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Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Patients Referring to Hospitals

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Background & Aims of the Study: The aim of this study was to detect and survey the antibiotic resistance pattern of *Pseudomonas (P.) aeruginosa* isolated from patients in Isfahan (located in central Iran) hospitals.

Materials & Methods: A Total of 50 clinical isolates of *P. aeruginosa* were collected from urine, wound, trachea, ear swab, and pus, and then were confirmed by standard tests. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method. Susceptibility data were compared by chi-square test using SPSS version 15.

Results: Among the isolated strains, resistance to oxacillin was seen in 100%, ceftriaxone in 76%, amikacin in 70%, ceftazidime in 68%, cefepime in 68%, tobramycin in 62%, gentamicin in 60%, ciprofloxacin in 58%, and imipenem in 58% of the isolates.

Conclusions: Comparison of the results showed that, patterns of antibiotic resistance are different from one hospital to another in various areas. Therefore, it is suggested that such studies should be performed in different hospitals. Also, prescribing correct medications is essential to prevent further increases in resistant bacteria.

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Background

Infectious diseases are an important cause of morbidity and mortality throughout life and in this regard, opportunistic pathogens play an important role. *Pseudomonas aeruginosa* is an aerobic gram-negative rod shaped bacteria that belongs to the *Pseudomonadaceae* family (1).

The rapid increase of drug resistance in clinical isolates of this opportunistic pathogen has been of worldwide concerns (2,3). *Pseudomonas aeruginosa* is an opportunistic pathogen in burned and cystic fibrosis patients all over the world, especially because of its natural resistance to many classes of antibiotics and also, for its potential virulence factors plus additional acquired resistance due to plasmids. It is also the most common gram-negative bacteria found in nosocomial infections (4-6).

Many *P. aeruginosa* infections occur after patients have been hospitalized. Several factors that account for the success of *P. aeruginosa are as follows*: it can utilize a wide range of nutrients; many strains have acquired resistance

factors; and it has tendency to form biofilms that further protects this pathogen from antibiotics and from the host immune defense.

The bacterium's virulence depends on numerous cell-associated and extracellular factors. Cell-to-cell signaling systems control the expression and allow a coordinated cell-density-dependent production of various extracellular virulence factors. *P. aeruginosa* is naturally resistant to a wide range of antibiotics and may show additional resistance after unsuccessful treatment, particularly via modification of a porin (7).

This pathogen is intrinsically resistant to most antibiotics such as β-lactams, quinolones, chloramphenicol, tetracycline, macrolides, trimethoprim–sulfamethoxazole, and rifampin (8). Resistance in *P. aeruginosa* may be due to outer membrane modifications, production of extended-spectrum beta-lactamase and efflux pumps, which confers various levels of resistance to expanded spectrum cephalosporins, such as cefotaxime, ceftazidime, and aztreonam (9,10).

Aims of the study: Therefore, the aim of this study was detection and survey of antibiotic resistance patterns of *P. aeruginosa* isolated from patients in Isfahan (located in central Iran).

Materials & Methods

A Total of 71 samples were collected (from February to June 2012) from "Al-Zahra" and "Shariati" Hospitals in Isfahan (located in central Iran). Among them, 50 strains of *P. aeruginosa* were isolated. The sample size was calculated using the following formula:

$n = pqz^2/d^2$; where, n=number of samples; pq=estimation of population; z=confidence level; and d=deviation of the ratios.

Information included patients' gender and age, cause of hospitalization, and origin of clinical samples, were collected. The bacteria were biochemically identified using biochemical tests including oxidation-fermentation, oxidase, catalase, green-blue pigment production, growth at 42°C, and production of colorless colonies on MacConkey agar.

Then, for further tests, the samples were kept in a rich environment, brain-heart infusion broth (BHI; Scharlau, Spain) and 30% glycerol, at -70°C. The strains were selected on the basis of their growth on MacConkey medium, which was shown by producingoxidase-positive lactose non-fermenting colonies. Antibiotic susceptibility was confirmed by Kirby-Bauer disc diffusion method on Mueller-Hinton medium, according to the Clinical Laboratory Standard Institute (CLSI) guidelines (11,12). Paper discs (HiMedia, Mumbai, India) were impregnated with the following antibiotics: oxacillin (1 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), imipenem (10 mcg), gentamicin (10 mcg), tobramycin (10 mcg), amikacin (30 mcg), and ciprofloxacin (5 mcg).

