

Prevalence, Biofilm-Forming Capacity and Expression of Matrix-Associated Genes in Clinical *Pseudomonas Aeruginosa* Isolates from Babylon, Iraq

Manar Fadhil Obaid¹ and Ali Hmood Al-Saadi²

Identify the Number of *Pseudomonas Aeruginosa* from Different Clinical Specimens and Quantify Biofilm Production

ABSTRACT

Objective: To identify the number of *Pseudomonas aeruginosa* from different clinical specimens and quantify biofilm production and to compare gene expression of *pslA*, *pelA* and *fliC* among biofilm and non-biofilm phenotypes by using reverse transcription-quantitative polymerase chain reaction

Study Design: Prospective cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Life Sciences, College of Science, University of Babylon, Iraq from 1st November 2025 to 30th April 2026.

Methods: Fifty samples from laboratory confirmed *Pseudomonas aeruginosa* were collected from 50 patients; 87 isolates were also cultured on Cetrimide agar and confirmed as *Pseudomonas aeruginosa* via standard polymerase chain reaction utilizing the *azu* gene (476 bp). Biofilm development was determined based on OD₅₉₀ readings from stained crystal violet biofilms formed following 24 hr of static culture in 96 well plates. Gene expression was measured via reverse transcription-quantitative polymerase chain reaction on an Mx3005P with SYBR Green utilizing *rpoD* as a normalizing reference gene and the 2^{-ΔΔC_T} method for data analysis.

Results: Seventy six (87.4%) were confirmed as *Pseudomonas aeruginosa*; wound sample comprised 30% of total samples; overall confirmatory increase across each type ranged from 81.8% (urine) to 100% (blood). Biofilms were created by 80.3% of confirmed isolates; of these, strongly biofilm-producing (vs weak) isolates constituted the majority (42.1%). Gene expression of *pslA* (13.5-62.5-fold; F=121,038; P<0.0001) and *pelA* (1,116-5,181-fold; F=903,770,772; P<0.0001) were significantly downregulated in all biofilm-producing phenotypes relative to non-biofilm-producing phenotypes; *fliC* was significantly upregulated (4.2-19.6-fold; F=497.9; P<0.0001).

Conclusion: *P. aeruginosa* clinical specimens from Babylon are highly capable of biofilm formation; however, the unexpected expression profile of biofilm producing specimens suggests that there is a significant dissociation between phenotypic expression of biofilm components and the mechanism of biofilm persistence.

Key Words: *Pseudomonas aeruginosa*, Biofilm, Exopolysaccharide, Prevalence, Nosocomial infection.

Citation of article: Obaid MF, Al-Saadi AH. Prevalence, Biofilm-Forming Capacity and Expression of Matrix-Associated Genes in Clinical *Pseudomonas Aeruginosa* Isolates from Babylon, Iraq. Med Forum 2026;37(9):47-51. doi:10.60110/medforum.370909.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacillus that can grow in a wide range of different environments and survive in harsh and damaging environments of the human body. *P. aeruginosa*, one of the leading causes of health-care associated infection in the world, causes significant morbidity and mortality around the world and is

associated with a large volume of cases in patients who are immune compromised or require prolonged hospitalization, or patients with an indwelling medical device^{1,2}. The clinical importance of *P. aeruginosa* increases due to its inherent resistance to many antibiotic classes (e.g., β-lactams, aminoglycosides, and fluoroquinolones) in addition to its ability to acquire new resistance factors through horizontal gene transfer or mutation³.

The virulence determinants of *P. aeruginosa* are extremely complex and include surface structures used for adhesion to the host and evasion of the immune response, an extensive number of secreted toxins and proteases, and sophisticated regulatory networks that allow for coordinated behavioral changes at the population level in response to environmental or host-derived signals^{4,5}. A central regulatory system governing the virulence of *P. aeruginosa* is the process of quorum sensing. Quorum sensing allows for the interaction of multiple *P. aeruginosa* cells by utilizing a set of chemical molecules, such as the N-acyl

¹. M.Sc. Student / Professor² / Department of Life Sciences, College of Sciences, University of Babylon, Iraq.

