



Molecular Profiling of *pqsABCD* Quorum Sensing Genes in Multidrug-Resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a major cause of nosocomial infections due to its high drug resistance. From 180 clinical specimens, 50 isolates were obtained from ear swabs, burn wounds, sputum, urine, and wound samples. This study investigated quorum sensing genes (*pqsA*, *pqsB*, *pqsC*, *pqsD*) and their association with multidrug resistance (MDR). Nineteen isolates (38%) were multi-drug resistant (MDR) and 31 (62%) were non-MDR. PCR analysis demonstrated detection rates of 89.5% for *pqsA*, 94.7% for *pqsB*, 94.7% for *pqsC*, and 73.7% for *pqsD*. Antibiotic susceptibility testing revealed the highest resistance to colistin (84%) and imipenem (76%), with moderate resistance to ciprofloxacin (32%) and tobramycin (30%). Lower resistance was observed for meropenem (16%), piperacillin/tazobactam (12%), gentamicin (10%), amikacin (8%), and ticarcillin/clavulanic acid (4%). The presence of *Pseudomonas* Quinolone Signal (PQS) genes was significantly associated with increased resistance to all tested antibiotics ($p \leq 0.01$). These findings highlight the contribution of the PQS system to virulence and biofilm formation, indirectly supporting antibiotic tolerance and persistence in MDR *P. aeruginosa*. This study provides the first molecular evidence from Iraq linking *pqsABCD* quorum sensing genes with resistance to antibiotics in clinical *Pseudomonas aeruginosa* isolates, offering new insight into the contribution of the PQS system to antibiotic tolerance and persistence.

Keywords: *Pseudomonas aeruginosa*, Multidrug resistant, *pqsA*, *pqsB*, *pqsC* and *pqsD* genes, Quorum sensing

1. Introduction

Pseudomonas aeruginosa, a Gram-negative bacterium that is highly adaptable, is frequently associated with hospital-acquired infections, particularly in patients with compromised immune systems. It is a significant problem to therapy worldwide due to its remarkable ability to develop multidrug resistance (MDR). In addition to classical resistance mechanisms such as efflux pumps and β -lactamase formation, *P. aeruginosa* uses complex regulatory systems, such as quorum sensing (QS), to coordinate virulence, biofilm formation, and antibiotic tolerance^{1,2}. One of the most significant challenges that modern medicine faces are the presence of antibiotic resistance in *P. aeruginosa*, particularly MDR, which may be the primary factor contributing to the high mortality rates among humans³⁻⁵. The *Pseudomonas* Quinolone Signal (PQS) pathway, which comprises the genes *pqsR*, *pqsA*, *pqsB*, *pqsC*, and *pqsD*, is extremely important and contributes to pathological behavior. The synthesis of alkyl quinolones, such as HHQ and PQS, molecules regulated by these genes, which in turn stimulate transcriptional responses that facilitate the production of virulence factors and the growth of biofilms⁶. PQS signaling has been shown to indirectly contribute to antibiotic resistance through increasing biofilm-mediated protection and stress tolerance⁷. The presence and expression of PQS-related genes are essential for determining the molecular basis of resistance and pathogenicity in the context of the

development of MDR strains, particularly in nosocomial environments, is to establish a foundation for future therapeutic options that target quorum sensing pathways by examining the presence of *pqsA*, *pqsB*, *pqsC*, and *pqsD* genes in multidrug-resistant *P. aeruginosa* isolates^{7,8}. The PQS system regulates the expression of the *pqsABCDE* operon via a PqsR-dependent feedback loop, contributing to adaptive responses in clinical strains⁶. The genes *pqsR*, *pqsA*, *pqsB*, *pqsC*, and *pqsD* are important components of the *Pseudomonas aeruginosa* quinolone signaling system. These genes are involved in quorum sensing, a bacterial communication process that regulates virulence, biofilm formation, and secondary metabolite production. It provides an auto-regulatory loop that links the las quorum sensing system to PQS signaling⁹. *pqsA*: The synthesis of PQS is initiated by converting anthranilate to anthraniloyl-CoA. This step prepares the molecule for entry into the alkylquinolone pathway^{9,10}. *pqsB* and *pqsC*: form a heterodimer that catalyzes the condensation of octanoyl-CoA with 2-aminobenzoylacetate (2-ABA), a key step in the biosynthesis of PQS; *pqsD*: converts anthraniloyl-CoA and malonyl-CoA into 2-ABA, which is used by *PqsBC* to produce HHQ (2-heptyl-4-quinolone), a precursor to PQS⁹. *P. aeruginosa* is a pathogen that poses a significant risk due to its capacity to cause severe, life-threatening infections. It is a significant cause of difficult-to-treat infections due to its ability to survive in clinical environments, undergo rapid mutations, and develop resistance to many antibiotic classes¹¹⁻¹³.

The aim of this study was to investigate the presence of quorum sensing genes (*pqsA*, *pqsB*, *pqsC*, *pqsD*) in *Pseudomonas aeruginosa* clinical isolates and to evaluate their association with multidrug resistance to antibiotics, thereby clarifying the role of the PQS genes in antibiotic resistance, and persistence of *P. aeruginosa*.

2. Materials and Methods

2.1. Isolation and Identification

This study was included one hundred eighty clinical samples of Burn, Urine, Wound, Sputum, blood and Ear swab were collected for inpatients and outpatient from Samples were collected from two hospitals in Baghdad including Al- Yarmouk Teaching hospital and AL-Mahmmodiya hospital for the purpose of outwardly from the period February to July 2025 All of the samples were cultured using a sterile loop. blood agar appeared, MacConkey agar and cetrimide agar media for identifying *P. aeruginosa* growth with fluorescent green color, elevated colonies, and grape-like odor. All isolates had the ability to grow on this media. They were further identified and characterized by performing gram staining traditional biochemical tests, including oxidase, catalase¹⁴⁻¹⁶. Additionally, the identification of *P. aeruginosa* was validated using the VITEK compact system^{17,18}.

