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Multidrug Resistance, Biofilm Formation, and oprL Gene in Pseudomonas Aeruginosa from Iraqi Surgical Wounds

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Annotation: Background & Objective: Pseudomonas aeruginosa is a Gram negative nosocomial wound infection opportunistic pathogen highly resistant and virulent. The present study was conducted to determine the prevalence of P. aeruginosa in surgical wounds, and to investigate biofilm formation, antibiotic resistance profiles and the presence of oprL gene and their association with one another.

Materials and Methods: A cross-sectional study was performed from April to October 2025 on 172 surgical wound samples in Azadi hospital and Kirkuk Teaching Hospital, Iraq. Identification was made with biochemical tests and VITEK 2. Antibiotic sensitivity was determined by Kirby-Bauer disk diffusion and colistin microdilution. Biofilm production was measured by 96-well microtiter plate and oprL gene was detected by PCR.

Results: P. aeruginosa was isolated from 33.7% of samples. High resistance was observed to piperacillin (90%), ceftazidime (87%), and tobramycin (86%), while imipenem (71%) and colistin (50%) were most effective. MDR and XDR phenotypes were 55% and 26%, respectively. Biofilm

analysis showed 36% strong, 31% moderate, and 33% weak/non-producers. The oprL gene was detected in 65% of tested isolates. Strong biofilm formation correlated with higher MDR/XDR rates.

Conclusion: P. aeruginosa types are prevalent in surgical site infections and most isolates were MDR/XDR strains. Opportunistic Pseudomonas raw milk Isolates are likely to harbour biofilm and virulence, oprL genes which may play a role in persistence and antibiotic resistance.

Keywords: P. aeruginosa; Biofilm; Antibiotic Resistance; oprL gene; Wound.

Introduction

Pseudomonas aeruginosa is an adaptable Gram-negative opportunistic pathogen and one of the most prevalent pathogens in healthcare-associated infections, including surgical wound, burn wound and chronic ulcer care (1,2). Its clinical significance is due to a combination of intrinsic mechanisms of resistance, which bring along rapid enzyme acquisition genes and the bacterium's ability to survive in hostile environments in the human body (3). These characteristics render P. aeruginosa one of the most prevalent causes of extended hospital stay, therapeutic failure, and morbidity/mortality in humans worldwide (4). Biofilm, a structured microbial community encased in an extracellular polymeric matrix made up of alginate and Pel and Psl polysaccharides predominately (5, 6) is one of the main reasons why it remains for a long time. Bacterial cells in biofilms are physiologically different from their planktonic counterparts, possessing limited growth, altered nutritional conditions and stress responses, reduced antibiotic penetration into the biofilm matrix and up-regulation of resistance genes, which can lead to 1,000-fold greater levels of tolerance than are seen in planktonically grown cells (7,8). As a result, biofilm formation is increasingly seen as a critical factor in the chronicity and recurrence of wound infections (9). Clinically, P. aeruginosa exhibits resistance to a broad range of antibiotics, such as β-lactams, aminoglycosides, and fluoroquinolones, which are mostly caused by efflux pumps, β-lactamases and target alterations in outer membrane (10, 11). The rise of multidrug resistant (MDR) and extensively drug resistant (XDR) strains has further complicated treatment, resulting in colistin and certain carbapenems being used as options of last resort in many patients (12). At molecular level, oprL, an outer membrane lipoprotein gene, is commonly used as a target for species-specific detection of P. aeruginosa by PCR and has also been reported to be related to membrane stability and environmental adaptation activities (13). Nonetheless, it has been poorly described in relation to biofilm formation and pattern of antibiotic resistance among clinical wound isolates. Regionally, studies in Iraq found P. aeruginosa to be a major pathogen: about 32% of wound isolates in Baghdad (14), approximately 35% in diabetic foot ulcers in Erbil (15), and up to 67% from mixed clinical samples collected from Nasiriyah city (16) with high MDR rates and variable biofilm formation ability. These results underscore the importance of combined phenotypic–genotypic analyses associating biofilm production, resistance profiles and molecular markers including oprL in wound related isolates. The actual quantitative biofilm strength and multidrug resistance capability also does not have a strong correlation with the presence of oprL gene

amongst wound isolated P. aeruginosa isolates. Thus, the study objective was to assess The relationship between oprL gene, biofilm production and profile of antibiotic resistant in Pseudomonas aeruginosa isolated from wound infection.

