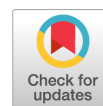


PHENOTYPIC AND GENOTYPIC PROFILING OF EFFLUX PUMP-MEDIATED MULTIDRUG RESISTANCE IN *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES



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Abstract. *Pseudomonas aeruginosa* is an opportunistic pathogen of major global health concern, due to its increasing multidrug resistance (MDR). One of the principal mechanism contributing to its MDR phenotype is the overexpression of efflux pump systems, particularly the MexAB-OprM complex. This study aimed to assess the prevalence, antibiotic susceptibility patterns, and phenotypic and genotypic profiles of efflux pump-mediated resistance in *P. aeruginosa* clinical isolates. A total of one hundred clinical specimens were collected from patients admitted to Al-Khidmat Hospital, Peshawar. Antibiotic susceptibility was performed against ten antibiotics using the Kirby–Bauer disc diffusion method. Efflux pump activity was assessed using the ethidium bromide-agar cartwheel method, while the presence of the *mexA* and *mexB* genes was detected by polymerase chain reaction (PCR). Forty percent of clinical specimens yielded bacterial growth, with *P. aeruginosa* isolates more prevalent in males (54%). High resistance rates were observed against ceftazidime (82%), ciprofloxacin (70%), cefepime (61%), and levofloxacin (66%), whereas higher susceptibility was recorded for imipenem (76%), amikacin (73%), gentamicin (70%), and colistin (100%). Among the isolates, 62.5% were classified as MDR, of which 68% demonstrated phenotypic efflux pump activity. Genotypic analysis revealed that 71% and 82% of MDR isolates harbored the *mexA* and *mexB* genes, respectively. These findings indicate a strong association between efflux pump overexpression and the MDR phenotype in *P. aeruginosa*. The high prevalence of *mexA* and *mexB* genes supports the major contribution of the MexAB-OprM efflux system to antimicrobial resistance in these clinical isolates. This study underscores the importance of routine molecular surveillance and suggests that efflux pump inhibitors (EPIs), in combination with conventional antibiotics, may represent a promising therapeutic strategy. Furthermore, these findings emphasize the need for strengthened antibiotic stewardship and targeted resistance monitoring to effectively manage MDR *P. aeruginosa* infections.

Keywords: multidrug resistance, antibiotic susceptibility testing, MexAB-OprM complex, hospital-acquired infections, bacterial disease, antibiotics.

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Для цитирования:

Улла А., Башир К., Хан А.В., Хан М., Тарик З., Фалак Н., Рехман Ф.У.
Фенотипические и генотипические профили множественной
лекарственной устойчивости, опосредованной эффлюксными
насосами, в клинических изолятах *Pseudomonas aeruginosa* //
Инфекция и иммунитет. 2026. Т. 16, № 2. С. 288–296. doi: 10.15789/2220-
7619-EOE-18024

Citation:

Ullah A., Bashir K., Khan A.W., Khan M., Tariq Z., Falak N., Rehman F.U.
Phenotypic and genotypic profiling of efflux pump-mediated multidrug
resistance in *Pseudomonas aeruginosa* clinical isolates // Russian Journal
of Infection and Immunity = Infektsiya i immunitet, 2026, vol. 16, no. 2,
pp. 288–296. doi: 10.15789/2220-7619-EOE-18024

ФЕНОТИПИЧЕСКИЕ И ГЕНОТИПИЧЕСКИЕ ПРОФИЛИ МНОЖЕСТВЕННОЙ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТИ, ОПОСРЕДОВАННОЙ ЭФФЛЮКСНЫМИ НАСОСАМИ, В КЛИНИЧЕСКИХ ИЗОЛЯТАХ *PSEUDOMONAS AERUGINOSA*

