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Isolation and diagnosis of *Pseudomonas aeruginosa* using traditional and Virtek-2 techniques

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ABSTRACT

This study has been conducted in order to isolate and identify *Pseudomonas aeruginosa* from human urinary tract infections using traditional methods and the VITEK-2 system. Where , 120 patients with urinary tract infections participated. 20 samples were taken using a catheter and 100 urine samples were taken without a catheter. The samples were incubated at 37°C for 24 hours using brain heart infusion broth, then Pseudo agar, Cetrimide agar, and MacConkey agar. The bacterial isolates were purified and diagnosed by conventional biochemical tests. Then the pure cultures underwent examination by the Vitek-2 system. Bacterial isolates on MacConkey agar showed small, pale colonies, while Cetrimide agar showed bacterial isolates as smooth, mucous-shaped colonies. The use of the Vitek-2 system confirmed that all suspected colonies were isolates of *Pseudomonas aeruginosa.* The results of the current study revealed the isolation of sixty-eight (68 isolates) *Pseudomonas aeruginosa* isolates. In Conclusion, Vitek-2 technology can quickly and accurately identify clinically important germs, including *Pseudomonas aeruginosa*.

1. Introduction

Pseudomonas aeruginosa is considered one of the most important genera of acute and chronic pathogenic bacteria of the urinary tract (1). It is frequently the cause of acquired infections from hospitals (2). Because of its extensive antibiotic resistance, *Pseudomonas aeruginosa* has been identified as a major source of nosocomial infections (3, 4). These Gram-negative facultatively anaerobic bacteria live in a variety of environments, including catheters and medical equipment (5). It is one of the most commonly encountered disorders in intensive care units (ICUs). Furthermore, it is the primary cause of infections that endanger the health and life of burn victims (6). They have weakened immune

systems and are vulnerable to *Pseudomonas* infections, which can be lethal, as a result, it is a significant contributor to clinical infections globally, particularly in patients admitted to intensive care units recuperating from wounds, burns, trauma, and pre-existing lung diseases such as cystic fibrosis (7). Catheters become infected with germs while being used by medical personnel, particularly those with the ability to develop a biofilm and employ specialized medical tools (9,10)

As the urinary catheter enters the bacteria into the bladder, it provides a surface on which the bacteria adhere and irritates the mucous membrane. The incidence of urinary tract infections increases. Between 10-30% of patients are exposed within 2-4 days, while 100% of patients exposed for long periods are at risk of infection (11). The study aimed to isolate *Pseudomonas aeruginosa* from urinary tract infections in humans, through diagnosis using traditional methods and confirming the diagnosis with the VITEK system-2 CD, detection of virulence factors, and investigation of histopathological changes of *Pseudomonas aeruginosa* in some rats organs.

2. Material and Methods:

2.1 Bacterial isolates
Urine specimens collection

This study comprised 120 participants of both sexes and ages with urinary tract infections. 25 urine samples were collected from hospitalized patients with catheters, and 95 midstream urine samples were collected from patients without catheters admitted to teaching hospitals (Al-Salam, Al-Jumhuri, Ibn Sina, and Mosul) between December 2022 and April 2023.

2.2 Culture specimens

The urine samples were centrifuged and the sediment was cultured in heart and brain infusion broth at a temperature of 37°C for 24 hours. Then, they were cultured on cetrimide agar and pseudomonas agar and incubated aerobically at a temperature of 37°C for 24 hours. The bacterial colonies were purified by culturing a second time for the suspicious Pseudomonas aeruginosa colonies. Colonies were then grown on MacConkey agar and blood agar.

2.3 Diagnosis of bacterial isolates

2.4 Morphological examination

The cultural characteristics of the colonies growing on the culture media were recorded, which included the shape of the colonies, their size, pigment production, hemolysis, and lactose fermentation. Pseudomonas agar helped in the emergence of exogenous pigments such as pyocyanin and pyoverdine, and these pigments were among the inhibitory substances that prevented the growth of other bacteria (Levinson, 2016).

2.5 Microscopic examination

A bacterial smear was performed with a Gram stain to evaluate cell shape and to observe how the Gram stain interacted with the cells, allowing bacterial isolates to be studied under a microscope (11).

2.6 Diagnosis by biochemical identification:

Many biochemical tests were used to identify *Pseudomonas aeruginosa* isolates including oxidase, catalase, indole, methyl red, Voges- Proskauer, citrate utilization, urease, H_2S production on TSI agar and motility test.

