

DETECTION OF PSEUDOMONAS AERUGINOSA FROM VARIOUS CLINICAL SPECIMEN AND ANTIMICROBIAL RESISTANCE PATTERN OF HUMAN

Rasha Khalid Abd Al-Baky 1 , Hawraa Riyadh Omran 2 ,
Haneen Ali Abbas 3 , Baraa Hussein Ali 3 , Samer Hassan
Alwan 2 , Rand Jawad Jalebawi 4

1 General Directorate Of Wasit Education \ Iraq Wasit

2 Biotechnology Department , Al-Qasim Qreen University \ Iraq
Dewania

3 Biotechnology Department , Al-Qadisiyah University \ Iraq
Babylon

4 Middle Technical University \ Iraq Wasit

Received: Mar 22, 2024; Accepted: Apr 29, 2024; Published: May 28, 2024;

Abstract: In this study, 60 samples were collected from different sources in Al-Zahra Teaching Hospital and Karma Hospital . The random sampling process was conducted to identify the foci of pollution in P.aeruginosa, which was one of the objectives of this study and obtained diagnostic procedures. 45 samples were collected from Al-Zahra Teaching Hospital and collected from 15 samples Karma Hospital . Results of agricultural tests and tests biochemistry 10 isolates of P.aeruginosa bacteria . The pharmacological sensitivity of 10 samples was tested against 10 types of antibiotics in the method of spreading the disk to Kirby_bauer. All isolates were almost resistant . All antibiotics were resistant to severe resistance.

Keywords: Pseudomonas aeruginosa, human, Api20E, Antibiotics.



This is an open-access article under the [CC-BY 4.0](https://creativecommons.org/licenses/by/4.0/) license

Introduction

In view of the reports of the World Health Organization, it has been taken into consideration that P. aeruginosa is a bacteria that raises great and serious concern for human health in particular and for public health in general . P.aeruginosa infection does not necessarily cause serious infections in healthy people, but it is linked to the length of time that the patient is in the hospital and is considered one of the invasive bacteria in hospitals, especially patients who suffer from serious diseases and people who take a lot of antibiotics and people. Those undergoing chemotherapy, as well as those using artificial respirators (World Health Organization, 2017).

P. aeruginosa resistance has been found to increase over the years in many regions of the world due to the indiscriminate use of antibiotics. It has been observed that the resistance of bacteria in the intensive care unit increases within a few days due to the incorrect use of antibiotics for patients, which results in mutations resistant to these treatments. In addition, there are many factors that contribute to P. aeruginosa infection, such as total dependence on parenteral nutrition, the incidence of some immune diseases such as malignant tumors, admission to the intensive care unit, weak

immunity, hereditary diseases, and obstructive coronary artery diseases. All of these risk factors contribute In bacterial infection. (Alhussain et al, 2019).

It was found that the mechanism of treatment with antibiotics used against *P. aeruginosa* is limited due to the presence of multiple resistance mechanisms in the organism, especially those at sites of infection such as those in the respiratory system, cardiac catheters, and surgical instruments. (Kollef et al, 2014). *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* are some of the most predominant bacterial co-infections) Faddin .2000) . *P. aeruginosa* can produce many cellular virulence factors, which are the main cause of the pathogenicity of this bacterium. These factors can be summarized as: alkaline protease, exotoxin U (exoU), secretion protein III, exoenzyme A (exoA), exotoxin S (exoS), elastase, and protease IV, each of which has a toxic effect on mammalian cells. (Khalifa et al .,2011, de Bentzmann et al ., 2000) .

P. aeruginosa can develop resistance to antibiotics through various routes, the main resistance of which is acquired by the production of enzymes. The production of metallo-beta-lactamase (MBL) is considered one of the most serious threats to life due to its rapid release and strong carbapenemase activity, as well as resistance to beta-lactamase inhibitors and its ability to degrade beta-lactam antimicrobials except aztreonam. (Gupta & Shrestha .2019; Ansari et al .,2016 ; Abdullah et al ., 2024)

Aim of the study

1. Isolation and identification of *Pseudomonas aeruginosa* from clinical samples.
2. Study the extent of resistance of bacteria *Pseudomonas aeruginosa* to some antibiotics.

