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Characterization of Quinolone Resistance Mechanisms in Pseudomonas aeruginosa Isolates

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Abstract :

Pseudomonas aeruginosa is an opportunistic bacterium known for its capacity to resist multiple antibiotics, particularly quinolones, through both inherent and acquired mechanisms. Quinolone resistance primarily arises from mutations in chromosomal genes like *gyrA* and *parC*, as well as through the presence of plasmid-encoded *qnr* genes. These factors reduce the effectiveness of fluoroquinolone treatments, complicating clinical management. A thorough understanding of these genetic resistance determinants, chromosomal and plasmid-associated, is vital for guiding appropriate antimicrobial therapy choices.

Keywords:
(Pseudomonas aeruginosa, VITEK 2 system, Quinolone, gyrA and parC)

Aims: This study aims to detect the molecular mechanisms of quinolone resistance in clinical isolates of *Pseudomonas aeruginosa* by detecting chromosomal mutations and plasmid-mediated resistance genes.

Methodology: From a total of 80 clinical samples, 20 isolates (25%) were identified as *Pseudomonas aeruginosa*. These isolates originated from burn (9 isolates, 45%), wound (5 isolates, 25%), and sputum (6 isolates, 30%) specimens. Patient ages ranged from 9

months to 60 years, including both male and female individuals. The identification by using the (VITEK®-2)automated machine and CHROMID® selective media. Biofilm formation was assessed by the tissue culture plate method with detection enhanced via ELISA. Conventional PCR assays were applied to detect resistance genes associated with quinolone resistance, targeting both chromosomal and plasmid factors.

Results: The twenty *Pseudomonas aeruginosa* isolates were distributed as follows: 45% from burn samples, 25% from wounds, and 30% from sputum. The highest infection rate was found in the 31–40 year age group (40%). Females showed a significantly greater infection frequency (80%) compared to males (20%) with a p-value of 0.0034. Antibiotic susceptibility testing by VITEK®-2 revealed complete resistance (100%) to cefazolin and tigecycline, while imipenem and gentamicin demonstrated 55% sensitivity. Strong biofilm production was observed in 45% of isolates and was significantly associated with increased antibiotic resistance ($p = 0.009$). The prevalence of biofilm producers was highest among isolates from burn specimens (58.3%), suggesting a correlation between biofilm formation and sample origin. PCR detection confirmed the presence of *16SrRNA*, *gyrA*, and *parC* genes in all isolates (100%). Plasmid-mediated *qnrS* genes were found in 5% of isolates (1 isolate), *qnrA* in 20% (4 isolates), while *qnrB* was absent in any samples.

Conclusion: The findings emphasise that both chromosomal mutations and plasmid-mediated resistance genes play a critical role in quinolone resistance among *Pseudomonas aeruginosa* strains. This underscores the necessity for ongoing surveillance and careful antibiotic usage policies to effectively manage infections caused by this pathogen.

Introduction :

Pseudomonas aeruginosa is an opportunistic pathogen that frequently causes infections in immunocompromised patients or those with underlying health conditions (1). Its remarkable adaptability to diverse environments, coupled with both intrinsic and acquired resistance to numerous antimicrobial agents, poses significant challenges in clinical settings. Quinolones, synthetic broad-spectrum antibiotics, exert bactericidal activity by inhibiting DNA gyrase and topoisomerase IV—enzymes critical for bacterial DNA replication and transcription (2). Their effectiveness has made quinolones widely used for treating various bacterial infections (3).However, the global emergence of

quinolone-resistant *P. aeruginosa* strains has become a major clinical concern, often leading to treatment failures. Resistance primarily arises through two mechanisms: chromosomal mutations and plasmid-mediated quinolone resistance (PMQR) (4), which can coexist to amplify resistance levels. Chromosomal mutations typically occur within the quinolone resistance-determining regions (QRDRs) of genes such as gyrA, gyrB, parC, and parE, resulting in structural alterations of target enzymes that reduce drug binding efficiency (5,6). Some mutations additionally enhance the expression of multidrug efflux pumps, including MexAB-OprM, MexCD-OprJ, and MexEF-OprN, actively extruding quinolones from the bacterial cell and lowering intracellular drug concentrations. Alterations in outer membrane porins, such as OprD, further contribute by decreasing antibiotic uptake (7).Plasmid-mediated mechanisms facilitate the horizontal transfer of resistance genes across strains and species (8,9). Plasmids commonly carry qnr genes (qnrA, qnrB, qnrS), which encode proteins that protect DNA gyrase and topoisomerase IV from quinolone inhibition (10). Some plasmids also encode quinolone-modifying enzymes or efflux pump components, further enhancing resistance (11). The frequent co-occurrence of chromosomal mutations and PMQR genes in a single isolate often results in high-level resistance, severely restricting effective therapeutic options (12). Clinically, this underscores the urgent need for strengthened antimicrobial stewardship and enhanced surveillance programs to promptly detect resistance patterns, optimize treatment strategies, and preserve quinolone efficacy (13).The present study focuses on elucidating the molecular mechanisms underlying quinolone resistance in clinical *P. aeruginosa* isolates. It aims to identify mutations in key chromosomal targets (gyrA and parC) and detect the presence of plasmid-encoded quinolone resistance determinants, particularly members of the qnr gene family.

2. **Materials and Methods :**

Isolation of *P. aeruginosa*.

Eighty Clinical specimens were collected from patients admitted to Al-Hilla Teaching Hospital and were cultured on conventional culture media using basic laboratory methods. twenty clinical specimens were then cultured on a selective agar(Cetrimide agar) for this bacterium and identified using the (Vitek system) as *P.aeruginosa*.

