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ORIGINAL ARTICLE

# Molecular characterization of plasmid-mediated quinolone resistance genes in clinical isolates of *Pseudomonas aeruginosa* from Al-Muthanna Province, Iraq

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## ABSTRACT

**Background and Objectives:** Fluoroquinolones represent a class of antibiotics commonly used to manage *Pseudomonas aeruginosa* infections. The appearance of fluoroquinolone resistance in *P. aeruginosa* represents a critical challenge to healthcare systems.

**Materials and Methods:** Between January and December 2024, 650 clinical specimens were collected from Al-Hussein Teaching Hospital and Al-Rumaitha Hospital in Al-Muthanna, Iraq. *P. aeruginosa* was identified by conventional biochemical assays and confirmed with the Vitek 2® system. Susceptibility testing was performed by disk diffusion. PCR was conducted to detect plasmid-mediated quinolone resistance determinants, namely *qnrA*, *qnrB*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, and *qepA*.

**Results:** Among the 650 specimens analyzed, 374 (57.54%) were positive for bacterial culture, with *P. aeruginosa* representing 40.10% (150/374) of the identified isolates. Among these, 39 (26%) exhibited resistance to at least one fluoroquinolone used. The most frequently detected gene was *qnrB* (67.65%), followed by *qnrD* (61.76%), *qnrS* (55.88%), *aac(6')-Ib-cr* (47.00%), and *qepA* (41.17%), while *qnrA* and *qnrC* were not detected in any isolate.

**Conclusion:** The fluoroquinolone resistance and widespread occurrence in isolates of *P. aeruginosa* from Al-Muthanna Province pose a challenge to infection management, as mobile genetic elements facilitate the rapid dissemination of resistance and limit available therapeutic options, emphasizing the necessity for genetic monitoring and effective antibiotic management.

**Keywords:** *Pseudomonas aeruginosa*; Drug resistance; Fluoroquinolones; Ciprofloxacin; Levofloxacin; Polymerase chain reaction; Ofloxacin

## INTRODUCTION

*Pseudomonas aeruginosa* is classified as a facultative aerobic rod. This microorganism has a wide range of habitats in nature and can adapt to many different environments; it can be found in almost any healthcare facility (1). This pathogen contributes to a broad spectrum of infections, including community-onset and hospital-acquired diseases, with substantially higher mortality and morbidity rates than

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those produced by other bacteria (2). Treatment of *P. aeruginosa* infections represents a challenge because the bacteria are often multidrug-resistant (MDR) and exhibit a high risk of developing resistance during therapy (3).

Fluoroquinolones are synthetic antibiotics that work on a wide range of bacteria. They are active against various pathogenic bacteria; however, their extensive utilization has led to a worldwide rise in resistance to this class of antibiotics (4). The genes that encode gyrase and topoisomerase, which possess changes in the quinolone resistance-determining regions, are a key factor that allows bacteria to become resistant to fluoroquinolones (5). Another well-known mechanism of fluoroquinolone resistance in bacteria involves efflux pumps or porins in the outer membrane (6).

Since 1998, reports have emerged of plasmid-mediated quinolone resistance (PMQR) development. PMQR is a kind of quinolone resistance mediated by genes carried on plasmids (7). The three main types of PMQR determinants include genes encoding efflux pumps (*oqxAB* and *qepA*), the *aac(6')-Ib-cr* acetyltransferase gene, and *qnr* quinolone-resistance determinants (8). Notably, plasmid-mediated *qnr* genes encode proteins containing pentapeptide repeat motifs that shield DNA gyrase and topoisomerase from fluoroquinolone inhibition. Furthermore, a bifunctional form of aminoglycoside acetyltransferase, *aac(6')-Ib-cr*, can alter fluoroquinolones with an amino nitrogen on the C7 of the piperazinyl ring, such as ciprofloxacin and norfloxacin, to compromise drug efficacy (9). However, it does not affect other fluoroquinolones that lack an unsubstituted piperazinyl nitrogen (10). Furthermore, the main facilitator superfamily, which consists of the efflux pump genes *oqxAB* and *qepA* , reduces vulnerability to hydrophilic fluoroquinolones, particularly ciprofloxacin (11).