2.7 Confirmation by VITEK-2 system:

The Vitek-2 compact device is an automated microbiological analysis

system using growth-based technology to identify bacterial isolates to support biochemical tests. This device is used to diagnose bacterial isolates after confirming them through preliminary biochemical tests. The test was conducted according to the manufacturer's instructions (bioMérieux - France) as follows:

Several pure colonies were transferred from the culture dish to a 3ml saline solution (4.50-5.00%) tube and mixed well until the solution became turbid. The turbidity of the suspension was measured using a DensiChek device. Then the suspension and the card were transferred to the device holder and placed in the places designated for them. Then the card and the suspension were connected using a very thin micro-channel, and the card code was entered using the scanner.

Place the holder in a special vacuum chamber, as the air vacuum process transfers the germs to the card as well as distributing them in the holes in it. The delivery channel was cut off automatically by the device within a period of 10-15 minutes and the card was sealed (i.e. the channel port was tightly closed to prevent any leakage), then it was transferred to the Carousel incubator and the cards were incubated at 35.5+1.0°C. After that, the results were recorded, which were in the form of a card containing the names of the biochemical tests, along with the positive or negative result for each test. After that, the level of diagnosis of the organism was determined through a map of its tests, where the organism was given a probability percentage, and the approved percentage was 96-99%.

Results:

3. 1Macroscopic, Microscopic and Biochemical Tests:

Bacterial isolates on MacConkey agar show small pale colonies, indicating that they are not lactose fermentative. Cetrimide agar shows bacterial isolates on cetrimide agar medium as smooth, mucous-shaped colonies with flat edges, raised center, fruity (grape) odor, and fluorescent green in color (Table 1, Figure1).

Table 1: Characteristics of Pseudomonas aeruginosa isolates on different media and surfaces

Agars	Characteristic
MacConkey	**small, pale pink colonies, non-lactose fermenting**
Cetrimide agar	**Mucous, smooth in appearance, with flat edges, a raised center, a fruity (grape) odor, and a fluorescent green hue**.
Blood agar	**Large, flat colonies, white to grey in odor, with a grape-like odor, somewhat showing beta hemolysis**.
Muller-Hinton agar	**Used to diagnose Pseudomonas aeruginosa pigments, including pyocyanin (blue), pyoveridin (yellow-green), pyorubin (red) and pyomelanin (brown).**

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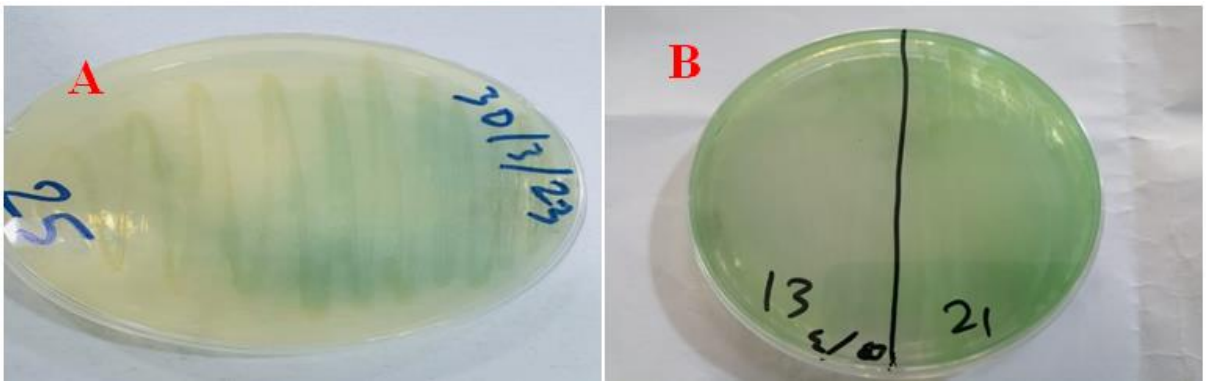

Figure 1: Growth and production of pigments by *Pseudomonas aeruginosa* isolates on Cetrimide agar (A) and Pseudo agar (B).

