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Prescence and Verification of *LasI* and *LasR* Genes in *Pseudomonas aeruginosa* Causing Urinary Tract Infection in Dogs

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Abstract | *Pseudomonas aeruginosa* is a pathogenic bacterium and has virulence factors under the quorum-sensing (QS) control increasing its pathogenicity. The purpose of this study was to detect some of the QS genes including *LasI* and *LasR* in *P. aeruginosa* isolated from dogs with urinary tract infection (UTI). Additionally, we aimed to determine histopathological changes in mice experimentally infected with bacteria. A group of mice was injected with phosphate buffer saline as a negative control, second group was infected with *P. aeruginosa* produced biofilm, and third group was infected with *P. aeruginosa* non produced biofilm. A total of 108 urine samples were collected from UTI dogs, obtained from the veterinary hospital and veterinary clinics. The identification of suspected isolates was confirmed by VITEK-2; and the genetic determination of *16S rRNA*, *LasI* and *LasR* gene was performed. Detection of biofilm layer by tube method and congo red agar was performed. The results showed that 6/108 (5.5%) of isolates are positive for *P. aeruginosa*, five isolates (83.33%) produced biofilm layer which carried both *LasI* and *LasR* genes, and one isolate 1/6 (16.66%) had no biofilm product and missing *LasI* and *LasR* gene. In addition, presence of changes in nucleotide sequence of *16S rRNA* in all *P. aeruginosa* isolates were assessed. *LasI* gene showed transition mutation in nucleotide sequence when compared to other globally known genes. The histopathological results revealed that the *P. aeruginosa* isolates produced biofilm layer severely affected mice compared to the isolates from non- biofilm layer. Taken together, molecular surveillance identifies that utilizing the *LasR* and *LasI* genes at the same time improves the accuracy of detection of *P. aeruginosa* producing biofilm.

Keywords | *Pseudomonas aeruginosa,* dogs, quorum sensing, *LasI/LasR* genes, histopathological changes

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INTRODUCTION

Pseudomonas aeruginosa is frequently cause localized or system-specific infections, such as urinary tract infection, ears, eyes, and occasionally the respiratory system (Mohammad *et al.*, 2024), mastitis in cattle (Al-Taee *et al.*, 2019). It is widely known to cause otitis externa in dogs (Mohammad *et al.*, 2024), wound infections (Raheem and Abdalshheed, 2023), and isolated from urine of dogs affected with malignant tumor (Sahar *et al.*, 2009). Urinary tract infections (UTIs) are the most prevalent diseases in dogs, which are caused by bacterial pathogens (Yu *et al.*, 2020), occurs frequently causing chronic kidney diseases with significant effects on the kidney functions (Mohmoud and Al-Dujaily, 2018). This bacterium regarded as a significant pathogen because its wide variety of resistance mechanisms (Yaseen and Ahmed, 2023; Khames and Ahmed, 2024). It has a vast genome that gives several

virulence characteristics, including the ability to form biofilm (Ali *et al*., 2024), adhered to surfaces by extracellular polymer substances (EPS) called matrix, enables bacteria to survive in a harsh condition, and permits the cells within the biofilm to communicate with each other (Al-Barhawee and Al-Rubyee, 2024). The microbial biofilm is the most efficient and competing expression of the genome in prokaryotic biofilm cells, and being more protected and metabolically efficient, demonstrating tolerance to many stressors through a signaling system controlled by quorum-sensing (QS) system (Lazar and Chifiriuc, 2010; Razzaq *et al*., 2017) which is a bacterial cell-cell communication system that uses chemical signals to control gene expression levels depended on the cell density manner, it is crucial for pathogenicity, biofilm formation, and antibiotic resistance (Lafta and Sadeq, 2024). Thes chemical signals known as autoinducers (AIs) that bind to specific receptors (Shalata, 2022). Interestingly the genes that contribute to the composition of this system are about 10% of *P. aeruginosa* genome and for this status are responsible for most of the cellular physiology processes and virulence phenotypes (Schuster and Greenberg, 2006).

In *P. aeruginos*, a there are seven genes associated with the occurrence of QS which are *rhlA, rhlR, rhlI, lasR, lasI, lasB, phzA1* (Mohammed and Zgair, 2022), and the main highly interconnected QS systems are *Las, Rhl, Pqs,* and *Iqs* (Shalata, 2022), which are channels interlinked together that used transcriptional regulators such as *LasR, RhlR, PqsR, and IqsR*, respectively and connected to specific AIs leading to aggravating the expression of the selected genes that cause bacterial virulence (Bhardwaj *et al*., 2021). This bacterium has two AHL-depended on systems known as *LasR/LasI* and *RhlR/RhlI*, which regulate the synthesis and signal transduction of the N-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL) and N-butanoyl-L-homoserine lactone (BHL), respectively (Wu and Luo, 2021). The QS system has significant role in this bacterium by increasing its pathogenicity (Hemmati *et al*., 2024).

