

## Isolation and Identification of *Pseudomonas aeruginosa* from Bovine Urine in Diyala Governorate

Ali Lattef Hussain AL- Gburi and Amer Khazaal AL-Azzawi

Department Microbiology, College of Veterinary Medicine, Diyala University, Iraq

Email: [Amer.k@uodiyala.edu.iq](mailto:Amer.k@uodiyala.edu.iq)

ORCID: <https://orcid.org/0000-0002-4422-5442>

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### Abstract:

*Pseudomonas aeruginosa* is a Gram-negative, aerobic rod from the Gamma-proteobacteria, widespread in humans and the environment. Its genetic diversity, adaptability, antibiotic resistance, and biofilm formation make it a clinically important pathogen causing urinary, wound, respiratory, and gastrointestinal infections. This study aimed to isolate and characterize *Pseudomonas aeruginosa* from cow urine samples collected between September 1, 2024, and May 1, 2025, in different regions of Diyala Governorate. Isolation was performed using cetrimide agar and MacConkey agar, and the isolates were confirmed by the VITEK 2 system. A total of 93 urine samples were collected from different regions of Diyala Governorates, 85 (91.4%) showed positive growth, while 8 samples (8.6%) showed no growth. Among all the positive samples, *Pseudomonas aeruginosa* was identified in 7 isolates (8.23%) through morphological and biochemical tests, and confirmation was achieved using VITEK 2. In conclusion, urine samples from cows are a common source of *Pseudomonas aeruginosa*, and the VITEK2 system is reliable for identifying the bacteria.

**Key words:** pseudomonas aeruginosa, VITEK2, cetrimide agar, Diyala Governorate



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### Introduction:

One of the most intriguing and clinically significant pathogens associated with urinary tract infections is *Pseudomonas aeruginosa* (*P. aeruginosa*). It is notorious for acute and chronic urinary tract infections (UTIs). Also, it is widely distributed in multiple ecologies because it can endure

them. Animals, plants, humans, soils, and bodies of water can all harbor it. The genetic variation within certain species of these microorganisms allows them to occupy different environments (Morales-Espinosa *et al.*, 2024).

*P. aeruginosa* is the causative agent for a significant number of multicentric and transmittable illness infections within the field of veterinary pathology. (Abd El-Ghany, 2021). It is worth mentioning that one of the primary routes of *P. aeruginosa* infection is through incipient zoonotic transmission and contaminated water sources (Silby *et al.*, 2011). This organism can cause secondary keratitis, conjunctivitis, and chronic otitis media. Infections have also been reported in farm animals such as sheep, goats, and cattle. Notably, *P. aeruginosa* infections are particularly prevalent in immunocompromised animals (Gharieb *et al.*, 2022). The exogenous infections that they suffer will depend on the geographical sources of these pasture weeds (Morales-Espinosa *et al.*, 2024).

Treating infections caused by these species of bacteria is highly complicated due to the *P. aeruginosa* infections being antibiotic resistant (Wijaya, 2021). Virulence factors associated with *P. aeruginosa* contribute to the development of serious diseases. These factors include exotoxins, proteases, phenazines, elastases, and various pigments (Liao *et al.*, 2022). The pigments produced by microorganisms serve to protect them from harmful influences and act as secondary metabolites with biological activities (Abdelaziz *et al.*, 2023).

*P. aeruginosa* is recognized globally as a significant opportunistic pathogen, capable of causing infections in a wide range of hosts, including humans and animals. Its ability to thrive in diverse environments, coupled with its resistance to multiple antibiotics, makes it a serious concern in both veterinary and human medicine (Pang *et al.*, 2019).

