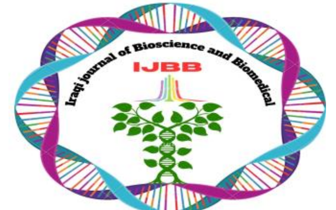


Iraqi journal of Bioscience and Biomedical
Published Online First: 31/ December /2023.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Identification, characterization and antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolated from clinical sources

Sumaya AL-Yasiri[1], Mohsen Hashem Risan[2], Okan Ürker[3], Emad Yousif[4]

[1, 3] School of Natural and Applied Sciences Çankırı Karatekin University
[2] College of Biotechnology, Al-Nahrain University, Baghdad-Iraq.
[4]College of Science, Al-Nahrain University, Baghdad- Iraq
*Corresponding Author Email. dr.muhsin20@nahrainuniv.edu.iq
https://orcid.org/0000-0001-5574-9156

Received 02/10/2023,
Accepted 28/11/2023,
Published 31/12/2023.

This work © 2024 by College of biotechnology/ Al-Nahrain university
This is an Open Access article distributed under the terms of the CC BY 4.0. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/ which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

In this study, hundred (100) specimens were collected from patients of both gender and different age groups in three government hospitals in Baghdad/Iraq from January 2022 to May 2022. Samples were diagnosed by routine, biochemical tests, and Vitek- 2 system device to diagnose the *Pseudomonas aeruginosa*, the results showed that 32 (32%) were *P. aeruginosa* bacteria. Antibiotic susceptibility testing was performed for all 32 isolated *P. aeruginosa* pathogenic bacteria and sensitivity was performed for the aminoglycoside group (Gentamycin, Amikacin, streptomycin) of antibiotics to detect isolates sensitivity or resistance to antibiotics circulating in health institutions, *P. aeruginosa* showed the highest antibiotic resistance to Gentamicin: 40.6%, streptomycin: 37.5%, Amikacin: 25%. In the present study our target isolates of *P. aeruginosa* which is resistant to Gentamicin, Amikacin and streptomycin according to our result we found the most isolates which resistant to Gentamicin, Amikacin and streptomycin are P1, P5, P8, P9, P14, P17, P18, P19, P22, P29.

Key word: *Pseudomonas aeruginosa,* antibiotic, susceptibility, clinical sources.

Introduction

Pseudomonas aeruginosa is a member of the Pseudomonadaceae bacterial family, a member of γ-proteobacteria. “Schroeter” was the first to propose the scientific name Bacterium *aeruginosa* for *Pseudomonas aeruginosa* in 1872, after he isolated it from suppurating wounds basis on its phenotypic characteristics. It is one of twelve subtypes of bacteria seen seldom as a component of the human micro-flora in healthy individuals. *P. aeruginosa* is widespread in nature and it is an opportunistic pathogen causing nosocomial infection in humans that causes a number of diseases like inflammation of urinary tract, burns, respiratory infections, and septicemia [7].

Pseudomonas aeruginosa is Grape-like or tortilla-like odor, motile, Gram-negative and straight or slightly curved rod-shaped, appearing as single pairs, and sometimes in short chains, that measures (0.5 to 0.8) µm by (1.5 to 3.0) µm, a sporogenous, and mono flagellated bacterium, Clinical isolates typically utilize pili, which may be antiphagocytic and possibly assist bacterial adhesion and colonization. It has capsules in some of its isolated strains of cystic fibrosis patients. *P. aeruginosa* aerobic and can grow anaerobically by the presence of arginine and it has limited fermentative abilities, nitrate performs as the

terminal electron acceptor while it grows anaerobically [9]. *P. aeruginosa* can grows well at 25℃ to 37℃, add to its ability to grow at 42℃ which is helps to distinguishes it from other *Pseudomonas* species (13). *P. aeruginosa* produces colonies that are spherical and smooth and have uneven borders. These colonies include a fluorescent pigment called pyoverdine that ranges in color from greenish to yellow. Furthermore, it is responsible for the creation of the non-fluorescent bluish pigment called pyocyanin, which permeate into the agar, adds the development of the red pigment pyoyrubin and the black pigment pyomelanin [4].

