Antibiotics susceptibility profile of multidrug resistant isolates of *Pseudomonas aeruginosa*

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Abstract:

Background: *Pseudomonas aeruginosa* is an opportunistic human pathogen cause most healthcare-associated infections and is considered a paradigm of antibiotic resistance.

Objective: The present study aimed to determine the antibiogram profile of multidrug-resistant isolates of *P. aeruginosa* isolates collected from Baghdad hospitals.

Methods: In a cross-sectional manner, *P. aeruginosa* isolates were collected from various clinical samples between December 2021 to June 2022. were defined as those resistant to at least one anti-pseudomonal agent in three or more classes. The Kirby-Bauer disk diffusion method tested one hundred isolates of *P. aeruginosa* for susceptibility to 24 anti-pseudomonal agents. Multiple-resistant isolates.

Results: The results showed that out of 2000 specimens, 100 isolates of *P. aeruginosa* were recovered and accounted for 5% of hospitalized infection isolates. The results showed high resistance against most antipseudomonal drugs. The results showed high resistance against Amikacine 77%, Ciprofloxacin 65%, Gentamicin 80%, Meropenem 82%, Levofloxacin 77%, Ofloxacin 78%, Tobramycin 80%

Conclusion: the present study proved that *P. aeruginosa* isolated had carbapenem-resistant genes that strongly correlated with antibiotic resistance according to phenotypic and genotypic characterization.

Introduction:

The finding *P. aeruginosa* has intrinsic resistance to numerous antimicrobial agents andalso easily acquires resistance to many antibiotics, including carbapenemsresistance is an ominous development that challenges this last resort antibiotic. Unfortunately, carbapenems resistant *P. aeruginosa* has now emerged and is disseminating worldwide (1,2).Over the last decade, an ever-growing number of P. aeruginosa isolates producing MBLs have been reported from many countries including Iraq (3,4), suggesting that these enzymes could become the predominant cause of carbapenem resistance in the near future.

Presently, β-lactamases are classified into four distinct classes based on structural similarities (classes A, B, C, and D) or four groups based on hydrolytic and inhibitor profiles. To date, twelve transferable MBL types that hydrolyze carbapenems have been identified in a number of nosocomial pathogens.To date, twelve transferable MBL types that hydrolyze carbapenems have been identified in a number of nosocomial pathogens (5,6).

However, routine antibiotics susceptibility tests done in the microbiology laboratory at Baghdad hospitals have observed that there is carbapenem resistance in a number of Gram-negative isolates including P. aeruginosa. The magnitude of this problem and the specific genes responsible for this resistance are not clear. However, MBLs have emerged as a major defense mechanism against carbapenems worldwide. The aim of the

present study was to evaluate the antibiotics susceptibility profile of P. aeruginosa isolated from different clinical isolates in hospitals of Bagdhad city.

Materials and Methods

Isolation and Identification of *P. aeruginosa*

A total of 2000 clinical samples were collected from inpatients of many hospitals in Baghdad city during the period from December 2020 to June 2021, which included: diabetic foot (50), otitis media (550), lower respiratory tract (150), urinary tract (725), wound (200), (75) Blood and burns (250). The clinical samples were transported to the laboratory without delay. All samples were cultivated, by using the standard loop of urine and sterile swabs of other samples, on the blood agar, MacConkey agar and cetrimide agar as selective media. *P. aeruginosa* and incubated overnight at 37˚C for 18- 24 hours. Initial diagnosis of isolates was made on the basis of Gram's staining of culture, colonial morphology on different media, hemolysis on blood agar, pigment production, odor in cultures, size, edge, and oxidase test. Suspected Pseudomonas colonies were further identified to species level using routine biochemical tests and selective culture media. In addition to these tests, the *P. aeruginosa* isolates were also confirmed biochemically with the Vitek-2 automated system. Production of pigments was detected by streaking of bacterial isolates onto the following selective medium. Cetrimide agar was used for the selective isolation and identification of *P. aeruginosa*. Isolates colonies were inoculated directly onto cetrimide agar using the streaking method. This selective medium was also used for the detection of pyocyanin production by *P. aeruginosa* isolates (7).

