

EFFLUX PUMPS AND ANTIBIOTIC RESISTANCE IN *P. AERUGINOSA* 10.15789/2220-7619-EOE-18024
EFFECT OF EFFLUX PUMP EXPRESSION ON ANTIBIOTIC SENSITIVITY PATTERNS IN PSEUDOMONAS AERUGINOSA: PHENOTYPIC AND GENOTYPIC PROFILING OF CLINICAL ISOLATES

AbdUllah A. ^a,

Bashir K. ^a,

Khan A.W. ^b,

Khan Moheb ^c,

Tariq Z. ^d,

Falak Niaz ^e,

Rehman F. U. ^f

^a Abasyn University, Khyber Pakhtunkhwa, Peshawar, Pakistan

^b Institute of Biotechnology & Microbiology, Bacha Khan University, Charsadda, Pakistan

^c Center for Circadian Clocks, Soochow University, Suzhou, Jiangsu, China

^d University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan

^e Riphah International University, Malakand Campus Chakdara, Khyber Pakhtunkhwa, Pakistan

^f Government Superior Science College Peshawar, Khyber Pakhtunkhwa, Pakistan

ВЛИЯНИЕ ЭКСПРЕССИИ ЭФФЛЮКС-НАСОСА НА ХАРАКТЕРИСТИКИ ЧУВСТВИТЕЛЬНОСТИ К АНТИБИОТИКАМ У PSEUDOMONAS AERUGINOSA: ФЕНОТИПИЧЕСКОЕ И ГЕНОТИПИЧЕСКОЕ ПРОФИЛИРОВАНИЕ КЛИНИЧЕСКИХ ИЗОЛЯТОВ

Абдулла А. ¹,

Башир К. ¹,

Хан А.В. ²,

Хан Мохеб ³,

Тарик З. ⁴,

Фалак Ниаз ⁵,

Рехман Ф. У. ⁶

¹ Университет Абасин, Хайбер-Пахтунхва, Пешавар, Пакистан

² Институт биотехнологии и микробиологии, Университет Бача Хана, Чарсадда, Пакистан

³ Центр циркадных ритмов, Сучжоуский университет, Сучжоу, Цзянсу, Китай

⁴ Университет науки и технологий, Банну, Хайбер-Пахтунхва, Пакистан

⁵ Международный университет Рифах, кампус Малаканд, Чакдара, Хайбер-Пахтунхва, Пакистан

⁶ Государственный высший научный колледж, Пешавар, Хайбер-Пахтунхва, Пакистан

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen of significant global health concern, largely due to its escalating resistance to multiple antibiotics. A major mechanism contributing to its multidrug resistance (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.

One hundred clinical specimens were collected from Al-Khidmat Hospital. Antibiotic susceptibility was tested against ten antibiotics using the Kirby-Bauer method, while efflux pump activity was assessed via the ethidium bromide–agar cartwheel method. Genotypic detection of mexA and mexB genes was performed by PCR.

Forty percent of clinical samples yielded bacterial growth, with *P. aeruginosa* isolates more prevalent in males (54%). High resistance rates were observed against several commonly used antibiotics, particularly cephalosporins, fluoroquinolones, ceftazidime (82%), ciprofloxacin (70%), cefepime (61%) and levofloxacin (66%). In contrast, imipenem (76%), amikacin (73%) and gentamicin (70%) colistin showing (100%) susceptibility demonstrated comparatively higher susceptibility. Among the isolates, 62.5% were classified as MDR strains. Notably, 68% of MDR isolates exhibited phenotypic efflux pump activity. Genotypic analysis confirmed the widespread presence of efflux pump genes, with 71% and 82% of MDR isolates positive for *mexA* and *mexB*, respectively. Efflux pump overexpression, particularly involving *mexA* and *mexB* is a major driver of MDR in *P. aeruginosa*. The widespread detection of these genes provides strong genetic evidence that the *mexAB-OprM* efflux system is the dominant resistance mechanism in this population. These findings underscore the need for routine molecular surveillance of efflux activity in clinical diagnostics and highlight the therapeutic potential of efflux pump inhibitors (EPIs). Combining otherwise resistance antibiotics, such as

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ciprofloxacin or ceftazidime, with EPIs may restore their efficacy and expand treatment options for MDR infections. Overall, the results emphasize the importance of antibiotic stewardship, targeted resistance monitoring and the development of EPI–antibiotic combinations as future strategies to manage *P. aeruginosa* resistance.

Keywords: Multidrug resistance, Antibiotic susceptibility testing, mexAB-OprM complex, Hospital-acquired infections, bacterial disease, Antibiotics.

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 было оценить распространенность, особенности чувствительности к антибиотикам, а также фенотипические и генотипические профили резистентности, опосредованной эффлюксными насосами, в клинических изолятах *P. aeruginosa*.

Было изучено сто клинических образцов из больницы Аль-Хидмат ea чувствительность к десяти антибиотикам с применением метода Кирби-Бауэра. 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 *mexAB-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 *mexAB-OprM*, Внутрибольничные инфекции, бактериальные заболевания, Антибиотики.

1 **1 Introduction**

2 *Pseudomonas aeruginosa* is a rod-shaped, motile, heterotrophic Gram-
3 negative bacterium, typically measuring 1–5 µm in length and 0.5–1 µm in width. It
4 exhibits metabolic versatility, including the ability to grow anaerobically when
5 supplied with arginine. This ubiquitous environmental bacterium is commonly
6 found in soil, freshwater and marine environments, where it can decompose
7 polycyclic aromatic hydrocarbons (PAH). It is also frequently isolated from
8 wastewater and sinks, both within and outside hospitals, often associated with
9 human and animal contamination [1]. Despite its broad environmental distribution
10 *P. aeruginosa* is often identified near coastal rivers, even in samples from the open
11 ocean [2]. *P. aeruginosa* is notorious for causing a wide array of healthcare-
12 associated infections (HAIs) in hospitalized patients, including urinary tract
13 infections (UTIs), bloodstream infections, surgical site infections and pneumonia.
14 Its remarkable adaptability enables it to contribute to a broad spectrum of infectious
15 diseases in the general population as well [3]. Infections are particularly common in
16 immunocompromised individuals, such as those with cystic fibrosis, neutropenia,
17 severe burns, cancer, organ transplants and diabetes mellitus, as well as patients in
18 intensive care units (ICUs) [4].

