



## Isolation and Detection of Biofilm Producing *Pseudomonas aeruginosa* from Suspected Urinary Tract Infections in Dogs and Its Resistance to Antibiotics

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### A B S T R A C T

This study aimed to determine the relationship between the biofilm formation in *Pseudomonas aeruginosa* and the protein concentration in it, as well as to determine the resistance of this bacterium to antibiotics. A total of 108 urine samples were collected from suspected urinary tract infections (UTIs) in dogs, conducting general urine examination (GUE) to detect the infection, isolation, and identification the bacteria based on traditional diagnostic tests, the VITEK-2 system, and polymerase chain reaction (PCR) targeting the 16S rRNA gene. The study involved determining the antibiotic susceptibility of the isolates against ten antibiotics, determination of the biofilm layer by the tube method and Congo red method, evaluation of the strength of biofilm by microtiter plate, and detection of the biofilm protein concentration by Bradford method. The total number of *P. aeruginosa* isolates was 6/108 (5.5%), among which the isolates that produced the biofilm were 5/6 (83.33%). These isolates were confirmed by the VITEK-2 assay followed by the PCR and sequencing of the amplicon that validated the identity of the isolates, with 99% similarity to *P. aeruginosa* reference sequences, and the sequences were deposited in NCBI GenBank (accession numbers PP979721.1-PP979726.1). Analysis showed a strong positive correlation ( $r = 0.998$ ) between the biofilm formation and the protein concentration. All isolates demonstrated 100% resistance to amikacin, trimethoprim, cefotaxime, amoxicillin/clavulanic acid, ampicillin, cephalixin, and lincomycin, and 33% were resistant to gentamicin, while 100% were sensitive to ciprofloxacin. In conclusion, these findings underscore a significant correlation between biofilm formation and protein concentration. The knowledge of bacterial ability to form biofilms and their antibiotic resistance pattern is important to improve veterinary practices and prescription of the appropriate antibiotic in the context of UTIs in dogs.

**Keywords:** *Pseudomonas aeruginosa*, urinary tract infection, dogs, biofilm formation, antibiotic resistance

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### INTRODUCTION

*Pseudomonas aeruginosa* is a non-fermenting Gram-negative bacterium, unable to ferment sugars (hence the name of the group) (1). It is rod-shaped, obligated aerobic, motile, no spore-forming, oxidase and catalase positive (2), and regarded as an important pathogen of chronic wounds and burns, eye infections, otitis, respiratory, and urinary tract infections (UTIs) (3).

Its ability to resist multiple antibiotics arises from both intrinsic resistance mechanisms and the acquisition of resistance-conferring plasmids, classifying it as a multidrug-resistant (MDR) pathogen (4). The MDR pathogen is defined as being non-susceptible to at least one agent in three or more antimicrobial groups (5). *P. aeruginosa* is resistant to various antimicrobial agents, this resistance is caused by many different mechanisms, such as low permeability of its outer membrane leading to difficult

crossing of these antibiotics (6), production of antibiotic-inactivating enzymes that destroy the antibiotic molecules before their arrival to the intracellular target position (7), also has efflux pumps (8), which expel antibiotics out of the bacterial cell and other toxic molecules produced by other bacteria or the host (9).

Moreover, *P. aeruginosa* has different virulence factors, among these the ability to produce one or more pigments, such as pyoverdine, pyocyanin, and pyorubin (10), these pigments are implicated in antioxidant, quorum sensing (QS), and iron acquiring properties (11). In addition, biofilm production is one of the most significant virulence factors of *P. aeruginosa*. Biofilms facilitate the adherence of microorganisms to various surfaces, providing protection against harsh environmental conditions (e.g., desiccation, nutrient deprivation) and the host's immune defenses (e.g., phagocytes, natural killer cells, and complement proteins) (12), and there is a strong correlation exists between the bacterium capacity to form biofilms and its resistance to antibiotics (13). The unique characteristics of biofilms complicate infection treatment, often leading to the progression of acute infections into chronic forms (14).

Biofilms have heterogeneous components, formed by accumulation of sessile bacterial communities, exopolysaccharides, extracellular DNA (eDNA), carbohydrates, proteins, surfactants, lipids, various ions, and water (1, 15). Biofilm-associated proteins may constitute a crucial and highly regulated system that supports pathogenesis, nutrition uptake, stress tolerance, and biofilm stability (16).

On the other hand, a group of virulence factors in *P. aeruginosa* is under the control of the QS system (17), which is a bacterial cell-cell communication that regulates the expression levels of genes in a cell density dependent manner through chemical signals that play an essential role in pathogenesis, biofilm formation as well as antibiotic resistance (18).

The UTI is an inflammation of any portion of the urinary system (19), and bacterial UTIs are estimated to affect about 14% to 15% of dogs over their lifetime (20). The most common bacterial causes in pets are *E. coli*, *S. aureus* and *Klebsiella* spp. followed by *P. mirabilis* and *P. aeruginosa* (21). UTI in dogs occurs frequently with significant effects on the kidney's functions, causing chronic kidney diseases (22). Among the bacterial agents that cause UTI in dogs is *P. aeruginosa* (23). The symptoms of UTI depend on the infected site, duration of the disease, presence or absence of influencing causes, animal response, virulence, and the microbial load of pathogenic agents (20). However, many dogs with UTI are asymptomatic (24).

