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DETECTION OF OPRD, MEXA AND MEXB GENES IN THE CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* AND THEIR ANTIMICROBIAL SUSPICIBILITY PATTERNS

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Abstract. Background: Pseudomonas aeruginosa is an important opportunistic pathogen associated with hospital acquired infection, particularly in wound and respiratory specimens. Objective: This is study aimed to detect the presence of oprD, mexA and mexB genes in clinical isolates of Pseudomonas aeruginosa and to describe their antimicrobial resistance patterns. Material and methods: One hundred clinical specimens from wound and respiratory infections were collected and processed using standard bacteriological methods. Presumptive P. aeruginosa isolates were identified phenotypically and then confirmed by PCR amplification of the 16S rRNA gene. The resistance -associated genes mexA, mexB, and oprD were screened by PCR to asses the molecular profile of the confirmed isolates. Results: out of 100 samples, 20 isolates were confirmed as P. aeruginosa giving an isolation rate of 20%. All confirmed isolates were positive for 16S rRNA, mexA, mexB, and oprD. The universal detection of these genes indicates successful molecular confirmation and suggests the presence of efflux pump -mediated resistance and outer membrane permeability -related resistance mechanisms. Conclusion: The study confirmed the presence of 16S rRNA, mexA, mexB and oprD genes in the clinical isolates of Pseudomonas arginosa Obtained from wound and respiratory samples. The isolates is also showed varying antimicrobial susceptibility patterns. However, PCR detection alone does not demonstrate gene expression or functional resistance mechanism. Therefore, the result should be interpreted as molecular detection of resistance-associated genes, not as direct proof of carbapenem resistance or multidrug resistance. Further studies using sequencing, gene expression analysis, and function assays are needed to clarify the role of these genes in antimicrobial resistance.

Keywords: Pseudomonas aeruginosa, Porins, Carbapenems, Over-expressio

Introduction

Pseudomonas aeruginosa has been the leading give rise of hospital-acquired infections, with carbapenems such as imipenem and meropenem, oftentimes representing the last line of defense. Nevertheless, non-enzymatic mechanisms notably missing of the OprD porin and over expression of the mexA, mexB, efflux pump, have appeared as predominant motivation of carbapenem resistance in this kinds, all these chromosomal amendments reduce effectiveness drug uptake and further antibiotic efflux, and by extension can act synergistically to give high level resistance or multidrug resistance phenotypes [1], [2]. In P. aeruginosa the OprD represent the primary channel for uptake of carbapenem, frameshift mutation in the oprD gene, with transcription as a downregulation, thus it will suppress or reduce carbapenem permeability. Through the clinical isolates, are observed it in extensive -drug resistance was downregulation of oprD up to more than 68% of strains, through this mechanism which alone rises minimum inhibitory concentration (MICs) for meropenem and imipenem, but nor does it extend to confer broad -spectrum resistance [3]. Over-expression of mexA and mexB genes are often created from mutation, a study reported of quantitative of RT-PCR surveys of carbapenem -resistant isolates about mexB over-expression in 82% of resistant strains against 33% of carbapenem -susceptible controls [4]. Over-expression of mexA and mexB genes are often created from mutation, a study reported quantitative of RT-PCR surveys of carbapenem -resistant isolates about mexB over-expression in 82% of resistant strains against 33% of carbapenem -susceptible controls, this indicate that up-regulation elevating of MIC through several drug classes which may contribute to multidrug-resistance profile [5], [6]. Recent studies show that concurrent oprD inactivation and MexAB -OprM come from overexpression synergize to get high-level carbapenem resistance and wide MDR phenotype [7]. Whole genome and expression analyses findings confirm all the clinical isolates both mechanisms appearance MICs for imipenem and meropenem exceed clinical breakpoints, where often complained with fluoroquinolones and cephalosporins [8]. In Iraq, the molecular studies about oprD porin antimicrobial diagnostic are limited, recent studies have initiated clarified these mechanisms in clinical isolates, there are reported mentioned the most of carbapenem resistance P. aeruginosa isolations from burn wound infections in Al-Najaf hospitals included mutation in oprD gene these refer it to porin loss as a main resistance determinate in this government [9]. Given the high rate of carbapenem-resistant P. aeruginosa (CRPA) in Iraq, Given the high rate of carbapenem-resistant P. aeruginosa (CRPA) in Iraq, this is study aimed to detect the presence of oprD, mexA and mexB genes in clinical isolates of Pseudomonas aeruginosa and to describe their antimicrobial resistance patterns.

