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Analysis of Gene Expression Linked to Efflux Pumps and Porins in *Pseudomonas Aeruginosa* Isolates

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Abstract

Background: *Efflux pumps (EPs) are essential in bacterial resistance, actively expelling antibiotics to reduce susceptibility. In Pseudomonas aeruginosa, they show a role in multi-drug resistance. Porin modifications, are serious for carbapenem uptake, and enhance resistance by restricting antibiotic entry.*

Objective: *This study investigates the roles of efflux pumps and porins in antibiotic resistance, aiming to guide more effective treatment strategies for Pseudomonas aeruginosa infections.*

Materials and Methods: *A total of 300 clinical specimens were collected from Hilla Teaching Hospitals between January and June 2024, comprising wounds (40%), burns (40%), blood (12%), and urine (8%). Bacteria were cultured, identified through Gram staining and biochemical tests, and confirmed as Pseudomonas aeruginosa (8.33%) via the Vitek-2 system. Antimicrobial susceptibility testing was conducted for 16 antibiotics. Efflux pump genes (adeB, adeJ) were detected using PCR, and their expression was quantified through RT-qPCR.*

Results: *Pseudomonas aeruginosa isolates showed high resistance to β-lactams, carbapenems, and aminoglycosides, with colistin remaining effective. The adeB gene was present in all isolates, while adeJ was absent. Overexpression of OperD (80%) and adeB (44%) was observed, alongside complete downregulation of the CarO gene (100%).*

Conclusion: *This study identified Pseudomonas aeruginosa in 8.33% of clinical specimens, revealing key resistance mechanisms, including OperD and adeB overexpression and CarO downregulation.*

Keywords: Pseudomonas Aeruginosa In, Porins, AdeB, AdeJ, OprD, CarO, RT QPCR, Efflux Pump

1. Introduction

Pseudomonas aeruginosa is a highly resistant pathogen and a major concern in healthcare settings due to its rapid acquisition of resistance mechanisms against a broad range of antibiotics, including carbapenems. Resistance is primarily driven by efflux pumps, porin alterations, and beta-lactamase production, which collectively reduce treatment efficacy. Its ability to thrive in harsh environments and resist disinfectants exacerbates its spread, particularly among immunocompromised patients [1]. Efflux pumps, such as AdeABC, AdeIJK, and AdeFGH, actively expel antibiotics, lowering intracellular drug concentrations and contributing to multidrug resistance [2]. These systems are often overexpressed in resistant strains. Additionally, porins like OprD and CarO, critical for carbapenem uptake, are frequently altered through mutations, deletions, or downregulation, reducing membrane permeability and antibiotic entry [3]. The interplay between efflux pump overexpression and porin modifications creates a robust defense mechanism, rendering *Pseudomonas aeruginosa* exceptionally difficult to treat [4,5]. These mechanisms reduce the efficacy of intracellular-acting antibiotics, making *Pseudomonas aeruginosa* infections difficult to treat [6-8]. Their interplay creates robust resistance, complicating clinical management [9].

2. Material and Methods

2.1 Study Design, Data, and Specimen Collection

A total of 25 (8.33%) *Pseudomonas aeruginosa* isolates were obtained from clinical specimens collected at Hilla Teaching Hospital, Iraq, between January and June 2024. The specimens included urine (8%), blood (12%), burns (40%), and wounds (40%), collected using sterile swabs and cultured on MacConkey agar, blood agar, and HI-chrom agar (Merck, Germany). Gram stain microscopy confirmed Gram-negative bacteria, followed by biochemical tests (citrate,

oxidase, motility, and growth at 42 °C) and VITEK 2 system identification, as shown in Table 1.

2.2 Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the VITEK-2 system (Biomerieux, France) with Gram-negative susceptibility cards, following the manufacturer's protocol. Susceptibility interpretation was based on CLSI 2024 guidelines. *Acinetobacter baumannii* (ATCC) served as the control strain. Antibiotic efficacy criteria for different infections are detailed in Table 2.

2.3 CartWheel -Method

Efflux pump activity was assessed using the ethidium bromide (EtBr) agar cartwheel method described by Martins et al. (2011). The minimum inhibitory concentration (MIC) of EtBr was determined in duplicate following CLSI (2024) guidelines. *A. baumannii* isolates were cultured overnight in brain heart infusion broth at 37°C, centrifuged, and adjusted to a McFarland standard (1.5 × 10^8 CFU/ml). A 1 µl bacterial suspension was inoculated onto TSA plates with increasing EtBr concentrations (0.25–2 mg/ml), prepared in light-protected conditions. Plates were incubated at 37°C overnight, then examined under UV light. Isolates fluorescing at higher EtBr concentrations were considered to possess more active efflux systems.

2.4 Genotypic Detection of Genes Encoding with Efflux Pump and Porins

The detection of genes encoding efflux pumps (AdeB, AdeJ), porins (oprD, carO), and 16SrRNA was performed using PCR. The primers used for amplification are listed in Table 3. Each PCR reaction mixture contained a 2.5 mM dNTP mixture, 1 unit (0.2 µL) of Taq Polymerase, 10 pmol of each primer, and 1 µL of bacterial DNA, resulting in a final reaction volume of 25 µL. The thermal cycling parameters were as follows:: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, annealing at 55–60 °C for 30 seconds, extension at 72 °C for 1 minute, and a final elongation at 72 °C for 5 minutes. The PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide (EtBr), and visualized under a UV transilluminator, with gel images captured for documentation.