Antibiotics Susceptibility Test:

Pure colonies of *P.aeruginosa* from overnight culture on nutrient broth were transferred to a tube containing 5.0 mL of normal saline to obtain suspension with 1.5 × 108 CFU/mL by adjusting to the Dosimeter system to give an equivalent suspension. The antimicrobial susceptibility testing was done by the agar discs' diffusion method depending on the (CLSI, 2021) The inhibition zones were calculated in mm by using a transparent ruler, and the diameter of the inhibition zones for individual antimicrobial agents was translated into terms of Sensitive (S), Intermediate (I) and Resistant (R) categories by comparison with the standard values of inhibition zone. The Kirby-Bauer disk diffusion method tested one hundred isolates of *P. aeruginosa* for susceptibility to 24 anti-pseudomonal agents. Multiple-resistant isolates. The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics against which the isolate showed resistance over the total number of antibiotics tested (8).

$$\text{MAR index} = \frac{\text{Number of antibiotics against which isolate showed resistance}}{\text{Total number of antibiotics tested}}$$

MAR index higher than 0.3 indicates wide use of this antibiotic in the originating environment of this isolate.

Statistical Analysis

The results of the current study were statistically analyzed using the Statistical Package for Social Science SPSS Twenty-Third Edition, and the statistical program was used according to the data of the study results.

Results and Discussion

Isolation and identification of *P.aeruginosa*

According to the results of the present study, the overall count constitutes a total of 100/2000 samples, *P. aeruginosa* representing (5%) of all the collected

samples in this study. The results revealed that the samples from burn injury were 23(23%), followed by urine samples 22 (22. %) wound 18 (18%), ear swabs 17 (17%), sputum swabs10 (10%), diabetic foot 5(5%) and blood 5(5%), It's well known that *P. aeruginosa* considers as an important nosocomial pathogen in many medical centers throughout the world and source of infections in any part of the body. Also, this bacterium is able to cause infection in a healthy individual at a low rate and creates a serious public health disaster resulting in an enormous burden of morbidity, and mortality in both developing and developed countries (9). One of the reasons for the high pathogenicity of P. aeruginosa is the intrinsic high resistance to several antibiotics, as well as the development of multiple drug resistance (10).

In the current study, the susceptibility patterns of 100 clinical *P. aeruginosa* isolates were tested by a panel of antipseudomonal agents according to the disk diffusion method and CLSI (2021) recommendations. It was found that the vast majority of P. aeruginosa isolates showed high levels of resistance to most of the commonly-used antibiotics and there was no antibiotic that inhibits all tested P. aeruginosa isolates (Table 1).

Table (1): Number and percentage of resistant and sensitive of *P. aeruginosa* isolates to different antibiotics

Antibiotics	***P. aeruginosa* isolates (Number =100)**			
	Resistant		**Sensitive**	
	No.	**%**	**No.**	**%**
Azetreonam(AT)	51	51	49	49
Amikacine (AK)	77	77	23	23
Ceftazidium(CAZ)	53	53	47	47
Ciprofloxacin (CIP)	65	65	35	35
Cefepime (CPM)	28	28	72	72
Gentamicin (GM)	80	80	20	20
Imipenem (IMI)	51	51	49	49
Meropenem (MEM)	82	82	18	18
Doripenem (DOR)	48	48	52	52
Levofloxacin (LEV)	77	77	23	23
Lomefloxine (LOM) only for urine isolates	1	1	99	99
Ofloxacin (OFX)	78	78	22	22
Norfloxacine (NOR)	43	43	57	57
Gatifloxacine (GAT)	41	41	59	59
Tobramycin (TOB)	80	80	20	20

