



## Isolation and Identification of *Pseudomonas Aeruginosa* from Enteritis in Pigeons and Study Their Antibiotic Sensitivity

### Article Info.

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### Article History

Received: Jan. 29, 2026

Accepted: March 3, 2026

Published: March 31, 2026

Article type: Research Article

<https://doi.org/10.23975/bjvr.2026.168889.1279>

### Abstract

Given the scarcity of published sources on *Pseudomonas aeruginosa* in birds and the significant health threat it poses as a zoonotic disease, the present study aimed to detect *Pseudomonas aeruginosa* as a cause of enteritis in pigeons, and to study their antibiotic sensitivity. Between August and November of 2025, fifty samples (part of the intestine and diarrhea) were obtained from pigeons with intestinal inflammation. Transfer samples by aseptic tubes to the laboratory of Microbiology, College of Vet Medicine, Campus of Mosul. All samples were subjected to culturing on enrichment media (brain heart infusion broth (BHIB) and blood agar) and selective media (cetrimide agar), morphological (Gram stain), tube conventional biochemical tests, and confirmed by the VITEK2 system and the Render MA120 Identity System operation (Kits contain a group of biochemical tests). 10 samples from 50 samples (20%) show a positive reaction for *Pseudomonas*, while 40 samples from 50 (80%) samples show a negative reaction for *Pseudomonas*. Furthermore, these isolates undergo antibiotic susceptibility tests, which showed complete resistance to cloxacillin, metronidazole, penicillin, sulfa-trimethoprim, tetracycline, and amoxicillin and showed complete sensitivity to neomycin, gentamicin, tobramycin, ciprofloxacin, and imipenem. In conclusion, *Pseudomonas aeruginosa* is one of the reasons for enteritis in pigeons and is highly resistant to many antibiotics, posing a real threat to public health.

**Key words:** Bacteria, *Pseudomonas aeruginosa*, Enteritis, Pigeon, Avian

## Introduction

The name *Pseudomonas* comes from the Greek language, composed of two parts; the first, pseudo, is used to denote a deceptive appearance or something unreal, while the second part, monas, refers to a single entity or unit. Regarding the distinctive pigmentation, the species name *aeruginosa* is used, which is of Latin origin, derived from the word aerūgō, describing a blue-green color or the color of oxidized copper(1).

In avian pathology, *Pseudomonas aeruginosa* is considered the most important bacterium (2). To identify the causes of epidemic diseases, bird behavior and migration patterns contribute significantly to determining the geographical distribution of infectious diseases(3). *Pseudomonas aeruginosa* infection is a zoonotic disease with the ability to be transmitted between animals and humans or Vice versa (4). Most serious infectious diseases that affect humans are transmitted through animals or animal products (5).

*Pseudomonas aeruginosa* are free-living organisms whose primary habitats are soil, water, and colonize plants and animals. They are characterized by their ability to adapt to different environments and can reproduce in water. This ability explains their continuous living in the surrounding environment (6). *Pseudomonas spp.* is an opportunistic bacterium that can cause disease under conditions of environmental challenge or immunodeficiency (7). *Pseudomonas aeruginosa* constitutes a Gram-negative aerobic bacterium noted by its rod-like shape in addition to motility(8,9). It is positive for catalase and oxidase tests and does not form endospores(10). These species are collectively known as ESKAPE, which include (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*). Because this infection may be resistant to antibiotics, the term is also derived from a word meaning escape(11). *P.aeruginosa* is an important member of the ESKAPE pathogen group (12). *Pseudomonas aeruginosa* has a normal ability to develop resistance to antibiotics, limiting the effectiveness of some antibiotics in eradicating it. According to scientific reports, its resistance to antibiotics is attributed to its liposaccharide outer membrane, which has low permeability(13).

Horizontal gene transfer in *Pseudomonas aeruginosa* and its adaptation mechanisms represent a combination that contributes to its enhanced resistance to antibiotics(14). It can also form biofilms that help it increase its resistance to antibiotics (15,16).

## Materials and methods

### Data collection permit

The certificate with the number UM.VET 2025.034 on 7/8/2025, which was given by the Commission of Scientific Morals, was used to collect data and provided the moral cover to carry out the research in the College of Veterinary Medicine.