This study aimed to investigate biofilm production in clinical *P. aeruginosa* isolates from dogs suffering UTI, and to detect the occurrence of mutations in*16S rRNA* in all six *P. aeruginosa* isolates. Additionally, we aim to determine if experimental infection of mice with diverse *P. aeruginosa* could induce differing histopathological changes.

MATERIALS AND METHODS

Samples collection

Samples were collected from dogs with suspected urinary tract infection (UTI) of different ages, both genders, and different breads, during the period from February 2024 to June 2024, obtained from the veterinary hospital and veterinary clinics located in various areas of Baghdad. This included 108 urine samples from 108 dogs with suspected UTI by spontaneous urination, collected in a sterile container, marked, and kept in cooling box to perform examination and bacterial diagnosing in the microbiology laboratory in the College of Veterinary Medicine in Baghdad.

General urine examination

This method was performed according to Punia *et al*., 2018 to detect the UTI in dogs. Taking 5 mL of the urine sample into a test tube and centrifuged at 3000 rpm for 7 min. The supernatant was discarded, placed the sediment on a glass slide for examination under a light microscope at 40× magnification.

Isolation and identification

Based on a loopful of urine spread onto blood agar and MacConkey agars (HiMedia, India), incubated aerobically at 37°C for 24 hrs. Then, isolated the colonies on selective media cetrimide agar and pseudomonas agars (HiMedia, India) to get pure growth (Devnath *et al*., 2017), after that identified by biochemical tests such as oxidase and catalase, confirmation the isolates using VITEK-2 compact system according to the manufacturer company VITEK-2 technique (BioMerieux, France) was done using a GN colorimetric identification card to conformation the isolates (Hakim *et al*., 2024; Abed *et al*., 2024).

Biofilm detection methods

Tube method (qualitative assay): A loopful of the isolated bacteria was inoculated into 5 mL of tryptic soy broth supplemented with 1% glucose and incubated at 37°C for 72 h. The tube was washed with normal saline and air-dried. The dried tube was 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 layer on the inner walls and at the bottom of the tube (Furtuna *et al*., 2018).

Congo red method: On Congo red agar, *P. aeruginosa* isolate was cultured for 48 h at 37°C, black colonies indicated the biofilm formation, while the red colonies indicated non-biofilm formation (Ciocan *et al*., 2016).

Polymerase chain reaction (PCR): DNA was extracted according to the commercial purification system using (Genomic DNA Mini Kit, Korea) to detect *16S rRNA, LasR* and *LasI* genes. The primers designed are mentioned in Table 1, and the optimum conditions of PCR cycles were: firstly one cycle of initial denaturation at 95°C for 5 min, then 35 cycles of denaturation at 95°C for 45 sec, annealing (*16S rRNA* at 58°C/ *LasR* and *LasI* at 57°C)

Table 1: The sequence of primers used in this study.

Primer	Sequence	Primer sequence	GC%	Size of product (bp)	Reference
16s RNA	27F	5'- AGAGTTTGATCCTGGCTCAG- 3'	50.0	1250 bp	(Srinivasan*et al.*, 2015)
	1392R	5'- GGTTACCTTGTTACGACTT- 3'	42.1		
LasI	F	5'- CGTGCTCAAGTGTTCAAGG-3'	52.63	295 bp	(Lima *et al.*, 2018)
	R	5'- TACAGTCGGAAAAGCCCAG-3'	52.63		
LasR	F	5'- ATGGCCTTGGTTGACGGT -3'	55.56	700 bp	(Azemin *et al.*, 2022)
	R	5'- GCAAGATCAGAGAGTAATAAGACC -3'	44.00		

for 45 sec, and extension at 72°C for 1 min, at last, final extension at 72°C for 5 min. The PCR products were stained with red stain (Intron, Korea), then electrophoresis on 1.5% agarose and observed under UV trans illuminator. The amplified products were sent for sequencing to Macrogen Inc. (Geumcheon-gu, Seoul, South Korea) using the Sanger's 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*.

Animal experimental design

A total of 24 mice were divided into three groups each one contains eight mice: first group (G1) of mice was injected with (PBS) as a negative control, second group (G2) mice were infected with biofilm producing *P. aeruginosa*, third group (G3) mice were infected with non-biofilm producing *P. aeruginosa*. The dose was given intraperitonially (I/P) (Al-Rasheed *et al.*, 2023), at 0.5 McFarland tube (1.5×10^8 CFU/ml) 0.25 ml, then sacrificed on 5th days (Ali, 2019).

Histological examination

Specimens were taken from internal organs (kidney, liver and spleen) of mice, fixed in 10% buffered neutral formalin. According to (Suvarna *et al.*, 2018) a technique of several steps was done for histological examination.

Statistical analysis

Data were analyzed using SAS (Statistical Analysis System - version 9.1). One-way analysis of variance (ANOVA) program was used to detect the percentage of difference groups in study parameters.