The role of *P. aeruginosa* in bovine urinary tract infections in cattle, *P. aeruginosa* is increasingly implicated in UTIs, leading to economic losses in the livestock industry. These infections are often associated with compromised health, poor hygiene practices, and environmental stressors. Bacterium virulence factors, such as biofilm formation and the production of exoenzymes, enhance its ability to colonize and persist in the urinary tract, posing a significant threat to bovine health. (Constable *et al.*, 2016)

## Materials and Methods:

This study involved the collection of ninety-three cow urine samples from various age groups between October 1, 2024, and February 28, 2025, and from deferent locations in the Diyala region. Cow urine samples were collected either from the midstream urine stream during normal urination or by catheterization. Then, these samples were collected in screw-cap containers for subsequent cryogenic storage and subjected to bacteriological analysis. Analyses were performed at the Department of Veterinary Microbiology, College of Veterinary Medicine, and University of Diyala, Iraq.

**Table (1):** Shows the number of samples collected from different areas.

Sampling regions	Number of samples
Al-khalis	22
Baqubah	17
Jalwalaa	16
Buhrez	14
Qarataba	13
Khanaqine	11
<b>Total</b>	<b>93</b>

## Bacterial Isolation and identification

Urine samples were collected and immediately transported to the laboratory under sterile conditions. The next step involved the inoculation of MacConkey agar plates and then incubated at 37 °C for 24 hours to differentially isolate and purify *P. aeruginosa* and to assess the lactose-fermenting ability of each isolate (Mahmood, 2023; Shaymaa and Aida, 2023).

### Growth on Cetrimide agar

For the purpose of isolating *P. aeruginosa* from clinical specimens, cetrimide agar was used as selective media to enhance the production of fluorescein and pyocyanin pigments (Levinson, 2016).

### Bacterial Identification

The bacterial isolates were identified based on colony characteristics, microscopic examination, and biochemical tests as presented in Table (1). Final confirmation of *P. aeruginosa* isolates was achieved using the VITEK 2 compact system (Hassoon *et al.*, 2023).

### Diagnosis of *P. aeruginosa* Isolates Using the VITEK 2 Compact System

Identification of bacterial isolates was performed using the VITEK 2 compact system with the GN identification card according to the manufacturer's instructions. Two integrated systems of the VITEK2 device and with fluorogenic methodology for each of the systems identify live organisms. The VITEK2 system utilizes the turbidimetric method for identification and performs sensitivity testing, emphasizing accurate and precise diagnosis of specific organisms. The VITEK2

system antibiotic sensitivity testing results are released in less than 18 hours. The VITEK2 cards pinpoint and differentiate some anaerobic bacteria and select non-fermenting Gram-negative organisms in less than 10 hours. The system is enhanced by the use of color-coded detector cards which are stored, the results of which are stored and interpreted. (Hernández Durán *et al.*, 2017).

In the current study, the VITEK2 system was established in several steps as follows;

- 1) The isolate *P. aeruginosa* was cultured for 24 hours and was then kept on MacConkey agar for 37 °C.
- 2) The gram stain was performed on the VITEK2 card.
- 3) A sterile tube with saline was then taken and a single, pure colony of bacteria was picked and diluted with 3ml.
- 4) The VITEK DENSICHEK calibrator was calculated, and systems standards were kept. The VITEK DENSICHEK then measures clearances of the turbidity streams.
- 5) Within the tubes, the suspension was inserted in a VITEK2 cassette, with each tube having a 'negative bacterial card' inside.
- 6) The VITEK2 cassette was plugged into the device so that it could find the name of the bacterium in each of the 64 wells. Each well contained dehydrated medium, along with a color marker to perform the biochemical tests. The system then analyzes the color variations for bacteria and their corresponding outcomes, which are retrieved after 24 hours.

## Statistical Analysis

The statistical analyses of this study were carried out using the GraphPad Prism 8 software. The software was also plotted figures. The software established the variance-controlling factors involved in the study.

