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PvcABCD Operon Genetic Diversity in Different Clinical P. aeruginosa Isolates

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Abstract

Introduction. Pseudomonas aeruginosa is an opportunistic gram-negative microorganism that can cause infections requiring medical attention and is characterized by pronounced resistance to antibiotics.
Purpose. To evaluate genetic diversity with the gene expression of the pvcABCD operon in clinical P. aeruginosa isolates obtained in Baghdad, Iraq.
Materials and methods. A total of 137 clinical samples were collected from various sources, from which (26) P. aeruginosa isolates were confirmed by PCR targeting the 16S rRNA and gyrB genes. Expression of pvcA – pvcD was assessed by RT-qPCR using rpoD as a reference gene and the $2^{-\Delta\Delta Ct}$ method. Genetic relatedness and sequence variation were evaluated using ERIC-PCR and Sanger sequencing of pvcA and pvcC.
Results. This study demonstrates that clinical P. aeruginosa isolates exhibit differential regulation of the pvcABCD operon, characterized by down-regulation of pvcA and up-regulation of pvcB, pvcC, and pvcD. The limited silent and missense mutations identified in pvcA and pvcC suggest that bacterial adaptation relies primarily on compensatory regulatory mechanisms that fine – tune gene expression under clinical stress conditions. In parallel, the observed genetic diversity and clustering of multidrug-resistant isolates, particularly from burn and diabetic foot ulcer sources, support the potential for hospital-associated transmission of adapted strains and highlight their epidemiological significance.
Conclusion. Clinical P. aeruginosa isolates showed differential regulation of the pvcABCD operon, with down-regulation of pvcA and up-regulation of pvcB, pvcC, and pvcD. ERIC-PCR revealed genetic diversity with clustering of burn and diabetic foot ulcer isolates sharing similar resistance profiles, while pvcA and pvcC sequencing indicated high conservation with limited mutations linked to increased gene expression.
Keywords: Pseudomonas aeruginosa, pvc genes, Sequencing, Antibiotic susceptibility, RT-qPCR, ERIC-PCR

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Генетическое разнообразие оперона PvcABCD в различных клинических изолятах P. aeruginosa

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Резюме

Введение. Pseudomonas aeruginosa – условно-патогенный грамотрицательный микроорганизм, способный вызвать инфекции, требующие оказания медицинской помощи и отличающийся выраженной устойчивостью к антибиотикам.
Цель. Провести анализ генетического разнообразия по экспрессии гена оперона pvcABCD в клинических изолятах P. aeruginosa, полученных в Багдаде, Ирак.
Материалы и методы. В общей сложности было отобрано 137 клинических образцов из различных источников, из которых в 26 изолятах было подтверждено наличие P. aeruginosa методом ПЦР с использованием генов 16S рРНК и gyrB в качестве мишеней. Экспрессию pvcA – pvcD оценивали методом ПЦР в реальном времени с использованием rpoD в качестве референсного гена, а также методом 2–ΔΔCt. Генетическое родство и вариабельность последовательностей оценивали с помощью повторяющейся межгенной консенсусной ПЦР энтеробактерий и секвенирования по Сэнгеру генов pvcA и pvcC.
Результаты. Согласно полученным данным, клинические изоляты P. aeruginosa проявляют дифференциальную регуляцию оперона pvcABCD, характеризующуюся снижением экспрессии гена pvcA и повышением экспрессии генов pvcB, pvcC и pvcD. Ограниченное количество молчащих и миссенс-мутаций, выявленных в pvcA и pvcC, предполагает, что адаптация бактерий базируется в основном на компенсаторных регуляторных механизмах, которые «тонко» настраивают экспрессию генов в условиях клинического стресса. Параллельно наблюдаемое генетическое разнообразие и кластеризация мультирезистентных изолятов, особенно из ожоговых ран и диабетических язв стопы, подтверждают потенциальную возможность внутрибольничной передачи адаптированных штаммов и подчеркивают их эпидемиологическое значение.
Заключение. Исследование продемонстрировало дифференциальную регуляцию оперона pvcABCD в клинических изолятах P. aeruginosa с понижением экспрессии гена pvcA и повышением экспрессии генов pvcB, pvcC и pvcD. Повторяющаяся

