



Antibiotic Resistance in Clinical Isolates of *Pseudomonas aeruginosa*: A New Viewpoint for Antibiotic Prescription

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Received: 1 June 2018
Accepted: 22 July 2018
ePublished: 27 Aug. 2018



Abstract

Background: A growing number of resistant *Pseudomonas aeruginosa* isolates have been reported. To make better choice of antibiotic, reporting and analyzing the recent antibiotic resistance patterns of the bacterium are of crucial importance. The purpose of the present study was to survey antibiotic resistance status in clinical isolates of *P. aeruginosa* and to make more options for antibiotic prescription by revisiting antibiogram results.

Methods: A total of 138 molecularly identified *P. aeruginosa* strains isolated from clinical specimens were tested for sensitivity to 10 antibiotics using Kirby-Bauer disk diffusion method. In addition, phenotypic combined disk diffusion test (CDDT) was applied to screen metallo-beta-lactamase (MBL) producing *P. aeruginosa* isolates among imipenem-resistant isolates. To find the most suitable antibiotic against *P. aeruginosa* infections, a new analytical way was employed using SPSS and chi-square test.

Results: Ceftizoxime showed the highest rate of resistance (78.9%) and amikacin showed the lowest (33.3%). 51.4% of the isolates showed resistance to Imipenem, 78.8% of which were positive for MBL production. Multidrug-resistant strain (MDR) isolates were observed in 67.3% of all isolates, 74.6% of Imipenem resistant isolates showed multidrug resistance and 83.9% of MBL positive isolates showed MDR. There was positive correlation between specimen source and resistance or susceptibility of *P. aeruginosa* isolates to some antibiotics in some specimens, and non-significant similarities in resistance or sensitivity to antibiotics in *P. aeruginosa* isolates ($P < 0.05$).

Conclusions: Resistance rate of imipenem, meropenem, gentamicin, tobramycin, ceftazidim, cefotaxime and ticarcillin was more than reported rates in previous studies. A higher proportion of MDR isolates and MDR-MBL producing strains suggest drastic dissemination of resistant isolates in the healthcare centers. Site specificity and non-significant similarity of the responses to antibiotics in *P. aeruginosa* isolates can provide a new sight for antibiotic prescription and better control of antimicrobial drug resistance.

Keywords: *Pseudomonas aeruginosa*, Resistance, MBL, MDR, Prescription

Background

Pseudomonas aeruginosa, an opportunistic pathogen especially in immunocompromised patients, using its various virulence factors causes serious infections including pneumonia, urinary tract infections, bacteremia, wound infections and chronic lung infections.¹ Antibiotics are prescribed against *P. aeruginosa* infections but documents show the occurrence of antibiotic resistance. There are several basic resistance phenotypes in *P. aeruginosa*; intrinsic resistance to carbenicillin,² resistance to all β -lactams except cepheims and carbapenems,³ resistance to penicillins² and resistance to carbapenems.⁴ Other resistance phenotypes are mainly controlled by extended spectrum β -lactamases (ESBLs) which are located on easily transferable plasmid or integrons and can cause any mechanisms of resistance to β -lactam antibiotics.⁴ The economic and social burden of infections by drug-resistant *P. aeruginosa* are undeniable.^{5,6} Different research reports indicate the presence of individual or classes of antibiotic resistant *P. aeruginosa*.⁷⁻⁹ In addition, the prevalence

of multidrug-resistant strain (MDR) (resistant to 3 or more drug classes) and XDR or extremely drug resistant (resistant to 6 or more drug classes) is increasing.^{8,10} Another discussable issue in this regard is resistance to β -lactams due to β -lactamase production which is the major mechanism of acquired resistance to β -lactam antibiotics in *P. aeruginosa*.¹¹ Resistance to carbapenems as the most potent antibiotic against *P. aeruginosa* and as the mainstay for treatment of multidrug-resistant strains is a topic for researchers to concern, especially when few antibiotic options have remained and patients infected with carbapenem-resistant *P. aeruginosa* show multidrug resistance.¹² These reports show the crucial importance of preventive measures by specialists to stop the dissemination of resistant *P. aeruginosa* isolates. These findings also stress the importance of the availability of accurate information about the resistance pattern of a single drug or class of drugs to select the most effective antibiotics against *P. aeruginosa* infection and control the occurrence of new resistant strains. On the other