19 Preventing *P. aeruginosa* infections is vital as medical literature documents
20 numerous outbreaks of nosocomial infections, some traceable to persistent carriage
21 states in healthcare personnel [5].

22 Treatment of *P. aeruginosa* infections generally encompasses three
23 primary kinds of antibiotics: fluoroquinolones, beta-lactams and aminoglycosides.
24 However, this bacterium can develop high levels of resistance through chromosomal
25 mutations and the acquisition of resistance genes via genomic islands and
26 transposons [6]. Treatment options are limited by antibiotic resistance. Globally,
27 antibiotic-resistant bacteria cause approximately 700,000 fatalities annually with
28 projections indicating this number could reach 10 million by 2050. In the United
29 States alone, antibiotic-resistant bacteria lead to over 2.9 million illnesses and

30 36,212 fatalities annually [7]. Specific environmental exposures, such as to triclosan,
31 have also been shown to select for multidrug-resistant (MDR) *P. aeruginosa* strains
32 [8].

33 *P. aeruginosa* resists antimicrobials through various mechanisms,
34 including intrinsic resistance, acquired resistance and adaptive resistance. Key
35 intrinsic mechanisms involve a low-permeability outer membrane and constitutively
36 expressed efflux pumps that actively expel antibiotics. Resistance can also arise
37 from chromosomal mutations or the acquisition of resistance genes via horizontal
38 gene transfer [9]. This inherent resistance to multiple antimicrobial agents,
39 combined with its propensity to develop resistance during therapy, profoundly limits
40 treatment options [10]. *P. aeruginosa* is notably characterized by the expression of
41 robust efflux pump systems, which confer resistance to multiple classes of
42 antibiotics and are a major contributor to its prominent antibiotic resistance
43 phenotype [11].

44 Efflux pumps membrane-bound protein complexes that actively expel
45 antibiotics and other toxic compounds from the bacterial cell are critical in
46 exacerbating antibiotic resistance. Overexpression of specific efflux pump systems,
47 such as *mexdD-OprJ*, *mexDF-OprNN*, *mexBA-OprNM* and *mexYX* (-OprA),
48 significantly decreases antibiotic susceptibility [12]. Among these the *mexAB-OprM*
49 efflux pump is a prominent system in *P. aeruginosa*, responsible for expelling a wide
50 range of antimicrobial agents. This complex consists *mexA*, a membrane-fusion
51 protein; *mexB*, a membrane transport factor; and *oprM* an outer membrane channel,
52 make up this complex [13].

53 According to Centers for Disease Control and Prevention (CDC), an
54 estimated 50,985 cases of *P. aeruginosa* infections linked to healthcare occur in the
55 US each year. More than 6,100 (13%) of these cases are caused by bacterial strains
56 that are resistant to various medications [14]. Similar resistance patterns have been
57 reported in other countries, including Australia, the UK and Denmark. These trends
58 have prompted extensive research into novel therapeutic options, including

59 combination therapies and aerosolized antibiotics such as aztreonam, tobramycin,
60 levofloxacin and liposomal amikacin particularly for patients with cystic fibrosis
61 [15, 16]. In addition to antibiotic resistance *P. aeruginosa* utilizes a Type III
62 Secretion System (T3SS) to inject effector exotoxins (*exoS*, *exoT*, *exoU* and *exoY*)
63 into host cells. These toxins mimic host proteins and interfere with cellular signaling
64 promoting immune evasion and disease progression [17].

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

75 **2 Materials and methods**

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

79 **Sample Collection**

80 One hundred clinical samples (blood, urine, and pus) were collected from
81 patients admitted to Al-Khidmat Hospital, Peshawar, during the period from
82 September 2023 to February 2024. All samples were immediately placed in sterile
83 containers, transported in an icebox to the Microbiology Research Laboratory at
84 Abasyn University, Peshawar, and stored at -20°C in a laboratory freezer to preserve
85 bacterial viability until processing. All samples were processed within 24 hours of
86 collection to minimize degradation and ensure reliable microbiological analysis. To
87 avoid duplicate sampling and ensure independence of isolates, only the first culture-

88 positive *P. aeruginosa* isolate from each patient was included in the analysis.
89 Patients of both sexes and all age groups with clinically suspected bacterial
90 infections were eligible for inclusion.

91 Bacterial Isolation and Identification

92 For bacterial isolation, samples were inoculated onto Blood Agar and
93 MacConkey Agar and incubated at 37 °C for 24 to 48 hours. Bacterial isolates were
94 initially identified based on colony morphology and Gram staining. Further
95 confirmation of *P. aeruginosa* was performed using standard biochemical assays,
96 including catalase and oxidase tests, and growth on Cefrimide Agar, a selective
97 medium that inhibits most other bacteria while promoting *P. aeruginosa* growth and
98 pyocyanin production [18,19].