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Резюме. *Pseudomonas aeruginosa* — условно-патогенный микроорганизм, представляющий существенную мировую угрозу для здоровья, главным образом из-за растущей мультирезистентности к антибиотикам. Одним из основных механизмов, способствующих развитию фенотипа множественной лекарственной устойчивости (МЛУ), является гиперэкспрессия систем эффлюксных насосов, в частности комплекса *texAB-OprM*. Целью данного исследования была оценка распространенности, чувствительности к антибиотикам, а также определение фенотипических и генотипических профилей резистентности, опосредованных эффлюксными насосами, в клинических изолятах *P. aeruginosa*. На чувствительность к 10 антибиотикам с применением метода Кирби–Бауэра было изучено 100 клинических образцов из больницы Аль-Хидмат. Активность эффлюксных насосов оценивали методом «колеса тележки» на агаре с бромидом этидия (EtBr). Генотипическое определение генов *texA* и *texB* проводили методом ПЦР. В 40% клинических образцов наблюдался рост бактерий, при этом изоляты *P. aeruginosa* чаще встречались у мужчин (54%). Высокий уровень резистентности наблюдался к нескольким распространенным антибиотикам, в частности к цефалоспорином, фторхинолонам, цефтазидиму (82%), ципрофлоксацину (70%), цефепиму (61%) и левофлоксацину (66%). В отличие от этого, имипенем (76%), амикацин (73%) и гентамицин (70%), а также колистин (100%) продемонстрировали сравнительно более высокую чувствительность. Среди изолятов 62,5% были классифицированы как мультирезистентные штаммы (MDR). Примечательно, что 68% MDR-изолятов проявляли фенотипическую активность эффлюксных насосов. Генотипический анализ подтвердил широкую представленность генов эффлюксных насосов: 71 и 82% MDR-изолятов были положительными на наличие генов *texA* и *texB* соответственно. Гиперэкспрессия эффлюксных насосов, особенно *MexA* и *MexB*, является главным фактором развития множественной лекарственной устойчивости (МЛУ) у *P. aeruginosa*. Выраженная представленность этих генов служит убедительным генетическим доказательством того, что система эффлюкса *texAB-OprM* является доминирующим механизмом резистентности. Полученные результаты подчеркивают необходимость рутинного молекулярного мониторинга активности эффлюксных насосов в клинической диагностике и указывают на терапевтический потенциал ингибиторов эффлюксных насосов (ИЭН). Комбинирование антибиотиков без устойчивости, таких как ципрофлоксацин или цефтазидим, с ИЭН может восстановить их эффективность и расширить возможности лечения инфекций с МЛУ. В целом результаты подчеркивают важность рационального использования антибиотиков, целенаправленного мониторинга резистентности и разработки комбинаций ИЭН-антибиотиков в качестве перспективных стратегий борьбы с резистентными *P. aeruginosa*.

Ключевые слова: множественная лекарственная устойчивость, тестирование чувствительности к антибиотикам, комплекс *texAB-OprM*, внутрибольничные инфекции, бактериальные заболевания, антибиотики.

Introduction

Pseudomonas aeruginosa is a rod-shaped, motile, heterotrophic Gram-negative bacterium, typically measuring 1–5 µm in length and 0.5–1 µm in width. It exhibits metabolic versatility, including the ability to grow anaerobically when supplied with arginine. This ubiquitous environmental bacterium is commonly found in soil, freshwater and marine environments, where it can decompose polycyclic aromatic hydrocarbons (PAH). It is also frequently isolated from wastewater and sinks, both within and outside hospitals, often associated with human and animal contamination [16]. Despite its broad environmental distribution *P. aeruginosa* is often identified near coastal rivers, even in samples from the open

ocean [30]. *P. aeruginosa* is notorious for causing a wide array of healthcare-associated infections (HAIs) in hospitalized patients, including urinary tract infections (UTIs), bloodstream infections, surgical site infections and pneumonia. Its remarkable adaptability enables it to contribute to a broad spectrum of infectious diseases in the general population as well [37]. Infections are particularly common in immunocompromised individuals, such as those with cystic fibrosis, neutropenia, severe burns, cancer, organ transplants and diabetes mellitus, as well as patients in intensive care units (ICUs) [24].

Preventing *P. aeruginosa* infections is vital as medical literature documents numerous outbreaks of nosocomial infections, some traceable to persistent carriage states in healthcare personnel [31].