RESULTS

In the present study according to the microscopic analysis, the urine samples from which *P. aeruginosa* was isolated contained pus and bacteria, that is indicative of a urinary tract infection. The bacterial isolation and identification revealed out of 108 urine samples with UTIs the *P. aeruginosa isolates* were found in 6/108 (5.5%) (Figure 1). The incidence rate of infection in male was higher than females with a rate of 66.6% and 33.33%, respectively (Figure 2). The rate of infection occurred at a high infection rate 100% in adult > 1 year with no infection rate in young ≤1 year.

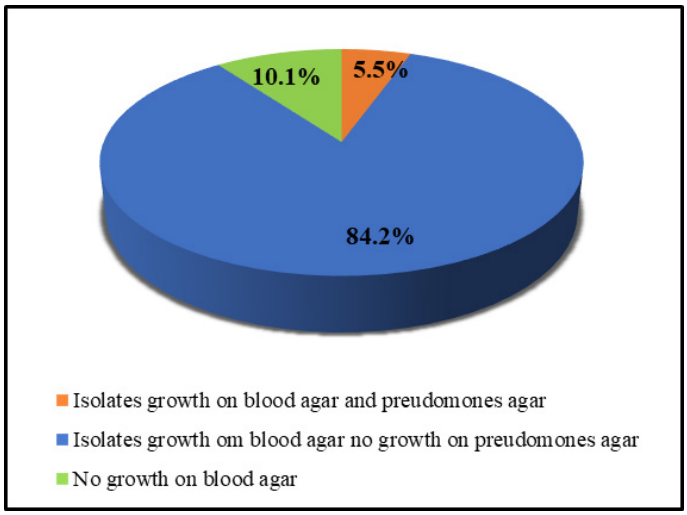

Figure 1: Incidence rate of *UTIs* caused by *P. aeruginosa* isolated from urine dogs

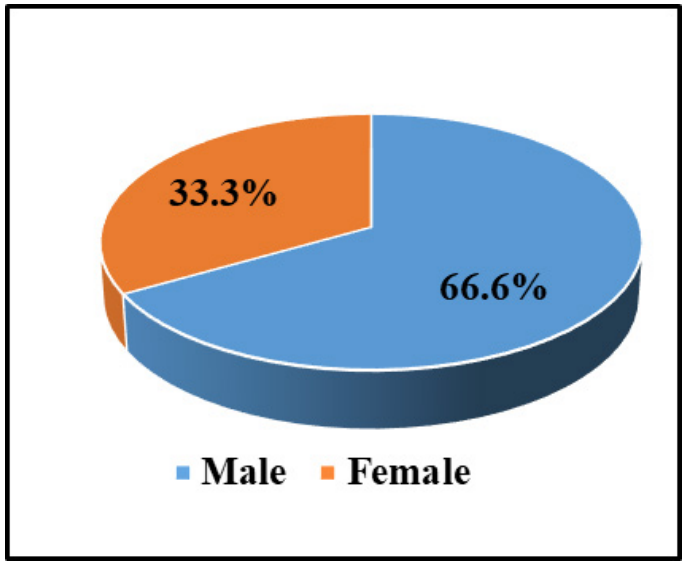

Figure 2: Incidence rate of *P. aeruginosa* from dogs with UTIs according to sex.

The biofilm formation was performed by using tube method and Congo red agar demonstrated that five out of six isolates (5/6, 83.33%) produced biofilm and only one

isolate (1/6, 16.66%) failed to produce. In tube method, the positive results to detect the biofilm formation were indicated by a violet-colored layer adhering to the tube wall, while a colorless tube indicated a negative result (Figure 3). Black colonies on Congo red agar indicated the presence of a biofilm layer, while non-biofilm colonies appeared red color (Figure 4).

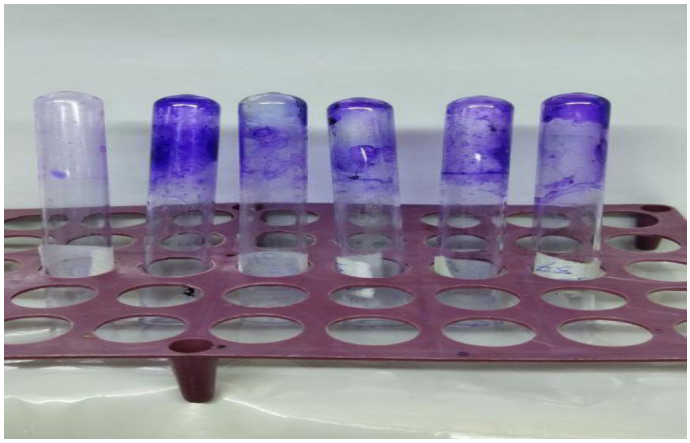

Figure 3: Tube method for detecting the biofilm formation by *P. aeruginosa*. A positive result is a violet-colored layer adhering to the tube wall, while a negative result is a colorless tube.