### Samples

Fifty samples of intestinal contents and associated diarrhea (swabs) from pigeons suffering from enteritis were collected between August and November 2025. These samples were collected from

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private veterinary clinics in Mosul. The swabs were placed in a sterile tube containing a broth of brain heart infusion, and were performed in the Laboratory of the Department of Microbiology at the College of Vet Medicine, Mosul Branch.

### **Isolation and biochemical identification of *P. aeruginosa***

The samples were taken after 24 hours of aerobic growth incubation at a temperature of 37° C and cultured on blood agar (Hi media/India). Then, single colonies were cultured on brain heart infusion agar (Scharlau/Spain), and subsequently cultured on Cetrimide agar (Scharlau/Spain), incubated for 24 hours at 37°C (17). After the incubation period ended, all morphological tests were performed, represented by Gram staining, and biochemical tests were performed, represented by 1-Indole, 2-Methyl Red, 3-Vocus-Proskauer, 4-Citrate Utilization, 5-Urea, and 6-Oxidase and 7-Catalase tests. The final diagnosis was confirmed using the VITEK 2 system (18), (19). and Render MA120 Identity System operation [20]. To validate bacterial isolates and identify pathogens, a Render MA120 instrument (Render Biotech, China) was used. The Render MA120 employs turbidity measurement for sensitivity testing and colorimetry for identification (17).

### **Antimicrobial susceptibility testing (AST).**

The antibiotic susceptibility testing of isolated bacteria was made using the modified Kirby- Bauer method (18).approved through the WHO(19). Muller-Hunton medium, prepared by the Oxoid company, was used. 16 types of antibiotics were used in Table 1. A suspension of isolated bacteria was prepared in phosphate-buffered saline (PBS), and the bacterial concentration was maintained at  $10^7 \times 1.5$  cfu/ml compared to the McFarland standard tube. Sterile cotton swabs were immersed in the suspension, and excess suspension was detached by wiping the swab against the inner walls of the tube. The suspension was then spread onto the plate with superficial agar, and the plates were left at room temperature to dry.

Antibiotic tablets were fixed to the plate surface using sterile forceps, and the plates were incubated at 37°C for 24 hours. The zone of inhibition for each disc was then measured. The diameter of the inhibition was measured using a transparent ruler in millimeters, and the isolates were divided into three categories: sensitive, intermediate and resistant, based on World Health Organization measurements (19).

## **Results**

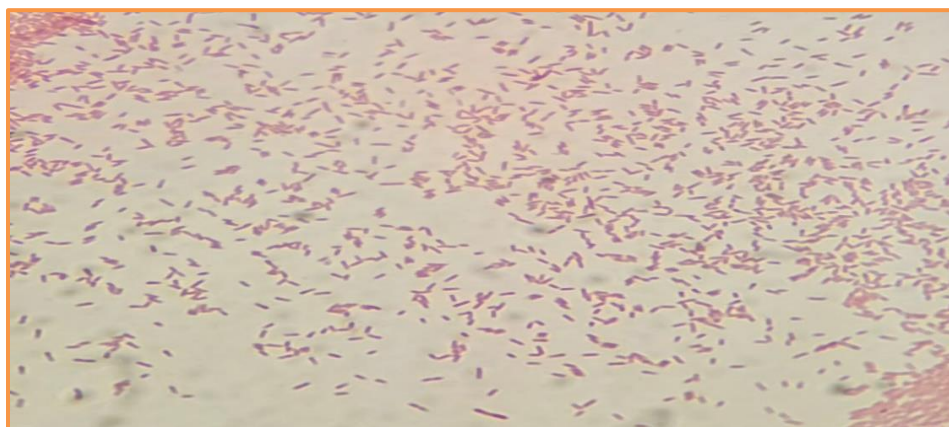
### **Bacteriological isolation**

The results of the current study revealed bacterial isolation of *Pseudomonas aeruginosa*, 10 isolates were obtained from a total of 50 samples of pigeon enteritis cases, representing an isolation rate of 20%. These samples were collected from private veterinary clinics in Mosul, and all were found to be *Pseudomonas aeruginosa* through the microscopic examination of Gram-stained bacterial smears, which revealed single or double Gram-negative rod-shaped bacteria, and by growing in brain infusion agar and heart infusion agar, producing the green pigment, which is the characteristic phenomenon for *P. aeruginosa* as shown in Figure 1.2,3.