Correspondence: Manar Fadhil Obaid, M. Sc. Student, Department of Life Sciences, College of Sciences, University of Babylon, Iraq.

Contact No: 07843718428

Email: sci712.mannar.f@student.uobabylon.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

homoserine lactones or the *Pseudomonas* quinolone signal, to activate the expression of several different virulence genes, including those encoding exotoxin A, the LasB elastase, alkaline protease, pyocyanin, and rhamnolipids, which all contribute to tissue damage, immune evasion, and ultimately the success of the bacterium in establishing an infection⁶. In addition to these planktonic virulence mechanisms, *P. aeruginosa* has the ability to form biofilms as a different way to survive as compared to free-living bacteria and significantly enhances the ability of bacteria to survive in the environment⁷. Biofilm formation occurs in *P. aeruginosa*. The developmental stages of *Pseudomonas aeruginosa* biofilms include reversible attachment and irreversible attachment (adhesion), microcolony formation, maturation into three-dimensional structures (tower), and dispersal of motile cells that can set up a new infection site at other locations^{8,9}.

METHODS

This prospective cross-sectional study was conducted at Department of Life Sciences, College of Science, University of Babylon, Iraq 1st November 2025 to 30th April 2026 vide letter 8251008 date 8-10-2025. Fifty samples from laboratory confirmed *Pseudomonas aeruginosa* were collected from 50 patients. The types of specimens collected were 15 wound swabs; 12 mid-stream urine specimens from patients with suspected urinary tract infections; 10 expectorated sputum specimens from patients with respiratory infections; 8 peripheral blood cultures; and 5 cerebrospinal fluid specimens obtained through lumbar punctures. All specimens were transported to the laboratory within 2 hours after collection using sterile transport media (i.e., Stuart's medium for wound swabs; sterile containers for liquid specimens). Using the streak plate method, all samples were plated onto Cetrimide agar plates (Liofilchem, Italy; Cat. No. 610041) and placed in a 37°C incubator for 24–48 hours of aerobic incubation. Colonial morphology, colour (pyocyanin blue-green, pyoverdine yellow-green fluorescent) and pattern of growth were noted. To maximise the amount of intra-specimen diversity captured, two morphologically different types of colonies were picked from plates with phenotypically diverse morphological could yield 87 total bacterial isolates from 50 specimens. Preliminary phenotypic identification was performed by Gram staining, KOH testing (3% KOH), and catalase (3% H₂O₂) and oxidase tests (1% tetramethyl-p-phenylenediamine dihydrochloride) following the protocols established by the authors.

Extracting genomic DNA from cultures grown 18-24 hours utilized the boiling method. One colony was diluted in 100 µl sterile distilled water, vortexed, boiled for 10-15 minutes, cooled quickly (on ice), centrifuged (10,000 rpm, 5 minutes); the supernatant was the template for PCR. The *azu* gene was amplified from

genomic DNA using species-specific PCR primers that produced 476 bp products (F: 5'-CAGTCGACGCCATCTTC-3'; R: 5'-AACCAGTTGTTCTTGCCG-3'). PCR reactions (25 µl) used Hot FirePol Master Mix (Solis BioDyne, Estonia) and were subjected to the following thermal cycling: 95°C × 5 mins; 35 × (95°C × 30 s, 55°C × 30 s, 72°C × 45 s); 72°C × 10 min. PCR products were resolved on 1.5% agarose gels containing RedSafe dye (Intron, Korea) at 75 V for 45 minutes using UV transillumination.

The standard crystal violet microtiter plate assay was used to quantify biofilm production 18 from overnight cultures.

A total of twelve biofilm representative isolates (three per category) were selected for transcriptional profiling according to their median OD₅₉₀ values in each category. Once the biofilm isolates were prepared in mid-log-phase cultures (OD₆₀₀ ≈ 0.5), total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) with on-column DNase I digestion to eliminate any genomic DNA contamination from the biofilm isolates. RNA quality was verified on a Quantus Fluorometer (Promega, USA) using A₂₆₀/A₂₈₀ ratios (1.8-2.1), and only those samples with RNA integrity numbers (RIN) ≥ 7.0 were used for downstream applications. Synthesis of complementary DNA (cDNA) was performed using 500 ng of total RNA and the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following manufacturer's recommendations to use random hexamer primers to create cDNA.