This study aimed to detect the relation between the presence of a biofilm layer and the concentration of its protein component with evaluating the resistance to antibiotics in *P. aeruginosa* isolated from dogs with UTIs.

## MATERIALS AND METHODS

### Ethical Approval

Ethical approval for this study was obtained from the Local Committee on Animal Care and Use at the College of Veterinary Medicine, University of Baghdad (Approval No. 279, dated 7/2/2024). Participation in the study was contingent upon informed consent from dog owners, who were provided with a detailed explanation of the study's objectives. Verbal consent from the owners was also secured prior to sample collection.

### Sample Collection

A total of 108 urine samples were collected from dogs suspected with UTIs with or without clinical signs and admitted to the Veterinary Hospital in Baghdad and private veterinary clinics located in different areas of Baghdad city (Alaadhama, Zaiuna, Almansor, Alkhadhray, Alghazalia, Algamiya) during the period from February 2024 to July 2024. The dogs included in the study were of different ages (21 dogs  $\leq$  1 year and 87 dogs  $>$  1 year), both genders (86 male and 22 female), and different breeds (37 German shepherd, 20 Belgian Malinois, 45 Terrier, 2 Husky, 2 *Canis coris*, 1 Pomeranian, and 1 Lolo fox). The samples were collected following spontaneous urination and manual urinary bladder compression after performing local sterilization. The urine was put in a sterile, labeled container in a cool box, and then sent to the Microbiology laboratory, at the College of Veterinary Medicine, University of Baghdad.

### General Urine Examination

A 5 mL aliquot of the urine sample was transferred into a test tube and centrifuged at 3000 rpm for 7 min. Following centrifugation, the supernatant was discarded, and the sediment was placed on a glass slide for examination under a light microscope at 40 $\times$  magnification (25).

### Isolation and Identification

A loopful of urine was inoculated onto 5% sheep blood agar and MacConkey agar (HiMedia, India), and the plates were incubated aerobically at 37 $^{\circ}$ C for 24 h. After incubation, a loopful of isolated colonies were selected and sub-cultured on selective media, including Cetrinide agar (Micromedia, USA) and *Pseudomonas* agar (HiMedia, India), to obtain pure bacterial growth (26).

The Gram-negative bacteria that were isolated by culture media were further diagnosed using the Vitek 2 system (BioMerieux, France) with specific Gram-negative cards. The cards contained 64 wells; each well represented a specific biochemical test (27).

### Polymerase Chain Reaction (PCR) and Sequencing

The PCR reaction was performed for the Vitek2 positive samples following the extraction of DNA using a commercial DNA extraction kit (Genomic DNA Mini Kit, Korea). Primers targeting the 16S rRNA gene were designed by Srinivasan et al. (28). The sequences of the primers were as follows: the forward primer (27F) 5'-

AGAGTTTGATCCTGGCTCAG-3' and the reverse primer (1392R) 5'-GGTTACCTTGTACGACTT-3', producing an amplicon of 1250 base pairs.

The PCR reaction mixture had a final volume of 25  $\mu$ L. The components included 5  $\mu$ L of Taq PCR PreMix (5U/ $\mu$ L), 1  $\mu$ L of the forward primer (10 picomole/ $\mu$ L), 1  $\mu$ L of the reverse primer (10 picomole/ $\mu$ L), 1.5  $\mu$ L of extracted DNA, and 16.5  $\mu$ L of distilled water. The PCR cycling conditions were as follows: the initial denaturation step was performed at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 58°C for 45 sec, and extension at 72°C for 1 min. A final extension step was conducted at 72°C for 5 min. The PCR products, with the targeted band size corresponding to the 16S rRNA gene, were visualized on a 1.5% agarose gel using a UV transilluminator (Vilber Lourmat Ste, France). The amplified products were sent for sequencing to Macrogen Inc. (Geumcheon-gu, Seoul, South Korea) using the Sanger method. The sequences were compared with the NCBI GenBank database using the basic local alignment search tool (BLAST) to confirm the isolates as *P. aeruginosa*.

## Biofilm Detection Methods

### Congo red method

Congo red agar (GCC, UK) was cultured with the isolated *P. aeruginosa* for 24-48 h at 37°C. Appearance of black colored colonies indicated the biofilm formation, while the red colonies indicated non-biofilm formation (29).

### Tube method (qualitative assay)

A loopful of the isolated bacteria was inoculated into 5 mL of tryptic soy broth (HiMedia, India) supplemented with 1% glucose and incubated at 37°C for 72 h. After incubation, the tube was washed with normal saline and air-dried. The dried tube was then heat-fixed by passing it through a flame three times. Subsequently, the biofilm was stained with 0.1% crystal violet for 1 h. Excess stain was removed, and the tube was rinsed with normal saline and dried in an inverted position. Biofilm formation was confirmed by the presence of a visible film on the inner walls and at the bottom of the tube (30).