VITEK-2 Automated Susceptibility Test Systems

An identification technique for bacterial species is known as the VITEK-2. The stages involved in the identification process were as follows: the isolates were cultured on MacConkey agar and incubated under aerobic conditions for a period of 24 hours. In a sterile tube, a single colony that was completely pure was suspended in 3 mm of normal saline. With the help of VITEK Densichek (bioMérieux), the turbidity of the bacterial suspension was adjusted so that it was in accordance with the McFarland standard, which was between 0.5 and 0.7, in 0.45% sodium chloride. It was necessary to manually load the suspension tubes and the VITEK 2 ID(Gram Negative) card into the machine. A computer that is connected to the VITEK-2 system receives rustle as data for each sample after approximately six to eight hours have passed since the instrument was first used. According to the VITEK2 Compact System, the identification of *P. aeruginosa* is dependent on the biochemical reactions that take place between the bacterial isolates that are suspended in their solutions and the medium that is included within the VITEK-2 Identification cards.

Biofilm Formation Detection in Tissue Culture Plate

Biofilm formation assay was done. A 96-well flat-bottom polystyrene microplate was used. Crystal violet (C.V 0.1%) was the staining agent. Procedure went as follows well was aseptically inoculated with one hundred eighty microliters of sterile Brain Heart Infusion (BHI) broth, followed by the addition of twenty microliters of a twenty-four-hour-old bacterial culture. The broth was supplemented with two per cent sucrose, equivalent to zero point five grams of sucrose per twenty-five millilitres of medium. Negative control wells contained two hundred microliters of sucrose-enriched BHI broth without bacterial inoculation. All plates were sealed with Parafilm and covered with lids, then incubated at thirty-seven degrees Celsius for twenty-four hours. Post-incubation and washed three times. Only the attached ones remained. Plates were air-dried. Room temperature. About 15 minutes. Then came staining added to the wells. Incubated at room temp. For(15) minutes. After that, the stain was removed. Wells were rinsed three times with distilled water. Gentle washing—important. Excess dye gone. Plates dried again. Slowly. At room temperature.Next step. Ethanol 95%—200 µl per well. This removed the bound dye. This reflects biofilm mass. Finally, the measurement of O.D. at 630 nm by the reader was used. To get adjusted OD, negative control values were subtracted from test values. This gave real biofilm readings. Based on OD, biofilm-forming ability was classified according to Table 1.

Extraction of Deoxyribonucleic acid:

The EasyPure® Bacteria Genomic Deoxyribonucleic acid Kit. The extracted DNA is suitable for applications such as PCR.

Preparation of primers

Primers were prepared as per the manufacturer's instructions. Simple steps. Lyophilised primers got dissolved in TE buffer (Tris-EDTA). The goal was to reach a stock concentration of 100 pmol/mL. After dissolving, the tubes were spun down. Quick centrifuge. Then left overnight. At 4°C. Cold helps stabilisation.Next step—dilution. TE buffer was used again. Final concentration was set at 20 pmol/mL. That's for the working solution. Primers were sequenced and are shown in table 2), PCR Reaction Mixture and Conditions(3), Table 4: PCR Thermal Cycling Conditions for Gene Amplification for *gyrA, parC* and qnr(A,B,S), respectively.

Table 2: Primer Sequences with amplicon and Annealing Temperature used in this study.

Primers	**SEQUENCES**	**product**	**Annealing Temperature**	**Reference**
Pseudomonas aeruginosa (16S rRNA gene)	**F 5-TGCCTGGTAGTGGGGGATAA -3 R 5-GGATGCAGTTCCCAGGTTGA -3**	**505bp**	**55 °C**	**(14)**
gyrA	**5'AGTCCTATCTCGACTACGCGAT'3 5'AGTCGACGGTTTCCTTTTCCAG'3**	**324bp**	**60°C**	**(15)**

parC	5'CGAGCAGGCCTATCTGAACTAT'3 5'GAAGGACTTGGGATCGTCCGGA'3	282bp	52°C	(15)
qnrA	5´-ATTTCTCACGCCAGGATTTG-3´ 5´-GATCGGCAAAGGTTAGGTCA-3´	516 bp	*55*°C	*(16)*
qnrB	5'GGCATTGAAATTCGCCACTG '3 5'TTTGCTGCTCGCCAGTCGA-'3	360bp	*61*°C	*(17)*
qnrS	5'GCAAGTTCATTGAACAGGGT '3 5'TCTAAACCGTCGAGTTCGGCG'3	428bp	*64*°C	*(18)*

Statistical analysis :

The data analysis was using Fisher's exact test and Pearson's chi-squared test , and the Social Sciences (SPSS) version 22.

Ethical approval :

Ethical approval for this study was obtained from the ethical committee at Hilla Surgical Teaching Hospital. The hospital ethics committee under document number [[IRB: 390-4/3/2025].