Despite increasing reports of fluoroquinolone resistance in *P. aeruginosa* across different regions of Iraq, critical gaps remain in understanding the genetic determinants and local patterns of quinolone resistance, particularly in Al-Muthanna Province. This study aimed to investigate the distribution of PMQR-associated genes (*qnrA, qnrB, qnrC, qnrD, qnrS, aac(6')-Ib-cr,* and *qepA*) amongst *P. aeruginosa* strains derived from various specimen types in Al-Muthanna Province, Iraq.

## MATERIALS AND METHODS

**Collection and identification of bacterial isolates.** A total of 650 clinical specimens were collected from two major hospitals in Al-Muthanna, Iraq—Al-Hussein Teaching Hospital and Al-Rumaitha Hospital—between January and December 2024. Specimens were obtained from microbiology laboratories with patient consent. Patient information was kept confidential.

The specimens were divided into categories: urine, 206 (31.69%); burns, 180 (27.69%); wounds, 161 (24.77%); ear swabs, 58 (8.92%); and sputum, 45 (6.92%). All specimens were collected in aseptic containers. Each specimen was sent to the microbiology laboratory for processing as described previously (12). All specimens were cultured on growth media: MacConkey agar, blood agar, and Cetrimide agar (Himedia, India).

Subsequently, the culture media were incubated at 37°C for 24 hours. Confirmation of *P. aeruginosa* involved the assessment of colony shape, Gram staining, and biochemical assays (catalase, oxidase, triple sugar iron). Furthermore, *P. aeruginosa* isolates were subjected to confirmatory diagnosis with the Vitek 2 system (BioMérieux, France).

**Antibiotic susceptibility assessments.** The antibiotic susceptibility of each *P. aeruginosa* isolate was evaluated using the Kirby-Bauer disc diffusion technique on Mueller-Hinton medium (13), and the results were analyzed in accordance with CLSI guidelines (CLSI, 2024) (14). The antibiotics used included piperacillin-tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (10 µg), cefoxitin (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and ofloxacin (5 µg). These were obtained from Himedia Laboratories, India. The antimicrobial susceptibility test employed *P. aeruginosa* ATCC 27853 as a control.

**DNA extraction.** The genomic DNA of *P. aeruginosa* isolates was extracted using the Presto™ Mini gDNA Bacteria Kit (Geneaid, USA), following the manufacturer's instructions. The quality of the extracted DNA was assessed by electrophoresis on a 1.5% agarose gel, and its purity and concentration were determined by spectrophotometry ($A_{260}/A_{280}$ ratio). The DNA was then stored at -80°C.

**Polymerase chain reaction (PCR) assay.** The thermocycling conditions for each gene were applied according to the protocols referenced in Table 1. Amplification was conducted using a conventional PCR thermocycler (BioRad, USA), as indicated in Table 2. PCR reactions were prepared in a final volume of 25 µl using GoTaq Green Master Mix (Promega, USA), containing template DNA (4 µl, ~100 ng/µl), primers (2 µl each, 10 pmol/µl), and nuclease-free water according to the manufacturer's recommendations. Based on the reference approach, the optimal conditions were determined. A PCR instrument was used for the detection of the PMQR genes. All PCR components were mixed in a PCR tube, which was then placed on ice under sterile conditions. The amplified PCR products were separated on a 1.5% agarose gel using an electrophoresis apparatus (Bioneer, Korea) and subsequently visualized under an ultraviolet transilluminator (Wised, Korea).

**Statistical analysis.** Statistical analysis was conducted in SPSS v24 (IBM Corp., Chicago, IL, USA). Proportions were compared between groups using the chi-square test, with significance set at $p < 0.05$.

