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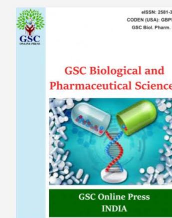

(Research Article)

Isolation and identification of pathogenic species of the genus Pseudomonas and study of antibiotic resistance

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Abstract

The bacteria *Pseudomonas aeruginosa* were isolated and diagnosed and compared with *Pseudomonas fluorescens* for the period between September 2021 and March 2022, in Ghazi Hariri Hospital, Yarmouk and the Medical City.) to investigate Pseudomonas aeruginosa and *Pseudomonas fluorescens* and other species study antibiotic resistance.

(90) samples were collected using a cotton swab and a sterile container. The diagnosis was made based on the culture characteristics and biochemical tests, after which the diagnosis was confirmed at the qualitative level using the phytic technique. Carry out a sensitivity test for antibiotics, depending on the method of dissemination of the disc (Kypri Power).

As (25 bacterial isolates were isolated from Pseudomonas aeruginosa, with a percentage of (21.7%)., and (10) bacterial isolates from Pseudomonas fluorescens, with a percentage (8.7%). As for P. putida and P.stutz, the total number of isolates for each of them was (3). Isolates with a percentage of (2.7%).The infection of males with these bacteria was higher than that of females out of a total of 41 isolates of interest to our study, as it appeared that there were (24) infected males, i.e. (58.5%), and (17) infected females, representing a percentage of (41.5%). The sensitivity of these isolates was tested against a group of antibiotics, including 7 antibiotics. The results of the current study showed that the Pseudomonas aeruginosa isolates were highly resistant to Piperacillin, Tetracycline, and Gentamicin, where it formed a high resistance rate of 100%, while the study isolates showed sensitivity to Tobramycin antibiotics. Ciprofloxaci by (90%). As for Pseudomonas fluorescens, the percentage of resistance to Co-trimoxazole was 80%, Tetracycline 50%, tobramycin 40%, cefepime 60%, Ciprofloxacin 60%, Piperacillin 40%, Gentamicin 70%.

Keywords: *Pseudomonas aeruginosa; Pseudomonas fluorescens*; *P. putida* and *P. stutz*

1. Introduction

The genus *Pseudomonas* includes many species of bacteria everywhere, and to date, it includes 272 species isolated from many different environments, such as soil, water, air, and sediment, as well as from many species of hosts, such as animals, plants, fungi, and algae [1].

Pseudomonas aeruginosa is one of the types *of Pseudomonas spp.* It is an opportunistic pathogen that possesses a group of virulence factors that make it a subject of interest and study for researchers because of the damage it causes to human health and is responsible for some of the injuries that affect it, as it causes many acute and chronic infections such as burns, wounds and ear infections [2,3] . As for the bacteria *Pseudomonas fluorescens*, it is a non-pathogenic bacterium found in soil and plants [4], and it is a chemo-organotroph for nutrition and respiration, and this bacterium is considered one of the most important factors of biological resistance [5] , these bacteria are characterized by being adapted to survive in the soil or settle inside plants, and have a positive effect on both the pathosystem and growth components

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such as increasing plant height, wet and dry weight, and production, and are characterized by being biofilm producers, sometimes need advanced techniques to detect and to remove from the environment [6,7]. *Pseudomonas putida* is a negative *Pseudomonas stutzeri* found in soil and plants and is an opportunistic pathogen that causes infections in immunocompromised hosts [8,9].

2. Material and methods

2.1. Preparation of culture media

2.1.1. Preparation of ready and synthetic culture media

The agricultural media was prepared according to the company's instructions, which are equipped and fixed on the box, where the sterilization process was carried out using the autoclave at a temperature of 121 ° C for 15 minutes and under a pressure of 15 pounds / inch2, then the medium was cooled to a degree (45-50 ° C), after which the process was carried out. Pouring into dishes as needed, then those dishes were placed in the incubator upside down at a temperature of 37 ° C for a period of 24 hours to ensure that there is no contamination of the medium, then it was kept in the refrigerator at a temperature of 4 ° C until use.

2.2. Bacterial isolates

2.2.1. Collection of Pseudomonas aeruginosa isolates

90 samples were collected from patients attending and inpatients in Ghazi Hariri, Yarmouk and Medical City hospitals from the first of September 2021 until the end of March 2020, and the collected samples included (burns, wounds and urination) and a sample of both sexes and different ages, where sterile cotton swabs were used Swabs were used to take burn and wound samples. As for sputum and urine, sterile plastic containers were used.

2.2.2. Identification of bacterial isolates

Microscopic and agronomic characteristics

Microscopic examination

A microscopic examination of the bacterial isolates under study was carried out by taking a part of the bacterial colony and transferring it to a clean glass slide and staining it with Gram stain to observe its interaction with Gram stain, the shape of the cells and the method of their aggregation.

Agronomic characteristics

The culture characteristics were studied through the initial diagnosis of the bacterial colonies on the media of the Citerimide agar, the blood agar and the MacConkey agar, where the morphological characteristics of the colonies, color, shape and texture were noted as well as their size, height, odor and the shape of the edges as well as their ability to decompose blood through the production of hemolysin on the media of the blood agar. Biochemical tests were carried out (oxidase test, catalase test, indole test, methyl red test, Voge's - Proskauer test, and citrate utilization test).

