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Molecular Identification of MexB Efflux Pump Gene in Clinical Isolates of *Pseudomonas aeruginosa*

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Abstract **Background:** *Pseudomonas aeruginosa* is a significant nosocomial pathogen notorious for its multidrug resistance (MDR), often mediated by efflux pumps like MexAB-OprM. This study aimed to molecularly identify the mexB gene within clinical isolates of *P. aeruginosa* and assess its correlation with antibiotic resistance profiles. This study aims to detect the presence of the MexB efflux pump gene in clinical isolates of *Pseudomonas aeruginosa* and to investigate its association with antibiotic resistance patterns, particularly multidrug resistance, to better understand the molecular mechanisms underlying antimicrobial resistance and support effective therapeutic strategies. **Methodology:** All clinical isolates of *P. aeruginosa* from patients at Teaching Ba'aqubah Hospital and Teaching Albatool Hospital were investigated in this study. identified with the Vitek 2 compact, MexB efflux pump genes are detected by the PCR technique and primers are designed in the biology department of the College of Education of Pure Science in Iraq. Conventional PCR results showed that the gene, MexB, was present in all seven clinical isolates of *P. aeruginosa*. It was concluded that carrying efflux pump genes can make *P. aeruginosa* more resistant to several drugs. **Result:** From the 100 clinical specimens collected *P. aeruginosa* recorded a high percentage of isolated 67(67%), *P. aeruginosa* isolates Including; Chronic burns swab 23 (34.3%), Wounds swab 15 (22.4%), Urine from UTI patients 12 (17.9%), Sputum 10 (14.9%) and Ear swabs 7 (10.5%). All isolates were obtained by cultural characterisation These clinical isolates were on cetrimide agar, which is a selective medium for *Pseudomonas* spp.at 37°C for 24 hours. the VITEK2 compact System to confirm the diagnosis of *Pseudomonas aeruginosa.* **Conclusion**: According to our research, any *P. aeruginosa* that has the *Mex*B gene of the efflux pump in the MexABOprM operon may enhance drug expulsion and antibiotic resistance, which could cause health issues for patients, particularly those with compromised immune systems.

Key Words Mexab-Oprm operon, PCR, *Pseudomonas aeruginosa*, Efflux pump

INTRODUCTION

A formidable opportunistic pathogen that causes serious infections in clinical settings, *Pseudomonas aeruginosa* is a Gram-negative bacterium known for its adaptability and inherent resistance to a wide range of medications. One of the biggest obstacles to preventing and treating infections brought on by this microbe is its innate and acquired antibiotic resistance [1]. *P. aeruginosa* has been connected to several antibiotic resistance mechanisms, including the innate resistance-related development of bacterial efflux pumps, which transport substrates from the bacterial core to the cell surface [2,3].

Pseudomonas aeruginosa is a powerful opportunistic pathogen in clinical settings, causing increasing hospital-acquired *Pseudomonas aeruginosa* infections and its inherent resistance to several medications. This resistance is largely caused by the action of multidrug efflux pumps, particularly the MexAB-OprM system [4]. MexB is the inner membrane transporter in this tripartite complex, aggressively removing many antibiotics and harmful substances from the bacterial cell to reduce their intracellular concentrations and effectiveness. The defence of *P. aeruginosa* against several antibiotics, such as beta-lactams, fluoroquinolones and aminoglycosides, depends on the MexAB-OprM efflux pump. Treatment of infections brought on by this pathogen is made extremely difficult by the overexpression or mutations in the genes encoding this efflux mechanism, such as mexB, which have been connected to multidrug-resistant (MDR) phenotypes [5].

The defense of *P. aeruginosa* against several antibiotics, such as beta-lactams, fluoroquinolones and aminoglycosides, depends on the MexAB-OprM efflux pump. Treatment of

infections brought on by this pathogen is made extremely difficult by the overexpression or mutations in the genes encoding this efflux mechanism, such as mexB, which have been connected to multidrug-resistant (MDR) phenotypes [6].

In conclusion, the MexB efflux pump is an important variable in *P. aeruginosa* drug-resistant bacteria". To effectively address antibiotic resistance in this adaptive pathogen, molecular investigations that concentrate on the identification and functional study of the mexB gene are crucial [7,8].

This study aims to detect the presence of the MexB efflux pump gene in clinical isolates of *P. aeruginosa* and to investigate its association with antibiotic resistance patterns, particularly multidrug resistance, to better understand the molecular mechanisms underlying antimicrobial resistance and support effective therapeutic strategies.

