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Detection of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) *Pseudomonas Aeruginosa* Isolated from Patients in Tehran, Iran

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ABSTRACT

Background: This study was done to detect multidrug resistant (MDR) and extremely drug resistant (XDR) of *Pseudomonas aeruginosa* among strains isolated from patients in Tehran, Iran, due to importance of these phenotypes in treatment of human infections.

Methods: Eighty eight *P. aeruginosa* were isolated from patients in Tehran, Iran, and identified by routine methods and PCR for oprL gene. Their antimicrobial susceptibility to 16 antimicrobial agents from 7 antimicrobial categories (aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins/ß-lactamase inhibitors, monobactams, polymyxins) were determined by disk diffusion method, according to recommendation of Clinical and Laboratory Standards Institute. Characterization of *P. aeruginosa* isolates as MDR and XDR was done according to standardized international terminology presented by European Centre for Disease Prevention and Control as well as the Centers for Disease Control and Prevention in 2011. MDR was defined as acquired non-susceptibility to at least one agent in ≥3 antimicrobial categories and XDR was defined as non-susceptibility to at least one agent in ≥6 antimicrobial categories.

Results: The rates of susceptibility to antimicrobials were as follows: gentamicin 27.3%, tobramycin 54.5%, amikacin 56.8%, netilmicin 36.4%, imipenem 55.7%, meropenem 55.7%, doripenem 60.2%, ceftazidime 63.6%, cefepime 56.8%, ciprofloxacin 59.1%, levofloxacin 60.2%, ticarcillin-clavulanic acid 37.5%, piperacillin-tazobactam 63.6%, aztreonam 43.2%, colistin 90.9%, polymyxin 95.5%. Altogether, 48 (54.5%) and 29 (33%) isolates were characterized as MDR and XDR, respectively.

Discussion: The high frequency of antibiotic resistance in clinical isolates of *P. aeruginosa* in Iran makes epidemiological surveillance of susceptibility of this bacterium more essential for the best selection of empirical antibiotics.

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Introduction

Pseudomonas aeruginosa is an opportunistic pathogen in human (1). The most worrisome characteristic of this bacterium is its low antibiotic susceptibility, which is attributable to low permeability of the bacterial cellular envelopes and action of multidrug efflux pumps. In addition to this intrinsic resistance, *P. aeruginosa* can get resistance by mutation either in chromosomally

encoded genes or by the horizontal gene transfer of antibiotic resistance determinants (2, 3). Unfortunately, rates of antibiotic resistance in *P. aeruginosa* are increasing worldwide (1, 2).

Besides, some of strains have shown resistance to multiple antibiotics, which could be mediated by several mechanisms including production of hydrolyzing enzyme, loss of outer membrane protein, efflux systems and target mutations (4). These isolates were named multidrug resistant (MDR), extremely drug resistant (XDR) and pandrug resistant (PDR), according the extreme of their resistance. Infections with these resistant isolates may be associated with increased morbidity and mortality, which can attributed to limited effective antimicrobial options (4, 5).

Review of literature on MDR *P. aeruginosa* has revealed considerably different definitions (6, 7). The absence of specific definitions for MDR in clinical study protocols makes difficult the comparison of data (8). In addition, the true prevalence of MDR isolates cannot well establish (4). However, in the majority of the published studies, multidrug resistance was defined as resistance to at least three drugs from a variety of antibiotic classes, mainly aminoglycosides, antipseudomonal penicillins, cephalosporins, carbapenems and fluoroquinolones (4).

In 2011, a group of international experts came together to create a standardized international terminology with which to describe acquired resistance profiles in bacteria often responsible for healthcare-associated infections and prone to multidrug resistance including *P. aeruginosa*. List of proposed antimicrobial categories for characterization of MDR, XDR and PDR in *P. aeruginosa* are shown in Table 1. MDR was defined as "acquired non-susceptibility to at least one agent in three or more antimicrobial categories", XDR was defined as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories" (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as "non-susceptibility to all agents in all antimicrobial categories" (8).

Since most studies performed in Iran about MDR *P. aeruginosa* do not include these criteria and there is a lack of study about presence of XDR *P. aeruginosa*, this research was designed to detect these phenotypes among *P. aeruginosa* isolated from patients in Tehran, Iran.