Treatment of *P. aeruginosa* infections generally encompasses three primary kinds of antibiotics: fluoroquinolones, beta-lactams and aminoglycosides. However, this bacterium can develop high levels of resistance through chromosomal mutations and the acquisition of resistance genes via genomic islands and transposons [27]. Treatment options are limited by antibiotic resistance. Globally, antibiotic-resistant bacteria cause approximately 700 000 fatalities annually with projections indicating this number could reach 10 million by 2050. In the United States alone, antibiotic-resistant bacteria lead to over 2.9 million illnesses and 36 212 fatalities annually [40]. Specific environmental exposures, such as to triclosan, have also been shown to select for multidrug-resistant (MDR) *P. aeruginosa* strains [12].

P. aeruginosa resists antimicrobials through various mechanisms, including intrinsic resistance, acquired resistance and adaptive resistance. Key intrinsic mechanisms involve a low-permeability outer membrane and constitutively expressed efflux pumps that actively expel antibiotics. Resistance can also arise from chromosomal mutations or the acquisition of resistance genes via horizontal gene transfer [8]. This inherent resistance to multiple antimicrobial agents, combined with its propensity to develop resistance during therapy, profoundly limits treatment options [2]. *P. aeruginosa* is notably characterized by the expression of robust efflux pump systems, which confer resistance to multiple classes of antibiotics and are a major contributor to its prominent antibiotic resistance phenotype [7].

Efflux pumps membrane-bound protein complexes that actively expel antibiotics and other toxic compounds from the bacterial cell are critical in exacerbating antibiotic resistance. Overexpression of specific efflux pump systems, such as MexdD-OprJ, MexDF-OprNN, MexBA-OprNM and MexYX-OprA, significantly decreases antibiotic susceptibility [13]. Among these the MexAB-OprM efflux pump is a prominent system in *P. aeruginosa*, responsible for expelling a wide range of antimicrobial agents. This complex consists MexA, a membrane-fusion protein; MexB, a membrane transport factor; and OprM an outer membrane channel, make up this complex [28].

According to Centers for Disease Control and Prevention (CDC), an estimated 50 985 cases of *P. aeruginosa* infections linked to healthcare occur in the US each year. More than 6100 (13%) of these cases are caused by bacterial strains that are resistant to various medications [33]. Similar resistance patterns have been reported in other countries, including Australia, the UK and Denmark. These trends have prompted extensive research into novel therapeutic options, including combination therapies and aerosolized antibiotics such as aztreonam, tobramycin, levofloxacin and liposomal amikacin particularly for patients with cystic fibrosis [11, 38]. In ad-

dition to antibiotic resistance *P. aeruginosa* utilizes a Type III Secretion System (T3SS) to inject effector exotoxins (ExoS, ExoT, ExoU and ExoY) into host cells. These toxins mimic host proteins and interfere with cellular signaling promoting immune evasion and disease progression [22].

Despite ongoing efforts to combat antimicrobial resistance the role of efflux pump overexpression in contributing to MDR phenotypes in clinical isolates of *P. aeruginosa* remains underinvestigated in Pakistan, particularly in the Khyber Pakhtunkhwa region. We hypothesized that efflux pump overexpression, particularly involving the MexAB-OprM system, is a major contributor to the MDR phenotype in local clinical isolates. Therefore, the present study aimed to: Determine the prevalence of MDR among *P. aeruginosa* isolates from a tertiary care hospital in Peshawar. Phenotypically assess efflux pump activity among MDR isolates. Genotypically detect the presence of MexA and MexB efflux pump genes in these isolates.

Materials and methods

This study was conducted at the Microbiology Research Laboratory, Abasyn University, Peshawar. The samples were collected during September 2023 to February 2024.

Sample collection. One hundred clinical samples (blood, urine, and pus) were collected from patients admitted to Al-Khidmat Hospital, Peshawar, during the period from September 2023 to February 2024. All samples were immediately placed in sterile containers, transported in an icebox to the Microbiology Research Laboratory at Abasyn University, Peshawar, and stored at -20°C in a laboratory freezer to preserve bacterial viability until processing. All samples were processed within 24 hours of collection to minimize degradation and ensure reliable microbiological analysis. To avoid duplicate sampling and ensure independence of isolates, only the first culture-positive *P. aeruginosa* isolate from each patient was included in the analysis. Patients of both sexes and all age groups with clinically suspected bacterial infections were eligible for inclusion.

