Journal of Medical Bacteriology

Antimicrobial Resistance Pattern and Presence of Beta-Lactamase Genes in *Pseudomonas aeruginosa* Strains Isolated from Hospitalized Patients, Babol-Iran

Akram Amini [1], Amirmorteza Ebrahimzadeh Namvar [2]*

[1] *Student Research Committee, Babol University of Medical Sciences, Babol, Iran.*

[2] *Department of Microbiology, School of Medicine, Babol University of Medical Sciences, Babol, Iran.*

ARTICLE INFO	ABSTRACT
Article type: Original Article	***Background***: *Pseudomonas aeruginosa* has recently emerged as one of the main causes of nosocomial infections in the intensive care unit due to vast antibiotic resistance potency. The aim of this study was to investigate the antimicrobial resistance pattern and evaluation of the frequency of beta-lactamase genes in *Pseudomonas aeruginosa* strains.
	Methods: Generally, 42 *P. aeruginosa* strains were collected from different clinical specimens. The phenotypic and genotypic tests such as disc diffusion, Modified Hodge test and molecular detection of *blaIMP*, *blaIMP1*, *blaVIM2*, and *blaKPC* genes were performed. ***Results*** According to the results, by disk diffusion method 33 (78.58%) to Cefotaxime, 31 (73.8%) of strains were resistant to Ceftriaxone, and 31 (73.8%) to Co-trimoxazole, while the lowest resistance was observed in case of Polymyxin B 3 (7.15%). On the other hand 4 (9.52%) of strains were recognized as MHT positive. Moreover, 26 (61.9%) of isolates were detected as multi drug resistance strains. In addition, 2 (4.7%) of isolates harbored *blaIMP*, 2 (4.7%) blaIMP1 and 10 (23.8%) blaVIM2 genes, whereas the *blaKPC* gene was not reported.
	Conclusion: According to the results, the prevalence of beta-lactamase genes and antibiotic resistance pattern in patients with high levels of hospitalization is essential. Therefore, it is necessary to identify the strains containing β-lactamase genes for better control and treatment.

*Corresponding Author: Amirmorteza Ebrahimzadeh Namvar, Department of Microbiology, School of Medicine, Babol University of Medical Sciences, Babol, Iran.

Tel: +98- 11-32199592, *E-mail:* amirmorteza.namvar@gmail.com

Introduction

Pseudomonas aeruginosa is A Gram-negative obligate aerobic, spore-free and motile bacilli that can be found in water, soil and the human natural flora (1). This microorganism is regarded as one of the human opportunistic pathogens, which may lead to widespread diseases such as septicemia, pneumonia, wound, respiratory and urinary tract infections, also responsible for approximate 10% of hospital infections, moreover is a major contributor to chronic pulmonary infections with cystic fibrosis patients (2, 3).

The presence of efflux pumps, reduced permeability of the outer membrane and secretion of the β-lactamase enzyme are the main prominent features of this bacteria for the inherent resistance to many antibiotics, including third-generation cephalosporins, imipenem, and aztreonam (4). According to the Ambler's classification, the beta-lactamases are divided into four categories (A to D), in which types A, C and D are serine β-lactamase, while type B is metallo-beta-lactamase (MBL), which are located within the integron and have the ability to integrate into the plasmid or chromosome, so can transfer to other bacteria family, including Enterobacteriaceae sp. It should be noted that MBL have zinc binding motifs around the active site and these active sites are inhibited by the ethylene diamine tetraacetic acid (EDTA), but, clavulanate/tazobactam. The MBL enzymes (Type B) are subdivided into B1-B3 that according to molecular structure the subgroup B1 is classified into four categories: *blaIMP*, *blaVIM*, *blaGIM* and *blaSPM* (5-7). For the first time the *blaIMP1* enzyme was identified in *Serratia marcescens* in Japan, and blaVIM2 was reported in Italy (8). Class A of bacterial β-lactamases which are widely identified in Enterobacteriaceae family are resistant to cephalosporins and inhibited by clavulanic acid and tazobactam. The *blaPER*, *blaGES*, *blaVEBs*, *blaTEMs*, *blaSHVs* and *blaCTX-Ms* are the most known common extended-spectrum beta-lactamases (ESBLs).

However blaKPC with an intrinsic carbapenemases activity is classified in class A (9).

Carbapenems (imipenem and meropenem) are the most important antimicrobial antibiotics used to treat *P. aeruginosa* strains (10). In the past two decades antibiotic resistance is a critical clinical problem and a serious threat to national health. Infections caused by antibiotic-resistant microorganisms lead to prolonged hospitalization and an increase in mortality rates and higher medical costs compared with antibiotic susceptible microorganisms. Most of the studies that researchers have done on this bacterium are about aspects of drug resistance, which is an important treatment factor to limit the hospital infections.