Materials and Methods

sample collection

A prospective cross sectional study was done on 172 clinical isolates from surgical wound infections. Material and methods Patients admitted in Azadi Teaching Hospital and Kirkuk Teaching Hospital (Kirkuk, Iraq) were collected from April to October 2025. Exudates and wound swabs were collected aseptically by sterile cotton swab after gargling the surface of the wound with 0.9% sterile saline. Specimens were transported to the microbiology laboratory in 2–4 h and stored in transport medium until promptly processed. Demographic and clinical data, age, sex, type of surgery, and wound condition were registered in each patient.

Exclusion criteria

Samples were only excluded if a patient had received systemic antibiotics for >72 hours prior to sampling, cultures revealed heavy polymicrobial contamination that precluded reliable isolation of a solitary pathogen or where the specimens represented superficial skin colonization as opposed to true SSI. Furthermore, samples inadequately collected or transported were rejected, and in the case of multiple isolates obtained from the same patient we selected only one (the earliest) isolate for analysis.

Isolation and identification

Wound specimens were inoculated by streak plate technique aseptically on Mueller-Hinton agar, MacConkey agar and Cetrimide agar aerobically at 37 °C for 24–48 h.. The biochemical tests conducted were oxidase test, catalase test, growth at 42 °C, motility and pyocyanin production. Pure cultures were maintained on brain heart fusion agar slants at 4 °C and as 20% glycerol stocks at –80 °C for further studies.

identification using VITEK 2 system

Further confirmation was made by VITEK 2 Compact system (bioMérieux, France) with GN identification card following the manufacturer protocol. Only isolates identified as Pseudomonas aeruginosa with ≥95% confidence were subjected to further tests.

Antibiotic susceptibility testing

Antibiotic sensitivity was assessed using a disk diffusion method of Kirby–Bauer on Mueller–Hinton agar, and the results were interpreted according to The Clinical & Laboratory Standards Institute 2025 [17]. The 10 antibiotics used were Piperacillin–tazobactam (TZP, 100/10 µg), Ceftazidime (CAZ, 30 µg), Cefepime (FEP, 30 µg), Imipenem (IPM, 10 µg), Meropenem (MEM, 10 µg), Amikacin (AK, 30 µg), Gentamicin (CN, 10 µg), Tobramycin (TOB, 10µg) and Ciprofloxacin (CIP5µ g). Colistin was estimated by broth micro dilution. Isolates were categorized as Susceptible, Intermediate and Resistant, while Multidrug resistance (MDR) was considered against three or more classes of antibiotics.

Biofilm assay – Microtiter plate technique

Biofilm assay Biofilm formation was evaluated according to the method of 96-well microtiter plate. Bacterial suspension was adjusted to 0.5 Mc Farland and diluted in a ratio of 1:100 with tryptic soy broth with 1% glucose and incubated at 37 °C for 24 h, the wells were washed three times with PBS and air dried followed by staining with crystal violent (0.1%) for 15 min, then rinsed in water and solubilized in 95% ethanol. The absorbance was recorded at 570 nm. Isolates were classified as non, weak, moderate or strong biofilm producers according to the threshold values defined [18].

Genomic DNA extraction

Genomic DNA was extracted from confirmed isolates as per the manufacturer's protocol by using Geneaid Genomic DNA Extraction Kit (Taiwan). After being lyzed with lysis buffer and proteinase K, bacterial cells were loaded onto a silica spin column for DNA binding to the membrane. The column was washed stepwise to remove impurities and DNA was eluted in nuclease-free buffer. DNA was checked for quality and concentration using NanoDrop (A260/A280) and stored at –20 °C.

PCR Amplification of oprL gene

PCR was used to amplify the oprL gene, which serves as a species-specific molecular marker for the identification of *Pseudomonas aeruginosa*. The reaction mixture (25 µL) contained 12.5 µL of 2× Master Mix, 1 µL of forward primer, 1 µL of reverse primer (Table 1), 2 µL of template DNA extracted from clinical isolates, and 8.5 µL of nuclease-free water (Table 2). The PCR was performed using a standard thermal cycling program in a thermocycler, which included an initial denaturation step at 95 °C to separate the DNA strands completely, followed by 35 cycles of denaturation at 95 °C, annealing at 55 °C for optimal primer binding, and extension at 72 °C. A final extension at 72 °C was included to ensure complete synthesis of all PCR products (Table 3). The amplified products were then analyzed on a 1.5% agarose gel, and the presence of a 504 bp band confirmed the amplification of the oprL gene.