Methods

1. Study Design and Setting: This was a cross- sectional, laboratory-based study was conducted for 8 months, between February–December 2025, at Baquba Teaching Hospital, Diyala Governorate, Iraq. A total of 100 clinical samples were all non-duplicate, collected from hospitalized patients using predefined inclusion and exclusion criteria. The samples were processed by standard microbiology methods for the isolation and identification of *Pseudomonas aeruginosa*. Molecular detection of the resistance -associated genes oprD, mexA and mexB was performed by PCR. The present study was designed as descriptive cross-sectional investigations conducted to assess feasibility and generate preliminary data for a larger future study.

2. Isolate Collection and Identification: Clinical specimens: Burn-wound swabs, respiratory secretions and urine samples from in-patients with suspicion *Pseudomonas aeruginosa* infections. Culture and species identification, samples were plated on cetrimide agar with incubation at 37°C 24h, colonies manifesting characteristic blue-green pigment, oxidase tests were positive and confirmed as a *Pseudomonas aeruginosa* by MALDI-TOF MS (Bruker Biotyper).

3. Antimicrobial Susceptibility Testing: Broth microdilution was minimum inhibitory concentration (MICs) for meropenem, imipenem, ciprofloxacin and ceftazidime were

determined by BMD according to CLSI 2024 guidelines. The interpretation depended by CLSI breakpoint were utilized to categorize isolates as susceptible, intermediate or resistant. The carbapenem resistant *Pseudomonas aeruginosa* (CRPA) was profiled as MIC ≥ 8 µg/mL for meropenem or

4. Molecular Detection of Resistance Genes: Molecular screening: Multiplex PCR targeting 16SRrna gene of Pseudomonas aeruginosa samples species can be detected and distinguished from other bacteria by sequencing and investigating its 16S ribosomal RNA (rRNA) gene. This method leverages the balance of conserved and variable regions in the ~956 bp 16S rRNA sequence. Also, oprD, mexA and mexB, primers/conditions per protocols PCR for detection of oprD, mexA and mexB genes. amplification of 16SRrna gene of Pseudomonas aeruginosa samples species.

Table 1. Kits

Kits	Company/Origin
ABIOpureTM Total DNA	ABIOpure, USA
Agarose, Ethidium Bromide Solution (10mg/ml)	
GoTag Green Master Mix, Nuclease Free Water	Promega, USA
TAE 40X, Quantifluor dsDNA System	
Absolute Ethanol	ROMIL pure chemistry, UK
Primers	Macrogen, Korea

Table 2. Genes Target

Gene Target	Primer Name	Sequence (5'→3')	Product Size (bp)	Annealing Temp. (°C)
oprD	oprD-F	ATGAAAGTGATGAAGTGGAGCG	1326	
	oprD -R	TTACAGGATCGACAGCGGATAG		
mexA	mexA-F	ATCGACGGGATCCTTCGGC	303	58
	mexA-R	TTCAGGGTGGCGAAGTTG		
mexB	mexB-F	GTGTTCGGCTCGCAGTACTC	214	56
	mexB-R	GACCTCGGTTTAGTTCACAGA		
16S	16S Rrna-F	GGGGGATCTTCGGACCTCA	956	60
rRNA	16S Rrna-R	TCCTTAGAGTGCCCACCCG-3		

Tabe 3. Primer preparation

Primer Name	Volume of Nuclease-Free Water (µl)	Concentration (pmol/µl)
oprD-F	300	100
oprD -R	290	100
mexA-F	300	100
mexA-R	320	100
mexB-F	300	100
mexB-R	300	100
16S Rrna-F	320	100
16S Rrna-R	300	100

These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.

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Table 4. PCR Reaction Setup

Parameter	Details
Number of reactions	10 rxn
Reaction volume per run	20 µl
Annealing temperature (°C)	55,51,53
PCR product length (bp)	1538,309,621,585

Table 5. PCR Master Mix Components (per 1 reaction, 20 µl total

Component	Stock Concentration	Final Concentration	Volume (µl)
Master Mix (2X)	2X	1X	10
Forward Primer	10 µM	0.5 µM	1
Revers Primer	10 µM	0.5 µM	1
Nuclease-Free Water	-----	----	6
DNA Template	ng/µl	ng/µl	2
Total Volume			**20**

Aliquoting: 18µl of Master mix per tube and add 2µl of Template.

Table 6. PCR Thermal Cycling Program

Step	Temperature (°C)	Time (min:sec)	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	58 OR 60	00:30	30
Extension	72	00:30	30
Final Extension	72	07:00	1
Hold	10	10:00	1

Ethical Approval

The study design was approved by the local research ethics committee of the Department of Medical Laboratories at Yarmouk University College, under Order No. 11 dated January 25, 2025. Informed consent was obtained from the participants.

Results and Discussion

A total of 100 wound and respiratory clinical samples were examined, of which 20 (20%) were confirmed as *Pseudomonas aeruginosa,* indicating an overall isolation rate of 20%. As shown in table 7.

