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Association between Antimicrobial Resistance and Biofilm Formation in Pseudomonas aeruginosa Isolated from Urinary Tract Infections

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Abstract :

Background

Pseudomonas aeruginosa is an important opportunistic pathogen associated with urinary tract infections (UTIs), particularly in healthcare settings, and is characterized by a high capacity for antimicrobial resistance. Its ability to form biofilms may enhance bacterial persistence within the urinary tract and reduce susceptibility to antimicrobial therapy. Molecular characterization of biofilm- and antimicrobial resistance-associated genes may provide additional information about the pathogenic and adaptive characteristics of clinical *P. aeruginosa* isolates.

Objectives

This study aimed to investigate the antimicrobial susceptibility patterns and biofilm-forming capacity of *P. aeruginosa* isolates recovered from patients with UTIs, assess the association between biofilm formation and antimicrobial resistance, and detect selected biofilm- and resistance-associated genes, including ***lasR, pelA, and mexA***, using conventional PCR.

Keywords:

Biofilm; TCP, lasR, pelA, and mexA, Conventional PCR.

Materials and Methods

A cross-sectional study was conducted from March to July 2026 among patients with suspected UTIs attending Al-Surgical Teaching Hospital and Mergan Hospital, Hilla, Iraq. A total of 150 urine specimens were collected. *P. aeruginosa* isolates were identified using conventional microbiological methods and confirmed using the VITEK 2 Compact system. Antimicrobial susceptibility testing was performed using the VITEK 2 Compact system. Biofilm production was evaluated using the tissue culture plate method with crystal violet staining. Genomic DNA was extracted from confirmed *P. aeruginosa* isolates, and conventional PCR was performed to detect the *lasR, pelA, and mexA* genes, followed by agarose gel electrophoresis. The association between the detected genes, biofilm-forming capacity, and antimicrobial susceptibility patterns was subsequently assessed.

Results

Fifteen *P. aeruginosa* isolates were recovered from 150 urine specimens, representing a recovery rate of 10.0%. High levels of antimicrobial resistance were observed, with complete resistance to ticarcillin, ticarcillin-clavulanic acid, piperacillin, and amikacin. Resistance to ceftazidime, cefepime, imipenem, gentamicin, meropenem, and ciprofloxacin ranged from 86.67% to 93.33%, whereas colistin showed the highest susceptibility rate (66.67%). Biofilm analysis revealed that 12 isolates (80.0%) were strong biofilm producers, two (13.33%) were moderate producers, and one (6.67%) was a very strong biofilm producer. Molecular analysis was performed to determine the distribution of the *lasR, pelA, and mexA* genes among the clinical isolates. The relationships between these molecular markers, biofilm-forming capacity, and antimicrobial resistance patterns were also evaluated.

Conclusion

Clinical *P. aeruginosa* isolates associated with UTIs demonstrated high levels of antimicrobial resistance and a high capacity for biofilm formation. The molecular detection of *lasR, pelA, and mexA* may provide additional insight into the genetic characteristics associated with biofilm development, bacterial adaptation, and antimicrobial resistance. Combining antimicrobial susceptibility testing, phenotypic

biofilm assessment, and molecular characterization may provide a more comprehensive understanding of *P. aeruginosa* isolates recovered from urinary tract infections.

1. Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen of considerable clinical importance and is responsible for a wide range of acute and chronic infections. It is frequently associated with healthcare-related infections, including urinary tract infections (UTIs), particularly among patients with underlying conditions, impaired host defenses, or prolonged exposure to healthcare settings and invasive medical procedures [1,2]. In the urinary tract, *P. aeruginosa* can present significant therapeutic challenges because of its remarkable ability to adapt to environmental stress and withstand antimicrobial treatment. This adaptability is supported by a broad range of virulence and survival mechanisms that facilitate bacterial persistence and infection.Antimicrobial resistance is one of the major characteristics contributing to the clinical significance of *P. aeruginosa*. The organism can develop resistance through several mechanisms, including reduced outer-membrane permeability, overexpression of efflux pumps, modification of antimicrobial targets, and enzymatic degradation or modification of antimicrobial agents [3,4]. The simultaneous occurrence of these mechanisms may result in resistance to multiple antimicrobial classes, thereby restricting available treatment options for *P. aeruginosa* UTIs. Consequently, antimicrobial susceptibility testing and continuous surveillance of resistance patterns remain important components of clinical microbiology and the management of urinary tract infections caused by resistant *P. aeruginosa* [11,20].

