

Phenotypic and Molecular Analysis of Fosfomycin Resistance Among *P. aeruginosa* Isolates from Cystic Fibrosis Patients

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Introduction: This study evaluated the prevalence of *P. aeruginosa* in cystic fibrosis (CF) patients, assessed its resistance patterns to commonly used antibiotics with an emphasis on fosfomycin, and examined the presence of resistance genes (*glpT*, *fosA3*, *blaCTX-M*) in these isolates.

Material and Methods: A cross-sectional study was conducted at Shahid Beheshti University of Medical Sciences, Tehran, from January to June 2022. Sixty sputum samples from CF-confirmed patients were collected and cultured. Antibiotic susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method according to CLSI guidelines. Minimum inhibitory concentrations (MICs) of fosfomycin were determined using E-test strips. PCR was employed to detect the presence of *glpT*, *fosA3*, and *blaCTX-M* resistance genes. Data were analyzed using SPSS version 21.

Results: *P. aeruginosa* was isolated from 71.6% (43/60) of samples. Based on AST, significant antibiotic resistance was observed, particularly against fosfomycin (77.2%), imipenem, and amikacin (53.5%). Using the combined disk diffusion test (CDDT), 46% of isolates were identified as ESBL producers. PCR analysis revealed the presence of *glpT* gene in all isolates, *fosA3* gene in 39.5%, and *blaCTX-M* gene in 30.2%.

Discussion: The *fosA3* gene showed a strong correlation with fosfomycin resistance, while *blaCTX-M* was associated with beta-lactam resistance. Molecular diagnostics targeting resistance genes such as *glpT*, *fosA3*, and *blaCTX-M* are essential for guiding antibiotic therapy. The resistance patterns observed, particularly against fosfomycin, highlight the need for innovative therapeutic approaches.

Conclusion: This study reveals a high rate of multidrug resistance in *P. aeruginosa* isolates from CF patients, particularly to fosfomycin, imipenem, and amikacin. The presence of resistance genes suggests a genetic basis for these patterns, emphasizing the importance of developing new strategies to manage *P. aeruginosa* infections effectively. Future studies should focus on targeted inhibitors for these genes to overcome resistance and improve clinical outcomes.

Plain Language Summary:

- (1) The high prevalence of *P. aeruginosa* in CF patients (71.6%) underscores the bacterium's significant role in chronic respiratory infections in this population. This high prevalence necessitates routine surveillance and aggressive antimicrobial stewardship to manage infections effectively.
- (2) The multi-drug resistance (MDR) observed, particularly to antibiotics like fosfomycin, imipenem and amikacin complicates treatment regimens and highlights the need for alternative therapeutic strategies and novel antibiotics to combat these resistant strains.
- (3) The detection of resistance genes such as *blaCTX-M*, *glpT*, and *fosA3* in a significant proportion of isolates highlights the genetic basis for the observed resistance patterns. The correlation between these genes and antibiotic resistance underscores the importance of molecular diagnostics in guiding effective therapy.
- (4) The specific mutations identified in the resistance genes provide insight into resistance mechanisms and can inform the development of targeted inhibitors or alternative therapeutic approaches to overcome resistance. A summary of the study design and process is provided in Figure 1.

Keywords: cystic fibrosis, *P. aeruginosa*, fosfomycin, drug resistance

Introduction

Cystic fibrosis (CF) is a genetic disorder inherited in an autosomal recessive manner, resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This gene encodes a protein responsible for transporting chloride ions across epithelial cell membranes. Mutations in *CFTR* disrupt the regulation of chloride and sodium ions, leading to the production of thick, sticky mucus in various organs, most notably the lungs and digestive system.¹ The defective *CFTR* protein can cause airway surface liquid (ASL) depletion.² Besides, it can alter bicarbonate, glutathione, thiocyanate transport, pH homeostasis, and innate immunity.³

CF has complications in various systems of the body, with lung infections being the most prominent ones causing morbidity and mortality.⁴ *Pseudomonas aeruginosa* (*P. aeruginosa*), surpassing other pathogens, is the dominant pathogen in CF lung infections, particularly during the second decade of life.⁵ Other pathogens associated with CF include *Staphylococcus aureus*, fungi like *Aspergillus fumigatus*, and the *Burkholderia cepacia* complex.^{6,7}

Pseudomonas aeruginosa, with a prevalence of 40.6% in Iran, is one of the most important pathogens seen in cystic fibrosis (CF) patients.⁸ It can cause recurrent infection and diminish pulmonary function in CF patients.⁹ *P. aeruginosa* employs various mechanisms to evade eradication and accelerate the deterioration of lung function. It forms biofilms and quickly develops antibiotic resistance.^{10,11} One of the ways that *P. aeruginosa* can escalate its resistance against antimicrobial agents and pathogenicity is through its interactions with other microorganisms in the airways of CF patients.¹² To highlight the global urgency of this issue, according to the World Health Organization, bacterial antimicrobial resistance (AMR) was directly responsible for 1.27 million deaths and associated with 4.95 million deaths worldwide in 2019.¹³ Additionally, *P. aeruginosa* exploits other resistance mechanisms, such as overexpression of efflux pumps, particularly the resistance-nodulation-division (RND) family systems like MexAB-OprM (which expels beta-lactams, quinolones, and macrolides) and MexXY (involved in aminoglycoside resistance). These pumps actively transport antibiotics out of the cell, reducing intracellular concentrations and contributing significantly to multidrug