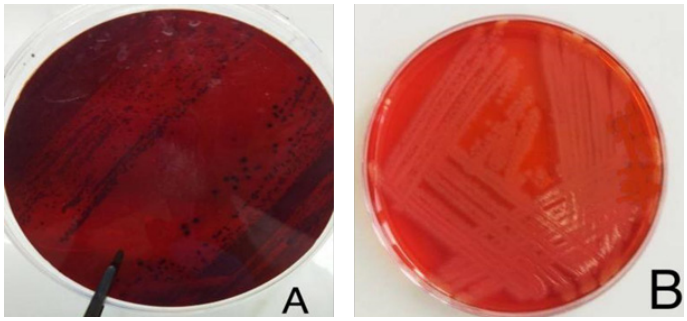

Figure 4: Pseudomonas aeruginosa colonies on the Congo red agar. (A) black colonies indicate the biofilm formation. (B) red color colonies indicate non-biofilm formation.

The *P. aeruginosa* confirmed by VITEK-2 system microbiology showed a match 99% (Figure 5), and the bacteria were genetically diagnosed by PCR and automated DNA sequencing to all isolates of *P. aeruginosa* amplified directed the *16S rRNA* gene as in (Figure 6).

The identity of isolates was subsequently confirmed by sequencing the PCR. BLAST analysis showed 99% similarity to reference sequences of *P. aeruginosa*, including strain OR793893.1. The sequences have been added to the NCBI GenBank database and are available under the accession codes: PP979721.1, PP979722.1, PP979723.1, PP979724.1, PP979725.1, and PP979726.1. In this study the detection of QS genes *LasR* and *LasI* was found in 100% of biofilm forming isolates and 100% missing in isolate that do not contain biofilm (Figure 7).

bioMérieux Customer: Microbiology Chart Report Printed March 22, 2022 1:44:49 PM GMT-06:00

Lab ID: shatha39 Isolate Number: 1

Organism Quantity:

Comments:

Identification Information	Analysis Time:	6.82 hours	Status:	Final
Selected Organism	99% Probability	Pseudomonas aeruginosa		
	Bionumber:	0043051103500252		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+		
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-		
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	ILATa	+					

Figure 5: VITEK 2 microbiology chart report for *P. aeruginosa* identification.

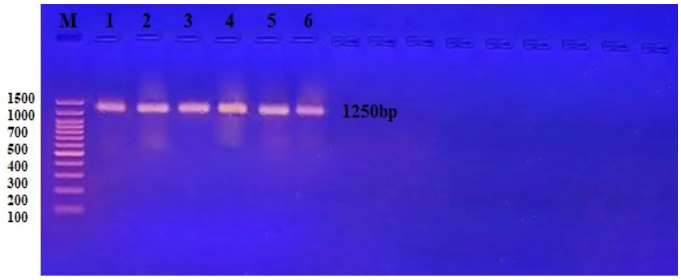

Figure 6: PCR products with a band size of approximately 1250 bp of 16S rRNA. The products were subjected to electrophoresis on 1.5% agarose at 5 volt/cm^2 for 1:30 h. M: DNA ladder (100 bp).

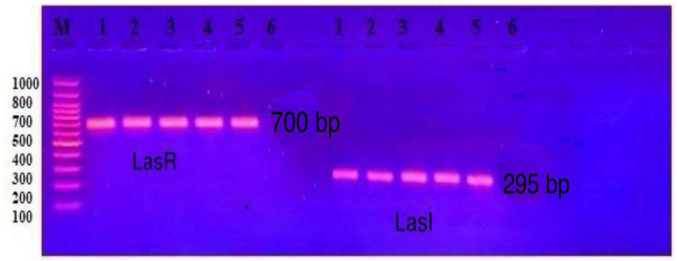

Figure 7: PCR product of the band size in LasR and LasI. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

Sequencing analysis of *P. aeruginosa 16S rRNA* gene was performed by MEGA6 software and showed the changes in nucleotide sequence compared to other globally known sequences. These changes were transition or transversion and are listed in Table 2.

Sequencing analysis of QS genes *LasR* and *LasI* in *P. aeruginosa* (ID: PP979723.1) was performed in MEGA6 software and has no changes in nucleotide sequence of *LasR* gene and had 100.

Introduction

Identity when compared to other globally known (ID: CP137940.1). On the other hands, *LasI* gene showed transition mutation in nucleotide sequence when compared to other globally known (ID: CP137912.1) as listed in Table 3.