Regarding the importance of *P. aeruginosa* infections and the frequency of multidrug resistance strains (MDR), the phenotypic and genotypic identification is concerned; so, the aim of this study was to investigate the antibiotic resistance patterns and beta lactamase genes such as *bla-IMP1*, *blaIMP*, *blaKPC* and *bla-VIM2* in *P. aeruginosa* strains isolated from hospitalized patients

Materials and Methods
Sampling and isolation of bacteria

In this descriptive cross-sectional study, in the period from November 2016 to August 2017, a total of 42 *Pseudomonas aeruginosa* strains were collected from ICU ward of educational hospitals in Babol city. Thereafter, identification and confirmation of strains were performed by using microbiological tests and finally stored at -20 °C on BHI broth containing 15% glycerol.

Antimicrobial Resistance testing

To determine the multidrug resistance phenotype, a disk diffusion method (Kirby-Bauer) was used according to Clinical and Laboratory Standards Institute 2016 by following antibiotics

(11): Imipenem (10µg), Meropenem (10µg), Ceftazidime (30µg), Cefotaxime (30µg), Polymyxin_ B (300µg), Tobramycin (10µg), Levofloxacin (5µg), Ceftriaxone (30µg), Fosfomycin (200µg) and Co-Trimoxazole (25µg) (Rosco-Denmark). *Escherichia coli* ATCC 25922 was used as a quality control (Babol University of medical sciences, Iran).

Modified Hodge Test (MHT)

At first a bacterial suspension equivalent to a 0.5 McFarland standard of *E. coli* ATCC25922 was inoculated on Muller-Hinton agar medium. The Ertapenem (10µg) antibiotic disc was placed in the center of the culture medium. The test organism, positive and negative controls were cultured in a straight line from the disc edge to the edge of the plate and incubated for 24 hours at 37 °C. A cloverleaf-like indentation of the *E. coli* ATCC 25922 growing along the test organism growth streak within the disc diffusion zone was considered as a positive MHT.

Genotypic tests

In order to perform the molecular reaction test, the bacterial genomic DNA was first extracted by a unique commercial kit (Yektatajhiz, Iran), and then the PCR test was conducted by specific primers which were listed in (Table 1). Each PCR reaction was carried out in a final volume of 25µl using a Mastermix prepared by the Gene Fanavaran (Tehran, Iran). Finally, 1.5% agarose gel electrophoresis was used for identification of DNA fragments. The PCR program is shown in (Table 2).

Result

During 10 months of study a total of 42 *P. aeruginosa* strains were collected from ICU ward of educational hospitals in Babol city. Out of 42 *P. aeruginosa* strains, %62 and %38 were isolated from male and female respectively. The specimens included urine (n=17, 40/47%), blood

(n= 11, 26/19%), tracheal aspirate (n=9, 21/42%), wound (n=3, 7/1%), catheter (n=1, 2/38%) and peritoneal fluid (n=1, 2/38%).

In accordance to disk diffusion method the most resistance was achieved in Cefotaxime 33 (78.58%), Ceftriaxone 31(73.8%), and Co-Trimoxazole 31 (73.8%), respectively. In addition the lowest resistance was belonged to Polymyxin B 3 (7.15%) (Figure 1).

Also, 26 (61/9%) of isolates were distinguished as MDR strains. On the other hand 4) 9.52% (of isolates were recognized as MHT positive. Among 42 isolates, 10 (23.8%) harbored *blaVIM2*, 2 (4.7%) *blaIMP* and 2 (4.7%) *blaIMP1* due to our results none of the strains were positive for *blaKPC* gene (Figure 2).

Discussion

Carbapenems are one of the best options for treating infections caused by gram-negative bacteria resistant to multiple drugs. In the present study, a high resistance to all available antimicrobials was observed, which is regarded as a serious threat to limit the treatment options in our hospitals.

This can be explained by the increasing use of antimicrobial agents over the past decade that has been led to using the selective antibiotics in *P. aeruginosa*. The most resistance genes are located in transportable elements such as plasmids and integrons, thus can easily be transmitted between different strains of a bacterium and even different bacteria. Due to genetic continuity the resistance to the other family of antibiotics including Aminoglycosides, Sulfonamides, and Fluoroquinolones can be occurred.

In a conducted research by Mirbagheri et al. (48.5%) were resistant to imipenem, (100%) to Tobramycin, (62.6%) to ceftazidime, (88.4%) to Meropenem (64.4%) to ceftriaxone and (54.3%) to gentamicin. The frequency of *blaVIM1* and *blaVIM2* genes were (58.7%) and (3.17%) among imipenem-resistant isolates (16). However, in the present study, the highest resistance to cefotaxime (78.58%) and the lowest resistance to Meropenem

Table 1.   Primers used for detection of *blaIMP*, *blaIMP1*, *blaVIM2*, and *blaKPC*.