Suspension of bacteria (0.5 McFarland) was prepared and inoculated on Mueller-Hinton agar (HiMedia, Mumbai, India) plates using sterile swabs, and then antibiotic discs were placed on it. The plates were incubated overnight at 37°C for 24 hours.

The diameter of the zone of inhibition was measured and compared to that of standard strain, and the results were interpreted as sensitive, intermediate resistant, or resistant, according to the CLSI guidelines.

Quality control was performed utilizing strains from the Iranian Type Culture Collection (PTCC), *P. aeruginosa* 1074.

Data analysis: Susceptibility data were compared by chi-square test using SPSS software version 15 for Windows. Both susceptibility and resistance were calculated as percentages with 95% confidence intervals. The analysis was performed on the cross-tabulated values of the presence of the resistant/intermediate/susceptible isolates, according to the categories of the selected

variable. A p-value of <0.05 was considered to be statistically significant.

Results

Among the 71 samples, a total of 50 *P. aeruginosa* strains were isolated from patients admitted during the study period. Bacteria that were lactose-negative, oxidase positive produced blue-green pigment on Mueller Hinton agar, lactose-negative, and colorless colonies on MacConkey agar were selected as *P. aeruginosa* strains.

Twenty eight patients (56%) were men and 22 patients (44%) were women, and the mean age of all patients was 45 years. The origin of specimens is shown in table 1.

The results of the antibiotic resistance test (Kirby-Bauer disc diffusion method) are shown in table 2. As the results of this study showed, to the higest resistance rate was found for oxacillin (100%), followed by ceftriaxone (76%), amikacin (70%), cefepime (68%), ceftazidime (68%), tobramycin (62%), and gentamicin (60%). The lowest resistance rates were found for ciprofloxacin (58%), and imipenem (58%).

Table 1) Distribution of samples isolated from patients referring to hospitals

Samples	Number	Percentage
Urine	18	36
Ear swab	2	4
Trachea	9	18
Wound	17	34
Pus	4	8
Total	**50**	**100**

Table 2) Antibiotic resistance pattern in *Pseudomonas aeruginosa* strains isolated from clinical specimens

Result / Antibiotic	Sensitive		Semi Sensitive		Resistant	
	Number	Percentage	Number	Percentage	Number	Percentage
Amikacin	14	28	1	2	35	70
Cefepime	13	26	3	6	34	68
Ceftazidime	14	28	2	4	34	68
Ceftriaxone	12	24	0	0	38	76
Ciprofloxacin	20	40	1	2	29	58
Gentamicin	20	40	0	0	30	60
Imipenem	19	38	2	4	29	58
Oxacillin	0	0	0	0	50	100
Tobramycin	16	32	3	6	31	62

Discussion

P. aeruginosa isolates due to inherent resistance to many antibacterial agents, are associated with a higher morbidity and mortality. This resistance is due to the synergy between multi-drug efflux systems or β-lactamase and low outer membrane permeability (13). Also resistance may be due to the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated. The carbapenem hydrolyzing enzyme may be class B metalloβ-lactamases, class D oxacillinase, or class A clavulanic acid inhibitory enzymes (14). In addition, *P. aeruginosa* resistance to imipenem is attributed to diminished expression of certain outer membrane proteins (15).

Maximum resistant isolates of *P. aeruginosa* were isolated from urine samples. In our study, all of the isolates of *P. aeruginosa* were resistant to oxacillin (100%) followed by ceftriaxone (76%), amikacin (70%), ceftazidime (68%), cefepime (68%), tobramycin (62%), gentamicin (60%), ciprofloxacin (58%), and imipenem (58%).