межгенная консенсусная ПЦР энтеробактерий выявила генетическое разнообразие с кластеризацией в изолятах, полученных из ожоговых ран и диабетических язв стопы, имеющих схожие профили резистентности. В то же время секвенирование pvcA и pvcC показало высокую консервативность с ограниченным количеством мутаций, связанных с повышенной экспрессией генов.
Ключевые слова: Pseudomonas aeruginosa, гены pvc, секвенирование, чувствительность к антибиотикам, КПЦР в реальном времени (RT-qPCR), повторяющаяся межгенная консенсусная ПЦР энтеробактерий (ERIC-PCR)

■ INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, rod shaped bacterium belonging to the family Pseudomonadaceae and is recognized as an opportunistic human pathogen capable of causing a wide spectrum of infections involving the bloodstream, skin, respiratory tract, and urinary tract [1, 2]. These infections may be acute or chronic and occur predominantly in immunocompromised patients, posing a major clinical challenge due to the organism's pronounced antibiotic resistance and virulence potential [3]. The pathogenicity of P. aeruginosa is mediated by numerous virulence factors, including lipopolysaccharide, outer membrane proteins, flagella, pili, and exopolysaccharides, which facilitate adhesion, immune evasion, and biofilm formation. In addition, the bacterium secretes a variety of effectors through specialized secretion systems [4, 5]. Biofilm formation represents a key pathogenic strategy, as it enhances bacterial adherence and confers protection against antimicrobial agents and host immune responses, thereby contributing to chronic and difficult to treat infections [6, 7] Moreover, P. aeruginosa exhibits high genomic plasticity that enables rapid adaptation to diverse clinical environments and favors the emergence of high risk antibiotic-resistant clones, a capacity supported by its large genome of approximately 6.3 Mb [8]. Operons represent a major gene regulatory mechanism in prokaryotes, allowing coordinated gene expression and efficient metabolic adaptation, with nearly half of bacterial protein coding genes organized in multigene operons [9]. The pvcABCD operon in P. aeruginosa consists of four genes clustered between 2,482,045 and 2,486,118 bp on the (PAO1) chromosome and was initially associated with pyoverdine chromophore biosynthesis [10–12]. Later studies demonstrated that this operon has broader metabolic roles, as PvcA and PvcB catalyze key reactions leading to the production of the secondary metabolite paerucumarin, with PvcC and PvcD completing the biosynthetic pathway [13, 14]. The instability of isonitrile containing metabolites and variability among pvc clusters explain earlier difficulties in detecting paerucumarin [13], operon expression is negatively regulated by iron and positively controlled by PvdS and the transcriptional regulator PtxR [10]. Furthermore, local gene expression studies revealed a strong association between pvcABCD expression and biofilm formation, where pvcA and pvcB are up-regulated in biofilm producing isolates, whereas pvcC and pvcD are down-regulated, supporting both enhanced adhesion and the presence of two distinct operons within this gene cluster [11, 15].

■ PURPOSE OF THE STUDY

To evaluate genetic diversity with the gene expression of the pvcABCD operon in clinical P. aeruginosa isolates obtained in Baghdad, Iraq.

■ MATERIALS AND METHODS

Sample Collection

A total of 137 clinical samples were collected from various age groups and sexes, including (48 burn swabs, 40 wound swabs, 22 diabetic foot ulcers, and 27 urine) samples, between November 2024 and February 2025.

Bacterial Isolates Identification

Molecular identification of the bacterial isolates was performed using the 16S rRNA and gyrB genes, which were considered reliable diagnostic genes for confirming the identity of P. aeruginosa. Out of 137 clinical swabs collected, PCR results confirmed 26 isolates as belonging to P. aeruginosa, genomic DNA was extracted by using the EasyPure® Bacteria Genomic DNA Kit (TransGen Biotech, China), according to the manufacturer's instructions. The bacterial cultures were grown overnight, harvested by centrifugation, and the resulting pellets were processed for DNA purification and elution as described in the kit protocol.