The purified isolates were typical for *Pseudomonas aeruginosa,* and appeared as Gram-negative bacteria (Figure 2), that produced catalase and oxidase enzymes, indicating that it can decompose water into oxygen and carbon dioxide. They did not create indole. *Pseudomonas aeruginosa* can grow at 4°C, indicating the growing at lower temperatures (Table 2, Figure 3). The biochemical test results revealed the isolation of sixty-eight (68 isolates) *Pseudomonas aeruginosa* isolates

Figure 2: *Pseudomonas aeruginosa* bacteria stained by Gram stain

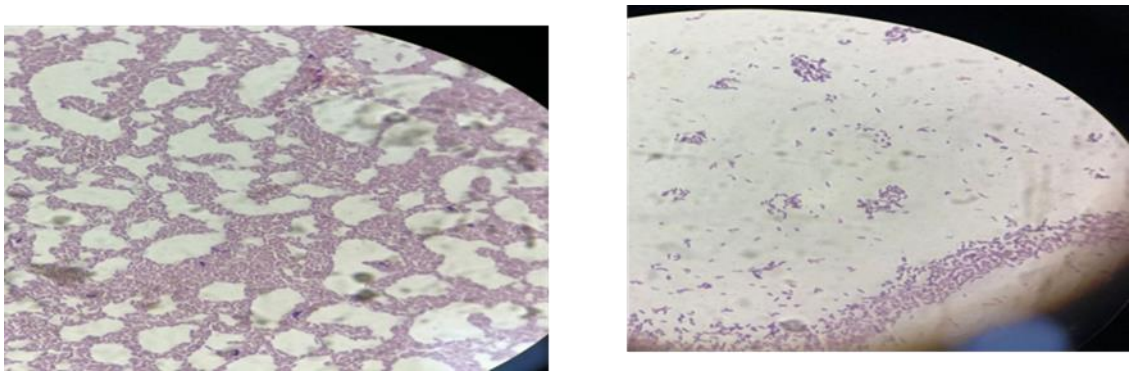

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Table 2: Biochemical tests for *Pseudomonas aeruginosa* bacteria in urinary tract infections

Type of test result	Pseudomonas aeruginosa bacteria
Gram dye	-
Catalase enzyme test	+
Oxidase enzyme test	-
Production of indole	-
Methyl red	-
Voges- Proskauer	+
Citrate utilization	-/+
Urease test	+
Movement test	-
Lactose fermentation	-
Production of H_2S	+

+ Positive result -Negative result -/+Varied result

Figure 3: IMVC results of Pseudomonas aeruginosa

3.2 VITEK-2 system

Use the Vitek-2 compact device to support the results of biochemical tests and also for a more accurate diagnosis. The results of the Vitek-2 device confirmed the results of biochemical tests in identifying *Pseudomonas aeruginosa* in all sixty-eight isolates (68 isolates), and the percentage of identity of the isolated bacterial isolates to *Pseudomonas aeruginosa* bacteria ranged from (95-99%) (Figures 4-5).

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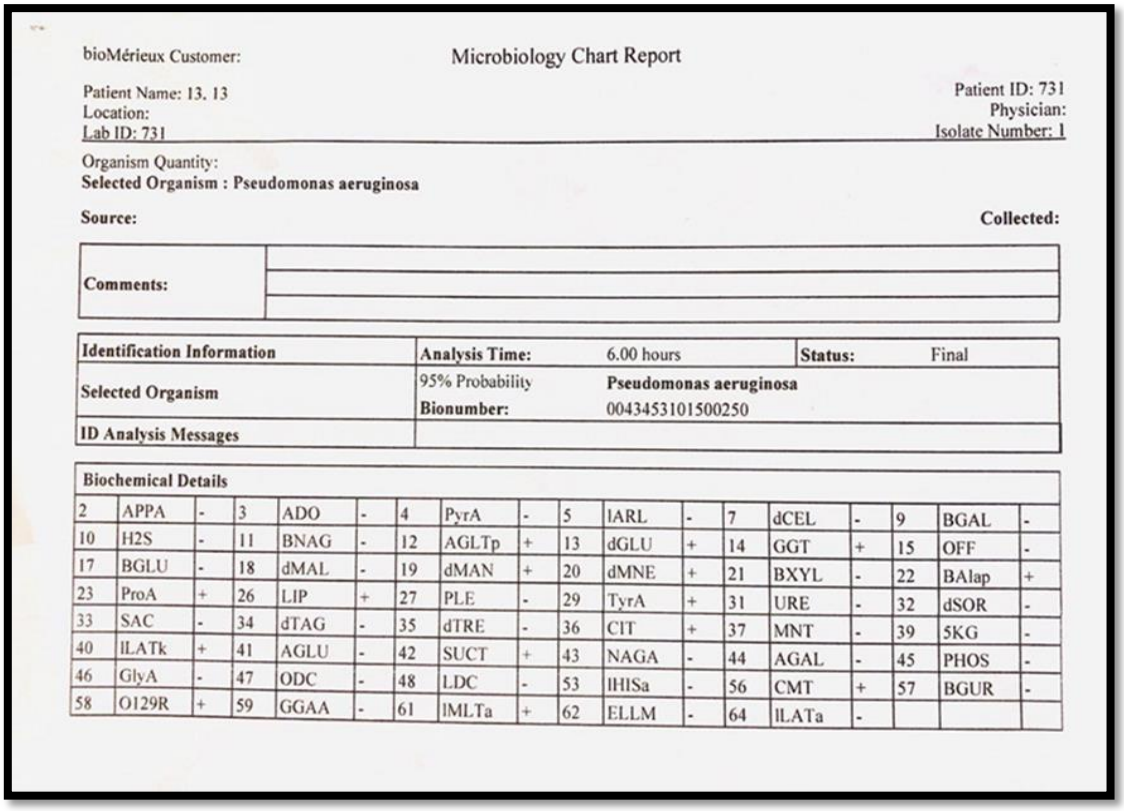