Gene Name	Primer Sequence (5´- 3´)	Amplicon(bp)	References
16SrRNA-F 16SrRNA-R	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	505	[10]
adeB-RT-F adeB-RT-R	TTAACGATAGCGTTGTAACC TGAGCAGACAATGGAATAGT	541	
adeJ -F adeJ -R	ATTGCACCACCAACCGTAAC TAGCTGGATCAAGCCAGATA	453	
carO-RT-F carO-RT-R	GGCGGATGAAGCTGTTGTTC GCACCACCGTAACCTGTAGT	102	
oprD-RT-F oprD-RT-R	TGCTGCGGATGGTATAGCTG ACACTATGTGGACCAGTCGC	84	

Table 1: Primer and Sequences Used in this Study

2.5 Isolation of (RNA) & cDNA Preparation

Bacterial strains were overnight growth on MHA plates cultured, using MHA plates and fresh Luria-Bertani media, from the late logarithmic phase bacterial samples total RNA was extracted using High Purity RNA Isolation kit. To rule out DNA contamination the following controls were performed with excluding RTase PCR sensitivity was tested PCR and reverse transcription were performed with the reagents included in the kit while RNA was used to synthesize cDNA. This cDNA obtained was stocked at -20°C for using in RT-PCR experiment later as well.

2.6 Quantitative Real-Time PCR

Relative real time quantity was done by absorbing salt pump genes (adeB, adeJ) and porin genes (oprD, carO) to SYBR Green I. Concentration of all PCR reactions contained 10 µL of primers, 10 µL of cDNA, 10 µL of SYBR Green I and the rest of nuclease free water. PCR parameters were as follows: 5 minutes at 94°C with once 40 cycles of 20 seconds at 94°C, 20 seconds at 60°C and 30 seconds at 72°C. Therefore to ascertain the presence of a single amplicon, melting analysis was carried out. The control strain was Acinetobacter baumannii taken from the ATCC collection and the housekeeping gene for this study was rpoB. All the reactions were done in triplicate and the CT value observed for each of the repeats was used to assess the level of the gene expression.

2.7 Analysis of Gene Expression

The 2^-ΔCT method was used to calculate gene expression fold changes, with the housekeeping 16S rRNA gene as a control and *Pseudomonas aeruginosa* ATCC 19606 as the reference strain. An RQ value of 1 indicated equal expression between test and reference strains, while values of 2 or 0.5 signified significant upregulation or downregulation, respectively. Log2 fold change distributions were analyzed, and descriptive statistics (mean, SE, SD, median, IQR, minimum, maximum) were calculated. Categorical variables were presented as frequencies and percentages, and compared using Chi-square or Fisher's Exact Test. Differences in mean gene expression were assessed via the Kruskal-Wallis H test. Statistical analysis was performed using SPSS 28.0, and graphics were created in Excel 2021. A P-value < 0.05 was considered significant.

2.8 Ethical Approval

Ethical approval was obtained from the Hilla Surgical Teaching Hospital Ethics Committee and the College of Medicine, University of Babylon (IRB: 4-27, 3/1/2024). Informed consent was obtained from all participants, who were informed about the study's purpose and use of results.

3. Results

3.1 Bacterial Isolates and Identification as *Pseudomonas Aeruginosa*

25(8.33%) out of 300 specimens collected from different clinical specimens. The clinical specimens including Urine (8%), blood (12%), burns (40%), and wounds (40%) Specimens were collected from patients using sterile swabs and cultured on MacConkey agar, blood agar, and Cetrimide agar (Merck, Germany) to confirm the presence of Gram-negative bacteria through Gram stain microscopy. Additionally, standard biochemical and microbiological tests, including citrate, oxidase, motility tests, and growth at 42 °C, were performed to identify and confirm the 25 isolates for inclusion in this study. The identification of strains using the VITEK 2 system is presented in Table 2.

Test type	Result	Test type	Result	Test type	Result	Test type	Result	Test type	Result
APPA	-ve	URE SAC	-ve	AGLTp	-ve	CIT	+ve	CMT O129R	+ve
ADO	-ve	dTRE	-ve	BGAL	-ve	BXYL ProA	-ve	ILATa	+ve
PyrA	-ve	dSOR	-ve	dMAN	-ve	LIP	-ve	SUCT	+ve
IARL	-ve	MNT ILATk	+ve	dMNE	+ve	PLE	-ve	BGUR	-ve
Dcel	-ve	AGLU	-ve	dGLU	+ve	TyrA	+ve	dMNE	+ve
NAGA	-ve	ILATa	+ve	BGUR	-ve	IHISa	+ve	dMAL	-ve
SAC	-ve	Balap	-ve	PHOS	-ve	Dmal	-ve	ADO	-ve
O129R	-ve	ILATK	+ve	Dtag	-ve	IARL	-ve	IMLTa	-ve
BGAL	-ve	CMT	+ve	GGAA	+ve	SUCT	+ve	Dglu	+ve

Table 2: Identification of Pseudomonas Aeruginosa by VITEK-2 Compact System

3.2 *+ve Positive result,* - ve Negative result

3.2.1 Distribution of *Pseudomonas Aeruginosa* among Different Clinical Specimens

8.33% of Pseudomonas aeruginosa was collected from different specimens as in Fig (1). The Chi-square value of 9.08 and a p-value of 0.028 indicate a statistically significant difference in the distribution of Pseudomonas aeruginosa among these clinical sources, with the highest prevalence found in wound and burn swabs.