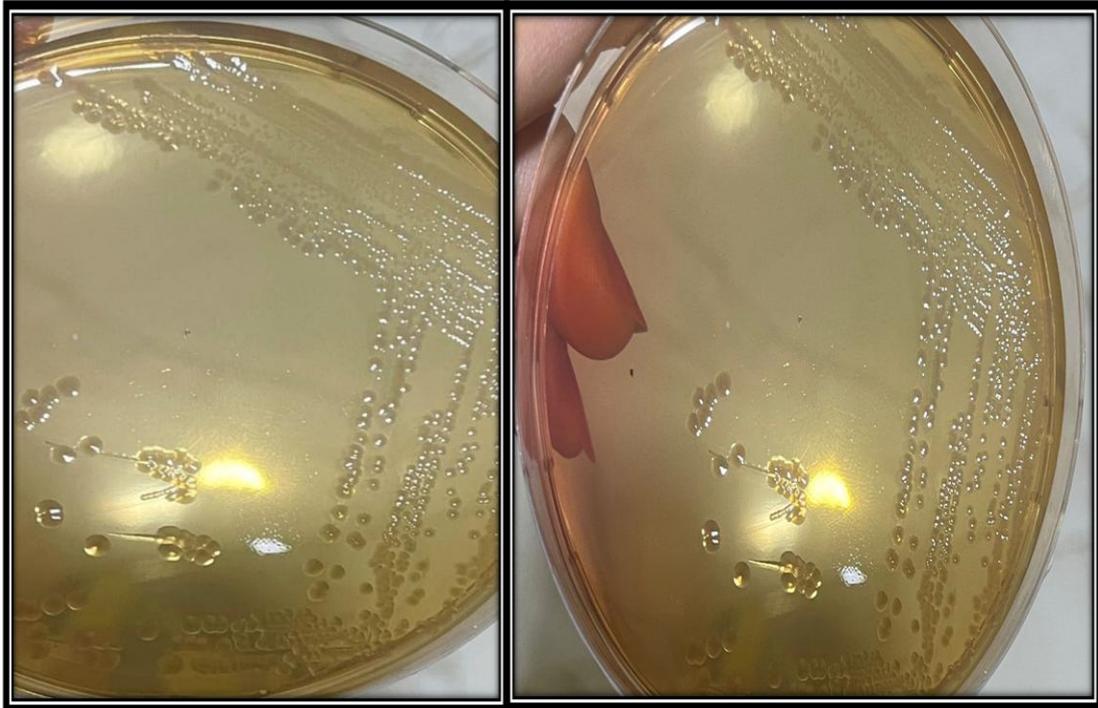
## Results:

Out of the 93 urine samples that collected from different regions of Diyala Governorate including Khalis, Baqubah, Jalawla, Buhriz, Qaratapa, and Khanaqin, 85 (91.4%) showed positive growth, while 8 samples (8.6%) showed no growth. Among all the positive samples, *P. aeruginosa* was identified in 7 isolates (8.23%) out of 85 of the total number of positive growths. *P. aeruginosa* was isolated purely with a high percentage in Baqubgh (3.5%), Alkalis (2.35), Qarataba and Jalwalaa (1.2) and Khanaqine and Buhrez (0%). These results are illustrated in Figure (1) and Table (2).

**Table (2):** Sampling regions and the positive values by VITTEK2

Sampling regions	N0. specimens	Positive growth	NO. of <i>P. aeruginosa</i>	Percentage of <i>P. aeruginosa</i>
Alkalis	22	19	2	2.35
Baqubgh	17	16	3	3.5
Jalawla	16	15	1	1.17
Buhrez	14	12	0	0
Qara taba	13	13	1	1.17
Khanaqine	11	10	0	0
<b>Total</b>	<b>93</b>	<b>85</b>	<b>7</b>	<b>8.23</b>

Isolates were identified as *P. aeruginosa* on MacConkey agar plates (Figure 2), which is indicated by their failure to produce acid and thus they did not turn the medium pink but instead left it colorless or yellowish. MacConkey agar was used for growing pseudomonas because it differentiates it from other Gram-negative species and it contains all required nutrients for pseudomonas growth.