Conventional PCR amplification was carried out using specific primers targeting the 16S rRNA and gyrB genes gene in the table 1. Each 25 µL reaction mixture contained 12.5 µL of 2× EasyTaq® PCR SuperMix, 1 µL of each primer (10 pmol), 3 µL of DNA template, and 7.5 µL of nuclease-free water. PCR cycling program was optimized as follows: an initial denaturation at 94 °C for 1–9 minutes, followed by 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 63 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was carried out at 72 °C for 5–15 minutes to ensure complete elongation of all amplicons. PCR products were subsequently separated by agarose gel electrophoresis and visualized to confirm the presence of the expected amplification bands for the 16S rRNA and gyrB genes.

Checking for Antibiotic Resistance

We used the Kirby – Bauer disc diffusion method to ascertain if they were resistant to antibiotics [16]. say that this was done in line with the rules specified by the Clinical and Laboratory Standards Institute [17]. We collected some colonies from a culture that had been growing overnight and put them in 2 cc of saline solution. This lowered the concentration to the 0.5 McFarland standard. A sterile cotton swab was used to evenly spread the bacterial suspension across the surfaces of all the Mueller – Hinton agar plates in three directions (about 60° each time) to make sure that all of them were infected. The dishes were left out at room temperature for 30 minutes to soak up any extra moisture. After that, sterile forceps were used to put antibiotic discs on the agar surface. The plates were then stored at 37 °C for 24 hours. We used a ruler to measure the diameter of the inhibitory zones surrounding each disc. Then we put the isolates into three groups: susceptible, intermediate, or resistant.

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Primers Design

The primers used in this study are listed in Table 1. All primers were synthesized in lyophilized form by Macrogen (Korea). Upon arrival, they were spun down and dissolved in DNase- and RNase-free water according to the manufacturer's instructions to prepare a stock solution (100 µM). A working solution (10 µM) was then prepared by diluting 10 µL of the stock solution in 90 µL of nuclease-free water. Both stock and working solutions were gently mixed and stored at –23 °C until use.

RT-qPCR Quantification of Pvc Operon Genes

The expression levels of the pvcA, pvcB, pvcC, and pvcD genes belonging to the pvcABCD operon, along with the housekeeping gene rpoD, were evaluated in P. aeruginosa clinical isolates using real-time quantitative PCR (RT-qPCR). Total RNA was extracted from the bacterial pellets using the TransZol Up Plus RNA Extraction Kit (TransGen Biotech, China) following the manufacturer's recommendations. To avoid genomic DNA contamination, the extracted RNA was treated with DNase enzyme and immediately stored at –20 °C

Table 1
A list of primers used during this study

Primers	Primer sequence (5´-3´)	Annealing, Tm (°C)	Templet length, bp	Reference
Housekeeping primers				
rpoD RNA polymerase sigma factor	F-ACAAGATCCGCAAGGTACTGAA R-ATCGCCCAGGTGCGAAT	60	180	[18]
RT-qPCR primers				
PvcA	F-CCTGTCGCTGTCGTTCCT R-CCTGGTAGGCGCTGATGT	59	143	[11]
PvcB	F-ATTCCCCATCCTGCGTT R-CGTGCAACAGGGTCAGG	57	212	Second author
PvcC	F-AAGGCGATCCACGAGATG R-CGAAGAACACAACAGCGA	56	187	Second author
PvcD	F-CCTCTCGGCCCTGCTT R-TCGGGTAGCTGCGGTTC	58	158	[11]
Detection primers				
16Sr RNA	F-GCACTTTAAGTTGGGAGGAA R-CTTTACGCCCARTRAWTCCG	58	144	[19]
Gyr B	F-CCTGACCATCCGTCGCCACAAC R-CGCAGCAGGATGCCGACGCC	66	220	[20]
Sequencing primers				
PvcA	F-AGCGCATCCAGCTGTTCTAT R-ATCATGTGGATGCCGAACTT	56	978	Second author
PvcC	F-GTCGCCAGGTCTACCTCAAC R-AGCGGATAGTCGAAGGGACT	58	1499	Second author
ERIC-PCR primers				
ERIC	F-CACTTAGGGGTCCTCGAATGTA R-AAGTAAGTGACTGGGGTGAGCG	60	Variable	[21]