## Biofilm Evaluation by Microtiter Plate Assay (Quantitative Assay)

The biofilm production by *P. aeruginosa* isolates was quantitatively assessed using the microtiter plate method (31). *P. aeruginosa* isolated from fresh agar plates were inoculated in 5 mL of tryptic soy broth with 4% sucrose, and the broth was incubated for 24 h at 37°C. Then, 96-microtiter plate with flat wells was used to inoculate 200  $\mu$ L of the bacterial suspension equivalent to 0.5 McFarland standard (1.5 x10<sup>8</sup> CFU/mL) in three wells for each isolate, and un-inoculated tryptic soy broth with 4% sucrose was used as a negative control. The 96-well plate was covered and incubated at 37°C for 24 h. After incubation, the plate was washed three times with phosphate buffered saline (PBS) to eliminate non-adherent cells after discarding the contents of the wells. To fix the adhered cells, 200  $\mu$ L of 99%

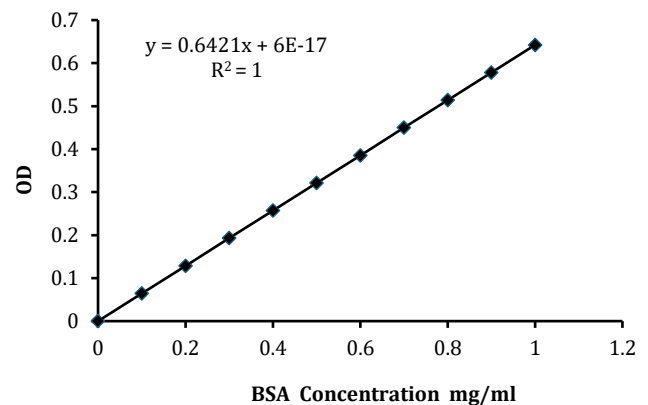
methanol per well was added for 20 min. The plate was dried at room temperature in an inverted position overnight. After that, the wells were stained with 180  $\mu$ L of 1% crystal violet tincture at room temperature for 15 min. Subsequently, the microtiter plate was washed and dried. Then, the attached dye was solubilized with 200  $\mu$ L of 96% ethanol, and the optical density (OD) was measured by a microtiter plate reader (Shimadzu, Japan) at 570 nm. The biofilm formation strength was evaluated according to (32) as described in Table 1.

**Table 1.** Evaluation of biofilm formation by the microtiter plate method

OD values	Biofilm evaluation
OD < OD <sub>c</sub>	Non
OD <sub>c</sub> < OD < 2 × OD <sub>c</sub>	Weak
2 × OD <sub>c</sub> < OD < 4 × OD <sub>c</sub>	Moderate
4 × OD <sub>c</sub> < OD	Strong

## Detection of Protein in the Biofilm

The concentration of protein in the biofilm was quantitatively determined using the Bradford method, as described by Bradford (33), with a standard curve generated by the use of bovine serum albumin (BSA) (Figure 1). Various concentrations of BSA stock solution (0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL) were prepared for this assay. A blank tube was prepared by adding 500  $\mu$ L of potassium phosphate buffer, while a sample tube was prepared by mixing 50  $\mu$ L of the sample with 450  $\mu$ L of potassium phosphate buffer. Subsequently, 2500  $\mu$ L of Coomassie Brilliant Blue G-250 dye (Thomas Baker, India) was added to both the blank and sample tubes. The tubes were allowed to stand at room temperature for 2 min before measuring the absorbance at 595 nm using a spectrophotometer.



**Figure 1.** Standard curve of BSA concentrations

## Antimicrobial Susceptibility Test

Antibacterial susceptibility test was done according to Kirby-Bauer disk diffusion susceptibility method (34) by using 10 different antibiotic disks. The antibiotics included ciprofloxacin (5  $\mu$ g), gentamicin (10  $\mu$ g), amikacin (30  $\mu$ g), trimethoprim (5  $\mu$ g), cefotaxime (30  $\mu$ g), amoxicillin/clavulanic acid (20/10  $\mu$ g), ampicillin (10  $\mu$ g), cephalixin (30  $\mu$ g), and lincomycin (10  $\mu$ g). These

antibiotics were chosen as they are the most common antibiotics described by veterinary clinics in Baghdad to treat most cases in dogs including UTIs.

The bacterial suspension was prepared by picking 4-5 pure colonies from a new culture of each isolate and suspending into a sterile test tube containing 4 mL of normal sterile saline. The turbidity was equal to 0.5 McFarland tube. Then, a sterile cotton swab was inserted into the suspension, carefully transferred, and equally spread over the surface of Mueller-Hinton agar (MHA, HiMedia, India) medium, which was left for 15 min to stand. The antibiotic disks were placed on the agar by using sterile forceps pressed firmly onto the medium to fix the disks. Then, the agar plates were incubated for 24 h at 37°C. The formation of the inhibition zone around these antibiotic disks was measured by a metric ruler with a millimeter (mm) unit according to CLSI (35). The readings were interpreted as resistant (R), intermediate (I), or sensitive (S) to specific antibiotics.