2.2. Antibiotic Sensitivity Test

The study included antibiotic susceptibility testing using collected bacterial colonies resuspended in sterile saline to a 0.5 McFarland standard turbidity, as determined by the Densi-Check method. This suspension was introduced into the Antimicrobial Susceptibility Testing (AST) No.222 VITEK -2. The resulting data were processed with integrated software for accuracy. This specimen was subsequently confirmed with the VITEK 2 compact system^{19,20}. Data were analyzed according to the CLSI criteria (2025)²¹. Each antibiotic was identified as being resistant, intermediate, or susceptible.

2.3. Molecular Assay

2.3.1. DNA Extraction and Purification:

The ABIOPure™ Total DNA Kit (ABIOPure, USA) was used for efficient and reliable genomic DNA extraction from bacterial cells. The quality and integrity of the isolated DNA were subsequently assessed through gel electrophoresis.

2.3.2. PCR amplification

The study utilized Polymerase Chain Reaction (PCR) to amplify the *pqs* genes (*pqsA*, *pqsB*, *pqsC*, and *pqsD*) in *Pseudomonas aeruginosa*. This process involved selecting specific primers

(Macrogen, Korea)²²⁻²⁴ and employing a thermal cycler to maintain precise temperature control throughout the procedure. During the denaturation phase, the double-stranded DNA template was divided into two single strands. The annealing phase followed, during which the primers bound to the target DNA. Finally, in the extension phase, Taq polymerase synthesized new DNA strands. The details of this process are presented in **Table 1**.

Table 1. Primers of genes used in PCR

Primer Name	Seq.3'→5'	Annealing Temp. (°C)	Product Size (bp)
<i>pqsA-F</i>	CCCGATACCGCCGTTTATCA	60	448
<i>pqsA-R</i>	AACCCGAGGTGTATTGCAGG		
<i>pqsB-F</i>	TGGCCGACACCCTTTATCAC	58	264
<i>pqsB-R</i>	TCGCGGTTCTCGATCAGATG		
<i>pqsC-F</i>	ACCGTCTGGATGAACTGCTG		586
<i>pqsC-R</i>	AGGTGAAGTCGAGCAGGTTG		
<i>pqsD-F</i>	TCCATCCCGTACACCCTGAT	60	761
<i>pqsD-R</i>	AGCAGGTCGAAGTAGTTGCC		

PCR amplifications were carried out in total reaction volumes of 25µl. Specifically, 1µl of each primer, 12.5µl of the premix, lyophilized powder master mix (Promega, USA), 2µl DNA template, and then the volume was completed by deionized distilled water, and the mixture was vortexed. The PCR reaction tubes were placed correctly into the thermocycler, where amplification was carried out according to the specifications outlined in **Tables 2 and 3**.

Table 2. Polymerase chain reaction components

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	
Forward primer	10	µM	0.5	µM	
Reverse primer	10	µM	0.5	µM	
Nuclease Free Water					
DNA		ng/µl		ng/µl	
Total volume					
Aliquot per single rxn	19µl of Master mix per tube and add 1µl of Template				

Table 3. Polymerase chain reaction program

Steps	(°C)	Min.:sec.	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	58 OR 60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

2.4. Statistical Analysis

The Statistical Packages of Social Sciences -SPSS (2019) program was used to detect the effect of difference groups/ Factors in study parameters. Least significant difference-LSD was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

3. Results

3.1. Isolation and Identification

From total of 180 specimens were collected and cultured on blood agar, MacConkey agar and cetrimide agar. However, after analysis, only 50 isolates were found to be associated with *P. aeruginosa*. Microscopically the isolated bacteria are gram negative bacteria. Therefore, they occurred as pale colonies on MacConkey agar. When the beta type of hemolysis was detected on

blood agar, and various strains of *P. aeruginosa* produced several species of pyocyanin on cetrimide agar, the *P. aeruginosa* isolates were positive for the oxidase test as shown in **Table 4** and **Figure 1**. And the recognition was established by VITEK 2 System

Table 4. Culture characteristics and biochemical reactions.

Culture and Biochemical Reaction	Results
Growth on Blood Agar	β -haemolysis
Growth on MacConkey Agar	Pale colonies
Growth on Cetrimide Agar	Appeared as elevated and blue-green colonies with a grape like odor
Gram Reaction	G-ve and rods
Catalase Reaction	Positive
Oxidase Reaction	Positive
Growth at 42°C	Growth

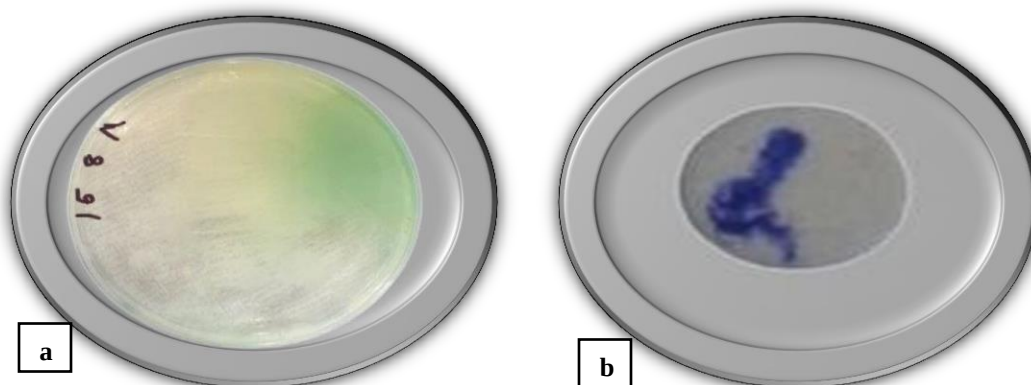


Figure 1. a: *P. aeruginosa* on Cetrimide agar , b: Oxidase test positive for *P. aeruginosa* .