P. aeruginosa is notoriously resistant to antimicrobials, which makes it exceedingly challenging to both treat and eradicate. Antibiotic resistance features in *P. aeruginosa* can be acquired, chromosomal, as well as transferred horizontally, which are frequently contained inside plasmids and integrons [6]

Material and methods

The collection of samples

All 100 samples collected from both sex at different ages of patients in Al-Yarmuk Hospital, Baghdad Teaching Hospital and Al-Kindi Teaching Hospital during the year 2021. The sample is taken by a sterile swab from wound and burns.

Culturing of the samples

Sample from burns and wounds were quickly cultured on Nutrient broth after that on blood agar, MacConkey agar, and Pseudomonas agar (Cetrimide agar) by streaking method. The agar plates incubated for at 37°C to24 h, then, for the microorganisms under investigation, biochemical tests and diagnostic tests were done [2].

Identification of bacteria

Isolates and identification of bacteria by studying the morphological properties of the colonies, bacterial cells, microscopic examination, and biochemical tests which were performed based on [22].

Cultural characterization

Isolation of bacterial and diagnosed during the study of the phenotypic characteristics of isolates dependent on the colony's shape and colony color, size, edges, and ability to hemolysis on blood agar and pigment produce.

Microscopic examination

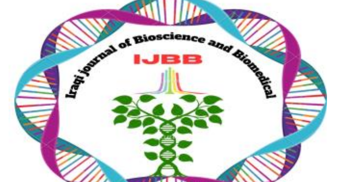
Swabs were prepared from bacterial isolates growing on the nutrient medium at 24 hours of age, after which the smears were stained with gram stain, then examined by optical convergence with a Rh lens, which observed the arrangement and shape of cells, as well as their interaction with gram stain [8].

Biochemical test

The *P. aeruginosa* isolates were subjected to a series of biochemical assays, including oxidase, indole, methyl red, Kovacs reagent, and catalase production, to verify their identification as the bacterium. The results of every test were consistent with [3].

Identification using Vitek-2 system

According to the information provided by the company, the VITEK-2 is an automated microbiological system that makes use of technology that is based on growth (BioMerieux-France). A sterile swab that is used to transfer a sufficient number of colonies from a pure culture



that has been grown for 20 hours and to suspend those colonies in three milliliters of normal saline (NaCl 0.45%, pH 5-7). Then, using a turbidity meter known as the DensiCheck, the turbidity was adjusted to match 0.5 McFarland, which is the appropriate inoculum density for Gram-negative and Gram-positive bacteria, as stated by the manufacturer. And manually placed within the VITEK 2 reader inoculator module before being automatically sealed. Every 15 minutes, fluorescence is monitored, and three hours later, the findings of identification are known. ID-GNB cards were used to identify users of the VITEK 2 system. The 41 tests on the 64-well plastic ID-GNB cards include 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 tests for decarboxylase, and 3 other tests [for urease, utilization of malonate, and tryptophane deaminase] using a vacuum cleaner [18].

Antibiotics susceptibility test

According to CLSI, the sensitivity test technique was carried out in the manner described below:

Single bacterial colonies of isolates were taken from isolate on culture medium from 18-24 hours to a test tube containing 3 mL Norma Saline, the turbidity and density of the bacterial suspension were controlled using 0.5 a standard MacFarland solution, which gives a density of $1.5x\ 10^{8}$ CFU/mL. Dip the cotton swab sterilizing into the culture tube which contain the suspension bacterial, and applied, on a Mullerhinton ager by wiping it on the surface of the medium to obtain a homogeneous growth, the dishes were dried in a lab environment for 10 minutes. Using sterile tweezers, the antibiotic disc was put on the surface of the inoculated Muller Hinton agar and gently pushed to be dimensioned on the surface. The panels were then incubated for 24 hours at a temperature of 37 pm. The diameter of the damping zone, expressed in mm, was measured around each intermediate disc to record the results, a scale ruler was then compared to standard rates of inhibition zone diameter for real antibiotics.

Result and discussion

Study Samples

A total number of 100 different clinical samples were collected from patients in some of Baghdad hospitals during the study period from January to May 2022. *P. aeruginosa* was determined according to conventional cultural and microscopic characteristics as well as biochemical tests. In addition, the identification is confirmed by Vitek 2 System.