3. Result

***Pseudomonas aeruginosa,* Distribution among different specimens**

In the present study, out of 80 specimens,20(25%) isolates of *Pseudomonas aeruginosa were* identified by subculturing on the selective media Cetramide agar by Vitek-2 system. The percentage rate of specimens included 5 (25%) wounds, 9(45%) burns, and 6(30%) sputum infections for each sex, with age groups from 9 months to 60 years, as in Figure 1. These specimens underwent aerobic culturing on different media. Of the 80 specimens, 20 (25%) had positive *Pseudomonas aeruginosa,* while no growth was observed in 45 (56.25%) specimens, and 15(18.75%) specimens suggested the presence of another microorganism(E. coli, Fungi). only 20 (25%) specimens tested positive as *Pseudomonas aeruginosa.*

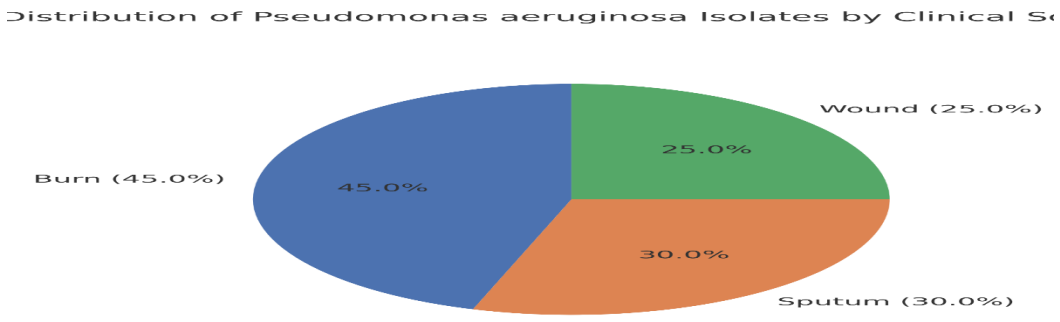


Figure 1: Distribution rate of *P. aeruginosa in* different clinical Specimens.

Positively rate of *P. aeruginosa* Among Different Clinical Specimens

In the present study, revealed that the Highest positivity rate was in burn specimens (45%), indicating a strong association of *P. aeruginosa* with burn infections, particularly in immunocompromised or hospitalised patients. wound and sputum specimens had similar positivity rates: 25% and 30**%,** respectively. This is consistent with *P.*

aeruginosa being a common nosocomial pathogen in wound units. The positivity rate across all specimens was 25%, suggesting a moderate prevalence of this pathogen in clinical settings. These findings underscore the pathogen's opportunistic behaviour and its preference for moist environments, making it a frequent cause of infections in respiratory, wound, and burn sites, as in Table 6.

Table(6): Percentage rate of *Pseudomonas aeruginosa* in Different Clinical Specimens.

Type of Specimens	**Total No.**	**Positive**	Positive (%)
Burn	40	9	45%
Wound	22	5	25%
Sputum	18	6	30%
Total	80	20	100%

Distribution Across Age Groups and Gender

Age Group Analysis :

In this study, the highest positivity rate was among patients aged 31–40 years (8 positives out of 20; 40%), followed by 21–30 years (33.3%). Children (1–10 years) and infants (9 months) had lower positivity rates, possibly due to less frequent exposure to hospital environments or fewer comorbidities. The p-value (0.443) in Table 7.

Gender Distribution :

In the current study, Females had a significantly higher positivity rate (80%) compared to males (20%) with a p-value =0.0034, indicating a statistically significant difference in Table 7.

Table (7): Distribution of *Pseudomonas aeruginosa* Positivity Across Age Groups and Gender with Statistical Analysis

Age Group	Total specimens	Positive	Percentage%	p-value
9 months	3	1	**5%**	
1–10 years	5	1	5%	
11–20 years	20	3	15%	**0.443**
21–30 years	15	5	25%	
31–40 years	20	8	40%	
40–50 years	7	1	5%	
50–60 years	10	1	5%	
Total	80	20	100%	
Gender	Total Specimens	Positive	Percentage (%)	
Female	45	16	80%	**0.0034**
Male	35	4	20%	

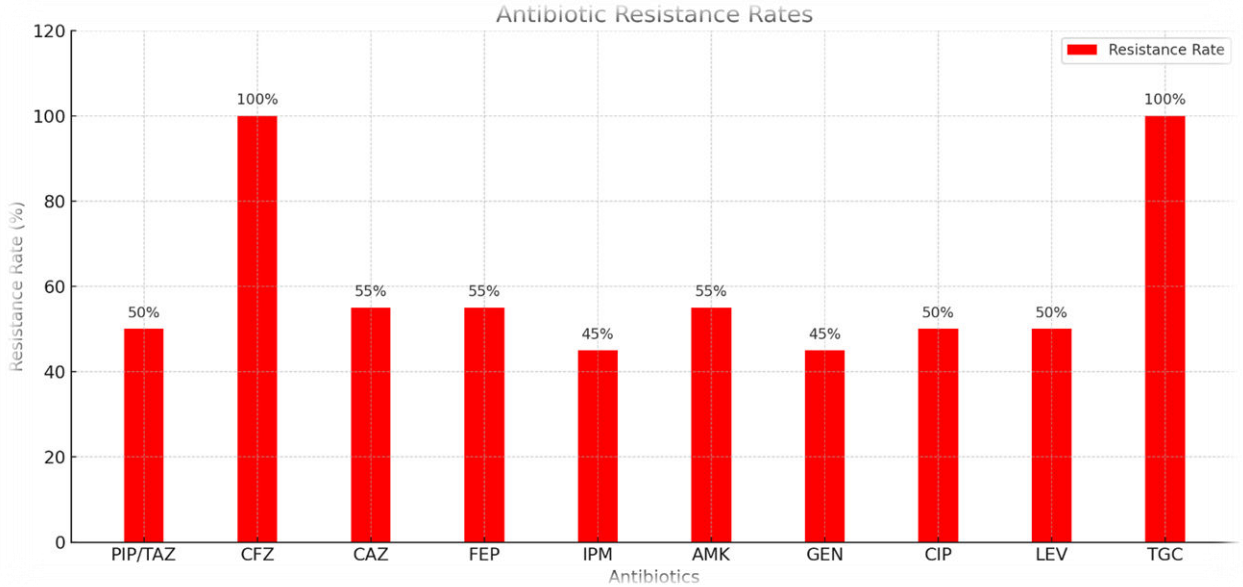
The p-value for the association between age group and positive *p. aeruginosa* cases is 0.443, and

p-value =0.0034 the difference in *p. aeruginosa* positivity between males and females was statistically significant.