Table 1. Primer sequences of oprL gene

Primer	Sequence (5′→3′)	Amplicon Size (bp)	Ref.
oprL-F	ATGGAAATGCTGAAATTCGGC	504	[19]
oprL-R	CTTCTTCAGCTCGACGCGACG		

Table 2. PCR reaction mixture (25 µL)

Component	Volume (µL)
2× Master Mix	12.5
oprL Forward (10 pmol)	1.0
oprL Reverse (10 pmol)	1.0
Template DNA	2.0
Nuclease-free water	8.5
Total	25 µL

Table 3. PCR thermal cycling conditions for oprL gene

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 s	35
Annealing	55	30 s	35
Extension	72	45 s	35
Final extension	72	7 min	1

Gel electrophoresis

PCR products were resolved on 1.5% agarose gel in 1× TBE buffer at 90–100 V for 60 min**,** stained with a safe DNA dye, and visualized under UV light. A 100 bp DNA ladder was used as a molecular marker. A distinct 504 bp band confirmed the presence of the oprL gene.

Results

Isolation

Out of the 172 surgical wound samples collected from Azadi and Kirkuk Teaching Hospitals

between April and October 2025, 58 isolates were confirmed as Pseudomonas aeruginosa, giving an isolation rate of 33.7%. The distribution of positive and negative samples is presented in Table 4, where 58 samples were positive (33.7%) and 114 samples were negative (66.3%). On Mueller-Hinton agar, the colonies appeared large, flat with irregular margins, producing blue-green pigmentation (pyocyanin) and a characteristic grape-like odor, as shown in Figure 1, confirming the presence of P. aeruginosa in the positive samples.

Table 4. Isolation rate of *Pseudomonas aeruginosa* from wound samples (n=172)

Sample outcome	Number of samples	Percentage (%)
Positive for *P. aeruginosa*	58	33.7
Negative for *P. aeruginosa*	114	66.3
Total	172	100

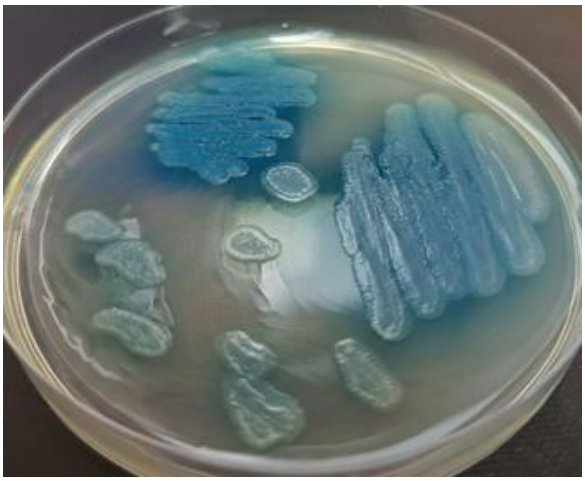

Figure 1. Colonies of *P. aeruginosa* on Mueller-Hinton agar, showing typical blue-green pigmentation and flat morphology.

Identification

All isolates of Pseudomonas aeruginosa were subjected to conventional biochemical tests to confirm their identity. All isolates were Gram-negative rods (-), oxidase positive (+), catalase positive (+), motile (+), and grew at 42 °C (+). In addition, pyocyanin production on Cetrimide agar (+) was observed for the majority of isolates, Table 5, Diagnosis was further confirmed using the VITEK 2 system, which validated all isolates as P. aeruginosa, giving a confirmation rate of 100%.

Table 5. Biochemical characteristics of *Pseudomonas aeruginosa* isolates

Biochemical test	Result
Gram stain (Gram-negative rods)	-
Oxidase test	+
Catalase test	+
Motility test	+
Growth at 42 °C	+
Pyocyanin production on Cetrimide agar	+

Antibiotic susceptibility

All confirmed Pseudomonas aeruginosa isolates (n=58) were tested for susceptibility to 12 commonly used antibiotics. Imipenem (S 71%) and Colistin (S 50%) showed the highest susceptibility, while Piperacillin (R 90%) and Ceftazidime (R 87%) were the least effective. Intermediate responses were generally low (5–10%), Table 6. These results indicate that carbapenems and colistin are the most effective therapeutic options for these isolates.