### Statistical Analysis

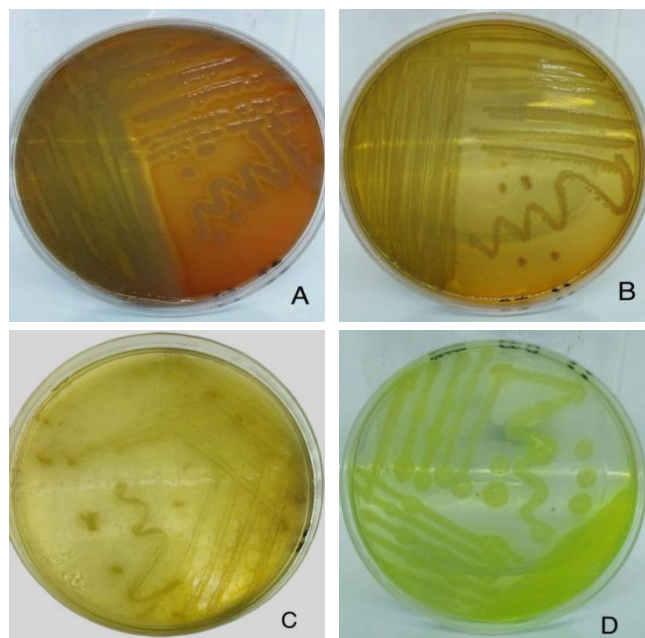
Data were analyzed using SAS (Statistical Analysis System - version 9.1). One-way analysis of variance (ANOVA) and least significant differences (LSD) test were performed to assess significant differences among means.  $P \leq 0.05$  was considered statistically significant. The Pearson correlation analysis was performed to detect the

relationship between the biofilm formation and protein concentration in *P. aeruginosa* samples at  $P \leq 0.05$ .

### RESULTS AND DISCUSSION

*P. aeruginosa* is widely regarded as a major causative agent of nosocomial infections infecting both humans and animals. It is a common pathogen that causes otitis externa (36) and wound infection (37), while it was isolated from the urine of dogs affected with malignant tumors by (38) at low percentage. Veterinary clinics and hospitals frequently lack comprehensive surveillance activity covering the most common bacterial species that cause UTIs in pets and their antimicrobial resistance profiles, which makes proper antibiotic management more difficult (39). The sample of this study was taken from dogs with UTIs from veterinary clinics and practices are presented in this study, to provide more information about the prevalence of *Pseudomonas* in urinary tract and their patterns of antibiotic resistance in dogs.

The results of general urine examination in this study as observed through the microscope demonstrated the presence of pus and bacteria in the urine samples where *P. aeruginosa* was isolated which indicates a UTI. *P. aeruginosa* isolates grew on the blood agar with beta-hemolysis, and on MacConkey agar where they produced non-lactose fermenting colonies. On selective media, such as cetrimide and pseudomonas agar, the colonies displayed a characteristic green pigment (Figure 2).

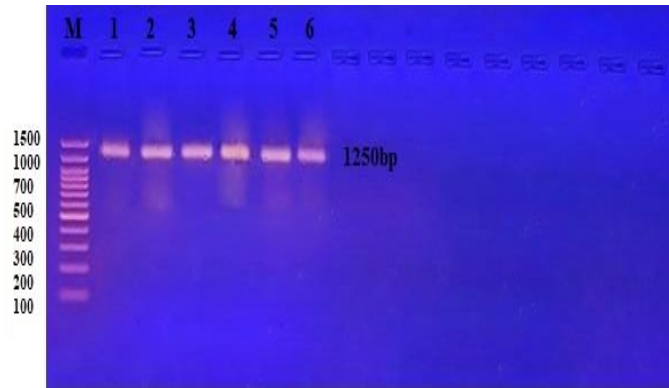


**Figure 2.** *P. aeruginosa* grown on various agar media. **(A)** Blood agar: Bacterial growth exhibiting beta-hemolysis. **(B)** MacConkey's agar: Bacterial growth with non-lactose fermenting colonies. **(C)** Cetrimide agar: Bacterial growth displaying green pigmentation. **(D)** Pseudomonas agar: Bacterial growth displaying green pigmentation.

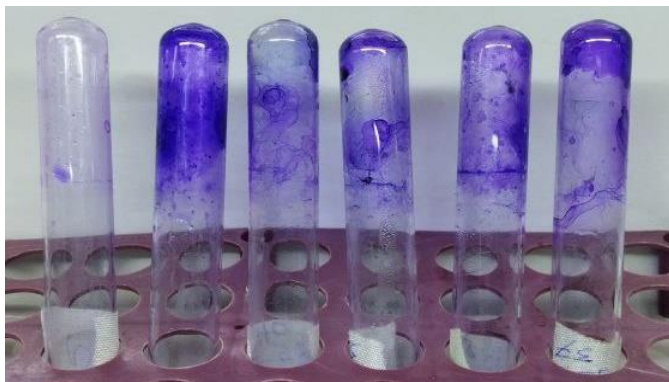
Additionally, the isolates were positive for oxidase and catalase activity (data not shown). The isolates were further confirmed as *P. aeruginosa* with 99% accuracy using the VITEK-2 system (data not shown). The PCR

amplification targeting the 16S rRNA gene successfully identified all six isolates as *P. aeruginosa* (Figure 3). Sequencing of the PCR products further validated the identity of the isolates, with BLAST analysis revealing 99%

similarity to *P. aeruginosa* reference sequences, including strain [OR793893.1](#). The sequences have been deposited in the NCBI GenBank database under the accession numbers [PP979721.1](#), [PP979722.1](#), [PP979723.1](#), [PP979724.1](#), [PP979725.1](#), and [PP979726.1](#).



