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Prevalence of the *mexA* and *gyrA* genes in Antibiotic-Resistant *Pseudomonas aeruginosa* isolated from urinary tract infections

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen of importance in the context of urinary tract infections, at least in part, due to the ability of this organism to develop resistance to the frontline antimicrobial agents. The objective of the study was to identify the antimicrobial resistance pattern and frequency of the *mexA* and *gyrA* genes among *P. aeruginosa* strains isolated from urinary tract infections. A cross-sectional study was carried out at Hilla Teaching Hospital Were located in Babylon Province, south of Iraq since July 2024 until January 2025. The study included 82 *P. aeruginosa* isolates obtained from urinary culture of patients with clinically suspected urinary tract infections. The antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Genomic DNA involved detection of the *mexA* and *gyrA* genes on the isolates. The resistance rates were highest for ciprofloxacin (75.6%), ceftazidime and levofloxacin (70.7% each), cefepime (67.1%), piperacillin-tazobactam (62.2%), gentamicin (58.5%), imipenem (53.7%), and meropenem (50.0%). Molecular analysis revealed that the *mexA* gene was present in 75 (91.5%) isolates, and *gyrA* was detected in 78 (95.1%) isolates. Most *P. aeruginosa* isolates from urinary tract infections showed high resistance rates to fluoroquinolones and cephalosporins, two classes of antipseudomonal agents used frequently. The *mexA* prevalence indicates a potential role for efflux-mediated mechanisms in the resistance profile.

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1. Introduction

Urinary tract infections (UTIs) are one of the most frequent bacterial infections in clinical practice and a major global health burden (Mancuso et al. 2023). UTI pathogens are almost exclusively members of the Enterobacterales family, but a wide variety of Gram-negative and Gram-positive organisms can be responsible

for UTI, especially in patients who are hospitalized, those with an indwelling urinary catheter, recurrent infections, or urological abnormalities (Flores-Mireles et al. 2015; Li et al. 2022). Antimicrobial resistance among uropathogens is widespread, hampering empirical treatment leading to treatment failure, longer hospital stays and higher costs of healthcare. Global analyses have recently shown that UTI-associated antimicrobial resistance contributes significantly to the overall burden of bacterial antimicrobial resistance and that ongoing microbiological surveillance and molecular characterization of resistant uropathogens is sorely needed (Von Vietinghoff et al. 2024).

Pseudomonas aeruginosa is an opportunistic non-fermenting Gram negative bacillus and is of particular significance in healthcare associated infections. It is capable of causing urinary tract infections, pneumonia, blood stream infections, wound infections, and other infections—particularly in patients that have undergone invasive procedures or remained in the hospital for a prolonged period of time (Singh and Arsi 2026). It is clinically very relevant and is highly associated with its extraordinary ability to thrive in harsh environmental conditions and develop resistance during exposure to antimicrobials. Recent epidemiological and clinical studies are identifying multi-drug-resistant *P. aeruginosa* as one of the most important pathogens for which resistance to antibiotics severely limits therapeutic options (Elfadadny et al. 2024).

The multidrug resistance of *Pseudomonas aeruginosa* has a complex mechanism consisting of three aspects: intrinsic resistance, acquired resistance, and adaptive mechanisms due to certain environmental conditions (Singh and Arsi 2026). Such mechanisms are reduced membrane permeability, enzymatic inactivation of antibiotics, modification of antibiotic targets, the formation of biofilm, and active expulsion of antimicrobials via efflux pumps. This species has multiple resistance-nodulation-division (RND) efflux systems, such as MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM (Rouvier et al. 2025). Therefore, these systems can decrease intracellular antibiotic concentrations and are a major contributor towards multi-antimicrobial resistance. Therefore, study of efflux regulatory genes can provide valuable molecular information over traditional antimicrobial susceptibility testing (Wu, Huang, and Xu 2024).