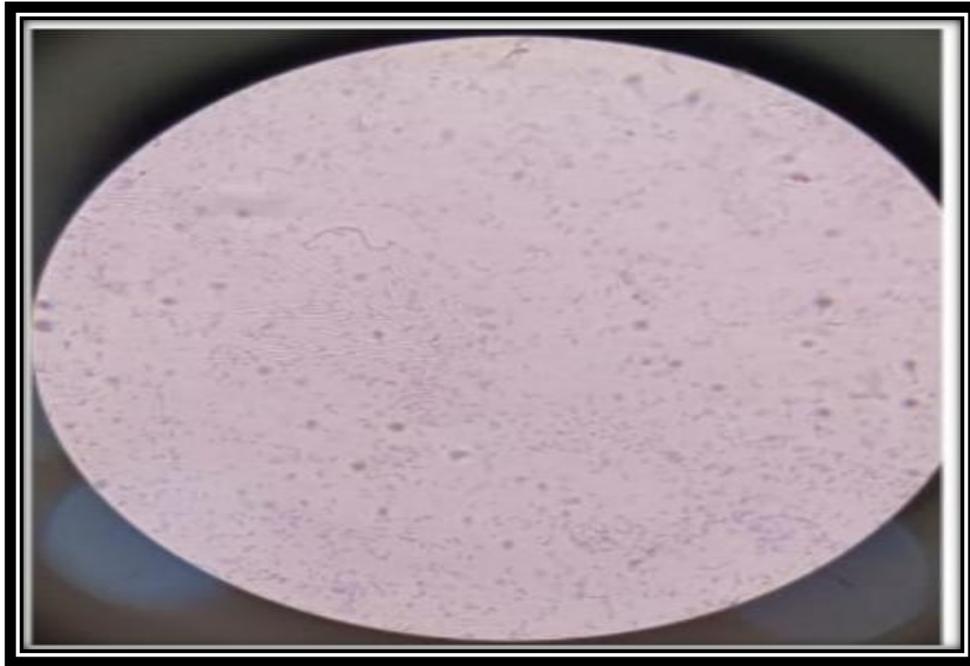


**Figure (1):** Growth *Pseudomonas aeruginosa* on MacConkey agar

### **Identification and characteristics of *Pseudomonas aeruginosa*:**

#### **Microscopic examination:**

Microscopic examination of the results showed that the bacterial cells isolate were negative to Gram stain, red color coccobacilli, variable in length, frequently occurred singly or in short chains and non-spores forming (Figure 2).



**Figure (2):** Gram stain of *Pseudomonas aeruginosa*

### Biochemical tests

Chemical tests were conducted on all eight isolates, including catalase, oxidase and IMVIC tests (indole, methyl red, Voges-Proskauer, and citrate). One of the characteristics of *P. aeruginosa* is that it is positive for the oxidase test. The dark purple color on its surface indicated the production of the enzyme cytochrome oxidase, which was used to detect bacteria that produce cytochrome oxidase. The formation of bubbles gave a positive result for the catalase chemical test, which was used to identify organisms that produce the enzyme catalase. The result of the citrate test was blue color of the medium, resulted from citrate consumption. All isolates tested negative for the indole, methyl red, and Vogess-Proskauer tests. These results were consistent with (Abbas and Al-Ethari, 2024) as shown in (Table 4).

**Table (4):** Shows the result of biochemical test for *Pseudomonas aeruginosa* bacteria.

Test type	Results/ <i>Pseudomonas aeruginosa</i>
Catalase test	+
Oxidase test	+
Indole test	-
Methyl-red	-
Voges-Proskauer	-
Simmon's citrate	+
Urease production	-

### Culturing on cetrinide agar

One colony from each isolate was selected and transferred to cetrinide agar. The colonies that grew on this medium were characterized by their regular round shape and the bluish-green color of the bacteria because it contains cetrinide, which inhibits all types of bacteria except *P. aeruginosa*. Following the incubation period, clear clusters were formed on the cetrinide plate, indicated that the samples belong to *P. aeruginosa* bacteria and that this medium is selective for the isolation and growth of Gram-negative *P. aeruginosa* bacteria (figure 4).



**Figure (3):** Growth *Pseudomonas aeruginosa* on cetrinide agar

These types of bacteria were distinguished by studying their growth at 4 and 42 °C. The bacteria were grown on a solid cultural medium, and the process was repeated for each isolate. Firstly, they were grown at 4°C and then at 42°C (Abbas and Al-Ethari, 2024). The results showed that all isolates grew at 42 °C.