Quantifying mRNA levels of *pslA*, *pelA* and *fliC* was accomplished using Mx3005P Real-Time PCR instruments (Stratagene, USA) which utilized the SYBR Green Master Mix (Qiagen, Germany) for detection. For each reaction (20 µl total volume), 10 µl of the SYBR Green Master Mix, 1 µl of the forward and reverse primers (10 pmol/µl), 1 µl of cDNA, and 7 µl of nuclease-free water were added to an optical well plate and the following thermocycler program was used: (1) 95°C for 10 min, (2) 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, and (3) final step of melt curve analysis from 60°C to 95°C (increments of 0.5°C). The housekeeping gene used as a reference for relative expression was the sigma factor gene *rpoD*. Each gene was quantified in technical duplicates and three biological replicates per treatment group were included. Relative mRNA expression was determined by the 2^{-ΔΔC_T} (Livak and Schmittgen) method where non-biofilm-producing strains were the calibrator strain for calculating relative expression. The data was entered and analyzed through SPSS-26.

RESULTS

Clinical samples were obtained from 50 patients with five different kinds of infections. Selective culture on Cetrimide agar yielded 87 bacterial isolates. Of these, the overall confirmation rate of *P. aeruginosa* by conventional PCR amplification of the *azu* gene was 76

out of 87 (an 87.4% confirmation rate). Confirmation rates were highest in samples from cerebrospinal fluid (100%) and lowest in urinary tract specimens (81.8%). The largest absolute number of confirmed *P. aeruginosa* isolates (23 isolates or 26.5%) were identified in wounds due to the high volume of specimens and the high prevalence of *P. aeruginosa* in cutaneous infections. Eleven isolates were excluded from all future analyses because they did not amplify the *azu* gene (Table 1). Figure 1 showed the agarose gel electrophoresis (1.5%, RedSafe stain, 75 V, 45 min) showing characteristic 476 bp *azu* gene amplicons from confirmed *P. aeruginosa* clinical isolates. Lanes 1-2: negative controls; positive control; M: 100 bp DNA ladder.

Of the 76 confirmed isolates, biofilm formation was found at an occurrence rate of 80.3% (61 of 76). Strong (maximum) biofilm producers represented the largest proportion of the overall number of isolates as 32 of 76

(42.1%), moderate biofilm producers represented the next largest proportion of the total number of isolates: 29 of 76 (38.2%), weak (minimum) biofilm producers 6 of 76 (7.9%) and non-producing biofilms 9 of 76 (11.8%). As for OD_{590} values, the strong biofilm producers are scored with a wide range from 0.404 to 2.173. Two wound specimen biofilm producers had an OD_{590} value greater than 1.4, yielding an approximate three to five times strong producer level hyper-biofilm phenotype. The vast majority of strong biofilm producers were found within the respiratory (43.8%) or the wound (43.5%) isolates. The overwhelming majority of non-producing biofilm isolates were found within the CSF isolates (28.6%); however, only a small number of CSF isolates (Table 2). The RT-qPCR Gene Expression of *pslA*, *pelA*, and *fliC* were shown in Tables 3-4.

Table No. 1: Distribution of clinical specimens, bacterial isolates, and confirmed *P. aeruginosa* across infection site categories

Specimen Type	Total Collected	Bacterial Isolates	Confirmed <i>P. aeruginosa</i>	Confirmation Rate (%)
Wound infections	15	26	23	88.5
Urinary tract infections	12	22	18	81.8
Respiratory (sputum)	10	18	16	88.9
Blood cultures	8	14	12	85.7
Cerebrospinal fluid	5	7	7	100.0
Total	50	87	76	87.4

Table No. 2: Biofilm-forming capacity of confirmed *P. aeruginosa* isolates (n=76) stratified by infection source