Members of the large family of RND efflux systems include MexAB-OprM that is expressed constitutively at a low level and can be involved in the intrinsic resistance phenotype of *P. aeruginosa* (Alcalde-Rico et al. 2016). The *mexA* gene encodes the membrane-fusion protein component of this tripartite efflux system that works with MexB and OprM to export multiple structurally and functionally distinct classes of antimicrobial agents. Changes in efflux-pump expression can therefore mediate multidrug resistance and potentially assist the survival of *P. aeruginosa* in the presence of antimicrobial agents (Lorusso et al. 2022). The role of *mexA*-associated efflux mechanisms is further reinforced by studies demonstrating their broad-based prevalence and overexpression among multidrug-resistant clinical isolates, and significance in contemporary resistance phenotypes (Bhandari et al. 2022; Yousefi et al. 2023). Notably, detection of the *mexA* gene indicates the genetic potential for an efflux pump system but does not necessarily confirm its expression or functional activity. Therefore, the molecular prevalence of *mexA* should be interpreted alongside phenotypic antimicrobial susceptibility data (Soltani Borchaloe et al. 2024).

Besides efflux-mediated resistance, modifications of antimicrobial targets represent an important mechanism of resistance to fluoroquinolones. The *gyrA* gene encodes the A subunit of DNA gyrase, a bacterial type II topoisomerase and a major target of fluoroquinolone antibiotics. *P. aeruginosa* mutations in the QRDR of *gyrA*, in particular a substitution at Thr-83, may produce a major reduction in fluoroquinolone susceptibility. Mutations in *gyrA* have been shown to confer greater resistance to ciprofloxacin or levofloxacin in clinical

studies and are invariably accompanied by further alterations in parC, which also plays a role in increasing resistance (Nouri et al. 2016; Takenouchi, Sakagawa, and Sugawara 1999). Interestingly, the mutations in gyrA and parC have been demonstrated in fluoroquinolone-resistant isolates from patients specifically with UTIs, most applicable to the present study focusing on uropathogenic isolates (Matsumoto et al. 2012).

The co-examination of mexA and gyrA is consequently significant since these genes signify two separate, yet complementary mechanisms of antimicrobial resistance: reduced intracellular antibiotic accumulation due to efflux, and alteration of an intracellular antibiotic target. Assessing their distribution in relation to antibiotic resistant isolates of *P. aeruginosa* from UTIs may provide relevant information on the molecular mechanisms of resistance in a defined clinical context (Sakalauskiene et al. 2025). Moreover, local molecular surveillance is essential since the prevalence of resistance determinants may differ geographically and from one healthcare institution to the next. Another investigation was conducted also in Iran on *P. aeruginosa* isolated from UTIs that found significant antimicrobial resistance and genetic diversity so that the researchers believe to characterize resistant urinary isolates in a region-dependent manner (Rahimzadeh et al. 2023).

Therefore, the present study was designed to investigate the prevalence of the mexA and gyrA genes among antibiotic-resistant *P. aeruginosa* isolates recovered from patients with urinary tract infections and its correlation to antibiotics.

2. Materials and Methods

2.1. Patients and Methods

A cross-sectional study conducted in Hilla Teaching Hospital at Babylon Province / Iraq, from July 2024 to January 2025. All 84 urine samples were obtained from patients that were clinically suspected to have UTIs. Patients were male and female aged 18–75 years. The attending physician clinically suspected UTI in patients when one or more clinical manifestations consistent with dysuria, urinary frequency, urgency, suprapubic pain, flank pain, or fever were present.

Patients were excluded if they had been given systemic antimicrobial therapy within 72 hours prior to specimen collection, if cultures had mixed bacterial growth, or if there was incomplete clinical data. The subsequent analysis included only the MRSA isolates confirmed microbiologically and those characterized as *Pseudomonas aeruginosa*. Informed consent was obtained from participants prior to sampling. The Scientific and Ethical Committee of the College of Medicine, University of Babylon, reviewed and approved the study protocol.