Samples were collected from Al-Yarmuk Hospital, Baghdad Teaching Hospital, and Al-Kindi Teaching Hospital in Baghdad / Iraq. Burns and wounds samples were collected from different age groups and gender Table 1. The collected specimens were burn swab (65), wound swab (35), the results showed that the number of positive samples for wounds and burns was 61(61%), while the number of negative samples was 39 (39 %). In general, burns infections are caused by the emergence of potential problematic gram-negative pathogen *P. aeruginosa* to the hospital environment and healthcare serve as potential reservoirs of *P. aeruginosa* according to [21].

Table 1. The number of specimens according to their sources

Clinical samples	Total samples	Positive (growth)	Negative (no growth)
Burns	65	42	23
Wounds	35	19	16
Total	100	61	39

Identification of *Pseudomonas* Species on Different Media

Cultured and microscopes test

A total of 100 samples were collected from patients suffering from burns and wounds that are cultured initially in the nutrient broth and incubated at 37°C. Then cultured on other mediums in order to identify and diagnose the microbial.

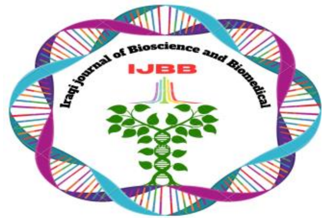
After that was cultured on MacConkey agar and it's incubated at 37°C for 24 hrs. Shown in Figure 1 (A), *P. aeruginosa* appeared non lactose ferment grew with pale yellowish color.

Bacteria were also cultured on blood agar. That is enriched medium was used for many pathogenic bacteria to examine and detect their ability to hemolysis blood because they have the ability to produce toxins that are capable of destroying or reducing the red blood cells. *P. aeruginosa* appeared beta hemolysis and grew with a metallic sheen shown in Figure 1 (B). The growth ability on selective medium Cetrimide agar (this medium was used to regulate the capability of an organism to grow in the presence of the 0.03% Cetrimide that acts as a quaternary ammonium cationic detergent (acetyl trimethyl ammonium bromide) and inhibits the development of other microorganisms) overnight at 37°C shown in Figure 1 (C), the production of blue greenish color pigments and had a fruity odor was especially associated with *P. aeruginosa* [21]. Besides, these pigments play role in *P. aeruginosa* pathogenicity as virulence factor they are playing as a character in pigmentation. Which remains a significant factor among the diagnostic traits in the genus of *Pseudomonas*, therefore, used by [12, 17] to diagnosis these bacteria in their study. These results are similar to other studies in Iraq by [1].

Depending on the phenotypic properties of the bacterial isolates the colonies appeared pale and non-fermented lactose, however in Blood agar, the colonies showed beta hemolysis in the blood agar, and Colonies appear green and yellow color. These dyes are known as bioferdin, biocyanin and have a fruity odor [19].

Also *P. aeruginosa* is able to grow at 42ºC - 4°C on nutrient agar. The ability to grow at high temperatures distinguishes *P. aeruginosa* from other *Pseudomonas* species.

A microscopic examination was done to distinguish the Gram-positive from Gram-negative bacteria and to determine the morphology of bacterial cells, Gram-negative bacteria. Been applied the most typical staining method for diagnostic purposes, is gram staining. This demonstrated motile, non-sporogenic gram-negative rod cells as shown in Figure 1 (D).