Antibiotic susceptibility test to *P. aeruginosa* isolates by the Vitek-2 system

In the present study, 20 isolates of *p. aeruginosa* were subjected to an antimicrobial susceptibility examination by the Vitek-2 system to determine against 10 antibiotics. In this study, *P. aeruginosa* were completely resistant to **cefazolin** (100%). Resistant to

ceftazidime and **cefepime** detected at 55% each, while **piperacillin–tazobactam** showed a resistance rate of 50%. Among carbapenems, **imipenem** exhibited a 45% resistance rate. Regarding aminoglycosides, resistance to **amikacin** was 55%, and **gentamicin** showed 45% resistance. For fluoroquinolones, both **ciprofloxacin** and **levofloxacin** demonstrated 50% resistance. Additionally, the tetracycline derivative **tigecycline** exhibited a high resistance rate of 100% as shown in Figure (2) .



Figure(2): The percentage of antibiotic-resistant tests detected by the Vitek-2 system on 20 *p. aeruginosa* isolates.

Detection of Biofilm Capacity in *P. aeruginosa* Isolates.

The assessment of biofilm capacity in *P.aeruginosa* clinical isolates revealed that (9/20) of the isolates exhibited strong formation,(8/20) showed moderate formation, and(3/20) as weak producers. The statistically significant difference in Table 8.

Table (8): Biofilm Formation Capacity of *Pseudomonas aeruginosa* Isolates.

Isolate No.	OD at 630 nm	Biofilm Strength	Isolate No.	OD at 630 nm	Biofilm Strength
PS.1	0.430	+++ (Strong)	PS.11	0.295	++ (Moderate)
PS.2	0.520	+++ (Strong)	PS.12	0.168	+ (Weak)
PS.3	0.512	+++ (Strong)	PS.13	0.139	++ (Moderate)
PS.4	0.470	+++ (Strong)	PS.14	0.166	+ (Weak)
PS.5	0.210	++ (Moderate)	PS.15	0.528	+++ (Strong)
PS.6	0.266	++ (Moderate)	PS.16	0.477	+++ (Strong)
PS.7	0.300	+++ (Strong)	PS.17	0.129	+ (Weak)
PS.8	0.456	+++ (Strong)	PS.18	0.200	++ (Moderate)
PS.9	0.489	+++ (Strong)	PS.19	0.230	++ (Moderate)
PS.10	0.298	++ (Moderate)	PS.20	0.277	++ (Moderate)
Control	0.134	—			

In Table 9, the p-value of 0.009 indicates that the differences in the distribution of biofilm formation strengths (strong, moderate, weak) among *Pseudomonas aeruginosa* clinical isolates are statistically significant ($p < 0.05$)with a significant toward stronger biofilm production in these isolates. This pattern may contribute to the increased resistance and persistence of infections caused by these strains.

Table (9): Percentage rate of Biofilm formation in 20 *Pseudomonas aeruginosa* Isolates.

Biofilm Strength	No. of Isolates (N)	Percentage (%)	p-value
Strong	9	45%	**0.009**
Moderate	8	40%	
Weak	3	15%	

Biofilm Formation Strength of *Pseudomonas aeruginosa* Isolates Across Different Clinical Specimen Types

The ability of *Pseudomonas aeruginosa* isolates to form biofilm was analysed in relation to the type of clinical specimen, as shown in Table 10.

Table (10): Biofilm Formation Strength of *Pseudomonas aeruginosa* Isolates Across Different Clinical Specimen Types

Specimen Types	Strong Biofilm (%)	Moderate Biofilm (%)	Weak Biofilm (%)
Burn	58.3% (7/12)	16.7% (2/12)	25.0% (3/12)
Wound	40.0% (2/5)	60.0% (3/5)	0.0% (0/5)
Sputum	0.0% (0/2)	100.0% (2/2)	0.0% (0/2)

Genetic profiling

Molecular Detection of *p. aeruginosa* by Conventional PCR

In the current study, *Pseudomonas aeruginosa* was identified by 16S rRNA gene using PCR for 20 ***p.*** *aeruginosa* isolates carrying this gene, each showing a single band at 505 bp, confirming the presence of *P. aeruginosa* DNA, as in Figure 3. These results demonstrate the specificity and accuracy of the identification of *P. aeruginosa*.

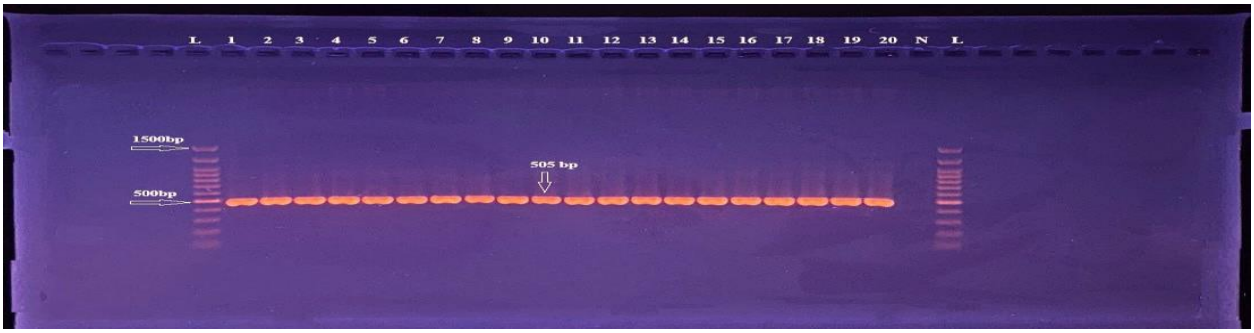


Fig3.Agarose gel electrophoresis of PCR products amplified with *16S rRNA* gene primers for *Pseudomonas aeruginosa* detection. All isolates 20 gave positive results of bp(505 bp).