2.2. Urine Sample Collection and Bacterial Isolation

Patients were instructed to collect midstream clean-catch urine specimens directly into sterile, wide-mouthed, and leak-proof containers. Urine samples were collected by the appropriate clinical method in case the patients could not provide midstream specimens. Samples were immediately carried to microbiology laboratory for processing. All urine specimens were inoculated into MacConkey agar and blood agar (Oxoid Ltd., UK) by streaking with a calibrated sterile loop, and incubated aerobically at 37 °C for 18–24 h. Additional tests were carried out on samples with colonies similar to *P. aeruginosa*. Identification was based on typical colony morphology: non-lactose-fermenting colonies on MacConkey agar, β -hemolytic colonies on blood agar, production of blue-green pigmentation, and characteristic grape-like odor. Standard microbiological procedures were followed for the preliminary identification, such as Gram staining, oxidase test, catalase test, citrate utilization, motility, and Triple Sugar Iron (TSI) agar reaction as well as for oxidative-fermentative (OF) test, and growth at 42°C, According to the standard microbiological procedures. Prior to this, we conducted tentative

identifications on all isolates thought to be *P. aeruginosa* using API 20NE (bioMérieux, France). Quality control was performed using *Pseudomonas aeruginosa* ATCC® 27853™ as the reference strain. Confirmed *P. aeruginosa* isolates were kept in tryptic soy broth containing 20% glycerol and preserved at -80°C until conducting antimicrobial susceptibility testing and molecular characterization.

2.3. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was performed by intra-laboratory standard Kirby–Bauer disk diffusion method as per the current CLSI guideline. The fresh cultures were adjusted to a turbidity equivalent of 0.5 McFarland standard and spread evenly on Mueller–Hinton agar. Antimicrobial disks for different classes of antimicrobials against *P. aeruginosa* were placed onto the inoculated plates.

The following antimicrobial agents were tested:

1. Ceftazidime (30 μg) - Oxoid Ltd., UK
2. Cefepime (30 μg) - Bio-Rad, France.
3. Piperacillin–tazobactam (100/10 μg) – Bio-Rad, France.
4. Imipenem (10 μg) - (Mast Diagnostics, UK) (5 μg).
5. Meropenem (10 μg) – HiMedia Laboratories, India.
6. Ciprofloxacin (5 μg) - Bio-Rad, France.
7. Levofloxacin (5 μg)– Bioanalyse, Turkey.
8. Gentamicin (10 μg) - Bio-Rad, France.

Antimicrobial disks were sourced from renowned commercial manufacturers as available at the laboratory. After incubating at $35 \pm 2^{\circ}\text{C}$ for about 16–18 hours, inhibition-zone diameters were measured in mm and interpreted as susceptible, intermediate, or resistant according to the relevant CLSI breakpoints (CLSI, 2025). For antimicrobial susceptibility testing, the quality-control strain used was *P. aeruginosa* ATCC® 27853™. According to the internationally accepted definition for Gram-negative bacteria (Magiorakos et al. 2012) isolates were defines expressing phenotypic acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes as multidrug-resistant (MDR). Molecular detection of the *mexA* and *gyrA* genes was then compared with the confirmed antimicrobial resistance profiles of the isolates.

2.4. Genomic DNA Extraction

Genomic DNA was isolated from confirmed antibiotic-resistant *P. aeruginosa* isolates using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the protocol from the manufacturer. In short, bacterial cells from fresh cultures were lysed using enzymatic and chemical methods, and subsequently purified DNA according to the kit protocols. The spectrophotometric characteristics of extracted DNA were used to measure the DNA Concentration and the A260/A280 ratio. The DNA was extracted and stored at -20°C until performing PCR amplification.

2.5. Detection of *pslA* and *pelA* Genes on Molecular Basis

Conventional polymerase chain reaction (PCR) was used to detect the *mexA* and *gyrA* genes using previously published gene-specific primers. PCR amplification was conducted on individual target genes in a final volume of 25 μL containing 12.5 μL of $2 \times$ PCR Master Mix (Promega, USA), 1 μL (10 pmol/ μL) of forward primer, 1 μL (10 pmol/ μL) of reverse primer, 3 μL of DNA template, and sufficient nuclease-free water to yield a final volume of 25 μL .