The prevalence of bacteria according to samples was (burn 26%, urine 20%, wound 22%, sputum 20%, and ear 12%) as shown in **Figure 2**.

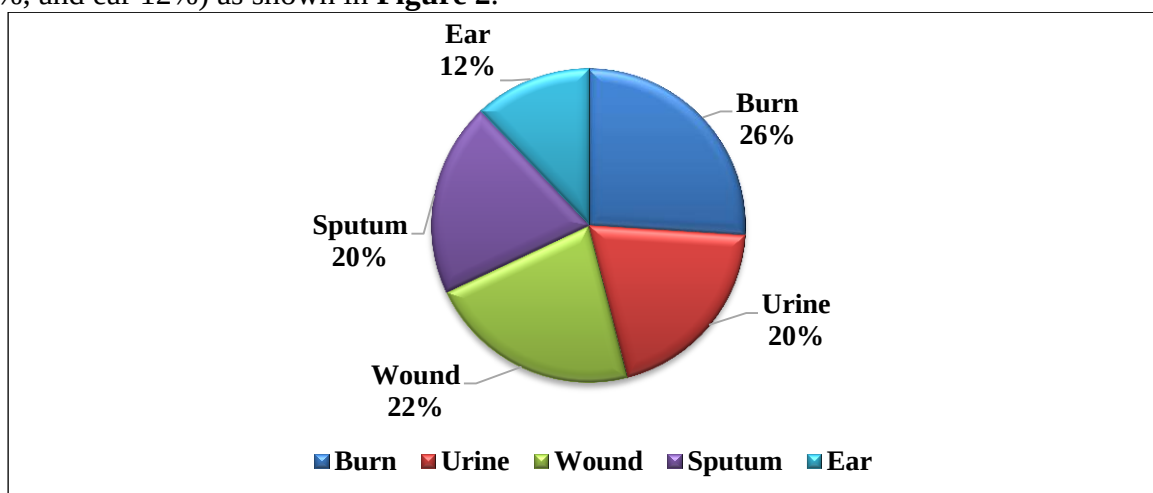


Figure 2. Prevalence of *P. aeruginosa* in different clinical specimen

3.2. Antibiotic Sensitivity

The antimicrobial susceptibility of fifty *Pseudomonas aeruginosa* isolates was evaluated using the VITEK2 compact system (GN222 kit). Among these, 19 isolates (38%) were identified as multidrug-resistant (MDR), although the association was not statistically significant ($p = 0.1573$). Resistance profiles against thirteen antibiotics were determined, as presented in **Figure 3**. The highest resistance was observed to colistin (84%), followed by imipenem (76%). Moderate resistance rates were recorded for piperacillin (52%) and ticarcillin (44%), while ciprofloxacin (32%) and tobramycin (30%) demonstrated comparatively lower resistance. Both ceftazidime and

cefepime exhibited equal resistance levels of 20%. Reduced resistance was noted for meropenem (16%), piperacillin/tazobactam (12%), and gentamicin (10%). The lowest resistance was detected against ticarcillin/clavulanic acid (8%) and amikacin (4%). Collectively, these findings underscore the substantial variability in antibiotic resistance among clinical isolates of *P. aeruginosa*, highlighting its continued threat in nosocomial infections.

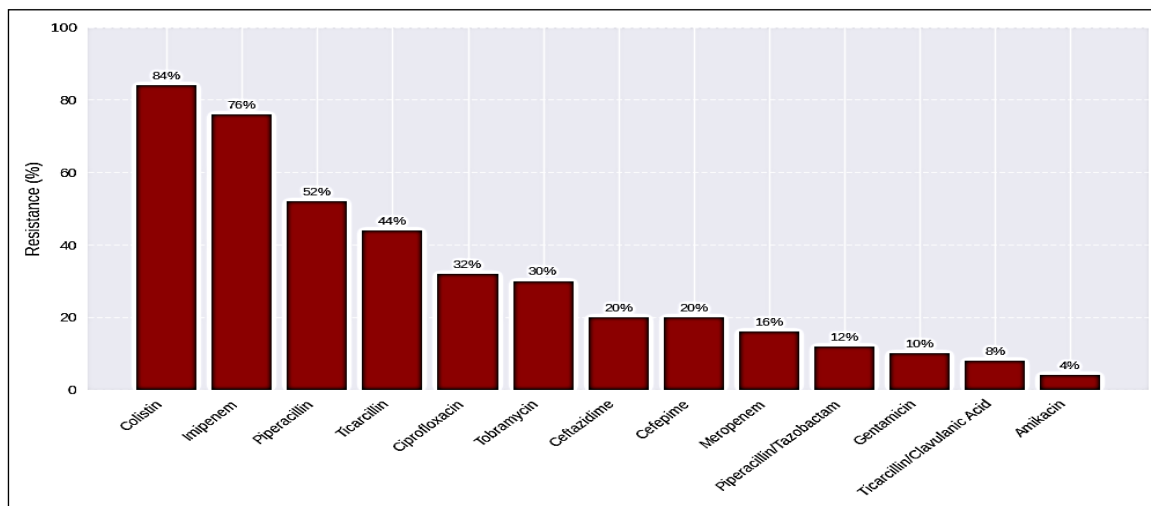


Figure 3. Resistance Rates of *Pseudomonas aeruginosa* to Selected Antibiotics

3.3. Molecular Detection of *pqsA*, *pqsB*, *pqsC* and *pqsD* Genes:

Among the 19 multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates, the *pqsA* gene was detected in 17 isolates (89.5%), the *pqsB* gene in 17 isolates (89.5%), the *pqsC* gene in 18 isolates (94.7%), and the *pqsD* gene in 14 isolates (73.7%). Statistical analysis revealed highly significant differences ($p \leq 0.01$), as illustrated in **Figures 4–7**.