In addition to antimicrobial resistance, biofilm formation is an important characteristic that may contribute to the persistence of *P. aeruginosa* in the urinary tract. Biofilms are organized microbial communities in which bacterial cells become attached to a surface and are embedded within an extracellular matrix composed of bacterial polysaccharides, proteins, extracellular DNA, and other components [5]. This lifestyle provides bacteria with a microenvironment that differs substantially from that of free-living planktonic cells and can enhance their ability to survive unfavorable environmental conditions. In clinical settings, biofilms may develop on host tissues as well as urinary catheters and other medical devices, providing a favorable environment for bacterial persistence.Biofilm formation can also reduce the effectiveness of antimicrobial therapy. Limited antimicrobial penetration, altered metabolic activity, physiological heterogeneity, and

stress-adaptation mechanisms may contribute to the increased tolerance of biofilm-associated bacterial cells [6,7]. This phenomenon is particularly relevant to *P. aeruginosa* UTIs, where biofilm formation may facilitate persistent colonization and recurrent infection, especially in patients exposed to urinary devices. Previous studies have reported a high prevalence of biofilm formation among clinical *P. aeruginosa* isolates and have described associations between biofilm production and antimicrobial resistance, although the magnitude of these relationships may vary according to the bacterial population and antimicrobial agent investigated [8,9].Molecular characterization of *P. aeruginosa* isolates can provide additional information about the genetic determinants underlying biofilm formation, virulence, and antimicrobial resistance. Among the genes of interest, *lasR* plays an important regulatory role in the quorum-sensing system of *P. aeruginosa* and is involved in the regulation of several virulence-associated functions and biofilm-related processes[10,11]. The *pelA* gene is associated with the *pel* operon and contributes to the production of components of the extracellular matrix, thereby supporting biofilm development and structural stability. In contrast, *mexA* encodes an essential component of the MexAB-OprM multidrug efflux system, which contributes to the extrusion of several antimicrobial agents and is therefore of particular interest in studies of antimicrobial resistance in *P. aeruginosa*.The investigation of *lasR, pelA, and mexA* may therefore provide complementary molecular information regarding important phenotypic characteristics of clinical *P. aeruginosa* isolates[12]. Evaluating these genes together with antimicrobial susceptibility and experimentally determined biofilm-forming capacity may help clarify the distribution of selected biofilm- and resistance-associated determinants among urinary isolates[13,14]. Such combined phenotypic and molecular characterization may be particularly useful for understanding *P. aeruginosa* isolates recovered from UTIs in local healthcare settings.Despite extensive investigation of antimicrobial resistance and biofilm production in *P. aeruginosa*, information combining antimicrobial susceptibility testing, phenotypic biofilm assessment, and detection of selected biofilm- and resistance-associated genes remains limited among urinary isolates from Hilla, Iraq. Investigating these characteristics within the same group of UTI-associated isolates may provide a more comprehensive description of locally circulating *P. aeruginosa* and may help determine whether *lasR, pelA, and mexA* occur alongside important antimicrobial resistance and biofilm phenotypes.Therefore, the present study aimed to determine the antimicrobial susceptibility profiles and biofilm-forming capacity of *P. aeruginosa* isolated from urinary tract infections and to investigate the occurrence of the selected biofilm- and

antimicrobial resistance-associated genes *lasR*, *pelA*, and *mexA* using conventional PCR[15,16,17].

Materials and Methods

Study Design and Bacterial Isolation

A cross-sectional study was conducted from March to July 2026 in Hilla City, Babil Governorate, Iraq. A total of 150 urine specimens were collected from patients with suspected urinary tract infections attending Al-Surgical Teaching Hospital and Mergan Hospital. Urine specimens were collected aseptically in sterile, leak-proof containers and immediately transported to the microbiology laboratory for bacteriological processing. Each urine specimen was inoculated onto MacConkey agar and nutrient agar using standard microbiological procedures and incubated aerobically at 37°C for 24 h. Following incubation, bacterial growth was initially evaluated based on colony morphology. Suspected Gram-negative isolates were examined by Gram staining, and Gram-negative bacilli showing characteristics compatible with *Pseudomonas* spp. were subcultured onto Cetrimide agar and incubated aerobically at 37°C for an additional 24 h. Presumptive *Pseudomonas aeruginosa* isolates were identified based on characteristic colony morphology and pigment production. Final identification was confirmed using the VITEK 2 Compact automated identification system (bioMérieux, France). Confirmed *P. aeruginosa* isolates were subsequently subjected to antimicrobial susceptibility testing, phenotypic assessment of biofilm formation, and molecular detection of the selected ***lasR, pelA, and mexA*** genes.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed for all confirmed *P. aeruginosa* isolates using the VITEK 2 Compact automated system (bioMérieux, France). Briefly, fresh bacterial colonies obtained from MacConkey agar were suspended in 3 mL of sterile normal saline, and the bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard. The standardized suspension was subsequently used to inoculate VITEK 2 AST-GN76 cards according to the manufacturer's instructions. The resulting antimicrobial susceptibility profiles were obtained from the automated system and interpreted according to the applicable antimicrobial susceptibility breakpoints.