Table 6. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolates (n=58)

Antibiotic	Susceptible (S)	Intermediate (I)	Resistant (R)
Piperacillin	0 (0%)	6 (10%)	52 (90%)
Ceftazidime	2 (3%)	6 (10%)	50 (87%)
Tobramycin	3 (5%)	5 (9%)	50 (86%)
Gentamicin	12 (21%)	3 (5%)	43 (74%)
Piperacillin–tazobactam	18 (31%)	6 (10%)	34 (59%)
Aztreonam	19 (33%)	4 (7%)	35 (60%)
Cefoperazone	19 (33%)	3 (5%)	36 (62%)
Amikacin	23 (40%)	3 (5%)	32 (55%)
Imipenem	41 (71%)	3 (5%)	14 (24%)
Meropenem	33 (57%)	4 (7%)	21 (36%)
Ciprofloxacin	27 (47%)	3 (5%)	28 (48%)
Colistin	29 (50%)	3 (5%)	26 (45%)

All Pseudomonas aeruginosa isolates (n=58) were classified according to their resistance profiles as multidrug-resistant (MDR), extensively drug-resistant (XDR), or pandrug-resistant (PDR). Among the isolates, 32 isolates (55%) were MDR, 15 isolates (26%) were XDR, and no isolates (0%) were PDR. The remaining 11 isolates (19%) were susceptible or non-MDR. Table 7 indicate a significant prevalence of multidrug resistance among wound-derived P. aeruginosa isolates.

Table 7. MDR, XDR, and PDR profiles of *Pseudomonas aeruginosa* isolates (n=58)

Resistance profile	Number of isolates	Percentage (%)
MDR	32	55%
XDR	15	26%
PDR	0	0%
Susceptible/Non-MDR	11	19%

All Pseudomonas aeruginosa isolates (n=58) were assessed for biofilm formation using the microtiter plate method. After staining with crystal violet, wells with deep purple color indicated strong biofilm formation, light purple indicated moderate biofilm, and nearly colorless wells indicated weak or no biofilm. Based on visual observation of the plate (Figure 2), 21 isolates (36%) were strong biofilm producers, 18 isolates (31%) were moderate, and 19 isolates (33%) were weak or non-producers.

Table 8. Biofilm formation profile of *Pseudomonas aeruginosa* isolates (n=58)

Biofilm category	Number of isolates	Percentage (%)
Strong	21	36%
Moderate	18	31%
Weak/Non-producer	19	33%

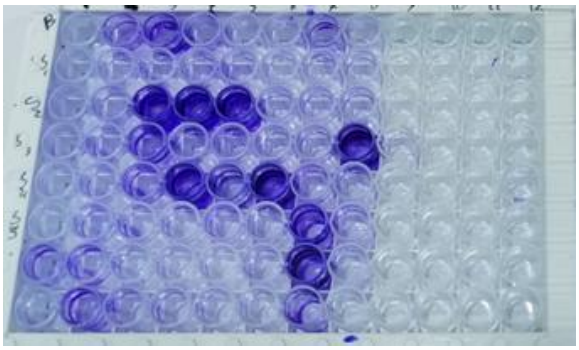

Figure 2. Biofilm formation of P. aeruginosa assessed by crystal violet staining

oprL gene

The presence of the oprL gene was evaluated in Pseudomonas aeruginosa isolates using PCR with specific primers. Out of 17 representative isolates selected for molecular analysis, 11 isolates (65%) tested positive for the oprL gene, while 6 isolates (35%) were negative. PCR amplification produced a 504 bp product for all positive isolates, confirming the species-specific detection of P. aeruginosa. Table 9, demonstrating the prevalence of the oprL gene among the selected wound-derived isolates.