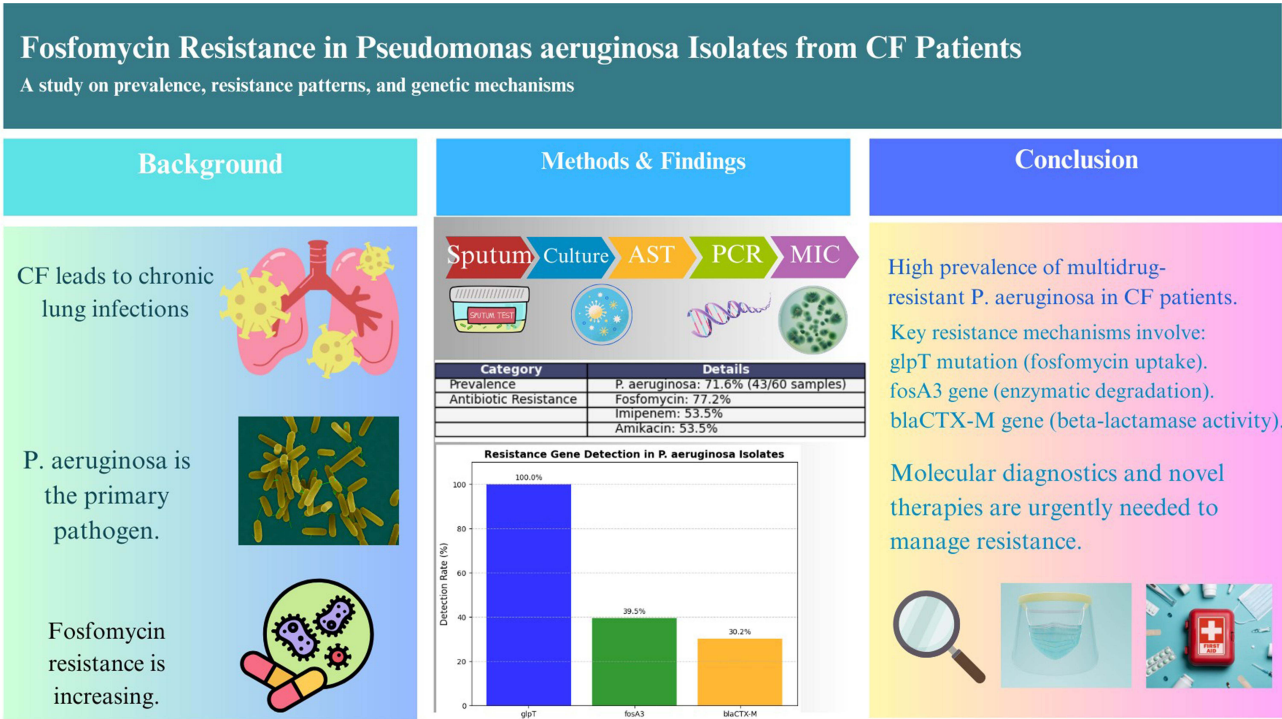


Figure 1 Schematic overview of the study process.

resistance in clinical isolates^{14,15} to expel antibiotics, modifications to porin channels to limit drug entry, and enzymatic degradation of antibiotics. These mechanisms profoundly contribute to CF morbidity and mortality by enabling colonization of the thick, sticky mucus in the respiratory tracts of these patients.^{10,16} The considerable existence of complications of *Pseudomonas aeruginosa* in cystic fibrosis patients requires us to start finding more effective therapeutic approaches.¹⁷ Some of the options for treatment of *Pseudomonas aeruginosa* include antibiotics, airway clearance techniques, and CFTR modulators.¹⁸ Recently, fosfomycin has gained a noteworthy amount of attention due to its unique mechanism of disrupting cell wall synthesis.¹⁹

Fosfomycin, a broad-spectrum antibiotic, disrupts cell wall synthesis by inhibiting the enzyme MurA (UDP-N-acetylglucosamine enolpyruvyl transferase). In *P. aeruginosa*, a key resistance mechanism involves loss-of-function mutations in the *glpT* gene, which encodes a fosfomycin transporter. Additionally, plasmid-mediated *fosA* genes confer fosfomycin resistance.^{17–19} This study aims to assess the prevalence of *P. aeruginosa* in CF patients, determine the resistance patterns of its isolates to commonly used antibiotics with an emphasis on fosfomycin, and examine the presence of resistance genes (*glpT*, *fosA3*, and *bla_{CTX-M}*) in *P. aeruginosa* isolates from CF patients.

Materials and Methods

Study Design

This cross-sectional, descriptive study was conducted at the Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Ethical approval was obtained from Shahid Beheshti University of Medical Sciences IR.SBMU.MSP.REC.1400. 050. This study complies with the Declaration of Helsinki. Informed consent was obtained from all participants, or their parents or legal Guardians.

Sample Collection

All the sputum samples for this cross-sectional study were collected from confirmed CF patients from an educational Mofid Children's Educational Hospital, Shahid Beheshti University of Medical Sciences Tehran-Iran, from January to June 2022. Sixty sputum samples were collected in sterile containers and transported to the laboratory within 2 hours. The inclusion criteria were CF-confirmed patients by sweat chloride test and genetic analysis, children aged 1–18 years, and no antibiotic treatment for at least two weeks before sample collection.

Sample Treatment and Bacterial Isolation

Each sputum sample was homogenized using a vortex mixer for 1 minute. Then, samples were inoculated onto blood agar, MacConkey agar and cetrimide agar. Plates were incubated at 37 °C for 24–48 hours. Then standard bacteriological tests including gram staining, oxidase test, Triple sugar Iron Agar (TSI), motility and growth at 42°C were done for *P. aeruginosa* confirmation.

Antibiotic Susceptibility Testing (AST)

AST was done by the Kirby Bauer disk diffusion method using Mueller-Hinton agar (Becton Dickinson, catalog no. 225250) based on CLSI 2022.²⁰ All antibiotic disks were: amikacin (30 µg), Ofloxacin (15 µg), imipenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), piperacillin (100 µg), aztreonam (30 µg), piperacillin-tazobactam (100/10 µg), and gentamicin (10 µg) were purchased from Mast Company, England (MASTDISCS[®] AST series), and Rosco Company, Denmark (Neo-Sensitabs series).

Bacterial suspensions were prepared in saline equivalent opacity to 0.5 McFarland standard (1.5×10^8 CFU/mL). A lawn of bacteria was prepared by a sterile swab on Mueller-Hinton agar. Antibiotic disks were placed on the surface of the inoculated agar with sterile forceps. Plates were incubated at 37°C for 24–48 hours. Zones of inhibition were measured, and the results were interpreted according to CLSI 2022 guidelines. *P. aeruginosa* ATCC 27853 was used as a positive control.