until use. The purified RNA was then reverse-transcribed into complementary DNA (cDNA) using the EasyScript® One-Step gDNA Removal and cDNA Synthesis Super Mix Kit (TransGen Biotech, China) according to the manufacturer's protocol. Each RT-qPCR reaction was prepared in a final volume of 20 µL, consisting of 10 µL of TransStart® Top Green qPCR Super Mix, 2 µL of cDNA template, 2 µL of primer mix (1 µL forward + 1 µL reverse), and 6 µL of nuclease-free water.

The thermal profile consisted of an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at gene-specific temperatures (60 °C for pvcA, pvcB, pvcD, and rpoD; 56 °C for pvcC), and extension at 72 °C for 20 s. Each reaction was carried out in triplicate to ensure accuracy. The rpoD gene, encoding the RNA polymerase sigma factor, served as the internal control for normalization of gene expression levels. Relative expression for each target gene was calculated using the comparative ΔΔCt method, based on the mean cycle threshold (Ct) values of the target and reference genes.

Genotyping of Pseudomonas aeruginosa Isolates by ERIC-PCR Technique

Genotyping of P. aeruginosa isolates was performed using ERIC-PCR to assess the genetic relationships among 19 clinical isolates. Amplification was performed using specific ERIC primers under defined amplification conditions. The PCR products were then separated by agarose gel electrophoresis and visualized under UV light. Banding patterns were scored as present or absent and analyzed using (UPGMA clustering) to generate dendrograms describing the genetic relationships among the isolates.

DNA Sequencing

Sanger sequencing was carried out on the PCR-amplified fragments using an ABI3730XL automated DNA sequencer (Macrogen Corporation, Korea). The extracted DNA from 17 P. aeruginosa isolates was subjected to direct sequencing, And the obtained nucleotide sequences were analyzed and compared with reference sequences using BLAST (Basic Local Alignment Search Tool) in the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov).

Analysis of Data

Data analysis involved processing results from Rotor-Gene Q Series Software, including Ct value recording, amplification plots, dissociation curves, and consolidated reports. Gene expression was quantified using the ΔCt method by subtracting the Ct of the housekeeping gene from the target gene for both control and patient samples. The normalized expression ratio was calculated as $2^{\wedge-\Delta\Delta Ct}$. The ΔΔCt method was then used to compare patient and control groups by subtracting the control ΔCt from the patient ΔCt, with relative fold change determined as $2^{\wedge-\Delta\Delta Ct}$, reflecting gene expression changes normalized to the housekeeping gene and relative to controls.

Statistical Analysis

ERIC-PCR data were analyzed using Dice and Jaccard coefficients, and clustered by the UPGMA method to generate dendrograms for genetic relatedness.

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■ RESULTS

Bacterial Isolates and Identification

The presence of 26 bacterial isolates was confirmed by Molecular detection was achieved using the 16S rRNA (144 bp) and gyrB (220 bp) genes, as in Figure 1, 2.

Gene Expression Analysis

Expression levels of pvcABCD operon genes were assessed in (26) clinical isolates of P. aeruginosa using RT-qPCR and analyzed using the Livak method (2^-ΔΔCt), with rpoD serving as the internal reference gene for normalization. According to the standard criterion, genes with a fold change value greater than one (>1) were considered up-regulated, while genes with a fold change value less than one (<1) were considered down-regulated [22]. The pvcABCD operon showed a differential gene expression pattern, with pvcA expression decreasing to (0.49), while pvc B, pvcC, and pvcD

Fig. 1. Amplified 16S rRNA products of P. aeruginosa isolates. Electrophoresed on 1% agarose for 60 min at 70 volt. The product size was 144 bp. M: DNA Ladder (100 bp)