Bacterial isolation and identification. For bacterial isolation, samples were inoculated onto Blood Agar and MacConkey Agar and incubated at 37°C for 24 to 48 hours. Bacterial isolates were initially identified based on colony morphology and Gram staining. Further confirmation of *P. aeruginosa* was performed using standard biochemical assays, including catalase and oxidase tests, and growth on Cetrimide Agar, a selective medium that inhibits most other bacteria while promoting *P. aeruginosa* growth and pyocyanin production [9, 15].

Antibiotic susceptibility testing (AST). Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton

Table 1. Characteristics of clinical samples and *P. aeruginosa* isolates

Parameter	Details	Numbers (%)	<i>P. aeruginosa</i> (+) (%)		<i>P. aeruginosa</i> (–) (%)	
			Male	Female	Male	Female
Sample	Blood	35%	8%	6%	10%	9%
	Urine	25%	6%	4%	9%	8%
	Pus	40%	8%	8%	13%	11%
Total		100%	22%	18%	32%	28%

Agar, strictly following the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines [13, 37]. The panel of antibiotics tested included ceftazidime (30 µg), cefepime (30 µg), piperacillin/tazobactam (110 µg), meropenem (10 µg), imipenem (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg) and gentamicin (10 µg). Isolates demonstrating resistance to three or more classes of antibiotics were categorized as multidrug-resistant (MDR) [10]. MDR strains were evaluated for efflux pump expression.

Phenotypic detection of efflux pump activity. Efflux pump activity was phenotypically assessed using the ethidium bromide (EtBr) agar cartwheel method. Plates prepared with increasing concentrations of EtBr (0.5–2.5 mg/L) were inoculated in a cartwheel pattern with bacterial isolates. Efflux pump activity was determined by observing the extent of fluorescence under UV light after incubation [5].

Genotypic detection of efflux pump genes. Genomic DNA was extracted from bacterial isolates using the thermal lysis method [6]. Polymerase chain reaction (PCR) was performed to detect the efflux pump genes *mexA* and *mexB* using gene-specific primers. For *mexA*, the forward primer was 5'-CAGGCCGTCAGCAAGCAG-3' and the reverse primer was 5'-CCTTGGTGTAGCGCAGGTTG-3, producing an amplicon of 100 bp [34]. For *mexB*, the forward primer was 5'-GTGTTCGGCTCGCAGTACTC-3' and the reverse primer was 5'-AACCGTCCGGGATTGACCTTG-3, generating a 244 bp product [3].

The 25 µL reaction mixture contained 12.5 µL of 4X Master Mix, 3 µL template DNA, 1 µL each of forward and reverse primers and 7.5 µL nuclease-free water. Amplification was carried out under the following cycling conditions: initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 35 seconds, and extension at 72°C for 2 minutes; followed by a final extension at 72°C for 5 minutes. PCR products were resolved by electrophoresis on 1.5% agarose gels stained with ethidium bromide, visualized under UV light, and documented using a gel imaging system [35].

Data analysis. Data obtained from phenotypic and genotypic assays were analyzed using descriptive and inferential statistical methods. Frequencies and percentages were calculated to summarize the distribution of isolates, antibiotic susceptibility patterns and efflux pump expression. Inferential analyses included

one-way ANOVA to determine the effect of patient age groups and gender on antibiotic resistance levels for each tested antibiotic. For the ANOVA, patients were categorized into four age groups: 1–20, 21–40, 41–60, and 61–80 years. Correlation analysis was performed to assess co-resistance patterns among different antibiotics. Additionally, the Chi-square test was used to evaluate the association between efflux pump activity and multidrug resistance (MDR) status. Statistical significance was set at a p-value < 0.05 for all analyses. All statistical tests were performed using SPSS 20 (20 IBM, USA) software.

