna Journal of Clinical Microbiology and Infection, (2017); 4(4): 1-6.
10. Yousef SA, Hassan IB, AlMusawi MT. Detection of multi-drug resistant and biofilm formation of Pseudomonas aeruginosa clinical isolates from Diyala Hospitals. Journal of Biomechanical Science and Engineering, (2023); 8(3S): 215-222.
11. Xavier DE, Picão RC, Girardello R, Fehlberg LC, Gales AC. Efflux pumps expression and its association with porin down-regulation and β-lactamase production among Pseudomonas aeruginosa causing bloodstream infections in Brazil. BMC microbiology, (2010); 10(1): 217.
12. Abbas HA, El-Ganiny A, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. African Health Sciences, (2018); 18(1): 11-21.
13. Murugan N, Malathi J, Therese KL, Madhavan HN. Application of six multiplex PCR's among 200 clinical isolates of Pseudomonas aeruginosa for the detection of 20 drug resistance encoding genes. The Kaohsiung Journal of Medical Sciences, (2018); 34(2): 79-88.
14. Pourakbari B, Yaslianifard S, Yaslianifard S, Mahmoudi S, Valian S, Mamishi S. Evaluation of efflux

pump gene expression in resistant Pseudomonas aeruginosa isolates in an Iranian referral hospital. Iranian Journal of Microbiology, (2016); 8(4): 249-256.
15. Abd NQ. Evaluation of the Activity of Quinoline-2-One Derivatives as Bacterial Antibiotic to Inhibit the mexB Efflux Protein of Pseudomonas aeruginosa. MSc. Thesis in Microbiology. Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq, (2018).
16. AL-Zwaid AJA, Al-Dahmoshimoshi HOM. Molecular investigation of Pseudomonas aeruginosa mexAB-oprM efflux pump genes from clinical samples and their correlation with antibiotic resistance. Journal of Applied and Natural Science, (2022); 14(1): 140-147.
17. Kishk RM, Abdalla MO, Hashish AA, Nemr NA, El Nahhas N,
18. Al-Absawe SAA, Tuwaij NSS. Molecular screening of RND-type efflux pump genes among Pseudomonas aeruginosa isolated from burn patients in Najaf City, Iraq. International Journal of Health Sciences, (2022); 6(S7): 3586-3594.
19. Alsaadi LAS. Molecular detection of the mex efflux pump genes in extensively drug-resistant and pandrug-resistant Pseudomonas aeruginosa isolated from Iraqi patients in Diyala. World Bulletin of Public Health, (2022); 10: 97-105.
20. Al-Jubori SS, Al-Jabiri HA, Al-Kadmy IM. Molecular Detection of Aminoglycoside Resistance Mediated by Efflux Pump and Modifying Enzymes in Pseudomonas aeruginosa Isolated From Iraqi Hospitals. International Conference on Medical Genetics, Cellular & Molecular Biology, Pharmaceutical & Food Sciences, (2015).