**Table 1: The antibiotics used for the antibiotic sensitivity test for isolates of *Pseudomonas aeruginosa*.**

<b>Antibiotic</b>	<b>Disk</b>	<b>Concentration (µg/disc)</b>
<b>Chloramphenicol</b>	C	10µg
<b>Neomycin</b>	N	10µg
<b>Gentamicin</b>	GEN	10µg
<b>Streptomycin</b>	S	25µg
<b>Tobramycin</b>	TOB	10µg
<b>Ofloxacin</b>	OF	5µg
<b>Ciprofloxacin</b>	CIP	5µg
<b>Cloxacillin</b>	CX	10µg
<b>Amoxicillin</b>	AX	10µg
<b>Penicillin</b>	P	10µg
<b>Metronidazole</b>	MET	30µg
<b>Azithromycin</b>	AZM	15µg
<b>Ceftriaxone</b>	CTR	30µg
<b>Imipenem</b>	IMP	10µg
<b>Sulfa/ Trimethoprim</b>	SXT	25µg
<b>Tetracycline</b>	TE	30µg

Biochemical tests showed that the ginger appendages yielded positive marks for oxidase, catalase, urea, and citrate consumption tests, and negative results for indole, methyl red, and Voges-Proskauer, as illustrated in Figures 4, 5, and 6. Final confirmation by using the Vitek2 compact system confirmed the 10 isolates with a purity of up to 98%, as shown in Figure 7. *Pseudomonas aeruginosa* bacteria showed a Gram-negative stain upon microscopic examination, as shown in Figure 7, The MA120 automated microbiological identification system (Rinder Group, China) was used to further characterize the purified *Pseudomonas* isolates according to the manufacturer's instructions, as shown in Figure 8.



**Figure 1: Gram stain for *Pseudomonas spp.***

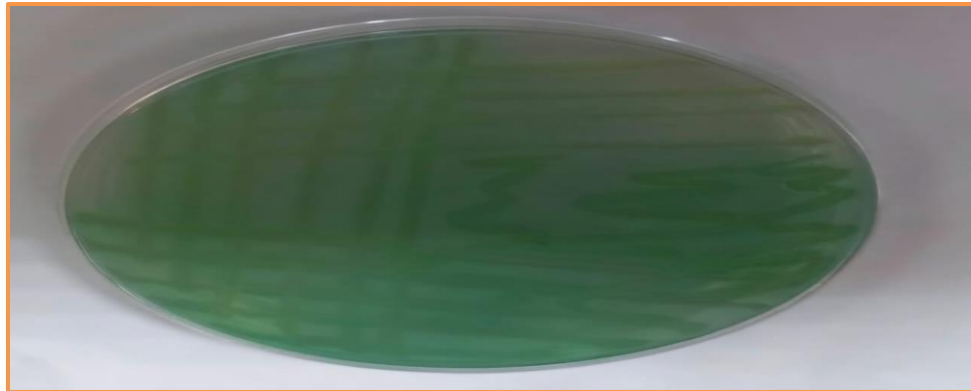


Figure 2: growing of *Pseudomonas aeruginosa* on Cetrimide agar.



Figure 3: growing of *Pseudomonas aeruginosa* on Brain heart infusion agar.