**Figure 3.** PCR products with a band size of approximately 1250 bp of 16S rRNA. The products were subjected to electrophoresis on 1.5% agarose at 5 volts/cm<sup>2</sup> for 1:30 h. M: DNA ladder (100 bp)

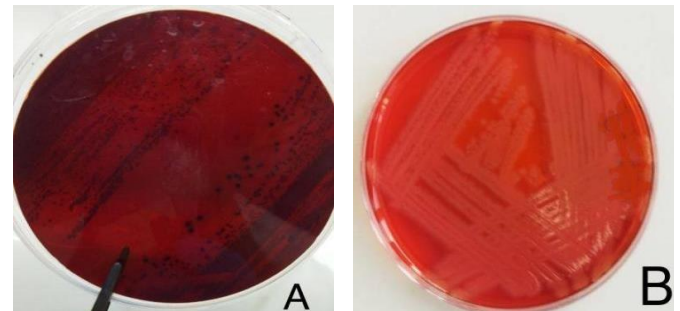


**Figure 4.** Tube method for detecting the biofilm formation by *P. aeruginosa*. A positive result is indicated by a violet-colored layer adhering to the tube wall, while a negative result is shown by a colorless tube with no visible layer.

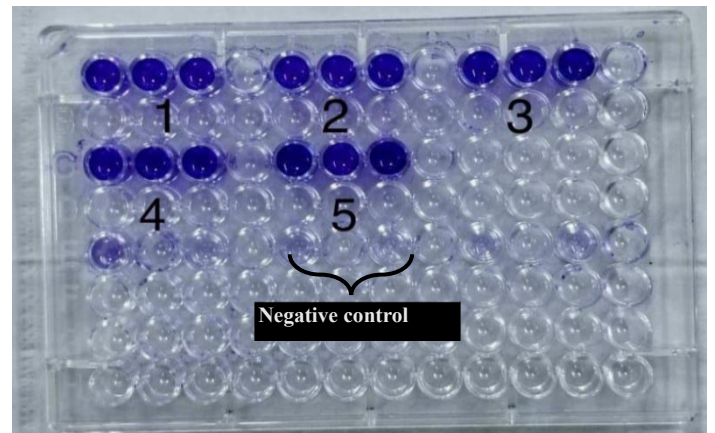
Basically, the results showed the occurrence of UTI due to *P. aeruginosa* in only six dogs (6/108, 5.5%). This corresponds with another study (27) where *P. aeruginosa* isolates constituted 5.3% of the dog cases represented by cystitis and lower UTIs. While another author (40) reported that 4.2% of the dogs with bladder infection had *P. aeruginosa*.

Biofilm formation was detected using the tube method and Congo red agar. As shown in Figure 4, the results of tube method revealed that five out of six isolates (5/6, 83.33%) produced biofilm versus one isolate (1/6, 16.66%) unable to produce it. Further examination for the biofilm formation of these isolates using the Congo red agar (in which the colonies with black color (Figure 5A) indicated biofilm production, whereas colonies that remained red (Figure 5B) signified the absence of biofilm production) revealed similar results. These findings differ from those of (40), who reported that 100% of the isolates produced a biofilm.

By using the microtiter plate assay (Figure 6) for the quantitative evaluation of biofilm production strength, the mean OD values of the biofilm-producing isolates were determined as presented in Table 2. The results indicate that all isolates had OD values greater than 0.224, classifying them as strong biofilm producers.



**Figure 5.** *P. aeruginosa* colonies on the Congo red agar. (A) black colonies indicate the biofilm formation. (B) red color colonies indicate non-biofilm formation



**Figure 6.** The microtiter plate assay used for the quantity evaluation of the biofilm formation strength. Numbers 1-5 represent the five isolates.

These results indicate that all samples (5/5, 100%) produced strong biofilm, a finding that differs from the study of Hattab et al. (40), which reported that all urine isolates from dogs were intermediate biofilm producers. Additionally, sample 5 appeared more significant among other samples at ( $P < 0.05$ ) as in (Table 2).

**Table 2.** Biofilm formation by *P. aeruginosa* isolates: Comparison of the OD values at 570 nm

Sample	Mean $\pm$ SE of OD
1	0.376 $\pm$ 0.06 b
2	0.359 $\pm$ 0.04 b
3	0.483 $\pm$ 0.06 b
4	0.708 $\pm$ 0.08 a
5	0.710 $\pm$ 0.07 a
Control	0.056 $\pm$ 0.01 c
L.S.D. value	0.2051 *

The highest protein concentration in the biofilm produced by *P. aeruginosa* isolates was 0.46 mg/mL, while