Molecular screening of (PMQR)Genes in *p. aeruginosa*:

In the current study, **Conventional PCR analysis of a total of 20** *P. aeruginosa* **isolates was screened for resistance genes (***qnrA***, *qnrS*, and *qnrB*). The distribution of the three resistance genes is illustrated in Figures 4, 5, and 6.**

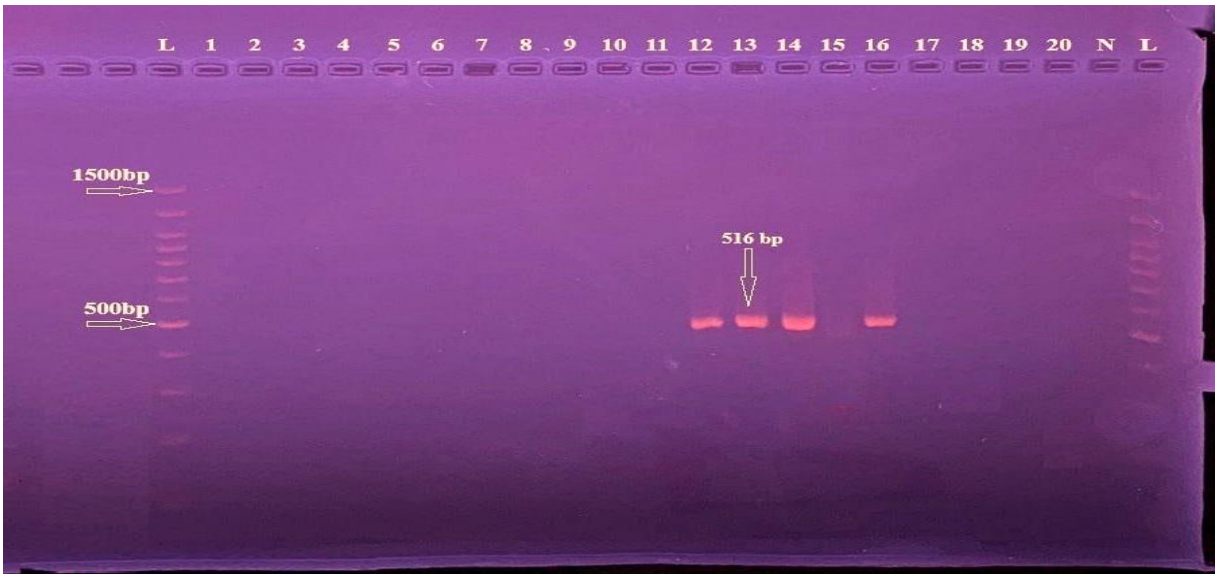


Figure (4) :- Agarose gel showing PCR amplification of the *qnrA* gene in *P. aeruginosa* at 324 bp . Lanes marked L contain molecular weight standards ranging from 500 to 1500 bp. The results demonstrate that *qnrA* is present in a few isolates and absent in most, supporting its variable distribution.

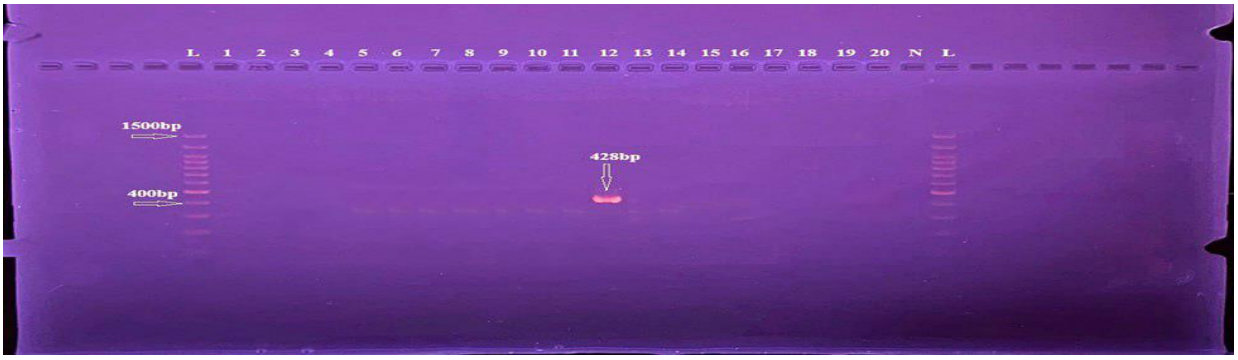


Figure (5) :- PCR products for *qnrS* gene detection in *P. aeruginosa*. A single, specific 428 bp band was detected in lane 12, confirming the presence of *qnrS* in that isolate. and lanes L show molecular weight markers ranging from 400–1500 bp.