Infection Source	N	Strong No. (%)	Moderate No. (%)	Weak No. (%)	None No. (%)
Wound	23	10 (43.5)	9 (39.1)	2 (8.7)	2 (8.7)
Urinary tract	18	7 (38.9)	7 (38.9)	2 (11.1)	2 (11.1)
Respiratory	16	7 (43.8)	7 (43.8)	1 (6.2)	1 (6.2)
Blood	12	5 (41.7)	4 (33.3)	1 (8.3)	2 (16.7)
CSF	7	3 (42.9)	2 (28.6)	0 (0.0)	2 (28.6)
Total (n=76)	76	32 (42.1)	29 (38.2)	6 (7.9)	9 (11.8)

Table No. 3: Mean CT values ($\pm$ SD) for *pslA*, *pelA*, *fliC* and reference gene *rpoD* across biofilm phenotype categories, with ANOVA F-statistics

Gene	Non-producers (CT $\pm$ SD)	Weak producers (CT $\pm$ SD)	Moderate producers (CT $\pm$ SD)	Strong producers (CT $\pm$ SD)	F-value (ANOVA)
<i>pslA</i>	28.31 $\pm$ 0.17	29.55 $\pm$ 0.25	26.61 $\pm$ 0.21	29.60 $\pm$ 0.36	121,038*
<i>pelA</i>	34.68 $\pm$ 0.23	33.48 $\pm$ 0.34	34.23 $\pm$ 0.06	35.37 $\pm$ 0.23	903,770,772*
<i>fliC</i>	20.27 $\pm$ 0.34	29.55 $\pm$ 0.10	19.53 $\pm$ 0.44	21.69 $\pm$ 0.10	497.9*
<i>rpoD</i>	23.58 $\pm$ 0.22	24.56 $\pm$ 0.09	22.34 $\pm$ 0.08	23.24 $\pm$ 0.03	Reference

Table No. 4: Relative fold-change expression ($2^{-\Delta\Delta C_T}$ method, non-producers as calibrator) of *pslA*, *pelA*, and *fliC* in biofilm-producing isolate categories

Gene	Weak producers (fold-change $\pm$ SD)	Moderate producers (fold-change $\pm$ SD)	Strong producers (fold-change $\pm$ SD)	Regulation Direction
<i>pslA</i>	0.074 $\pm$ 0.005	0.016 $\pm$ 0.001	0.030 $\pm$ 0.001	↓ Downregulated
<i>pelA</i>	0.0009 $\pm$ 0.0001	0.0002 $\pm$ 0.00001	0.0004 $\pm$ 0.00001	↓↓ Strongly down
<i>fliC</i>	19.59 $\pm$ 1.23	4.22 $\pm$ 0.22	7.87 $\pm$ 0.17	↑ Upregulated

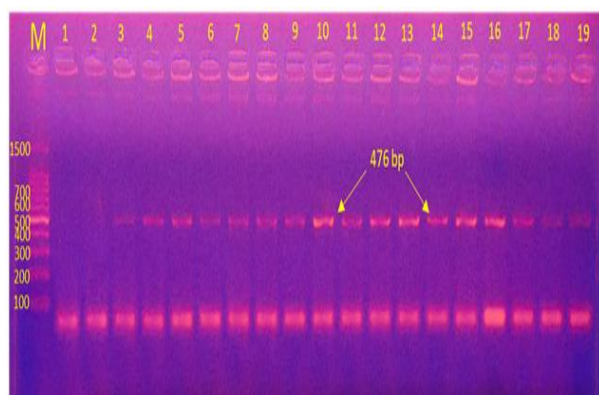


Figure No. 1: Gel electrophoresis image: *azu* gene PCR amplicons (476 bp) confirming *P. aeruginosa* from diverse clinical specimens. M = 100 bp ladder; NC = negative control (*E. coli*); PC = positive control (PAO1 reference strain)