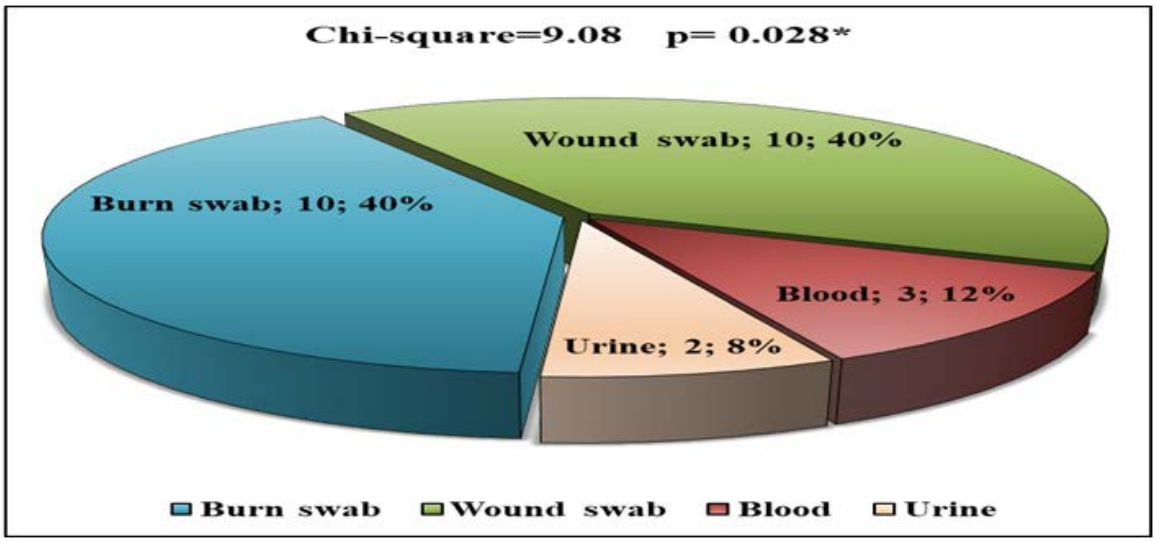

Figure 1: Percentage rate of Distribution Pseudomonas Aeruginosa among Different Clinical Specimens

3.3 Antimicrobial Susceptibility Test

The results of the Antimicrobial susceptibility Test indicate that Pseudomonas aeruginosa isolates demonstrate high levels of resistance to most of the tested antibiotics, particularly cefoxitin, tetracycline, aztreonam, imipenem, and colistin, with resistance rates of 80%. Colistin was the most effective antibiotic, with 96% of isolates being sensitive to it, highlighting its critical role in treating multidrug-resistant Pseudomonas aeruginosa infections as in Table (3).

Antibiotic	Resistant	Intermediate	Sensitive
Carbencillin	19 (76%)	1 (4 %)	5 (20 %)
Amoxi-clav	16 (64%)	1 (4 %)	8 (32 %)
cefepime	18 (72 %)	2 (8 %)	2 (20 %)
Meropenem	19 (76 %)	1 (4%)	5 (20%)
Ceftazidime	16 (64 %)	0 (0 %)	9 (36 %)
Cefotaxime	17 (68 %)	2 (8 %)	6 (24 %)
Ciprofloxacin	18 (72%)	1(4 %)	6 (24%)
cefoxitin	20 (80%)	0 (0 %)	5 (20 %)
Tetracycline	20(80 %)	0 (0%)	5 (20%)
Doxycycline	19 (76%)	2 (8 %)	4(16 %)
Aztreonam	20 (80%)	0 (0 %)	5 (20 %)
Ertapime	17 (68 %)	2 (8%)	6 (24%)
Oxacillin	13 (52 %)	2 (8 %)	10 (40%)
Imipenem	20 (80 %)	0 (0 %)	5 (20 %)
Tobramycin	16 (64%)	0 (0 %)	9(36 %)
Colistin	0 (0 %)	1 (4 %)	24 (96 %)

Table 3: Percentage of Antimicrobial Susceptibility Rate of Pseudomonas Aeruginosa. (25)Isolates Against 16 Antimicrobial Agents

3.4 Detection of Efflux pump by Cart Wheel Method

In the study, all (25) isolates of *Pseudomonas aeruginosa* showed a positive fluorescence at the EtBr concentration (0.25, 0.5, 1, 1.5, 2 mg/ml) as shown in Fig (2). With percentage (100%) because they have phenotypically efflux pumps that enable them to extrude EtBr. This method is considered a simple and rapid way for detection of phenotypic characterization of the examined isolates in extruding ethidium bromide and easy to perform, less time-consuming and can be utilized to screen large bacterial strain numbers, thus facilitating the fast identification of isolates exhibiting an MDR phenotype.