Figure (6): PCR amplification using qnrB primers in *Pseudomonas aeruginosa* isolates showed no detectable bands, indicating the absence of the qnrB gene. Lanes L contain DNA ladders; lane N is the negative control**.**

As shown in Figure 4, four isolates (numbers 12, 13, 14, and 15) produced distinct PCR bands at 516 bp, indicating the presence of the *qnrA* gene. In contrast, detection of the *qnrS* gene was limited to a single isolate (No. 12), which displayed a specific band at 428 bp, as depicted in Figure 5. No amplification was observed for

the *qnrB* gene in any of the isolates, as shown in Figure 6. These findings suggest a limited and variable distribution of PMQRE resistance genes among *P. aeruginosa* isolates.

Molecular Detection of (parC , GyrA) by PCR

PCR analysis of the *gyrA* gene was performed on 20(100%) *Pseudomonas aeruginosa* isolates. All isolates produced distinct bands at 324 bp, confirming successful amplification, as shown in Figure 7. Similarly, amplification of the *parC* gene in the same 20 isolates yielded specific bands at 282 bp, indicating consistent and specific amplification, as illustrated in Figure 8.

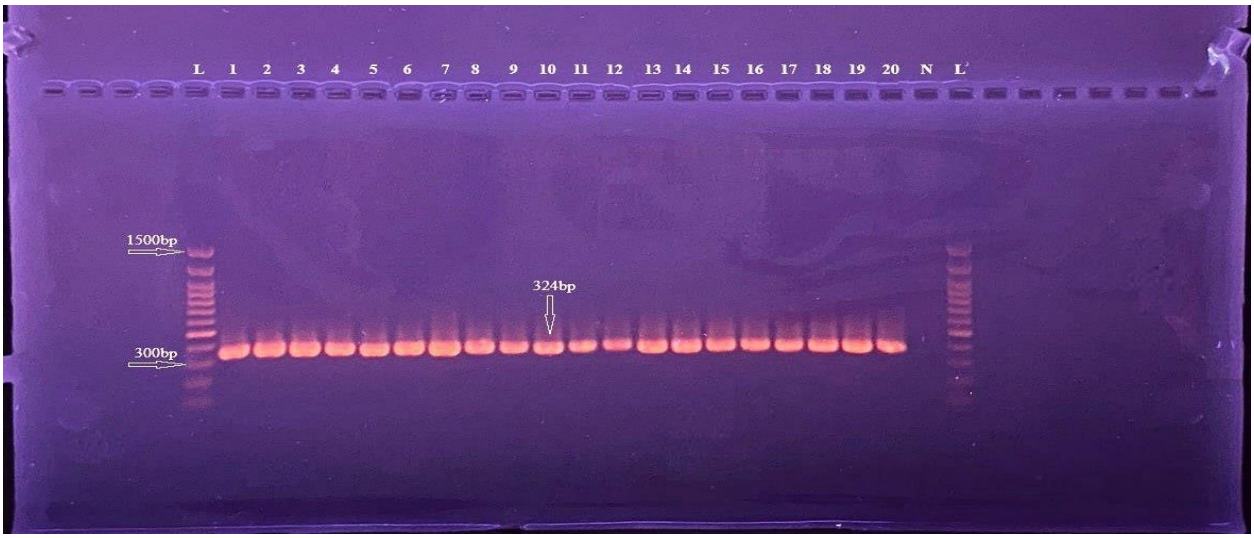


Figure (7) :- Agarose gel showing PCR amplification of the *gyrA* gene from *P. aeruginosa* isolates a single band at 324 bp,

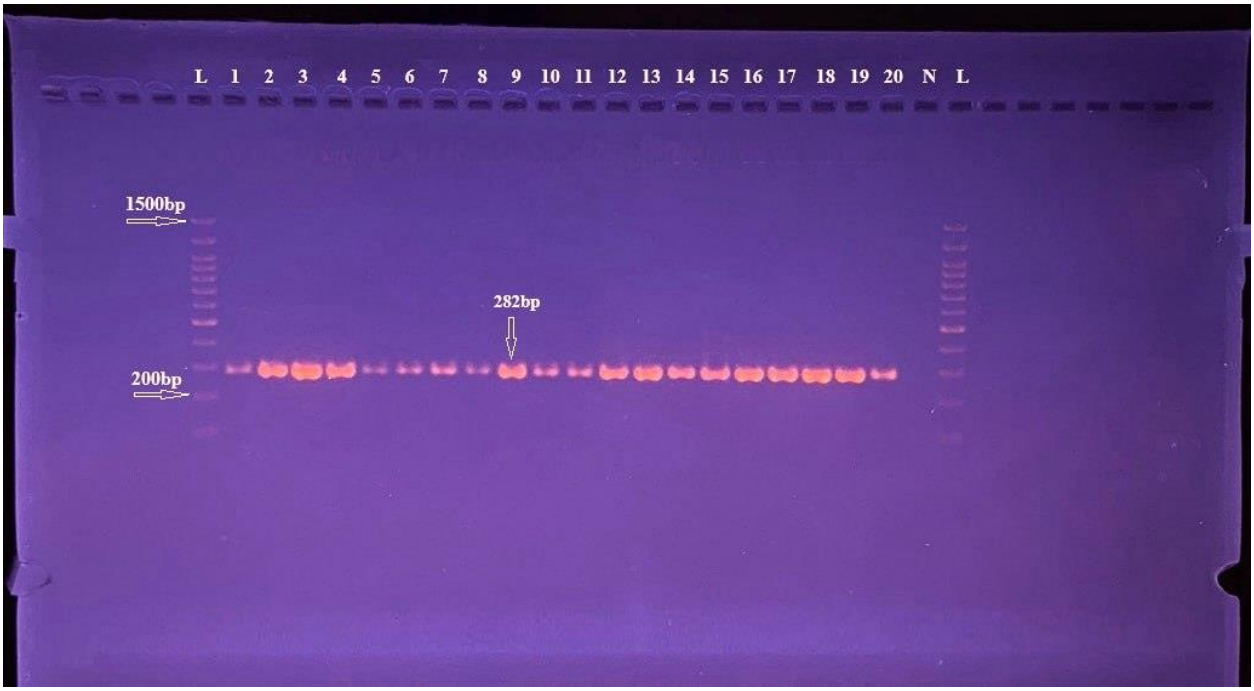


Figure (8) :- Agarose gel electrophoresis showing PCR amplification of the *parC* gene in *Pseudomonas aeruginosa* isolates and lanes L contain the molecular weight marker (200–1500 bp).