Phenotypic Assessment of Biofilm Formation

The biofilm-forming ability of the confirmed *P. aeruginosa* isolates was evaluated using the tissue culture plate (TCP) assay, a microtiter plate-based method for assessing adherent bacterial biomass [13,14]. Briefly, standardized bacterial suspensions were inoculated into sterile 96-well tissue culture plates and incubated under appropriate conditions to allow biofilm development. Following incubation, the wells were gently washed with sterile phosphate-buffered saline to remove planktonic and loosely adherent bacterial cells. The remaining attached biofilm was stained with crystal violet. Excess stain was removed by repeated washing, and the retained crystal violet was subsequently solubilized. The optical density (OD) of each well was measured at 630 nm using a microplate reader. Higher OD values indicated greater amounts of adherent biofilm biomass. Based on the obtained OD values and the established classification criteria, isolates were categorized according to their biofilm-forming capacity.

Genomic DNA Extraction

Genomic DNA was extracted from confirmed *P. aeruginosa* isolates using the Geneaid Bacteria DNA Extraction Kit (Geneaid, USA), according to the manufacturer's recommended protocol. The extracted DNA was stored under appropriate conditions and subsequently used as the template for conventional PCR amplification of the selected *lasR, pelA, and mexA* genes.

Primers and Target Genes

The primers used for molecular detection of the selected *lasR, pelA, and mexA* genes were synthesized by Macrogen, Korea. Prior to PCR amplification, the primers were prepared in TE buffer to obtain a working concentration of 10 pmol/µL and stored at −20°C until use.The investigated genes were selected based on their established biological relevance to *P. aeruginosa* biofilm formation, quorum-sensing regulation, and antimicrobial resistance. The *lasR* gene was selected as a representative quorum-sensing regulatory gene associated with the regulation of virulence and biofilm-related functions. The *pelA* gene was investigated because of its role in the production of extracellular matrix components involved in biofilm development. The *mexA* gene was selected as a component of the MexAB-OprM efflux system associated with antimicrobial resistance.Conventional PCR was performed using gene-specific primer sets for *lasR, pelA, and mexA*. The PCR

products were subsequently analyzed by agarose gel electrophoresis to determine the presence or absence of the respective target genes. The primer sequences, expected amplicon sizes, and annealing temperatures for the investigated genes are presented in Table 1.

Table 1. Primers used for molecular detection of CRISPR-Cas system subtypes in *Pseudomonas aeruginosa*

Target gene	Primer-Sequences	PCR-product	Annealing temperature
lasR	F:GCTGAATAACCAGCACAAACAGG R: GCCGTAAGGCACGATGTGCTT	**318 bp**	53°C
pelA	F: TCAACCATTTGTTGCGTCGCGA R: ATTGGGCGCCAGCCCTAACACA	**274 bp**	53°C
mexA	F: TGAATCTGGTCGAGGAGGATCG R: CATCCAGCCCAGATCACGCTC	**283 bp**	53°C

PCR Detection of Genes

The *lasR, pelA, and mexA* genes were investigated using conventional polymerase chain reaction (PCR). The amplification protocol was performed according to previously described procedures cited in the relevant literature. Each PCR reaction was prepared in a final volume of 25 µL using GoTaq® G2 Green Master Mix (Promega, USA). The reaction mixture consisted of 12.5 µL of GoTaq® G2 Green Master Mix, 2.5 µL of forward primer, 2.5 µL of reverse primer, 3.0 µL of template DNA, and 4.5 µL of nuclease-free water. Separate PCR reactions were performed for each target gene using the corresponding gene-specific primer pair. The complete reaction composition is presented in Table 2.

Table 2. PCR reaction mixture

Component	Volume (µL)
GoTaq® G2 Green Master Mix	12.5
Forward primer	2.5
Reverse primer	2.5
Template DNA	3.0
Nuclease-free water	4.5
Total volume	**25.0**

PCR amplification was performed with an initial denaturation step at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, primer annealing at 53°C, 54°C, or 55°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. The specific annealing temperature was selected according to the primer set used. The complete cycling protocol is summarized in Table (3).

Table 3. PCR Cycling Conditions

Step	Temperature (°C)	Time	Cycles
Initial denaturation	95	5 min	1
Denaturation	94	1 min	35
Annealing	53 / 54 / 55	1 min	35
Extension	72	1 min	35
Final extension	72	10 min	1

Following amplification, PCR products were separated by electrophoresis on 1.5% agarose gel containing Green Star stain. Electrophoresis was performed at 100 V for 35 min, after which the amplified DNA fragments were visualized using a gel documentation system. The presence of bands corresponding to the expected amplicon sizes was considered evidence of amplification of the respective target gene.