Minimum Inhibitory Concentration (MIC) Detection by E-Test Strips

The minimum inhibitory concentration (MIC) of fosfomycin was detected by E-test (bioMérieux, catalog no. 412390) on Mueller Hinton agar using the same protocol mentioned above. MICs were read after 24 hours of incubation at 37 °C. The CLSI criteria for Enterobacteriaceae ($S \leq 64 \mu\text{g/mL}$, $I = 128 \mu\text{g/mL}$, $R \geq 256 \mu\text{g/mL}$) were used for interpretation, as specific breakpoints for **P. aeruginosa** are not defined.

Combined Double Disk Synergy Test (CDDT) for Extended Spectrum Beta-Lactamase Detection

The ceftazidime resistant strains were candidates for ESBL detection by CDDT method based on CLSI protocol.²⁰ In this test, colonies from overnight cultures on blood agar plates were suspended in sterile normal saline and their turbidity was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL). Then, the suspension was streaked onto Mueller-Hinton agar plates (Qlab, Montreal, Canada, catalog no. QL-10225) CDDT test was performed by using plain ceftazidime disk (30 μg) and ceftazidime/clavulanic acid (30 μg /10 μg) and putting other third generation cephalosporins like cefotaxime, ceftriaxone, ceftazidime.

Detection of Resistance Genes Including *glpT*, *Bla*_{CTX-M} and *fosA3* by the PCR Method

DNA Extraction

DNA was extracted by the boiling method.²¹ Bacterial isolates were first cultured on a solid TSA medium, then inoculated in LB liquid medium and incubated at 37 °C for 24–48 hours. Subsequently, 1000 μL of each bacterial suspension was added to a sterile tube and centrifuged at 12000 rpm for 5 minutes. Then the supernatant (SN) was removed, 200 μL of sterile injectable distilled water was added to continue, and the samples were centrifuged at 12000 rpm for 5 minutes. The supernatant was removed, and 100 μL of sterile injectable distilled water was added and vortexed for 10 seconds. The samples were heated at 100 °C for 10 minutes and then transferred to an ice container for 5 minutes. After centrifugation at 12000 rpm, the supernatant containing DNA was transferred to 0.5 mL microtubes and stored at –20 °C. The OD of the extracted DNA samples at 260/280nm was evaluated using a nanodrop instrument (Thermo Scientific NanoDrop 2000, model no. ND-2000).

PCR Procedure

PCR was used to identify the *glpT*, *bla*_{CTX-M}, and *fosA3* genes. The PCR reaction mix included the following components:

The PCR reaction mix contained the following components: 12.5 μL Master Mix Amplicon, 1 μL of each primer (Table 1), 7.5 μL distilled water, and 3 μL DNA template, for a total volume of 25 μL . The primer sequences used for PCR are listed in Table 1. The PCR conditions for each gene are detailed in Table 2.

Table 1 Primers Used for the PCR Method in This Study

Gene	Sequence (5'-3')	Segment Length (bp)	Reference
<i>glpT</i>	F: AGCGGAGCTCGCGATGTTC	1366	[22]
	R: TCAGCCGGCTTGCTGCGG		
<i>fosA3</i>	F: CCTGGCATTATTCAGCAGT	234	[23]
	R: CGGTTATCTTCCATACCTC		
<i>bla</i> _{CTX-M}	F: TTGAGGCTGGCTGAAGT	761	[24]
	R: ACGCTGTTGTTAGGAAGTG		

Table 2 PCR Conditions Used in This Study

Gene	Initial Denaturation	Denaturation	Annealing Temp	Extension	Final Extension	Cycles
<i>glpT</i>	95°C, 3'	95°C, 30''	58°C, 30''	72°C, 30''	72°C, 1'	26
<i>fosA3</i>	94°C, 5'	94°C, 45''	58°C, 45''	72°C, 45''	72°C, 5'	36
<i>bla_{CTX-M}</i>	94°C, 5'	94°C, 45''	57°C, 45''	72°C, 45''	72°C, 5'	30

Gel Electrophoresis

PCR products were separated on a 1% agarose gel prepared with TBE 1x buffer. Electrophoresis was performed at 100 volts for 1 hour, and the results were visualized under UV light in the gel document system (Figure 1).

Sequencing

Sequencing was done by the Pishgam Company of Iran. The *glpT* gene sequence of *Pseudomonas aeruginosa* PAO1 was used as a control to investigate mutations. Sequencing analysis of the resistance genes identified specific mutations in the *glpT* gene that confer fosfomycin resistance.²⁵ The sequences were aligned using Clustal in BioEdit software to identify conserved and variable regions. The alignment revealed a total sequence length of 1052 base pairs (bp), with no gaps or missing data. The aligned sequences were saved in FASTA format for further analysis. The sequences obtained from the samples were compared with the control sequence through pairwise BLAST analysis using NCBI BLASTn with default parameters (Expect threshold: 10; Word size: 28; Match/Mismatch scores: 1/-2; Gap costs: Existence 5, Extension 2).

Statistical Data Analysis

The data were analyzed using SPSS version 21. Descriptive statistics were used to summarize patient demographics and the prevalence of resistance genes. Chi-square tests evaluated associations between gene presence and antibiotic resistance patterns. A p-value of <0.05 was considered statistically significant.

Results

Prevalence of *Pseudomonas aeruginosa* in CF Patients

Based on standard biochemical tests, out of the 60 sputum samples collected from cystic fibrosis (CF) patients and 43 (71.6%) samples confirmed to be positive for *Pseudomonas aeruginosa*. The age distribution of the patients was as follows: 37.20% (n=16) 1–5 years, 32.55%¹⁴ 6–10 years and 30.23%¹³ 11–15 years old.