Table 1
Antimicrobial categories and agents proposed for characterization of MDR, XDR and PDR in *P. aeruginosa* (8)

Antimicrobial categories	Antimicrobial agents
Aminoglycosides	Gentamicin, tobramycin, amikacin, netilmicin
Carbapenems	Imipenem, meropenem, doripenem
Cephalosporins	Ceftazidime, cefepime
Fluoroquinolones	Ciprofloxacin, levofloxacin
Penicillins/ß-lactamase inhibitors	Ticarcillin-clavulanic acid, Piperacillin-tazobactam
Monobactams	Aztreonam
Phosphonic acids	Fosfomycin
Polymyxins	Colistin, polymyxin b

Materials & Methods

Bacterial strains

The study was performed with the approval of Ethics Committee of Shahed University. Clinical isolates of *P. aeruginosa,* identified as *P. aeruginosa* based on general phenotypic methods (morphology and pigmentation of colony, oxidase test, oxidative carbohydrate utilization, growth at 42 ˚C and on Cetrimide agar) were collected from laboratory of 3 hospitals in Tehran (Pars, Milad and Motahari) in 2013.

PCR using selective *oprL* gene primers (5'-ATGGAAATGCTGAAATTCGGC-3' and

5'-CTTCTTCAGCTCGACGCGACG-3') was used for molecular identification of *P.aeruginosa* (9). Product length of amplicon was 504 base pair. Genomic DNA was extracted from overnight cultures of *P. aeruginosa* by boiling.

Antimicrobial susceptibility test

Disk diffusion method was used for detection of antimicrobial susceptibility in clinical isolates of *P. aeruginosa* according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (10). The following antibiotics disks from MAST Categories Ltd., Merseyside, UK, were used: gentamicin (GM, 10µg), tobramycin (TN, 10µg), amikacin (AK, 30µg), netilmicin (NET, 30µg), imipenem (IMI, 10µg), meropenem (MEM, 10µg), doripenem (DOR, 10µg), ceftazidime (CAZ, 30µg), cefepime (CPM, 30µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), ticarcillin-clavulanic acid (TIM, 85 µg), piperacillin-tazobactam (PTZ, 110µg), aztreonam (ATM, 30µg), colistin (CO, 10µg), and polymyxin B (PB, 300U). Control strain used for all antibiotics disks was *P. aeruginosa* ATCC27853, except for penicillins/ß-lactamase inhibitors, which was *E. coli* ATCC35218.

Detection of MDR and XDR isolates

Defining of MDR and XDR in *P. aeruginosa* isolates were done according to new standardized international document (8), by the results of antimicrobial susceptibility of *P. aeruginosa* to all antimicrobial agents listed in Table 1 except fosfomycin, since interpretive criterion recommendation by CLSI and EUCAST for fosfomycin disk diffusion test of *P. aeruginosa* is not available yet (10, 11).

Therefore, isolates of *P. aeruginosa*, which have shown non-susceptibility to at least one agent in ≥3 antimicrobial categories considered MDR, and isolates exhibit non-susceptibility to at least one agent in ≥6 antimicrobial categories known as XDR.

Results

Antimicrobial susceptibility of 88 *P. aeruginosa* isolates against 16 agents from 7

Table 2
Antimicrobial susceptibility of 88 *P. aeruginosa* isolated from patients in Tehran, Iran

Antimicrobial categories	Antimicrobial agents	Number of isolates (%)		
		Resistant	Intermediate	Susceptible
Aminoglycosides	Gentamicin	44 (50)	20 (22.7)	24 (27.3)
	Tobramycin	39 (44.3)	1 (1.1)	48 (54.5)
	Amikacin	32 (36.4)	6 (6.8)	50 (56.8)
	Netilmicin	41 (46.6)	15 (17)	32 (36.4)
Carbapenems	Imipenem	30 (34.1)	9 (10.2)	49 (55.7)
	Meropenem	39 (44.3)	-	49 (55.7)
	Doripenem	32 (36.4)	3 (3.4)	53 (60.2)
Cephalosporins	Ceftazidime	31 (35.2)	1 (1.1)	56 (63.6)
	Cefepime	34 (38.6)	4 (4.5)	50 (56.8)
Fluoroquinolones	Ciprofloxacin	34 (38.6)	2 (2.3)	52 (59.1)
	Levofloxacin	34 (38.6)	1 (1.1)	53 (60.2)
Penicillins/ß-lactamase inhibitors	Ticarcillin-clavulanic acid	25 (28.4)	30 (34.1)	33 (37.5)
	Piperacillin-tazobactam	25 (28.4)	7 (8)	56 (63.6)
Monobactams	Aztreonam	32 (36.4)	18 (20.5)	38 (43.2)
Polymyxins	Colistin	8 (9.1)	-	80 (90.9)
	Polymyxin B	4 (4.5)	-	84 (95.5)