## RESULTS

**Bacterial isolates.** Among the 650 clinical samples, 374 (57.54%) were positive for bacterial cultures, while 276 (42.46%) were negative. Among the positive cultures, 150 (40.10%) were *P. aeruginosa*. Isolate identification was performed by assessing morphology and biochemical profiles using the Vitek 2® (bioMérieux) system.

**Baseline demographic properties of the study population.** According to the demographic data (Table 3), burn samples had the largest proportion of

**Table 1.** Oligonucleotide primers and PCR product sizes (Macrogen, Korea).

| Selected gene | Sequence (5′–3′) | Product size (bp) | Reference |
|---|---|---|---|
| *qnrA* | TCAGCAAGAGGATTTCTC<br>GGCAGCACTATTACTCCCA | 516 | 15 |
| *qnrB* | GATCGTGAAAGCCAGAAAGG<br>ACGATG CCTGGTAGTTGTCC | 469 | 15 |
| *qnrC* | GGGTTGTACATTTATTGAATCG<br>CACCTACCCATTTATTTTCA | 307 | 16 |
| *qnrD* | CGAGATCAATTTACGGGGAAT<br>AACAAGCTGAAGCGCCTG | 582 | 16 |
| *qnrS* | ACGACATTCGTCAACTGCAA<br>TAAATTGGCACCCTGTAGGC | 417 | 17 |
| *aac-6-Ib-cr* | TTGCGATGCTCTATGAGTGGCTA<br>CTCGAATGCCTGGCGTGTTT | 482 | 18 |
| *qepA* | GCAGGTCCAGCAGCGGGTAG<br>CTTCCTGCCCGAGTATCGTG | 218 | 19 |

**Table 2.** Optimized PCR conditions for PMQR gene detection.

| Target gene | Initial | Cycling Conditions | | | | No. of cycles |
|---|---|---|---|---|---|---|
| | Denaturation | Denaturation | Annealing | Extension | Final Extension | |
| *qnrA* | 94°C 10 min | 94°C 30 sec | 52°C 30s | 72°C 1 min | 72°C 10 min | 35 |
| *qnrB* | 94°C 10 min | 94°C 30 sec | 55°C 30s | 72°C 1 min | 72°C 10 min | 35 |
| *qnrC* | 95°C 5 min | 95°C 1 min | 55°C 1 min | 72°C 5 min | 72°C 5 min | 30 |
| *qnrD* | 94°C 5 min | 94°C 1 min | 50°C 1min | 72°C 1 min | 72°C 10 min | 30 |
| *qnrS* | 94°C 4 min | 95°C 30 sec | 53°C 30s | 72°C 1 min | 72°C 10 min | 35 |
| *aac(6)-Ib-cr* | 95°C 5 min | 95°C 15 sec | 58°C 15 s | 72°C 40 sec | 72°C 4 min | 30 |
| *qepA* | 96°C 5 min | 96°C 1 min | 60°C 1 min | 72°C 1 min | 72°C 10 min | 30 |

**Table 3.** Distribution and demographic characteristics of patients infected with *P. aeruginosa* (n =150)

| Study variable | Group | No. (%) of *P. aeruginosa* isolates |
|---|---|---|
| Type of specimen | Burns | 67 (44.67%) |
| | Ear swab | 8 (5.33%) |
| | sputum | 12 (8%) |
| | Urine | 19 (12.67%) |
| | Wounds | 44 (29.33%) |
| Age (Years) | 1-20 | 15 (10%) |
| | 21-40 | 41 (27.33%) |
| | 41-60 | 63 (42%) |
| | > 60 | 31 (20.67%) |
| Gender | Male | 87 (58%) |
| | Female | 63 (42%) |
| Hospitalization | Inpatient | 89 (59.33%) |
| | Outpatient | 61 (40.67%) |

*P. aeruginosa* isolates (44.67%), followed by wounds (29.33%). Analysis of age and gender in the current study revealed a higher isolation rate of *P. aeruginosa* among males (58%) compared with females (42%). The highest isolation rate was observed in patients aged 41-60 years (42%), followed by those aged 21-40 years (27.33%) and those over 60 years (20.67%). However, the *P. aeruginosa* isolates were recovered mainly from inpatients (59.33%), while (40.67%) of the isolates were from the outpatients.