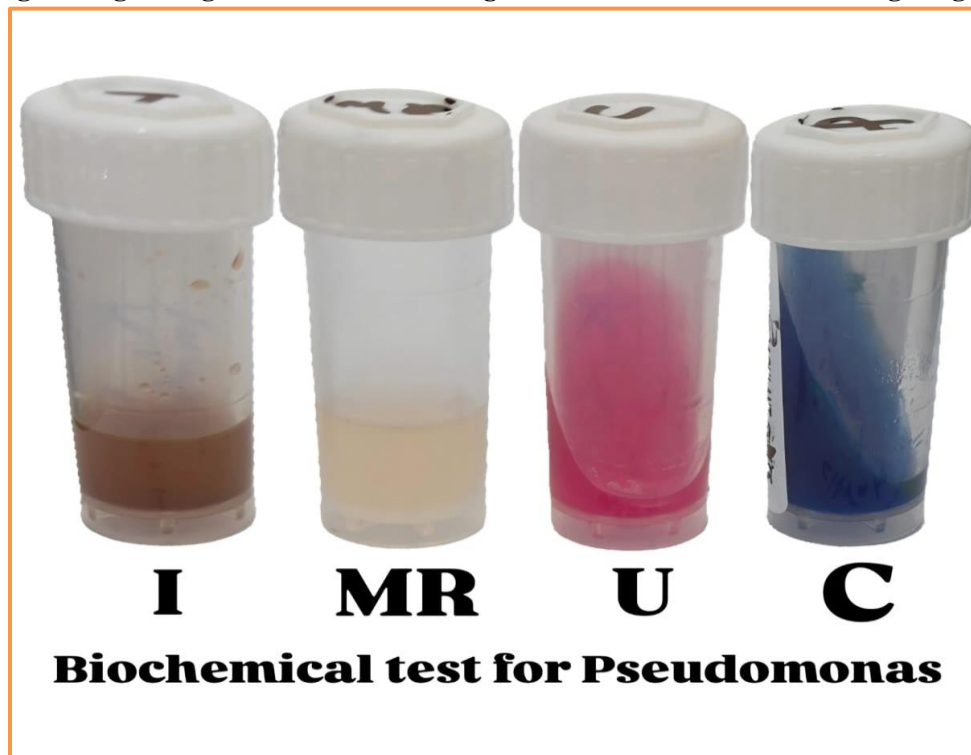


Figure 4: Basic biochemical tests for the diagnosis of *Pseudomonas aeruginosa* showed negative results for indole and methyl red, and positive results for urea and citrate.

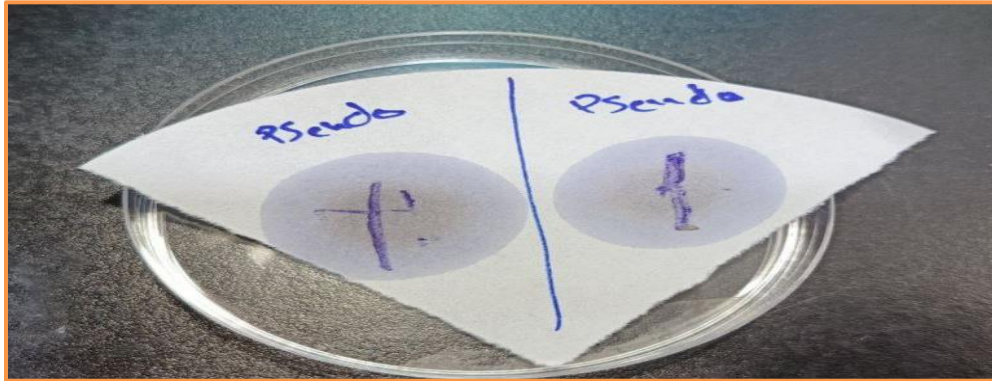


Figure 5: positive result to oxidase test for *P.aeruginosa*.