### Diagnosis of bacteria using VITEK2 system

The diagnosis of *P. aeruginosa* isolates was confirmed using The VITEK2 compact system enhances biochemical test results and improves diagnostic accuracy. The VITEK2 system confirmed the biochemical test results in detecting *P. aeruginosa* in all eight isolates. The percentage of bacterial samples that matched *P. aeruginosa* was estimated to be between 95% and 99%. The accuracy of this device, which can reach 100%, has led to its widespread adoption in the identification and bio-classification of bacteria. This device was designed to be a standard biochemical characterization method and is used to confirm and identify various types of microorganisms (figure, 5).

### Discussion

*P. aeruginosa* is an opportunistic pathogen that may infect almost all bodily tissues. It has an extremely large genome, which helps it survive in varied habitats. It also contains a variety of gene regulatory functions that aid in adaptability to new environmental situations. As a result, timely and precise identification of *P. aeruginosa* from culture samples is extremely critical. However, identification of this species may be difficult due to significant phenotypic diversity among isolates and the presence of several closely related species (Qin *et al.*, 2022) The results of the current study indicated that *P. aeruginosa* is a common cause of urinary tract infections in cattle. Surveys in the United States have revealed that *P. aeruginosa* was the third leading cause of urinary tract infections (Weiner *et al.*, 2016). In recent years, infections caused by *P. aeruginosa* have emerged as a major problem in hospitals and are associated with high mortality rates and morbidity rates ranging from 18% to 20% (Usman *et al.*, 2023).

In the current study, *P. aeruginosa* isolation and identification were performed according to morphological characteristics, microscopic examination, biochemical tests and using the VITEK2 system, The results of this study are consistent with the results of other studies, including the study conducted by Kazem and Ali. (2014) in Baghdad Governorate, which resulted in the isolation of sixty samples of *P. aeruginosa* from different areas in Baghdad.

Saven (7) *P. aeruginosa* strains were generated for their investigation, and phenotypic analysis, biochemical testing, and Vitek-2 system were carried out. Following cultivating the bacteria on MacConkey agar and cetrimide agar, their phenotypic characteristics were used to identify the investigated microorganisms. According to the data, *P. aeruginosa* isolates from the Gram stain are negative, which is consistent with (Cappuccino, 2018). After being tested under 100 x magnifications using a light microscope, it emerged as short rods without capsule but flagellated. Regarding pigment production, colonies growing on cetrimide medium were identified by a greenish color - suggesting the development of pyocene pigment (fluorescent green-yellow). Its growth on the MacConkey agar

was yellow in color, which was consistent with the findings of Hassoon et al. (2023) and Hasan, (2025) about its incapacity to ferment lactose. Although *P. aeruginosa* can withstand temperatures ranging from 4 to 42 °C, it thrives best around 37 °C. One of the key distinguishing characteristics of *P. aeruginosa* from the other *Pseudomonas* species is its ability to be developed at a temperature of 42 °C (Procop *et al.*, 2017).

In the current study, bioMerieux GN card for Gram-negative bacteria was used to identify isolates (Al-Shamaa *et al.*, 2016). The VITEK2 method is rapidly and accurately identify clinically important bacteria. The VITEK2 system's operating mechanism enables it to effectively detect clinical or medical conditions with extreme speed and high accuracy. This method, the VITEK2, has been adopted as the standard method for estimating the efficiency of traditional and semi-automatic methods. VITEK2 also boasts high accuracy, with accuracy rates ranging between 97.8% and 98.02%. This percentage depended on sources on the sample conditions (collection season, source isolation, sampling period, and collection area).

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### **Competing interests:**

The authors declare no conflict of interest.

### **Authors' Contributions:**

Ali Lattef Hussain AL- Gburi and Amer Al-Azzawi co-developed the hypothesis, designed the study, and collected samples of cow urine from 93 animals from various regions of Diyala Governorate. Ali Lattef and Amer Al-Azzawi contributed to the manuscript preparation and approved the final version of the article.

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