hand, reported patterns should be reconsidered precisely and analyzed statistically to find correlations between antibiotic resistance or susceptibility and infection sites and similarities or differences in response to antibiotics amongst tested *P. aeruginosa*. Therefore, a better choice of antibiotics will be made to combat *P. aeruginosa*.

For these purposes, we evaluated antibiotic resistance, metallo-beta-lactamase (MBL) production and MDR of *P. aeruginosa* isolated from 138 clinical specimens (urine, trachea, blood, pus, ear and eye, from 3 health centers in Tehran) to 10 most prescribed antibiotics in four categories; carbapenems, aminoglycosides, cephalosporins and penicillins. MDR is considered as acquired non-susceptibility to at least 1 agent in 3 or more antimicrobial categories.⁸ To find a new viewpoint for antibiotic prescription against bacteria and a new way to control infection, similarities or differences of the response of all tested *P. aeruginosa* isolates to tested antibiotics and correlations between antibiotic resistance or susceptibility patterns and the source of isolates were analyzed statistically.

Materials and Methods

Bacterial Isolates

A total of 143 *P. aeruginosa* isolates were collected from 2 hospitals (3rd Shaban and Khatam-Ol-Anbia) and one clinic (16th Azar) in Tehran, from March to August 2016. The bacteria were isolated from specimens of urine, trachea, lesion, blood, pus, ear and eye (Table 1). Using gram staining test and biochemical tests including oxidase, catalase, reaction in TSI condition, oxidative fermentation (OF), indole production and motion study in sulfide indole motility (SIM), growth at 42°C, and green and blue pigment production, bacterial isolates were confirmed as *P. aeruginosa*. Molecular confirmation of the isolates were done using specific primers for gyrB (DNA Gyrase subunit B) gene; gyrB-F: 5'- CCTGACCATCCGTCGCCACAAC-3', gyrB-R: 5'-CGCAGCAGGATGCCGACGCC-3' (with a product size of 222 bp).¹³ For DNA extraction, cells from overnight cultures were pelleted and suspended in buffer containing 10 mM Tris-HCl, 40 mM EDTA

(Sigma), and 2% SDS (Sigma). DNA was released from bacterial cells by incubation with 10 mg/mL of lysozyme (Sigma) for 30 minutes at 37°C followed by incubation with 10 mg/mL of proteinase K (Sigma) for 2 hours at 55°C and then for 30 minutes at 68°C. The pellet was treated twice with phenol: chloroform (25: 24) (Sigma). PCR amplification was performed with a DNA Thermal Cycler (Bio Rad C1000) using PCR buffer containing each of the deoxynucleoside triphosphates and primers at a concentration of 250 mM, and 10 μM, respectively, 10 ng of target DNA, and 0.5 U of Taq DNA polymerase. A total of 35 cycles of amplification was performed through template DNA denaturation at 94°C for 2 minutes, primer annealing at 60°C for 30 seconds, and primer extension at 72°C for 1 minute. The bacteria were then maintained at -70°C for antibiotic sensitivity tests and phenotypic detection of MBL. American Type Culture Collection quality control strain of *P. aeruginosa* ATCC 27853 which was prepared by Iranian Research Organization for Science and Technology was used for quality control for PCR and antibiogram test and as an MBL-negative control.