Results

Sample distribution and *Pseudomonas aeruginosa* isolation. A total of 100 clinical specimens comprising pus (n = 40), blood (n = 35), and urine (n = 25) — were collected and processed. Of these, 40 samples (40%) yielded positive growth for *P. aeruginosa*, resulting in 40 distinct isolates. The distribution of positive isolates across sample types was as follows: 40% from pus, 35% from blood, and 25% from urine. Among all samples, males accounted for 54% (n = 54) and females for 46% (n = 46), with a slightly higher prevalence of *P. aeruginosa* isolation observed in males (Table 1).

Patients were categorized into four age groups: 1–20 years, 21–40 years, 41–60 years and 61–80 years. The mean age of patients was 56.8±15.4 years (range: 12–80 years). The majority of isolates (57%) were from the 61–80 year age group, followed by 28% from 41–60 years, 9% from 21–40 years and 6% from 1–20 years.

Antibiotic susceptibility test of *P. aeruginosa* isolates. Among the 40% *P. aeruginosa* isolates significant antibacterial activity was observed for colistin, showing 100% susceptibility against all isolates. Other highly active antibiotics included imipenem (76% susceptibility), meropenem (75%), and amikacin (73%). In contrast, high resistance rates were noted against several commonly used antibiotics, particularly cephalosporins and fluoroquinolones. The highest resistance was observed against ceftazidime (82%), followed by ciprofloxacin (70%), levofloxacin (66%), and cefepime (61%) (Fig. 1).

One-way ANOVA revealed that patient age had a statistically significant effect on antibiotic resistance patterns for all tested antibiotics (p < 0.05). Resistance levels were generally higher in older pa-

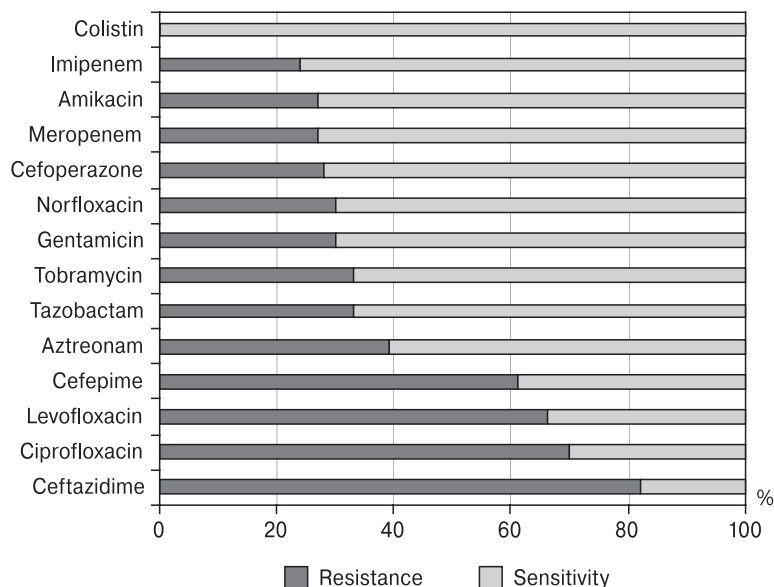


Figure 1. Antibiotic resistance and susceptibility profiles of *P. aeruginosa* isolates (n = 40)

tients (61–80 years). Gender-based analysis showed significant associations between patient gender and resistance to ceftazidime ($p < 0.001$), cefepime ($p = 0.011$), amikacin ($p = 0.011$), and tobramycin ($p = 0.013$). No significant associations were found for gentamicin, tazobactam, or cefoperazone.

Detection of MDR strains. Among the 40 isolates, 25 (62.5%) were multidrug-resistant (MDR; resistant to 3 antibiotic classes), while 15 (37.5%) were non-MDR (Fig. 2). Correlation analysis was performed to identify relationships between the resistance patterns of different antibiotics. The results demonstrated strong positive correlations indicating the presence of co-resistance and multidrug resistance (MDR) phenomena. A very strong positive correlation was observed between resistance to cefepime and amikacin ($r = 0.848$). Similarly, high correlations were found among the fluoroquinolone antibiotics ciprofloxacin, norfloxacin, and levofloxacin with all showing a Pearson correlation coefficient of $r = 0.929$ with each other. These findings suggest that isolates resistant to one antibiotic are highly likely to also ex-

hibit resistance to others, possibly due to shared resistance mechanisms including efflux pump activity. In contrast, resistance to colistin showed no correlation with any other antibiotic and the data exhibited zero variance. This result is consistent with the observation that 100% of the isolates were sensitive to colistin indicating no detected resistance to this antibiotic.