Ticarcillin\clavulanic acid (TCC)	16	16	84	84
Imipenem relebactam (I\R)	17	17	83	83
Ceftazidime\avibactam (C\A)	18	18	82	82
Ceftolozane\tazobactam (C\T)	17	17	83	83
Pipercillin\tazo-bactam (P\T)	17	17	83	83
Colistin (CO)	3	3	97	97
Mezlocillin (MEZ)	25	25	75	75
Netilmicine (NET)	20	20	80	80
Cefiderocol (CEFD)	13	13	87	87

Virulence Factors of *P. aeruginosa*:

Hemolysis pattern

The hemolysis pattern of tested isolates of *P.aeruginosa* was beta hemolysis 47 (78.33%) while 13(21.66%) did not produce hemolysis. The study of (11) who reported the analysis of virulence factors revealed that out of the 61of P. aeruginosa isolates studied, 57(93.4%) were beta-hemolytic, while (12) noticed that all the isolates 100 (100%) had the ability to produce beta hemolysis.

Pigment production:

The results revealed that 72/100 (72%) isolates of *P.aeruginosa*were able to produce pyocyanin (a blue-green, water-soluble, non-fluorescent, phenazine pigment), 28/100 (28%) isolates were able to produce Pyoverdine, fluorescein (greenish-yellow) stain . These results agreed with updated local studies. Pyocyanin is a blue-green pigment with a strong antibiotic effect against other bacterial species. Several infections associated with pyocyanin cytotoxic effects have been reported, and they involve pro-inflammatory and free radical production resulting in cellular damage and necrosis, pyocyanin detection can be employed as a rapid approach for detecting P. aeruginosa infections in patients (13).

Production of *Exotoxin A*:

In the current study,100 isolates were tested for the identification exoA gene using PCR where *exoA* was identified in all *P. aeruginosa* isolates (100%), the size of the PCR product was 454 base pairs. These results agreed with (14) who found *exoA* gene in isolates 100%. Whereas differ from (15) who observed that the presence of *exoA* gene in isolates from burn isolates was 97/100(97%), while the results of (16) referred to 90% of isolates having *exoA* gene. On the other hand, (17) *exoA* gene in the *P. aeruginosa* isolates was (69.56%), so that, these results differed from the data of the present study. This may be attributed to the differences in the type and virulence of isolates those collected in both studies. *exoA* in *P. aeuroginosa*produces a variety of

extracellular metabolites which are potentially causing damage to ocular tissues. (18). In summary, table (2) showed the relationship between the virulence factors and the number of antibiotic resistance.

Table (2): Virulence factor of *P.aeruginosa* isolated from different clinical samples (n= 100)

clinical samples	No. of isolates	*exoA* +	No of pigment in		Beta Hemolysis	Number of Antibiotic resistance
			pyocyanine	pyoverdin		
Diabetic foot	5	5	4	1	5	7-14
Blood	5	5	4	1	5	7-23
Sputum	10	10	8	2	10	3-22
Ear	17	17	11	6	15	6-21
Urine	22	22	14	8	20	3-24
Burn	23	23	17	6	21	3-24
Wound	18	18	13	5	16	4-14
Total	100	100	71	29	92	24 agent

The diversity of antibiotic resistance mechanisms contributes to the development of multiple antibiotic-resistant isolates and makes conventional antibiotics ineffective for the treatment of *P. aeruginosa* infections (19). In recent years, Iraq has been considered among the countries that reported high rates of antimicrobial resistance. (20 -23). The periodic surveillance and monitoring of antibiotic resistance of P. aeruginosa is very important in order to provide updated information about the current activity of commonly used antipseudomonal antibiotics in Baghdad hospitals and would enable the physicians to detect the trends in the resistance pattern to the commonly prescribed antibiotics in a given organism. Therefore, one objective of the present survey was planned to assess the susceptibility of *P. aeruginosa* isolates, as well as determine the magnitude, frequency, and current trends of antibiotic resistance development among isolates against various commonly used antibiotics.