MATERIALS AND METHODS

Sample Collection

During the period from 4 January 2025 to 28 March 2025. 100 clinical specimens, including burn swabs, wound swabs, ear swabs, throat swabs, Urinary Tract Infection (UTI) infections, sputum and urethral swabs) were collected from patients' attending (Teaching Ba'aqubah Hospital and Teaching Albatool Hospital in Diyala\Iraq). Bacterial isolates and identification. The identification of all bacterial isolates was conducted using morphological techniques, which involved the utilisation of culture media, including Cetrimide Agar and other media, as well as biochemical assays, including oxidase, catalase, TSI and indole [9,10].

VITEK2 compact system through *Pseudomonas aeruginosa* identification. The Vitek-2 compact system (BioMérieux in France) was used to confirm the diagnosis for every clinical isolate [11].

Genomic DNA Extraction commercial PrestoTM Mini gDNA Bacteria Kit (Geneaid, Taiwan) was used to extract the genomic DNA of *P. aeruginosa* isolates [12-14) Using the EasyTaq® PCR SuperMix kit (Trans Gen, China), a conventional PCR experiment was performed to amplify the MexB gene of *P. aeruginosa*, as indicated in Table 1. Lyophilized primers from Macrogen, Korea, were used to make the primer stock solution. Table 2 lists the exact primers for the MexB gene used in this investigation methods.

Molecular Assay

MexB gene Detection

All the selected isolates of *P. aeruginosa* were identified with the *Mex*B gene. The positive gene result was subsequently confirmed through electrophoresis on a 1.5% agarose gel stained with ethidium bromide, electrophoresed at 75 volts for 50 minutes and visualized under an ultraviolet (UV) transilluminator. The present study revealed the presence of a sharp, singular and non-dispersed 180 bp MexB gene band, which was clearly distinguished from the DNA ladder, as demonstrated in Figure 1. Notably, there was no evidence of DNA degradation, as indicated by the absence of any smearing of the gene band.

Quantitation of DNA

The concentration of extracted DNA was measured using a Quantus Fluorometer to assess the quality of the samples for use in subsequent processes. QuantiFluor Dye was diluted and combined with 1 µL of DNA. DNA concentration readings were found after 5 minutes of room temperature incubation.

Primer Preparation

The Macrogen Company provided these primers in a lyophilized form. Nuclease-free water was used to dissolve lyophilized primers, resulting in a stock solution with a final concentration of 100 pmol/µL. To create a workable primer solution of 10 pmol/µL, 10µL of primer stock solution (which was kept at -20°C in the freezer) was mixed with 90 µL of nuclease-free water. The table illustrates the Reaction Setup process.

The PCR reaction tubes were mixed briefly and placed into a thermocycler PCR instrument where DNA was amplified according to the PCR program as indicated in Table 2-4. The optimum temperature for annealing primers, specifically for *phzM* is 60°C.

Agarose Gel Electrophoresis

Agarose gel electrophoresis verified the existence of amplification following PCR amplification. According to the extracted DNA criteria, PCR was reliable.

Table 1: Components of PCR reaction with their volume

Components	Volume
2×EasyTaq® PCR Super Mix volume (dNTPs, Taq polymerase, MgCl2 and PCR buffer)	12.5 µL
Forward Primer (10 pmol/µL)	1 µL
Reverse Primer (10 pmol/µL)	1 µL
Template DNA	3 µL
Nuclease-free water	7.5 µL
Total volume	25 µL

Table 2: MexBreverse, forward primers and their housekeeping genes

Primer name	Sequence 5`-3`	Annealing temperature (°C)	Product size (bp)	References
MexB-F	GTACCGGCGTCATGCAGGGTTC	60	~180	Abbas *et al.* [1]
MexB-R	TTACTGTTGCGGCGCAGGTGACT	60	~180	