Table 2: Genetic variation at *16S rRNA* genes of *Pseudomonas aeruginosa.*

Identities	Sequence ID with submission	Sequence ID with compare	Nucleotide	Location	Type of substitution	No.
99%	ID: PP979721.1	ID: OR793893.1	G\A	133	Transition	1
99%	ID: PP979722.1	ID: OR793893.1	T\A	208	Transvertion	2
			G\A	536	Transition	
			T\A	537	Transvertion	
99%	ID: PP979723.1	ID: OR793893.1	T\C	222	Transition	3
			A\C	277	Transvertion	
99%	ID: PP979724.1	ID: OR793893.1	A\T	252	Transvertion	4
			C\T	253	Transition	
			C\G	563	Transvertion	
99%	ID: PP979725.1	ID: OR793893.1	G\T	539	Transvertion	5
99%	ID: PP979726.1	ID: OR793893.1	C\A	563	Transvertion	6

Source: *P. aeruginosa*; *16S rRNA* gene.

Table 3: Genetic variation at *LasR* and *LasI* genes of *Pseudomonas aeruginosa.*

Identities	Predicted effect	Amino acid change	Nucleotide change	Nucleotide	Location	Type of substitution	Gene	No. of gene
100%	----	------	------	------	-----	------	LasR	1
99%	Missense	Q(Glutamine)\ R(Arginine)	CTG\CCG	T\C	4101403	Transition	LasI	2

Source: *Pseudomonas aeruginosa* (ID: PP979723.1). Sequence ID with compare: 1: *LasR*=ID: CP137940.1; and 2: *LasI*=ID: CP137912.1

The result of protein conformation of *LasR* and *LasI* genes in *P. aeruginosa* (ID: PP979723.1) showing as three-dimensional (3D) modeling secondary structure, and exposes the location of mutation in only *LasI* protein as in Figure 8.

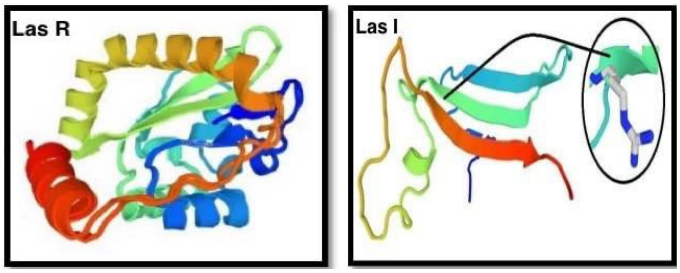

Figure 8: The ribbon representation of 3D secondary structure of *LasR*, and *LasI* proteins of *P. aeruginosa* producing biofilm (ID: PP979723.1), the enlarged part of *LasI* appears the location of mutation.

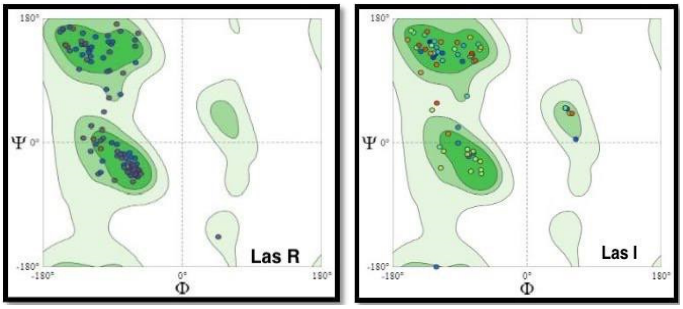

Figure 9: Distribution of the amino acid residues in the Ramachandran graph of *LasR* and *LasI* proteins of *P. aeruginosa* producing biofilm (ID: PP979723.1).

In Ramachandran graph for amino acid in general and local quality estimation with Z score of *LasR* and *LasI* genes, the results showed that model evaluation of the amino acid of query protein were in the most favorable energy regions for good quality model (Figure 9). The results also show that the model validation include the local quality estimation with Z score, located within the light or dark gray and blank areas (Figure 10).

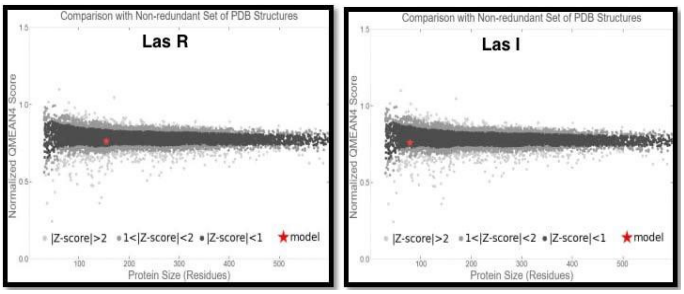

Figure 10: Representative *Z*-score graphs of the generated models, compared to X-ray (gray) and magnetic (dark gray) crystallography models deposited in the PDB. The dots in red refer to the generated models for the *LasR* and *LasI* protein of *P. aeruginosa* biofilm producing isolate (ID: PP979723.1).

In histological examination of G1 mice injection with PBS as a negative control showed that the kidney with normal appearance of glomerular tuffet surrounded by Boman space, and normal proximal convoluted tubule (Figure 11A). Liver tissue showed normal central vein surrounded by a regular arrangement of normal hepatocytes and

sinusoids (Figure 11B). The spleen has a normal (white pulp) consist of lymphoid follicles with central arteriols surrounded by lymphocytes, and the (red pulp) contains lymphocytes with blood vessels, all of them surrounded by a normal capsule (Figure 11C).