Antibiotic Susceptibility Test (AST)

The antibiotic resistance profiles of the *Pseudomonas aeruginosa* isolates were examined against a panel of commonly used antibiotics. The results indicate a worrying trend of multi-drug resistance (Table 3 and Figure 2). As illustrated in

Table 3 Frequency of Antibiotic Resistance of *Pseudomonas Aeruginosa* Isolates in This Study

Antibiotics	S	I	R	Resistance Percentage (%)
PIP-Tazo	25	3	15	41.86
CAZ	23	0	20	46.51
FEP	22	0	21	48.84
Azt	21	3	19	51.16
IPM	20	0	23	53.49
GM	26	1	16	39.53
AN	20	1	22	53.49
CP	21	2	20	51.16

(Continued)

Table 3 (Continued).

Antibiotics	S	I	R	Resistance Percentage (%)
FOS	10	9	24	76.74
FOS(ESBL+)	5	3	14	77.27
OFX	21	0	22	51.16
PIP	25	1	17	41.86

Abbreviations: Pip-Tazo, Piperacillin-Tazobactam; CAZ, Cefazidime; FEP, Cefepime; Azt, Aztreonam; IPM, Imipenem; GM, Gentamicin; AN, Amikacin; CP, Ciprofloxacin; OFX, Ofloxacin; PIP, Piperacillin; FOS, Fosfomycin; FOS (ESBL+), Fosfomycin against ESBL positive isolates.

Figure 3, the figure clearly shows the extent of resistance across different antibiotics. The isolates demonstrated particularly high resistance to fosfomycin (76.74% overall and 77.2% among ESBL-positive isolates), imipenem and amikacin (53.5%).

MIC of Fosfomycin by E-Test

The frequency of resistant to fosfomycin by E-test strips are mentioned in Figure 3.

Frequency of ESBL in *P. aeruginosa* Isolates from CF Patients by CDDT Test

Based on the results of CDDT 46% of *P. aeruginosa* isolates from CF patients identified as ESBL producers.

Detection of Resistance Genes by PCR Method

PCR analysis was performed to detect the presence of key resistance *bla*CTX-M, *glpT*, and the *fosA3* genes. The results are summarized below (Table 4 and Figure 4A–C).

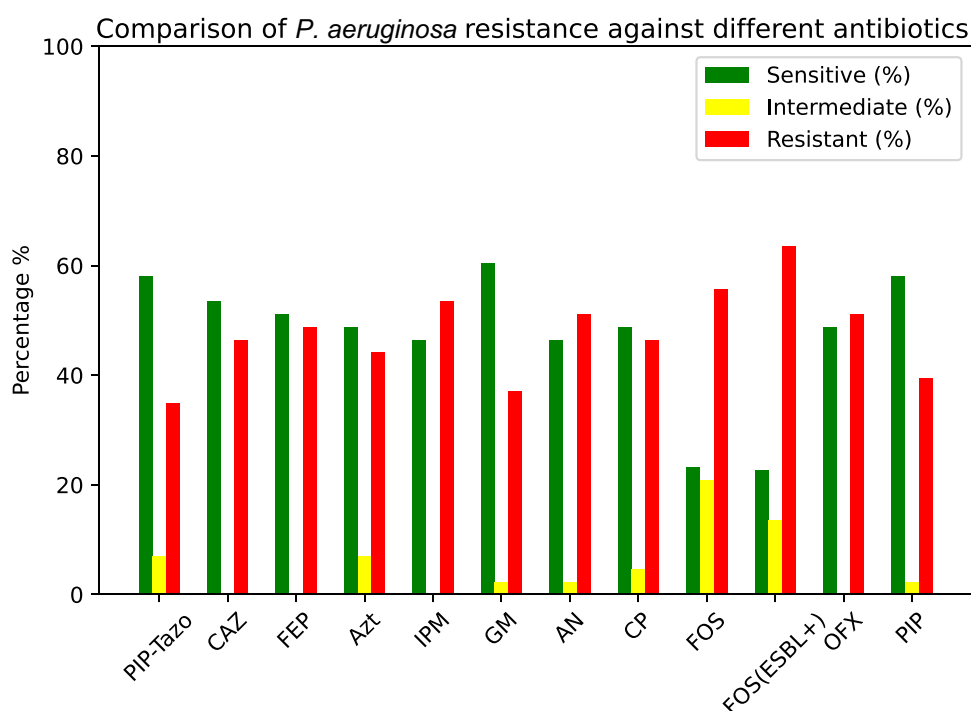


Figure 2 Resistance pattern of *Pseudomonas aeruginosa* isolates from CF in this study.

Abbreviations: Pip-Tazo, Piperacillin-Tazobactam; CAZ, Cefazidime; FEP, Cefepime; Azt, Aztreonam; IPM, Imipenem; GM, Gentamicin; AN, Amikacin; CP, Ciprofloxacin; OFX, Ofloxacin; PIP, Piperacillin; FOS, Fosfomycin; FOS (ESBL+), Fosfomycin against ESBL positive isolates.

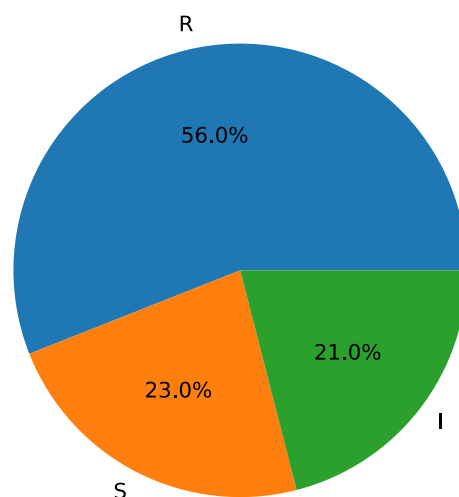


Figure 3 Frequency of fosfomycin resistant by E-test in *Pseudomonas aeruginosa* isolates from CF in this study (R = resistant, I = intermediate, S = sensitive).

Correlation Between Gene Presence and Antibiotic Resistance

The association between the presence of specific resistance genes and antibiotic resistance was evaluated. The presence of the *fosA3* gene showed a strong correlation with fosfomycin resistance, and the *bla_{CTX-M}* gene was significantly associated with resistance to beta-lactam antibiotics like ceftazidime and cefepime (Figures 5 and 6, Table 5).