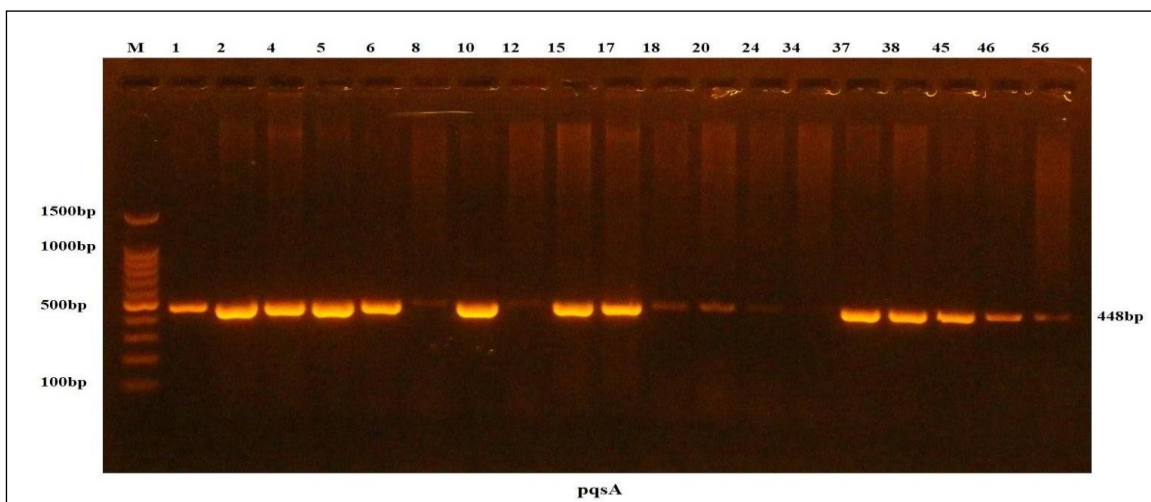


Figure 4. Results of the amplification of *pqA* of *Pseudomonas aeruginosa* were fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide at 100 volt/Amp for 75 min, M: 100bp ladder marker. Lanes 1,2,4,5,6,8,10,12,15,17,18,20,24,34,37,38,45,46and 56 resemble 448bp PCR product.

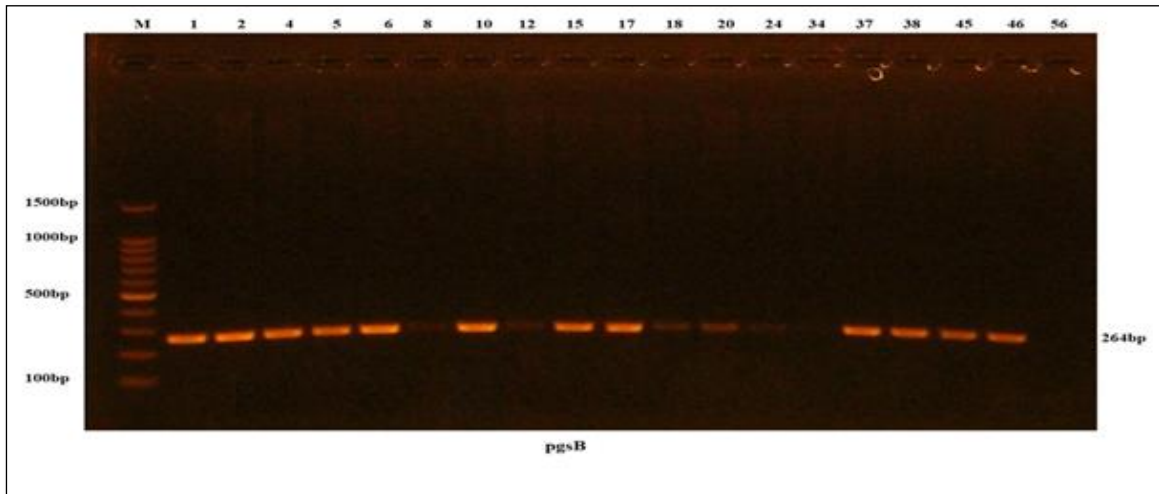


Figure 5. Results of the amplification of *pqB* of *Pseudomonas aeruginosa* were fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide at 100 volt/Amp for 75 min, M: 100bp ladder marker. Lanes 1,2,4,5,6,8,10,12,15,17,18,20,24,34,37,38,45,46and 56 resemble **264bp** PCR product