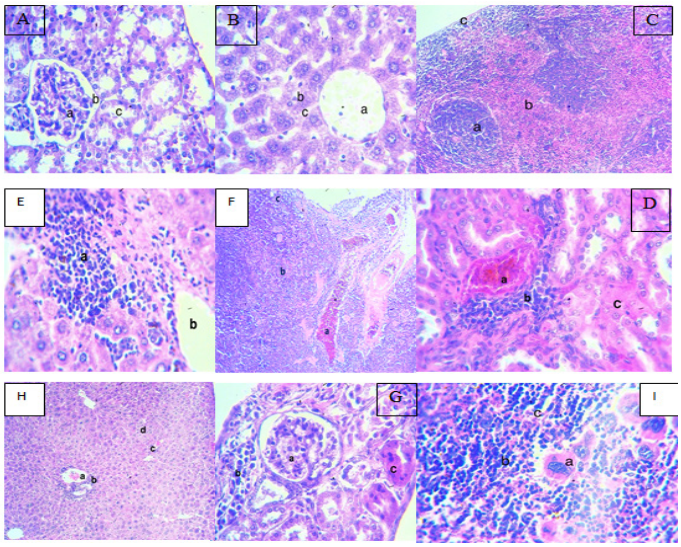

Figure 11: A: Histological section of normal kidney in G1 mice a- Glomerular tuffet, b-Boman space c-Proximal convoluted tubule. (H and E 400X), B: Histological section of normal liver in G1 mice a- Normal central vein b- hepatocytes c-sinusoids. (H and E 400X), C: Histological section of normal spleen in G1 mice a-Lymphoid follicle consists of central arterioles surrounded by lymphocytes (white pulp) b-Red pulp consists of lymphocytes with blood vessels c-spleen capsule. (H and E 400X), D: Histopathological section of kidney in G2 mice infected with *P. aeruginosa* produced biofilm. a-Severely congested blood vessels b- Mononuclear cells aggregation surrounding blood vessels c- Tubules cloudy swelling (H and E 400X) , E: Histopathological section of liver in G2 mice infected with *P. aeruginosa* produced biofilm. a- severe interstitial infiltration in hepatocyte with inflammatory cells (lymphocytes and macrophage) b-dilated central vein (H and E 400X) , F: Histopathological section of spleen in G2 mice infected with *P. aeruginosa* produced biofilm a- Extensive interstitial hemorrhage b-Depleted lymphoid follicle c-Necrotic lymphocytes. (H and E 200X), G: Histopathological section of kidney in G3 mice infected with *P. aeruginosa* non produced biofilm. a- Enlargement glomeruli b-Mononuclear cells Infiltration surrounded glomeruli c-Tubule cloudy swelling. (H and E 400X) , H: Histopathological section of liver in G3 mice infected with *P. aeruginosa* non produced biofilm. a- congested blood vessel b- lymphocytic cuffing central vein c-sinusoids hemorrhage d- apoptosis 200 X (H and E), and I: Histopathological section of spleen in G3 mice infected with *P. aeruginosa* non produced biofilm. a-Increase in the megakaryocytic cell b- Depleted lymphoid c- Hemorrhage. (H and E 400X).

In G2 mice infected with *P. aeruginosa,* produced biofilm there were histological changes in kidney demonstrates severely congested blood vessels, aggregation of mononuclear cells, and cloudy swelling tubules (Figure 11D), the liver showed severe interstitial infiltration with inflammatory cells (lymphocytes and macrophages) with dilated central vein (Figure 11E), in spleen there is an extensive interstitial hemorrhage with the depleted lymphoid follicle, and the presence of necrotic lymphocytes (Figure 11F). In G3 mice infected with *P. aeruginosa* not produced biofilm there were histological changes in kidney demonstrates enlarged glomeruli surrounded by infiltration of mononuclear cells and tubules cloudy swelling (Figure 11G), the liver showed congested blood vessels, lymphocytic cuffing central vein, sinusoid hemorrhage, and apoptosis (Figure 11H). In spleen there is an increase in megakaryocytic cell, depleted lymphoid follicle with hemorrhage (Figure 11I).

In general, according to the results of this study, only six dogs (6/108, 5.5%) had a UTI caused by *P. aeruginosa*. This is consistent with another study (Hakim *et al.*, 2024) where they have found 5.3% of dog cases with lower UTI and cystitis were caused by *P. aeruginosa* isolates. According to another study (Hattab *et al.*, 2021), *P. aeruginosa* was present in 4.2% of the dogs that had bladder infections, while (Rampacci *et al.*, 2018) isolated 13.4% *P. aeruginosa* from dogs with UTIs in Italy. The spread of *P. aeruginosa* in dogs with UTIs varies according to geographic area and underlying medical conditions (Ataya *et al.*, 2023). In this study, the incidence rate of males was higher than females (66.6%), and (33.3%), respectively, occurred at a high infection rate 100% in (adult > 1 Year). However, the study conducted by (Thompson *et al.*, 2011), presented that many reports showed UTIs are more prevalent in older female dogs with a mean age of about 7–8 years.