Sequence Analysis of *glpT* Gene

The *glpT* gene, responsible for glycerol-3-phosphate transport in *Pseudomonas aeruginosa*, was analyzed to assess genetic diversity, mutations, and sequence variations among six isolates. The analysis identified 7 polymorphic sites, including 6 singleton variable sites and 1 parsimony-informative site. Table 6 summarizes the sequence characteristics and polymorphic site positions.

Statistical Significance of the Study

Statistical significance of the study is shown in Figure 7

Discussion

The findings of this study highlight the pressing issue of antibiotic resistance in *P. aeruginosa* infections among CF patients, with our results indicating a substantial burden of this pathogen. In summary, our key findings include a 71.6% resistance rate to fosfomycin (63% in ESBL+ samples), 53.5% to amikacin and imipenem, with 100% of isolates harboring the *glpT* gene, 39.53% carrying the plasmid *fosA3* gene, and 30.23% containing the plasmid *bla_{CTX-M}* gene, showing strong genetic correlations to multi-drug resistance. These resistance patterns underscore the necessity for tailored therapeutic strategies and the development of new antibiotics to manage *P. aeruginosa* infections effectively in the CF population. Future research should explore resistance mechanisms at genomic and transcriptomic levels,

Table 4 Prevalence of Resistance Genes in *P. aeruginosa* Isolates From CF Patients

Gene	Positive Isolates	Prevalence (%)
<i>glpT</i>	43	100%
<i>fosA3</i>	17	39.53%
<i>bla_{CTX-M}</i>	13	30.23%

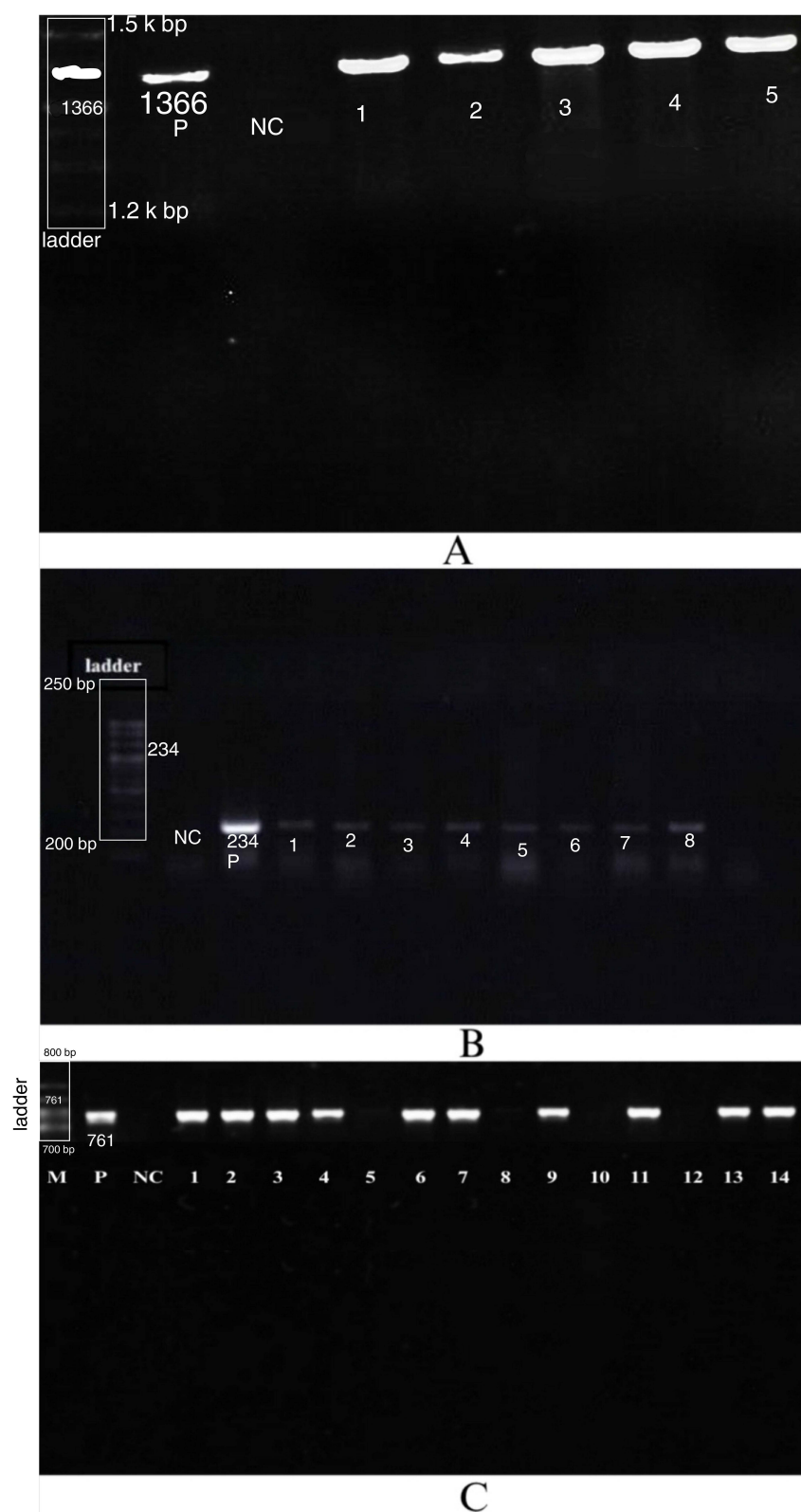


Figure 4 PCR amplification of the *glpT*, *fosA3* and *blaCTX-M* genes from *Pseudomonas aeruginosa* isolates from CF patients. **(A)** *glpT* gene with a band of 1366bp lanes 1–5 positive samples, **(B)** *fosA3* gene with a band of 234 bp, lanes 1–8 positive samples **(C)** *blaCTX-M* gene with a band at 761 bp, lanes 1–4, 6–7, 11, 13–14 positive samples, Lane M: Molecular weight marker (ladder size in bp indicated). Lane N: Negative control (no template DNA). Lane P: Positive control (template DNA with the target gene).