2.3. Antibiotic Sensitivity Test

2.3.1. Disk Diffusion Test

The standard Kirby & Bauer method was used to test the sensitivity of bacteria to antibiotics.

The bacterial suspension was prepared by transferring 3-5 young colonies to tubes containing the nutrient agar medium, and the tubes were incubated at a temperature of 37 °C for a period of 18-24 hours. After that, the growth of the bacterial suspension in the tubes was measured by comparing it with the turbidity of the standard MacFarland tube, which is constant turbidity equal to (1.5×10^{8} cells/ml).

Sterile cotton swabs were used to spread the bacterial inoculum on the prepared dishes containing Muller-Hinton agar medium by immersing the swab in the bacterial inoculum after shaking it well. The excess bacterial suspension is removed by pressing the cotton swab several times on the inner wall of the tube containing the medium vigorously to remove moisture. After that, the dishes were inoculated with a cotton swab using a brush method on the surface of the

agar, then the dishes were left at room temperature for 10-15 minutes, then the antibiotic tablets were transferred to the surface of the cultured dish with sterile forceps at equal distances suitable to prevent overlapping in the inhibition areas Taking into account the sterilization of forceps by (fire flame and then cool).

After that, the plates were incubated at 37 °C for 24 hours, then the readings were determined by measuring the diameter of the bacterial inhibition zone around each disk in millimeters using a transparent ruler. In comparison with the standard rates for the diameter of the inhibition zone given in the international laboratory tables of CLSI 2016.

3. Results and discussion

3.1. Isolation and identification of *Pseudomonas spp*.

90 samples were collected from patients attending and inpatients in Ghazi Hariri, Yarmouk and Medical City hospitals from the first of September 2021 until the end of March of the year 2022, and the collected samples included (burns, wounds and urination) and by a sample of both sexes and different ages, where sterile cotton swabs were used swabs for taking samples of burns and wounds. As for urine, sterile plastic containers were used.

(25) isolates from Pseudomonas aeruginosa, (10) isolates from P. fluorescens, (3) isolates from P. putida, (3) isolates from P.stutzi, i.e. (21.7%, 8.7%, 2.7% and 2.7%, respectively. (40) isolates representing Staph. aureus, (34) E.Coli bacteria, i.e. (34.7%) and (29.5%) out of a total of 115 samples, respectively. Isolates that did not represent the bacteria under study were also discarded. Where 41 isolates of interest to our study were taken and differences were found between Pseudomonas aeruginosa, P. fluorescens, P. putida and P.stutzi.

As the total number of bacterial isolates of Pseudomonas aeruginosa reached (25) bacterial isolates out of the total (115) isolates with a percentage of (21.7%), which included (5) isolates from urine, (7) isolates from wounds and (13) isolates from burns, which were represented by: (20%), (28%), and (52%), respectively, from a total of 25 isolates. As for P. fluorescens, the number of its isolates also reached (10) bacterial isolates out of the total (115) with a percentage of (8.7%), which included (2) isolates from urine, (5) isolates from wounds, (3) isolates from burns, where It was represented by (20%), (50%) and (30%), respectively, from a total of 10 isolates. As for P. putida, the total number of isolates was (3) isolates, with a percentage of (2.7%) out of a total of 115 samples, which included (1) isolate from wounds and (2) isolates from burns, which were (33.3%) and (66.7%). Consecutive from a total of 3 isolates. Also, P.stutzi, the total number of isolates reached (3) isolates from a total of (115) samples, with a percentage of (2.7%), which included (1) isolates from wounds and (2) isolates from burns, which represented (33.3%) and (66.7%). respectively from a total of 3 isolates. And as shown in Table (4-1). The results also showed that the infection of males with these germs was higher than the infection of females from a total of 41 isolates of interest to our study, as it appeared that there were (24) infected males, i.e. (58.5%), and (17) infected females, representing (41.5%), as shown in Table 4.2.

Table 1 Numbers and percentages of *Pseudomonas aeruginosa, P. fluorescens, P. putida, and P.stutzi* isolated from different regions of the human body

Pseudomonas spp								**isolation sours**
P.stutzi		***P. putida***		***P. fluorescens***		***Pseudomonas aeruginosa***		
(%)	**No.**	(%)	**No.**	(%)	**No.**	(%)	**No.**	
	0		0	% 20	2	%20	5	urine
%33.3	1	%33.3	1	%50	5	%28	7	wound
%66.7	2	%66.7	2	%30	3	%52	13	burn
%100	3	%100	3	%100	10	%100	25	total
41								total

Table 2 Distribution of infection with germs according to the sex of the patient.

(%)	**No.**	**sex**
%58.5	24	Male
%41.5	17	female
%100	41	total

Pseudomonas aeruginosa is the third most common pathogen associated with hospital-acquired infections, and the frequency of hospital-acquired infections by Gram-negative Enterobacteriaceae especially *P. aeruginosa* has increased over the past decade [10]. This bacteria one of the most pathogenic can be escape from disinfection of hospital into the wastewater of health facilities which also consider environmental threading source and its detection of pseudomonas can be examined according [11].

Burns become infected because the environment at the wound site is ideal for bacteria to multiply, so the burn wound is considered one of the major health problems in the world [12].