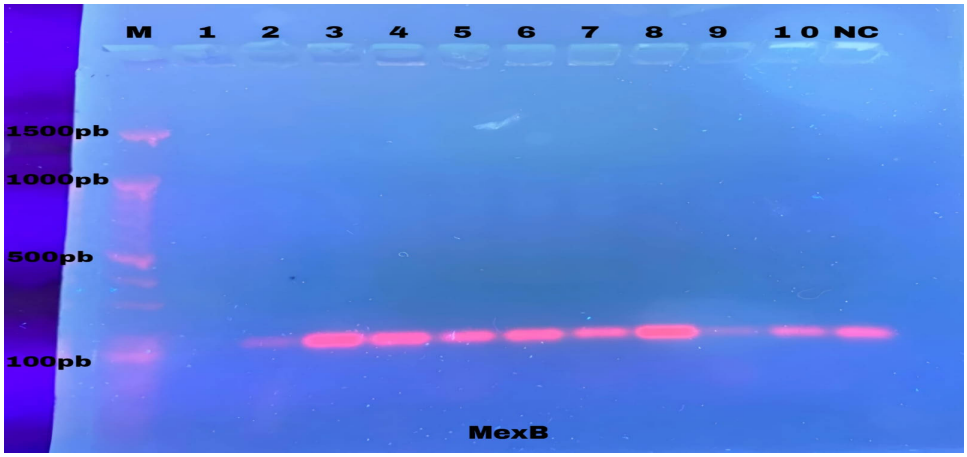

Figure 1: Results of the amplification of genes MexB of bacterial species were fractionated on a 2% agarose gel electrophoresis stained with Eth
Br M: 100 bp ladder marker, Lanes 1-10 resemble 180 bp PCR products, **(M = marker, P1-P10 = samples)

Table 3: Reaction setup

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	10
Forward primer	10	µM	0.5	µM	1
Reverse primer	10	µM	0.5	µM	1
Nuclease free water					6
DNA		ng/µL		ng/µL	2
Total volume					20
Aliquot per single rxn	18 µL of Master mix per tube and add 2 µL of Template				

Table 4: Conditions of PCR reaction for PhzM genes

Steps	Temperature (°C)	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	50,55, 60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Solutions
Ethidium bromide (10 mg/ml), a DNA ladder marker and 1 TAE buffer.

Preparation of Agarose

- A flask containing 100 cc of 1X TAE was taken
- The buffer was supplemented with 1.5 g (for 1.5%) agarose
- Using a microwave, the mixture was brought to a boil until all of the gel particles were dissolved
- To the agarose, 1 µL of Ethidium Bromide (10 mg/ml) was added
- To mix the agarose and prevent bubbles, it was swirled
- The mixture was allowed to cool between 50 and 60 degrees Celsius

Casting of the Horizontal Agarose Gel
After sealing both edges with cellophane tapes, the agarose solution was transferred into the gel tray and allowed to harden for half an hour at room temperature. After carefully removing the comb, the gel was put into the gel tray. 1X TAE-electrophoresis buffer was added to the tray until it covered the gel's surface by 3-5 mm [1].

DNA Loading
Direct loading of the PCR products was done. Five microliters of the PCR product were put straight into the well. For 60 minutes, electrical power was turned on at 100 v/m Amp. From the cathode to the positive anode poles, DNA travels. A gel imaging equipment was used to view the bands in the gel that were stained with ethidium bromide [15].

RESULTS

Sample Distribution :From the 100 clinical specimens collected, *P. aeruginosa* recorded a high percentage of isolates (67%), the number and percentage of *P. aeruginosa* isolates as shown in Table 5. All 67 isolates were obtained by cultural characterisation. These clinical isolates were on cetrimide agar, which is a selective medium for *Pseudomonas* spp. at 37°C for 24 hours.

Table 4: The sample distribution of *P. aeruginosa* isolates from different types of Specimens

Type of specimens	No. (Percentage)
Chronic burns swab	23 (34.3%)
Wounds swab	15 (22.4%)
Urine from UTI patients	12 (17.9%)
Sputum	10 (14.9%)
Ear swabs	7 (10.5%)
Total	67 (100.0 %)

Bacterial Detection: In this study, we used the VITEK2 compact System to confirm the diagnosis of *Pseudomona aeruginosa* probability is approximately 93%-98% that all seven isolates are identified as *Pseudomonas aeruginosa* detection.

Molecular Detection of *P. aeruginosa* MexB gene

Ten identical isolates of *P. aeruginosa* were used in this investigation. The presence of the mexB gene in 67 clinical isolates of *P. aeruginosa* has been found using PCR. An active efflux pump system was indicated by the analysis of 67 (100%) culture-positive isolates. All 67 isolates had the MexB gene. Notably, the gene band did not smear, indicating that there was no indication of DNA degradation.

DISCUSSION

This high prevalence indicates that *P. aeruginosa* remains a major opportunistic pathogen in clinical infections, especially in hospitalised patients. The isolates were initially cultured on Cetrimide agar, a selective medium that enhances the isolation of *Pseudomonas* spp. by inhibiting the growth of most other bacteria. The use of incubation at 37°C for 24 hours aligns with the optimal growth conditions for *P. aeruginosa*.