99 Antibiotic Susceptibility Testing (AST)

100 Antibiotic susceptibility testing was performed using the Kirby-Bauer disc
101 diffusion method on Mueller-Hinton Agar, strictly following the Clinical and
102 Laboratory Standards Institute (CLSI) 2023 guidelines [20, 21]. The panel of
103 antibiotics tested included ceftazidime (30µg), cefepime (30µg),
104 piperacillin/tazobactam (110 µg), meropenem (10µg), imipenem (10µg), aztreonam
105 (30µg), ciprofloxacin (05µg), levofloxacin (05µg), norfloxacin (10µg) and
106 gentamicin (10µg). Isolates demonstrating resistance to three or more classes of
107 antibiotics were categorized as multidrug-resistant (MDR) [22]. MDR strains were
108 evaluated for efflux pump expression.

109 Phenotypic Detection of Efflux Pump Activity

110 Efflux pump activity was phenotypically assessed using the ethidium bromide
111 (EtBr) agar cartwheel method. Plates prepared with increasing concentrations of
112 EtBr (0.5–2.5 mg/L) were inoculated in a cartwheel pattern with bacterial isolates.
113 Efflux pump activity was determined by observing the extent of fluorescence under
114 UV light after incubation [23].

115 Genotypic Detection of Efflux Pump Genes

116 Genomic DNA was extracted from bacterial isolates using the thermal lysis
117 method [24]. Polymerase Chain Reaction (PCR) was performed to detect the efflux
118 pump genes *mexA* and *mexB* using gene-specific primers. For *mexA*, the forward
119 primer was 5'-CAGGCCGTCAGCAAGCAG-3' and the reverse primer was 5'-
120 CCTTGGTGTAGCGCAGGTTG-3, producing an amplicon of 100 bp [25]. For
121 *mexB*, the forward primer was 5'-GTGTTCGGCTCGCAGTACTC-3' and the
122 reverse primer was 5'-AACCGTCGGGATTGACCTTG-3, generating a 244 bp
123 product [26].

124 The 25 μ L reaction mixture contained 12.5 μ L of 4X Master Mix, 3 μ L
125 template DNA, 1 μ L each of forward and reverse primers and 7.5 μ L nuclease-free
126 water. Amplification was carried out under the following cycling conditions: initial
127 denaturation at 94 °C for 5 minutes; 35 cycles of denaturation at 94 °C for 30
128 seconds, annealing at 56 °C for 35 seconds, and extension at 72 °C for 2 minutes;
129 followed by a final extension at 72 °C for 5 minutes. PCR products were resolved
130 by electrophoresis on 1.5% agarose gels stained with ethidium bromide, visualized
131 under UV light, and documented using a gel imaging system [27].

132 Data analysis

133 Data obtained from phenotypic and genotypic assays were analyzed using
134 descriptive and inferential statistical methods. Frequencies and percentages were
135 calculated to summarize the distribution of isolates, antibiotic susceptibility patterns
136 and efflux pump expression. Inferential analyses included one-way ANOVA to
137 determine the effect of patient age groups and gender on antibiotic resistance levels
138 for each tested antibiotic. For the ANOVA, patients were categorized into four age
139 groups: 1–20, 21–40, 41–60, and 61–80 years. Correlation analysis was performed
140 to assess co-resistance patterns among different antibiotics. Additionally, the Chi-
141 square test was used to evaluate the association between efflux pump activity and
142 multidrug resistance (MDR) status. Statistical significance was set at a p-value <
143 0.05 for all analyses. All statistical tests were performed using SPSS 20 (20 IBM,
144 USA) software.

145 **3 Results**

146 Sample Distribution and *Pseudomonas aeruginosa* Isolation

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

154 Patients were categorized into four age groups: 1–20 years, 21–40 years, 41–
155 60 years and 61–80 years. The mean age of patients was 56.8 ± 15.4 years (range:
156 12–80 years). The majority of isolates (57%) were from the 61–80 year age group,
157 followed by 28% from 41–60 years, 9% from 21–40 years and 6% from 1–20 years.

158 Antibiotic susceptibility test of *P. aeruginosa* isolates

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

166 One-way ANOVA revealed that patient age had a statistically significant
167 effect on antibiotic resistance patterns for all tested antibiotics ($p < 0.05$). Resistance
168 levels were generally higher in older patients (61–80 years). Gender-based analysis
169 showed significant associations between patient gender and resistance to ceftazidime
170 ($p < 0.001$), cefepime ($p = 0.011$), amikacin ($p = 0.011$), and tobramycin ($p = 0.013$).
171 No significant associations were found for gentamicin, tazobactam, or cefoperazone.

172 Detection of MDR strains

173 Among the 40 isolates, 25 (62.5%) were multidrug-resistant (MDR; resistant
174 to 3 antibiotic classes), while 15 (37.5%) were non-MDR (Figure 2). Correlation
175 analysis was performed to identify relationships between the resistance patterns of
176 different antibiotics. The results demonstrated strong positive correlations indicating
177 the presence of co-resistance and multidrug resistance (MDR) phenomena. A very
178 strong positive correlation was observed between resistance to cefepime and
179 amikacin ($r = 0.848$). Similarly, high correlations were found among the
180 fluoroquinolone antibiotics ciprofloxacin, norfloxacin, and levofloxacin with all
181 showing a Pearson correlation coefficient of $r = 0.929$ with each other. These
182 findings suggest that isolates resistant to one antibiotic are highly likely to also
183 exhibit resistance to others, possibly due to shared resistance mechanisms including
184 efflux pump activity. In contrast, resistance to colistin showed no correlation with
185 any other antibiotic and the data exhibited zero variance. This result is consistent
186 with the observation that 100% of the isolates were sensitive to colistin indicating
187 no detected resistance to this antibiotic.

