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Isolate and Identification of *Pseudomonas Aeruginosa* Isolates From Some Ruminants (Cow, Sheep and Goat) From Different Regions of Babylon

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Abstract

Despite being an environmental organism, P. aeruginosa is occasionally discovered in the feces, mucous membranes, and skin of certain healthy animals (wild, companion, or agricultural animals). Numerous toxins and enzymes produced by P. aeruginosa encourage tissue invasion and injury. Antimicrobial agent resistance was demonstrated by P. aeruginosa. It has a big impact on both human and animal medicine. The present study aimed to isolate and identification of P. aeruginosa isolates from some ruminants (cow, sheep and goat) from different regions of Babylon, Iraq, using the Vitek system. Swabs were taken from the rumen of cows, sheep, and goats and were transferred directly to the laboratory for the purpose of culturing. The swabs were cultured on blood, and cetrimide agars and incubated for 24 hours at a temperature of 37°C. 21 (18.3%) of the total samples showed positive results for P. aeruginosa growth when cultured on the most optimal culture media. 94(81.0%) of total samples appeared as negative results for bacterial growth. It is noted that sheep showed the highest percentage of positive isolation, reaching 11 (9.6%) out of a total of 115 isolates, while goats had the lowest percentage of isolation, reaching 3 (2.6%). The genus's bacterial isolates were identified based on microscopic traits such as Gram stain reactivity. Furthermore, isolates and genera were identified using colony attributes such as color, texture, metallic shine, and pigment production. It is concluded from the current study that there is contamination and infection of ruminants with P. aeruginosa, which was isolated from their rumen and is pathogenic to humans.

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1. Introduction

Pseudomonas aeruginosa is a major contributor to infections linked to healthcare settings, with a focus on critical care units. Numerous populations [1-4]. The World Health Organization has classified it as a "critical" bacterial infection, meaning that there is an immediate need for research and development of new antibiotics [5-6]. *P. aeruginosa* is a multipurpose opportunistic pathogen that can cause infections that are either acute or persistent. The pathogenic profile of *P. aeruginosa* is influenced by a wide range of virulence factors and antibiotic resistance determinants found in its genome. These factors allow the bacteria to exhibit remarkable metabolic flexibility and adaptability to a variety of conditions, including the host immune response [7-8]. The host-P. aeruginosa relationship is still poorly understood, which makes the creation of efficient treatments and vaccinations more difficult. Despite 50 years of study dedicated to this problem, there are currently no vaccines to prevent these illnesses, as a recent review found [9]. There are multiple underlying reasons for enzootic or epizootic mastitis epidemics in, bovines and small ruminants. *P. aeruginosa* would be one of the bacteria implicated in this instance. Veterinarians and animal scientists find ruminant mastitis to be quite important [10]. The growing human population on the planet has raised the demand for animal proteins, particularly small ruminant milk. As a result, veterinarians and animal scientists are working to

stop some of the harmful consequences of bacterial infections that lower milk production. When *P. aeruginosa* causes mastitis, the intoxications from milk, cheese, and yogurt cause severe health issues for consumers. Infections with *P. aeruginosa* can manifest as symptomatic or subclinical intra-mammary infections during the postpartum phase and occasionally after drying-off [11-12]. The current study aimed to isolate and diagnose *P. aeruginosa* from some ruminants, cows, sheep and goats, from different regions of Babylon, Iraq, using the Vitek system. This was done in order to demonstrate the seriousness of *P. aeruginosa* presence in the animal environment, as well as on healthy and infected animal organs where these bacteria are not expected to exist.

2. Materials and Methods

2.1 Swabs

1115 Swabs were taken from the rumen of cows, sheep, and goats and were transferred directly to the laboratory for the purpose of culturing. The swabs were cultured on blood, Cetrimide and MacConkey agars and incubated for 24 hours at a temperature of 37°C.

2.2 Bacterial Identification

Bacteria were diagnosed based on the following aspects:

2.3 Morphological diagnosis and media characteristics

Based on the culturing features of the *P. aeruginosa* colonies developing on blood agar, and MacConkey agar, were diagnosed after incubation for 24 hours at 37 0C.

2.4 Microscopic examination

By using a microscope to examine the morphological characteristics of bacterial cells—specifically, how they contacted the gram stain, which indicates the shape and arrangement of the bacterial cells—bacterial colonies were found.

2.5 Biochemical reaction and motility test

Numerous biochemical tests, such as methyl red, citrate, urease, Voges-Proskauer, catalase, oxidase, KIA, and indole test, were carried out in order to identify and diagnose bacteria.

2.6 Identification of bacteria isolates via VITEK2

The newest generation of colorimetric technology, VITEK 2, is the gold standard for microbial identification. Procedure: The following processes were completed in accordance with the guidelines provided by the manufacturer, Biomerieux.