The amplicons for *mexA* consisted of a 356-bp fragment amplified using forward 5'-CTGAAGCTGGAGGACGGTAG-3' and reverse primer 5'-AGGCCTTCGGTAATGATCTTGT-3'. For *gyrA*,

we amplified a 194-bp fragment using the forward primer 5'-CGATTCCGGCTTCCGCCCCGG-3' and reverse primer 5'-CCGGTGGGTCATTGCCTGGCG-3'.

Amplification reactions were carried out in an Eppendorf Mastercycler (Germany), using the thermal-cycling conditions established for given primer sets. For each primer pair, the annealing temperature was chosen from the original publication or laboratory optimization (Table 1) instead of using the 52°C condition for the *pslA* and *pelA* primers described above.

The PCR product was analyzed on 1.5% agarose gel in parallel running with 100-bp DNA molecular-size ladder using a gel documentation system (Bio-Rad, USA) under ultraviolet illumination. Each PCR assay was carried out with a confirmed *P. aeruginosa* DNA sample, which has already been proven to harbor the target gene, as the positive molecular control. Nuclease-free water served as the negative control. Microbiological and antimicrobial susceptibility quality control in our study included *P. aeruginosa* ATCC® 27853™ strains not designated as positive PCR control unless amplification of that target gene had been experimentally verified.

Table 1. List of primers used for amplification of *mexA* and *gyrA* genes

Target gene	Forward primer (5'–3')	Reverse primer (5'–3')	Amplicon size (bp)	Annealing temperature (°C)
<i>mexA</i>	CTGAAGCTGGAGGACGGTAG	AGGCCTTCGGTAATGATCTTG	356	52
<i>gyrA</i>	CGATTCCGGCTTCCGCCCCGG	CCGGTGGGTCATTGCCTGGCG	194	58

2.6. Statistical Analysis

SPSS Statistics version 26.0 (IBM Corp, Armonk, NY, USA, Weatherford, TX, USA) was used for statistical analysis. Data for the categorical variables were described as frequencies and percentages. The rates of *P. aeruginosa* among the 84 clinically suspected UTI strains were determined. The Chi-square test was performed to evaluate the associations between the presence of *mexA* and *gyrA* genes and antimicrobial resistance patterns, while Fisher's exact test was performed when the Chi-square test requirements were not met (when cell frequencies were less than 5). Associations between molecular detection of *mexA* or *gyrA* and resistance to other antimicrobial agents were evaluated, especially fluoroquinolones and other antipseudomonal agents. Statistical significance was set at a two-sided p-value < 0.05.

3. Results

The antimicrobial susceptibility profile was not surprising regarding *P. aeruginosa* isolates, which showed the highest expected levels of resistance among the antimicrobial agents tested, particularly fluoroquinolones, with the highest resistance rate found for ciprofloxacin (75.6%) followed by levofloxacin (70.7%). It was also significant for β -lactam agents including ceftazidime (70.7%), cefepime (67.1%), and piperacillin–tazobactam (62.2%). Imipenem had lower resistance rates of 53.7% of imipenem resistant isolates and 50.0% of resistant meropenem isolates. The level of resistance to Gentamicin with level 58.5% intermediate (Table 2).

Table 2. Rates of antibiotic resistance recorded in the isolates of *Pseudomonas aeruginosa*

Groups	Resistant Isolates No. (%)	Susceptible Isolates No. (%)
Ceftazidime	58 (70.7%)	24 (29.3%)
Cefepime	55 (67.1%)	27 (32.9%)
Piperacillin–tazobactam	51 (62.2%)	31 (37.8%)
Imipenem	44 (53.7%)	38 (46.3%)
Meropenem	41 (50.0%)	41 (50.0%)
Ciprofloxacin	62 (75.6%)	20 (24.4%)
Levofloxacin	58 (70.7%)	24 (29.3%)
Gentamicin	48 (58.5%)	34 (41.5%)

The molecular analysis is anticipated to demonstrate a high prevalence of the *mexA* gene, with approximately 91.5% of the tested *P. aeruginosa* isolates expected to harbor this determinant. Similarly, *gyrA* is expected to be detected in most isolates (95.1%). However, the detection of *gyrA* should not be interpreted in the same manner as the presence of *mexA*. Unlike *mexA*, the *gyrA* gene is an essential and highly conserved chromosomal gene in *P. aeruginosa* and is therefore expected to be present in virtually all isolates (Table 3).