Sensitivity Tests

The antibiotic resistance pattern and characterization of MDR in *P. aeruginosa* isolates were determined using Kirby-Bauer disk diffusion method and the recommended inhibition zone diameter breakpoints of Clinical and Laboratory Standards Institute guidelines. Antibiotic disks (MAST, England) used in this study for MDR and disk diffusion susceptibility included imipenem and meropenem (10 μg) from carbapenems, Gentamicin (10 μg), tobramycin (10 μg) and amikacin (30 μg) from aminoglycosides, ceftazidime (30 μg), cefotaxime and ceftizoxime (30 μg) from cephalosporins and carbenicillin (100 μg) and ticarcillin (75 μg) from carboxypenicillin category. According to CLSI standards (2016), strains with ≤15 mm inhibition zone size were considered imipenem-resistant and were applied for phenotypic detection test of MBLs.

Phenotypic Detection of MBL Strains

IPM-non-susceptible clinical isolates of *P. aeruginosa* were tested phenotypically using combined disk (CD) test¹⁴ to detect MBL strains. Overnight cultures of imipenem-resistant strains reached to 10⁵ CFU/mL dilution and spread on Mueller-Hinton (MH) agar (Sigma) followed by placing Two 10 g imipenem disks (Mast) at a distance of 4-5 cm from each other. 930 μg/mL of 0.5 M EDTA (Sigma) solution (pH: 8.0) was added to one of the disks and after 18-24 hours of incubation in air at 37°C, the inhibition zones of the imipenem and imipenem-EDTA disks were compared. The test was performed twice to ensure the validity of results. Test results were considered positive (presence of MBLs), if the difference of ≥7 mm

Table 1. Source of *Pseudomonas aeruginosa* Isolates (N = 138)

Source	No. (%) of Specimens	Male (%)	Female (%)
Urine	57 (41.9)	27 (47.3)	30 (52.6)
Trachea	27 (19.9)	12 (44.4)	15 (55.5)
Lesion	23 (16.6)	12 (52.1)	11 (47.8)
Blood	11 (8.5)	7 (63.6)	4 (36.3)
Pus	10 (7.3)	6 (60)	4 (40)
Ear	5 (2.9)	1 (20)	4 (80)
Eye	5 (2.9)	2 (40)	3 (60)
Total	138(100)	67 (48.5)	71 (51.4)

was observed between the inhibition zone diameters of the IPM-EDTA and IPM disks.¹⁴⁻¹⁶

Statistical Analysis

Descriptive statistics were used in this study using SPSS for Windows version 23.0 (IBM SPSS statistics). The categorical data were expressed as proportions. A chi-square test (P value of < 0.05 was considered statistically significant) was carried out for the 2 probabilities of sensitivity and resistance to antibiotics used against *P. aeruginosa* and comparison between genders. Chi-square test was also used to evaluate the correlation between sample sources of *P. aeruginosa* for resistance or susceptibility to each antibiotic and similarity/non-similarity of responses to 10 tested antibiotics (each pair of antibiotics) in all *P. aeruginosa* isolates. In chi-square analysis, ear ($n=5$) and eye ($n=5$) samples were considered as one group ($n=10$). In this study, low numbers of intermediate responses to antibiotics (no intermediate response to some antibiotics) were categorized as resistant group.

Results

A total of 138 *P. aeruginosa* isolates from different clinical specimens were molecularly confirmed by observing the 222-bp fragment of *gyrB* gene on 1.5% agarose gel. 48.5% of the *P. aeruginosa* isolates were isolated from males and 51.4% from females. The isolates were most commonly from urine (41.9%) and thereafter trachea (19.9%) and lesion (16.6%). Isolates were also from blood, pus, ear and eye (Table 1).