Ethical Considerations

The study received ethical approval from the relevant ethics committee at Hilla Surgical Teaching Hospital and the Ethical Committee of the Al- Hilla teaching Hospital , Iraq.

Participants were informed about the purpose and procedures of the study, and informed consent was obtained before specimen collection. The research was conducted in accordance with the applicable ethical requirements governing studies involving human participants. The study's ethical approval was documented under IRB No. 9-30, dated 7 January 2026.

Results

Recovery and distribution of *Pseudomonas aeruginosa* isolates

A total of 150 urine specimens were examined during the study period, from which 15 confirmed *Pseudomonas aeruginosa* isolates were recovered, representing an overall isolation rate of 10.0%. All confirmed isolates were recovered from urine specimens collected from patients with suspected urinary tract infections. The isolated *P. aeruginosa* strains were subsequently subjected to antimicrobial susceptibility testing, phenotypic assessment of biofilm-forming capacity, and molecular detection of the selected *lasR, pelA, and mexA* genes.

Antimicrobial susceptibility profile

The 15 isolates of *P. aeruginosa* showed high resistance rates as demonstrated by antimicrobial susceptibility testing. Total resistance (100.0%) was recorded against ticarcillin, ticarcillin/clavulanic acid, piperacillin and amikacin and none of the isolates was categorized as susceptible to these agents. Fourteen isolates (93.33%) were resistant to ceftazidime, cefepime and imipenem and only 1 isolate (6.67%) was susceptible to each agent. For meropenem, 13 isolates (86.67%) were resistant and 2 isolates (13.33%) were sensitive. Among the aminoglycosides, resistance to gentamicin was detected in 14 isolates (93.33%), whereas tobramycin showed resistance in 12 isolates (80.0%) and susceptibility in 3 isolates (20.0%). Ciprofloxacin showed resistance in 13 isolates (86.67%), with 2 isolates (13.33%) remaining susceptible.In contrast, colistin showed the highest susceptibility rate, with 10 isolates (66.67%) susceptible and 5 isolates (33.33%) resistant. The complete antimicrobial susceptibility profile is summarized in Table 4.

Table (4) showed Antibiotic susceptibility percentage of *p. aeruginosa* isolates .

Antimicrobial agent	Resistant, n (%)	Susceptible, n (%)
Ticarcillin	15 (100.0)	0 (0.0)
Ticarcillin/clavulanic acid	15 (100.0)	0 (0.0)

Antimicrobial agent	Resistant, n (%)	Susceptible, n (%)
Piperacillin	15 (100.0)	0 (0.0)
Ceftazidime	14 (93.33)	1 (6.67)
Cefepime	14 (93.33)	1 (6.67)
Imipenem	14 (93.33)	1 (6.67)
Meropenem	13 (86.67)	2 (13.33)
Amikacin	15 (100.0)	0 (0.0)
Gentamicin	14 (93.33)	1 (6.67)
Tobramycin	12 (80.0)	3 (20.0)
Ciprofloxacin	13 (86.67)	2 (13.33)
Colistin	5 (33.33)	10 (66.67)

Biofilm-forming capacity

All **15 clinical isolates** were evaluated for biofilm production using the tissue culture plate assay. Biofilm formation was detected among all tested isolates, although the intensity of production varied between isolates.The majority of isolates, 12 (80.0%), were classified as strong biofilm producers. Two isolates (13.33%) demonstrated moderate biofilm formation, while one isolate (6.67%) exhibited very strong biofilm production. No isolate was classified as a weak biofilm producer.The recorded optical density values at 630 nm ranged from 0.144 to 0.512. The highest optical density was recorded for isolate ST.12 (0.512), which was classified as a moderate biofilm producer according to the study's reported classification, whereas ST.15 also showed an OD of 0.512 and was categorized as a very strong producer. The individual OD measurements and biofilm classifications are presented in Table 5.

Table 5. Biofilm formation among clinical *P. aeruginosa* isolates

N0.of Isolates	O.D at 630 nm	Biofilm results

PS.1	0.287	+++ Strong
PS.2	0.144	+++ Strong
PS.3	0.158	+++ Strong
PS.4	0.479	+++ Strong
PS.5	0.345	+++ Strong
PS.6	0.250	+++ Strong
PS.7	0.383	+++ Strong
PS.8	0.501	+++ Strong
PS.9	0.446	+++ Strong
PS.10	0.476	++ Moderate
PS.11	0.145	+++ Strong
PS.12	0.512	++ Moderate
PS.13	0.168	+++ Strong
PS.14	0.287	+++ Strong
PS.15	0.512	++++ Very strong