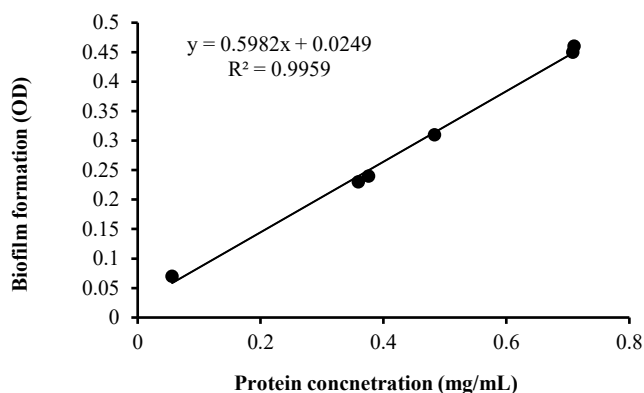
the lowest concentration was 0.24 mg/mL. This finding differed with the results of Alithawy (41), who reported that the highest protein concentration in the biofilm produced by *P. aeruginosa* isolates was 0.12 mg/mL, while the lowest concentration was 0.035 mg/mL. Also, these results differ from those of Altabakchally (42), who found that the protein concentration in the biofilm produced by *E. coli* was significantly higher, reaching 92 mg/mL. Furthermore, there was no significant difference in the protein concentration between samples 1, 2, and 3, nor between samples 4 and 5. However, a significant difference was observed between these samples and the control at the  $P < 0.05$  level (Table 3).

**Table 3.** Protein concentration in the biofilm produced by *P. aeruginosa* isolates

Sample	Protein concentration
1	0.24±0.01 <sup>b</sup>
2	0.23±0.01 <sup>b</sup>
3	0.31±0.05 <sup>b</sup>
4	0.45±0.08 <sup>a</sup>
5	0.46±0.08 <sup>a</sup>
Negative	0.07±0.01 <sup>c</sup>
LSD	0.12

\* ( $P < 0.05$ )

The relationship between the biofilm formation and protein concentration in *P. aeruginosa* samples was assessed using Pearson's correlation. The results indicate a strong positive correlation ( $r = 0.998$ ), suggesting that increased protein concentration is closely associated with higher biofilm formation (Figure 7). Specifically, the analysis demonstrates that as the protein concentration increases across samples, the biofilm formation also correspondingly increases.



**Figure 7.** Scatter plot showing the correlation between the protein concentration and the biofilm formation ( $r = 0.998$ ). A strong positive linear relationship was observed, indicating that protein levels are closely linked with biofilm formation in the samples

The antibiotic susceptibility test was conducted using 10 different antibiotic disks (Table 4 and Figure 8). All six *P. aeruginosa* isolates (6/6, 100%) exhibited resistance to chloramphenicol, trimethoprim, amikacin, cefotaxime, amoxicillin-clavulanic acid, ampicillin, cephalexin, and

lincomycin. In contrast, 2 out of 6 isolates (33%) were resistant to gentamicin, and all isolates (100%) were sensitive to ciprofloxacin. These results differ from the findings of Hakim et al. (27), who reported lower resistance rates in *P. aeruginosa* isolates from canines, including resistance to amikacin (7%), ampicillin (19%), cefotaxime (28%), ciprofloxacin (15%), and gentamicin (18%), but a much higher resistance rate to trimethoprim-sulfamethoxazole (92%). However, the findings are consistent with Hakim et al. (27) who revealed 100% resistance to amoxicillin-clavulanic acid. Additionally, the results of the present study align with Hariharan et al. (43), who found that 32.1% of isolates from canine UTIs were resistant to gentamicin. In contrast, only 10% resistance to ciprofloxacin, amikacin, and gentamicin in isolates from the urogenital system of dogs was reported in another study (44).

Furthermore, the findings of the current study differ from Darwich et al. (45), who observed resistance rates of  $\geq 50\%$  to amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, ampicillin and cephalexin in *P. aeruginosa* isolates from dogs with UTIs.