Phenotypic test for efflux pump expression. After assessment of MDR strains of *P. aeruginosa* all strains were analyzed for confirmation of efflux pump activity. Out of the 25 MDR strains, 17 (68%) were found to produce efflux pumps, which is directly related to multidrug resistance. The remaining 8 (32%) were found to not produce efflux pumps (Fig. 3). Chi-square analysis demonstrated a statistically significant association between efflux pump activity and MDR status ($\chi^2 = 22.7$, $df = 1$, $p < 0.0001$), indicating a strong correlation between efflux pump expression and multidrug resistance.

Correlation of efflux pump producers with MDR. The results of the analysis showed that efflux pump

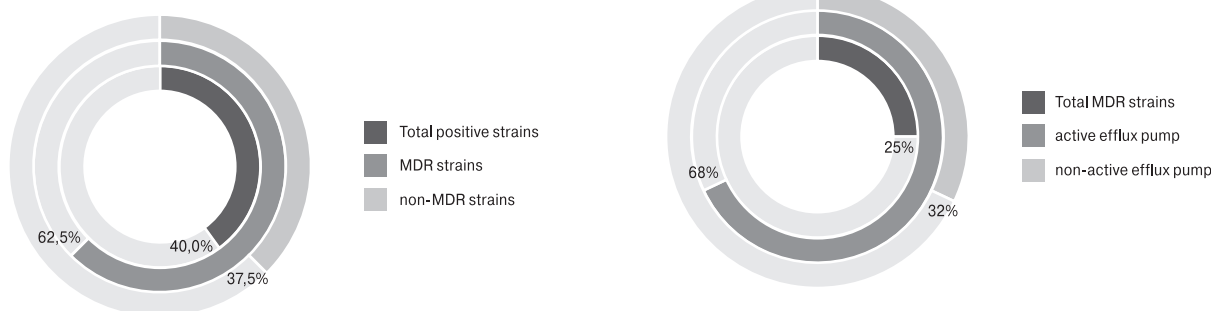


Figure 2. Prevalence of multidrug-resistant (MDR) and non-MDR *P. aeruginosa* strains (n = 40)

Figure 3. Phenotypic assessment of efflux pump activity in multidrug-resistant (MDR) *P. aeruginosa* isolates (n = 25 MDR strains)

expression and multidrug resistance were positively correlated with MDR strains being more likely to produce efflux pumps. 13 (76%) of the 17 strains that produced efflux pumps were resistant to the beta-lactam antibiotic ceftazidime, 11 (65%) to the fluoroquinolone ciprofloxacin, 8 (47%) to levofloxacin and 7 (41%) to Cefepime (Fig. 4).

Amplification of *mexA* and *mexB* gene. Among the 17 *P. aeruginosa* isolates, 12 (71%) tested positive for the *mexA* gene showing efflux pump expression, while 5 (29%) were negative. A 100 bp band was observed using a 100 bp DNA ladder. Similarly, 14 isolates (82%) were positive for the *mexB* gene with a 244 bp band detected, whereas 3 (18%) were negative (Table 2 and Fig. 5).

Discussion

The present study provides insight into the burden of multidrug resistance and efflux pump-mediated antibiotic resistance in *Pseudomonas aeruginosa* isolates recovered from a tertiary care hospital in Peshawar. *P. aeruginosa* was isolated from 40% of clinical specimens, highlighting its substantial contribution to healthcare-associated infections in this setting. Although this isolation rate is higher than that reported by Abdallah et al. (2021), who documented a prevalence of 29.4% across multiple specimen types, such variation is expected due to differences in patient populations, clinical settings, and specimen sources [1].