Distribution of *qnrA*, *qnrS*, and *qnrB*, ParC, *gyrA genes* among *P. aeruginosa* Isolates

PCR analysis of 20 isolates revealed the presence of resistance and housekeeping genes listed in Table 11.

Table 11.Distribution of *qnrA*, *qnrS*, and *qnrB*, ParC,*gyrA* Genes Among *P. aeruginosa* Isolates

Gene	No. of Positive Isolates	Percentage (%)
qnrA	4 / 20	20%
qnrS	1 / 20	5%
qnrB	0 / 20	0%
parC	20 / 20	100%
gyrA	20 / 20	100%
16SrRNA	20 / 20	100%

Association of Biofilm Formation and Antimicrobial Resistance in *Pseudomonas aeruginosa*

Twenty of the *P. aeruginosa isolates used in the present study were used to detect* the relationship between genotypic resistance genes and phenotypic biofilm virulence factors. AST, done by using the VITEK® 2 Compact system, detected widespread resistance among the isolates. All isolates (100%) were resistant to cefazolin and tigecycline. Moderate resistance levels were observed for the fluoroquinolones ciprofloxacin and levofloxacin, with resistance rates ranging from 45% to 55%.(PCR) Analysis revealed that the *qnrA* gene was present in 20% of isolates, while the *qnrS* gene was detected in 5%. The chromosomally encoded *gyrA gene* and *parC* gene, for fluoroquinolones, were detected in all isolates, indicating the presence of intrinsic resistance mechanisms. The detection of the 16S rRNA gene confirmed both species identity and the reliability of molecular testing. Biofilm production was measured using the tissue culture plate method, with optical density (OD) readings measured at 630 nm. Among the 20 isolates, 45% exhibited strong biofilm production, 40% showed moderate biofilm production, and 15% displayed weak biofilm formation. Statistical analysis ($p = 0.009$) demonstrated a significant difference in biofilm-forming ability, with a predominance of strong biofilm producers. This phenotype is frequently linked to enhanced antimicrobial resistance, as biofilm matrices hinder antibiotic penetration and promote bacterial survival under stress conditions.

Discussion

In the current study, 20 *P. aeruginosa* isolates were obtained from various clinical specimens. The highest isolation rate was observed in burn samples (forty-five percent), followed by sputum (thirty percent) and wound specimens (twenty-five percent). These findings indicate that burns represent a major source of *Pseudomonas aeruginosa* infections in the study population. Comparable observations have been reported in previous studies. A study by [19]documented an isolation rate of twenty-eight point two percent from wound samples in Al-Dewaniyah city. Similarly, a study by [twenty] reported high isolation rates from wound infections (sixty percent) and sputum samples (forty percent) compared with other infection sites, such as blood and burn wounds. Consistent with these results, [21] found *P. aeruginosa* isolates were recovered from pus specimens obtained from wounds, burns, urine and ear swabs. And study by [22] reported that *P. aeruginosa* accounted for 50% of cases in patients with otitis media, while [23] observed isolation rates of Twenty-seven percent of isolates were recovered from urine samples, nineteen point seven percent from ear infections, and thirteen point five percent from wound specimens. In the present study, twenty clinical isolates of *Pseudomonas aeruginosa* underwent antimicrobial susceptibility testing (AST) against ten antibiotics. All twenty isolates (one hundred percent) exhibited complete resistance to cefazolin and tigecycline. Additionally, elevated resistance rates were observed for cefepime (fifty-five percent), ceftazidime (forty-five percent), imipenem (fifty-five percent), gentamicin (fifty-five percent), and amikacin (fifty-five percent). Resistance to ciprofloxacin, levofloxacin, and piperacillin-tazobactam was documented at fifty percent. These resistance patterns align with recent global and regional studies, underscoring *P. aeruginosa* as a predominant multidrug-resistant pathogen in hospital settings. **(24)** in Syria reported that over 90% of *P. aeruginosa* isolates showed resistance to at least one β-lactam antibiotic, and over 50% were resistant to aminoglycosides and fluoroquinolones, in agreement with the present study. And a study by (25) The increasing trend of resistance to carbapenems, specifically imipenem and meropenem, with resistance rates ranging from forty to seventy percent across Middle Eastern countries, including Iraq, is concerning. The current finding of fifty-five percent imipenem resistance aligns with this regional pattern and underscores the urgent need for continuous surveillance of carbapenem resistance. Regarding fluoroquinolone

resistance, the fifty percent resistance rates to ciprofloxacin and levofloxacin observed in this study reflect an alarming global trend. According to [twenty-six], fluoroquinolone resistance in *Pseudomonas aeruginosa* has risen due to overuse in both clinical and community settings, with resistance levels varying between thirty-five and sixty percent depending on geographic location. Complete resistance to tigecycline and cefazolin (one hundred percent) is also significant, indicating the presence of both intrinsic and acquired resistance mechanisms. Although tigecycline is not routinely employed to treat *P. aeruginosa* because of intrinsic resistance, its inclusion here further highlights the multidrug-resistant nature of the isolates. Moreover, the observed aminoglycoside resistance to gentamicin and amikacin, each at fifty-five percent, is consistent with the findings study by [27] in Pakistan, where high aminoglycoside resistance was attributed to efflux pump overexpression and modifying enzymes.