antimicrobial categories is shown in Table 2. The highest susceptibility was shown to polymyxins categories, which was >90%, and the lowest to gentamicin (27.3%), netilmicin (36.4%),

Table 3
Antimicrobial susceptibility patterns of 88 *P. aeruginosa* isolated from patients in Tehran, Iran. Categories: A= aminoglycosides, B= carbapenems, C= cephalosporins, D= fluoroquinolones, E= penicillins/ß-lactamase inhibitors, F= monobactams, G= phosphonic acids, H= polymyxins, Symbols: □= the isolate was susceptible to all agents listed in categories; ◘= the isolate was non-susceptible to some, but not all agents listed in categories; ■= the isolate was non-susceptible to all agents listed in categories; NT= the isolate was not tested to agents listed in categories

Pattern	Antimicrobial categories								Number of isolates
	A	B	C	D	E	F	G	H	
1	□	□	□	□	□	□	NT	□	3
2	◘	□	□	□	□	□	NT	□	6
3	□	□	◘	□	□	□	NT	□	1
4	□	□	□	□	◘	□	NT	□	11
5	□	□	□	□	□	■	NT	□	3
6	□	◘	□	□	□	■	NT	□	1
7	□	□	□	□	◘	■	NT	□	1
8	◘	□	□	□	□	■	NT	□	2
9	◘	◘	□	□	□	□	NT	□	1
10	◘	■	□	□	□	□	NT	□	1
11	■	□	□	□	◘	□	NT	□	1
12	◘	□	□	□	◘	□	NT	□	9
13	□	□	◘	□	◘	■	NT	□	1
14	◘	□	□	□	◘	■	NT	□	3
15	◘	□	□	◘	◘	□	NT	□	1
16	◘	■	□	□	□	■	NT	□	1
17	◘	◘	□	□	◘	□	NT	□	2
18	□	□	◘	◘	◘	■	NT	□	1
19	◘	□	□	□	◘	■	NT	■	1
20	◘	◘	□	□	◘	■	NT	□	1
21	■	□	■	■	□	■	NT	□	1
22	◘	■	◘	□	□	■	NT	◘	1
23	◘	◘	□	□	◘	■	NT	◘	1
24	◘	◘	■	□	□	■	NT	■	1
25	■	■	■	■	■	□	NT	□	1
26	■	■	◘	■	■	□	NT	□	2
27	■	□	■	■	◘	■	NT	□	1
28	◘	■	■	■	■	■	NT	□	1
29	◘	■	◘	◘	□	■	NT	■	1
30	■	■	◘	■	◘	■	NT	□	2
31	■	■	■	■	◘	■	NT	□	5
32	■	■	◘	■	■	■	NT	□	1
33	■	◘	■	■	■	■	NT	□	1
34	■	■	■	■	■	■	NT	□	15
35	■	◘	■	■	◘	■	NT	◘	1
36	■	■	■	■	◘	■	NT	■	1
37	■	■	■	■	■	■	NT	◘	1

ticarcillin-clavulanic acid (37.5%) and aztreonam (43.2%). In three isolates (3.4%), there was not non-susceptibility to any tested antimicrobial categories.

Non-susceptibility to one and two categories were seen in 21 (23.9%) and 16 (18.2%) isolates, respectively. In addition, non-susceptibility to three, four, five, six and seven categories were seen in 8 (9.1%), 4 (4.5%), 7 (8%), 26 (29.5%) and 3 (3.4%) isolates, respectively. Therefore, non-susceptibility to ≥3 antimicrobial categories was seen in 48 isolates (54.5%), which characterized as MDR *P. aeruginosa*, and non-susceptibility to ≥6 antimicrobial categories were seen in 29 isolates (33%), which characterized as XDR *P. aeruginosa*. Pandrug *P. aeruginosa* was not detected, because non-susceptibility to all used agents was not seen in any isolates.