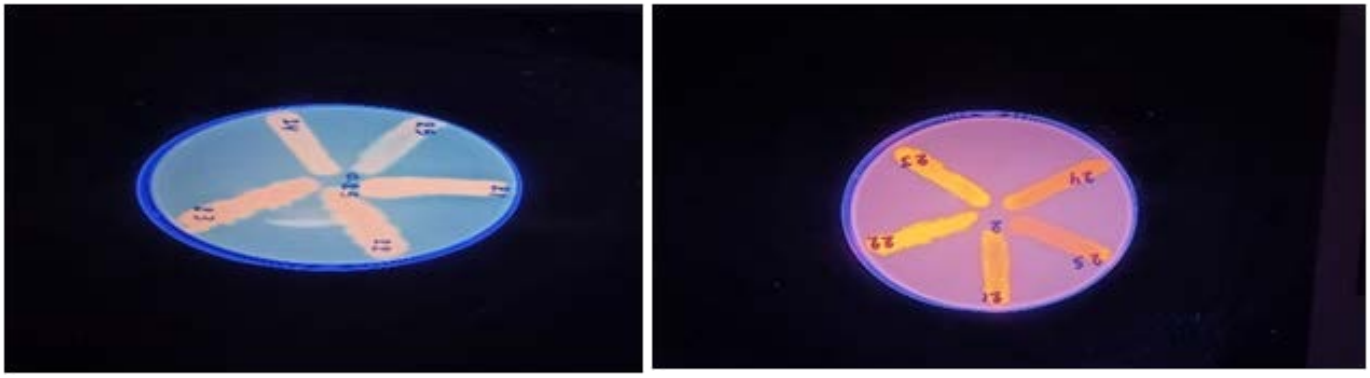

Figure 2: Cartwheel Methods for Detection of Efflux Pump in Different Concentrations (0.25-2 mg/ml) of Ethidium Bromide

4. Molecular Detection of Efflux Pump Genes

4.1 DNA Extraction

DNA extraction was performed with a DNA extraction kit (Pioneer Company Korea).

4.2 Identification of adeB and adeJ Gene via PCR

PCR was used to detect *16SrRNA, adeB, and adeJ* genes. Primer sequences are listed in Table 3. The PCR protocol included an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles: denaturation at 94 °C for 30 seconds, annealing at 58 °C for *blaOXA-51*, 55.5 °C for adeB, and 54.5 °C for adeJ for 30 seconds, extension at 72 °C for 90 seconds, and a final extension at 72 °C for 5 minutes. PCR products were analyzed on a 2% agarose gel with Syber Safe and visualized using a gel documentation system, as shown in Fig. 3, 4, and 5.

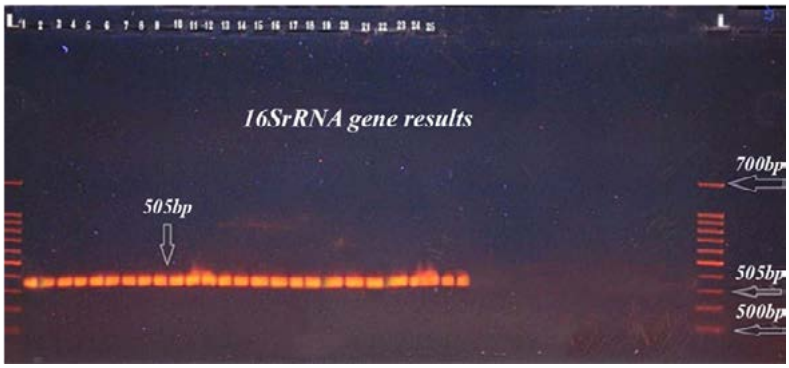

Figure 3: The 16SrRNA Gene Product (505 bp) was Detected Using Agarose Gel Electrophoresis. The DNA Isolated from Pseudomonas Aeruginosa Samples Tested Positive in Isolates 1 -25 Isolated

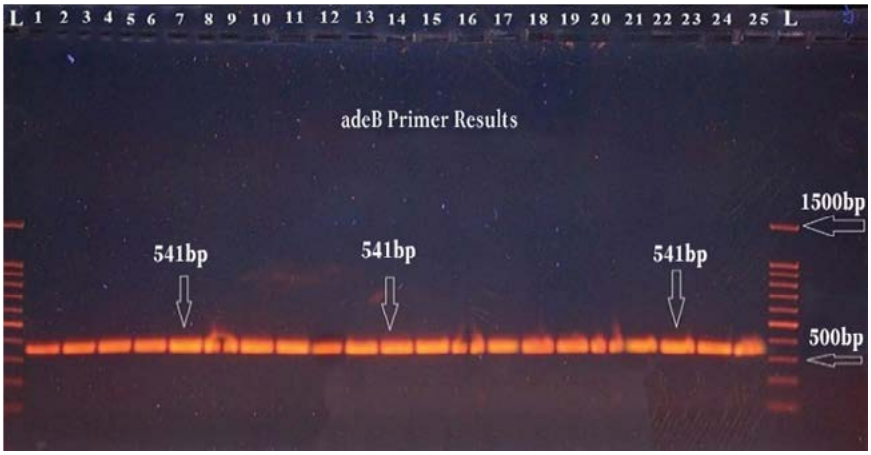

Figure 4: The AdeB Gene Product Size (band 541 bp) was Detected using Agarose gel Electrophoresis . DNA Isolated from Pseudomonas Aeruginosa Samples has Shown Positive from 1-25 Isolates