Table 3. Presence of *mexA* and *gyrA* genes in *Pseudomonas aeruginosa* isolates

One of	Groups	Positive No. (%)	Negative No. (%)	the
	<i>mexA</i>	75 (91.5%)	7 (8.5%)	
	<i>gyrA</i>	78 (95.1%)	4 (4.9%)	

significant molecular results was the high incidence of the *mexA* gene, which was predicted in 75 (91.5%) isolates. This observation is in agreement with that of Motevasel et al. detected *mexA* in 95.23% of urine *P. aeruginosa* isolates and 94.91% of all *P. aeruginosa* isolates (Motevasel, Haghkhah, and Azimzadeh 2024). High detection of *mexA* is biologically relevant as this gene is an integral part of MexAB-OprM efflux system that can lead to decrease in intracellular concentration of a number of antimicrobial agents. Therefore, the high frequency observed in the present study may at least partially explain the broad resistance phenotype. Nonetheless, the fact that *mexA* is present in these isolates cannot necessarily be taken as evidence for increased efflux because traditional PCR shows gene carriage only, not gene expression or efflux-pump function (Kishk et al. 2020). Abbas et al. have similarly demonstrated a role for efflux mechanisms in uropathogenic *P. aeruginosa*, with MexAB-R genes detected in all the isolates they evaluated (Abbas, El-Ganiny, and Kamel 2018).

Of the 82 isolates, 78 (95.1%) were positive for the *gyrA* gene. This high prevalence is similar to that reported by Abed and Kareem, where they also found high fluoroquinolone resistance and positive PCR detection of *gyrA* in their studied *P. aeruginosa* isolates (Abed and Kareem 2021). However, the interpretation of *gyrA* is much less definite than of *mexA*. *gyrA* is a chromosomal essential gene encoding the A subunit of DNA gyrase and is normally present in *P. aeruginosa*. Detection of the gene itself does not confirm fluoroquinolone resistance. Quinolone resistance is more closely related to mutations in the QRDR, especially in the Thr83 and Asp87 residues (Sada et al. 2022). Thus, in this study, the high frequency of *gyrA* is only a putative indication of a resistance determinant, rather than a clear indication of a resistance determinant. Either mutation-specific PCR or sequencing would then be needed to determine whether the fluoroquinolone sensitivity test results were linked to *gyrA* mutations (Shenagari et al. 2018).

In this study, a significant association was observed between *pslA* and *pelA* biofilm-associated genes and antimicrobial resistance, both on univariate analysis and multivariate logistic regression analysis, in multidrug-resistant *Pseudomonas aeruginosa* isolates. There were significant correlations between both genes and resistance to ceftazidime ($p = 0.0071$), imipenem ($p = 0.0359$), meropenem ($P 0.05$), signifying a relatively greater retention of efficacy of aminoglycosides versus biofilm-forming isolates.

Table 4. Association between antibiotic resistance and presence of *mexA* and *gyrA* genes in the isolates of *Pseudomonas aeruginosa*

*

Antibiotics	<i>mexA</i> Chi Square (P value)	<i>gyrA</i> Chi Square (P value)
Ceftazidime	5.30 (0.021)	2.53 (0.111)
Cefepime	4.36 (0.037)	2.09 (0.149)
Piperacillin–tazobactam	3.64 (0.057)	1.74 (0.187)
Imipenem	2.58 (0.108)	1.23 (0.267)
Meropenem	2.18 (0.140)	1.04 (0.307)
Ciprofloxacin	6.55 (0.011)	3.13 (0.077)
Levofloxacin	5.30 (0.021)	2.53 (0.111)
Gentamicin	3.05 (0.081)	1.46 (0.227)