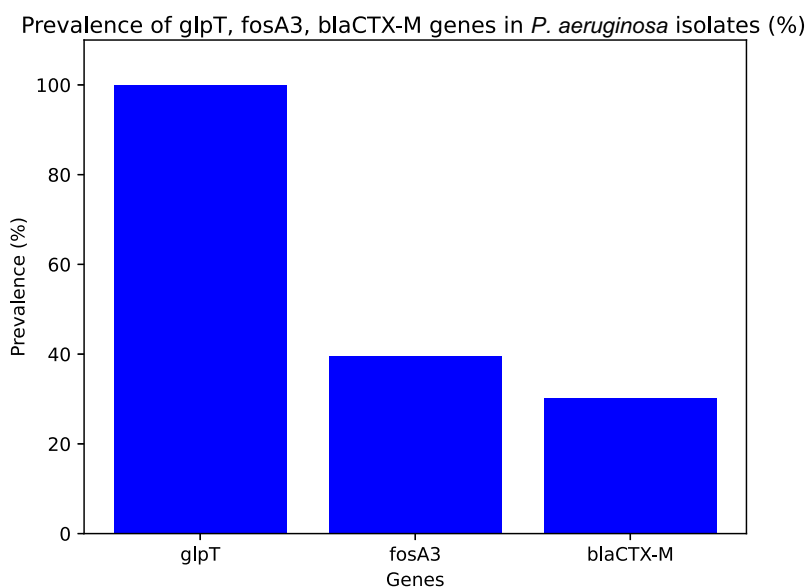


Figure 5 Association between Resistance Genes and Antibiotic Resistance.

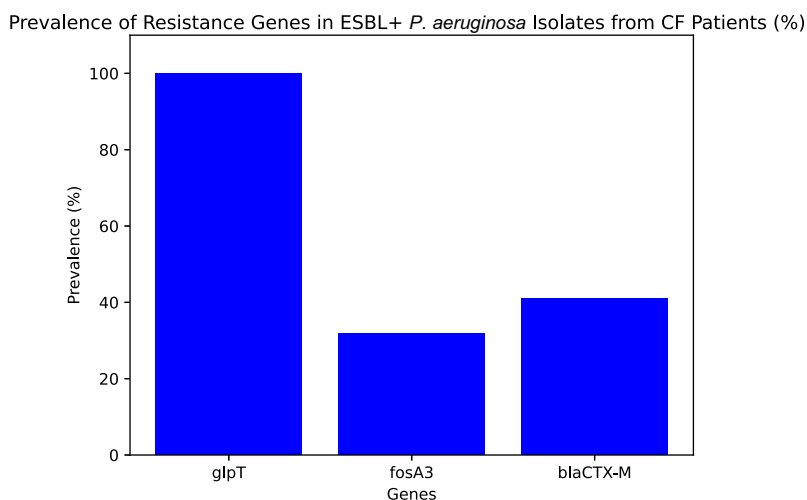


Figure 6 Association between Resistance Genes and Antibiotic Resistance in ESBLe *P. aeruginosa* isolates.

investigate the role of biofilm formation in enhancing fosfomycin resistance, and evaluate combination therapy strategies for CF-associated *P. aeruginosa* infections.

Chronic *Pseudomonas aeruginosa* infections impact cystic fibrosis (CF) patients undeniably, leading to rapid deterioration of lung function and elevated number of pulmonary exacerbations. This pathogen plays a key role in CF morbidity and mortality. Clonal or epidemic strains can be transmitted between CF patients and are associated with worse outcomes.²⁶

Table 5 Prevalence of Resistance Genes in ESBLe *P. aeruginosa* Isolates From CF Patients

Gene	Positive Isolates	Prevalence (%)
<i>glpT</i>	22	100%
<i>fosA3</i>	7	31.81%
<i>bla_{CTX-M}</i>	9	40.9%

Table 6 Sequence Characteristics of the *glpT* Gene in Six Isolates

Parameter	Value
Total number of sequences	6
Sequence length (bp)	1052
Variable (polymorphic) sites	7
Invariable (monomorphic) sites	1045
Singleton variable sites	6

Managing *P. aeruginosa* infections in CF patients remains challenging despite advances in treatment. Although the prevalence of *P. aeruginosa* has diminished with the emergence of CFTR modulators, chronic infections often persist.^{27,28} The incidence of multidrug-resistant *P. aeruginosa* (MDR-PA) has been increasing, with one study reporting a rise from 26% to 43% over five years.²⁹ Factors that cause antibiotic resistance include chronic inhaled antibiotic use and history of previous infection.³⁰ *P. aeruginosa* by itself poses a significant challenge in CF treatment due to its various antibiotic resistance mechanisms. The bacterium's ability to form biofilms and persist in hostile environments complicates eradication efforts even further.^{10,31}

Current treatment plans used for cystic fibrosis (CF) patients include combine antibiotics, airway clearance techniques, and CFTR modulators.¹⁸ Inhaled antibiotics such as tobramycin, aztreonam, levofloxacin, and colistin are key elements for long-term control of *P. aeruginosa*, even in patients under treatment with CFTR modulators. This is due to the higher drug concentrations achieved in the airways and lower systemic exposure, which improves efficacy while minimizing systemic side effects.^{1,32,33} However, *P. aeruginosa* strains isolated from CF patients after CFTR modulator therapy persist, showing continuous antibiotic resistance and chronic phenotypes.³⁴ Fosfomycin has recently attracted attention in treating multidrug-resistant (MDR) infections because of its unique mechanism of inhibiting cell wall synthesis.¹⁹ It has shown effectiveness against both Gram-positive and Gram-negative bacteria, with a favorable clinical response rate of 68% in