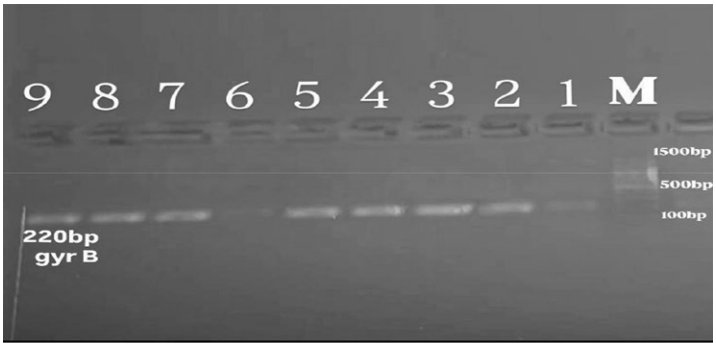

Fig. 2. Amplified Gyr B products of P. aeruginosa. isolates. Electrophoresed on 1% agarose for 60 min at 70 volt. The product size was 220 bp. M: DNA Ladder (100 bp)

Table 2
Fold of pvcABCD expression depending on $2^{\wedge-\Delta\Delta Ct}$ method

Study group	Mean of pvc ct	Mean of ct rpOD	Mean of Δct	Mean ΔΔct	Mean 2^- ΔΔct	Fold of gene expression
PvcA	28.38	23.14	5.24	–1.87	13.71	0.49
PvcB	22.82	23.14	–0.32	0.28	2.42	2.44
PvcC	27.15	23.14	4.01	–0.17	6.10	5.49
PvcD	26.44	23.14	3.30	–0.64	3.18	3.08

expression increased to 2.44, 5.49, and 3.08, respectively, particularly the pvcC and pvcD genes, as shown in Table 2.

ERIC-PCR Genotypes and Antibiotic Resistance Patterns

ERIC-PCR analysis of 19 clinical P. aeruginosa isolates revealed considerable genetic diversity, as indicated by distinct banding patterns on agarose gels Figure 3. Cluster analysis using UPGMA generated a dendrogram showing several genetic clusters at intermediate dissimilarity levels (≈25–30 units), alongside genetically distinct isolates Figure 4. Notably, certain clustered isolates exhibited similar antibiotic resistance profiles. Isolates 2 and 5 clustered at a distance of ≈14 and shared resistance to (TCC, IPM, CAZ), while isolates 20 and 12 clustered at ≈17 and showed multidrug resistance to (TCC, IPM, CAZ, PRL, ATM, AK). Similarly, isolates 17 and 14 clustered at ≈19 with comparable resistance patterns. ERIC-PCR analysis also showed genetic similarity between isolates 8 and 15 despite the difference in clinical source, with similarity in resistance patterns. In contrast, isolates 6 and 11 appeared as separate branches, indicating marked genetic divergence. These results demonstrate a relationship between ERIC-PCR genotypes and antibiotic resistance profiles among the studied isolates Table 3.

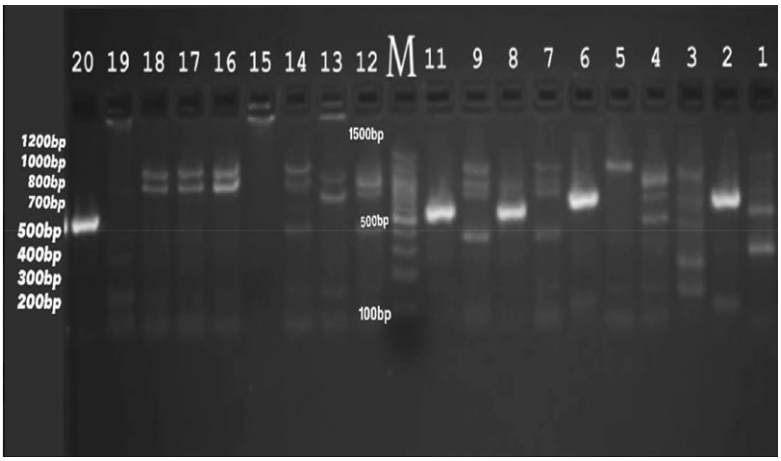

Fig. 3. ERIC-PCR banding patterns of P. aeruginosa isolates showing variability that reflects genetic diversity

