Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6073 - 6078
Received 25 April 2021; Accepted 08 May 2021.

Antibiotic Resistance Pattern to *Pseudomonas Aeruginosa* Isolated from Different Sample

Muna M. Kareem1*, Abeer F. Murad2*

1,2 Department of Biology, College of Science for women, University of Babylon, Iraq

***Corresponding author email : abeerbio15@gmail.com**

Abstract

Aim: The *P. aeruginosa* has become an important and frequent opportunistic nosocomial pathogen. Antimicrobial managers are often classified according to their principal mechanism of action. This study analysis the infections caused by *Ps. aeruginosa* and try to reveal the antimicrobial agents susceptibility against *Ps. aeruginosa.*

Material and Method: A cross-sectional study was carried out in different specimens (urine, sputum,tooth) were collected from Public Health Laboratory and private clinic lab, which transferred by using media or swab media between (October 2020 to 30 march 2021).

A total 50 *P. aeruginosa* isolates were obtained from 157 clinical samples.. Were (29, 58%) from urine ,(19, 38%)from sputum and (2, 4%) from tooth. These isolates were identified according to the traiditianal and molecular technique, such as culture and microscopic examination, biochemical tests, API 20E kit, Vitek2 system, and PCR

Result: In present study, isolates of *Ps. aeruginosa* isolated from various samples (29, 58%) urine ,(19, 38%) sputum and (2, 4%) from tooth. It was found out that (24.14% male and 75.86% female in urine sample) , (36.85% male and 63.15% female in sputum sample) and (50% male and 50% female in tooth sample), For *Pseudomonas aeruginosa* the highest resistance percentages were found to Ampicillin, ceftriaxone, Imipenem, ,Gentamicin, norfloxacin, levofloxacin, Ciproflaxin, Amikacin, cefoxitin, and the lowest level of antibiotics was pipracillin .

Conclusion: Steady educational programs on infection control for all healthcare workers to stop the range of nosocomial infections.the antidrug resistance will continue to be a problem with *Pseudomonas spp*. infections, there is an immediate need to replace these antibiotics with developing treatment strategies, to avoid and to exclude the infections.

Introduction

Infections with *Pseudomonas aeruginosa* have become a real concern in hospital-acquired infections, especially in critically ill and immunocompromised patients. The major problem leading to high mortality lies in the appearance of drug-resistant strains [1] .*Pseudomonas aeruginosa* is one of the six bacterial pathogens, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*., which are commonly associated with antimicrobial resistance, and denoted by their acronym ESKAPE,Several virulence's may cause pathogenicity that facilitates adhesion and/or disrupt host cell signaling pathways while targeting the extracellular matrix ,Among the pathogenicity caused by virulence factors, can be cited Lipopolysaccharide, Flagellum, Type IV Pili, Type III Secretion

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6073 - 6078
Received 25 April 2021; Accepted 08 May 2021.

System, Exotoxin A, Proteases, Alginate, Quorum Sensing, Biofilm Formation, Type VI Secretion Systems, Oxidant Generation in the Airspace. These are major virulence factors acting in different manners in the immune system [2].

MATERIALS AND METHODS

Isolation and identification of *P aeruginosa*

A total)50(*P. aeruginosa* isolates were obtained from 157 clinical samples The sources of *P. aeruginosa* . Were (29, 58%) from urine ,(19, 38%)from sputum and (2, 4%) from tooth ,. as summarized in the Table (1).for Identification of causative microorganisms was performed by classic microbiological methods

It was found out that (24.14% male and 75.86% female in urine sample) , (36.85% male and 63.15% female in sputum sample) and (50% male and 50% female in tooth sample), Fig. (1). Table (2).

These isolates were identified according to the traiditianal and molecular technique, such as culture and microscopic examination, biochemical tests, API 20E kit, Vitek2 system Fig. (2)(3) , and PCR.

Antimicrobial susceptibility test

The modified Kirby-Bauer method [3] was used for Antibiotic Susceptibility Testing .A total (50) *P arouginosa* isolates were exposed to susceptibility testing using different antibiotics such as, Ciproflaxin (cip) ,levofloxacin (levo) ,norfloxacin (norf) ,Ampicillin (Am),pipracillin (pip),ceftriaxone (cro), Imipenem (imip) ,Amikacin (Ak) , ,cefoxitin (fox) ,Gentamicin (GN).

Results and Discussion

The patterns of antimicrobial resistance were as followed: the highest resistance percentages were found to Ampicillin (98%) , ceftriaxone (62%) , Imipenem (58%) , ,Gentamicin (56%) , norfloxacin (56%) , levofloxacin (56%) , •Ciproflaxin (54%) , Amikacin (42%) , cefoxitin (42%) , and the lowest level of antibiotics was pipracillin (18%) . , as summarized in the Table (3). Fig (4).