188 Phenotypic test for Efflux pump expression

189 After assessment of MDR strains of *P. aeruginosa* all strains were analyzed
190 for confirmation of efflux pump activity. Out of the 25 MDR strains, 17 (68%) were
191 found to produce efflux pumps, which is directly related to multidrug resistance. The
192 remaining 8 (32%) were found to not produce efflux pumps. Result of the Ethidium
193 Bromide-Agar Cartwheel method is also shown in Figure 3. Chi-square analysis
194 demonstrated a statistically significant association between efflux pump activity and
195 MDR status ($\chi^2 = 22.7$, $df = 1$, $p < 0.0001$), indicating a strong correlation between
196 efflux pump expression and multidrug resistance.

197 Correlation of Efflux pump producers with MDR

198 The results of the analysis showed that efflux pump expression and multidrug
199 resistance were positively correlated with MDR strains being more likely to produce
200 efflux pumps. 13 (76%) of the 17 strains that produced efflux pumps were resistant

201 to the beta-lactam antibiotic ceftazidime, 11 (65%) to the fluoroquinolone
202 ciprofloxacin, 8 (47%) to levofloxacin and 7 (41%) to Cefepime (Figure 4).

203 Amplification of *Mex A* and *Mex B* gene

204 Among the 17 *P. aeruginosa* isolates, 12 (71%) tested positive for the *MexA*
205 gene showing efflux pump expression, while 5 (29%) were negative. A 100 bp band
206 was observed using a 100 bp DNA ladder. Similarly, 14 isolates (82%) were positive
207 for the *MexB* gene with a 244 bp band detected, whereas 3 (18%) were negative.
208 These findings are presented in (Figure 5).

209 **4 Discussion**

210 The present study provides insight into the burden of multidrug resistance and
211 efflux pump-mediated antibiotic resistance in *Pseudomonas aeruginosa* isolates
212 recovered from a tertiary care hospital in Peshawar. *P. aeruginosa* was isolated from
213 40% of clinical specimens, highlighting its substantial contribution to healthcare-
214 associated infections in this setting. Although this isolation rate is higher than that
215 reported by Abdallah *et al.* (2021), who documented a prevalence of 29.4% across
216 multiple specimen types, such variation is expected due to differences in patient
217 populations, clinical settings, and specimen sources [28].

218 In the present study, the antibiotic susceptibility profile revealed significant
219 variability. Colistin remained the most potent agent (100% susceptibility), followed
220 by imipenem (76%) and amikacin (73%), aligning with prior studies that have
221 identified carbapenems and aminoglycosides as among the most effective classes
222 [29]. In contrast, high resistance rates were observed against third and fourth
223 generation cephalosporins and fluoroquinolones, including ceftazidime, cefepime,
224 ciprofloxacin, and levofloxacin. These findings align with the resistance patterns
225 reported by Hirsch *et al.* (2010), who documented extensive resistance to
226 cephalosporins and β -lactam/ β -lactamase inhibitor combinations [30]. Collectively,
227 these findings underscore the critical importance of susceptibility-guided therapy to
228 improve clinical outcomes in *P. aeruginosa* infections.

229 The treatment of *P. aeruginosa* infections remains challenging due to both
230 intrinsic and acquired resistance mechanisms. Carbapenems, such as imipenem and
231 meropenem, are often considered first-line agents; notably, in our study, they
232 demonstrated relatively high susceptibility rates (76% and 75%, respectively).
233 However, the global rise in carbapenem resistance reported in 10–50% of isolates in
234 some countries [31], underscores the diminishing utility of even these critically
235 important agents. This trend highlights the urgent need to understand and address
236 underlying resistance mechanisms, such as efflux pump overexpression, which
237 contribute to multidrug-resistant phenotypes.

238 In the present study, 68% (17/25) of multidrug-resistant (MDR) *P. aeruginosa*
239 isolates showed efflux pump activity, and the overall MDR prevalence was 62.5%.
240 This rate is considerably higher than those reported from Canada (5.9–10%),
241 Germany (19%), and Malaysia (19.6%) [32, 33, 34]. However, direct numerical
242 comparisons should be interpreted cautiously, as antimicrobial resistance is heavily
243 influenced by local prescribing practices, infection-control measures, and healthcare
244 infrastructure. In Pakistan, factors such as widespread empirical antibiotic use, over-
245 the-counter antibiotic availability, and high patient burdens in tertiary care hospitals
246 may contribute to elevated MDR rates [35].

247 Efflux pump overexpression is a well-established mechanism contributing to
248 reduced antibiotic susceptibility in *P. aeruginosa* by actively expelling structurally
249 diverse antimicrobial agents. Previous studies have demonstrated high expression
250 frequencies of efflux pump components, including *mexB*, *mexC*, *mexE*, and *mexY*,
251 particularly among ICU isolates, with corresponding resistance to β -lactams,
252 fluoroquinolones, and carbapenems [36]. Similar observations were reported by
253 Rana *et al.* (2015), who identified active efflux in all MDR isolates examined and
254 confirmed the presence of the *mexABR* operon using multiplex PCR [37].

255 In the present study, among the 17 MDR efflux pump-producing isolates, 13
256 (76.47%) exhibited co-resistance to beta-lactams and fluoroquinolones, specifically
257 76.5% to ceftazidime and 64.7% to ciprofloxacin. These observations align with

258 studies highlighting efflux systems as major contributors to multidrug resistance in
259 *P. aeruginosa* [36, 37]. For instance, Rana *et al.* (2015) reported active efflux in all
260 MDR isolates examined [37], while other work has linked efflux pump
261 overexpression specifically to carbapenem and fluoroquinolone resistance [38].

262 Genotypic analysis in this study confirmed the presence of efflux pump
263 genes in these isolates: 70.6% were positive for *mexA* and 82.4% for *mexB*,
264 supporting the role of the *mexAB-OprM* system in the observed resistance
265 phenotype. This is consistent with studies that detected *mexAB-OprM* and related
266 genes in amoxicillin-clavulanate-resistant isolates and linked them to broad-
267 spectrum resistance beyond beta-lactams, including fluoroquinolones [39, 40, 41].
268 Another study reported the presence of the *mexAB-OprM* system in 80% of
269 ciprofloxacin-resistant isolates [42], emphasizing the potential for cross-resistance
270 driven by shared efflux mechanisms.