* Very strong ++++, Strong +++,Moderate++,Weak+, Control: 0.134

Molecular Detection of *lasR, pelA, and mexA* Genes

PCR-based molecular analysis was performed to investigate the presence of the selected biofilm- and antimicrobial resistance-associated genes, *lasR, pelA, and mexA*, among the 15 confirmed *P. aeruginosa* isolates recovered from urine specimens. The PCR products were analyzed by agarose gel electrophoresis according to the expected amplicon sizes of the respective target genes.The *lasR* gene was investigated as a quorum-sensing regulatory determinant associated with virulence and biofilm-related functions. The *pelA* gene was examined as a biofilm-associated gene involved in the production of extracellular matrix components, whereas *mexA* was investigated as a component of the MexAB-OprM efflux system associated with antimicrobial resistance.The molecular analysis demonstrated the distribution of *lasR, pelA, and mexA* among the investigated *P. aeruginosa* isolates. The corresponding PCR amplification patterns and expected fragment sizes are presented in Figures [1,2,3]. The presence or absence of each target gene was recorded for individual isolates and subsequently compared with their phenotypic biofilm-forming capacity and antimicrobial susceptibility profiles.Overall, molecular detection of *lasR, pelA*, **and** *mexA* provided additional genetic characterization of the urinary *P. aeruginosa* isolates and allowed assessment of the relationship between selected molecular determinants and the observed antimicrobial resistance and biofilm-forming phenotypes.

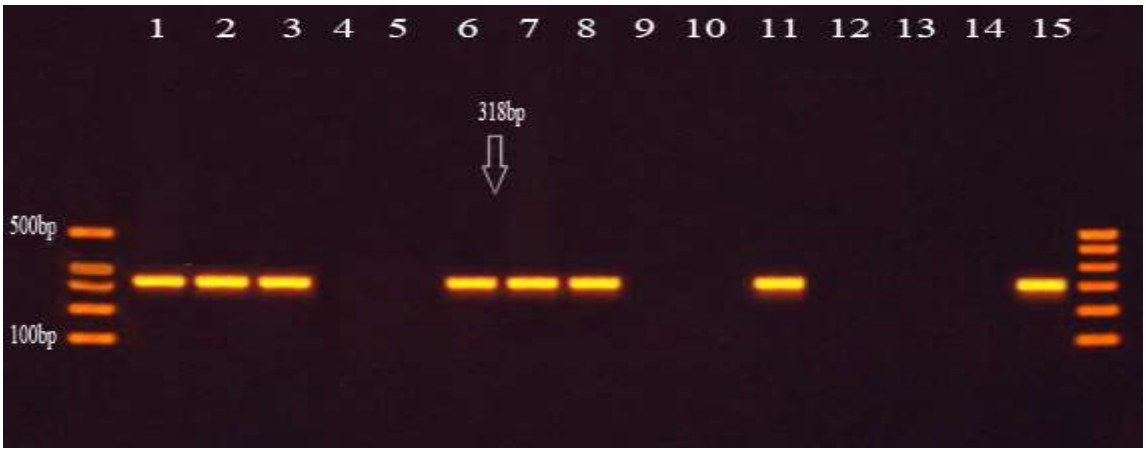
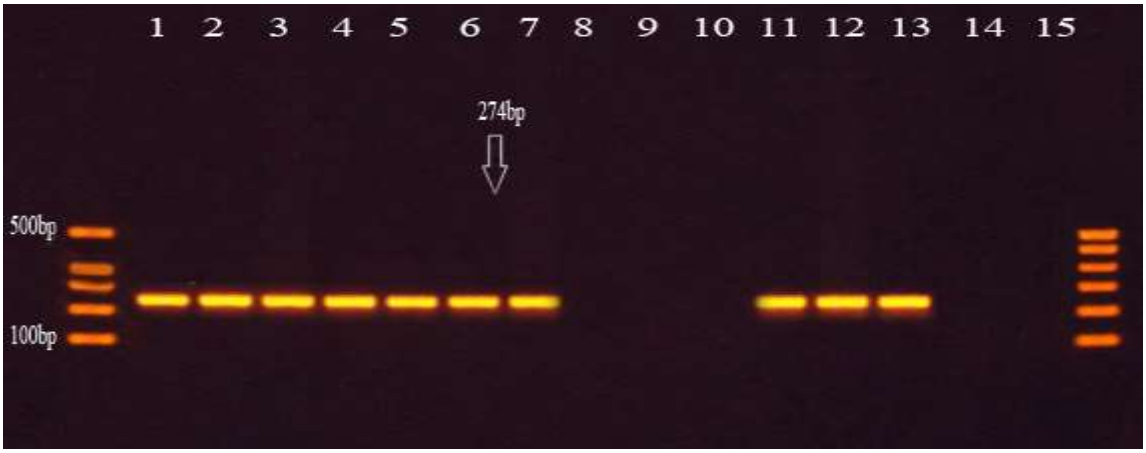
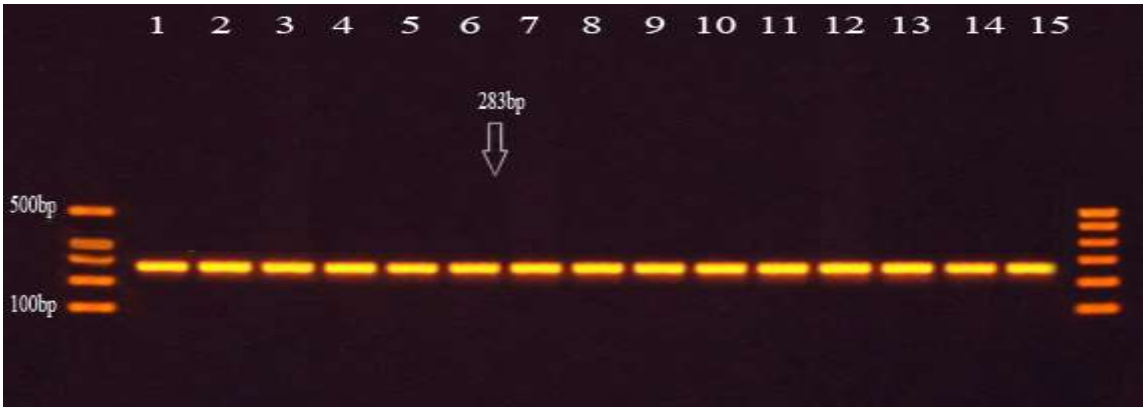