In our study, more than 38% of isolates were sensitive to imipenem and 58% were resistant to this antibiotic. However, in a study by Fazeli et al. (2011) in Isfahan hospitals, it was reported that among 79 isolates of *P.*

aeruginosa isolated from burned patients, resistance to imipenem were observed in 94.9%, ceftazidime in 100%, ciprpfloxacin in 98%, and tobramycin in 95% of isolates.. Therefore, in contrast to our results, the prevalence of resistance was higher in isolates of burns (20). Also, Forozesh fard et al. (2011) reported that among 11 *P. aeruginosa* isolates taken from cystic fibrosis patients of Al-zahra hospital, all isolates were susceptible to imipenem, ticarcillin, ciprofloxacin and piperacillin. The lowest scale of susceptibility belonged to ceftazidime (72.2%) followed by tobramycin (45.4%) (22). It seems that the prevalence of resistance genes in patients with cystic fibrosis is lower than other patients

Strateva et al. (2007) reported that in clinical isolates of *P. aeruginosa*, the resistance to clavulanic acid was 53% and to ticarcilin was 8.22% (16). Mohajeri (2004) reported antibiotic resistance to amikacin by nearly 38% (17). Streit et al. showed that the most active agents tested against *P. aeruginosa* were amikacin, cefepime, tobramycin, meropenem and piperacillin/tazobactam (3.1-13.0% resistance), in the United States (18). Cefepime, ceftazidime and levofloxacin were more resistant antibiotics against *P. aeruginosa*, in lower respiratory tract infections (LRTI) patients, with resistanceof 36.27, 35.30, and 32.35 percent, respectively. In Shahcheraghi et al.'s study in Tehran, the minimum and maximum antibiotic resistance was 9% for imipenem and 97% for ceftizoxime.

Also, 42% of the isolates were resistant to ceftazidime. Thirty five percent of isolates were resistance to amikacin and the result for other antibiotics was as follows: piperacillin-tazobactam, 34%; ciprofloxacin, 41%; ceftazidime, 42%; and piperacillin, 55% (19).

In Arak hospitals (2012), Rahimi and colleagues reported, that among 100 isolates of *P. aeruginosa*, resistance rate to ceftazidime, tobramycin, gentamicin, amikacin imipenem, and ciprofloxacine was 53%, 31%, 38%, 36%, 12%, and 46%, respectively (21). So, it seems that, rates of antibiotic resistance in Isfahan, were higher than those in Arak. This may be due to high antibiotic usage in this region.

Rajat Rakesh et al. (2012) isolated 100 strains of *P. aeruginosa* and found that resistance to tobramycin, gentamicin, piperacillin, ciprofloxacin, ceftazidime was 68%, 50%, 63%, 49%, and 43%, respectively (23). The results of our study were almost similar with those of Rajat Pakesh et al.'s study in India.

Comparing the results of various researches, we can say that the rate of antibiotic resistance in burned patients in Isfahan is higher than other patients, as demonstrated in the study by Fazeli et al. high consumption of antibiotics leads to the emergence of resistant strains of bacteria and as a result, treatments cannot be effective.

Comparison of the results of various studies, it can be stated that antibiotic resistance is different from one hospital to another, one city to another, and one country to another. Therefore, it is suggested that such studies should be carried out in different hospitals of the city.

Footnotes

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Conflict of Interest:

The authors declare no conflict of interest.

References

1. Pagani L, Mantengoli E, Migliavacca R, Nucleo E, Pollini S, Spalla M, et al. Multifocal Detection of Multidrug-Resistant *Pseudomonas aeruginosa*

Producing the PER-1 Extended- Spectrum β-Lactamase in Northern Italy. J Clin Microbiol 2004;42(6):2523–9.

2. Ling TKW, Xiong J, Yu Y, Lee CC, Ye H, Hawkey PM, et al. Multicenter Antimicrobial Susceptibility Survey of Gram-Negative Bacteria Isolated from Patients with Community-Acquired Infections in the People's Republic of China. Antimicrob Agents Chemother 2006;50(1):374–8.

3. Gupta V, Datta P, Agnihotri N, Chander J. Comparative in vitro Activities of Seven New beta-Lactams, Alone and in Combination with beta-Lactamase Inhibitors, Against Clinical Isolates Resistant to Third Generation Cephalosporins. Braz J Infect Dis 2006;10(1):22-5.

4. Lister PD, Wolter DJ, Hanson ND. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin Microbiol Rev 2009;22(4):582–610.

5. Shahid M, Malik A. Plasmid mediated amikacin resistance in clinical isolates of *Pseudomonas aeruginosa*. Indian J Med Microbiol 2004;22(3):182-4.

6. Song W, Woo HJ, Kim JS, Lee KM. In vitro activity of beta-lactams in combination with other antimicrobial agents against resistant strains of *Pseudomonas aeruginosa*. Int J Antimicrobiol Agents 2003;21(1):8-12.