**Resistance susceptibility test of *P. aeruginosa* bacteria.** Susceptibility profiling indicated that 39 of 150 (26%) *P. aeruginosa* isolates were resistant to the fluoroquinolones used. Among these isolates, 24 (61.54%) of 39 fluoroquinolone-resistant isolates exhibited resistance to ciprofloxacin, 25 (64.10%) to levofloxacin, and 31 (74.35%) to ofloxacin (Fig. 1).

High levels of resistance were observed among isolates against the following antibiotics: piperacillin (87.18%), piperacillin-tazobactam (84.62%), cefepime (94.87%), cefoxitin (94.87%), ceftazidime (94.87%), amikacin (79.49%), tobramycin (82.05%), and aztreonam (64.10%). Resistance rates were lower for meropenem (51.28%) and imipenem (48.71%).

**Fluroquinolone-resistant *P. aeruginosa* clinical isolates.** The highest proportion of fluoroquinolone-resistant *P. aeruginosa* isolates was found in ear swabs (37.50%), followed by burns (31.34%), wounds (29.55%), and sputum (16.67%). No fluoroquinolone-resistant isolates were identified in urine (0.00%), as shown in Table 4. No statistically significant association was observed between specimen type and the occurrence of fluoroquinolone resistance (p = 0.06).

**Genetic screening of PMQR-associated genes in *P. aeruginosa.*** Among fluoroquinolone-resistant *P. aeruginosa* isolates, 34 (87.17%) were positive for plasmid-mediated quinolone resistance genes by PCR, while 5 (12.82%) were negative for all PMQR genes tested. The predominant PMQR determinant was *qnrB* (67.65%), followed by *qnrD* (61.76%), *qnrS* (55.88%), *aac(6')-Ib-cr* (47.00%), and *qepA* (41.17%). In contrast, *qnrA* and *qnrC* were not present in any of the isolates screened (Table 5). Figs. 2-6 show the PCR amplification results for the analyzed genes.

Coexistence of multiple PMQR genes was common among the genotyped isolates (N = 34). Only 6 isolates (17.65%) carried a single PMQR gene, whereas 28 isolates (82.35%) harbored ≥2 genes, including 11 (32.35%) with three genes, 4 (11.76%) with four genes, and 4 (11.76%) carrying all five PMQR determinants *qnrB*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, and *qepA*.

## DISCUSSION

An increasing proportion of *P. aeruginosa* isolates exhibiting multidrug resistance underscores an urgent public health risk, particularly of critical antibiotic classes such as fluoroquinolones. This makes treatment more difficult and increases mortality rates, especially among vulnerable populations. Our research indicated that *P. aeruginosa* obtained from clinical specimens accounted for approximately 40.10%, highlighting its role as a predominant pathogen. In comparison with Iraqi studies, this rate is higher than a study in Sulaymaniyah Province that reported 8.67% (20) but lower than other studies carried out in Basrah Province, 50.6% (21).