Table 7. Samples type

Sample type	Total Samples	Positive Isolation	Isolation rate
Wounds and respiratory Samples	100	20	20%

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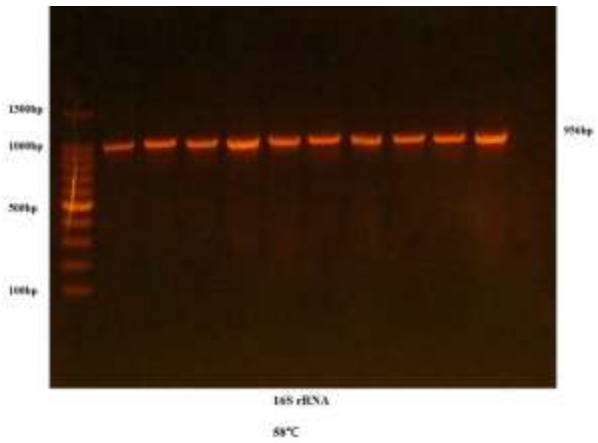

Figure 1. Results of the 16SRrna gene amplification in P. aeruginosa bacterial samples were separated by 1,5 % agarose gel electrophoresis and stained with ethidium bromide.

M indicates the 100 bp DNA ladder marker. Lanes P1-P10showed the expected 965 bp PCR product, while NC represents the negative control.
Based on (figure 1) the presence of a 956bp PCR product in all sample lanes and the absence of amplification in the negative control confirm successful and specific amplification of the 16S rRNA gene from *Pseudomonas aeruginosa* isolates.

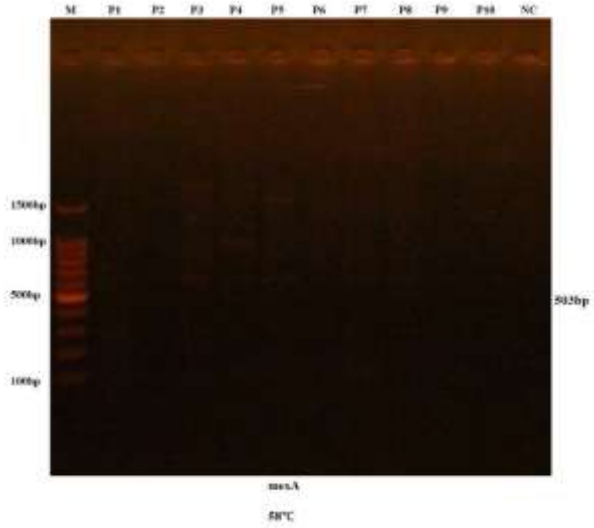

Figure 2. Results of the mexA gene amplification in P. aeruginosa bacterial samples were separated by 1,5 % agarose gel electrophoresis and stained with ethidium bromide.

M indicates the 100 bp DNA ladder marker. Lanes P1-P10showed the expected 503 bp PCR product, while NC represents the negative control.
As shown on (figure 2) PCR amplification of the mexA gen in *Pseudomonas aeruginosa* isolates was performed and the product was separated on a 1.5% gel agarose stained with ethidium bromide. A distance band of approximately 503 bp was observed in lanes. corresponding to the expected mexA amplification. The 100pb DNA ladder was used as the molecular size marker, and the negative control (NC) showed no amplification, indicating that the PCR assay was specific and free from contamination. The mexA band was weaker than the other gene band, probably because of lower PCR efficiency or lower DNA quality /quantity, but its presence at 503bp and the clean negative control confirm successful specific amplification.

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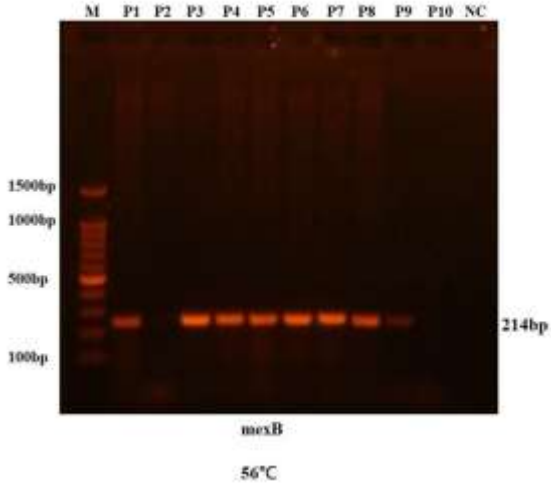

Figure 3. Results of the mexB gene amplification in P. aeruginosa bacterial samples were separated by 1,5 % agarose gel electrophoresis and stained with ethidium bromide.

M indicates the 100 bp DNA ladder marker. Lanes P1-P10showed the expected 214 bp PCR product, while NC represents the negative control.
As shown on (figure 3) PCR amplification of the mexB gen yielded clear 214bp in all *Pseudomonas aeruginosa* isolates. The negative control showed no band, indicating no contamination. These findings confirm successful and specific amplification of the presence target gene in *P. aeruginosa*.