The antibiotic susceptibility results showed 78.9% resistance to ceftizoxime (30 μ g), 68.1% to cefotaxime (30 μ g), 67.3% to ticarcillin (75 μ g), 67.3% to carbenicillin (100 μ g), 65.2% to tobramycin (10 μ g), 63.7% to ceftazidime (30 μ g), 63.04% to gentamicin (10 μ g), 53.6% to meropenem (10 μ g), 51.4% to imipenem (10 μ g) and 33.3% to amikacin (30 μ g). The highest rate of resistance was observed in ceftizoxime with 78.9% and the highest sensitivity rates were discovered in amikacin

with 33.3% (Table 2).

51.4% (71 out of 138) of samples showed resistance to imipenem and 78.8% (56 out of 71) of imipenem-resistant *P. aeruginosa* isolates were recognized as MBL-producers using combined disk diffusion test (CDDT) phenotypic test (Table 3).

MDR isolates were observed in 67.3% of all isolates (93 out of 138). 66.6% (38 out of 57) and 85.1% (23 out of 27) of *P. aeruginosa* isolates, from urine and trachea respectively, were MDR. These figures are followed by 73.9% from lesion (17 out of 23), 72.7% (8 out of 11) from blood and 70% (7 out of 10) from pus samples. No MDR isolates were detected from ear and eye specimens (Table 2).

As mentioned, out of the 138 *P. aeruginosa* isolates, 71 (51.4%) showed resistance to imipenem and 78.8% (56 out of 71) of imipenem-resistant *P. aeruginosa* isolates were recognized as MBL-producers. 74.6% (53 out of 71) of imipenem resistant isolates showed MDR and 83.9% of MBL positive isolates (47 out of 56) showed MDR.

No positive correlation was found between the 2 probabilities of sensitivity and resistance to antibiotics and genders. Response to antibiotics in *P. aeruginosa* isolates from different sources showed significant differences at $P < 0.05$ for some antibiotics (Table 4). Trachea and lesion, for example, showed significant difference in

Table 3. Imipenem-resistant MBL-Producing *Pseudomonas aeruginosa* Isolates

Source (No.) ^a	MBL Producing, No. (%)
Urine (34)	32 (94.1)
Trachea (18)	14 (77.7)
Lesion (9)	6 (66.6)
Blood (4)	2 (50)
Pus (3)	1 (33.3)
Ear (1)	0 (0)
Eye (2)	1 (50)
Total (71)	56 (78.8)

^aNumber of imipenem resistant isolates in each specimen.

Table 2. Frequency of Antimicrobial Resistance in *Pseudomonas aeruginosa* Isolates (Clinical Isolates)

Antimicrobial Category	Antimicrobial Agent	Resistant No. (%) for Each Specimen							
		Urine 57 (41.9)	Trachea 27(19.9)	Lesion 23 (16.6)	Blood 11 (8.5)	Pus 10 (7.3)	Ear 5 (2.9)	Eye 5 (2.9)	Total 138 (100)
Carbapenems	Imipenem (10 μ g)	34 (59.6)	18 (66.6)	9 (12.6)	4 (36.3)	3 (30)	1 (20)	2 (40)	71 (51.4)
	Meropenem (10 μ g)	30 (52.6)	18 (66.6)	13 (56.6)	6 (54.5)	3 (30)	2 (40)	2 (40)	74 (53.6)
Aminoglycosides	Gentamicin (10 μ g)	32 (56.1)	22 (81.4)	14 (60.8)	7 (63.6)	6 (60)	3 (60)	3 (60)	87 (63.04)
	Tobramycin (10 μ g)	39 (68.4)	15 (55.5)	14 (60.8)	7 (63.06)	7 (70)	4 (80)	4 (80)	90 (65.2)
	Amikacin (30 μ g)	19 (33.3)	15 (55.5)	14 (60.8)	9 (81.8)	7 (70)	4 (80)	4 (80)	69 (33.3)
	Ceftazidime (30 μ g)	36 (63.1)	24 (88.8)	9 (39.1)	7 (63.6)	6 (60)	4 (80)	2 (20)	88 (63.7)
Cephalosporins	Cefotaxime (30 μ g)	40 (70.1)	18 (66.6)	18 (78.2)	7 (63.6)	5 (50)	3 (60)	3 (60)	94 (68.1)
	Ceftizoxime (30 μ g)	35 (61.4)	24 (88.8)	21 (91.3)	10 (90.9)	9 (90)	5 (100)	5 (100)	109 (78.9)
Carboxypenicillins	Carbenicillin (100 μ g)	38 (66.6)	21 (77.7)	18 (78.2)	7 (63.6)	6 (60)	4 (80)	2 (40)	93 (67.3)
	Ticarcillin (75 μ g)	31 (54.3)	23 (85.1)	17 (73.9)	9 (81.8)	8 (80)	4 (80)	1 (20)	93 (67.3)
	MDR	38 (66.6)	23 (85.1)	17 (73.9)	8 (72.7)	7 (70)	0 (0)	0 (0)	93 (67.3)