Pseudomonas fluorescens is not generally considered a bacterial pathogen in humans [4]. However, infection with *Pseudomonas fluorescens* was recorded at a rate of 5 in wounds and 3 in burns, and *P. fluorescens* was characterized as an acute cause of infection (opportunistic or primary) at sites Other than blood, as previous researchers have recorded that *Pseudomonas fluorescens* is present in skin wounds and abscesses after dog bites [13].

Pseudomonas fluorescens is typically non-pathogenic, which means that it does not cause disease in humans. However, there have been cases of this bacterium affecting people with weakened immune systems, known as immunodeficiencies becoming infected by *Pseudomonas fluorescens*, usually through the bloodstream due to contaminated intravenous injections and blood transfusions. These bacteria can also enter the body through contaminated drinking water or products [14].

In the current study, 13 (52%) *P. aeruginosa* isolates were recovered from burn patients, and this result was close to the findings by [15,16] reported a higher percentage of sequestration rate (50%) and (68.3%). While [17] reported (35.2%) of burn patients. While the studies of [18,19] showed a lower prevalence of *P. aeruginosa* in burn infection (23.1%) and (8.55%)

The current study did not agree with the study conducted by the researcher [47]. *P.aeruginosa* bacteria were isolated from clinical samples by 70%, distributed as follows: 48.5% of burn infections, 41.4% of wound infections, and 5.7% of urinary tract infections. As for the percentage of isolation of these bacteria from burn samples, it reached 52% in the current study, and the reason may be due to the contamination of these burns from the hospital atmosphere, tools, beds, and hands of hospital workers contaminated with this bacteria. of *Pseudomonas aeruginosa* in the city of Najaf on 37 isolates of this bacteria, the distribution ratios reached 27 (67.6%) for burns, while the urinary system was 10 (9.9%).

P. stutzeri is an uncommon opportunistic pathogen found in the environment. Approximately 1% to 3% of *Pseudomonas* species detected from hospital isolates were identified as *P. stutzeri*. Human infection caused by this type of *Pseudomonas* was first reported in 1973 in a person with a cutaneous infection **[21].**

In this study, samples were collected from bacterial infections. *P. stutzeri P. putida* from burns and wounds accounted for 33.3% and 66.7% *P. stutzeri* is an opportunistic pathogen. It has been isolated from clinical samples and has been found to cause infection in humans, although rarely. Several *Pseudomonas species* have been shown to cause skin infections including *P. stutzeri*. Other cases include infections of the artificial bone replacement. All cases of *P. stutzeri* were treated with antibiotics [22]. Pseudomonas putida is an uncommon cause of skin and soft tissue infections. It is often associated with trauma or an immunocompromised state. We present the first fatal case of bacteremia due to skin and soft tissue infections, which includes malnutrition, immobility and peripheral vascular disease as risk factors [23].

In this study, samples of *Pseudomonas* bacterial infections were collected, showing that 24 males (58.5%) were females 17 (41.5%), meaning that the percentage of infection in males is more than that of females, as shown in Table 2.4.

This finding was inconsistent with the study of findings by Rajupt *et al.* (2008) who showed that the incidence of burns in females (60%) was more than in males (40%). Hamoudi's (2014) study did not agree with the current study, which

found that females are more common than males for burn injuries. This may be because females get burned more often than males.

It is noted from the comparison of the results obtained with the results of other studies that there is a convergence and difference in the percentage of bacteria isolation from different samples, and this is due to reasons including: the different places of isolation, such as hospitals and intensive care centers, and this discrepancy may also be due to the number of samples collected from Also, the percentages of distribution of isolates may vary according to the sites of infection, and other reasons are due to the degree of concern for cleanliness, and the type of sterilizers and various antiseptics used in hospitals, because P. aeruginosa bacteria are resistant to most sterilization materials and antibiotics, and they are a major cause of acquired infections From Shuwaikh Hospitals [24].

3.2. Identification of Pseudomonas spp.

3.2.1. Laboratory culture

The results of laboratory culture when cultivating samples on Pseudomonas agar medium (cetrimide agar) showed that 41 isolates have the ability to grow on this medium, [25], the colonies growing on this medium were characterized by their regular circular shape and bluish-green color, and they were characterized by an odor similar to that of ripe fruits, as shown in figure (4.1)A. Through development on solid MacConkey medium, it was found that 41 isolates had the ability to grow on this medium, but they did not ferment the sugar lactose, as shown in picture (1.4) B. In MacConkey Agar medium, the colonies of Pseudomonas aeruginosa are colorless due to the lack of lactose fermentation that It is of great importance in distinguishing P. aeruginosa from other bacteria present in the sample, especially from Gram-positive bacteria., [26]. *Pseudomonas aeruginosa* forms flat, smooth colonies 2 to 3 mm in diameter. Generally, these colonies have regular edges and have a crocodile-skin-like appearance when viewed from above. A single colony was selected from each isolate, and planted on solid blood medium, which is a rich, stimulating medium, to determine the bacteria's ability to analyze blood and determine the type of decomposition (Li *et al.,* 2022), and 23 isolates were completely β-hemolysis, as a transparent halo appeared around The colony is as shown in the picture (1.4) C.