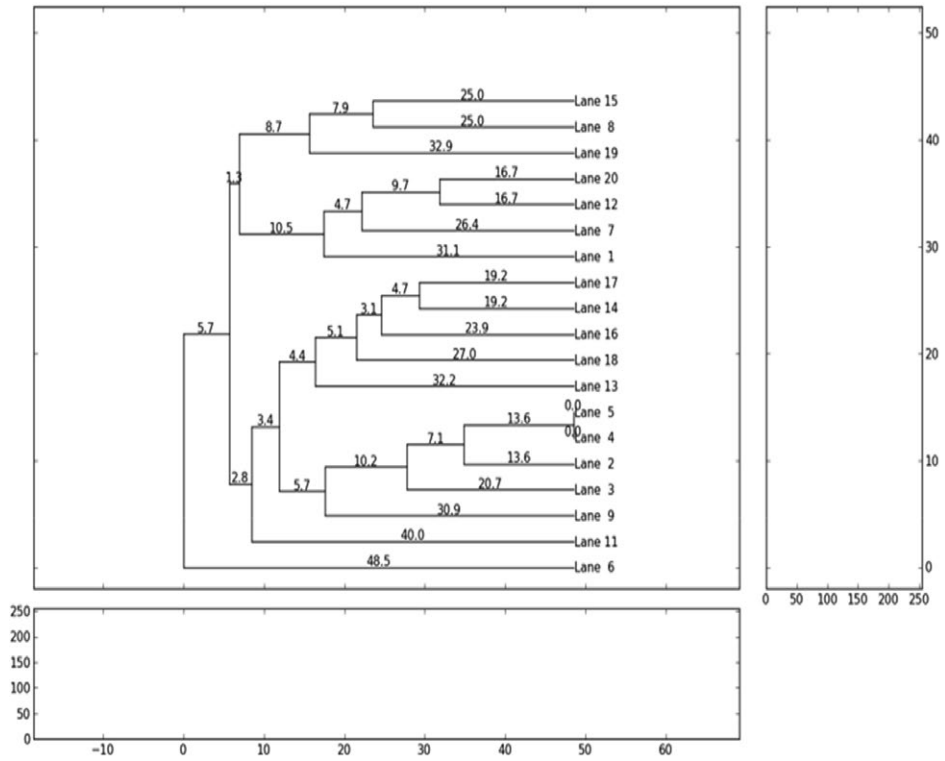

Fig. 4. ERIC-PCR dendrogram of 19 P. aeruginosa isolates

Table 3
Genetic distance, source of isolation, and antibiotic resistance types of P. aeruginosa

No. Of isolate	Genetic Distance (≈)	Source	Antibiotic resistance type
2	≈14	burns	TCC, IPM, CAZ, LEV, PRL, ATM, AK
4		DFU	TCC, IPM, LEV, ATM
5		Burns	TCC, IPM, CAZ
8	≈25	Burns	TCC, IPM, LEV
15		DFU	TCC, IPM, CAZ, LEV, PRL, ATM
12	≈17	Burns	TCC, IPM, CAZ, LEV, PRL, ATM, AK
20		Burns	TCC, IPM, CAZ, PRL, ATM, AK
14	≈20	Burns	TCC, IPM, CAZ, LEV, PRL, ATM, AK
17		Burns	TCC, IPM, CAZ, LEV, ATM, AK

Notes: DFU – Diabetic Foot Ulcer; AK – Amikacin; ATM – Aztreonam; PRL – Piperacillin; CAZ – Ceftazidime; IPM – Imipenem; LEV – Levofloxacin; TCC – Ticarcillin / Clavulanic acid.