bioMérieux Customer:

Microbiology Chart Report

Patient Name: 13, 13
Location:
Lab ID: 731

Patient ID: 731
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source:

Collected:

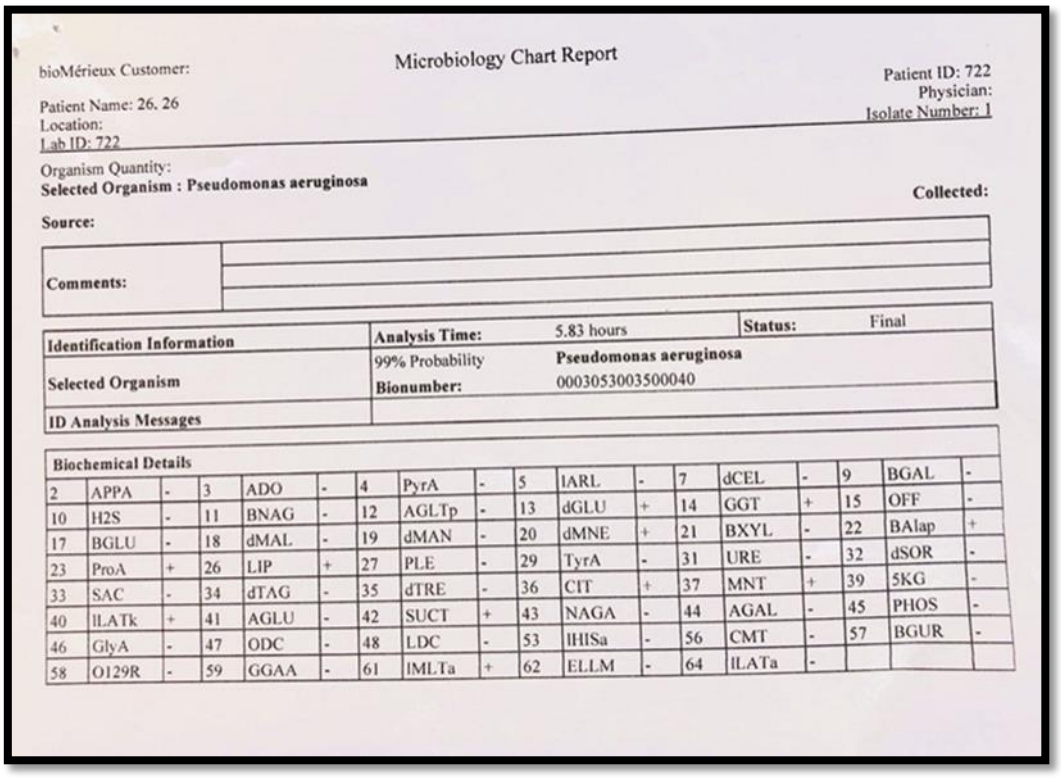
Comments:	

Identification Information	**Analysis Time:**	6.00 hours	**Status:**	Final
Selected Organism	95% Probability	**Pseudomonas aeruginosa**		
	Bionumber:	0043453101500250		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	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	lLATk	+	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	lLATa	-			

Figure 4: Vitek-2 card of Pseudomonas aeruginosa with a probability of 95%.



bioMérieux Customer:

Microbiology Chart Report

Patient Name: 26, 26
Location:
Lab ID: 722

Patient ID: 722
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Collected:

Source:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	99% Probability	**Pseudomonas aeruginosa**		
	Bionumber:	0003053003500040		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	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	lLATk	+	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	lLATa	-			

Figure 5: Vitek-2 card of Pseudomonas aeruginosa with a probability of 99%.