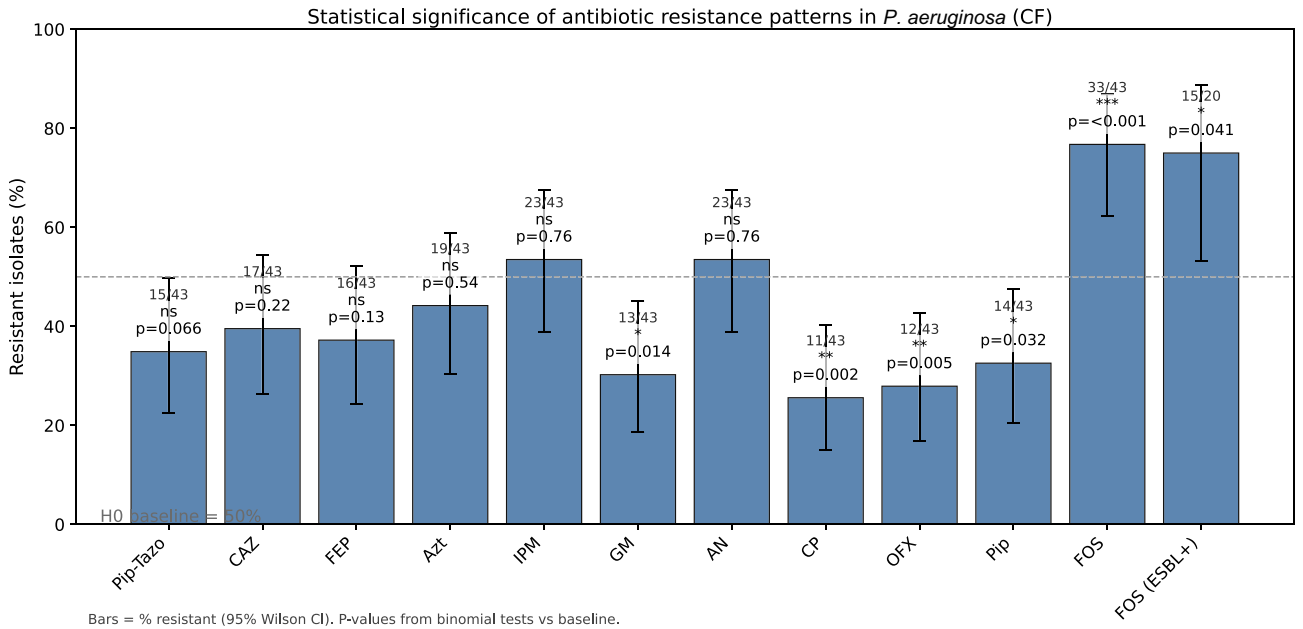


Figure 7 Statistical significance of antibiotic resistance patterns in *P. aeruginosa* isolates from cystic fibrosis (CF) patients. Bar graph depicts resistance percentages (%) against various antibiotics. High resistance noted for FOS (77%), IPM and AN (53%).
Abbreviations: Pip-Tazo, Piperacillin-Tazobactam; CAZ, Ceftazidime; FEP, Cefepime; Azt, Aztreonam; IPM, Imipenem; GM, Gentamicin; AN, Amikacin; CP, Ciprofloxacin; OFX, Ofloxacin; FOS, Fosfomycin; FOS(ESBL+), Fosfomycin against ESBL-positive isolates; Pip, Piperacillin.

one study. However, careful stewardship is crucial, especially since CLSI does not provide recommended breakpoints for *P. aeruginosa*, to preserve the efficacy of this treatment option.³⁵ Novel therapeutic approaches are being explored to combat *P. aeruginosa* infections, including nanotechnology-based drug delivery systems that can overcome the unique mucus conditions in CF patients.³⁶ Emerging therapies aim to disrupt biofilm formation and improve antibiotic efficacy. These include combined-enzyme approaches, such as using N-acylhomoserine lactonase AidH as a quorum-sensing inhibitor with glycosyl hydrolase PslG.³⁷ Virulence-attenuating combination therapy (VACT) shows promise by combining quorum-sensing inhibitors or extracellular polymeric substance repressors with traditional antibiotics to enhance efficacy and reduce resistance development.³⁸ Other emerging strategies include antimicrobial peptides, bacteriophage therapy, vaccines, gallium, and monoclonal antibodies targeting specific *P. aeruginosa* antigens.^{39,40} These innovative approaches offer new hope for treating resistant *P. aeruginosa* infections in CF patients though none are yet approved for widespread clinical use. Several studies, including the current one, have investigated the susceptibility of *Pseudomonas aeruginosa* to different antibiotics. A report by Kalurazi et al found that piperacillin-tazobactam had the lowest resistance rate at 7.3%, while ceftazidime had the highest resistance rate at 34.7%. There is a high level of antibiotic resistance against ceftazidime and gentamicin, which may be due to the severe complications caused by *P. aeruginosa* infections in CF patients. Piperacillin-tazobactam, tobramycin, and amikacin were identified as the most suitable antibiotics for treating respiratory infections caused by *P. aeruginosa* in the studied population.⁴¹

In another study by Bonyadi et al, *Pseudomonas aeruginosa* in CF patients showed high resistance to most antibiotics, with colistin being the most appropriate treatment choice. The strains exhibited the highest resistance to cefotaxime (67%), while colistin had the lowest resistance rate (5%). Overall, high resistance to most studied antibiotics was observed in *Pseudomonas aeruginosa* isolates from CF patients.⁴²

In a study done by Rajae Behbahani et al in 2019, they found that a high percentage of the *P. aeruginosa* isolates (65.91% phenotypically and 84.10% genotypically) were found to produce extended-spectrum beta-lactamases (ESBLs). A majority of the *P. aeruginosa* isolates (71.59%) were found to be multidrug-resistant.⁴³ Similarly, our study's findings confirmed the high resistance of *Pseudomonas aeruginosa* against different antibiotics.