271 The remarkable adaptive capacity of *P. aeruginosa* enables it to persist in
272 hostile environments and develop resistance to multiple antibiotic classes
273 simultaneously. Previous studies have shown that a large proportion of clinical
274 isolates harbor multiple efflux-related genes and exhibit resistance to β -lactams,
275 aminoglycosides, tetracyclines, and carbapenems [37]. Together, these findings
276 highlight the clinical relevance of efflux pump mediated resistance and underscore
277 the need for targeted antibiotic therapies and further genetic investigations to
278 effectively combat multidrug-resistant *P. aeruginosa* infections.

279 **5 Conclusion**

280 This study revealed a high prevalence of multidrug-resistant *Pseudomonas*
281 *aeruginosa* (62.5%) among clinical isolates from a tertiary care hospital in
282 Peshawar. A strong phenotypic association was observed between efflux pump
283 activity and the MDR phenotype, with 68% of MDR isolates exhibiting efflux pump
284 function. Genotypic analysis confirmed the frequent presence of the *mexA* and *mexB*
285 genes in these isolates. Collectively, these findings demonstrate the frequent
286 occurrence of efflux pump activity and efflux-related genes among MDR *P.*

287 *aeruginosa* and underscore the need for ongoing surveillance and antimicrobial
288 stewardship to address resistant infections. Acknowledgment

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291 **AUTHORS CONTRIBUTION**

292 Abdullah: Methodology

293 Kashif Bashir: Supervision

294 Abdul Waheed Khan: Investigation

295 Faiz Ur Rehman and Zarkish Tariq: Manuscript writing, Editing

296 Moheb Khan: Review

297 Khuzin Dinislam: Data Curation

298 Declaration

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309 Ethics approval and consent to participate

310 Studies have been approved by the Ethical committee of Department of health
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313 All procedures performed in studies involving human participants were in
314 accordance with the ethical standards of the institutional and/or national research

315 committee and with the 1964 Helsinki declaration and its later amendments or
316 comparable ethical standards

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

ТАБЛИЦЫ

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%

Table 2. Detection frequency of MexA and MexB genes in efflux pump-positive *P. aeruginosa* isolates (m=17).

Genes name	Total samples	Detected	Non detected
<i>Mex A</i>	17 (100%)	12 (71%)	05(29%)
<i>Mex B</i>	17(100%)	14(82%)	03(18%)

РИСУНКИ

Figure 1. Age group distribution of patients (n=100)

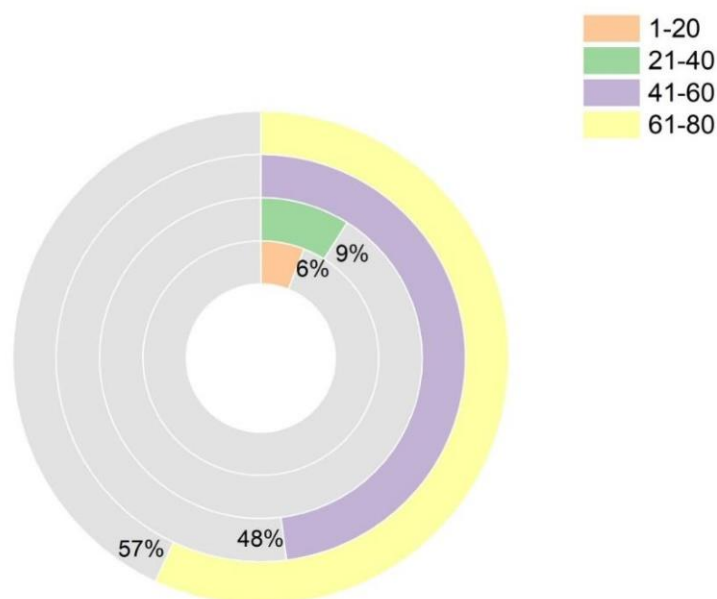


Figure 2. Antibiotic resistance and susceptibility profiles of *Pseudomonas aeruginosa* isolates (n = 40)