4. Discussion

Pseudomonas aeruginosa isolates were identified in the current study by traditional diagnostic methods, which included culturing on culture media, where cetrimide and Pseudo agar were used, both of which are considered selection media for the isolation and identification of *Pseudomonas aeruginosa* isolates, so the growing colonies were mucous-shaped, smooth, with flat edges, high in the center, with a distinct odour similar to that of grapefruit, and green pigment spread in the middle. These characteristics indicate that the bacterial cultures are of the genus *Pseudomonas* (12-13), since these media encourage the growth of *Pseudomonas* and the production of pigments. These blue and green pigments, such as pyocyanin, and fluorescent dyes, such as pyoverdine, act as inhibitors that prevent the growth of most other bacteria (13). The reference studies have indicated that *Pseudomonas aeruginosa* bacteria are biologically conserved bacteria and do not show changes in metabolic tests between their strains. Therefore, there are no differences between bacterial isolates isolated from different organs and tissues or even from different hosts in their biochemical interactions (12-13), so biochemical tests are important in the diagnosis of *Pseudomonas aeruginosa* (14, 15). The results of the Vitek-2 device confirmed the results of biochemical tests in identifying *Pseudomonas aeruginosa* in all sixty-eight isolates (68 isolates), and the percentage of identity of the isolated bacterial isolates to *Pseudomonas aeruginosa* bacteria ranged from (95-99%). According to (16, 17), the Vitek-2 method is better able to quickly and accurately identify clinically important bacteria, including *Pseudomonas aeruginosa*. The automatic Vitek-2 method was used as a standard method to evaluate the efficiency of manual and semi-automated methods. Also, the Vitek-2 method has a better accuracy range of 97.8% to 98.02%. The percentages change between the scientific references based on conditions (collection season, isolation of sources, sample period, area of collection area). Vitek-2 is also used to determine sensitivity to antibiotics and showed a sensitivity of 96.2% and a specificity of 89.6% for detecting Pseudomonas aeruginosa bacteria that produce carbapenemase enzymes (18).

5. Conclusion

Vitek-2 system is accurate for detecting *P. aeruginosa* and confirming the results of traditional biochemical tests, also, it may be use instead of the conventional methods.

6. Acknowledgement

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عزل وتشخيص جراثيم الزوائف الزنجارية باستخدام التقنيات التقليدية وتقنية الفايتك-2

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الملخص

استهدفت هذه الدراسة عزل وتشخيص الزائفة الزنجارية من المجرى البولي في الأنسان بالطرق التقليدية و نظام VITEK-2. أجريت الدراسة على العينات المجموعة من 120 شخصاً مصابين بمرض التهاب المجرى البولي. حيث تم أخذ 25 عينة باستخدام القسطرة و95 عينة بول بدون قسطرة. حضنت العينات عند 37 مْ خلال 24 ساعة بأستخدام مرق نقيع القلب والدماغ ثم أكار السيدو و أكار الستريمايد وأكار الماكونكي. تم تنقية العزلات البكتيرية وشخصت عن طريق الاختبارات البيوكيميائية التقليدية. ثم خضعت الزروعات النقية للفحص بواسطة نظام Vitek-2. اظهرت العزلات البكتيرية على وسط أكارماكونكي مستعمرات صغيرة شاحبة، وظهرت على أكار السيتريمايد بشكل مستعمرات مخاطية الشكل ناعمة. أظهر استخدام تقنية الفايتك-2 أن جميع المستعمرات المشتبه بها كانت من نوع الزوائف الزنجارية. أسفرت نتائج الدراسة الحالية عن عزل ثمانية وستين (68) عزلة زوائف زنجارية. واخيرا يمكن لتقنية الفايتك-2 تحديد الجراثيم المهمة سريريًا بسرعة وبدقة، بما في ذلك الزوائف الزنجارية.

الكلمات المفتاحية: الزوائف الزنجارية، التشخيص، الفايتك-2، عدوى المجاري البولية