Figure (١): Gel electrophoreses the detection of the ***lasR*** gene (318 bp) in 15 clinical isolates . ladder (100-500 bp ladder) . Lanes 1-3, 6-8, 11, and 15 indicate positive for gene While other negative isolates .



)٢Figure(

: Gel electrophoreses the detection of the ***pelA*** (274 bp) in clinical isolates . ladder (100-500 bp ladder) . Lanes1-7 and 11-13 positive for the gene While other negative isolates



.Figure (٣): Gel electrophoreses the detection of the ***mexA*** gene (283 bp) in clinical isolates . ladder (100-500 bp ladder) Lanes 1-15 signify positive of the ge

Discussion

In the present study, *Pseudomonas aeruginosa* was recovered from 15 of 150 urine specimens, giving an overall isolation rate of 10.0%. The recovery of *P. aeruginosa* from patients with suspected urinary tract infections confirms its relevance as a urinary pathogen, particularly in healthcare-associated settings. Previous investigations have similarly demonstrated that urinary *P. aeruginosa* isolates may combine antimicrobial resistance with biofilm-forming and virulence-associated characteristics [18,19]. Differences in isolation rates between studies may be related to variations in patient populations, hospitalization status, previous antimicrobial exposure, catheterization, specimen collection procedures, and local epidemiological conditions. Therefore, the relatively modest isolation rate observed in the present study should be interpreted within the context of the study population and the limited number of isolates.The antimicrobial susceptibility results demonstrated a pronounced resistance phenotype among the 15 urinary *P. aeruginosa* isolates. Complete resistance (100%) was observed against ticarcillin, ticarcillin/clavulanic acid, piperacillin, and amikacin. Resistance was also very high against ceftazidime, cefepime, imipenem, and gentamicin (93.33%), while meropenem and ciprofloxacin showed resistance rates of 86.67%. These findings indicate that the isolates circulating in the studied clinical setting exhibited resistance to several major antimicrobial classes commonly used against *P. aeruginosa* infections. Similar studies have reported substantial antimicrobial resistance among clinical *P. aeruginosa* isolates, although resistance profiles may differ according to geographic location, healthcare setting, antimicrobial exposure, and patient characteristics [20–22].

The high resistance observed in this study is consistent with the recognized ability of *P. aeruginosa* to develop resistance through multiple overlapping mechanisms, including reduced outer-membrane permeability, efflux pump activity, target modification, and

antimicrobial inactivation. The organism may accumulate several resistance mechanisms within the same strain, resulting in complex and difficult-to-treat phenotypes [23–25]. The high levels of resistance observed against β-lactam and carbapenem agents in the present study may therefore reflect the combined contribution of several mechanisms rather than a single resistance determinant. However, the present study did not investigate the individual molecular mechanisms responsible for resistance to each antimicrobial class.