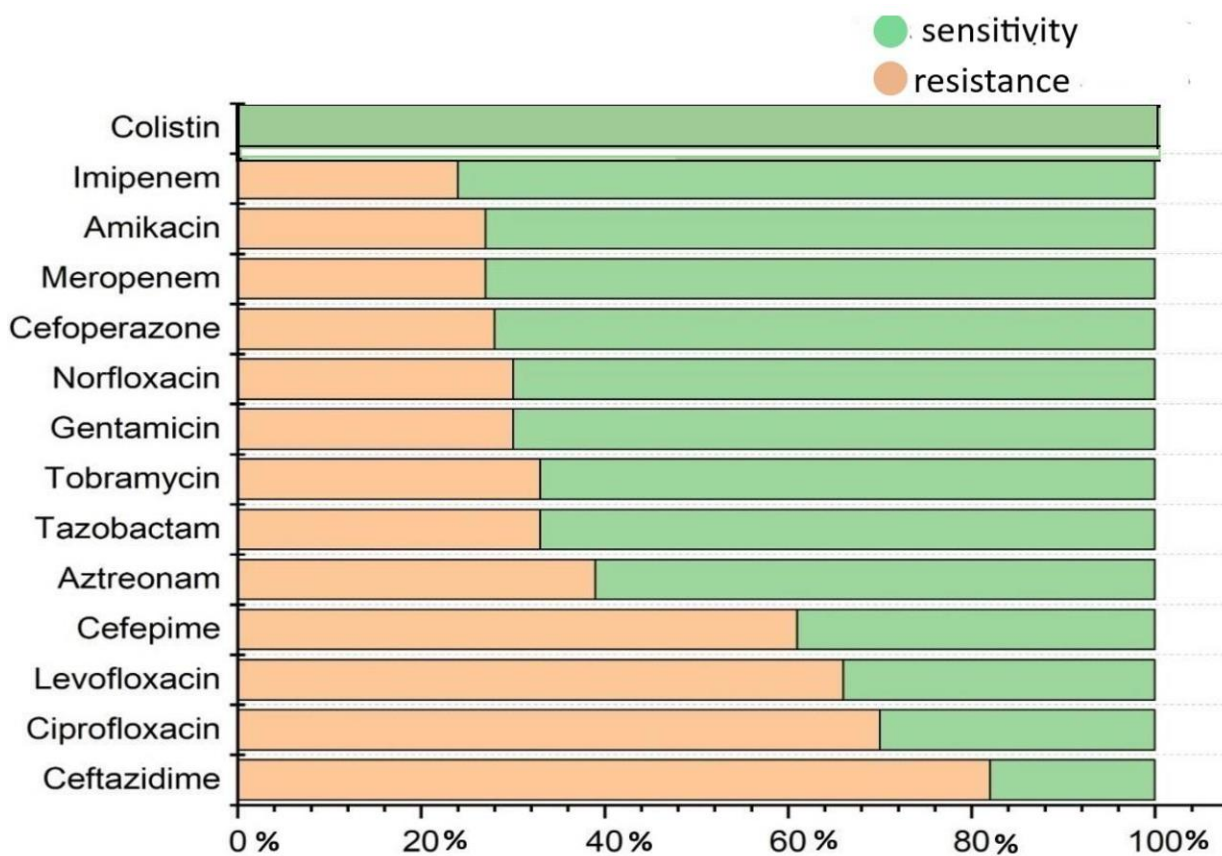


Figure 3. Prevalence of multidrug-resistant (MDR) and non-MDR *P. aeruginosa* strains (n=40)

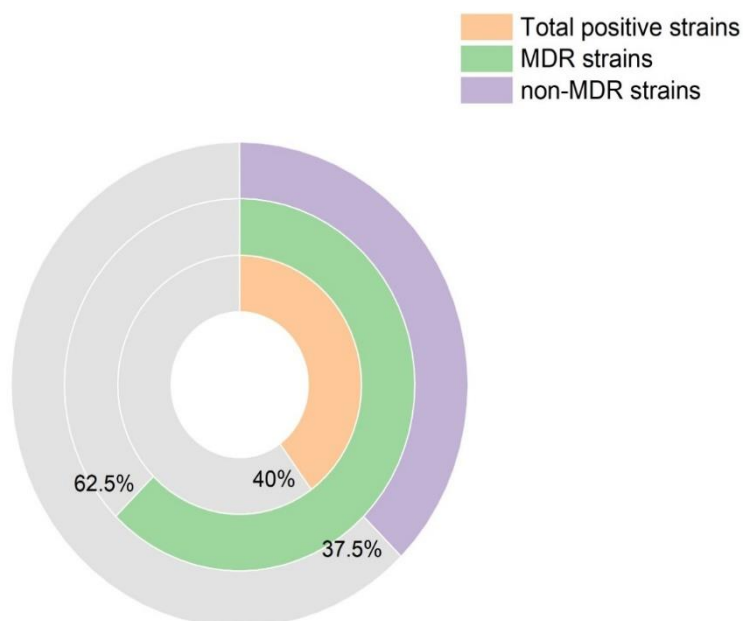


Figure 4. Phenotypic Assessment of Efflux Pump Activity in Multidrug-Resistant (MDR) *P. aeruginosa* Isolates (n=25 MDR strains)

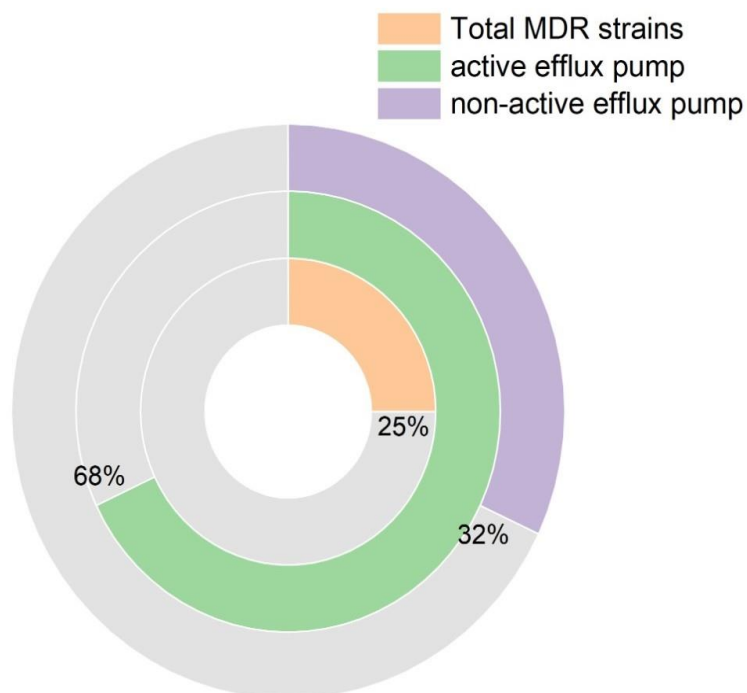


Figure 5. Antibiotic resistance profiles of Efflux pump-producing *P. aeruginosa* multidrug-resistant Strains (n=17 efflux pump producers)