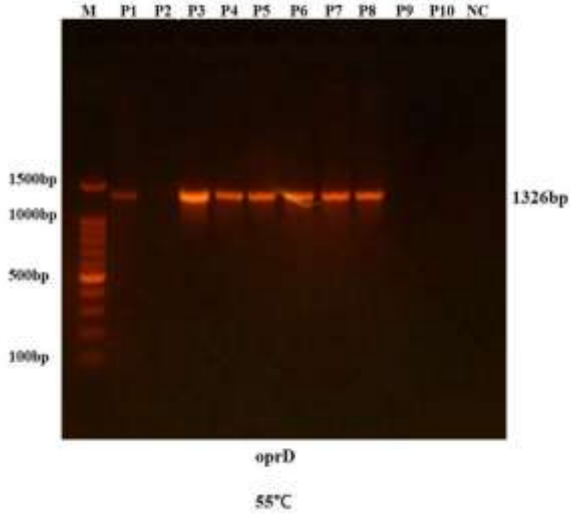

Figure 4. Results of the oprD gene amplification in P. aeruginosa bacterial samples were separated by 1,5 % agarose gel electrophoresis and stained with ethidium bromide.

M indicates the 100 bp DNA ladder marker. Lanes P1-P10showed the expected 1326 bp PCR product, while NC represents the negative control.
As shown on (figure 4), PCR amplification of the oprD gene yielded a specific 1326 in all *Pseudomonas aeruginosa* isolates. The 100pb ladder confirmed expected amplification size and the negative control showed no band, indicating no contamination. These findings confirm successful and specific amplification of the target gene. The presence of oprD-2 related amplification is consistence with the known role of OprD in Carbapenem uptake and resistance in *P. aeruginosa*.

The present study used PCR to detect16S rRNA, mexA, mexB and oprD in all clinical isolates of *Pseudomonas aeruginosa* recovered from wound and respiratory samples. The detection of the 16S rRNA confirmed the molecular identity of the isolates as Pseudomonas aeruginosa. in addition, the successful amplification of mexA, mexB and oprD indicates that these genes are present in the studied isolates.

The findings are in agreement with the previous studies reporting that *Pseudomonas aeruginosa* frequently harbors resistance -associated genes, including efflux pump components and outer membrane porin genes such as mexA, mexB and oprD [10]. However, PCR detection alone does not demonstrate gene expration, mutation, deletion, or functional contribute to antibiotic resistance. Therefore, the results of this study, should be interpreted as evidence of gene presence not as proof of resistance mechanism activity.

Although mexA and mexB components of the eflux system, their detection by conventional PCR in this study only demonstrate the presence of the genes and not establish their expression activity or direct contribution to antimicrobial resistance. This wording is consistent with studies that distinguish PCR based gene detection from gene expression analysis. For example, Bhandari et al (2022) reported PCR detection of mexA, mexB and and separately interpreted them in the context of efflux- pump presence [11].

Detection of oprD confirms genes presence but not functional integrity, because oprD -meditated resistance can result from mutation, deletion, or transcriptional downregulation [12]. Since no sequencing, expression analysis or functional assays were preformed no direct conclusion can be made about carbapenem resistance mechanism based on these PCR result alone.

Although the mexA amplicon appeared less and intens than the other bands, this finding should not be interpreted as reduce or altered gene expression. In conventional PCR, band intensity is not quantitative quantitatively intensity is not quantitative measure of transcriptional activity and may vary according template amount DNA integrity, reaction, efficiency and gel visualization conditions. Thus, the gel pattern confirm amplification only and cannot be used to infer mexA expression levels [13].

Overall, this study demonstrated the molecular detection and prevalence of selected resistance associated genes in *Pseudomonas arginosa* isolates recovered from clinical samples in Baquba teaching hospital. Therefore, further investigationi ntegrating antimicrobial susceptibility testing oprD equencing RT-PCR-based expression [14], [15].

However, the presence of these genes alone does not confirm their functional contribution to antimicrobial resistance, since gene expressions, sequence variation and regulatory mechanism may substantially influence the phenotypes. Analysis and functional efflux pump assays are necessary to clarify the actually role of these genes in resistance development.

Conclusion

The study confirmed the presence of 16S rRNA, mexA, mexB and oprD genes in the clinical isolates of Pseudomonas arginosa Obtained from wound and respiratory samples. The isolates is also showed varying antimicrobial susceptibility patterns. However, PCR detection alone does not demonstrate gene expression or functional resistance mechanism. Therefore, the result should be interpreted as molecular detection of resistance-associated genes, not as direct proof of carbapenem resistance or multidrug resistance.

Further studies using sequencing, gene expression analysis, and function assays are needed to clarify the role of these genes in antimicrobial resistance.

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