In local study done by [4] mentioned that isolate of *P. aeruginosa* were resistance rate Ampicillin (81.1%), Cefetriaxone and Amoxicillin-Clavulanic acid were (78.4%), Ampicillin – Sulbactam (75.6%), Cefepime (72.9%), Trimethoprime-Sulphamethoxazole (70.2 %), Nitrofurantoin (64.8%), Cefazoline and Tobromycin (62.2%) then moderate resistance to Ciprofloxacin (56.7%), Ceftazidime (51.4%), Imipenem (45.9%) and the lowest level of antibiotics was Amikacin (40.5%), which agreed with the results of the current study.

While another study, disagreed with this results and showed that level resist to Ciprofloxacin (14%), Amikcin (2%) and Imipenem (0%) [5].

The high antibiotic resistance of the *P. aeruginosa* bacterium might contribute to many different factors including: the widespread use of a broad spectrum antibiotics leading to the selective survival advantage of the bacteria [6]. and another study [7] making this bacterium is difficult to treat as well as the serious biofilm formation by *P. aeruginosa.* In addition to the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6073 - 6078
Received 25 April 2021; Accepted 08 May 2021.

capability of the bacterium to form a biofilm, which provides the physical protection to the bacterium, hence the biofilm formations retard the penetration of the antimicrobial agents [8]. A concerning trend towards multi-drug resistance is emerging worldwide, which has gave implications for the capacity of current therapies to eradicate *P.aeruginosa* infections in the future [9]. Infections by *P. aeruginosa* are notoriously difficult to treat due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance. All known mechanisms of antibiotic resistance can be displayed by this bacterium (intrinsic, acquired, and adaptive); sometimes all within the same isolate [10].

Table (1): Prevalence of *P. aeruginosa* among Different Clinical Samples

	Isolate	Urine	Sputum	Tooth	Number	
	Pseudomonas aeroginosa	29 (58%)	19 (38%)	2 (4%)	50	12.06 **
** (P≤0.01).						

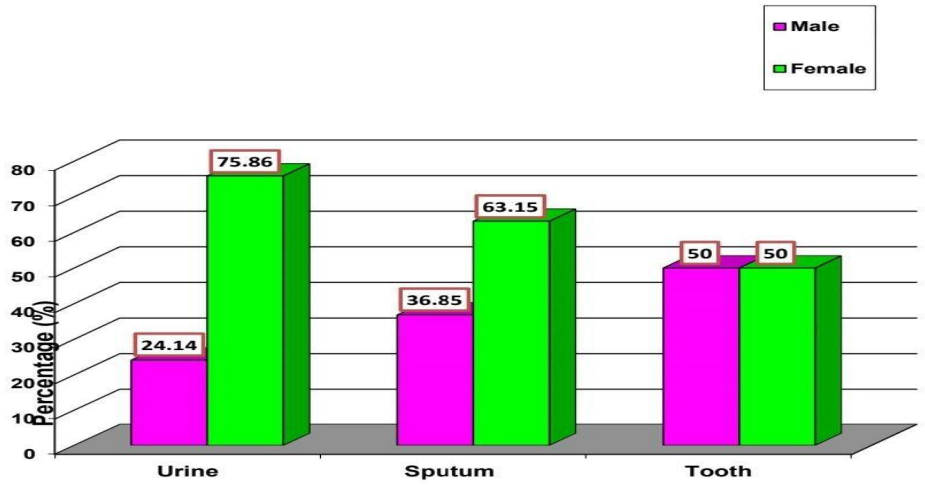

Figure (1).Distribution of Pseudomonas aeroginosa according to gender

Table (2) : Distribution of *Pseudomonas aeroginosa* according to gender

Sex	Urine NO(%)	Sputum NO(%)	Tooth NO(%)	Chi-Square (χ^2)
Male	7 (24.14%)	7 (36.85%)	1 (50.00%)	9.33 **
Female	22 (75.86%)	12 (63.15%)	1 (50.00%)	9.33 **
Total	29	19	2	---
Chi-Square (χ^2)	12.502 **	8.966 **	0.00 NS	----
** (P≤0.01), NS: Non-Significant.				

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6073 - 6078
Received 25 April 2021; Accepted 08 May 2021.