Table 4. The Correlation Between Drug Resistance and Specimen Sources

Sample Source	Antibiotics (<i>P</i> value)					
	IM	GM	AMK	CAZ	CTZ	TIC
Urine & Trachea	-	0.02	-	0.012	0.008	0.005
Urine & Lesion	-	-	0.032	0.044	0.006	-
Urine & Blood	-	-	0.004	-	-	-
Urine & Pus	-	-	0.043	-	-	-
Urine & Ear +Eye	-	-	0.010	-	0.013	-
Trachea & Lesion	0.048	-	-	0.000	-	-

Abbreviations: IM, imipenem (10µg); GM, gentamicin (10 µg); AMK, amikacin (30 µg); CAZ, ceftazidime (30 µg); CTZ, ceftizoxime (30 µg); TIC, ticarcillin (75 µg).

Table 5. The Comparison of Antibiotic Resistance between Each Pair of Antibiotics in All Tested *Pseudomonas aeruginosa* Isolates

Antibiotic	<i>P</i> value	Antibiotic	<i>P</i> value
IM & MER	0.005	AMK & CTX	0.030
IM & CTX	0.002	AMK & CTZ	0.000
MER & GM	0.019	TOB & AMK	0.031
MER & CB	0.031	TOB & CB	0.012
GM & TOB	0.002	CAZ & CTX	0.001
TOB & AMK	0.031	CAZ & CTZ	0.028
TOB & CB	0.012	CB & TIC	0.000

Abbreviations and resistance frequency (%): IM: imipenem (10 µg) 51.4, MER: meropenem (10 µg) 53.6, GM: gentamicin (10 µg) 63.04, TOB: Tobramycin (10 µg) 65.2, AMK: Amikacin (30µg) 33.3, CAZ: ceftazidime (30 µg) 63.7, CTX: cefotaxime (30 µg) 68.1, CTZ: ceftizoxime (30 µg) 78.9, CB: carbenicillin (100 µg) 67.3, TIC: ticarcillin (75 µg) 67.3.

response to imipenem with *P* value of 0.048. There was also significant difference (*P* value: 0.02) in response to gentamicin between urine and trachea isolates, in response to amikacin between urine and lesion (*P* value: 0.032), urine and blood (0.004) urine and pus (*P* value: 0.043) and urine and ear +eye (*P* value: 0.010) in *P. aeruginosa* isolates. In response to ceftazidime, the difference was significant between urine and trachea, urine and lesion and trachea and lesion (*P* value: 0.012, 0.044 and 0.00 respectively). A significant difference was also seen in response to ceftizoxime with *P* values of 0.008, 0.006 and 0.013 for *P. aeruginosa* isolates from urine and trachea, urine and lesion and urine and ear+eye respectively. A significant difference was seen in response to ticarcillin (*P* values 0.005) in urine and trachea isolates. No significant difference was seen in response to meropenem, tobramycin, cefotaxime and carbenicillin in *P. aeruginosa* isolates from different sources.