The genotypic results for the *16S rRNA* gene confirmed the diagnosis for all isolates, allowing to conclude that all of them were *P. aeruginosa*, as expected. Also, the detection of QS genes *LasR* and *LasI* was found in 100% of biofilm-forming isolates and 100% missing in isolate that do not contain biofilm. The genes *LasI* and *LasR* were tested to know the quorum sensing regulatory system, which is essential for coordinating gene expression related to virulence and biofilm formation (Fernandes *et al.*, 2023).

The substitutions in *16S rRNA* gene were found in *P. aeruginosa* in this study showed transition and transversion substitutions at different locations with 99% identity when compared to other globally known ones. Moreover, the substitution in *LasI* gene found in *P. aeruginosa* in this study showed one substitution of Q(Glutamine)/R(Arginine) at location 4101403 was a missense mutation and had been 99% identity when compared with (ID: CP137912.1). In

general, these genetic changes may result in a high degree of biofilm formation and serve a supporting role in the formation of high levels of pathogenicity in *P. aeruginosa*. In the study conducted by de Sousa *et al.* (2023) they suggest that biofilm production is common among *P. aeruginosa* isolates from dogs and may play a role in the pathogenesis of the disease, and found *LasI* gene within 90% of *P. aeruginosa* isolates from dogs and *lasR* gene was absence, whereas (Lima *et al.*, 2018) they found 100% of the isolates had *lasR* gene, and 97.5% had *lasI* genes.

From previous studies, found limited available data on the prevalence of *las*R, *lasI* virulence genes in *P. aeruginosa* specifically isolates from dogs, highlighting the importance of this study. Also, some studies investigated the distribution of *las*R, *lasI* genes in *P. aeruginosa* obtained from humans and the environment (Park and Koo, 2022), they showed the prevalence of virulence genes and found the genes *lasR* was present in a significant proportion of carbapenem-resistant *P. aeruginosa* (CRPA) isolates in 96.7% of the isolates. Another study conducted by (O'Connor *et al.*, 2021) investigated the prevalence of virulence genes in 90 environmental *P. aeruginosa and* found that *lasR* was presented in 50% of the isolates. The result of protein conformation of *LasR* and *LasI* protein in this study revealed there was no change in 3D secondary structure of *lasR and* distribution the amino acid of query protein were in the most favorable energy regions for a favorable quality model, and located within the black areas with graph of Z score indicated of an effective model, while *LasI* 3D structure showed the location of mutation, and distribution the amino acid of query protein were in the most favorable energy regions for good quality model and located within the black areas with graph of Z score, these results were differed from (Lima *et al.*, 2018) they revealed there is change in 3D structure of *LasR* protein with distribution the amino acid of query protein were in the unfavorable energy regions, and negative Z-score indicated of a favorable model.

Plokarz *et al.* (2022); Zhang *et al.* (2022) have shown that the studying *P. aeruginosa* biofilms in dogs enables the researchers to gain a better understanding of how these structures contribute to infection and develop more effective treatments (Rex *et al.*, 2023) revealed that *P. aeruginosa* possesses four complicated (QS) system used several signals and receptors to regulate virulence and pathogenicity, among which the *LasI/ LasR* and *RhlI/ LasR* system is regulated by (3-oxo-C12-HSL) and (C4-HSL) respectively, plays a major role in host pathogenesis. The las and rhl quorum-sensing circuitry operate in a hierarchical cascade responsible for regulating the expression of many virulence determinants, secondary metabolites, type 2 secretion system, stationary phase genes and genes involved in biofilm formation (Sánchez-Jiménez *et al.*, 2023). Therefore, the persistence of *P. aeruginosa* in various environments, including host tissues regulates the synthesis of proteases, toxins, and other exoproducts that contribute to pathogenicity.