DISCUSSION

We found a significant and consistent prevalence of *P. aeruginosa* in multiple types of clinical specimens. Second, we demonstrated the ability of the extracted *P. aeruginosa* isolates to produce biofilm, with strong producer isolates being a dominant phenotype among confirmed samples. Finally, we identified a very interesting profile of gene expression in the *P. aeruginosa* isolates that was marked by a down-regulation of biosynthesis-related genes, such as *pslA* and *pelA*, that are critical to biofilm formation and a concurrent up-regulation of the motility gene *fliC*. Overall, this study provides the most thorough molecular epidemiologic description of biofilm formation by *P. aeruginosa* in Babylon Province, Iraq and serves to challenge many fundamental concepts that are intrinsic to current biofilm formation theories^{10,11}.

The overall molecular confirmation rate (i.e., proportion of total isolates that were confirmed by molecular means) of all isolates from all specimen types (n=68) was 87.4%. The percentage of confirmed isolates is important to highlight the EPC of *P. aeruginosa* as a nosocomial pathogen in Babylon Province and is similar to the confirmation percentages reported in comparable studies from other provinces in Iraq. The prevalence of *P. aeruginosa* among Gram negative organisms isolated from clinically multi-drug resistant clinical collections in central Iraq by Al-Dahmoshi et al¹² was between 83-90%, while Hassan et al¹³ confirmed that their isolates of *P. aeruginosa* were also present at very high rates among patients in Baghdad area hospitals. The specimen-specific confirmation rates for the *P. aeruginosa* samples in the current study ranged from 81.8% for the urine specimens to 100% for the cerebrospinal fluid specimens, and reflect demonstrated trends of *P. aeruginosa* in regards to their ability to be pathogenic to different anatomical sites. It is particularly interesting to note that the high rate of confirmation from CSF specimens is high (though based on a small number of

specimens) and indicates the severe immunocompromised state of patients from whom lumbar puncture specimens are collected; this is an environment where biosphere organisms can colonize^{14,15}. The use of the *azu* gene as a molecular target for species-specific confirmation is methodologically validated; azurin is the copper-binding electron transfer protein encoded only in *P. aeruginosa*, without corresponding genomic equivalents in related *Pseudomonas* species, giving it exceptional specificity for diagnostic purposes^{16,17}.

The 80.3% prevalence of biofilm formation among confirmed strains is consistent with biofilm forming stats reported in the literature; for example, Al-Shammaree et al¹⁸ reported 82.4% of *P. aeruginosa* isolates from Najaf and Hassan et al¹³ reported 78.9% of isolates from Baghdad. It has been reported that biofilm formation among *P. aeruginosa* from clinical settings globally has fallen in a range between approximately 70%-90% depending on specimen type and methodology¹⁹. The predominance of highly biofilm-producing strains (42.1% of this cohort) is clinically significant due to the well-established positive correlation between biofilm forming strength, minimum inhibitory concentrations for antibiotics, failure rate and length of stay in a hospital. Hyper-biofilm-producing strains identified in this study were characterized by OD₅₉₀ measurements of >1.4, substantially above the strong producer threshold, and are especially worthy of further study because there is mechanistic linkage between hyper-biofilm phenotype and permanent activation of the RetS-GacS-GacA regulatory cascade leading to a chronic infection lifestyle characterized by high levels of matrix production, excessive alginate production and down-regulation of acute virulence factors²⁰. These strains may represent particularly hard to treat strains associated with persistent relapsing infections. The transcriptional results presented show that *pslA* and *pelA* were down-regulated in the biofilm-forming isolates. This is the opposite of what is predicted under traditional biofilm regulatory theory. Traditional beliefs about how biofilms form come from studies on the laboratory reference strains, *Pseudomonas aeruginosa* PAIO and PA14, which suggest that the transition from planktonic to biofilm form occurs when the c-di-GMP signal is elevated, which triggers the expression of both the *psl* and *pel* gene clusters and inhibits the expression of genes involved in flagellar motility (such as *fliC*)^{10,11}. These results suggest that this traditional model does not hold true for clinical isolates of *P. aeruginosa* and points to other mechanisms by which this may occur¹⁴.