Methods

2.1. Sample Collection

The current study included the collection of 60 samples of various clinical in Al-Zahra Teaching Hospital and Karma Hospital The random sampling was conducted to investigate the contamination of *P.aeruginosa* bacteria, resulting in diagnostic, preventive and therapeutic procedures. The samples were collected by ear from 10 ear swabs , burns 25 , and Sputum 10 and Urine 15 .And also taken from cats the samples were collected from different places . The samples were cultured using the planning method and the plates were incubated at 37°C for 24 hours on blood agar and MacConkey.

2.2. Detection of the Isolates

Based on biochemical and morphological tests and matching them with the results described by (Holt et al ., 1994), and according to API 20 E confirmatory test .

2.3. Antibiotic Ten Test (Qualitative Disk Method)

Twelve antibiotic disks (amikacin (AK), Amoxicillin- Clavulanic acid (AMC), Ceftriaxone (C), Trimethoprim-Sulfamethoxazole (TMP), Cefotaximase (CTX) , N-acetyl cysteine(NA) , Meropenem (MEM) , Azithromycin (ATM), gentamicin (CN) and rifampicin(RA)) were used to detect the sensitivity of isolates of *Pseudomonas aeruginosa* according to method described earlier (Bauer. 1966)

Results and Discussion

3.1. Isolation and Characterization of *Pseudomonas aeruginosa* .

Sixty specimen were collects from patients in wasit city hospitals. as shows in table (1).Ten sample local were characterized depending on cultural and microscopic characteristic. Genus and species were characterized by using biochemical tests and API 20 E confirmatory test.

Table 1: Types of sample, number and Percentage of *Pseudomonas aeruginosa* isolated from clinical samples of human .

Sample	Type	The number	Percentage
Clinical	Ear	10	16.66 %
Clinical	Burns	25	41.66 %
Clinical	Sputum	10	16.66 %
Clinical	Urine	15	25 %

3.2. Cultural Characteristics

The main objective of the collection of samples was to isolate the bacteria *P.aeruginosa* and isolated the isolates depending on the phenotypic characteristics of the developing colonies as the appearance of the center of Agar color pale color of the inability to ferment the sugar lactose located in the center of the plant and has a smell similar to the smell of grape fermented while the colonies appeared dark color and most surrounded by A clear halo on the center of the blood agars, indicating its ability to decompose blood The results of the biochemical tests showed positive results for the oxidase test. All the isolates were characterized by their inability to produce hydrogen sulfide gas and they were not fermented for sucrose and lactose. For accentuation of the biochemical results, the API 20 E confirmatory were used for Enterobacteriaceae detection . The results expose that the tested isolate were *P.aeruginosa*. shows in the Table (2) and Figure (1)

Table (2) : Api 20E technique of *P.aeruginosa*

No.	Active ingredients	Symbol test	Results
1.	Ortho NitroPhenyl-Bd-Galactopyranside	ONPG	-
2.	L-arginine	ADH	+
3.	L-Lysine	LDC	-
4.	L-Ornithin	ODC	+
5.	Trisodium citrate	CIT	-
6.	Sodium thiosulfate	H ₂ S	-
7.	Urea	URE	-
8.	L-tryptophane	TDA	-
9.	L-tryptophane (indole production)	IND	-
10.	Sodium pyruvate	VP	+
11.	Gelatin (bovine origin)	GEL	+
12.	D-Glucose	GLU	-
13.	D-Mannitol	MAN	-
14.	Inositol	INO	+
15.	D-Sorbitol	SOR	-
16.	L-Rhamnose	RHA	-
17.	D-Saccharose (sucrose)	SAC	-

18.	D-Melibiose	MEL	+
19.	Amygdaline	AMY	-
20.	L-Arabinose	ARA	-



Figure (1): Api 20 E technique for *P.aeruginosa*

3.3. Antimicrobial susceptibility

The *P.aeruginosa* antimicrobial susceptibility test isolates f shows that resistant to 100 % (amikacin, Amoxicillin- Clavulanic acid, Ceftriaxone , Trimethoprim-Sulfamethoxazole, Cefotaximase , N-acetyl cysteine, Meropenem , Azithromycin, gentamicin and rifampicin). The prevalence of MDR was 100%. Recorded the present study that increased significantly in ratios resistant isolates *P.aeruginosa* to antibody RA and CTX where resisted all isolates bacteria the proportion of resistant isolates to antibody C and MEM less than resistant isolates to antibody AMC and TMP . It can interpret the proportion of resistance highly expressed by isolates *P.aeruginosa* to this antibodies vital in the present study to use the random to this antibiotics Besides evolution in resistance, which brought this bacteria because of the use of doses under the therapeutic contributed to the emergence of isolation mutation (magent and balanchard, 2005 ., Abdullah et al ., 2024).