Most Pseudomonas can be identified. *P. aeruginosa* easily based on their distinctive colony morphology, pigment and odor production, and their ability to grow at 42 °C. *While P. fluorescens* and *P. putida* do not have a distinct colony shape or odor. And their inability to reduce nitrate to nitrogen gas and their inability to produce xylose acid, which was produced in the two species than in other *pseudomonads* [27].

P. fluorescens can be distinguished from *P. putida* by its ability to hydrolyze gelatin. *P. fluorescens* isolates can require 4–7 days of incubation for accurate detection of gelatin hydrolysis [28] . *P. fluorescens* can be distinguished from *P. stutzeri* by its ability to grow at 4 °C [28]. *P. stutzeri* can grow in the presence of 6.5% NaCl. This biochemical property, along with the ability to reduce nitrate to nitrogen gas, the ability to oxidize glucose but not lactose, and the characteristic dry and wrinkled colony morphology (1-6 mm in diameter), distinguish the group *P. stutzeri* from other *Pseudomonas spp*. It forms brown colonies [29]. *P. stutzeri* colony morphology and pigment production on nutrient agar after 24 h, and again after 1 week at room temperature produced pyocyanin and fluorescein. A colony of *P. putida* on nutrient agars appeared dark brown, with a flattened shape and irregular edges [29].

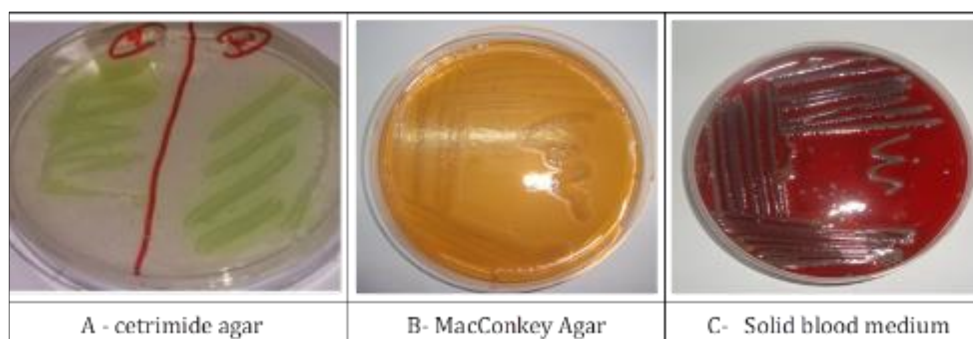

Figure 1 Colonies of *P. aeruginosa* bacteria

Table 3 Characteristics of *P. aeruginosa, P. fluorescents*

***P. fluorescents* NO.10**	***P. aeruginosa* NO.25**	**Characteristic**
10	25	Fluorescin
10	25	Oxidase
0	25	Nitrate reduction
10	25	Gelatin liquefaction
10	25	Growth on cetrimide
10	0	Growth on 4C.
0	25	Growth on 42C

The growth of the bacteria was monitored at 4ºC and 42ºC, as the bacteria were grown on the solid nutrient media and repeated for each isolate, the first incubated at 4ºC and the other at 42ºC, the results showed the ability of all isolates to grow at 42ºC, which is an important diagnostic characteristic for the type *Pseudomonas aeruginosa* over the rest of the species of the genus *Pseudomonas* However, not all isolates grew at 4 °C, and this is in agreement with [30] .

3.2.2. Microscopic examination

When conducting a microscopic examination of bacterial swabs stained with Gram stain, the bacteria (*Pseudomonas aeruginosa*) appeared negative for this stain [31] and in the form of single bacilli or short chains, as shown in the figure (2.4).

Figure 2 Gram stain of P. aeruginosa under magnification (X100) after 24 hours of growth on cultured culture medium, which shows Gram-negative bacillus

P. fluorescens, under light microscopy, showed rod-shaped cells, single in arrangement, and not connected to each other in chains. They are also motile bacteria, which means they have flagella [32] as shown in Figure (3.4).

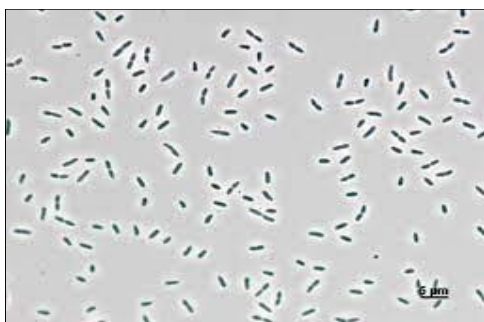

Figure 3 of a Gram stain of *Pseudomonas fluorescens* under a light microscope

Pseudomonas stutzeri is a Gram-negative, rod-shaped, non-spore-forming bacterium that is typically 1–3 μm long and 0.5–0.8 μm wide under a light microscope as shown in figure (4.4) [33].