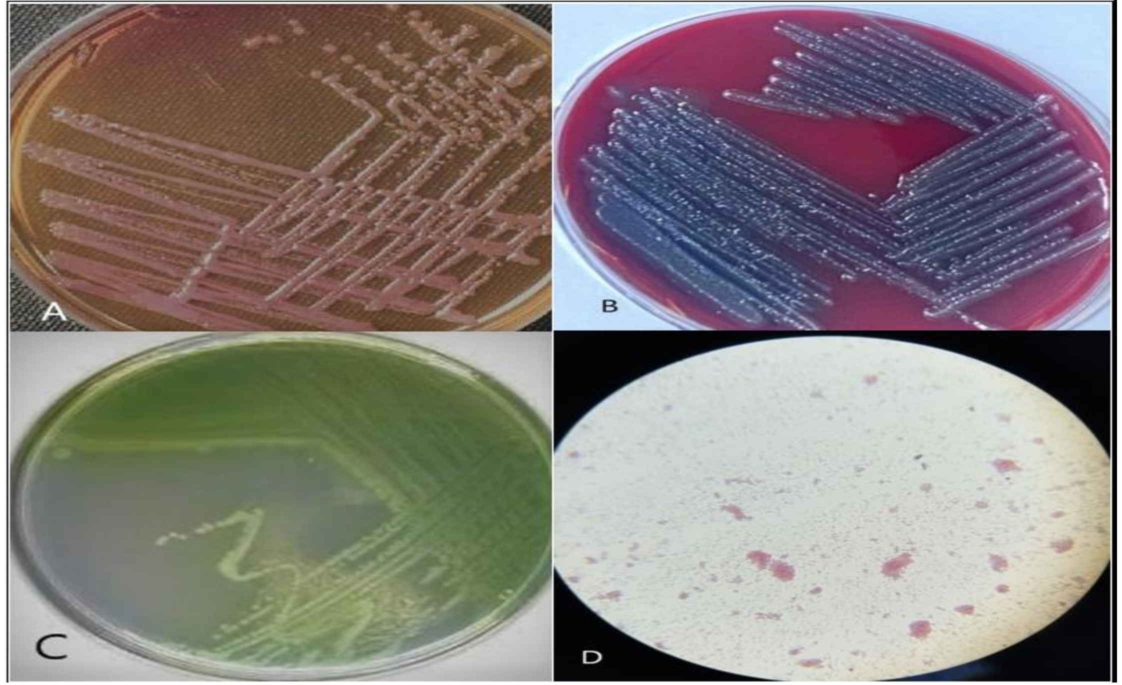


Figure 1. Identification of *P. aeruginosa*: **(a)** MacConkey, **(b)** blood agar, **(c)** cetrimide agar, **(d)** microscopic examination.

Biochemical test to identification of *P. aeruginosa*

The identification by biochemical tests showed a positive result for both oxidase and catalase tests, While IMVIC tests show negative results for each indole Figure 2, methyl red, and Voges Proskauer. While it's positive to Citrate utilization test as shown in the Table 2. These results were similar to [20].

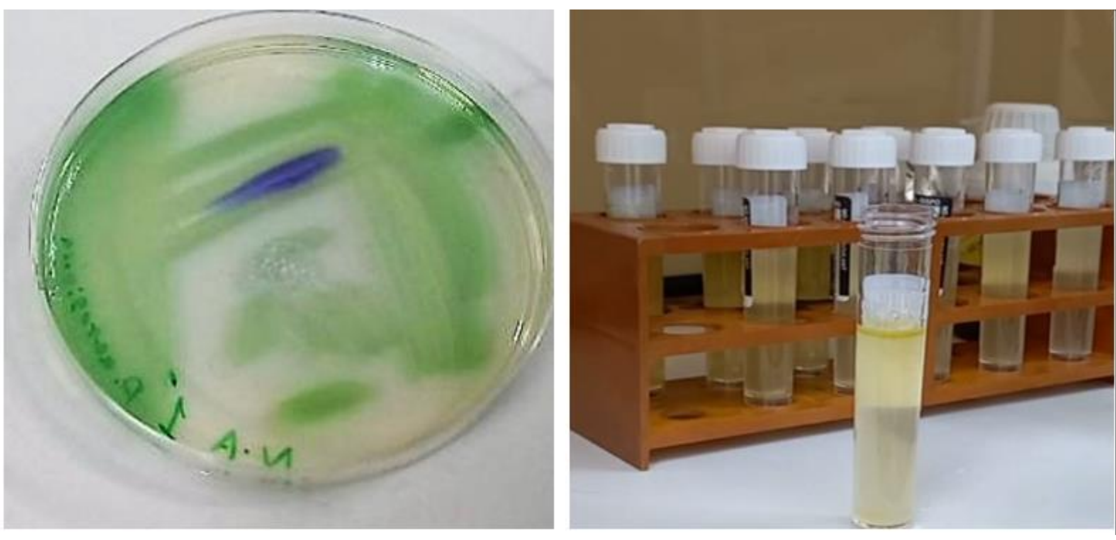


Figure 2. Biochemical test

Table 2. Phenotypic and biochemical laboratory examinations to *P. aeruginosa*

Test	Result
Gram-stain	G- rods
Growth at 42°C	+
Hemolysis(ß-hemolysis)	+/-
Cetrimide agar	+
Pigments production	+
Catalase test	+
Oxidase test	+
Indole test	-
Methyl-red	-
vogas proskauer.	-
Citrate test	+