In Iraqi healthcare settings, *P. aeruginosa* is increasingly reported as a major cause of healthcare-associated infections, likely driven by gaps in infection prevention and control, extensive empirical antibiotic use, and limited public health awareness (20). The predominance of isolates from burns and wounds in the current study is attributed to the bacteria's ability

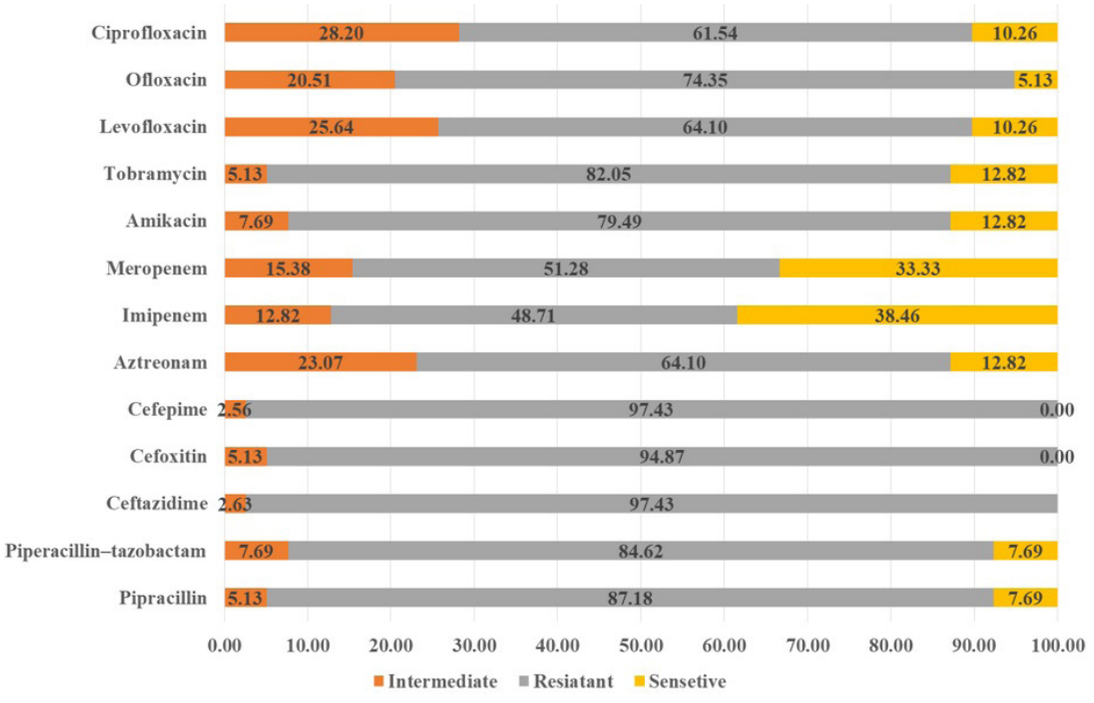


**Fig. 1.** In vitro susceptibility test of *P. aeruginosa* isolates to selected antibiotics (n = 39)

**Table 4.** Distribution of fluoroquinolone-resistant *P. aeruginosa* isolates by specimen type (n=39).

| Source of specimen | No. of *P. aeruginosa* isolates | No. FQ-resistant *P. aeruginosa* |
|---|---|---|
| Burns | 67 (44.67%) | 21 (31.34%) |
| Ear swab | 8 (5.33%) | 3 (37.50%) |
| Sputum | 12 (8.00%) | 2 (16.67%) |
| Urine | 19 (12.67%) | 0 (0.00%) |
| Wounds | 44 (29.33%) | 13 (29.55%) |
| Total | 150 (100.00%) | 39 (26.00%) |
| Chi-Square | X2=9.05, Df=4, P≤0.05 | P-value= 0.06 |

**Table 5.** Frequency of PMQR determinants across *P. aeruginosa* isolates (n = 34)

| PMQR genes | No. of isolates |
|---|---|
| *qnrA* | 0 |
| *qnrB* | (23) 67.65% |
| *qnrC* | 0 |
| *qnrD* | (21) 61.76% |
| *qnrS* | (19) 55.88% |
| *aac-6-Ib-cr* | (16) 47.00% |
| *qepA* | (14) 41.17% |

to thrive in damaged tissues, persist in moist hospital environments, form biofilms, and colonize surgical sites—all of which facilitate transmission and recurrent infection (22, 23).