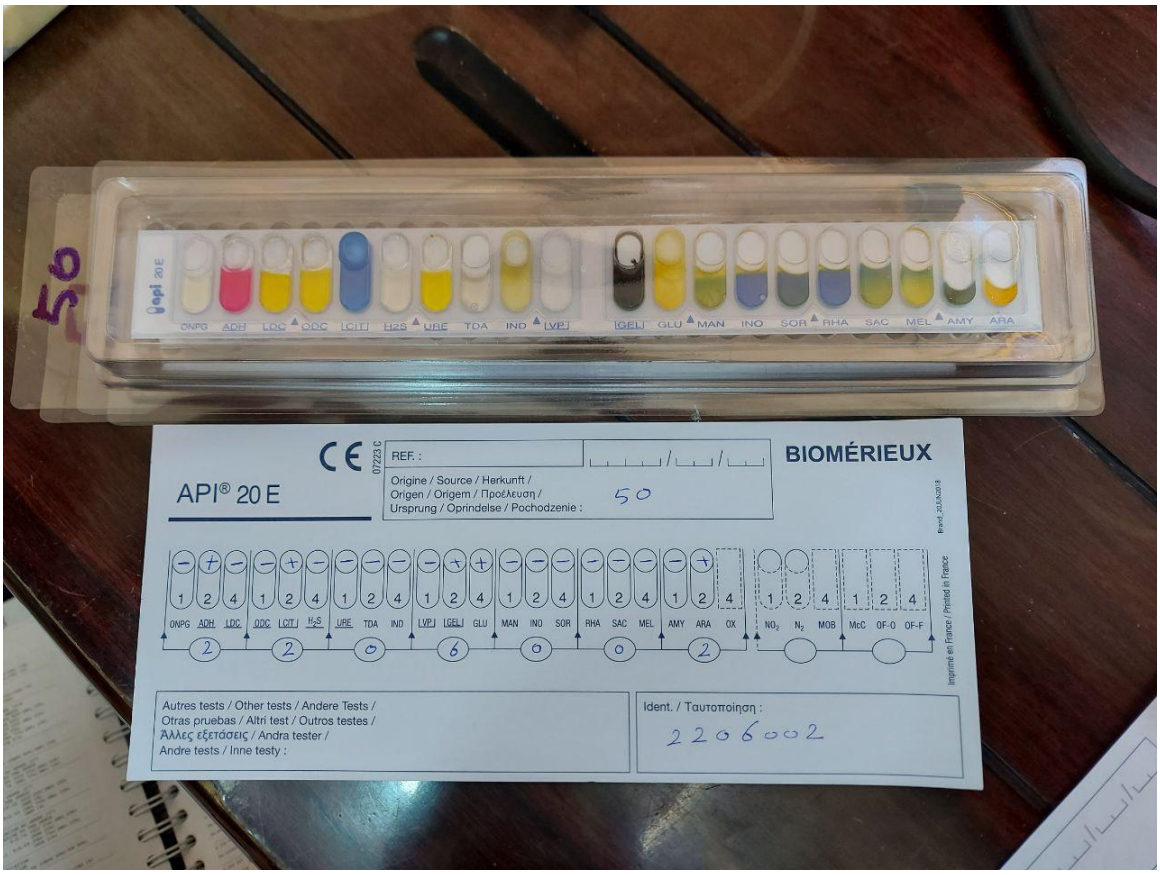

Figure (2): API 20E system Control for identification gram negative bacteria

bioMérieux Customer: Microbiology Chart Report Printed Nov 22, 2020 07:04 CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: Muna Mohammed Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 5.35 hours	**Status:** Final
Selected Organism	**Pseudomonas aeruginosa**	
ID Analysis Messages		

Susceptibility Information	**Analysis Time:** 14.62 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Imipenem	2	S
Ampicillin			Amikacin	4	S
Piperacillin/Tazobactam	8	S	Gentamicin	8	I
Cefazolin	>= 64	R	Ciprofloxacin	<= 0.25	S
Cefoxitin			Levofloxacin	0.5	S
Ceftazidime	8	S	Tigecycline	>= 8	R
Ceftriaxone			Nitrofurantoin		
Cefepime	4	S	Trimethoprim/Sulfamethoxazole		
Ertapenem					

+= Deduced drug *= AES modified **= User modified

AES Findings	
Confidence:	Consistent

Figure (3): Identification of *P.aeruginosa* by VITEK 2 System

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6073 - 6078
Received 25 April 2021; Accepted 08 May 2021.