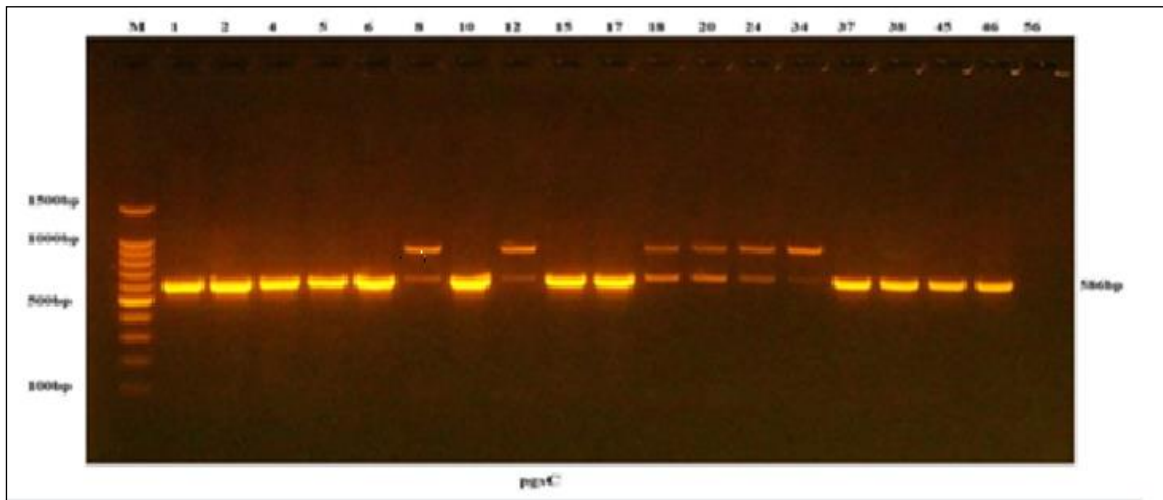


Figure 6 . Results of the amplification of *pgsC* gene of *Pseudomonas aeruginosa* samples species were fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide at 100 volt/Amp for 75 min, M: 100bp ladder marker. Lanes 1,2,4,5,6,8,10,12,15,17,18,20,24,34,37,38,45,46and 56 resemble 568 bp PCR products.

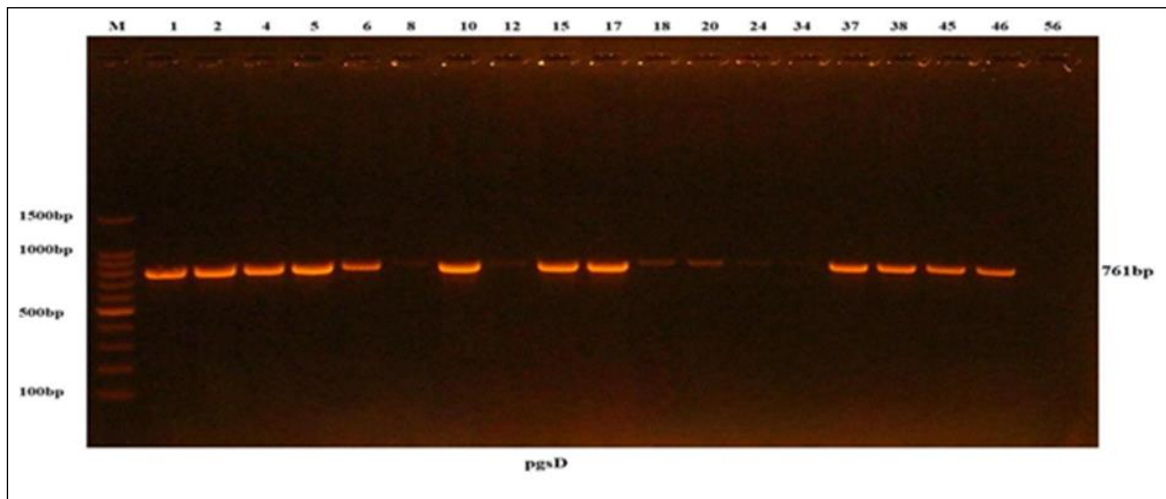


Figure7. Results of the amplification of *pgsD* gene of *Pseudomonas aeruginosa* samples species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1,2,4,5,6,8,10,12,15,17,18,20,24,34,37,38,45,46and 56 resemble 761bp PCR product

3.4 Relationship of Presence of *pqsA*, *pqsB*, *pqsC* and *pqsD* and Resistance to Antibiotics in MDR *P. aeruginosa*:

Recent studies have revealed that the presence of these genes makes bacteria more resistant to antibiotics and the isolates that are sensitive to many antibiotics don't have these genes such as, *pqsA*, *pqsB*, *pqsC* and *pqsD*. The results shown in **Table 4** refer to the relation between antibiotic resistance and the presence of *pqsA*, *pqsB*, *pqsC* and *pqsD* genes ($P \leq 0.01$). These results, which were highly significant, confirmed the importance of *pqsA*, *pqsB*, *pqsC* and *pqsD* genes in resistance to antibiotics which increases in bacteria with the above mentioned four genes.

Table 4: Relationships of *pqs* Genes and Antibiotic Resistance (R/I/S Profile) in MDR *P. aeruginosa*

Antibiotics	<i>pqsA</i> (+/-)	<i>pqsB</i> (+/-)	<i>pqsC</i> (+/-)	<i>pqsD</i> (+/-)	P-value
Ticarcillin	R: 14/2	R: 14/2	R: 15/1	R: 11/5	0.001**
Tic/Clav	S: 13/2	S: 13/2	S: 14/1	S: 10/5	0.001**
Piperacillin	R: 15/2	R: 15/2	R: 16/1	R: 12/5	0.001**
Pip/Tazo	S: 11/2	S: 11/2	S: 12/1	S: 9/4	0.001**
Ceftazidime	R: 7/0	R: 7/0	R: 7/0	R: 6/1	0.001**
Cefepime	R: 6/1	R: 7/0	R: 7/0	R: 5/2	0.001**
Imipenem	I: 10/0	I: 10/0	I: 10/0	S: 10/0	0.001**
Meropenem	I: 6/0	I: 6/0	S: 6/1	R: 6/0	0.001**
Amikacin	S: 15/2	S: 15/2	S: 16/1	S: 12/5	0.001**
Gentamicin	I: 10/2	I: 10/2	I: 11/1	I: 7/5	0.001**
Tobramycin	R: 13/2	R: 13/2	R: 14/1	R: 10/5	0.001**
Ciprofloxacin	R: 14/2	R: 14/2	R: 15/1	R: 11/5	0.001**
Colistin	R: 16/2	R: 16/2	R: 17/1	R: 13/5	0.001**

- (+/-): Gene Positive / Gene Negative.
- R/I/S: The dominant clinical category (Resistant, Intermediate, or Sensitive)
- **: Statistically significant ($P \leq 0.01$)