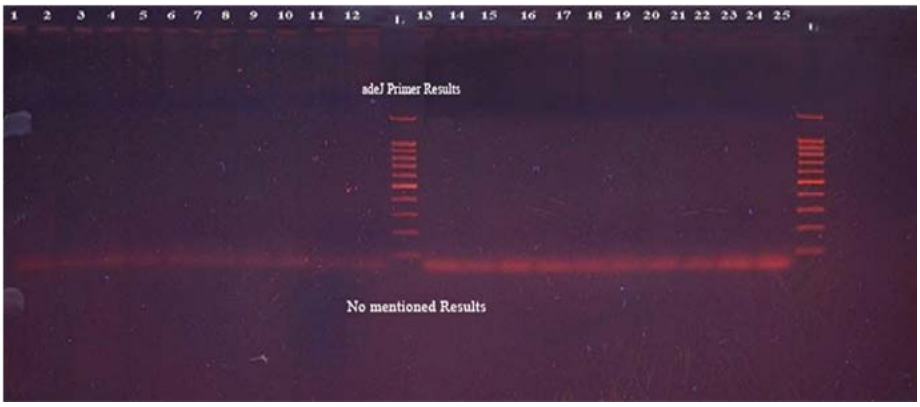

Figure 5: AdeJ Gene was Not Detected in all Isolates Using Agarose Gel Electrophoresis DNA Isolated from Pseudomonas Aeruginosa Samples has Shown Negative from 1-25 isolates

4.3 Gene Expression Analysis by using qRT PCR Technique

4.3.1 Extraction of Total RNA

In start extraction of RNA from clinical isolates to measure the gene expression level. In highly precise conditions avoid any contamination especially RNase and the protection came from using TRIzoL (guanidinthiocynate) with the ready kit. The quantity was measured by the Quantus Fluorometer. In this technique SYBR green dye was used as an indicator for qPCR, this dye binds with double-strand DNA and emits green light, and this light can be measured by real-time PCR at the end of each cycle, the result of amplification of each cycle was determined as CT (cycling threshold). The quantitative RT-PCR reaction was completed by using Pseudomonas aeruginosa -resistant isolates obtained from different specimens.

4.4 Gene Expression

The determine the fold of expression for adeB, CarO, OprD genes by determining delta CT by decreasing the mean CT of each isolate from the mean CT of the housekeeping gene and the delta CT was determined by decreasing the delta CT of each isolate from the delta CT of the control. The fold of expression was determined from the equation (2-(ΔΔCt)).

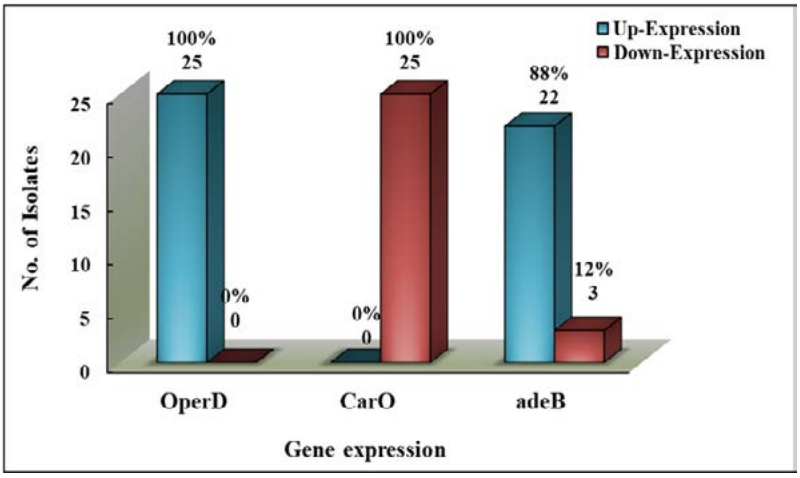

Figure 6: Genes Expression (up Expression, Down Expression) among Isolates

Gene expression		OperD		CarO		adeB	
		N	%	N	%	N	%
Log2 Fold change	Down expression	1	4.0%	25	100.0%	5	20.0%
	Overexpression	20	80.0%	0	0.0%	11	44.0%
	Non-significant expression	4	16.0%	0	0.0%	9	36.0%
Total	25	25	100.0%	25	100.0%	25	100.0%

Table 4: Genes Expression (Down, Over, Non-Significant Expression) among Isolates

In Table(4), Fig(6): The results indicate that a significant portion of *Pseudomonas aeruginosa* isolates exhibit overexpression of the OperD gene (80%) and adeB gene (44%), suggesting a potential role in antibiotic resistance through increased efflux pump activity. The universal downregulation of the CarO gene (100%) across all isolates highlights its critical loss, likely contributing to decreased antibiotic uptake and further resistance.

4.5 Genes Expression among Different Specimens

The significant overexpression of the OperD gene in burn and wound swabs suggests that *Pseudomonas aeruginosa* isolates from these sources may have a more robust efflux pump activity as in Table (5), potentially contributing to higher antibiotic resistance. The consistently low expression of CarO across all samples points to its likely downregulation, which is associated with reduced antibiotic uptake. Meanwhile, the adeB gene expression does not significantly differ among the samples, indicating a consistent role in antibiotic resistance across different clinical isolates.