In the present study, the antibiotic susceptibility profile revealed significant variability. Colistin remained the most potent agent (100% susceptibility), followed by imipenem (76%) and amikacin (73%), aligning with prior studies that have identified carbapenems and aminoglycosides as among the most effective classes [18]. In contrast, high resistance rates were observed against third and fourth generation cephalosporins and fluoroquinolones, including ceftazidime, cefepime, ciprofloxacin, and levofloxacin. These findings align with the resistance patterns reported by Hirsch et al. (2010), who documented

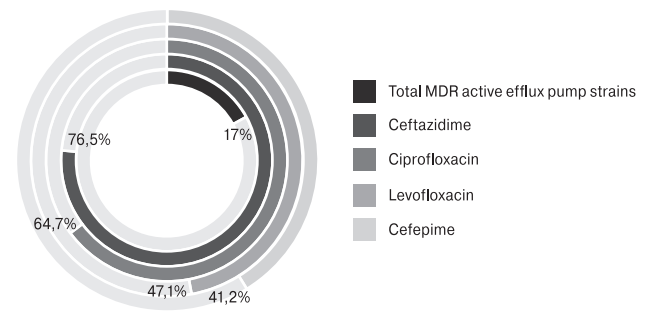


Figure 4. Antibiotic resistance profiles of efflux pump-producing *P. aeruginosa* multidrug-resistant strains (n = 17 efflux pump producers)

extensive resistance to cephalosporins and β -lactam/ β -lactamase inhibitor combinations [20]. Collectively, these findings underscore the critical importance of susceptibility-guided therapy to improve clinical outcomes in *P. aeruginosa* infections.

The treatment of *P. aeruginosa* infections remains challenging due to both intrinsic and acquired resistance mechanisms. Carbapenems, such as imipenem and meropenem, are often considered first-line agents; notably, in our study, they demonstrated relatively high susceptibility rates (76% and 75%, respectively). However, the global rise in carbapenem resistance reported in 10–50% of isolates in some countries [14], underscores the diminishing utility of even these critically important agents. This trend highlights the urgent need to understand and address underlying resistance mechanisms, such as efflux pump overexpression, which contribute to multidrug-resistant phenotypes.

In the present study, 68% (17/25) of multidrug-resistant (MDR) *P. aeruginosa* isolates showed efflux pump activity, and the overall MDR prevalence was 62.5%. This rate is considerably higher than those reported from Canada (5.9–10%), Germany (19%), and Malaysia (19.6%) [25, 26, 42]. However, direct numerical comparisons should be interpreted cautiously, as antimicrobial resistance is heavily influenced by local prescribing practices, infection-control measures, and healthcare infrastructure.

Table 2. Detection frequency of *mexA* and *mexB* genes in efflux pump-positive *P. aeruginosa* isolates (n = 17)

Genes name	Total samples	Detected	Non detected
<i>mex A</i>	17 (100%)	12 (71%)	5 (29%)
<i>mex B</i>	17 (100%)	14 (82%)	3 (18%)

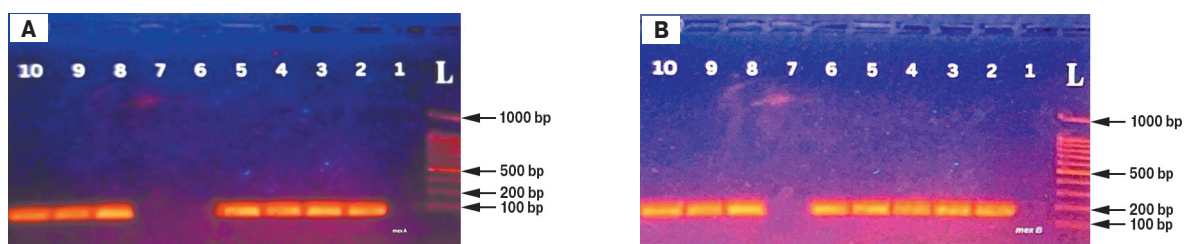


Figure 5. Gel electrophoresis of amplified gene. (A) *mexA* gene (100 bp); (B) *mexB* gene (244 bp)

In Pakistan, factors such as widespread empirical antibiotic use, over-the-counter antibiotic availability, and high patient burdens in tertiary care hospitals may contribute to elevated MDR rates [4].