The histopathological finding in this study, the G2 mice infected with *P. aeruginosa* forming biofilm there were histopathological changes in kidney tissue demonstrates severely congested blood vessels, aggregation of mononuclear cells, and cloudy swelling tubules, the liver section showed severe interstitial infiltration with inflammatory cells (lymphocytes and macrophages) with dilated central vein, while spleen tissue shows an extensive interstitial hemorrhage with the depleted lymphoid follicle with the presence of necrotic lymphocytes. In G3 mice infected with *P. aeruginosa* non-forming biofilm the histopathological changes in kidney tissue demonstrates enlarged glomeruli surrounded by infiltration of mononuclear cells and tubules cloudy swelling, the liver showed congested blood vessels, lymphocytic cuffing central vein, sinusoid hemorrhage, and apoptosis, in addition the spleen section showed an increase in megakaryocytic cell, depleted lymphoid follicle with hemorrhage. These results corresponds to (Mittal *et al.*, 2009) found in their study the severity of lesions induced by four-days old biofilm cells was more as compared to planktonic cells. Although, that planktonic as well as biofilm cells both the cell forms of *P. aeruginosa* were able to induce chronic renal inflammation in the experimental animals, more significantly, when bacteria and neutrophils interact during the planktonic stage of an infection, the pathogen is typically killed, inflammatory mediators are regulated, neutrophils undergo apoptosis, and acute inflammation is resolved, conversely, the bacterial infection biofilm stage promotes neutrophil necrosis and results in chronic inflammation (Lenda *et al.*, 2019). The reasons for these histopathological changes may be due to the virulence factors of *P. aeruginosa* that counteract host defenses causing direct damage to the organ tissues or making the bacteria more competitive, this agrees with (Gellatly and Hancock, 2013). Also, this results corresponds to (Alrabia *et al.*, 2024) they showed the presence of cystic dilatation of some renal tubules with cloudy swelling of others, atrophy of some glomeruli with degeneration of some renal tubules, constriction of renal blood vessels, and degenerative changes of some renal tubules were all observed in the kidney tissues of the mice given strain PAO1 injection, also in the liver tissue showed diffuse vacuolation of certain hepatocytes, significant hepatic blood vessel congestion, and focal regions with inflammatory cell infiltration in their liver tissues, and in the spleen there was a localized area of extravasated blood (hemorrhage), splenic blood vessel congestion, and a decrease in white pulp lymphocytes. As

well as (Abd Al-Rubai, 2013) appeared kidney aggregation of neutrophils and lymphocytes around congested blood vessels and also between kidney tubules with severe cytoplasmic vacuolation of endothelium cells and others explained congestion of blood vessels with filtration neutrophils between kidney tubules, and showed spleen depletion of white pulp with neutrophil infiltration in the congested red pulp, however, the liver showed granuloma with congestion of the central vein and sinusoids with neutrophils in the lumen as well as necrosis of hepatocytes with an explanation of sinusoids with neutrophils in the lumen added to necrosis hepatocytes (Abd Al-Rubai, 2013). The researchers (Al-Rasheed *et al*., 2023) presented that the spleen of the mice in the *P. aeruginosa* infected group showed signs of severe inflammation, the red and white pulps were enlarged, and the characteristic germinal center structures were lost. There were also hemorrhages, clogged blood vessels, inflammatory cell infiltration, and many erythrocytes in the red pulp. The study of (Panpetch *et al*., 2024) showed that increased gut LPS produced by *P. aeruginosa* promotes gut permeability destruction resulting in the direct transfer of LPS to hepatocytes in part via portal veins that promote systemic inflammation (inflammation, apoptosis, and increasing oxidative stressors in hepatocytes), adding to that LecA binds to glycoprotein oligosaccharides on the surface of epithelial and endothelial cells to help *P. aeruginosa* adhere to host cells (Grishin *et al*., 2015). While the researchers (Hickey, *et al*., 2018) revealed that the virulence factors promoted by RpoN are siderophore pyoverdine, and the phenazine compound pyocyanin, these factors are a redox-active molecule that causes oxidative stress that damages target cells. On the other hand, it has been demonstrated that the (T3SS) secretion system effectors ExoS and ExoY, cause regions of cell death, which results in defects in the barrier of the epithelial cells (Golovkine *et al*., 2016).

CONCLUSION

In conclusion, this study highlights the biofilm forming abilities, importance of QS genes, and mutation occurrence of *P. aeruginosa* isolates from canine UTIs. The relatively low frequency of *P. aeruginosa* in dogs UTIs indicates that the epidemiology of this bacterium is not significantly impacted by domestic dogs, probably act as an ecological reservoir for the virulent *P. aeruginosa* that can be harmful. The identification of *LasI/ LasR* genes was found in *P. aeruginosa* isolates resulting in thought that these genes are connected to varying levels of intrinsic virulence and pathogenicity that prompting the need for further studies. The main histopathological finding emphasizing that the biofilm-forming strain caused more severe histological changes than non-biofilm-forming strain.

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NOVELTY STATEMENT

The study revealed that the *P. aeruginosa* isolates produced biofilm layer severely affected mice compared to the isolates from non- biofilm layer. Taken together, molecular surveillance identifies that utilizing the *LasR* and *LasI* genes at the same time improves the accuracy of detection of *P. aeruginosa* producing biofilm.

AUTHOR'S CONTRIBUTION

SEJ: Conceptualization, methodology, validation, writing original draft, writing review and editing.
SMH: Conceptualization, methodology, review and editing, supervisor.

Ethical approval

Ethical approval was obtained through the local committee of animal care in the College of Veterinary Medicine, University of Baghdad (Number 279 on 7/2/2024) before starting this study.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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