3. Results and Discussion

3.1 Samples distribution

The current study included 115 swab samples collected from the rumen of cows, sheep, and goats (table 1). The findings were found that 21(18.3%) of total samples appeared as positive results for *P. aeruginosa* growth that cultured optimal media such as blood agar, and MacConkey agar. 94(81.7%) of total samples appeared as negative results for bacterial growth.

Table 1. Distribution of study samples according to UTI

	No. (%) +ve culture	No. (%) -ve culture	Total No.(%)	P value
Number	21(18.3%)	94(81.7%)	115(100.0%)	0.001

Table (2) shows the number and types of animals from which swabs were taken and *P. aeruginosa* isolated for the purpose of conducting the biodegradation process. It is noted that sheep showed the highest percentage of positive isolation, reaching 11 (9.6%) out of a total of 115 isolates, while goats had the lowest percentage of isolation, reaching 3 (2.6%).

Table 2. Number and percentage of bacterial isolates

Isolate type	Positive results	Negative results	Total
Cow	7(6.1%)	28(24.3%)	35(30.4%)
Sheep	11(9.6%)	34(29.6%)	45(39.2%)
Goat	3(2.6%)	32(27.8%)	35(30.4%)
Total	21(18.3%)	94(81.7%)	115(100.0%)

The number of samples reached (115) samples, which were collected from the slaughterhouses in Kirkuk city. They were distributed between (35) samples of cows, (45) samples of sheep and (35) samples of goat. All of these samples were

grown on blood agar and MacConkey agar. The primary culture was conducted on this medium for the purpose of isolating and purifying the study samples. The number of P. aeruginosa isolates was 21(18.3%), which is higher than the study by [13] in Bangladesh where it was isolated two isolates of P. aeruginosa from cattle using nutrient agar and Macconkey agar. Approach to a study by [14], where P. aeruginosa was isolated from the rumen of three types of ruminants (cows, sheep and goats) and their average was 9%. As for Duncan et al. (1999) were able to isolate P. aeruginosa from sheep's rumen. Their study produced 11 strains of P. aeruginosa and then performed phenotypic examination and biochemical and molecular tests. The studied bacteria were identified by their phenotypic properties by growing on Bushnell Haas medium, Nutrient agar and MacConkey agar.

3.2 Identification

On blood agar and MacConkey agar, the morphology, diameter, and forms of the bacterial isolates were as certified. Additionally, the results of the biochemical identification were confirmed by means of the System's small Vitek-2 equipment, microscopic and biochemical exams, which comprised the particular tests for each kind. The Vitek-2 results were in line with the findings of the biochemical testing. The form and diameter of the primary isolate of *Pseudomonas aeruginosa* on MacConkey and Cetrimide agars are displayed in Figure (1). The genus's bacterial isolates were identified according to microscopic characteristics like Gram stain reactivity. Additionally, colony characteristics like color, texture, metallic shine, and pigment production were used to identify isolates and genera. The *Pseudomonas aeruginosa* biochemical assays As indicated in Table (3), it tested negative for urease, indole, lactose ferment, M-R, and V-P, but positive for oxidase, citrate, motility, and catalase.

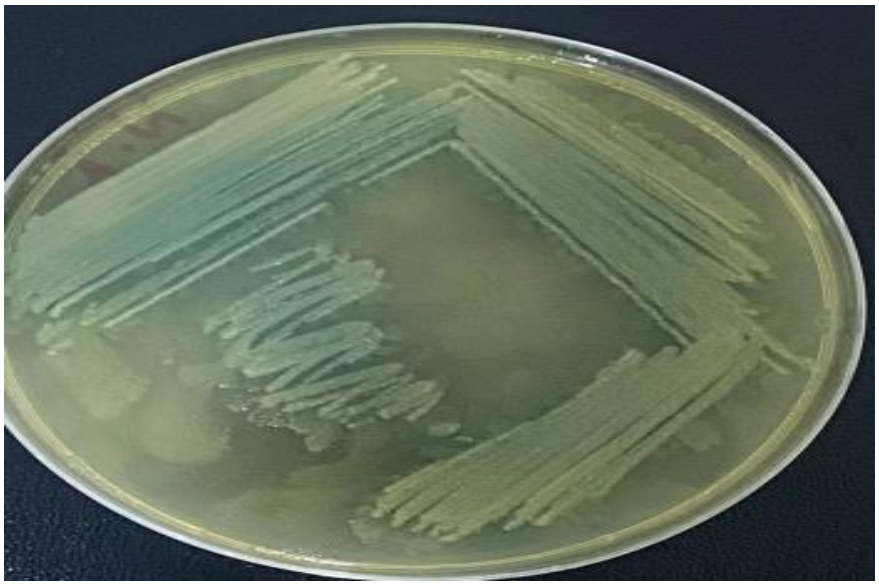

Figure 1. *P. aeruginosa* colonies on Cetrimide agar

Table 3. biochemical tests of bacteria isolates

Bacteria isolates / Biochemical tests	Pseudomonas
Gram stain	+
Catalase	+
Oxidase	-
MR	-
Indole	-
VP	-
Urase test	-
Citrate	-
Hemolysis type	Beta
Motility test	+