Conclusion

- 1- *P. aeruginosa* can be isolated from mucous membranes in every clinical disease
- 2- It was found that *P. aeruginosa* bacteria are subject to many antibiotic resistances and the effect of drug administration on their rate.
- 3- The similarity resulting in antibiotic resistance in all *P. aeruginosa* isolates indicates that hospital equipment and people working there may have a role in transmitting *P. aeruginosa*.

4- The API 20 E test represents one of the most important means of diagnosing *P. aeruginosa*, and it can also be considered as a source of contamination. It can help people who suffer from weak immunity and is considered a diagnostic method for them in early detection of the infection.

References

- [1] Alhussain F, Yenugadhati N, Al Eidan F, Al Johani S and Badri M(2019) Risk factors, antimicrobial susceptibility pattern and patient outcomes of *Pseudomonas aeruginosa* infection: A matched case-control study. *J. Infect. Publ. Hlth.* 14(1), 152-157.
- [2] Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am J clin pathol*, 45, 149-158.
- [3] Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T., and Williams, S. T. (1994). *Bergey's Manual of determinate bacteriology*.
- [4] Kollef M, Shorr A, Tabak Y, Gupta V, Liu L and Johannes R (2005) Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. *Chest* 128(6), 3854-3862.
- [5] Kollef M, Shorr A, Tabak Y, Gupta V, Liu L and Johannes R (2005) Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. *Chest* 128(6), 3854-3862.
- [6] Magnet, S. and Blanchard, J.S. (2005). Molecular insights into aminoglycoside action and resistance. *Chem. Rev.*, 105(2):477_498.
- [7] World Health Organization (WHO) (2017) Publishes list of bacteria for which new antibiotics are urgently needed. Short Summary 25 Feb ET NM WHO.pdf/. Accessed 24 January 2019
- [8] Gupta, R. S., & Shrestha, N. (2019). Clinical significance and antibiogram of *Pseudomonas aeruginosa* isolated from tertiary care hospital of Birgunj, Nepal. *Indian J Med Res Pharm Sci*, 6(8), 7-10.
- [9] Ansari, S., Dhital, R., Shrestha, S., Thapa, S., Puri, R., Chaudhary, N., ... & Gautam, R. (2016). Growing menace of antibacterial resistance in clinical isolates of *Pseudomonas aeruginosa* in Nepal: an insight of beta-lactamase production. *BioMed Research International*, 2016.
- [10] Khalifa, A. B. H., Moissenet, D., Thien, H. V., & Khedher, M. (2011, August). Virulence factors in *Pseudomonas aeruginosa*: mechanisms and modes of regulation. In *Annales de biologie clinique* (Vol. 69, No. 4, pp. 393-403).
- [11] de Bentzmann, S., Polette, M., Zahm, J. M., Hinnrasky, J., Kileztky, C., Bajolet, O., ... & Puchelle, E. (2000). *Pseudomonas aeruginosa* virulence factors delay airway epithelial wound repair by altering the actin cytoskeleton and inducing overactivation of epithelial matrix metalloproteinase-2. *Laboratory investigation*, 80(2), 209-219.
- [12] Abdullah, R. M., Dölarslan, M., & Raouf, T. F. R. (2024). Detection of (*pslA*, and *PA-SS*) Genes in *Pseudomonas aeruginosa* Isolated from Clinical Cases. *Ibn AL-Haitham Journal For Pure and Applied Sciences*, 37(2), 101-111.
- [13] Faddin JFM. Lippincott Williams & Wilkins: *Biochemical Tests for Identification of Medical Bacteria*; 2000..