		Mean	SE	SD	Mean Rank	Kruskal-Wallis H
Burn swab	OperD	9.611	2.045	6.467	23.00	20.968 <0.001**
	CarO	0.112	0.040	0.127	5.50	
	adeB	4.529	1.007	3.185	18.00	
Wound swab	OperD	11.753	3.830	12.113	23.20	20.921 <0.001**
	CarO	0.142	0.089	0.281	5.60	
	adeB	4.772	1.853	5.860	17.70	
Blood	OperD	7.904	2.844	4.926	7.33	5.956 0.050*
	CarO	0.087	0.048	0.083	2.00	
	adeB	3.882	1.769	3.064	5.67	
Urine	OperD	8.946	1.141	1.613	5.50	4.571 0.102
	CarO	0.432	0.313	0.442	1.50	
	adeB	5.413	2.002	2.831	3.50	

Table 5: Genes Expression (Significantly, Not Significantly) among Different Specimens

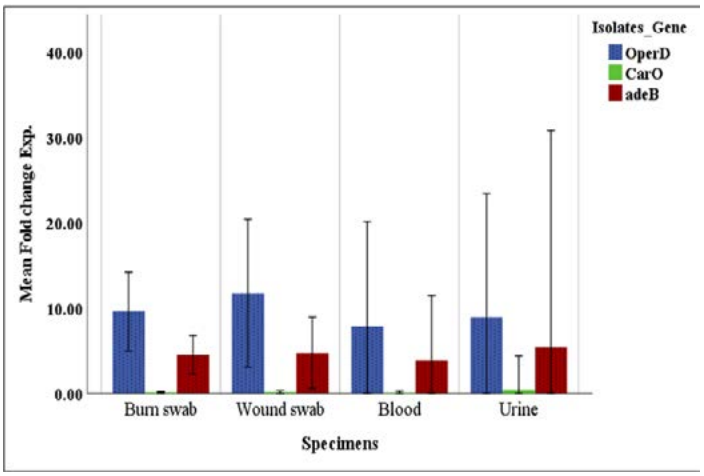

Figure 7: Distribution of OprD, CarO, and adeB Genes among Different Specimens

In Fig (7): The results suggest that OperD is notably overexpressed in burn and wound swabs, indicating a potential role in antibiotic resistance in these specimens. The consistent downregulation of CarO across all specimen types could contribute to decreased antibiotic uptake, reinforcing resistance. AdeB shows moderate expression, particularly in burn and wound swabs, which may also contribute to the resistance phenotype through active efflux mechanisms. The variability in expression across samples highlights the complex nature of gene regulation in Pseudomonas aeruginosa related to different clinical sources.

5. Discussion

This study collected 8.33% of *Pseudomonas aeruginosa* from different clinical specimens and agreement with the study who found *Pseudomonas aeruginosa* in isolate rate of 8.71% from different specimens and the study found *Pseudomonas aeruginosa* isolates rate 9.12% while a study who found 97% *Pseudomonas aeruginosa* isolate [10-13]. In the present study different specimens were collected including wounds75%, burns80%, blood12% and urine 8% agreement with the study collected 25 patients including 15 from wounds (25%), 2 from burns (20%), and 8 from urine (13.3%) [14]. Antimicrobial susceptibility Test for detection of resistance or sensitivity to antibiotics. All isolates were resistant to at least one antibiotic, with the following resistance rates: Ceftazidime (76%), Amoxicillin-clavulanate (64%), Carbenicillin (76%), Ertapenem (68%), Cefepime (72%), Cefoxitin (80%), Tobramycin (64%), Doxycycline (76%), Imipenem (80%), Meropenem (76%), Cefotaxime (68%), Ciprofloxacin (72%), Tetracycline (80%), Aztreonam (80%), Oxacillin (52%), and Colistin (0%).. In agreement with the study who found all the isolates were resistant to at least one antibiotic with the following breakdown: gentamicin (45%), amikacin (96.7%), imipenem (70%), meropenem (66.7%), ceftazidime (96.7%), ciprofloxacin (56.7%), trimethoprim-sulfamethoxazole combination (55%), tetracycline (98.3%), and ceftriaxone (83.3%) [14]. Phenotypic detection of the efflux pump was conducted in the present study using the Cartwheel method this study was acceptable to the local studies Conventional PCR for detection of *16SrRNA,adeB,adeJ* found 25(100%),25(100%) and0(0%) respectively, The results of this study align with findings from local studies conducted in different years, such as those [11,15,16,22].

These studies reported that all ***Pseudomonas aeruginosa*** isolates possessed efflux pumps, with detection rates of 99–100% using the EtBr-agar cartwheel method. In this study, DNA extraction was performed on all 25 bacterial isolates to detect the presence of the efflux pump genes AdeB and AdeJ in ***Pseudomonas aeruginosa*** and to examine the correlation of these genes with antibiotic resistance. The detection of RND-efflux pump in ***Pseudomonas aeruginosa*** was done by using the molecular technique. In this experiment, the conventional PCR technique used to detect two genes of efflux pumps in ***Pseudomonas aeruginosa***, primers were designed as we illustrate in Table (3). The detection assay was applied to (25) isolates of ***Pseudomonas aeruginosa***. The amplified products were resolved by gel electrophoresis with 1.5% agarose in the presence of molecular marker DNA ladder size (100- 1500bp). In molecular biology, gel electrophoresis is one of the most developed and popular technique that use to separate nucleic acids based on their charge. This technique offers many advantages in the laboratory. Mainly, the casing of agarose gel is easy, it is suitable for DNA with large and moderate sizes, and can be used to separate wide range of molecular sizes [17].