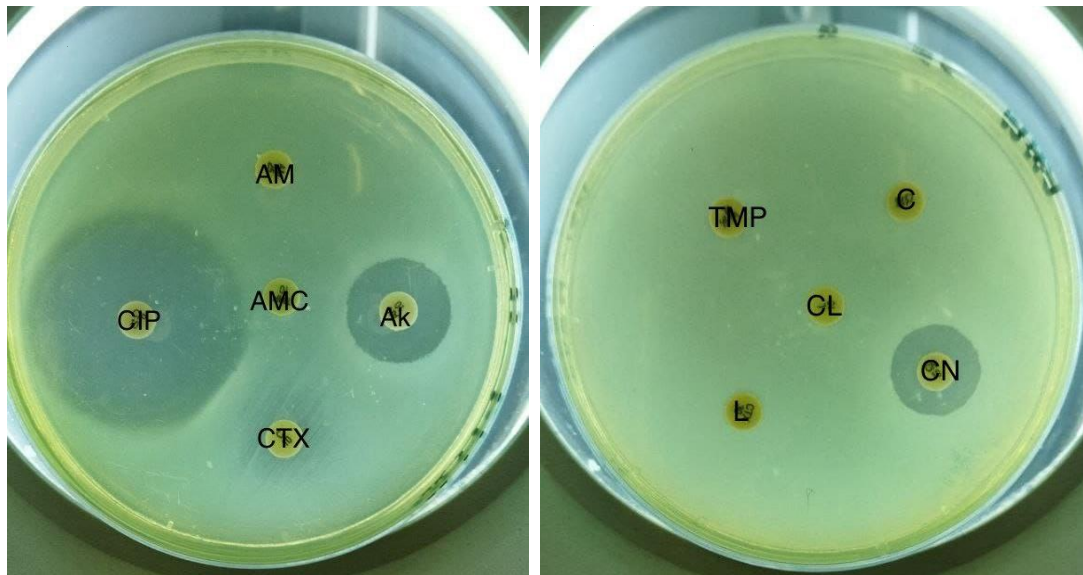
This study revealed that there was a significant correlation between the protein concentration within biofilm layers and the strength of biofilm formation among *P. aeruginosa* isolates. All isolates that produced biofilms demonstrated strong biofilm-formation capabilities. According to the researcher (46), proteins are known to play crucial roles in biofilm development by facilitating bacterial adhesion, surface binding, and the formation of an extracellular matrix that stabilizes and maintains biofilm architecture. Supporting literature highlights that biofilm-associated proteins are integral to biofilm resilience, nutrient acquisition, stress resistance, and the persistence of bacterial communities (16). The researcher (47) clarified that protein levels changed during different stages of biofilm cell adhesion. While the researchers (48) pointed out in his research that the overproduction of Fap amyloids, which are insoluble fibrous proteins support the structural stability of biofilms, causes cell aggregation and increased biofilm development. Others mentioned in their work that metal ion-binding exoprotein was identified and demonstrated to enhance ionic bridges in EPS, thereby promoting biofilm formation (49). In contrast, the authors Ahimou et al (50) declared that the protein concentration did not affect cohesion and remained relatively consistent throughout the biofilm depth. On the other hand, the current findings showed no relationship between the biofilm formation and antibiotic sensitivity, as all *P. aeruginosa* isolates, regardless of biofilm production, exhibited resistance to more than three classes of antibiotics, classifying them as MDR. This aligns with the findings of Gajdacs (51), who reported no significant differences were present in the biofilm formation between MDR and non-MDR *P. aeruginosa* isolates. Similarly, the capacity for biofilm formation in Gram-negative bacteria does not necessarily correlate with phenotypic antibiotic resistance (52). The researcher (53) pointed out that resistance may arise as a result of changes in expression or

lifestyle through the formation of biofilm and tolerance. However, these findings contrast with another research in which a significant relationship between the biofilm

formation and the antibiotic resistance was observed in *P. aeruginosa* isolates (54).

**Table 4.** Antibiotic resistance patterns of *P. aeruginosa* isolates from canine UTIs

Class	Antimicrobial Agent ( $\mu\text{g}/\text{disk}$ )	Susceptibility Number (%)		
		Sensitive	Intermediate	Resistant
Beta-lactams	Amoxicillin-Clavulanate 20/10 (AMC)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Aminoglycosides	Amikacin 30 (AK)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
	Gentamicin 10 (CN)	0/6 (0.00)	4/6 (66.7)	2/6 (33.3)
Folate Pathway Antagonist	Trimethoprim 5 (TMP)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Phenicol	Chloramphenicol 30 (C)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Cephems (subclass: Cephalosporin I)	Cephalexin 30 (CL)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Cephems (subclass: Cephalosporin III)	Cefotaxime 30 (CTX)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Penicillin	Ampicillin 10 (AM)	0/6 (0.00)	0/6 (0.00)	6/6 (100)
Quinolones (subclass: Fluoroquinolones)	Ciprofloxacin 5 (CIP)	6/6 (100)	0/6 (0.00)	0/6 (0.00)
Lincosamides	Lincomycin 10 (L)	0/6 (0.00)	0/6 (0.00)	6/6 (100)



**Figure 8.** Antibiotic sensitivity test shows the zone of inhibition on Mueller Hinton agar using ten antibiotic disks: (A) AM=Ampicillin 10, CIP=Ciprofloxacin 5, AMC=Amoxicillin-Clavulanate 20/10, AK=Amikacin 30, CTX=Cefotaxime 30. (B) TMP=Trimethoprim 5, C=Chloramphenicol 30, CL=Cephalexin 30, L=Lincomycin 10, CN=Gentamicin 10

The findings of this study are consistent with earlier investigations conducted in different parts of the world, showing that *P. aeruginosa* resistance patterns vary according to population, region, and antibiotic use (6; 55). Also, other researchers emphasize the importance of comprehend *P. aeruginosa* resistance patterns across various populations and geographical areas to stop the development and spread of MDR *P. aeruginosa* strains and highlight the necessity of using antibiotics responsibly and closely keeping to infection control protocols (56). In another study, the researchers mentioned several different mechanisms that contribute to the occurrence of antibiotic resistance in *P. aeruginosa*, including reducing the permeability of the outer membrane to antibiotics more than the rest of the Gram-negative bacteria (57). In a study conducted by (58), it revealed that reducing permeability in turn leads to intrinsic resistance to several types of

antibiotics, as the outer membrane contains different types of proteins that affect the movement of these antibiotics in and out of the bacterial cell. Also, the efflux pumps increase the activity of pumping antibiotics out of the cell. Add to that acquired resistance is formed through the transfer of genetic material for example, plasmids, as well as it occurs as a result of mutations in the genes, leading to functional changes.