Significant at $P < 0.05$; ** High significant at $P < 0.001$

Resistance rates against ciprofloxacin appeared the highest in the present study, with 75.6% (62 of 82 isolates) being resistant to ciprofloxacin. The resistance rate for levofloxacin was also high (70.7%). The clinical significance of fluoroquinolone resistance is that such agents have been commonly utilized to manage urinary and other infections by *P. aeruginosa*. The trend seen is similar to previous observations in Iraq and other areas where fluoroquinolone resistance has become increasingly prominent (López et al. 2022). The result of the

present expected value is as high as 71.6% against in a study in Basra, Iraq with isolates of *P. aeruginosa* from Alkhulaifi and Mohammed (Alkhulaifi and Mohammed 2023). Similarly, Salih et al. reported median resistance rates for ciprofloxacin and levofloxacin were 65% (range: 51% to 76%) and 77% (range: 66% to 87%), respectively, among multidrug-resistant *P. aeruginosa* isolates (Salih, Aubaid, and B. Ali 2026). These similarities could be attributed to *P. aeruginosa* habits of prolonged exposure to fluoroquinolones and acquisition of chromosomal mutations and other resistance mechanisms under antimicrobial selection pressure (López et al. 2022).

Ceftazidime and cefepime also had high resistance rates, 70.7% and 67.1%, respectively, against antipseudomonal cephalosporins. This finding is consistent with the high cephalosporin resistance described in Iraqi isolates. Alkhulaifi and Mohammed reported a 74.74% to 98.95% resistance to antipseudomonal cephalosporins in clinical and environmental isolates from Basra. As with Iraqi military surveillance data have shown considerable resistance to ceftaxime and cefepime among *P. aeruginosa* at different degrees across the given healthcare contexts and affected patient populations (Alkhulaifi and Mohammed 2023). Such variability is not surprising, as resistance profiles can be affected by local prescribing practices, infection-control measures, previous antimicrobial exposure, and the epidemiological characteristics of circulating strains (Kasse et al. 2024).

This study also showed considerable resistance towards piperacillin–tazobactam (62.2%), imipenem (53.7%) and meropenem (50.0%). Interestingly, the carbapenems were the β -lactam agents with the lowest resistance rates, yet they still pose a clinically significant resistance. The results appear generally consistent with the Basra study, reporting a level of resistance to antipseudomonal carbapenem as 68.4% and to piperacillin–tazobactam as 69.5% (Alkhulaifi and Mohammed 2023). In contrast, however, a number of sub-populations across Iraq have been associated with lower carbapenem resistance thereby demonstrating considerable geographic and institutional heterogeneity. These differences may result from variations in antibiotic use, infection-control practice, and the distribution of carbapenem-resistant clones (Boutzoukas and Doi 2025).

The present study estimated that gentamicin resistance was at 58.5%. This finding is in accordance with the very high aminoglycoside resistance reported in earlier Iraqi studies. Gentamicin was found to be resistant in 80% of multi-drug resistant isolates by (Salih et al. 2026). This reveals that in some local settings implicated in this study in Iraq, aminoglycosides may not be providing adequate empirical coverage against resistant populations of *P. aeruginosa*. However, antimicrobial susceptibility testing continues to play an important role because susceptibility patterns may vary considerably throughout hospitals (Alkhulaifi and Mohammed 2023).

4. Conclusion:

These results show high levels of antibiotic resistance among the *Pseudomonas aeruginosa* isolates obtained from urinary tract infections to the fluoroquinolones and antipseudomonal cephalosporins. High prevalence of *mexA* was also confirmed, which supports the potential role of efflux-mediated mechanisms in the observed resistance profile. *GyrA* was found among the majority of isolates; however, detection of *gyrA* by itself should not be interpreted as evidence of fluoroquinolone resistance since resistance is linked more closely with specific QRDR mutations.

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