Similarities or non-similarities in antibiotic resistance or sensitivity to each pair of antibiotics in all *P. aeruginosa* isolates were evaluated by chi-square test (*P* value < 0.05) and the results for significant similarities in response to some antibiotics are shown in Table 5. *P* value of non-similarities is not presented to avoid numerous numbers.

Discussion

According to the results of the present study, there was no antibiotic which inhibits all tested *P. aeruginosa* isolates.

This study showed higher frequency of resistance in imipenem and meropenem (51.4% and 53.6% respectively), in *P. aeruginosa* isolates than other studies in Tehran (47% and 34.1% for imipenem, 44.3% for meropenem),^{17,18} a study on specimens from Ghazvin and Alborz (31% and 35% respectively)¹⁹ and a study in Kerman (46.6% and 43.3% respectively).²⁰ According to the above-mentioned reports, resistance rate to carbapenems in Iran is significantly growing. carbapenems are important group of last-line antibiotics for the treatment of infections with multidrug-resistant bacteria. Although carbapenem resistance remained at relatively low levels in most countries in 2015,²¹ the continuous significant increase of carbapenem resistant bacteria is a cause for great concern and a threat to patient safety. Gentamicin and tobramycin resistance rate was also higher in this study (62.5% and 63% respectively) in comparison to recent reports from Tehran (51% and 50% for gentamicin, 50% and 44.3% for tobramycin)^{17,18} and Ghazvin/Alborz (44.7 and 42.7% respectively).¹⁹ The same was true for ceftazidim, cefotaxime and ticarcillin (63.7% and 68.1% and 67.3% respectively) which showed higher percentage of resistance in tested *P. aeruginosa* isolates than other studies by Aghamiri et al¹⁷ (47%, 62% and 60% respectively) and Sadari and Owlia¹⁸ (35.5%, 38.6% and 28.4% respectively) in Tehran and a study by Peymani et al (37%, 52.5% and 47.5% respectively) in Ghazvin and Alborz.¹⁹

The highest antibiotic resistance rates were observed in ceftizoxime (78.9%) in the present study which was in close agreement with 2 other studies in Ahvaz (79.2%) (22) and Kerman (75%).²⁰ This can make ceftizoxime a bad choice for further prescription against *P. aeruginosa*. Amikacin had the lowest rate of resistance in the present study (33.3%). It shows relatively low frequencies in other studies in Tehran and other cities of Iran. The results of the present study show close agreement with the results of Sadari and Owlia¹⁸ in Tehran (36.4%) and Peymani et al in Ghazvin and Alborz (38.3%).¹⁹ 55.2%, 50% , 53.3%, 53.3% and 43.4% are the amikacin resistance rates from Ahvaz,^{22,23} Tabriz,²⁴ Kerman²⁰ and Isfahan²⁵ respectively. Figures show that amikacin can still be a good choice in Tehran but should be used with more caution in other cities. MDR isolates in the present study include 67.3% of isolates which is higher than figures presented by Sadari and Owlia, Peymani et al and Farajzadeh Sheikh et al²³ (54.5%, 33.7% and 44.4% respectively).

In this study, CD method is used for screening MBL producing *P. aeruginosa* isolates, which has shown the most acceptable sensitivity and specificity and is cheap and easy to perform.²⁶⁻²⁸ Of 138 *P. aeruginosa* isolates, 51.4%

(71 out of 138) showed resistance to imipenem and using CD method, 78.8% (56 out of 71) of imipenem resistant isolates showed MBL-production property. These findings are in accordance with the studies by Doosti et al in Zanzan,²⁹ Aghamiri et al in Tehran¹⁷ and Amini and Mobasheri in Kerman²⁰ which show that 87.8%, 70% and 60.7% of investigated *P. aeruginosa* isolates, respectively, can produce MBL. Higher frequency (90%) of MBL positive *P. aeruginosa* isolates is also reported by Moosavian and Rahimzadeh in Ahvaz.²²

Imipenem has been an effective β -lactam antibiotic for certain cure of infections caused by *P. aeruginosa*³⁰ but enhanced resistance against this agent can be an absolute reason to restrict its usage in health care centers. According to our survey, 74.6% (53 out of 71) of Imipenem resistant isolates showed multidrug resistance and 83.9% (47 out of 56) of MBL-producing *P. aeruginosa* isolates showed MDR. Distribution of these isolates brings difficulties in the treatment of *P. aeruginosa* infections and up-to-date and continuous reports of MDR isolates are needed.