Depending on morphology of bacterial isolates on cultured media and the outcome of biochemical test clarified that only thirty-six isolates from one hundred bacterial isolates was *P. aeruginosa*. The results identified by classical routine bacteriology for clinical specimens, the outcome was 36 (36%) isolates belong to *P. aeruginosa* which is depending on the sampling time, collection season, limited period, scope of collecting region and other conditions that differ among studies of this type, also which is special and unique for each study, Where this study was Compared with other local studies in Iraq there were Similar to the percentages observed of *P. aeruginosa* isolates from burn patients to researcher; Ismail and Altaai[11] was (٣٧.93%) from 145 samples isolated as *P. aeruginosa.*

Identification *P. aeruginosa* by VITEK-2 system

VITEK 2 Compact was used to certify all 36 clinical isolates that were identified as *Pseudomonas aeruginosa*. Using the identification card (GN Card) for gram negative isolates, the VITEK-2 system identified the clinical samples as *P. aeruginosa*. This approach was used in much earlier studies and produced good results for the identification and validation of biochemical tests (10). The results in detailing of Vitek-2 system tests, isolates that were diagnosed with. This device as a confirmation test showed 32 (88.8%) isolates were identified as *P. aeruginosa* spp from 36 isolates.

Antibiotic Susceptibility Test of Isolated Bacteria

Antibiotic susceptibility testing was performed for all 32 isolated *Pseudomonas aeruginosa* pathogenic bacteria as shown in Table 3, Figure 3, and sensitivity was performed for the aminoglycoside group (Gentamycin, Amikacin, streptomycin) of antibiotics to detect isolates sensitivity or resistance to antibiotics circulating in health institutions, these antibiotics were selected because the target gene in the

research under study is responsible for bacterial resistance to these antibiotics through Production of protein aminoglycoside-modifying enzymes (AMEs) and Overexpression of efflux pump [16].

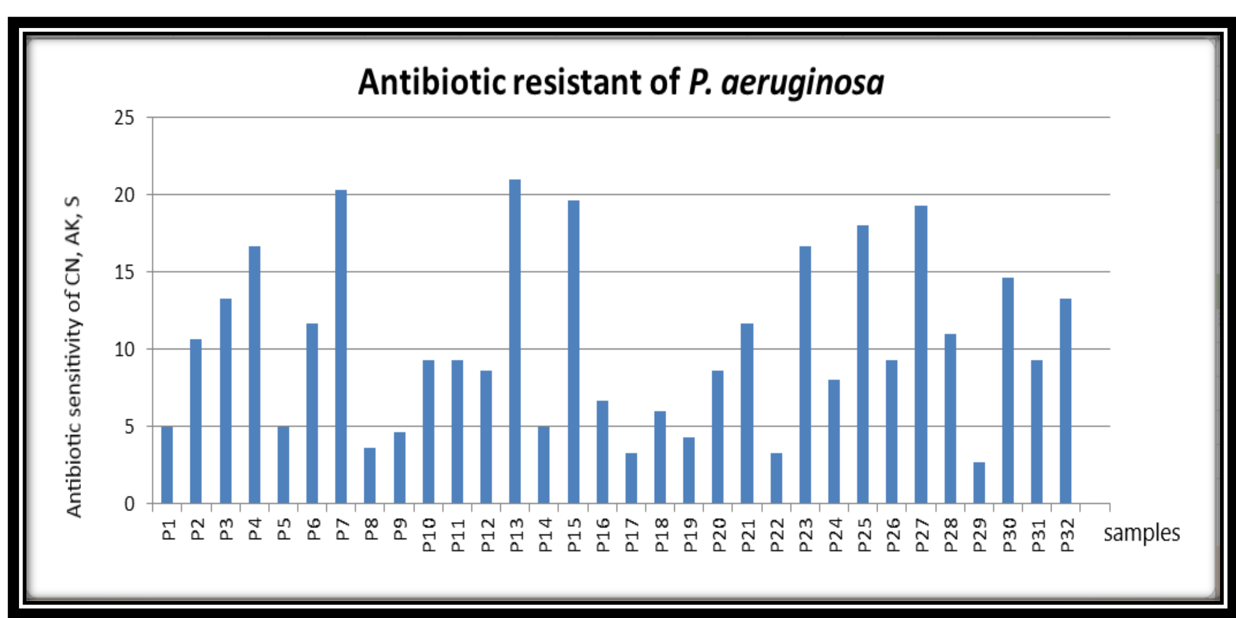
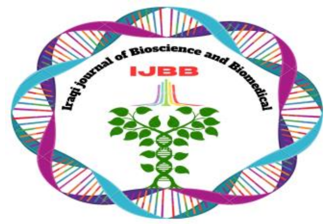