According to (27), *P. aeruginosa* is generally thought to have an inherent resistance mechanism to several antimicrobial drugs, including tetracycline, trimethoprim-sulfamethoxazole, cephalosporins,  $\beta$ -lactamases, chloramphenicol, and less resistant to imipenem, aminoglycosides, and quinolones. While (59) revealed that the higher susceptibility to ciprofloxacin of the isolates in the present study is probably due to its very limited use in veterinary practice.

Therefore, with appropriate antibiotic use and therapeutic techniques, it is essential to carry out recommended procedures to effectively maintain the antimicrobial susceptibility pattern of bacteria (60). Because of a low frequency of *P. aeruginosa* causing UTI in dogs this study clearly demonstrated that the epidemiology of this bacterium is not significantly impacted but in case MDR *P. aeruginosa*, these dogs can be crucial hosts that act as an ecological reservoir for antibiotic-resistant strains of *P. aeruginosa* that can be harmful.

In conclusion, this study highlights the biofilm-forming capabilities, importance of protein composition, and antibiotic resistance of *P. aeruginosa* isolates from canine UTIs. A significant link was observed between protein levels and biofilm strength. All isolates were MDR, which were resistant to multiple antibiotics except ciprofloxacin. The lack of correlation between biofilm formation and antibiotic resistance suggests that resistance mechanisms may involve genetic adaptations beyond biofilm defenses. These findings emphasize the need for targeted diagnostics and therapies in veterinary medicine.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## العزل والكشف عن الزائفة الزنجارية المكونة للغشاء الحيوي المعزولة من ادرار الكلاب المشتبه اصابتها بالتهاب المسالك البولية ومقاومتها للمضادات الحيوية

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### الخلاصة

تعتبر الزائفة الزنجارية نموذجاً بكتيريا لتكوين الأغشية الحيوية وعلى الرغم من الدور الرئيسي للبروتين في تكوين الأغشية الحيوية إلا أنه لا توجد دراسات كثيرة حول هذا الموضوع لذلك هدفت هذه الدراسة الى تحديد العلاقة بين قوة تكوين طبقة الغشاء الحيوي وتركيز البروتين فيها وكذلك تحديد مقاومة هذه البكتيريا للمضادات الحيوية. تم جمع ١٠٨ عينة بول من كلاب مشتبه في إصابتها بعدوى المسالك البولية من مستشفى بيطري في بغداد ومن عيادات بيطرية خاصة في مناطق مختلفة من محافظة بغداد/العراق. خضعت الكلاب لفحص عام للادرار (GUE) للكشف عن العدوى وعزل البكتيريا التي تم تحديدها بناءً على الاختبارات التشخيصية التقليدية ونظام VITEK-2 تلاه اختبار تفاعل البلمرة المتسلسل (PCR). تم إجراء اختبار حساسية المضادات الحيوية للعزلات ضد عشرة مضادات حيوية. تم تحديد طبقة الأغشية الحيوية التي تنتجها الزائفة الزنجارية بطريقة الأنبوب وطريقة الكونكو الحمراء وتم تقييم قوة الأغشية الحيوية بواسطة لوحة المايكرو تابترو للكشف عن تركيز بروتين الأغشية الحيوية بطريقة برادفورد. أظهرت النتائج أن العدد الإجمالي لعزلات الزائفة الزنجارية ١٠٨/٦ (٥٠,٥٪)، وكانت العزلات المنتجة للغشاء الحيوي ٦/٥ (٨٣,٣٪) والغير منتجة للغشاء الحيوي ٦/١ (٦٦,٦٪). وأظهرت نتائج تأكيد هذه العينات من خلال اختبار VITEK-2 و(PCR) تشابه العزلات بنسبة ٩٩٪ مع تسلسلات لعزلات *P. aeruginosa*. تم ايداع هذه التسلسلات في بنك الجينات NCBI المرقمة (PP979726.1-PP979721.1) أظهرت التحليلات وجود علاقة إيجابية قوية ( $r = 0.998$ ) بين تكوين الغشاء الحيوي وتركيز البروتين، مما يشير إلى أن زيادة محتوى البروتين ترتبط ارتباطاً وثيقاً بزيادة إنتاج الغشاء الحيوي. أظهرت جميع العزلات مقاومة متعددة للأدوية (MDR)، حيث أظهرت مقاومة بنسبة ١٠٠٪ للاميكاسين والتريميثوبريم والسيوفوتاكسيم والاموكسيسيلين/حمض الكلافولانك والأمبيسلين والسيفالين واللينكوميسين، وكانت ٣٣٪ من العزلات مقاومة للجنتاميسين بينما كانت جميع العزلات حساسة للسايبيروفلوكساسين بنسبة ١٠٠٪. تؤكد هذه النتائج على العلاقة الإيجابية بين قوة تكوين الغشاء الحيوي وتركيز البروتين، وكذلك أهمية معرفة سلوك البكتيريا لتكوين الأغشية الحيوية ومقاومتها للمضادات الحيوية للمساهمة في تحسين الممارسات البيطرية ووصف المضادات الحيوية في حالة عدوى المسالك البولية لدى الكلاب.

**الكلمات المفاحية:** الزائفة الزنجارية، عدوى الجهاز البولي، الكلاب، تكوين الأغشية الحيوية، مقاومة المضادات الحيوية