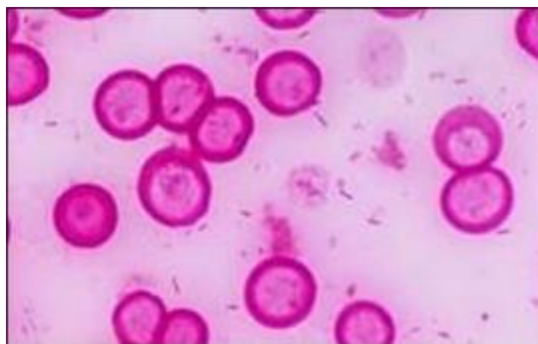

figure 4 of a gram stain of *Pseudomonas stutzeri* under a light microscope

Microscopically, 3 isolates were found to be motile by single pole flagella. Two main types of colonies were observed on nutrient agar: dry, firm, elongated ("rough") colony with concentric areas of spread and mucous ("smooth") colony with different intermediate types. Colonial range A variety of colonial appearance was obtained, for example, *Pseudomonas stutzeri* could be confused with other species of the genera [34]. Pseudomonas putida showed a Gram-negative rod shape, 0.5 to 1.0 μm long. The length of the cells may vary depending on the age of the cell in the culture medium, as young cells tend to be longer [35] as shown in the figure (4.5).

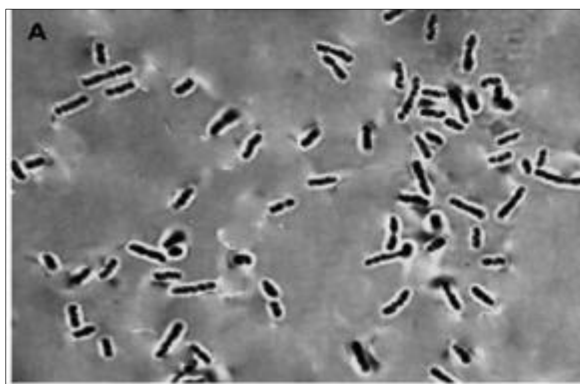

Figure 5 of a gram stain of *Pseudomonas putida* under a light microscope

Our study agrees with the study of [36] in terms of the difficulty of examining the pathogenicity of Pseudomonas putida and *Pseudomonas stutzeri* in diseased cases in terms of isolating them from wounds, burns and urine. It rarely occurs in humans and is transmitted through soil, plants and animals to humans. It was recently isolated from pathogenic material obtained from human sources in this country.

3.2.3. Biochemical tests Pseudomonas spp.

Biochemical tests for *Pseudomonas aeruginosa*

Table 4 Tests that were used in the diagnosis of P.aeruginosa bacteria isolated from different disease cases

Number (%) of positive isolate	Biochemical tests
Gram-negative bacillus	Gram stain
+	Growth test at 42c
_	Growth test at 4c
+	Oxidase assay
(%42.7) 32	urease
+	catalase
+	hymolysin
_	lactose fermentation
K/K	KIA
_	produceH_2S
_	Indole
_	MR
_	-V-P
+	Citrale reductase

Biochemical tests for *P. fluorescens*

Table 5 Biochemical characteristics of *Pseudomonas fluorescens*

P. fluorescens	Biochemal characteristics	No
-	Hydrolysis of starch	1
+	Gelatin liquefaction	2
+	fluorescent dye	3
+	test Catalase	4
+	Oxidase test	5

Biochemical tests for *P. stutzeri* and *P. putida*

Table 6 Biochemical characterization of*P. stutzeri* and *P. putida*

No\3 P.stutzi	***No\3 P. putida***	***No\10 P. fluorescens***	***No\25 P. aeruginosa***	**Biochemical tests**
3	0	0	25	Pyocyanin dye
0	3	10	25	fluorescent
0	0	0	25	Nitrite reduction
0	0	10	25	Gelatin liquefies
3	3	0	0	brown tint
0	3	10	0	4c growth
3	0	0	25	42c growth
3	0	0	25	Nitrogen gas