Figure 3. The percentages of antibiotic resistant of *P. aeruginosa* to gentamicin (CN), amikacin (AK) and streptomycin (S)

Table 3. Antibiotic susceptibility test of isolated bacteria

IsolateNo / Antibiotic	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
CN	R	S	S	S	R	S	S	S	R	R
AK	S	S	S	R	S	S	S	S	S	S
S	S	R	R	S	R	R	S	R	R	S
IsolateNo / Antibiotic	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**	**P18**	**P19**	**P20**
CN	S	S	S	S	S	S	R	R	R	S
AK	R	S	S	R	S	R	R	R	S	S
S	S	R	S	R	S	R	S	S	R	R
IsolateNo	**P21**	**P22**	**P23**	**P24**	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**



Antibiotic										
CN	R	R	S	R	S	S	S	R	R	S
AK	S	S	S	S	S	R	S	S	S	S
S	S	R	S	S	S	S	S	S	R	S
IsolateNo / **Antibiotic**	**P31**	**P32**								
CN	R	S								
AK	S	S								
S	S	S								

P. aeruginosa showed the highest antibiotic resistance to CN: Gentamicin: 40.6%, S streptomycin: 37.5%, AK: Amikacin: 25%. In the present study our target isolate of *P. aeruginosa* which is resistant to CN: Gentamicin, AK: Amikacin and S: streptomycin according to our result we found the most isolates which resistant to CN, AK, and S is P1, P5, P8, P9, P14, P17, P18, P19, P22, P29 shown in Figure 4. Depending on these results we chose the most six resist isolates and in the same time contains a genes (*aac6* or *mexz*) in this is isolates P1, P5, P9, P17, P18, P29.