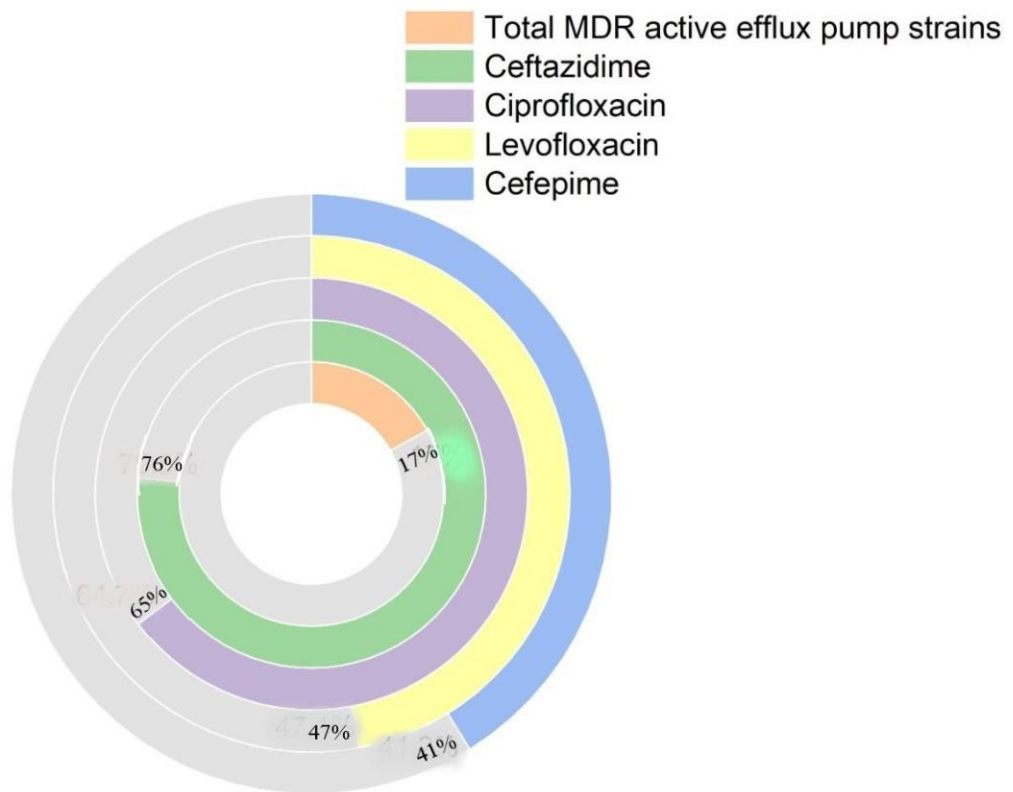
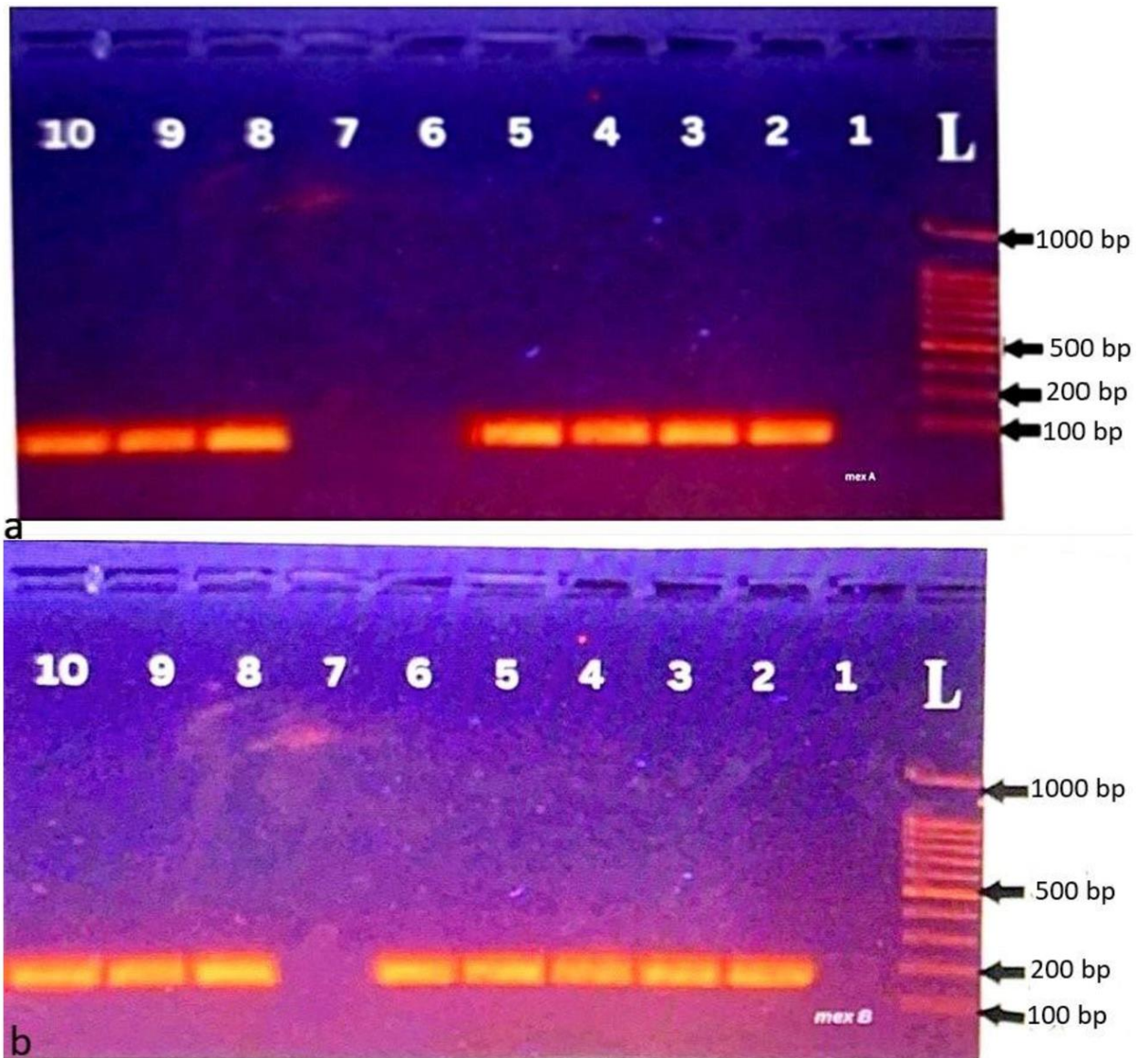