A total of 115 samples were obtained from the livestock in Babylon City. They were split up amongst (45) sheep samples, (35) cow samples, and (13) goat samples. Bushnell Haas medium was used to grow each of these specimens. In order to isolate and purify the study materials, this medium was used for the primary culture. There were 21 isolates of *P. aeruginosa* (18.3%), which is larger than the findings of a study conducted in Bangladesh by [16] in which two isolates of the bacteria were obtained from cattle using nutritional agar and Macconkey agar. Methodology for a study by [17], in which *P. aeruginosa* was isolated with an average of 9% from the rumen of three different types of ruminants: goats, sheep, and cows. Concerning Duncan et al. [18], they succeeded in separating P. aeruginosa from the rumen of sheep. Eleven P. aeruginosa strains were generated for their investigation, and phenotypic analysis, biochemical testing, and molecular analyses were carried out. By cultivating the bacteria in Bushnell Haas medium, Nutrient agar, and MacConkey agar, their phenotypic characteristics were used to identify the investigated microorganisms. According to the data, Pseudomonas aeruginosa isolates from the gram stain are negative, which is consistent with [19]. After being tested under 100x magnification using a light microscope, it emerged as short rods without a capsule and with flagellates inside that were employed for locomotion. Regarding pigment production, colonies grew on Nutrient agar medium were identified by a greenish or greenish-yellow color, which suggests the development of pyocene and bioferidine pigment (fluorescent green-yellow). Its growth on the MacConkey agar medium was yellow in color, which is consistent with the findings of Brown et al. [20] and Forbes et al. [21] about its incapacity to ferment lactose. Although P. aeruginosa can withstand temperatures ranging from 4 to 42 °C, it thrives best around 37 °C [22]. One of the key distinguishing characteristics of Pseudomonas aeruginosa from the other Pseudomonas species is its ability to develop at a temperature of 42 °C. In line with Procop et al. [23], this.

4. Conclusions

We conclude from the current study that samples collected from the rumen of some ruminants contain P. aeruginosa, which is considered a bacterial species pathogenic to both humans and animals.

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عزل وتشخيص عزلات الزائفة الزنجارية من بعض المجترات (الأبقار والأغنام والماعز)

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المستخلص:

على الرغم من كونها كائنات مجهرية بيئية، إلا أن P. aeruginosa يتم اكتشافها أحيانًا في براز وأغشية مخاطية وجلد بعض الحيوانات السليمة (البرية أو الحيوانات الأليفة أو الزراعية). تعزز العديد من السموم والإنزيمات التي تنتجها P. aeruginosa غزو الأنسجة وإصابتها. وقد أثبتت P. aeruginosa مقاومة العديد من العوامل المضادة للميكروبات. ولها تأثير كبير على صحة البشر والحيونات. تهدف الدراسة الحالية إلى عزل وتشخيص عزلات P. aeruginosa من معدة بعض المجترات (الأبقار والأغنام والماعز) من مناطق مختلفة من بابل، العراق، باستخدام نظام Vitek, تم أخذ مسحات من معدة الأبقار والأغنام والماعز ونقلها مباشرة إلى المختبر حيث تمت زراعة المسحات على اكار الدم، واكار السيتريميد وحضنت لمدة 24 ساعة في درجة حرارة 37 درجة مئوية. أظهرت 21 (18.3%) من إجمالي العينات نمو ايجابي لبكتريا P. aeruginosa . في حين اظهرت 94 (81.0%) من إجمالي العينات كنتائج سلبية لنمو البكتيريا. ويلاحظ أن العينات التي تم اخذها من الأغنام أظهرت أعلى نسبة عزل إيجابية، حيث بلغت 11 (9.6%) من إجمالي 115 عزلة، في حين اظهرت العينات التي تم اخذها من الماعز أقل نسبة عزل، حيث بلغت 3 (2.6%). تم تشخيص عزلات البكتيريا بناءً على الصفات المجهرية كونها سالبة لصبغة كرام. علاوة على ذلك، تم التعرف على العزلات باستخدام الصفات المظهرية للمستعمرات مثل اللون والملمس واللمعان المعدني وإنتاج الصبغة. يستنتج من الدراسة الحالية أن هناك تلوث وإصابة المجترات بـP. aeruginosa ، التي تم عزلها من المعدة والتي تسبب أمراضًا للإنسان.