The pictures were obtained after screening with UV-Light. The results of this experiment demonstrated that genes of the RND superfamily efflux pump in ***Pseudomonas aeruginosa*** are widely distributed globally and are present in approximately 100% of clinical strains. These findings are consistent with those of, who both observed that the RND superfamily gene (adeB) was the most prevalent, being found in nearly all isolates (100%). The overproduction of efflux pumps may be a significant factor contributing to drug resistance, as these pumps can expel a variety of antimicrobial agents, reducing the accumulation of antibiotics in bacteria [17,22]. QRT-PCR analysis was performed to assess the upregulation of the efflux pump gene adeB in the isolates. All 25 ***Pseudomonas aeruginosa*** isolates exhibited efflux pump activity and expressed the adeB gene. The study found that 100% of the ***Pseudomonas aeruginosa*** isolates carried the adeB gene, aligning with previous research

by Japoni Nejad et al. (2014), which also reported a 100% prevalence of the adeB gene in ***Pseudomonas aeruginosa*.** The 16SrRNA is considered a better molecular marker for the study of phylogenetic and taxonomic relationships at the species level precisely for this bacterium reported that 16SrRNA genes were used for the identification of isolates giving a similar result as compared with the current study [16,21,22]. While adeB was Carried in all isolates adeJ was not found in all isolates. Gene expression for Efflux pump adeB,porins CarO,OprD recorded in the present study,OperD and adeB show a high level of overexpression in a majority of isolates, suggesting their potential role in resistance mechanisms.

Study reported that 16SrRNA genes were used for the identification of isolates giving a similar result as compared with the current study [22]. While study reported that Very high levels of expression of AdeJ have been shown to be toxic in both ***Pseudomonas aeruginosa*** [23]. AdeJ, providing intrinsic resistance, and adeB, synergistically providing even higher resistance. It is uncommon for adeJ to be overexpressed, implying the functions it carries out are important and need to be tightly regulated disagrees with the current study that is not detect adeJ in all isolates.CarO is universally down-expressed, possibly indicating a consistent strategy among the isolates to reduce permeability, which may contribute to resistance.While study conducted that changes in carO gene expression in resistant ***Pseudomonas aeruginosa*** [24]. The variation in adeB expression suggests that different isolates may rely on different mechanisms or levels of efflux pump activity in response to environmental pressures or antibiotic exposure and a study recorded CarO in 25% *Pseudomonas aeruginosa* isolates down expression disagreed with current study [25]. In the present study overexpression of the OperD gene in burn and wound swabs, as shown in Table (4), suggests that *Pseudomonas aeruginosa*isolates from these sources may exhibit enhanced efflux pump activity, potentially contributing to increased antibiotic resistance. Conversely, the consistently low expression of the CarO gene across all samples indicates its likely downregulation, which is associated with decreased antibiotic uptake. In contrast, the expression of the adeB gene remains consistent among the samples, suggesting a stable role in antibiotic resistance across different clinical isolates. This study was acceptable to the studies who recorded that resistance mediated by efflux pumps in clinical strains of Acinetobacter baumannii isolated from burn, wound, blood and urine infections [26]. Thus, it is proven that the resistance differs according to the specimens.

6. Conclusion

This study supports the role of efflux pump activity, particularly involving the OperD and adeB genes, plays a crucial role in the antibiotic resistance of *Pseudomonas aeruginosa*. and underscores the variation in resistance mechanisms depending on the type of clinical specimen.

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References

1. Wood, S. J., Kuzel, T. M., & Shafikhani, S. H. (2023). Pseudomonas aeruginosa: infections, animal modeling, and therapeutics. *Cells, 12*(1), 199.
2. Vadakkan, K., Ngangbam, A. K., Sathishkumar, K., Rumjit, N. P., & Cheruvathur, M. K. (2024). A review of chemical signaling pathways in the quorum sensing circuit of Pseudomonas aeruginosa. *International Journal of Biological Macromolecules,* 254, 127861.
3. Sathe, N., Beech, P., Croft, L., Suphioglu, C., Kapat, A., & Athan, E. (2023). Pseudomonas aeruginosa: Infections and novel approaches to treatment “Knowing the enemy” the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment. *Infectious Medicine, 2*(3), 178-194.
4. Ng, Q. X., Ong, N. Y., Lee, D. Y. X., Yau, C. E., Lim, Y. L., Kwa, A. L. H., & Tan, B. H. (2023). Trends in Pseudomonas aeruginosa (P. aeruginosa) bacteremia during the COVID-19 pandemic: a systematic review. *Antibiotics, 12*(2), 409.
5. Cao, P., Fleming, D., Moustafa, D. A., Dolan, S. K., Szymanik, K. H., Redman, W. K., ... & Whiteley, M. (2023). A Pseudomonas aeruginosa small RNA regulates chronic and acute infection. *Nature, 618*(7964), 358-364.
6. Manner, C., Dias Teixeira, R., Saha, D., Kaczmarczyk, A., Zemp, R., Wyss, F., ... & Jenal, U. (2023). A genetic switch controls Pseudomonas aeruginosa surface colonization. *Nature Microbiology, 8*(8), 1520-1533.
7. Sanya, D. R. A., Onésime, D., Vizzarro, G., & Jacquier, N. (2023). Recent advances in therapeutic targets identification and development of treatment strategies towards Pseudomonas aeruginosa infections. *BMC microbiology, 23*(1), 86.
8. Zhao, K., Yang, X., Zeng, Q., Zhang, Y., Li, H., Yan, C., ... & Zhou, X. (2023). Evolution of lasR mutants in polymorphic Pseudomonas aeruginosa populations facilitates chronic infection of the lung. *Nature Communications, 14*(1), 5976.
9. Naz, S., & Kashyap, G. S. (2024). Enhancing the predictive capability of a mathematical model for pseudomonas aeruginosa through artificial neural networks. *International Journal of Information Technology, 16*(4), 2025-2034.
10. Fernández-Billón, M., Llambías-Cabot, A. E., Jordana-Lluch, E., Oliver, A., & Macià, M. D. (2023). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa biofilms. *Biofilm*, 5, 100129.
11. Jeong, G. J., Khan, F., Tabassum, N., & Kim, Y. M. (2024). Natural and synthetic molecules with potential to enhance biofilm formation and virulence properties in Pseudomonas aeruginosa. *Critical Reviews in Microbiology, 50*(5), 830-858.
12. Reyes, J., Komarow, L., Chen, L., Ge, L., Hanson, B. M., Cober, E., ... & Satlin, M. (2023). Global epidemiology and clinical outcomes of carbapenem-resistant Pseudomonas aeruginosa and associated carbapenemases (POP): a prospective cohort study. *The Lancet Microbe, 4*(3),