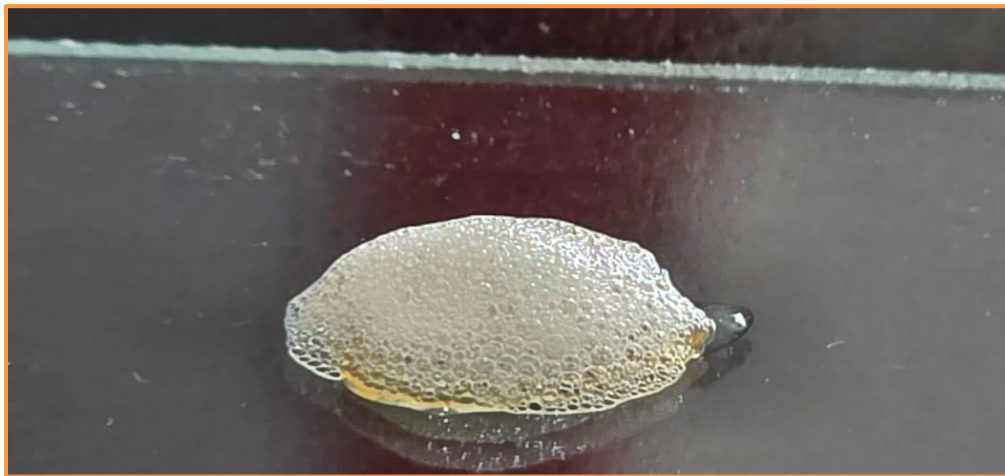


Figure 6: positive result to catalase test for *P.aeruginosa*

bioMérieux Customer:		Microbiology Chart Report		Printed October 9, 2025 3:54:53 PM AST													
Patient Name: ., Abd 22				Patient ID: 1387													
Location:				Physician:													
Lab ID: 1387				Isolate Number: 1													
Organism Quantity:																	
Selected Organism : <i>Pseudomonas aeruginosa</i>																	
Source: Research				Collected:													
Comments:																	
<b>Identification Information</b>		<b>Analysis Time:</b> 4.98 hours		<b>Status:</b> Final													
<b>Selected Organism</b>		98% Probability <i>Pseudomonas aeruginosa</i>															
<b>ID Analysis Messages</b>		Bionumber: 0043053203500250															
<b>Biochemical Details</b>																	
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	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHSa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Figure 7: diagnosis of *P.aeruginosa* and sample purity of up to 98% using the Vitek 2 system.

Al-Salam Teaching Hospital Microbiology Chart Report					
NAME: AB	WARD:	Sp. code: 192			
GENDER:	Department: Orthopedics Department	Bed No.:	Dr. Name:		
AGE:	Specimen: Aspirate	DIAGNOSIS:	Sampling Date: 2025/12/03		
REMARK:					
Culture results					
Concentration: % <i>P.aeruginosa</i> ( <i>Pseudomonas</i> )					
Antibiotic Susceptibility Testing :					
(Group A) First choice for allergic reactions			(Group B) Choose when Group A Resistant/Useless		
Drug Name	Range	MIC R	Drug Name	Range	MIC R
Gentamicin		<=2 S	Levofloxacin		<=2 S
Piperacillin/Tazobac		=8/4 S	Amikacin		<=4 S
Tobramycin		<=1 S	Aztreonam		<=4 S
Ceftazidime		=4 S	Meropenem		<=1 S
			Ciprofloxacin		<=1 S
			Imipenem		=2 S
			Cefepime		<=2 S
(Group C) Substitute when Group A			(Group U) For urinary system infection only		
Drug Name	Range	MIC R	Drug Name	Range	MIC R
(Group O) With clinical indications. Usually useless			(Group Inv) Has not yet been clinically verified		
Drug Name	Range	MIC R	Drug Name	Range	MIC R
Colistin		<=2 S	Cefoperazone/Sulbact		<=4/2
Polymyxin B		<=2 S			
Piperacillin		<=8 S			
Ticarcillin/CA		=16/2 S			
Remark:					
1. MIC: minimum inhibitory concentration.					

Figure 8: diagnosis of *P.aeruginosa* by using the Render MA120 ID&AST reading.

### Antimicrobial susceptibility testing (AST)

*Pseudomonas aeruginosa* has shown high resistance to amoxicillin, as well as to tetracyclines, penicillin, and sulfonamides. In contrast, it has shown high sensitivity to ciprofloxacin, gentamicin, and Imipenem and tobramycin. Table 2, figure 9.