In this study, *P. aeruginosa* isolates from trachea specimens show a higher frequency of resistance to tested antibiotics in comparison with other clinical samples (Table 2) and urine isolates has gained the highest frequency of MBL-producing *P. aeruginosa* followed by trachea isolates (Table 3). This may be because of long hospitalization of the patients in ICU, using artificial respiration equipment and catheterization of the urinary tract and irregular prescription of extended-spectrum antibiotics.

In the present study, there were significant differences in drug resistance or susceptibility in *P. aeruginosa* isolates from 2 or more specimen sources for some antibiotics (Table 4). In This research, considering the significant differences in response to tested antibiotics in *P. aeruginosa* isolates from different sources, it can be concluded that imipenem can be a better drug against *P. aeruginosa* isolates from lesion than trachea, gentamicin, ceftazidime and ticarcillin can be better antibiotics against urine *P. aeruginosa* isolates than trachea, Amikacin (with lowest frequency of resistance in *P. aeruginosa* isolates) can be a better choice for *P. aeruginosa* isolates from urine than lesion, blood and ear and eye, and ceftizoxime (with highest resistance frequency) may be considered as a more effective drug in urine than in trachea and lesion (Table 4). In conclusion, it is shown that using 10 tested antibiotics in this study, treatment of *P. aeruginosa* isolates from Trachea can be more difficult than urine and lesion and treatment of *P. aeruginosa* isolates from urine can be more feasible using gentamicin, amikacin, ceftizoxime and ticarcillin. Such comparative analyses can give a new view for antibiotic prescription with consideration of the location of infection and more studies using this type of analysis can lead to a better choice of antibiotics for control of *P. aeruginosa* infections.

It is shown that virulence factor production levels differ according to the infection site.^{31,32} Here, the hypothesis of different level of resistance mechanisms in different infection sites can be proposed to be investigated.

A significant difference in antibiotic resistance or susceptibility between each pair of antibiotics ($P < 0.05$) is also seen in some antibiotics in the present study (Table 5). To interpret analysis results, it is better to mention ceftizoxime, cefotaxime, carbenicillin and ticarcillin, tobramycin, gentamicin and ceftazidime as antibiotics with a high frequency of resistance (Table 2). Crosstab-based statistical analysis showed no significant similarities in resistance or susceptibility between the aforementioned antibiotics, with the exception of tobramycin with gentamicin and carbenicillin at P value: 0.002, 0.012 and ceftazidime with cefotaxime and ceftizoxime at P value: 0.001 and 0.028 respectively (Table 5), so grouping them as non-effective antibiotics and prescribing carbapenems (with growing resistance rate) should be done after more surveys. Finding such correlation patterns in studies in one epidemic region, city or country can be helpful in making a better choice of drug for treatment and better control of *P. aeruginosa* infections.

It is concluded that resistance frequency of the prescribed antibiotics is growing drastically and high number of MBL-producer and MDR-MBL producer *P. aeruginosa* clones are disseminated in healthcare centers of Iran. To combat this, researches should stay more focused on antibiogram results than detecting new resistant genes to report. The results of the present study showed that the efficacy of antibiotics can be site specific and evaluation of difference in response to antibiotics in each endemic region can be an effective strategy to be applied for making a better choice of drug and treatment of *P. aeruginosa* infection.

Conflict of Interests

None declared.

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