4. Discussion

An extensive array of innate, adaptive, and acquired resistance mechanisms against contemporary antimicrobial agents is exhibited by *Pseudomonas aeruginosa*. The development of multidrug resistance is frequently accelerated by the synergistic application of these drugs, which significantly contributes to therapeutic failures in both clinical and hospital settings²⁵⁻²⁸. The present study found that common drugs were becoming increasingly resistant as a result of the excessive use of antibiotics against bacteria. The current findings indicate the presence of MDR *P. aeruginosa*, which is in line with the results of²⁹, which showed *P. aeruginosa*'s multidrug resistance to a wide range of antibiotics consistent with previous reports³⁰⁻³². The isolates of *P. aeruginosa* in this study exhibited various levels of resistance to a variety of antibiotics, including (Ticarcillin, Ticarcillin/clavulanic acid, Piperacillin, Piperacillin/Tazobactam, Ceftazidime, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, and Colistin). This finding is consistent with the reported high resistance of *P. aeruginosa* to Colistin (84%) and Imipenem (76%), which is aligned with recent global data. *P. aeruginosa* has acquired an alarming resistance to last-resort antibiotics such as Colistin and carbapenems as a result of mechanisms such as biofilm formation, efflux pumps, and horizontal gene transfer, as per a 2025 review published in Microorganisms³³. Multidrug-resistant strains are particularly prevalent in ICU settings, which is why this trend is particularly concerned. Furthermore, the moderate resistance levels to Ciprofloxacin (32%) and Tobramycin (30%) are in agreement with surveillance studies that indicate the decreasing effectiveness of fluoroquinolones and aminoglycosides as a result of overuse and adaptive resistance mechanisms³⁴. Therefore, this current result disagreed with the Finding low resistance to Amikacin (4%) and Ticarcillin/clavulanic acid (8%) may not reflect broader global trends²⁹. The fact that aminoglycoside-modifying enzymes are prevalent in hospital-acquired infections indicates that amikacin resistance is increasing in many areas. Similar to this, β -lactamase production

frequently reduces the effectiveness of ticarcillin/clavulanic acid, and testing methods and local antibiotic stewardship may result in underestimated resistance rates. In addition, in environments where extended-spectrum β -lactamase (ESBL)-producing strains are highly prevalent, the comparatively low resistance to piperacillin/tazobactam (12%) may not hold true. The same study warns that β -lactam/ β -lactamase inhibitor combinations are becoming less effective worldwide³³.

The present study demonstrates that the PQS quorum sensing system, particularly the genes *pqsA*, *pqsB*, *pqsC*, and *pqsD*, plays a pivotal role in mediating resistance to the antibiotics investigated in this work³⁵. This finding underscores the critical importance of quorum sensing in regulating both antibiotic resistance and virulence, especially given the widespread prevalence of multidrug-resistant (MDR) *Pseudomonas aeruginosa*. The results revealed that nearly all MDR *P. aeruginosa* isolates resistant to the studied antibiotic classes harbored one or more of the *pqs* genes, which are known to regulate biofilm formation, virulence factor production, and efflux pump expression³⁶. These results indicate that targeting the *pqs* system may be a promising approach to improve antibiotic efficacy and reduce resistance. *P. aeruginosa* has become known for its high drug resistance. In fact, *P. aeruginosa* makes the majority of antimicrobials ineffective, leaving polymyxins, quinolones, and aminoglycosides to the role of last resort drugs. Despite the identification of numerous resistance mechanisms to these medications, several studies have identified elements and methods for the development of new approaches to enhance their efficacy against antibiotics.

PCR analysis confirmed the detection of all four target genes (*pqsA*, *pqsB*, *pqsC*, and *pqsD*) among the multidrug-resistant *Pseudomonas aeruginosa* isolates, though with variable frequencies. The *pqsA* and *pqsB* genes were identified in 89.5% of isolates, *pqsC* in 94.7%, and *pqsD* in 73.7%. These results demonstrate that each gene was present separately across the specimens, indicating their broad distribution among MDR strains.

The high prevalence of these quorum-sensing-associated genes suggests that the PQS system is highly conserved and may play a pivotal role in the pathogenicity and persistence of *P. aeruginosa*. The statistically significant differences observed ($p \leq 0.01$) imply that, although all genes are represented, their relative frequencies vary, potentially reflecting differential regulation or selective adaptation under antimicrobial pressure.

Collectively, these findings underscore that the PQS system is not only widespread but also likely essential for the survival and virulence of MDR *P. aeruginosa*, thereby reinforcing its relevance as a promising therapeutic target in the management of resistant infections.

5. Conclusion

P. aeruginosa possesses a wide range of innate, adaptive resistance mechanisms to the antibiotics. One of these mechanisms is *pqs* system. For that, these bacteria were resistant to almost all antibiotics used in this study. MDR of *P. aeruginosa* harbored *pqs* genes such as *pqsA*, *pqsB*, *pqsC* and *pqsD*. The study revealed a statistically significant association ($p \leq 0.01$) between the presence of specific genes and antibiotic resistance in multidrug-resistant *P. aeruginosa*. This indicates that isolates harboring these genes tend to exhibit heightened resistance to antimicrobial agents. These genetic factors play a crucial role in the resistance mechanisms of MDR *P. aeruginosa*. As a result, the growing resistance of this pathogen directly contributes to increased mortality rates.

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Conflict of Interest

Conflict of Interest: The authors declare that they have no conflicts of interest.

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Ethical Clearance

The study was conducted following the receipt of participant consent and ethical approval from the Ethics Committee of the Biology Department at the University of Baghdad's College of Science (Ref.CSEC/0125/0009) on January 20, 2025. This procedure is in line with the guidelines set forth by the Iraqi Ministry of Health and Environment.