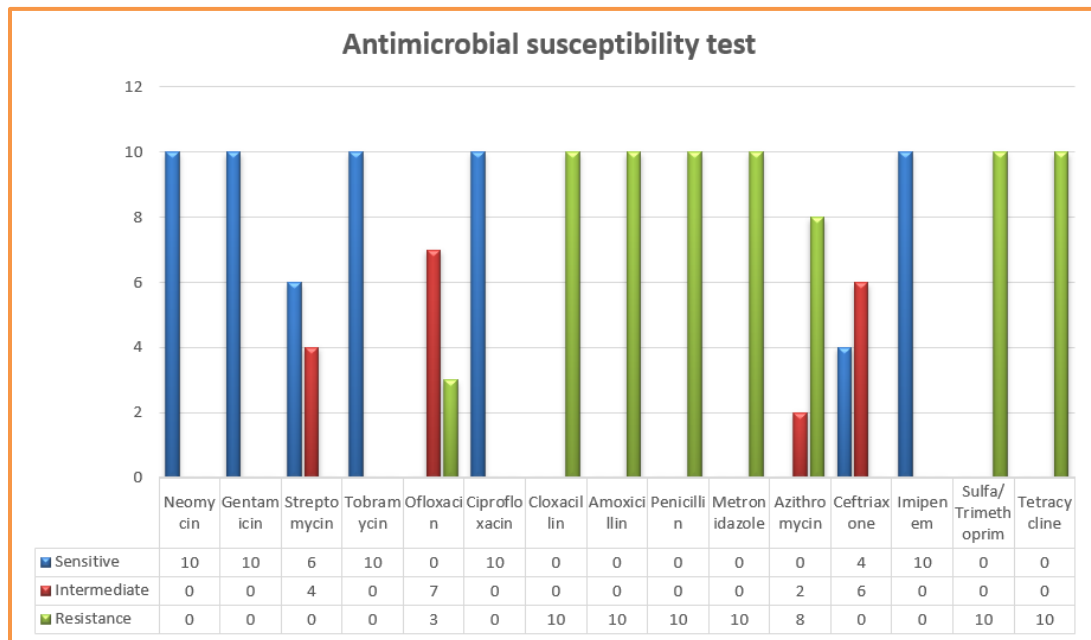


Figure 9: Results of the antibiotics sensitivity test for *Pseudomonas aeruginosa*

**Table 2: *Pseudomonas aeruginosa*: sensitive, resistant, or intermediately sensitive.**

Antimicrobial class	Antimicrobial agent	Disk power	Sensitive number (%)	Intermediate number (%)	Resistance number (%)
Amphenicols	Chloramphenicol	C-10µg	0(0)	2(20)	8(80)
	Neomycin	N-	10(100)	0(0)	0(0)
Aminoglycoside	Gentamicin	10µg	10(100)	0(0)	0(0)
		GEN-10µg	10(100)	0(0)	0(0)
	Streptomycin	S-25µg	6(60)	4(40)	0(0)
	Tobramycin	TOB-10µg	10(100)	0(0)	0(0)
Fluoroquinolone group	Ofloxacin	OF-5µg	0(0)	7(70)	3(30)
	Ciprofloxacin	CIP-5µg	10(100)	0(0)	0(0)
β-lactam	Cloxacillin	CX-10µg	0(0)	0(0)	10(100)
	Amoxicillin	AX-10µg	0(0)	0(0)	10(100)
	Penicillin	P-10µg	0(0)	0(0)	10(100)
Nitroimidazole	Metronidazole	MET-30µg	0(0)	0(0)	10(100)
Macrolide	Azithromycin	AZM-15µg	0(0)	2(20)	8(80)
Cephalosporin	Ceftriaxone	CTR-30µg	4(40)	6(60)	0(0)
Carbapenem	Imipenem	IMP-10µg	10(100)	0(0)	0(0)
Sulfonamide	Sulfa/ Trimethoprim	SXT-25µg	0(0)	0(0)	10(100)
Glycylcycline	Tetracycline	TE-30µg	0(0)	0(0)	10(100)

## Discussion

Many health organizations have classified *Pseudomonas aeruginosa* as a critical priority pathogen of global concern because it is believed to be a major cause of hospital-acquired or healthcare-associated infections (20). *P.aeruginosa* can develop biofilm and develop drug resistance, both of which enable it to endure in challenging conditions(21). Antibiotic resistance in *Pseudomonas*