Antibiogram Profile of *P. aeruginosa* Isolates:

As it is shown in table (3), all isolates differentiated into 20 patterns according to resistance by number and similarity of antibiotic resistance. The highest rate of XDR was observed with pattern 20, in which this isolate was able to resist 24 antibiotics. While the lowest isolates were noticed with pattern 1 in which the total number of resisted antibiotics was only 2 antibiotics, followed by patterns 19, and 18 in which the total number of resisted antibiotics was only (23,22) antibiotics. Pattern 17 in this style total number of resisted antibiotics was (21) antibiotics.

In pattern 16 in this style total number of resisted antibiotics was (17) antibiotics, in Pattern 15 in this style total number of resisted antibiotics was (16) antibiotics, and in Pattern 14 in this style total number of resisted antibiotics was (15) antibiotics.

Pattern 13 in this style total number of resisted antibiotics was (14) antibiotics, isolates from burns and one from urine were resistant to all antibiotics.

Also, the present results Also pattern (19) resisted 23 antibiotics (8 isolates from burns and five from urine). Therefore, the highest resistance isolate was from burns because emerging opportunistic isolates of XDR endemic seen mainly in nosocomial infections in hospital environments especially burn and wound departments are difficult to eradicate and the contamination and this department contain many types of MDR from light resistance to high resistance, as well as the patient in burn department treated with MDR to emergency and difficult of patient health

While the lowest multiple drug-resistant noticed with Pattern 10,11,12 in which the total number of resisted antibiotics was only (11,12,13), While the lowest multiple drug-resistant noticed with pattern 8,9 in this style total number of resisted antibiotics was (9,10), While the lowest multiple drug-resistant noticed with pattern16,7 in this style total number of resisted antibiotics were (7,8) and then lowest MDR noticed with Pattern 2,3,4,5 in which the total number of resisted antibiotics were only (3,4,5,6,7)antibiotics.

This study found that pattern (20) of *P. aeruginosa* (3 from burns samples, was XDR which resist all antibiotics that were used. These results are in agreement with (23) who found the highest resistance to burns and agreed with (12) who found 8

Table (3): Anti-biotyping of *P.aeruginosa* isolates from different clinical isolates.

Pattern	Total number of isolates	Site of infecton							Antibiotics resistance pattern	Number of antibiotics resistance
		Burns	Wounds	Sputum	Diabetic foot	Ear	Urine	Blood		
1	1	1		-	-				CAZ, TIM	2
2	6	1	-	2		-	3		MEM, LEV, NOR, CAZ, MEZ, TIM, AT, NET, DOR,CPM, GM, OFX.	3
3	5	3	1				1		AT, NET, MEM<, LEV, NOR, TOB	4
4	6	1	4	1	-	-	-		CPM, GM, OFX, TOB, AT, CIP, MEM, DOR, GAT, TN LEV,NOR,CAZ, TIM	5
5	8	3	-	2	-	2	1		CPM, GM, OFX, TOB, AT, CIP, MEM,TIM, LEV,IMI, DOR, GAT, TN	6
6	12		1	4	2	3	-	2	CPM,CAZ,MEM,TIM, CIP , IMI,TIM, OFX, GM, , TOB, AT, AK, LEV,PTZ	7
7	5	3	-	-	-	2	-		CPM, MEM, TIM, OFX, LEV, GM, TOB, AK, MEZ	8
8	3		1	-	-	-	2		CIP, MEM, DOR, GAT, AT, TN, GM, OFX, LEV, CIP,	9
9	2	2	-	-	-	-	-		MEM, CAZ, ATM, MEZ, IMI, TIM, OFX, LEV, GM, TOB,PTZ, TOB, CPM	10
10	4					4			CIP, DOR, GAT, AT, GM, OFX, LEV, CAZ, NOR, AK, CEF, MEM, TIM, CIP, CPM,AK, PTZ,TOB	11