DNA Sequencing with Gene Folding for the PvcA and PvcC Genes

This study integrated gene expression data with sequence analysis by selecting the pvc A and pvcC genes. sequence analysis was performed on 13 isolates for pvcA and

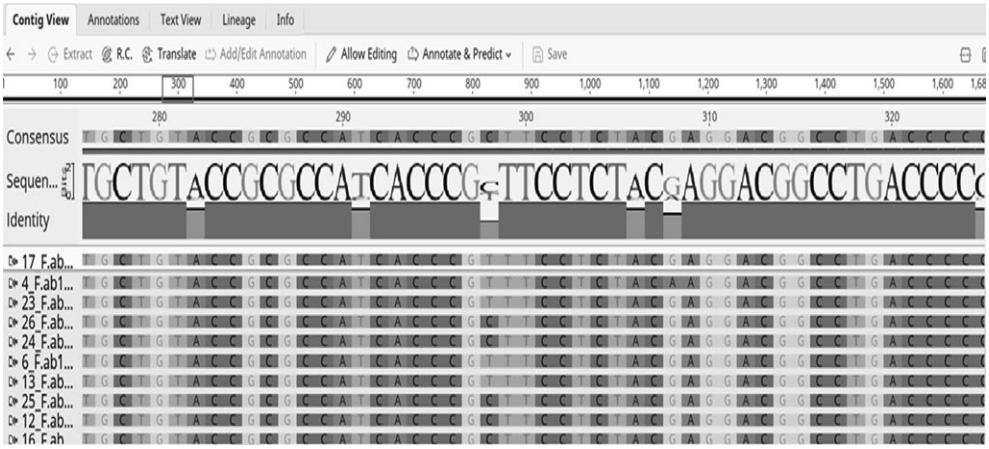

Fig. 5. Alignment of the pvcA gene sequence of P. aeruginosa isolates with the reference strain A31628 (GenBank: CP166030.1) using Geneious Prime software

Fig. 6. Alignment of the pvcC gene sequence of P. aeruginosa isolates with the reference strain by using Geneious Prime software

9 isolates for pvcC. The amplified products were subjected to Sanger sequencing, and the resulting sequences were compared with reference sequences deposited in the (NCBI) GenBank database to assess potential sequence variations in relation to gene expression patterns, as in Figures 5 and 6 and Tables 4 and 5.

■ DISCUSSION

The 16S rRNA gene is considered the gold standard for bacterial identification because it is present in all bacteria and contains conserved and variable regions that enable genus-level discrimination using PCR [23, 24]. A previous study demonstrated the high efficiency of the 16S rRNA gene in identifying P. aeruginosa and distinguishing it from

Table 4
Sequence variation with gene folding for the pvcA gene

No. Of sample	Fold pvcA	Type of substitution	Location	Nucleotide change	Codon change	Amino acid change	Predicted effect
1	21.41	Transversion	2482503	G→C	GCC→CCC	Alanine→Proline	Missense
14	17.88						
12	25.99	Transition	4004780	T→C	CGT→CGC	Arginine→Arginine	Silent
16	19.16						
24	36.76		4004828	T→C	ACT→ACC	Threonine→Threonine	
25	22.94						
26	29.65						
4	17.15						
6	16.22						
7	0.03						
13	11.39						
17	0.04						
23	27.47						

Table 5
Sequence variation with gene folding for the pvcC gene

No. Of sample	fold pvcC	Sequence
2	0.16	
3	0.90	
4	1.00	
5	4.92	
6	43.71	
7	0.24	
9	2.93	
10	1.32	
1	6.11	Mutant

other Pseudomonas species, with most isolates yielding positive results [25, 26]. A local Iraqi study conducted in 2019 reported a high sensitivity of (96%) for the molecular detection of P. aeruginosa bacteria using the 16S rRNA gene [27]. In contrast, the gyrB gene exhibits greater sequence diversity than 16S rRNA, allowing more accurate species – level discrimination, particularly among closely related species [28–30]. Recent studies further confirmed that the combined use of 16S rRNA and gyrB genes provides high reliability for the molecular diagnosis of P. aeruginosa by PCR, as each gene complements the other in precise bacterial identification [31].