7. Brown PD, Izundu A. Antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa* in Jamaica. Rev Panam Salud Publica/Pan Am J Public Health 2004;16(2):125-30.

8. Dundar D, Otkun M. In-Vitro Efficacy of Synergistic Antibiotic Combinations in Multidrug Resistant *Pseudomonas Aeruginosa* Strains. Yonsei Med J 2010;51(1):111-6.

9. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, McCaskey LA, et al. Prevalence, Resistance Mechanisms, and Susceptibility of Multidrug-Resistant Bloodstream Isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2010;54(3):1160-4.

10. Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli F, Goli H, Kalantari N. Detection of VEB-1, OXA-10 and PER-1genotypes in extended-spectrum beta-lactamase- producing *Pseudomonas aeruginosa* strains isolated from burn patients. Burns.2010;36(1):70-4.

11. Patzer JA, Dzierzanowska D. Increase of imipenem resistance among *Pseudomonas aeruginosa* isolates from a Polish paediatric hospital (1993-2002). Int J Antimicrob Agents 2007;29(2):153-8.

12. Walkty A, Decorby M, Nichol K, Mulvey MR, Hoban D, Zhanel G; Canadian Antimicrobial Resistance Alliance. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates obtained from patients in Canadian intensive care units as part of the Canadian National Intensive Care Unit study. Diagn Microbiol Infect Dis 2008;61(2):217-21.

13. Livermore DM. Of *Pseudomonas*, porins, pumps and carbapenems. J Antimicrob Chemother 2001;47(3):247-50.

14. Navneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metalloβ- lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. Indian J Med Res 2002;116:26, 4-7.

15. Büscher KH, Cullmann W, Dick W, Opferkuch W. Imipenem resistance in *Pseudomonas aeruginosa* resulting from diminished expression of an outer membrane protein. Antimicrob Agents Chemother 1987;31(5):703–8.

16. Strateva T, Ouzounova-Raykova V, Markova B, Todorova A, Marteva-Proevska Y, Mitov I. Problematic clinical isolates of *Pseudomonas aeruginosa* from the university hospitals in Sofia, Bulgaria: current status of antimicrobial resistance and prevailing resistance mechanisms. J Med Microbiol 2007;56(7):956-63.

17. Mohajeri P. Antibiotic susceptibility and resistance patterns of *pseudomonas aeruginosa* strains isolated from different clinical specimens in patients referred to the teaching hospitals in Kermanshah (2001-2). Behbood Res J Kermanshah Univ Med Sci 2004;7(4):11-20. (Full Text in Persian)

18. Streit JM, Jones RN, Sader HS, Fritsche TR. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the Sentry Antimicrobial Surveillance Program (North America, 2001). Int J Antimicrob Agents 2004;24(2):111-8.

19. Shacheraghi F, Shakibaie MR, Noveiri H. Molecular Identification of ESBL Genes blaGES-1, blaVEB-1, blaCTX-M blaOXA-1, blaOXA-4, blaOXA-10 and blaPER-1 in *Pseudomonas aeruginosa* Strains Isolated from Burn Patients by PCR, RFLP and Sequencing Techniques. Int J Biol life Sci 2010;3(6):138-42.

20. Fazeli H, Moslehi Tekantapeh Z, Irajian GHR, Salehi M. Determination of drug resistance patterns and detection of bla-vim gene in *pseudomonas aeruginosa* strains isolated from burned patients in the Imam Mosa Kazem Hospital, Esfahan, Iran (2008-9). Iran J Med Microbiol 2010;3(4);1-8. (Full Text in Persian)

21. Rahimi B, Shojapour M, Sadeghi A, Pourbabayi A. The study of the antibiotic resistance pattern of

Pseudomonas aeruginosa strains isolated from hospitalized patients in Arak. Arak Univ Med Sci J 2012;15(3):8-14. (Full Text in Persian)

22. Forozesh Fard M, Irajian G, Moslehi Takantape Z, Fazeli H, Salehi M, Rezania S. Drug resistance pattern of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients at Isfahan AL Zahra hospital, Iran (2009-2010). Iran J Microbiol 2012;4(2):94-7. (Full Text in Persian)

23. Rajat Rakesh M, Ninama Govind L, Mistry K, Parmar R, Patel K, Vegad MM, Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care hospital, Ahmadabad. Natl J Med Res 2012;2(2):156-9.