*aeruginosa* bacteria poses a health threat, as birds act as biological vectors due to their ability to move easily(22). Infection with *Pseudomonas aeruginosa* leads to many symptoms, including enteritis(23). The results of this study are consistent with what the researcher (24)concluded, as the percentage reached 20.9%. This agreement is attributed to the similarity of the experimental conditions, the method of work, and the type of samples. Unlike earlier research, which reported prevalence rates between 2% – 10%, (25–27). This discrepancy may be due to variations in environmental conditions, methodological differences, or the source of the samples. The identity of the isolates was confirmed by the Vitek2 instrument for assessing the performance of modern *Pseudomonas aeruginosa* isolates, using state-of-the-art software and antibiotic susceptibility test cards from bioMérieux(28,29). *Pseudomonas aeruginosa* shows resistance to amoxicillin, as well as to tetracyclines, penicillin, and sulfonamides(30). In contrast, it has shown complete sensitivity to imipenem, ciprofloxacin, gentamicin, and levofloxacin (31,32). Using the disc diffusion method, all sensitivity tests were performed according to the standards of the Clinical Laboratory Institute CLSI(33).

### **Conclusion**

This study demonstrated the severity of *Pseudomonas aeruginosa* infection, highlighting its zoonotic nature and dangerous resistance to many antibiotics, posing a real threat to public health. Therefore, laws restricting the indiscriminate use of antibiotics must be enforced.

### **Acknowledgments**

The authors would like to thank the journal for contributing to the publication of this research.

### **Conflicts of interest**

The authors declare that there is no conflict of interest.

### **Ethical Clearance**

This work is approved by The Research Ethical Committee.

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## عزل وتحديد بكتيريا الزائفة الزنجارية من التهاب الأمعاء في الحمام ودراسة حساسيتها للمضادات الحيوية.

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### الخلاصة

نظراً لقلّة المصادر المنشورة حول بكتيريا الزائفة الزنجارية في الطيور، وما تشكّله من خطر صحي كبير كمرض حيواني المنشأ، هدفت هذه الدراسة إلى الكشف عن بكتيريا الزائفة الزنجارية المسببة لالتهاب الأمعاء في الحمام، ودراسة حساسيتها للمضادات الحيوية. في الفترة ما بين أغسطس ونوفمبر من عام 2025، جُمعت خمسون عينة (أجزاء من الأمعاء وعينات من الإسهال) من حمام مصاب بالتهاب معوي. نُقلت العينات في أنابيب معقمة إلى مختبر الأحياء الدقيقة، كلية الطب البيطري، فرع الموصل. خضعت جميع العينات للزراعة على أوساط إثراء (مرق مستخلص القلب والدماغ (BHIB) وأجار الدم) وأوساط انتقائية (أجار السيتريميد)، بالإضافة إلى الفحص المورفولوجي (صبغة غرام)، والاختبارات الكيميائية الحيوية التقليدية في الأنابيب، وتم تأكيد النتائج باستخدام نظام VITEK2 ونظام Render MA120 Identity System (تحتوي هذه المجموعات على مجموعة من الاختبارات الكيميائية الحيوية). أظهرت 10 عينات من أصل 50 عينة (20%) تفاعلاً إيجابياً لبكتيريا الزائفة الزنجارية، بينما أظهرت 40 عينة من أصل 50 عينة (80%) تفاعلاً سلبياً. كما خضعت هذه العزلات لاختبارات حساسية المضادات الحيوية، والتي أظهرت مقاومة كاملة للكلوكساسيلين، والميترونيدازول، والبنسلين، والسلفا-تريمثوبريم، والتنتراسيكلين، والأموكسيسيلين، وحساسية كاملة للنيومايسين، والجنتاميسين، والتوبرايسين، والسيبروفلوكساسين، والإيميبينيم. وختاماً، تُعد بكتيريا الزائفة الزنجارية أحد أسباب التهاب الأمعاء لدى الحمام، كما أنها تُشكل مقاومة خطيرة للعديد من المضادات الحيوية، مما يُشكل تهديداً حقيقياً للصحة العامة.

**الكلمات المفتاحية:** جراثيم، الزائفة الزنجارية، التهاب الامعاء، الحمام، الطيور.