Although burn and wound specimens were the principal specimen sources in the present study, a study from Kirkuk, Iraq reported a higher isolation rate from these specimen types (24). This variation may be attributable to differences in hospital settings, sampling strategies, and local antimicrobial use patterns. The higher incidence among males and individuals aged 41-60 years may relate to increased

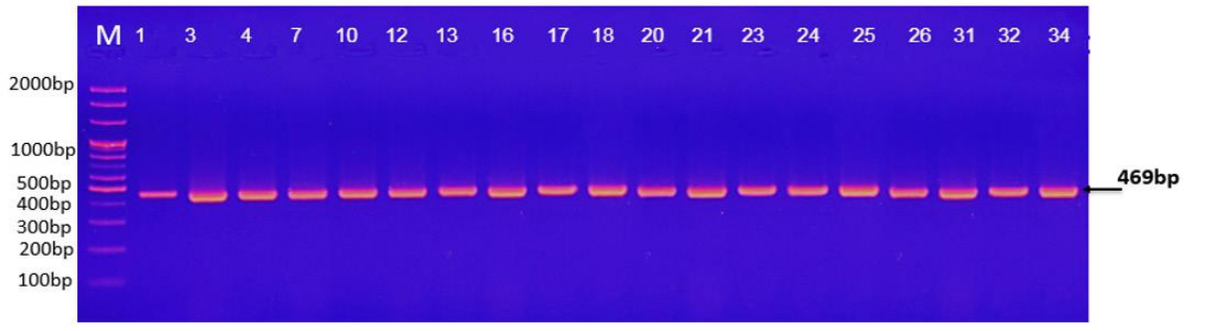


**Fig. 2.** PCR product of the *qnrB* gene (469 bp), Lane (M): Marker ladder (2000-100 bp), Lane (1-34): Positive detection of *qnrB* gene.

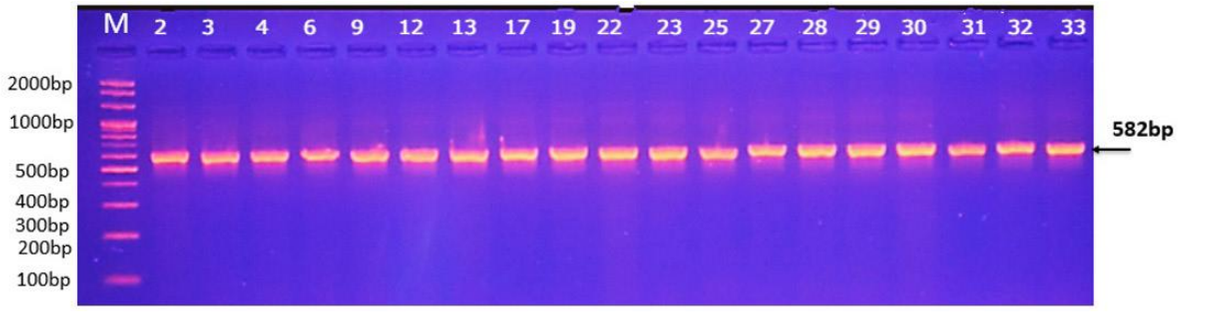


**Fig. 3.** PCR product of *qnrD* gene (582 bp), Lane (M): Marker ladder (2000-100 bp), Lane (2-33): Positive detection of *qnrD* gene.