Antimicrobial susceptibility patterns of studied *P. aeruginosa* isolates and their frequency were shown in Table 3. The most prevalent patterns were as follows and other patterns were seen in ≤3 isolates.

- Susceptibility to all 7 tested categories except aminoglycosides categories, which there is non-susceptibility to at least one of them (pattern 2, 6 isolates)
- Susceptibility to all 7 tested categories except penicillins/ß-lactamase inhibitors categories, which there is non-susceptibility to one of them (pattern 4, 11 isolates)
- Susceptibility to all 7 tested categories except penicillins/ß-lactamase inhibitors categories, which there is non-susceptibility to one of them, and aminoglycosides categories, which there is non-susceptibility to at least one of them (pattern 12, 9 isolates)
- Non-susceptibility to all 7 tested categories except polymyxins categories, which there is susceptibility to both of them and penicillins/ß-lactamase inhibitors categories, which there is non-susceptibility to one of them (pattern 31, 5 isolates)
- Non-susceptibility to all 7 tested categories except polymyxins categories, which there is susceptibility to both of them (pattern 34, 15 isolates)

In XDR *P. aeruginosa* isolates, the two later were the most common pattern and *P. aeruginosa* to all 7 tested categories (patterns 35-37) was shown only in three isolates. All XDR isolates, except four isolates, exhibit susceptibility to polymyxin B and colistin.

Discussion

In this study, the antimicrobial susceptibility of 88 *P. aeruginosa* isolates against 16 agents from 7 antimicrobial categories was determined. Altogether, the highest susceptibility was shown for polymyxin antimicrobials (90.9% and 95.5%, respectively, for colistin, and polymyxin B). Resistance of *P. aeruginosa* clinical isolates to all antibiotics except the polymyxins was shown in many medical centers (3). These agents may not be as effective as first-line agents and may be associated with more significant adverse effects (12, 13).

The difference between the rates of susceptibility of *P. aeruginosa* isolates to different agents in aminoglycosides and penicillins/ß-lactamase inhibitors categories was also shown in this study. Susceptibility to gentamicin and netilmicin was low (27.3% and 36.4%, respectively) comparing susceptibility to amikacin and tobramycin (55% and 51%, respectively), while intermediate phenotype were high to gentamicin and netilmicin. In addition, susceptibility to ticarcillin-clavulanic acid was much less than piperacillin-tazobactam (37.5% and 63.6%, respectively), probably due to antagonism of the bactericidal activity of clavulanate with ticarcillin, which has been shown by other researches (14).

In this study, 48 isolates (54.5%) was recognized as MDR, 29 isolates (33%) as XDR

and there was not PDR among 88 clinical isolates of *P. aeruginosa* isolated from patients in Tehran, Iran. There is few published literature about multidrug resistance in clinical isolates of *P. aeruginosa* in Iran with proper definition of MDR, and the study with our used criteria was not found. However, high prevalence of MDR was reported in the studies defined MDR as resistance to ≥ 3 classes of antibiotics; 100% by Moazami-Goudarzi et al. and Ranjbar et al., 60% by Bayani et al., 45.3% by Nikokar et al. and 33.1% by Salimi et al.(15- 19). In the studies in other countries, lower prevalence was usually reported; Morales et al. 5.46%, De Francesco et al. 20% and Tacconelli et al. 14% (5, 20, 21). Geographic differences in antimicrobial resistance was shown in other studies and population demographics, access to medical care and illicit drug use are some of the variables explaining such differences (4, 22).

One limitation of our study was the absence fosfomycin susceptibility test results of isolates, because we used only disk diffusion test, which interpretive criterion of fosfomycin for *P. aeruginosa* recommended by CLSI and EUCAST is not available yet (10, 11). Since we used all antimicrobial agents listed in Table 1 except this antibiotic, some of MDR and XDR *P. aeruginosa* isolates were not detected in this study.

Conclusion

The observation that high percentage of studied clinical isolates of *P. aeruginosa* in Iran are multidrug and extremely drug resistant (46.6% and 33%, respectively), is worrisome and requires proper methods to prevent the spread of these strains.

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Conflict of interest

The authors declare that there is no conflict of interests.

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