Efflux pump overexpression is a well-established mechanism contributing to reduced antibiotic susceptibility in *P. aeruginosa* by actively expelling structurally diverse antimicrobial agents. Previous studies have demonstrated high expression frequencies of efflux pump components, including MexB, MexC, MexE, and MexY, particularly among ICU isolates, with corresponding resistance to β -lactams, fluoroquinolones, and carbapenems [41].

In the present study, among the 17 MDR efflux pump-producing isolates, 13 (76.47%) exhibited co-resistance to β -lactams and fluoroquinolones, with resistance rates of 76.5% to ceftazidime and 64.7% to ciprofloxacin. These findings are consistent with previous reports highlighting efflux systems as major contributors to multidrug resistance in *P. aeruginosa* [19, 41]. For instance, Habib et al. (2025) reported active efflux in the majority of MDR isolates examined [19], while other studies have specifically linked efflux pump overexpression to carbapenem and fluoroquinolone resistance [21].

Genotypic analysis in this study confirmed the presence of efflux pump genes in these isolates: 70.6% were positive for *mexA* and 82.4% for *mexB*, supporting the role of the MexAB-OprM system in the observed resistance phenotype. This is consistent with studies that detected *mexAB-oprM* and related genes in amoxicillin-clavulanate-resistant isolates and linked them to broad-spectrum resistance beyond β -lactams, including fluoroquinolones [17, 23, 29]. Another study reported the presence of the MexAB-OprM system in 80% of ciprofloxacin-resistant isolates [32], emphasizing the potential for cross-resistance driven by shared efflux mechanisms.

The remarkable adaptive capacity of *P. aeruginosa* enables it to persist in hostile environments and develop resistance to multiple antibiotic classes. Previous studies, including the present findings, demonstrate that a substantial proportion of clinical isolates harbor efflux pump related genes such as *mexB* and exhibit multidrug resistance to β -lactams, aminoglycosides, fluoroquinolones, and carbapenems [19, 23]. Together, these findings highlight the clinical relevance of efflux pump mediated resistance and underscore the need for targeted antibiotic therapies and further genetic investigations to effectively combat multidrug-resistant *P. aeruginosa* infections.

Conclusion

This study revealed a high prevalence of multidrug-resistant *Pseudomonas aeruginosa* (62.5%) among clinical isolates from a tertiary care hospital in Peshawar. A strong phenotypic association was observed between efflux pump activity and the MDR phenotype, with 68% of MDR isolates exhibiting efflux pump function. Genotypic analysis confirmed the frequent presence of the *mexA* and *mexB* genes in these isolates. Collectively, these findings demonstrate the frequent occurrence of efflux pump activity and efflux-related genes among MDR *P. aeruginosa* and underscore the need for ongoing surveillance and antimicrobial stewardship to address resistant infections.

Acknowledgment

We acknowledge the Abasyn university, Peshawar for providing the necessary facilities and resources to conduct this research

Additional information

Authors contribution. Abdullah: Methodology; Kashif Bashir: Supervision; Abdul Waheed Khan: Investigation; Faiz Ur Rehman and Zarkish Tariq: Manuscript writing, Editing; Moheb Khan: Review; Khuzin Dinislam: Data Curation.

Competing interest. The authors have declared that there is no conflict of interest regarding the publication of this article.

Availability of data and materials. Data is used within manuscript.

Consent for publication. Not Applicable.

Funding. The authors received no financial support for the research, authorship, and/or publication of this article.

Ethics approval and consent to participate. Studies have been approved by the Ethical committee of Department of health and biological sciences Abasyn university (Date: 25.12.2023/Approval Number: 135).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

All methods used in this study were performed in accordance with the relevant guidelines and regulations.

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Поступила в редакцию 27.09.2025
 Отправлена на доработку 26.11.2025
 Принята к печати 05.03.2026

Received 27.09.2025
 Revision received 26.11.2025
 Accepted 05.03.2026