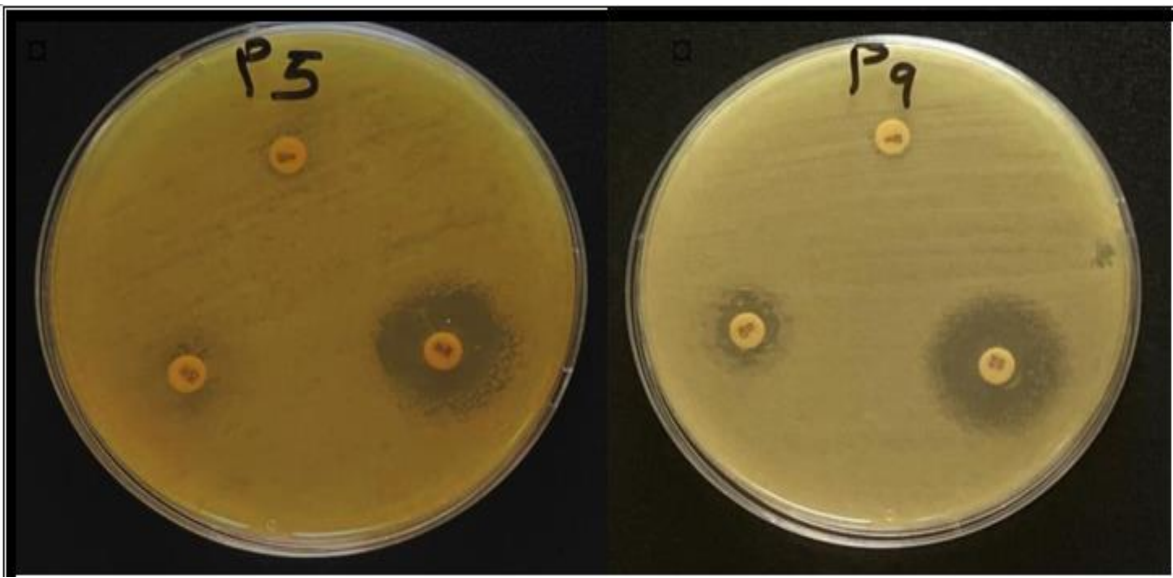


Figure 4. Antibiotic susceptibility test

In general *P. aeruginosa* have high intrinsic resistance to toxic molecules, *P. aeruginosa* is surrounded by a poorly permeable outer membrane that allows extrusion mechanisms, such as efflux systems, to operate efficiently. Some of these transmembrane machineries are able to export antibiotics and thus to impair the interaction of drugs with their cellular targets. At least four efflux pumps can significantly increase the

resistance of *P. aeruginosa* to antibiotics when overproduced upon mutations [14]. In addition to other mechanisms such as modifying enzymes the bacterial resistance to aminoglycosides is most likely owing to the presence of plasmids that encode for the development of modified enzymes represented by the tiny ribosomal unit 30, which prevents these antibiotics from binding to ribosomes [15].

Conclusion

The results showed that 32 (32%) were *P. aeruginosa* bacteria. Antibiotic susceptibility testing was performed for all 32 isolated *P. aeruginosa* pathogenic bacteria and sensitivity was performed for the aminoglycoside group (Gentamycin, Amikacin, streptomycin) of antibiotics to detect isolates sensitivity or resistance to antibiotics circulating in health institutions, *P. aeruginosa* showed the highest antibiotic resistance to Gentamicin: 40.6%, streptomycin: 37.5%, Amikacin: 25%. In the present study our target isolates of *P. aeruginosa* which is resistant to Gentamicin, Amikacin and streptomycin according to our result we found the most isolates which resistant to Gentamicin, Amikacin and streptomycin are P1, P5, P8, P9, P14, P17, P18, P19, P22, P29.

Acknowledgment

We express our profound gratitude to all those who contributed to the successful completion of this study. Special thanks to the laboratory staff at college of biotechnology and Al-Nahrain University research center lab, whose expertise and support were invaluable in the execution of our experiments. Our appreciation also extends to the participants for their willingness to contribute to this research. This work was supported by the authors.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours.

Authors' Contribution Statement

[First Author]: Contributed to the conception and design of the study, conducted the experiments, data analysis, and drafted the initial manuscript.

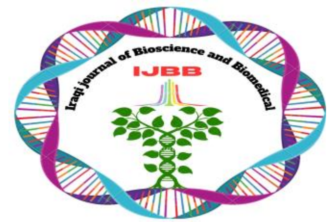
[Second Author]: Assisted in the preparation of the media and solutions used for identification, characterization and antibiotic susceptibility testing of *Pseudomonas aeruginosa.*

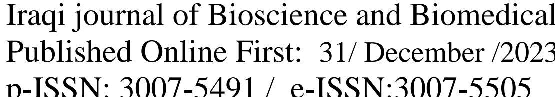
[Third and fourth Author's]: Played a crucial role in the writing and statistical analysis of the data and interpretation of the results.

References:

1- Al-Rubaye, D. S., Albassam, W. W., Al-habobi, H. M., & AL-Rubaye, I. A. 2015. Frequency of blaOxa10 Beta-lactamase gene in *Pseudomonas aeruginosa* isolated from different clinical swabs. Iraqi Journal of Science, 56(4C): 3405-3412.

2- Amézquita-López, B. A., Soto-Beltrán, M., Lee, B. G., Yambao, J. C., & Quiñones, B. 2018. Isolation, genotyping and antimicrobial resistance of Shiga toxin-producing Escherichia coli. Journal of Microbiology, Immunology and Infection, 51(4): 425-434.





3- Chauhan, A., & Jindal, T. 2020. Biochemical and molecular methods for bacterial identification. In Microbiological Methods for Environment, Food and Pharmaceutical Analysis, Springer, 425: 68.

4- De Melo, A. G., Rousseau, G. M., Tremblay, D. M., Labrie, S. J., & Moineau, S. 2020. DNA tandem repeats contribute to the genetic diversity of Brevibacterium aurantiacum phages. Environmental Microbiology, 22(8): 3413-3428.