The pvcABCD operon is considered one of the major secondary metabolic pathways in P. aeruginosa, as it participates in the biosynthesis of paerucumarin through a sequential series of enzymatic reactions involving PvcA, PvcB, PvcC, and PvcD [13, 32]. Paerucumarin has been reported to play multiple roles associated with bacterial virulence, including enhancement of adhesion via regulation of cup genes, contribution to iron homeostasis, and indirect effects on antibiotic resistance mechanisms [11, 32, 33].

Local study has shown that increased expression of pvcB is associated with enhanced biofilm development and adaptation to clinical environments [15, 34]. Furthermore, suggest that the pvcAB and pvcCD gene clusters may be regulated as independent transcriptional units, which may explain the pronounced expression of pvcC and pvcD observed in clinical isolates [15].

The ERIC-PCR analysis revealed the presence of distinct genetic clusters among P. aeruginosa isolates, where genetic relatedness among certain isolates was accompanied by clear similarities in antibiotic resistance patterns, particularly among burn isolates, indicating the possible dissemination of epidemic strains within the same clinical environment [35]. In addition, clustering of isolates originating from different clinical sources within the same genetic group was observed, suggesting that some resistant strains are capable of spreading throughout the hospital environment rather than being confined to a single clinical source [36]. In contrast, several isolates appeared as genetically distant, independent branches, reflecting the high genetic diversity of this pathogen and the presence of distinct strains not linked to direct epidemiological transmission chains [37, 38]. The findings of this study are consistent with previous research demonstrating clear genetic relatedness among multidrug-resistant P. aeruginosa isolates, particularly those recovered from burns and wounds, indicating the circulation of epidemic strains within the same clinical environment [39]. Recent reports have further indicated that identical genotypes detected among isolates from different patients provide strong evidence of in hospital transmission through patients or healthcare associated environmental sources rather than random similarity [40, 41]. Hospital acquired outbreaks caused by multidrug-resistant P. aeruginosa therefore represent a serious epidemiological challenge, particularly in high-risk units such as burn wards, underscoring the importance of molecular surveillance to support infection control programs and identify transmission routes [9, 42, 43]. Although ERIC-PCR is a rapid, cost effective, and practical method for assessing genetic relatedness in molecular epidemiological studies, its limitations in discriminating closely related strains highlight the need to complement it with higher-resolution approaches, such as whole genome sequencing, for a more comprehensive interpretation of genetic diversity [44].

Gene sequencing and expression analyses of pvcA and pvcC in P. aeruginosa isolates revealed elevated transcript levels, particularly in isolates harboring the G→C mutation in pvcA at position 2,482,503, resulting in an Ala to Pro substitution predicted to affect protein folding. This pattern is consistent with stress induced mutagenesis models, in which environmental stress triggers transcriptional reprogramming that up-regulates survival related genes despite the presence of structurally detrimental mutations, serving as a compensatory mechanism to maintain pathway function [45–47]. Sequencing analysis identified recurrent silent mutations in the pvcA gene among five Pseudomonas aeruginosa clinical isolates, characterized by synonymous T→C substitutions that did not alter the encoded amino acids but were associated with marked up regulation of pvcA. This pattern supports evidence that silent mutations can influence gene expression through effects on mRNA structure, stability, and translation efficiency [48]. In contrast, isolates lacking mutations in pvcA or pvcC showed variable expression levels, reflecting the complex regulation of the pvcABCD operon by iron availability, regulatory factors such as PvdS and PtxR, and environmental stress signals, as well as intrinsic transcriptional and post transcriptional heterogeneity within bacterial operons [11, 15, 49].

CONCLUSION

Clinical P. aeruginosa isolates exhibited differential regulation of the pvcABCD operon, characterized by down-regulation of pvcA and up-regulation of pvcB, pvcC, and pvcD, reflecting transcriptional adaptation to clinical stress. Mutations in pvcA and pvcC were associated with increased gene expression, suggesting compensatory regulatory mechanisms, while ERIC-PCR analysis revealed genetic diversity with clustering of burn and diabetic foot ulcer isolates, supporting the possibility of hospital – acquired transmission of multidrug-resistant strains.

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