The particularly high resistance to aminoglycosides in the present study is noteworthy. All isolates were resistant to amikacin, while 93.33% and 80.0% were resistant to gentamicin and tobramycin, respectively. Such a pattern may reflect the accumulation of resistance mechanisms affecting this antimicrobial class, including enzymatic modification, altered uptake, target-related mechanisms, and efflux. Similar high aminoglycoside resistance has been reported among multidrug-resistant clinical *P. aeruginosa* isolates [26,27]. Nevertheless, because the present study did not investigate aminoglycoside-resistance genes, the observed phenotypic resistance should not be attributed to a specific genetic determinant without additional molecular evidence.Among the tested antimicrobial agents, colistin showed the highest susceptibility rate, with 66.67% of isolates remaining susceptible. Although this finding indicates greater in-vitro activity against the studied isolates compared with the other tested agents, resistance was still detected in one-third of the isolates. This finding is clinically relevant because increasing resistance to last-line agents may further restrict therapeutic options for infections caused by highly resistant *P. aeruginosa* [28,29]. The susceptibility pattern observed in the present study should, however, be interpreted cautiously because the number of isolates was relatively small.All 15 *P. aeruginosa* isolates demonstrated biofilm formation in the tissue culture plate assay. Most isolates (80.0%) were classified as strong biofilm producers, while 13.33% were moderate producers and 6.67% were very strong producers. Thus, all isolates exhibited at least moderate biofilm-forming activity according to the classification used in this study. The high frequency of biofilm formation is biologically relevant to urinary tract infections because biofilms can facilitate bacterial persistence on biological and abiotic surfaces, including urinary devices. Biofilm-associated cells may exhibit increased tolerance to antimicrobial exposure as a consequence of restricted antimicrobial penetration, altered metabolic activity, physiological heterogeneity, and stress-response mechanisms [30–32].

Previous clinical investigations have also reported high frequencies of biofilm production among *P. aeruginosa* isolates. A meta-analysis reported a high overall frequency of biofilm formation among clinical isolates, although the strength of biofilm production varied substantially between individual studies [33]. Studies specifically involving urinary isolates have likewise demonstrated a high prevalence of biofilm production, supporting the importance of this phenotype in urinary infections [19,34]. The predominance of

strong biofilm producers in the present study may therefore be particularly relevant to healthcare-associated UTIs.The relatively small number of isolates limits the extent to which the observed distribution can be generalized to the wider population. In addition, biofilm classification depends on assay conditions, inoculum preparation, incubation period, staining procedure, and interpretation thresholds, which can make direct comparisons between studies difficult. In the present study, OD630 values ranged from 0.144 to 0.512, indicating considerable variation in the amount of adherent biomass produced by individual isolates despite the overall predominance of strong biofilm formation. This variation supports the concept that biofilm production is a strain-dependent phenotype rather than a completely uniform characteristic among clinical *P. aeruginosa* isolates [35].

The molecular component of the present study provided additional characterization of the urinary isolates by investigating three genes with different biological roles. The *lasR* gene is an important component of the Las quorum-sensing system. LasR functions as a transcriptional regulator after interaction with its cognate autoinducer and influences the expression of several virulence-associated and biofilm-related functions. Previous clinical studies have reported the presence of *lasR* among *P. aeruginosa* isolates and have demonstrated its relevance to quorum sensing and biofilm-associated phenotypes [36,37]. Therefore, detection of *lasR* in urinary isolates may provide additional information regarding their genetic potential for quorum-sensing-related behavior.Nevertheless, the presence of *lasR* alone does not establish functional quorum sensing. Sequence variation, mutations, regulatory interactions, and gene expression can influence the activity of the Las system. Naturally occurring LasR-deficient clinical isolates have also been described, demonstrating that PCR detection should not be interpreted as evidence of functional quorum-sensing activity [38]. Accordingly, the molecular findings in the present study should be considered evidence of gene carriage rather than direct evidence of gene expression or functional activity.The *pelA* gene was selected because it is associated with the Pel exopolysaccharide system, which contributes to the extracellular matrix of *P. aeruginosa* biofilms. Pel contributes to the structural organization and stability of biofilms and interacts with other matrix components during biofilm development [39,40]. The detection of *pelA* therefore provides molecular information complementary to the phenotypic biofilm assay. However, the presence of a biofilm-associated gene does not necessarily predict the exact intensity of biofilm formation because biofilm development is controlled by multiple interconnected regulatory, structural, and environmental pathways. Thus, the TCP results in the present study provide important phenotypic information that complements the PCR findings.The *mexA* gene represents another important aspect of *P. aeruginosa* adaptation. MexA is a component of the MexAB-OprM efflux system, which contributes to the extrusion of multiple antimicrobial compounds. Efflux systems represent important mechanisms involved in both intrinsic and acquired