Table 9. Detection of oprL gene in *Pseudomonas aeruginosa* isolates (n=17)

oprL gene	Number of isolates	Percentage (%)
Positive (+)	11	65%
Negative (−)	6	35%

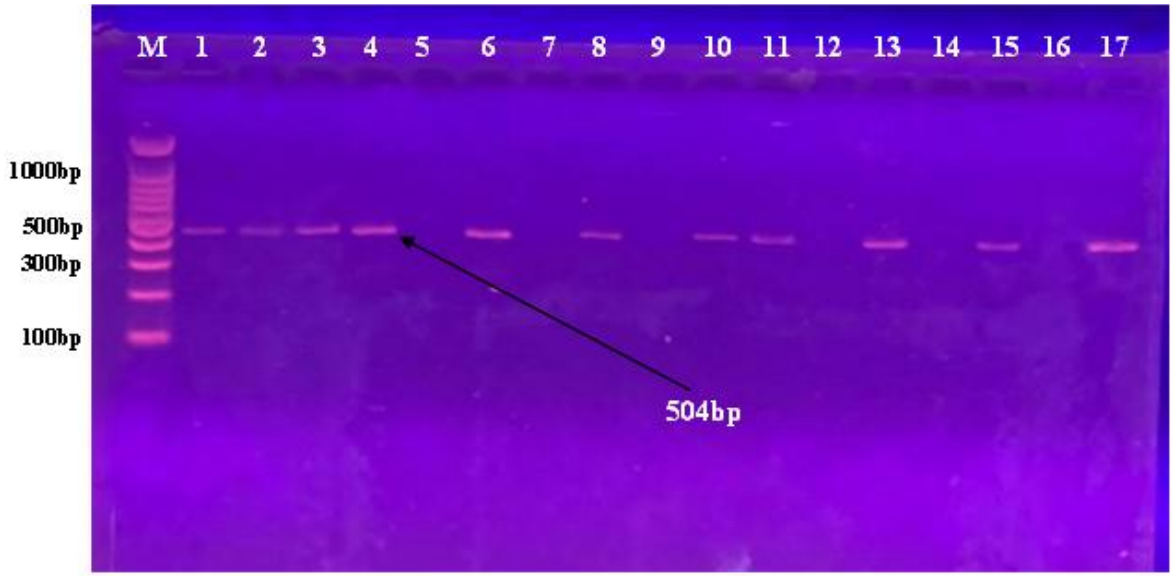

Figure 3. PCR amplification of the oprL gene using 1.5% agarose gel electrophoresis at 75 V, showing a 504 bp product, M: 100bp Ladder, 1-17: bacterial isolates.

Relationship between biofilm formation and **antibiotic resistance profiles**

The relationship between biofilm formation and antibiotic resistance profiles was evaluated to determine whether stronger biofilm-producing Pseudomonas aeruginosa isolates were associated with higher multidrug resistance. Among the 58 isolates, strong biofilm producers (n=21) showed a higher proportion of MDR/XDR profiles, whereas weak or non-biofilm producers (n=19) were predominantly susceptible or non-MDR. Moderate biofilm producers (n=18) exhibited intermediate resistance patterns. These results are summarized in Table 10, demonstrating a clear trend of increased resistance with stronger biofilm formation.

Table 10. Association between biofilm formation and MDR/XDR profiles of *Pseudomonas aeruginosa* isolates (n=58)

Biofilm category	MDR	XDR	Susceptible/Non-MDR	Total
Strong	15	5	1	21
Moderate	12	3	3	18
Weak/Non-producer	5	7	7	19
Total	32	15	11	58

Discussion

In the present work, P. aeruginosa was predominant in surgical wound 33.7% of Azadi and Kirkuk Teaching Hospitals-General /Kirkuk- city, showing the high risk for opportunistic pathogen to