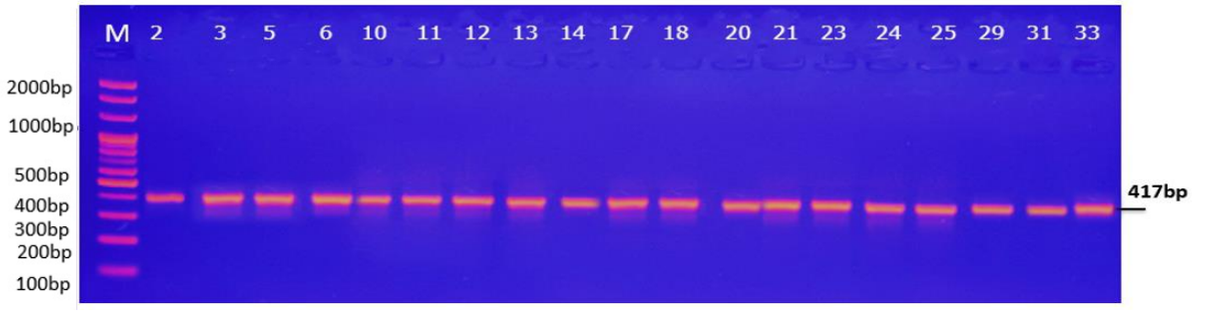


**Fig. 4.** PCR product of *qnrS* gene (417 bp), Lane (M): Marker ladder (2000-100 bp), Lane (2-33): Positive detection of *qnrS* gene.

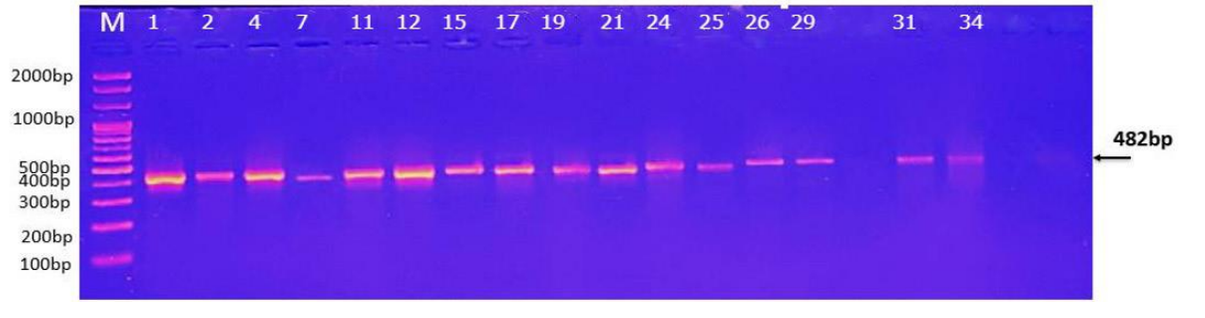


**Fig. 5.** PCR product of *aac (6')-Ib-cr* gene (482 bp), Lane (M): Marker ladder (2000-100 bp), Lane (1-34): Positive detection of *aac (6')-Ib-cr* gene.

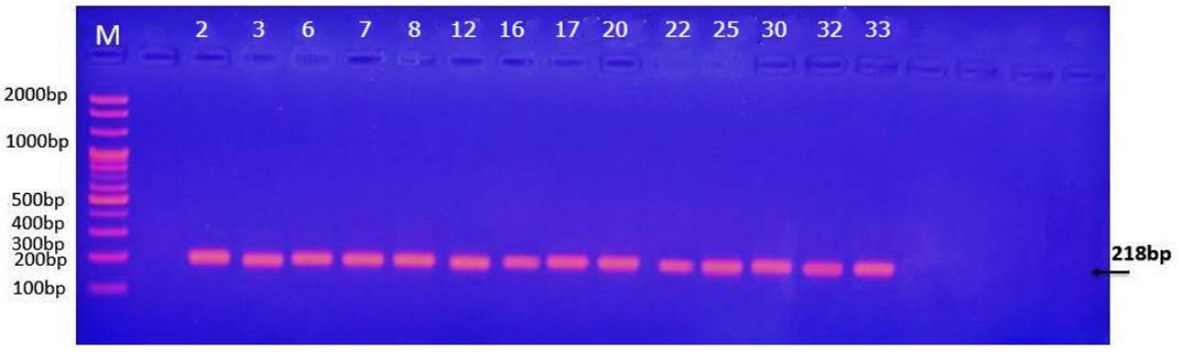


**Fig. 6.** PCR product of *qepA* gene (218 bp), Lane (M): Marker ladder (2000-100 bp), Lane (2-33): Positive detection of *qepA* gene.