In the study of Berghea et al, the least effective antibiotic against *P. aeruginosa* was gentamicin with 50% sensitivity, and the most effective was colistin with 100% susceptibility. Half of the patients gained antibiotic resistance during the study period. They noticed the most significant increase in antibiotic resistance was to ciprofloxacin. And 28.5% of *P. aeruginosa* isolates were MDR-PA.⁴⁴

Saadh et al reported that the overall proportion of carbapenem resistance (imipenem, meropenem, and doripenem) in CF patients was high, ranging from 28% to 48%. However, carbapenem resistance showed a gradual decrease over time from 1979 to 2021, likely due to the limited clinical effectiveness of these antibiotics in treating CF cases. The pathogens with the highest carbapenem resistance rates in CF patients were *Stenotrophomonas maltophilia*, *Burkholderia* spp., *P. aeruginosa*, and *Staphylococcus aureus*.⁴⁵

Through studies conducted by Bressan et al (2021), it was revealed that fosfomycin exhibits similar activity against both mucoid and non-mucoid strains of *Pseudomonas aeruginosa*. The MIC₅₀ and MIC₉₀ values for fosfomycin against *P. aeruginosa* were determined to be 32 µg/mL and 64 µg/mL, respectively, using the agar dilution method. The automated system used to measure fosfomycin MICs showed a high level of agreement (73%) with the gold standard agar dilution method, indicating that the sensitivity level in this study was 73%.⁴⁶

In the study by Abdulwahab et al (2017), it was found that 19.7% of the CF patients studied were MDR-PA. The MDR-PA isolates exhibited high resistance rates to several common antibiotic classes, including gentamycin, amikacin, and cefepime. Some of the CF patients in the study were receiving inhaled antibiotic treatments, which may have contributed to the emergence of MDR-PA.⁴⁷

Another study by Pallam et al (2019) investigated the efficacy of fosfomycin against MDR-*P. aeruginosa*. Fosfomycin demonstrated good inhibitory effects on biofilms produced by MDR *P. aeruginosa*, inhibiting 88% of strains at concentrations below the minimum inhibitory concentration (MIC).⁴⁸

In a study by Slade-Vitković et al (2022), fosfomycin exhibited synergistic effects when combined with other antipseudomonal antibiotics, particularly ceftazidime, and gentamicin, and enhanced the post-antibiotic effect.⁴⁹

Chavan et al (2022) reported that in a large-scale study of Indian clinical isolates, fosfomycin demonstrated excellent in vitro activity against *Pseudomonas* spp., with a susceptibility rate of 72.4%, outperforming comparator drugs like imipenem and meropenem.⁵⁰

Regarding genes associated with resistance in *P. aeruginosa*, Jafari Sales et al (2017) found that the highest gene frequencies of ESBL genes in the isolates were *bla*_{CTX-M1} (27.27%), *bla*_{CTX-M2} (23.63%), and *bla*_{CTX-M3} (9.09%). The isolates showed the highest resistance to amikacin (81.81%), nalidixic acid (89.09%), and ceftriaxone (75.45%), and the lowest resistance to tetracycline (44.54%) and gentamicin (50.09%). These resistance patterns were associated with extended-spectrum beta-lactamase production. However, other studies found other genes associated with high resistance in *P. aeruginosa* patients. The most common ESBL genes found in the isolates in another study were *bla*_{TEM} (78.41%), *bla*_{SHV} (47.73%), *bla*_{GES} (5.58%), *bla*_{OXA-10} (3.41%), and *bla*_{PSE} (4.55%).⁴³

Mutations in the *bla*_{CTX-M} and *glpT* genes result in a truncated *GlpT* permease, leading to fosfomycin resistance. The fosfomycin-tobramycin combination shows synergistic and bactericidal activity against susceptible isolates and those with low-level tobramycin resistance, potentially preventing the emergence of resistant mutants in both aerobic and anaerobic environments.²² Despite this study, in the recent study, only the frequency of *bla*_{CTX-M} gene is evaluated in front of 2 other fosfomycin-resistant genes, but, similarly, the role of mutated *glpT* and *bla*_{CTX-M} genes among fosfomycin resistant *P. aeruginosa* isolates is remarkable.

Similarly, Ejaz et al (2020) found a high prevalence (18.8%) of ESBL-producing *P. aeruginosa* isolates, with the majority containing the *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} genes. Patients infected with ESBL-producing *P. aeruginosa*, especially neonates, had significantly higher mortality rates. The isolates showed high resistance to cephalosporins but relatively lower resistance to cefoperazone-sulbactam, carbapenems, and piperacillin-tazobactam.⁵¹

The current study's limitations include geographical differences in the strains of *P. aeruginosa* and the potential variation in isolates found in other parts of the world, as well as potential variations in clinical outcomes and resistance levels in Iran due to regional antibiotic prescribing practices.

Finding the resistance patterns in *Pseudomonas aeruginosa* and the genetic basis of it can help the clinicians structure specific targeted therapies for such a rapidly changing bacteria and get a clearer understanding of how different antibiotics work against this pathogen in order to add or delete specific antibiotics from the routine regimen of antibiotics in different medical facilities to ensure optimal treatment options for the patient, least unnecessary expenses for medical facilities and better prognosis and quality of life for CF patients.

Finally, further research is needed to evaluate the long-term impact of these resistance patterns on clinical outcomes in CF patients. Though the high rate of resistant to fosfomycin and other common antibiotics among *P. aeruginosa* isolates in this study, developing alternative therapeutic strategies and new antibiotics is crucial to addressing the growing challenge of antibiotic resistance in *P. aeruginosa*.

Conclusion

This study identified two significant genetic factors associated with resistance in *P. aeruginosa* the *bla*_{CTX-M} gene and the *fosA3* gene (metalloenzyme). Seventeen isolates carried the plasmid *fosA3* gene, indicating its significant role in fosfomycin resistance. The detection of *glpT* mutations in all fosfomycin-resistant isolates, alongside the *fosA3* gene and the frequent coexistence of *bla*_{CTX-M}, underscores the importance of both chromosomal and plasmid-mediated resistance genes. However, the role of plasmidic genes seems more important because of their ability to spread conveniently. Combining antibiotic therapy with careful monitoring of these genes in clinical settings could help manage *P. aeruginosa* infections more effectively. Future studies should focus on genomic and transcriptomic analyses of resistance mechanisms, the impact of biofilms on fosfomycin resistance, and the efficacy of combination therapies to improve treatment outcomes in CF patients.

Disclosure

The authors declare that there are no conflicts of interest for this work.

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