postoperative infection this results similar with previous studies that done at Iraq (20,21) and Nigeria (22,23). This high occurrence might be because the organism is able to adapt in hospital environments, has viability in medical equipment, and colonizes wet sites (21,22). All isolates were determined to be gram-negative rods that were oxidase and catalase positive, motile, capable of growing at 42°C, and produced pyocyanin phenotypically by classical biochemical identification and in accordance with previous studies of burn and wound isolates (22, 24). Phenotypically, the oprL gene was detected in 65% of selected isolates, thereby supporting species-specific detection (22,23,24), and negative detections in some isolates was probably due to genetic variation or primer binding site mutations, thus phenotypical and molecular diagnostics were complementary (22,24). The majority of isolates exhibited resistance pattern of > 85 per cent towards piperacillin (90%) > ceftazidime (87%) and tobramycin (86%), whereas the lowest rate was recorded for imipenem (71%) followed by colistin (50%). These findings are partially consistent with Al Mohammed and Mahmood (25) and Saudi et al. (21), who found comparable β-lactam and aminoglycoside resistance rates, but are unlike Khan and Faiz (26) who reported lower resistance rates in Saudi hospitals, which may relate to regional variance in antibiotic stewardship and consumption. MDR and XDR were observed in 55% and 26% of isolates, respectively, with no PDR strains identified in this collection, similar to what has been reported among isolates from Iraq before (21,25,27), emphasizing the increasing clinical impact of MDR P. aeruginosa. Biofilm production In biofilm formation analysis, 36% strong, 31% moderate and 33% weak/non producers was detected. The majority of strong biofilm producers were MDR/XDR, and conversely the majority of weak biofilm formers were susceptible/non-MDR, indicating a significant relationship between formation of biofilms and antimicrobial resistance (28,29,30). This is consistent with earlier studies demonstrating the protective role of biofilm matrices against antibiotics and host defenses which prompted persistence in chronic wounds (25,28,29). The presence of other virulence genes, such as exoS, exoT, exoY, exoU and oprI and oprL supports a multifactorial virulence spectrum (Veetilvalappil et al. (31), Chadha et al. (32), Ali and Abdulrahman (33), Degaim et al. (34), Al-Mayyahi (35), and Gholami, et al. (24). Since the prevalence of virulence genes varies among isolates, this might also contribute to genomic diversity and therefore different disease severity and biofilm-associated resistance found (22,24). In general, these results indicate a high prevalence of MDR and XDR P. aeruginosa associated with surgical wound infections in Iraq, limiting therapeutic options for infected individuals due to biofilm formation and virulence factors, suggestions that underscore the necessity of surveillance strategies and targeted antimicrobial stewardship (20,27, 35).

Conclusions

Most SWIs were caused by Pseudomonas aeruginosa (33.7%), with a high prevalence of MDR (55%) and XDR strains (26%). Biofilm formation and the presence of essential virulence genes (oprL, oprI, exoS, exoT, exoY and exoU) were both related with high-level of antimicrobial resistance suggesting involvement in disease persistence as well as treatment failure. Carbapenems, particularly imipenem, and colistin were the most active agents. These data underline the necessity of active surveillance, reinforcement of infection control and appropriate antibiotic utilization for preventing MDR and XDR P. aeruginosa dissemination.

Limitations

The study was performed in 2 hospitals only, and findings may not be generalized. A subset of isolates was not analyzed at the molecular level and, hence, some variation in virulence genes might have been missed. Environmental contributors to biofilm formation and resistance were not evaluated. Larger nationwide, multi-center studies are needed to confirm these findings and explore other environmental-and host-related resistance and virulence determinants.

References

1. Gellatly SL, Hancock RE. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Nat Rev Microbiol. 2013;11:490–502.

2. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017;7:39.
3. Poole K. Pseudomonas aeruginosa: resistance to the max. Front Microbiol. 2011;2:65.
4. Strateva T, Yordanov D. Pseudomonas aeruginosa—a phenomenon of bacterial resistance. J Med Microbiol. 2009;58:1133–1148.
5. Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in P. aeruginosa biofilm development. Curr Opin Microbiol. 2007;10:644–650.
6. Ma L, Conover M, Lu H, et al. Assembly and development of the Pseudomonas aeruginosa biofilm matrix. PLoS Pathog. 2009;5:e1000354.
7. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358:135–138.
8. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2:95–108.
9. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. Wound Repair Regen. 2008;16:37–44.
10. Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev. 2015;28:337–418.
11. Breidenstein EB, de la Fuente-Núñez C, Hancock RE. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 2011;19:419–426.
12. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drugs Context. 2018;7:212527.
13. De Vos D, Lim A Jr, Pirnay JP, et al. Species-specific PCR detection of P. aeruginosa using oprL. J Clin Microbiol. 1997;35:1040–1044.
14. Al-Ajeeli MN et al. Antibiotic sensitivity and biofilm formation in P. aeruginosa from wound infections in Baghdad. J Coll Basic Educ. 2024.
15. Saeed RH et al. P. aeruginosa in diabetic foot ulcers in Erbil: resistance patterns. Med J Tikrit Univ. 2023.
16. Kareem AA et al. Biofilm formation and resistance in clinical isolates from Nasiriyah. Univ Thi-Qar J Sci. 2025.
17. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.
18. Christensen GD, Simpson WA, Younger JJ, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for biofilm formation. J Clin Microbiol. 1985;22(6):996–1006.
19. Okafor CN, Mel D, et al. Identification of efflux pump mutations in Pseudomonas aeruginosa from clinical samples; Antibiotics (Basel). 2023;12(3):486.
20. Alkhulaifi ZMM, Mohammed KAS. Prevalence and molecular analysis of antibiotic resistance of Pseudomonas aeruginosa isolated from clinical and environmental specimens in Basra, Iraq. Iran J Microbiol. 2023;15(1):119–127.
21. Saud A, Hussein RH, Jameel YM. Antibiotic resistance and biofilm formation in Pseudomonas aeruginosa from clinical samples in Nasiriya, Iraq. Univ Thi-Qar J Sci. 2025;12(1):216–223.