antimicrobial resistance in *P. aeruginosa* [41,42]. Previous clinical studies have reported frequent detection of efflux-associated determinants among resistant *P. aeruginosa* isolates, supporting their relevance when investigating the relationship between antimicrobial resistance and bacterial adaptation [43].In the present study, the molecular results should therefore be interpreted as evidence of the presence of selected genetic determinants rather than direct evidence that these genes caused the observed resistance or biofilm phenotype. Conventional PCR detects gene carriage but does not determine whether a gene is actively expressed or whether sequence variation affects its encoded protein. This distinction is particularly important for *lasR* and *mexA*, for which functional activity may depend on regulatory mechanisms and expression levels. Demonstrating a direct relationship between gene carriage and phenotype would require additional approaches, such as quantitative real-time PCR, sequencing, or functional assays.

The simultaneous observation of extensive antimicrobial resistance and frequent strong biofilm formation is an important finding of the present study. All isolates formed biofilms, while most displayed resistance to several antipseudomonal agents. Previous research has described an association between biofilm formation and antimicrobial resistance in *P. aeruginosa*, although this relationship is not necessarily linear or universal for every antimicrobial agent [33,44]. Biofilm-associated antimicrobial tolerance can arise from physiological and structural characteristics of the biofilm and should therefore be distinguished from genetically determined antimicrobial resistance measured by conventional susceptibility testing.The detection of *mexA* together with the high resistance observed in the present study is biologically compatible with a possible contribution of efflux-mediated mechanisms to the resistance phenotype. However, detection of *mexA* should not be interpreted as proof that MexAB-OprM overexpression caused the resistance observed in the VITEK 2 results. Establishing such a relationship would require gene-expression analysis and/or functional efflux-pump assays. Similarly, detection of *lasR* and *pelA* alongside strong biofilm formation is compatible with their established biological roles in quorum sensing and extracellular matrix-associated biofilm development, but conventional PCR alone cannot establish a direct causal relationship.Overall, the findings support a multifactorial model in which antimicrobial resistance and biofilm formation represent interconnected but partially independent characteristics of *P. aeruginosa*. The combination of antimicrobial resistance, biofilm production, and selected molecular determinants may contribute to the persistence of urinary isolates and complicate antimicrobial management. Larger studies involving more urinary isolates, quantitative gene-expression analysis, sequencing, and functional assays are required to clarify the relationships between *lasR*, *pelA*, *mexA*, biofilm formation, and antimicrobial resistance in urinary *P. aeruginosa* populations.

Study significance and limitations

The main strength of the present study is the combined evaluation of antimicrobial susceptibility, quantitative phenotypic biofilm formation, and molecular detection of selected genes in *P. aeruginosa* isolated specifically from urinary specimens. This integrated approach provides more information than antimicrobial susceptibility testing alone and allows phenotypic observations to be considered alongside selected genetic characteristics.Nevertheless, several limitations should be acknowledged. First, the study included only 15 confirmed *P. aeruginosa* isolates, which substantially limits statistical power and generalizability. Second, the cross-sectional design does not establish temporal or causal relationships between antimicrobial resistance, biofilm formation, and gene carriage. Third, conventional PCR demonstrates the presence or absence of target genes but does not assess gene expression, mutations, or functional activity. Finally, the study was conducted in hospitals in Hilla, Iraq; therefore, the findings may not represent all *P. aeruginosa* isolates from other Iraqi healthcare settings.Overall, the findings demonstrate that the urinary *P. aeruginosa* isolates investigated in this study were characterized by extensive antimicrobial resistance and a high capacity for biofilm formation. The additional detection of ***lasR, pelA, and mexA*** provides molecular information that complements the phenotypic findings and supports further investigation of the genetic and regulatory mechanisms underlying persistence and antimicrobial resistance in urinary *P. aeruginosa*.

Conclusion

The present study demonstrated that *Pseudomonas aeruginosa* isolated from urinary tract infections exhibited high levels of antimicrobial resistance and a strong capacity for biofilm formation. Most isolates were resistant to multiple commonly used antipseudomonal agents, while colistin showed the highest susceptibility rate among the tested antimicrobials. Biofilm production was observed in all isolates, with the majority classified as strong biofilm producers, highlighting the potential contribution of biofilm formation to bacterial persistence in urinary infections.Molecular detection of the selected *lasR, pelA, and mexA* genes provided additional characterization of the urinary *P. aeruginosa* isolates. The detection of these genes, together with the observed antimicrobial resistance and biofilm phenotypes, supports the importance of investigating both phenotypic and molecular characteristics of *P. aeruginosa* in UTIs. However, PCR detection alone does not confirm gene expression or establish a direct causal relationship with resistance or biofilm formation. Further studies involving larger numbers of isolates and gene-expression or functional assays are recommended to clarify these relationships.

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