3.3. Antibiotic Resistance of Isolates

The sensitivity of (20) isolates was tested against each of the following antibiotics Co-trimoxazole, cefepime, piperacillin, ciprofloxacin, tobramycin, tetracycline, gentamicin by Kirby-Bauer method using tablets and depending on the results of the examination (measurement of the diameter of the inhibition zone) around the antibody disk that was compared. With international standard tables [49]. The results of the current study showed that the Pseudomonas aeruginosa isolates were highly resistant to Piperacillin, Tetracycline, and Gentamicin, where it formed a high resistance rate of 100%. , as the rate of resistance to Piperacillin antibiotics reached 100%, and the result of resistance to the antibiotic Piperacillin in our study did not agree with the findings of [37], as the rate of resistance reached 77% and came close to the study of the researcher Faidah, 2015, which The resistance rate of 91% to the anti-tetracycline was also a result of convergence with the results obtained by the researcher [48] when he noticed that the resistance rate of 92% to the anti-Gentamicin was also close to the results of the study conducted by the researcher [36]. Whereas, the rate of resistance to Gentamicin was 98%. The resistance of these bacteria to Gentamicin, which is one of the types of Aminoglycosides, can be attributed to its ability to produce enzymes that modify and neutralize antibiotics. These are called Aminoglycoside modifying enzymes (AMEs). such as aminoglycoside N acetyltransferase(AACs) , aminoglycoside-o-phosphptransferases (APHs) Or the resistance to these antibiotics may be due to a change in the permeability of the wall, as in the resistance to Amikacin [38] . As for the Tetracycline antagonist, the resistance to this antibody is due to the reduction of the permeability of the bacterial outer membrane, which leads to a decrease in the permeability of the antibody inside the bacterial cells and its accumulation. inside the cell as well as stimulating the active efflux system, which leads to an increase in the efflux of antigens outside the cell [39]. As for *Pseudomonas fluorescens*, the percentage of resistance to Co-trimoxazole was 80%, Tetracycline 50%, tobramycin 40.% cefepime , 60%, Ciprofloxacin 60%, Piperacillin 40%, Gentamicin 70%. This study was in agreement [40]. Where the study conducted by the researcher [41] showed that Pseudomonas fluorescens is resistant to a group of antibiotics such as cefepime, Piperacillin, Co-trimoxazole, but it is sensitive to anti-fluoroquinolones and aminoglycosides. These results did not agree with the researchers [42], where the study showed resistance to Tetracycline 44%, cefepime 6.3%, and Piperacillin 11%. Resistance to antibiotics can be attributed to an important phenomenon, which is the indiscriminate use of them, whether through unjustified or incorrect use in terms of dose and duration of time, in addition to other essential factors related to multiple resistance, including the ability of bacteria to form a biofilm if basic materials are available. Within the surrounding environment, it takes for granted the creation of a biofilm, which is the first building block in the beginning of antibiotic resistance through the mechanisms of transport, communication, and cohesion between cells (cell-to-cell) and the mechanisms of the sensor (Quorum Sensing (QS), which has proven its effective role in antibiotic resistance Another physiological mechanism is the Efflux System (Efflux Pumps Multidrug), which works to reduce the antibody concentration in the cell, as the efflux systems work to remove Macrolides, Fluoroquinolones, β-lactams, and aminoglycosides [43; 44], as well as the different virulence factors that characterize P. aeruginosa bacteria that contribute to the increase in resistance to various antibiotics, as well as the presence of genetic factors that contributed to the epidemic This bacteria and its virulence, which made it more resistant to antibiotics[45].

The increasing of resistance open the rout to find and apply alternatives remedies for example using nanoparticles combined with other chemical material [46].

4. Conclusion

- Isolation of the four species of the *genus Pseudomonas aeruginosa, P. fluorescens, P. putida, P.stutzi* isolated from different parts of the human body.
- *P.stutzi* does not produce the fluorescent pigments that distinguish *P. stutzeri* from other species of the pseudo-fluorescent group
- The results of the current study showed that the *Pseudomonas aeruginosa* isolates were highly resistant to Piperacillin, Tetracycline, and Gentamicin, where it formed a high resistance rate of 100%.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that no conflict of interest.

References

[1] Govarthanan, M., Selvankumar, T., Mythili, R., Srinivasan, P., Ameen, F., AlYahya, S. A., & Kamala-Kannan, S. (2019). Biogreen remediation of chromium-contaminated soil using Pseudomonas sp.(RPT) and neem (Azadirachta indica) oil cake. International Journal of Environmental Science and Technology, 16(8), 4595-4600.

[2] Haghi, F., Zeighami, H., Monazami, A., Toutouchi, F., Nazaralian, S., & Naderi, G. (2018). Diversity of virulence genes in multidrug resistant Pseudomonas aeruginosa isolated from burn wound infections. Microbial pathogenesis, 115, 251-256.

[3] Hasannejad-Bibalan, M., Jafari, A., Sabati, H., Goswami, R., Jafaryparvar, Z., Sedaghat, F., & Ebrahim-Saraie, H. S. (2021). Risk of type III secretion systems in burn patients with Pseudomonas aeruginosa wound infection: A systematic review and meta-analysis. Burns, 47(3), 538-544.

[4] Scales, B. S., Dickson, R. P., LiPuma, J. J., & Huffnagle, G. B. (2014). Microbiology, genomics, and clinical significance of the Pseudomonas fluorescens species complex, an unappreciated colonizer of humans. Clinical microbiology reviews, 27(4), 927-948.

[5] Kumar, H., Franzetti, L., Kaushal, A., & Kumar, D. (2019). Pseudomonas fluorescens: A potential food spoiler and challenges and advances in its detection. Annals of microbiology, 69(9), 873-883.

[6] Li, T., Wang, D., Liu, N., Ma, Y., Ding, T., Mei, Y., & Li, J. (2018). Inhibition of quorum sensing-controlled virulence factors and biofilm formation in Pseudomonas fluorescens by cinnamaldehyde. International journal of food microbiology, 269, 98-106.

[7] Sadiq, S.T., & Yaşa, İ. (2019). New Techniques Used for Removing Antibiotic Residues and Antibiotic Resistance Genes from Water. Recent Advances in Biology and Medicine.

[8] Yu, Q., Yuan, Y., Feng, L., Sun, W., Lin, K., Zhang, J., & Peng, Q. (2022). Highly efficient immobilization of environmental uranium contamination with Pseudomonas stutzeri by biosorption, biomineralization, and bioreduction. Journal of Hazardous Materials, 424, 127758.

[9] Volke, D. C., Calero, P., & Nikel, P. I. (2020). Pseudomonas putida. Trends Microbiol, 28(6), 512-513.

[10] Farhan, S. M., Ibrahim, R. A., Mahran, K. M., Hetta, H. F., & Abd El-Baky, R. M. (2019). Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing Pseudomonas aeruginosa isolated from hospitals in Minia, Egypt. Infection and Drug Resistance, 12, 2125.