11	**4**	**1**	**1**	**-**	**1**	**-**	**1**		**DOR, GAT, AT, GM, OFX, LEV, CAZ, NOR, AK, CEF, T\C, TOB,MEM, MET, TIM, CIP, CPM, GM, AT, PTZ, TOB**	**12**
12	**10**	**1**	**7**	**-**	**1**	**1**	**-**	**-**	**CAZ, MEM, ATM, MEZ, IMI, TIM, CIP, OFX, LEV, NOR, AK, GM, TOB,CAZ, CPM, AT, DOR,**	**13**
13	**12**	**2**	**2**		**1**	**3**	**3**	**1**	**CAZ, MEM, ATM, MEZ, TIM, CIP, OFX, LEV, CPM, IMI, AK, GM, TOB, PTZ, DOR, AT,PTZ, C\A**	**14**
14	**1**						**1**		**CIP,OFX,LEV, MEM, IMI, CEFD, DOR, GAT, AT, TN, CAZ, NOR, AK, CEF, TOB,GM,**	**15**
15	**1**						**1**		**AT, MEM, IMI, DOR, GM, AK, CIP, LEV, NOR, OFX, GAT, TIM, LOM, CAZ< CEF**	**16**
16							**3**		**AT, MEM, IMI, DOR, TOB, GM, AK, CIP, LEV, NOR, OFX, GAT, TIM, CIP, T\C, CEF, CAZ**	**17**
17	**6**	**3**				**2**			**AT, MEM, IMI, DOR, TOB, GM, AK, CIP, LOV, NOR,OFX, GAT, TIM,CAZ,CEF, NET, I\R,TCC,C\A, P\T,TCC**	**21**
18				**1**					**AT, MEM, IMI, DOR, TOB, GM, AK, CIP, LOV, NOR,OFX, GAT, TIM,CAZ,CEF, NET, I\R,TCC,C\A, P\T,TCC, CEFD.**	**22**
19	**8**	**1**					**5**	**2**	**AT, Cip, MEM, IMI, DOR, TOB, GM, AK, LEV, NOR,OFX, GAT, TIM,CAZ,CEF, CEFD, NET, I\R, TCC, C\A, C\T, P\T,TCC.**	**23**
20	**3**	**1**				**1**	**1**		**AT, MEM, IMI, DOR, TOB, GM, AK, CIP, LOV, NOR,OFX, GAT, TIM,CAZ,CEF, CEFD, NET, I\R,TCC,C\A, C\T, P\T,TCC,CO.**	**24**

The high occurrence of MDR and XDR P. aeruginosa isolates in Baghdad hospitals is a serious concern. The growing resistance of P. aeruginosa to different classes of antimicrobial agents is a major concern worldwide.

Relationship between Virulence Factor, Antibiotic Resistance, and MDR, XDR in *P. aeruginosa*:

In summary, the relationship among the tested virulence factors pigment production, hemolysis, exotoxin A production, and antibiotic resistance with 49(49%) MDR isolates and 18(18%) XDR isolates patterns in 100 of *P. aeruginosa* according to the source of isolates and there was no PDR among isolates tested. The high occurrence of MDR and XDR P. aeruginosa isolates in Baghdad hospitals is a serious concern. The growing resistance of *P. aeruginosa* to different classes of antimicrobial agents is a major concern worldwide.

In recent reports in Iraq, the most common carbapenemases are the MBL and OXA variant enzymes of Ambler class D (24,25,26,23). However, this study was carried out to detect the diversity and distribution of the main carbapenemase genes amongst the MDR

and XDR *P. aeruginosa* isolates obtained from Baghdad hospitals during a 6-months period.

The early detection of MBL-producing P. aeruginosa may avoid the future spread of these isolates. At this time, little is known about the dissemination of MBL-producing P. aeruginosa isolates in the Baghdad hospitals. Therefore, one objective of the current study pointed to detect the presence of blaNDM, blaIMP, blaVIM-1, blaGIM, SPM and blaIMI, and blaGIM genes among P. aeruginosa isolates(27-31).

Ethics Consideration

This study is in accordance with the ethics committee of Al-Diwaniaya teaching hospital, Iraq. Verbal agreement was obtained from participants in the study and the relative's pre-taking samples.

Conflict of interest: No known conflict of interest correlated with this publication.

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