Figure 6. Gel electrophoresis of amplified gene. A. MexA Gene (100 bp).b. MexB gene (244 bp).



Блок 1. Информация об авторе ответственном за переписку

Faiz Ur Rehman, M.Phil, Lecturer;

Affiliation: Department of Zoology, Government Superior Science College
Peshawar, 25000, Khyber Pakhtunkhwa, Pakistan;

Tel 00923481195739;

E-mails: Faiz02140@gmail.com

Блок 2. Информация об авторах

AbdUllah A., M.Phil, Student;

Affiliation: Department of health and biological sciences Abasyn university 25000,
Khyber Pakhtunkhwa, Pakistan;

Kashif Bashir, PhD, Lecturer;

Affiliation: Department of health and biological sciences Abasyn university 25000,
Khyber Pakhtunkhwa, Pakistan;

Abdul Waheed Khan, PhD, Student

Affiliation: Institute of Biotechnology & Microbiology, Bacha Khan University,
Charsadda, Pakistan;

Moheb Khan, PhD, Scholar;

Affiliation: Center for Circadian Clocks, Soochow University, Suzhou 215123,
Jiangsu, China

Zarkish Tariq, Student

Affiliation: Department of Zoology, University of Science and Technology, Bannu,
Khyber Pakhtunkhwa, Pakistan

Falak Niaz, M.Phil

Affiliation: Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, Malakand Campus Chakdara 18800, Khyber Pakhtunkhwa, Pakistan

Faiz Ur Rehman, M.Phil, Lecturer

Affiliation: Department of Zoology, Government Superior Science College Peshawar, 25000, Khyber Pakhtunkhwa, Pakistan

Блок 1. Информация об авторе ответственном за переписку

Фаиз Ур Рехман, магистр филологии, преподаватель;

Место работы: Кафедра зоологии, Государственный высший научный колледж Пешавара, 25000, Хайбер-Пахтунхва, Пакистан;

Тел: 00923481195739;

Электронная почта: Faiz02140@gmail.com

Блок 2. Информация об авторах

Абдулла А., магистр философии, студент;

Место работы: Факультет здравоохранения и биологических наук Абасинского университета 25000, Хайбер-Пахтунхва, Пакистан;

Кашиф Башир, доктор философии, преподаватель;

Место работы: Факультет здравоохранения и биологических наук Абасинского университета 25000, Хайбер-Пахтунхва, Пакистан;

Абдул Вахид Хан, доктор философии, студент

Место работы: Институт биотехнологии и микробиологии, Университет Бача Хана, Чарсадда, Пакистан;

Мохеб Хан, доктор философии, исследователь;

Место работы: Центр циркадных ритмов, Сучжоуский университет, Сучжоу 215123, провинция Цзянсу, Китай

Заркиш Тарик, студент

Место работы: Кафедра зоологии, Университет науки и технологий, Банну, провинция Хайбер-Пахтунхва, Пакистан

Фалак Ниаз, магистр наук

Место работы: Факультет реабилитации и смежных медицинских наук,
Международный университет Рифах, кампус Малаканд, Чакдара 18800,
провинция Хайбер-Пахтунхва, Пакистан

Фаиз Ур Рехман, магистр наук, преподаватель

Место работы: Кафедра зоологии, Государственный высший научный
колледж Пешавара, 25000, провинция Хайбер-Пахтунхва, Пакистан

Блок 3. Метаданные статьи

EFFECT OF EFFLUX PUMP EXPRESSION ON ANTIBIOTIC SENSITIVITY PATTERNS IN *PSEUDOMONAS AERUGINOSA*: PHENOTYPIC AND GENOTYPIC PROFILING OF CLINICAL ISOLATES
ВЛИЯНИЕ ЭКСПРЕССИИ ЭФФЛЮКС-НАСОСА НА ХАРАКТЕРИСТИКИ ЧУВСТВИТЕЛЬНОСТИ К АНТИБИОТИКАМ У *PSEUDOMONAS AERUGINOSA*: ФЕНОТИПИЧЕСКОЕ И ГЕНОТИПИЧЕСКОЕ ПРОФИЛИРОВАНИЕ КЛИНИЧЕСКИХ ИЗОЛЯТОВ

Сокращенное название статьи для верхнего колонтитула:

EFFLUX PUMPS AND ANTIBIOTIC RESISTANCE IN *P. AERUGINOSA*
ЭФФЛЮКСНЫЕ НАСОСЫ И АНТИБИОТИКОРЕЗИСТЕНТНОСТЬ У *PSEUDOMONAS AERUGINOSA*

Keywords: Multidrug resistance, Antibiotic susceptibility testing, mexAB-OprM complex, Hospital-acquired infections, bacterial disease, Antibiotics.

Ключевые слова: Множественная лекарственная устойчивость, Тестирование чувствительности к антибиотикам, комплекс mexAB-OprM, Внутрибольничные инфекции, бактериальные заболевания, Антибиотики.

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