e159-e170.
13. Swart, A. L., Laventie, B. J., Sütterlin, R., Junne, T., Lauer, L., Manfredi, P., ... & Jenal, U. (2024). Pseudomonas aeruginosa breaches respiratory epithelia through goblet cell invasion in a microtissue model. *Nature microbiology, 9*(7), 1725-1737.
14. Weimann, A., Dinan, A. M., Ruis, C., Bernut, A., Pont, S., Brown, K., ... & Floto, R. A. (2024). Evolution and host-specific adaptation of Pseudomonas aeruginosa. *Science, 385*(6704), eadi0908.
15. Mugunthan, S., Wong, L. L., Winnerdy, F. R., Summers, S., Bin Ismail, M. H., Foo, Y. H., ... & Seviour, T. (2023). RNA is a key component of extracellular DNA networks in Pseudomonas aeruginosa biofilms. *Nature Communications, 14*(1), 7772.
16. Marouf, S., Li, X., Salem, H. M., Ahmed, Z. S., Nader, S. M., Shaalan, M., ... & Cheang, T. (2023). Molecular detection of multidrug-resistant Pseudomonas aeruginosa of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against multidrug-resistant *P. aeruginosa. Poultry Science, 102*(10), 102995.
17. Li, X., Gu, N., Huang, T. Y., Zhong, F., & Peng, G. (2023). Pseudomonas aeruginosa: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. *Frontiers in Microbiology, 13,* 1114199.
18. Phan, S., Feng, C. H., Huang, R., Lee, Z. X., Moua, Y., Phung, O. J., & Lenhard, J. R. (2023). Relative abundance and detection of Pseudomonas aeruginosa from Chronic Wound Infections globally. *Microorganisms, 11*(5), 1210.
19. Abdelghafar, A., El-Ganiny, A., Shaker, G., & Askoura, M. (2023). Isolation of a bacteriophage targeting Pseudomonas aeruginosa and exhibits a promising in vivo efficacy. *AMB Express, 13*(1), 79.
20. Li, Y., Roberts, J. A., Walker, M. M., Aslan, A. T., Harris, P. N., & Sime, F. B. (2024). The global epidemiology of ventilator-associated pneumonia caused by multi-drug resistant Pseudomonas aeruginosa: A systematic review and meta-analysis. *International Journal of Infectious Diseases,* 139, 78-85.
21. Gales, A. C., Stone, G., Sahm, D. F., Wise, M. G., & Utt, E. (2023). Incidence of ESBLs and carbapenemases among Enterobacterales and carbapenemases in Pseudomonas aeruginosa isolates collected globally: results from ATLAS 2017–2019. *Journal of Antimicrobial Chemotherapy, 78*(7), 1606-1615.
22. Zhao, J. S., Ahmad, N., Li, S., & Zhou, C. H. (2024). Hydrazyl hydroxycoumarins as new potential conquerors towards Pseudomonas aeruginosa. *Bioorganic & Medicinal Chemistry Letters,* 103, 129709.
23. Zhao, J. S., Ahmad, N., Li, S., & Zhou, C. H. (2024). Hydrazyl hydroxycoumarins as new potential conquerors towards Pseudomonas aeruginosa. *Bioorganic & Medicinal Chemistry Letters,* 103, 129709.
24. Maffei, E., Woischnig, A. K., Burkolter, M. R., Heyer, Y., Humolli, D., Thürkauf, N., ... & Harms, A. (2024). Phage Paride can kill dormant, antibiotic-tolerant cells of Pseudomonas aeruginosa by direct lytic replication. *Nature Communications, 15*(1), 175.
25. Wang, C., Lin, Y., Huang, J., Song, H., Zhang, Y., Zhang, Y., ... & Liu, J. (2023). Pseudomonas aeruginosa targeting cascade photodynamic nanoassemblies for efficient antibacterial and anti-inflammatory therapy. *Nano Today,* 51, 101892.
26. Minns, M. S., Liboro, K., Lima, T. S., Abbondante, S., Miller, B. A., Marshall, M. E., ... & Pearlman, E. (2023). NLRP3 selectively drives IL-1β secretion by Pseudomonas aeruginosa infected neutrophils and regulates corneal disease severity. *Nature Communications, 14*(1), 5832.