Table(3) : Sensitivity test for *Pseudomonas aeroginosa*

Antibiotic	Sensitive	Resist	Total for isolate NO.	Chi-Square (χ^2)
CIP	23 (46%)	27 (54%)	50	2.96 NS
LEVO	22 (22%)	28 (56%)	50	9.52 **
NORF	22 (44%)	28 (56%)	50	4.75 *
AM	1 (2%)	49 (98%)	50	14.89 **
PIP	41 (82%)	9 (18%)	50	13.66 **
CRO	19 (38%)	31 (62%)	50	8.94 **
IMIP	21 (42%)	29 (58%)	50	0.921 NS
AK	29 (58%)	21 (42%)	50	6.02 **
FOX	9 (18%)	41 (42%)	50	10.46 **
GN	22 (44%)	28 (56%)	50	4.75 *

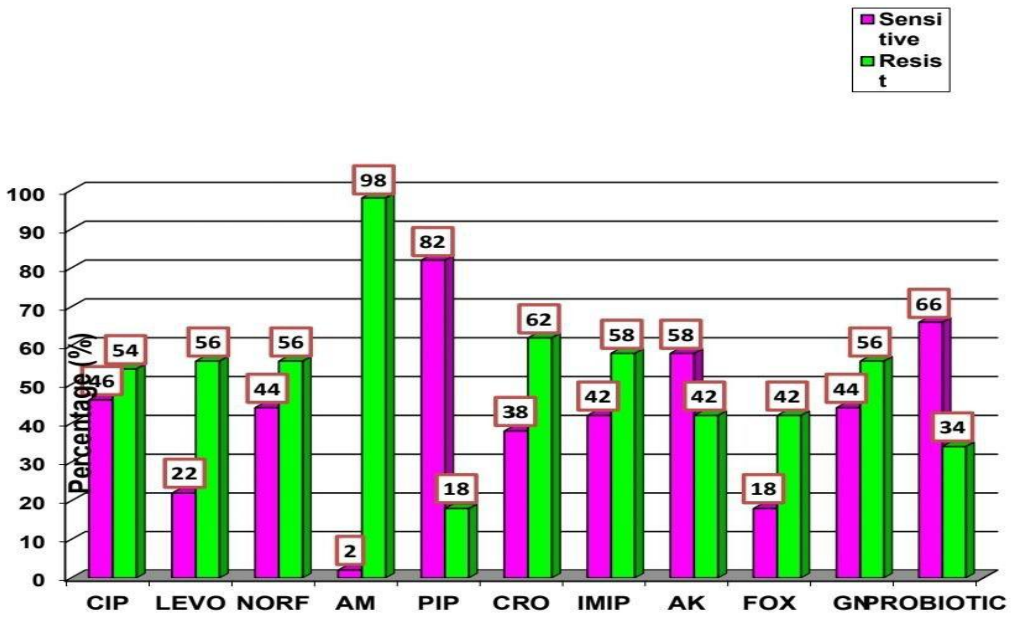

Figure (4). The Antibiogram pattern of isolates towards antimicrobials used in this study.

References:

1. **Bassetti**, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. (2018). How to manage Pseudomonas aeruginosa infections. Drugs in context, 7.
2. **Skariyachan**, S., Sridhar, V. S., Packirisamy, S., Kumargowda, S. T., & Challapilli, S. B. (2018). Recent perspectives on the molecular basis of biofilm formation by Pseudomonas aeruginosa and approaches for treatment and biofilm dispersal . Folia microbiologica , 63(4), 413-432.
3. **Vandepitte,** J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., Heuck, C. C., and Heuck, C. C. (2003). *Basic laboratory procedures in clinical bacteriology*. World Health Organization.
4. **Mahdi**, L. H., Jabbar, H. S., & Auda, I. G. (2019). Antibacterial immunomodulatory and

antibiofilm triple effect of Salivaricin LHM against Pseudomonas aeruginosa urinary tract infection model. International journal of biological macromolecules, 134, 1132-1144.

5. **Al-Kubaisy**, R.S.S. (2018). Effect of some nanomaterials on virulence factors controlled by quorum sensing genes in clinical isolates of pseudomonas aeruginosa. Ph.D. thesis, College of Science, Mustansiriyah University, Iraq.
6. **Henrichfreise**, B.; Wiegand, I.; Pfister, W. and Wiedemann, B. (2007). Resistance mechanisms of multiresistant Pseudomonas aeruginosa strains from Germany and correlation with hypermutation. Antimicrob. Agents Chemother. 51(11): 4062-4070
7. **Hirsch**, E. B., & Tam, V. H. (2010). Impact of multidrug -resistant Pseudomonas aeruginosa infection on patient outcomes . Expert review of pharmacoeconomics & outcomes research, 10(4), 441-451.
8. **Mah,** T. F. C., & O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology*, *9*(1), 34-39.
9. **Kiska**, D. L., and Gilligan, P. H. (2003). Pseudomonas. The Manual of Clinical Microbiology. P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller and R. H. Yolken. Washington, D.C., ASM Press. 1: 719-728.
10. **Moore**, N. M., and Flaws, M. L. (2011). Antimicrobial resistance mechanisms in *Pseudomonas aeruginosa*. *Clinical Laboratory Science*, *24*(1), 47-52.