5- Doughari, J. H., & Ahmad, A. A. 2018. Characterization and Antimicrobial Resistance Profile of Pseudomonas Aeruginosa Isolated from Clinical and Environmental Samples. Journal of Diseases, 5(1): 24-32.

6- Dubois, V., Poirel, L., Marie, C., Arpin, C., Nordmann, P., & Quentin, C. 2002. Molecular Characterization of a Novel class 1 integron containing bla GES-1 and a fused product of aac (3)-Ib/aac (6")-Ib" gene cassettes in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy, 46(3): 638-645.

7- Fazzeli, H., Akbari, R., Moghim, S., Narimani, T., Arabestani, M. R., & Ghoddousi, A. R. 2012. *Pseudomonas aeruginosa* infections in patients, hospital means, and personnel's specimens. Journal of Research in Medical Sciences 17(4): 332–37.

8- Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. 2016. Study Guide for Bailey and Scott's Diagnostic Microbiology-E-Book. Elsevier Health Sciences, 200 page, Amsterdam.

9- Gaviard, C., Jouenne, T., & Hardouin, J. 2018. Proteomics of *Pseudomonas aeruginosa*: The increasing role of post-translational modifications. Expert Review of Proteomics, 15(9): 757-772.

10- Guckan, R., Kilinc, C., Demir, A. D., Capraz, A., & Yanik, K. 2015. Antimicrobial susceptibility of *Acinetobacter baumannii* complex isolated from different clinical samples in a tertiary care hospital. The Journal of Antibiotics Research, 1(1): 1-5.

11- Ismail, S. T. and Altaai, M. I. (2021). Study ndvB gene expression in *Pseudomonas aeruginosa* Producing Biofilm, Medico-Legal Update 21(1): 961–965.

12- Jayaseelan, S., Ramaswamy, D., & Dharmaraj, S. 2014. Pyocyanin: production, applications, challenges and new insights. World Journal of Microbiology and Biotechnology, 30(4): 1159-1168.

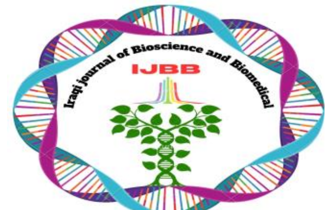
13- Kämpfer, P., & Glaeser, S. P. 2012. Prokaryotic taxonomy in the sequencing era–the polyphasic approach revisited. Environmental Microbiology, 14(2): 291-317.

14- Li, X. Z., Plésiat, P., & Nikaido, H. 2015. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clinical Microbiology Reviews, 28(2): 337-418.

15- Lin, M. F., & Lan, C. Y. 2014. Antimicrobial resistance in *Acinetobacter baumannii*: From bench to bedside. World Journal of Clinical Cases: WJCC, 2(12): 787.

16- Mao, W., Warren, M. S., Lee, A., Mistry, A., & Lomovskaya, O. 2001. MexXY-OprM efflux pump is required for antagonism of aminoglycosides by divalent cations in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy, 45(7): 2001-2007.

17- Novik, G., Savich, V., & Kiseleva, E. 2015. An insight into beneficial *Pseudomonas* bacteria. Microbiology in Agriculture and Human Health, 1(5): 73-105.



18- Pincus, D. H. 2006. Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association, 1-32.

19- Seviour, T., Doyle, L. E., Lauw, S. J. L., Hinks, J., Rice, S. A., Nesatyy, V. J., & Marsili, E. 2015. Voltammetric profiling of redox-active metabolites expressed by Pseudomonas aeruginosa for diagnostic purposes. Chemical Communications, 51(18): 3789-3792.

20- Tadesse, A., & Alem, M. 2006. Medical Bacteriology. Ethiopia Public Health Training Initiative, 6(1): 44-444.

21- Tang, Y. W., Stratton, C. W., & Tang, Y. W. 2013. Advanced techniques in diagnostic microbiology. Springer Science & Business Media, 957 page, New York.

22- Váradi, L., Luo, J. L., Hibbs, D. E., Perry, J. D., Anderson, R. J., Orenga, S., & Groundwater, P. W. 2017. Methods for the detection and identification of pathogenic bacteria: past, present, and future. Chemical Society Reviews, 46(16): 4818-4832.