CONCLUSION

87.4% of bacterial isolates from humans are *Pseudomonas aeruginosa*. Wound infections were the most prevalent source of infection, and 80.3% of confirmed isolates had evidence of biofilm formation.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Manar Fadhil Obaid, Ali Hmood Al-Saadi
Drafting or Revising Critically:	Manar Fadhil Obaid, Ali Hmood Al-Saadi
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 8251008 Dated 08.10.2025

REFERENCES

- Jurado-Martín I, Sainz-Mejías M, McClean S. *Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenal of virulence factors. *Int J Mol Sci* 2021;22(6):3128.
- Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: An update. *Drugs* 2021; 81(18):2117-31.
- Botelho J, Grosso F, Peixe L. Antibiotic resistance in *Pseudomonas aeruginosa* - Mechanisms, epidemiology and evolution. *Drug Resist Updat* 2020; 44:100640.
- Javed H, Srivastava A, Yadav A, Yadav N, Tyagi RK, Pandey LM. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther* 2024; 9(1):63.
- Moradali MF, Ghods S, Rehm BH. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol* 2021;7:39.
- Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, et al. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther* 2022;7(1):199.
- Maurice NM, Bedi B, Sadikot RT. *Pseudomonas aeruginosa* biofilms: Host response and clinical implications in lung infections. *Am J Respir Cell Mol Biol* 2020;58(4):428-39.
- Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, et al. Bacterial biofilm and associated infections. *J Chin Med Assoc* 2021;81(1):7-11.
- Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. *Pathog Dis* 2020;67(3):159-73.
- Ahmed MN, Abdelsamad A, Wassermann T, Porse A, Becker J, Sommer MOA, et al. The evolutionary trajectories of *P. aeruginosa* in biofilm and planktonic growth modes exposed to ciprofloxacin: Beyond selection of antibiotic resistance. *npj Biofilms Microbiomes* 2023;9(1):28.
- Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW. *Pseudomonas* genomes: Diverse and adaptable. *FEMS Microbiol Rev* 2021;35(4):652-80.
- Al-Dahmoshi HOM, Al-Khafaji NS, Jeyad AH, et al. Molecular characterization of virulence factors in multidrug resistant *Pseudomonas aeruginosa* isolates from Iraqi hospitals. *J Pure Appl Microbiol* 2020;14(2):1383-92.
- Hassan RH, Al-Marjani MF, Kadhim HS. Molecular detection of virulence and biofilm formation genes in multidrug-resistant *Pseudomonas aeruginosa* isolates collected from Baghdad hospitals. *J Pure Appl Microbiol* 2021;15(3):1484-95.
- Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage *Pseudomonas aeruginosa* infections. *Drugs Context* 2021;7:212527.
- Freschi L, Vincent AT, Jeukens J, Emond-Rheault JG, Kukavica-Ibrulj I, Dupont MJ, et al. The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer, and pathogenicity. *Genome Biol Evol* 2020;11(1):109-20.
- Mohammed SH, Saleh IY, Abdul-Hassan IA. Prevalence and antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolated from burn patients in Baghdad, Iraq. *Burns* 2023;49(4):892-9.
- Olewi MH, Jasim MH, Al-Halbosi MMF. Ventilator-associated pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa* in intensive care units: A cross-sectional study. *Med J Babylon* 2021;18(3):320-26.
- Al-Shammaree SA, Al-Ghanimi MA, Sadiq SK. Biofilm formation and antimicrobial resistance patterns of *Pseudomonas aeruginosa* isolated from patients in Najaf province, Iraq. *J Pure Appl Microbiol* 2023;17(1):543-53.
- Al-Rubaye AF, Tariq ZN, Alrubaiy L. Molecular characterization and phylogenetic analysis of multidrug resistant *Pseudomonas aeruginosa* isolated from Iraqi patients. *Gene Rep* 2022;26:101481.
- Khalil MA, Al-Jebory KN, Alzubaidy TH. Virulence genes and their association with clinical outcomes in *Pseudomonas aeruginosa* bacteremia in Iraq. *Microb Pathog* 2021;160:105173.