References

1. Arami N, Tajani AS, Hashemi M, Rezaei T, Ghodsi R, Soheili V, Fazly Bazzaz BS. Targeted inhibition of *PqsR* in *Pseudomonas aeruginosa* PAO1 quorum-sensing network by chalcones as promising antibacterial compounds. *Mol Biol Rep.* 2025;52(1):175. <https://doi.org/10.1007/s11033-025-10270-7>
2. Ramesh R, Rekha ND, Gopal S. *Pseudomonas aeruginosa* biofilm: treatment strategies to combat infection. *Arch Microbiol.* 2025;207(141). <https://doi.org/10.1007/s00203-025-04346-8>
3. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage *Pseudomonas aeruginosa* infections. *Drugs Context.* 2018;29(7):212527. <https://doi.org/10.7573/dic.212527>
4. Nasser AS, Hussein RH, Jameel YM. Type 3 secretion system virulotypes in clinical isolates of multidrug resistant *Pseudomonas aeruginosa*. *Diyala J Med.* 2025;28(2). <https://doi.org/10.26505/djm.v28i2.1367>
5. Fatima N, Amin A, Hussain MB, Al-Sameeai NAD, Raza M. Molecular profiling of multidrug-resistant *Pseudomonas aeruginosa* isolated from health care settings for targeted antibiotic development. *Afr J Biol Sci.* 2025;7(10):112-131. <https://doi.org/10.48047/AFJBS.7.10.2025.112-131>
6. Rampioni G, Falcone M, Heeb S, Frangipani E, Fletcher MP, Dubern JF, Diggle SP, Leoni L, Williams P. Unravelling the genome-wide contributions of specific 2-alkyl-4-quinolones and *PqsE* to quorum sensing in *Pseudomonas aeruginosa*. *PLoS Pathog.* 2016;12(11):e1006029. <https://doi.org/10.1371/journal.ppat.1006029>
7. Jia T, Bi X, Li M, Zhang C, Ren A, Li S, Wang Y, Liu H, Chen J, Zhao Q. Hfq-binding small RNA *PqsS* regulates *Pseudomonas aeruginosa* pqs quorum sensing system and virulence. *NPJ Biofilms Microbiomes.* 2024;10(1):82. <https://doi.org/10.1038/s41522-024-00550-4>
8. Tchadi BV, Derringer JJ, Detweiler AK, Taylor IR. *PqsE* adapts the activity of the *Pseudomonas aeruginosa* quorum-sensing transcription factor RhlR to both autoinducer concentration and promoter sequence identity. *J Bacteriol.* 2025;207(5):e00516-24. <https://doi.org/10.1128/jb.00516-24>
9. Aziz M, Ahmed K, Ravichandran V. *PqsR*-specific quorum sensing inhibitors targeting *Pseudomonas aeruginosa*: current advances and future directions. *J Chemother.* 2025;:1-18. <https://doi.org/10.1080/1120009x.2025.2510093>
10. Coleman JP, Hudson LL, McKnight SL, Farrow JM, Calfee MW, Lindsey CA, Pesci EC. *Pseudomonas aeruginosa* *PqsA* is an anthranilate-coenzyme A ligase. *J Bacteriol.* 2008;190(4):1247-55. <https://doi.org/10.1128/JB.01368-07>
11. Sahu A, Ruhel R. Immune system dynamics in response to *Pseudomonas aeruginosa* biofilms. *NPJ Biofilms Microbiomes.* 2025;11:738. <https://doi.org/10.1038/s41522-025-00738>
12. Vidal-Cortés P, Campos-Fernández S, Cuenca-Fito E, del Río-Carbajo L, Fernández-Ugidos P, López-Ciudad VJ, Garnacho-Montero J. Difficult-to-treat *Pseudomonas aeruginosa* infections in critically ill patients: a comprehensive review and treatment proposal. *Antibiotics.* 2025;14(2):178. <https://doi.org/10.3390/antibiotics14020178>
13. Ahmed YK, Hamadailla MJ. The burden of *Pseudomonas aeruginosa* infections in Iraq: a review. *Microbes Infect Dis.* 2025. <https://doi.org/10.21608/mid.2025.407682.3054>
14. Al Mathkhury H. In vivo lung injury caused by *Pseudomonas aeruginosa* DNA. *Iraqi J Sci.* 2012;53(4):794-8. <https://doi.org/10.25196/ij.s.2012.53.4.14>
15. AL-Mojamaee NAH, ALtaii HAJ. Comparison of two methods for the detection of *Pseudomonas aeruginosa* biofilm formation isolated from different clinical samples. *Iraqi J Humanit Soc Sci Res.* 2023;11:651–68. <https://doi.org/10.36327/ijhssr.v11i2.290357>