[11] Sadiq, S.T., Jasim, S.T., AL-Bayati, A.A., Hasaanzadeh, S., Eren, A.E., & Tunç, G. (2019). Practical Notes for Quantification of Antibiotics Resistance Bacteria and Antibiotics Resistance Genes in Wastewater.

[12] Sarker, R. R., Tsunoi, Y., Haruyama, Y., Sato, S., & Nishidate, I. (2022). Depth distributions of bacteria for the Pseudomonas aeruginosa-infected burn wounds treated by methylene blue-mediated photodynamic therapy in rats: effects of additives to photosensitizer. Journal of Biomedical Optics, 27(1), 018001.

[13] Wang, Z. F., Ren, S. X., Li, W., & Gao, G. H. (2018). Frequency of the acquired resistant mutation T790 M in non-small cell lung cancer patients with active exon 19Del and exon 21 L858R: a systematic review and meta-analysis. BMC cancer, 18(1), 1-7.

[14] Nishimura, T., Hattori, K., Inoue, A., Ishii, T., Yumoto, T., Tsukahara, K., ... & Nakayama, S. (2017). Bacteremia or pseudobacteremia? Review of Pseudomonas fluorescens infections. World journal of emergency medicine, 8(2), 151.

[15] Song, W., Lee, K. M., Kang, H. J., Shin D.H., and Kim D.K. (2001). Microbiological aspects of predominant bacteria isolated from the burn patients in Korea. J. Burns. 27(2): 136-139.

[16] Mansour, A., Klantar, E. (2004). Bacteriological monitoring of hospital borne septicemia in burn patients in Ahvaz, Iran. J. Burns & Surg. Wound Care. 3(1): 4.

[17] Abeer. Hamoudi Jabbar, & Seyouf Khoman Al-Ramahi. (2017). From different clinical samples with pseudomonas aeruginosa isolated and diagnosed bacteria to study their sensitivity to some antibiotics. Al-Qadisiyah Journal of pure science, 22 (3).

[18] Haleem, H., Kadhim, J., Ilham, T. and Banyan, A. (2011). Isolation of Pseudomonas aeruginosa from Clinical Cases and Environmental Samples, and Analysis of its Antibiotic Resistant Spectrum at Hilla Teaching Hospital Medical. J. Babylon. 8(4).

[19] Naqvi, Z., Hashmi, K., Rizwan, Q., and Kharal, S. A. (2005). Multi durg resistant Pseudomonas aeruginosa: anosocomial infection threat in burn patients. Pakistan J. Pharma. 2(22): 9-15.

[20] Bashar, S., Sanyal, S. K., Sultana, M., & Hossain, M. A. (2017). Emergence of IntI1 associated bla VIM-2 gene cassette-mediated carbapenem resistance in opportunistic pathogen Pseudomonas stutzeri. Emerging microbes & infections, 6(1), 1-3.

[21] Alwazzeh, Marwan J., Feras A. Alkuwaiti, Moammer Alqasim, Sarah Alwarthan, and Yasser El-Ghoneimy. "Infective endocarditis caused by Pseudomonas stutzeri: a case report and literature review." Infectious Disease Reports 12, no. 3 (2020): 105-109.

[22] Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature reviews microbiology, 3(9), 711-721.

[23] Al-Shuwaikh, Rana Mujahid Abdullah. (2006). Production and characterization of Protease from Pseudomonas aeruginosa isolated from diseased cases and its relation to some antibiotics. PhD dissertation. College of Science . Mustansiriya University .

[24] Al-samamari, N. M. H. (2019). Isolation and Identification of Pseudomonas aeruginosa from Some Clinical And Environmental Samples And Study It, s Activity for The Production of Pyocyanin and Protease. Journal Of Education and Science, 28(4), 93-107.

[25] Zainab Faleh. (2015). Investigation of some genes of resistance to aminoglycosides and quinolines in Pseudomonas aeruginosa isolated from different clinical sources in Al-Diwaniya city. Master Thesis, College of Science - Al-Qadisiyah University.

[26] Wickramasinghe, N. N., Ravensdale, J., Coorey, R., Chandry, S. P., & Dykes, G. A. (2019). The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. Comprehensive Reviews in Food Science and Food Safety, 18(5), 1622-1635.

[27] Reichler, S. J., Trmčić, A., Martin, N. H., Boor, K. J., & Wiedmann, M. (2018). Pseudomonas fluorescens group bacterial strains are responsible for repeat and sporadic postpasteurization contamination and reduced fluid milk shelf life. Journal of Dairy Science, 101(9), 7780-7800.

[28] Halabi, Z., Mocadie, M., El Zein, S., & Kanj, S. S. (2019). Pseudomonas stutzeri prosthetic valve endocarditis: a case report and review of the literature. Journal of infection and public health, 12(3), 434-437.

[29] Ciofu, O., & Tolker-Nielsen, T. (2019). Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—how P. aeruginosa can escape antibiotics. Frontiers in microbiology, 10, 913.