occupational exposure, trauma risk, and comorbidity burden in this group. The results are similar to another study conducted in India (25). This association requires confirmation in larger multicenter studies. With respect to fluoroquinolones, the resistance level observed in our isolates is comparable to that reported in an Iraqi study by Ismail and Mahmoud (2018) (26). In contrast, markedly higher rates have been reported in burn-unit-associated isolates in Iran (27), whereas lower rates were observed in other international datasets, likely reflecting differences in patient populations, antibiotic exposure, and infection-control practices (28). Mechanistically, fluoroquinolone resistance in *P. aeruginosa* is typically multifactorial, involving QRDR mutations (*gyrA* and *parC*), efflux pump overexpression, and reduced outer-membrane permeability, which collectively limit drug activity and complicate treatment in hospital-acquired infections (29).

In the present study, fluoroquinolone-resistant *P. aeruginosa* isolates exhibited extensive cross-resistance across the fluoroquinolones tested (Fig. 1), which is expected given that fluoroquinolone resistance in *P. aeruginosa* is commonly driven by QRDR mutations and efflux-mediated mechanisms, often coexisting with multidrug-resistant phenotypes. A similar pattern of resistance to multiple quinolones among quinolone-resistant *Pseudomonas isolates* has been reported in Saudi Arabia, supporting that resistant subpopulations frequently exhibit reduced susceptibility across the entire drug class rather than to a single agent (30). Notably, although burns contributed the highest absolute number of fluoroquinolone-resistant isolates, the highest proportion was observed in ear swab specimens, suggesting that otologic infections may represent a niche where local antibiotic exposure, including topical fluoroquinolones, can select for resistant *P. aeruginosa* strains. This is supported by reports of chronic suppurative otitis media with emergence of ciprofloxacin-resistant Pseudomonas and by studies describing the microbiology and antimicrobial susceptibility patterns in otitis externa, including the clinical challenge of ciprofloxacin-resistant ear infections (31, 32).

Genotypic screening showed that PMQR determinants were common within the fluoroquinolone-resistant *P. aeruginosa* groups, with frequent detection of *qnrB* and *qnrD.* This pattern differs from reports in the region. For example, research in Saudi Arabia has identified *aac(6')-Ib-cr* as the predominant mechanism (30); conversely, Iranian reports have found *qnrS* and *qnrA* to be more common, with no *qnrD* detected in the study reported by Saki et al. (33). The widespread occurrence of *qnrB* and *qnrD* in this investigation suggests a unique local epidemiology of transferable genetic elements, possibly arising from specific antimicrobial selective pressures or the clonal spread of successful MDR strains carrying these plasmids in Iraqi hospitals. These resistance patterns may increase treatment failure and restrict therapeutic options. Therefore, susceptibility-guided therapy, strict infection control, and antimicrobial stewardship with ongoing molecular surveillance are essential.

## CONCLUSION

This study demonstrated a substantial burden of plasmid-mediated quinolone resistance among clinical *P. aeruginosa* isolates collected from hospitals

in Al-Muthanna, Iraq, chiefly dominated by *qnrB, qnrD, qnrS, aac(6´)-Ib-cr,* and *qepA.* The coexistence of multiple PMQR determinants in the same isolate may partly explain the pronounced fluoroquinolone resistance observed, and the dissemination of these transferable resistance elements can further restrict effective treatment choices for *P. aeruginosa* infections. Collectively, these findings provide deeper insight into quinolone resistance pathways in *P. aeruginosa* and may support improved antimicrobial stewardship and clinical management of healthcare-associated infections caused by this organism.

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