16. Ismaieel MS, Hassan MH, Flayyih MT. Cytotoxic activity of partially purified ExoT toxin produced by *Pseudomonas aeruginosa* against MCF-7 and PC3 cancer cell lines. Egypt J Med Microbiol. 2025;34(2):435-8. <https://doi.org/10.21608/ejmm.2025.372841.1550>
17. Mais E. Ahmed, Ali H. Alhammer, Rasha K. Mohammed, Fadhel M. Lafta. Synergistic effects of neem oil and gentamicin on *Pseudomonas aeruginosa* via *phzM* gene downregulation: a comprehensive review. J Microbiol Biotech Food Sci. 2024; 14 (2) e11095. <https://doi.org/10.55251/jmbfs.11095>
18. Cavalieri SJ, Rankin ID, Harbeck RJ, McCarter YS, Sharp SE, Ortez JH, Carroll KC. Effect of antimicrobial stewardship with rapid MALDI-TOF identification and VITEK® 2 antimicrobial susceptibility testing on hospitalization outcome. Diagn Microbiol Infect Dis. 2019;92(2):208-11. <https://doi.org/10.1016/j.diagmicrobio.2019.07.010>
19. Khan M, Al-Mutairi T, Rahman S. Evaluation of multidrug-resistant *Pseudomonas aeruginosa* isolates using VITEK 2 Compact AST-GN system in clinical microbiology laboratories. J Glob Antimicrob Resist. 2025; 33:45-53. <https://doi.org/10.1016/j.jgar.2025.01.00626>
20. Ali AA, Sweedan EG. Role of *higB-higA* novel genes in antibiotic resistance of *Pseudomonas aeruginosa*. Int J Drug Deliv Technol. 2023;13(2):605-609. <https://doi.org/10.25258/ijddt.13.2.22>
21. Clinical and Laboratory Standards Institute (CLSI) 35th ed. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Wayne (PA): Clinical and laboratory standards institute; 2025. <https://clsi.org/shop/standards/m100-ed35>
22. Bahrami N, Mohammadi M, Sabzi N, Ghanei M. The correlation between quorum sensing genes (*pqsR* and *lasR*) in antibiotic resistance of *Pseudomonas aeruginosa* isolated from burned patients. Arch Med Lab Sci. 2020;4(4). <https://doi.org/10.22037/amls.v4i4.28281>
23. Kabir RB, Ahsan T, Rahman MF, Jobayer M, Shamsuzzaman SM. Biofilm-producing and specific antibiotic resistance genes in *Pseudomonas aeruginosa* isolated from patients admitted to a tertiary care hospital, Bangladesh. IJID Reg. 2024;11:100369. <https://doi.org/10.1016/j.ijregi.2024.100369>
24. Faisal AJ, Ali MR, Said LA. Co-existence of *lasI*, *rhI*, and *Pseudomonas* quinolone signal quorum-sensing genes in clinical *Pseudomonas aeruginosa* isolates. Int J Drug Deliv Technol. 2020;10:338–43. <https://doi.org/10.25258/ijddt.10.3.15>
25. Zhao Y, Xu H, Wang H, Wang P, Chen S. Multidrug resistance in *Pseudomonas aeruginosa*: genetic control mechanisms and therapeutic advances. Mol Biomed. 2024;5:62. <http://doi:10.1186/s43556-024-00221-y>
26. Jones HA, Baxter MJ, Zimmermann N, Li A, Perrault Uptmor KA, Taylor IR. Screening of FDA-approved small molecules to discover inhibitors of the *Pseudomonas aeruginosa* quorum-sensing enzyme, *PqsE*. bioRxiv. 2025. <https://doi.org/10.1101/2025.07.25.666791>
27. Schwartz B, Klammer K, Zimmerman J, Kale-Pradhan PB, Bhargava A. Multidrug-resistant *Pseudomonas aeruginosa* in clinical settings: a review of resistance mechanisms and treatment strategies. Pathogens. 2025;13(11):975. <https://doi.org/10.3390/pathogens13110975>
28. Shahreen N, Shahid SA, Subhani M, Al-Siyabi A, Saha R. Minimal gene signatures enable high-accuracy prediction of antibiotic resistance in *Pseudomonas aeruginosa*. NPJ Syst Biol Appl. 2025;11(1):108. <https://doi.org/10.1038/s41540-024-00352-4>
29. Zhang X, Li S, Zhang D, Zheng S, Zhou D, Hou Q, Li G, Han H. A comprehensive review of the pathogenic mechanisms of *Pseudomonas aeruginosa*: synergistic effects of virulence factors, quorum sensing, and biofilm formation. Front Microbiol. 2025;16:1619626. <https://doi.org/10.3389/fmicb.2025.1619626>
30. Dunphy LJ, Yen P, Papin JA. Integrated experimental and computational analyses reveal differential metabolic functionality in antibiotic-resistant *Pseudomonas aeruginosa*. Cell Syst. 2019;8(1):3–14. <https://doi.org/10.1016/j.cels.2018.12.002>
31. Jayaraman M, Gosu V, Kumar R, Jeyaraman J, Lee HK, Shin D. Exploring marine natural products as potential quorum sensing inhibitors by targeting the *PqsR* in *Pseudomonas aeruginosa*: virtual screening assisted structural dynamics study. PLoS One. 2025;20(3):e0319352. <https://doi.org/10.1371/journal.pone.0319352>
32. Sadoq BE, Boulaamane Y, Touati I, Britel MR, Maurady A. Virtual screening and identification of natural molecules as promising quorum sensing inhibitors against *Pseudomonas aeruginosa*. Nano Biomed Eng Lett. 2025;14(2):90. <http://doi:10.33263/LIANBS142.090>

- 33.Sweedan EG. The antimicrobial effects of alcoholic leaves extract of *Salvia officinalis* against multidrug resistant *Pseudomonas aeruginosa*. Iraqi J Sci. 2021;62(2):441–8. <https://doi.org/10.24996/ij.s.2021.62.2.9>
- 34.Hu M, Chua SL. Antibiotic-resistant *Pseudomonas aeruginosa*: current challenges and emerging alternative therapies. Microorganisms. 2025;13(4):913. <https://doi.org/10.3390/microorganisms13040913>
- 35.Ramatla T, Nkhebenyane J, Lekota KE, Thekisoe O, Monyama M, Achilonu CC, Oladipo OO, Adewumi TS. Global prevalence and antibiotic resistance profiles of carbapenem-resistant *Pseudomonas aeruginosa* reported from 2014 to 2024: a systematic review and meta-analysis. Front Microbiol. 2025;16:1599070. <https://doi.org/10.3389/fmicb.2025.1599070>
- 36.Hemmati J, Nazari M, Abolhasani FS, Ahmadi A, Asghari B. In vitro investigation of relationship between quorum-sensing system genes, biofilm forming ability, and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. BMC Microbiol. 2024;24(1):99. <http://doi:10.1186/s12866-024-03249-w>