[30] Wood, T. E., Howard, S. A., Förster, A., Nolan, L. M., Manoli, E., Bullen, N. P., ... & Filloux, A. (2019). The Pseudomonas aeruginosa T6SS delivers a periplasmic toxin that disrupts bacterial cell morphology. Cell reports, 29(1), 187-201.

[31] Sun, X., Lu, Q., Boluk, Y., & Liu, Y. (2014). The impact of cellulose nanocrystals on the aggregation and initial adhesion of Pseudomonas fluorescens bacteria. Soft Matter, 10(44), 8923-8931.

[32] Qing, H., Donde, O. O., Tian, C., Wang, C., Wu, X., Feng, S., & Xiao, B. (2018). Novel heterotrophic nitrogen removal and assimilation characteristic of the newly isolated bacterium Pseudomonas stutzeri AD-1. Journal of bioscience and bioengineering, 126(3), 339-345.

[33] Zhao, B., Cheng, D. Y., Tan, P., An, Q., & Guo, J. S. (2018). Characterization of an aerobic denitrifier Pseudomonas stutzeri strain XL-2 to achieve efficient nitrate removal. Bioresource technology, 250, 564-573.

[34] Carvalho-Assef, A. P. D., Gomes, M. Z., Silva, A. R., Werneck, L., Rodrigues, C. A., Souza, M. J., & Asensi, M. D. (2010). IMP-16 in Pseudomonas putida and Pseudomonas stutzeri: potential reservoirs of multidrug resistance. Journal of medical microbiology, 59(9), 1130-1131.

[35] Lalucat, J., Bennasar, A., Bosch, R., García-Valdés, E., & Palleroni, N. J. (2006). Biology of Pseudomonas stutzeri. Microbiology and molecular biology reviews, 70(2), 510-547.

[36] Fazeli, H.; Sadighian, H.; Esfahani, B. N. & Pourmand, M. R. (2015). Genetic characterization of Pseudomonas aeruginosa-resistant isolates at the university teaching hospital in Iran. Adv. Biomed Res., 27 (4):156.

[37] Kilinc, C., Guckan, R., & Coskun, U. S. S. (2018). Investigation of antibiotic susceptibility profile and minimal inhibitor concentration changes in Pseudomonas aeruginosa isolates that exposed to subinhibitory concentrations of antibiotic. Medical Science and Discovery, 5(9), 312-319.

[38] Zheng, Z., Tharmalingam, N., Liu, Q., Jayamani, E., Kim, W., Fuchs, B. B., ... & Mylonakis, E. (2017). Synergistic efficacy of Aedes aegypti antimicrobial peptide cecropin A2 and tetracycline against Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy, 61(7), e00686-17.

[39] Naghmouchi, K., Le Lay, C., Baah, J., & Drider, D. (2012). Antibiotic and antimicrobial peptide combinations: synergistic inhibition of Pseudomonas fluorescens and antibiotic-resistant variants. Research in microbiology, 163(2), 101-108.

[40] Pappas, G., Karavasilis, V., Christou, L., & Tsianos, E. V. (2006). Pseudomonas fluorescens infections in clinical practice. Scandinavian journal of infectious diseases, 38(1), 68-70.

[41] Sader, H. S., & Jones, R. N. (2005). Antimicrobial susceptibility of uncommonly isolated non-enteric Gram-negative bacilli. International journal of antimicrobial agents, 25(2), 95-109.

[42] Li, X. & Nikaido, H. (2012). Efflux-Mediated Drug Resistance in Bacteria: an Update. Drugs., 69(12): 1555-1623.

[43] Hocquet, D.; Nordmann, P.; El Garch, F.; Cabanne, L. & Plésiat, P. (2006). Involvement of the MexXY-OprM efflux system in emergence of cefepime resistance in clinical strains of Pseudomonas aeruginosa. Antimicrob Agents Chemother., 50(4):1347-1351.

[44] Khalil, M. A.; Ibrahim Sonbol, F.; Mohamed, A. F. & Ali, S. S. (2015). Comparative study of virulence factors among ESβL-producing and nonproducing Pseudomonas aeruginosa clinical isolates. Turk J. Med. Sci., 45(1):60-69.

[45] Jasim, S.T., Noori, A.M., Sadiq, S.T., & Flayyih, M.T. (2020). Antimicrobial and Antibiofilm Activity of D-amino acids Combined with Nanoparticles against Candida albicans. Systematic Reviews in Pharmacy.123597.

[46] Al-Muhammadawi, Khawla Jabr Khalaf. (2006). Study of protein A- as a virulence factor of Pseudomonas aeruginosa isolated from different environments with an emphasis on the productive molecular nature. PhD thesis. College of Science, Al-Mustansiriya University.

[47] Hasan, S. A., Najati, A. M., & Abass, K. S. (2019). Isolation and identification of multi-drug resistant" pseudomonas aeruginosa" from burn wound infection in Kirkuk City, Iraq. EurAsian Journal of BioSciences, 13(2), 1045-1050.

[48] Shah, S. H. A., Ali, W., Shah, F. A., Falah, S. F., Rehman, E., Umar, A& Ullah, I. (2022). Multi Drug Resistance Pseudomonas Aeruginosa Frequency and Antibiogram in A Tertiary Teaching Care Hospital in Pakistan: Frequency and Antibiogram of Pseudomonas aeruginosa. Pakistan BioMedical Journal, 231-235