For confirmation, the VITEK2 Compact System was employed, yielding a high probability of identification (93%-98%) for *P. aeruginosa*. This level of diagnostic accuracy reflects the reliability of automated identification systems, especially when coupled with initial cultural characteristics.

These findings are consistent with several previous studies. For example, a study conducted by Al-Muhanna *et al.* [16] in Iraq reported that *P. aeruginosa* was isolated in 65% of clinical samples, especially from wound infections and respiratory tract specimens. Similarly, Khan *et al.* [17] found a prevalence rate of 70% for *P. aeruginosa* among nosocomial infections, confirming the organism's significant role in hospital-acquired infections [18].

The high isolation rate in our study might be attributed to several factors, including:

- The intrinsic resistance of *P. aeruginosa* to multiple antibiotics
- Its ability to survive in moist hospital environments

The compromised immune status of many hospitalized patients, particularly those with burns, ventilator-associated pneumonia, or urinary catheters [19].

Moreover, the successful use of selective media such as Cetrimide agar and confirmatory tools like VITEK2 enhances the accuracy of detection, reducing false positives or negatives compared to traditional biochemical testing. In contrast, some studies report slightly lower isolation rates, likely due to differences in sample types, patient demographics, or hospital infection control practices. For instance, Ahmed *et al.* [20] reported a 50% isolation rate in a study conducted in a tertiary care centre in Jordan.

Overall, the results of this study align closely with regional and international findings, highlighting *P. aeruginosa* as a critical pathogen that requires continuous monitoring and strict infection control strategies.

Molecular Detection of *P. aeruginosa* MexB gene:In the present study, the MexB gene, a key component of the MexAB-OprM efflux pump system, was detected in all 67 clinical isolates of *Pseudomonas aeruginosa* using PCR analysis. The amplification results showed clear, sharp DNA bands without smearing, confirming high-quality DNA extraction and integrity with no significant degradation.

The detection of the MexB gene in 100% of the isolates strongly suggests that the MexAB-OprM efflux system is universally present among the clinical strains investigated. This finding is consistent with the known role of MexAB-OprM as a constitutively expressed efflux pump in *P. aeruginosa*, which is crucial for intrinsic resistance to multiple classes of antibiotics, including β-lactams, fluoroquinolones, tetracyclines and chloramphenicol [17].

The presence of an active efflux system across all isolates indicates that efflux-mediated antibiotic resistance is a widespread and significant mechanism in clinical *P. aeruginosa* strains. The efficiency of this pump contributes heavily to the bacterium's ability to survive antibiotic pressure in hospital settings, leading to therapeutic challenges and persistent infections.

These findings are in agreement with previous studies, such as Poole [21] reported that MexAB-OprM is the most consistently expressed efflux system in *P. aeruginosa* and plays a central role in intrinsic multidrug resistance. Zhang *et al.* [22] demonstrated that 95-100% of clinical *P. aeruginosa* isolates harboured the mexB gene, confirming its essential function in resistance patterns globally. In a local study from Iraq, Al-Muhanna *et al.* [16] similarly found a high prevalence of efflux-related genes, including mexB, among clinical isolates.

The absence of DNA smearing further validates the quality of molecular procedures, ensuring that PCR results are reliable [23,24]. Moreover, the robust presence of MexB across all isolates highlights the necessity of considering Efflux Pump Inhibitors (EPIs) as a potential adjunctive strategy to restore antibiotic susceptibility in resistant *P. aeruginosa* infections [25]. In contrast, some studies have noted occasional downregulation or mutation of the mexB gene, particularly under specific selective pressures or

mutations affecting the regulatory genes (e.g., mexR), although such phenomena were not observed in this study.

CONCLUSIONS

This study showed a high prevalence (67%) of *Pseudomonas aeruginosa* in clinical specimens, confirmed with high accuracy by the VITEK2 system. All isolates carried the MexB efflux pump gene, highlighting its major role in antibiotic resistance. The presence of MexB within the MexAB-OprM operon increases drug expulsion, contributing to multidrug resistance, especially dangerous for immunocompromised patients like burn victims, newborns and cancer patients. Detection of efflux pump genes by PCR provides rapid insight into emerging resistance.Effective infection control, continuous surveillance and development of therapies targeting efflux mechanisms are critical to managing *P. aeruginosa* infections.

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