Biofilm production was another critical factor observed in this study. Among 20 clinical isolates, 45% were strong biofilm producers, 40% moderate, and 15% weak, with statistically significant variation ($p = 0.009$). This is in agreement with [28], who reported that 60–70% of *P. aeruginosa* isolates from chronic infections exhibit strong biofilm-forming capacity. Burn wound isolates demonstrated the highest proportion of strong biofilm formation (58.3%), reflecting the ideal conditions in burn wounds—moisture, protein-rich exudates, and tissue damage—for colonisation and persistence. The respiratory isolates (from sputum) exhibited only moderate biofilm capacity, which differs from [29], who reported stronger biofilm formation in respiratory strains. This discrepancy may result from variations in clinical settings, patient populations, or biofilm quantification methods. Biofilms are known to enhance bacterial resistance by limiting antibiotic penetration, facilitating horizontal gene transfer, and altering bacterial metabolism [30]. The dominance of strong biofilm producers in this study likely contributes to the observed high resistance rates and suggests the need for anti-biofilm therapies as adjuncts to conventional antibiotics.In the current study, *Pseudomonas aeruginosa* isolates were confirmed using conventional PCR targeting the ***16S rRNA gene***, yielding a 505 bp product in all 20 isolates. This method provided a reliable and highly specific identification tool. A study by [31], reported the use of 16S rRNA gene for accurate identification of *P. aeruginosa* from clinical specimens in Iranian hospitals . Similarly, [32] emphasised that 16S rRNA PCR provides a rapid and specific method for distinguishing *P. aeruginosa* from other non-fermenters. This pattern is comparable to previous literature. For instance, [33] in Tehran found that qnrA was present in 15.3% of

P. aeruginosa isolates, while qnrS was rare (4.6%), and qnrB was absent, consistent with the current findings. Likewise, [34] noted a low prevalence of qnr genes in *P. aeruginosa* compared to Enterobacteriaceae, highlighting that plasmid-mediated resistance in *P. aeruginosa* is less frequent but may co-occur with chromosomal mutations. All isolates tested positively for the gyrA gene and parC gene, which are well-known chromosomal targets for fluoroquinolone action and resistance. These genes are commonly mutated in quinolone-resistant strains. While the current study did not investigate point mutations, the universal presence of these genes suggests their importance in intrinsic quinolone resistance pathways. This aligns with findings from [35], who reported that all fluoroquinolone-resistant *P. aeruginosa* isolates carried wild-type or mutated gyrA and parC genes. They emphasised that while PMQR genes contribute to resistance, chromosomal mechanisms like gyrA/parC mutations are the primary drivers in *P. aeruginosa* . Furthermore, [36] reported that 100% of their clinical *P. aeruginosa* isolates harboured gyrA and parC genes, supporting the universality of these genes as markers for quinolone susceptibility and resistance evaluation.n this study, molecular screening of 20 *Pseudomonas aeruginosa* clinical isolates from wound, burn, and sputum infections revealed the presence of plasmid-mediated quinolone resistance (*qnr*) genes and chromosomal quinolone resistance markers, along with a universal identification marker, *16S rRNA*. The findings were then compared with those reported in recent studies from different geographical regions.The universal presence (100%) of the ***16S rRNA*** gene in all isolates further confirms its utility as a reliable and specific molecular marker for the identification of *P. aeruginosa*. This is in agreement with the findings of [37] and [38], who validated the use of 16S rRNA-targeted PCR for accurate species-level identification. The conserved nature of this gene makes it a cornerstone for diagnostic and taxonomic studies in microbiology.All 20 isolates (100%) were positive for both the *gyrA* and *parC* genes. These results are consistent with those reported by [39] and [40], who also observed a 100% prevalence of these genes among quinolone-resistant *P. aeruginosa* isolates. The detection of the *qnrA* gene in 20% of isolates is consistent with the findings of[40], who reported a 15.3% prevalence among *P. aeruginosa* isolates in Iran. This suggests a moderate presence of this plasmid-borne resistance gene in clinical strains, which could be associated with the increasing dissemination of mobile genetic elements carrying *qnrA*.In contrast, the *qnrS* gene was detected in only 5% of isolates, a frequency similar to the 3% prevalence reported by [39] in Brazil. This reinforces the notion that *qnrS* is less prevalent in *P. aeruginosa*, potentially due to its more limited horizontal gene transfer in this species.The absence of

the qnrB gene in all tested isolates aligns with studies like [40], who also reported a 0% prevalence in Chinese clinical isolates.These genes encode essential subunits of the gyrase gene and topoisomerase IV, respectively, and are the primary targets of fluoroquinolones.The high prevalence of these chromosomal genes, regardless of their mutation status, underscores their essential role in *P. aeruginosa*'s core genome. Mutations within the *gyrA* and *parC* mechanisms contribute to high-level resistance in this species.

Conclusion

The current study shows that plasmid-mediated resistance remains uncommon in *P.aeruginosa*, while chromosomal resistance determinants, specifically *gyrA* and *parC*, were detected in all isolates (100%), indicating the predominant role of intrinsic resistance mechanisms in fluoroquinolone resistance. The chromosomal mechanisms over plasmid-mediated factors in the development of quinolone resistance in *P. aeruginosa*.

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