22. Onyi T, Doko MHI, Ella E. Molecular detection of oprL gene in Pseudomonas aeruginosa associated with surgical site infections in Bauchi, Nigeria. Microbes Infect Dis. 2025;6(3):5029.

23. Mohammed AG, Gharbi WA. Molecular detection of oprI and oprL virulence genes of Pseudomonas aeruginosa isolated from burns and wounds. Iraqi J Biotechnol. 2022;21(2):215–224.

24. Gholami A, Majidpour A, Talebi-Taher M, Boustanshenas M, Adabi M. PCR-based assay for the rapid and precise distinction of Pseudomonas aeruginosa from other Pseudomonas species recovered from burns patients. J Prev Med Hyg. 2016;57(2):E811.

25. Al-Mohammed TA, Mahmood HM. Carbapenem resistance related with biofilm formation and pilin genes in clinical Pseudomonas aeruginosa isolates. Iraqi J Pharm Sci. 2024;33(1):72–78.

26. Khan MA, Faiz A. Antimicrobial resistance patterns of Pseudomonas aeruginosa in tertiary care hospitals of Makah and Jeddah. Ann Saudi Med. 2016;36(1):23–28.

27. Ismail SJ, Mahmoud SS. First detection of New Delhi metallo-β-lactamases variants (NDM-1, NDM-2) among Pseudomonas aeruginosa isolated from Iraqi hospitals. Iran J Microbiol. 2018;10(2):98.

28. Amieva R, Gil-Gil T, Martínez JL, Alcalde-Rico M. The MexJK multidrug efflux pump is not involved in acquired or intrinsic antibiotic resistance in Pseudomonas aeruginosa, but modulates the bacterial quorum sensing response. Int J Mol Sci. 2022;23(14):7492.

29. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes Dis. 2019;6(2):109–119.

30. Al-Saeedi RHA, Raheema RH. Molecular diagnosis of some virulence genes in Pseudomonas aeruginosa clinical isolates in Wasit Province. Indian J Public Health. 2019;10(04).

31. Veetilvalappil VV, Manuel A, Aranjani JM, Tawale R, Koteshwara A. Pathogenic arsenal of Pseudomonas aeruginosa: an update on virulence factors. Future Microbiol. 2022;17(6):465–481.

32. Chadha J, Harjai K, Chhibber S. Revisiting the virulence hallmarks of Pseudomonas aeruginosa: a chronicle through the perspective of quorum sensing. Environ Microbiol. 2022;24(6):2630–2656.

33. Ali MDY, Abdulrahman ZF. Molecular identification, susceptibility pattern, and detection of some virulence genes in Pseudomonas aeruginosa isolated from burn patients. Plant Arch. 2020;20(1):2573–2580.

34. Degaim ZD, Al-Malk HK, Nassar AM, Nasir AA. Molecular detection of two virulence factors of Pseudomonas aeruginosa isolated from burn patients. Univ Thi-Qar J Sci. 2019;7(1):62–65.

35. Al-Mayyahi AW. Detection of (exoT, exoY, exoS and exoU) genes in Pseudomonas aeruginosa isolates from different clinical sources [MSc thesis]. Baghdad: University of Baghdad; 2018. p. 60–63.