Primer name	Primer sequence (5 to 3)	Product size (bp)	Reference
IMP1-F IMP1-R	TGAGCAAGTTATCTGTATTC TTAGTTGCTTGGTTTTGATG	740	(12)
VIM2-F VIM2-R	CAGATTGCCGATGGTGTTTGG AGGTGGGCCATTCAGCCAGA	523	(13)
IMP-F IMP-R	GGAATAGAGTGGCTTAATTCTC CCAAACCACTAGGTTATCT	188	(14)
KPC-F KPC-R	GTATCGCCGTCTAGTTCTGC GGTCGTGTTTCCCTTTAGCC	636	(15)

and Tobramycin (23.8%) were obtained. Various statistics have been reported on *Pseudomonas aeruginosa* resistance to carbapenems in Iran. Since some isolates were resistant to imipenem and meropenem in this study, the isolates studied in this study Belong to the same strain. Generally, in Iran resistance to Carbapenems is lower than other antibiotics; So that the effectiveness of these antibiotics could be promising in the treatment of *Pseudomonas* infection (17).

Improper use of antibiotics in medicine has led to a rise in resistant strains. This resistance induces changes in the receptor or the location of the antibiotic activity by induction of inactive antibiotics or mutation in the genes encoding the membrane proteins. In a study by Zafer et al, from 122 *P. aeruginosa* isolates, the following resistance was obtained: Imipenem (39.3%), ceftazidime (60.6%), gentamicin (50%) and ciprofloxacin (43.4%), also (27%) of strains were MBL producer, in which the prevalence of *blaVIM2* and *blaIMP1* gene were (58.3%) and (2.1%) respectively (18). The results of these studies are similar to those of the present study; therefore, antibiotic resistance seems to be *Pseudomonas aeruginosa* can vary from city to

city and from hospital to hospital. Rahimzadeh et al., examined 51 strains of *Pseudomonas aeruginosa* that 63% of the samples had the *blaIMP* gene (19), which is not readily available from the research findings. In Aghamiri et al. study,the frequency of *blaIMP* and *blaVIM* genes was reported %9, %33 respectively (20).
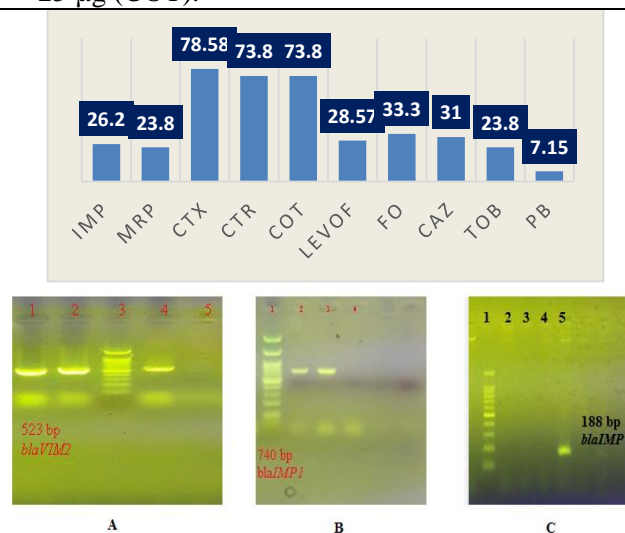
Figure 1.   Antibiotic Resistance pattern in *P. aeruginosa* isolates Imipenem 10µg (IMI), Meropenem 10µg (MRP), Ceftazidime 30µg (CAZ), Cefotaxime 30µg (CTX), Polymyxin B 300µg (PB), Tobramycin 10µg (TOB), Levofloxacin 5 µg (LVOF), Ceftriaxone 30 µg (CTR), Fosfomycin 200 µg (FO), Co-trimoxazole 25 µg (COT).

Figure 2.   A: Lanes 1, 2: positive strains for blaVIM2 gene; Lane 3: DNA Size Marker (100bp); Lane 4: Positive control; Lane 5: negative control. B: Lanes 1: DNA Size Marker (100bp); Lane 2: Positive strain for *blaIMP1*; Lane 3: Positive control; Lane 4: negative control. C: Lane 1: DNA Size Marker (100bp); Lane 2 & 3: Negative Strains; Lane 4: Negative control; Lane 5: Positive strain.

Table 2.   The PCR program for amplification of *blaIMP1*, *blaVIM2*, *blaIMP* and *blaKPC* genes.

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
bla_{IMP1}	94°C for 4min	94°C for 40Sec	56°C for 40 Sec	72°C for 45 Sec	72°C for 4min
		35 cycles			
bla_{VIM2}	94°C for 4min	94°C for 40Sec	54°C for 40 Sec	72°C for 45 Sec	72°C for 4min
		35 cycles			
bla_{IMP}	94°C for 4min	94°C for 40Sec	52°C for 40 Sec	72°C for 45 Sec	72°C for 4min
		35 cycles			
bla_{KPC}	94°C for 4min	94°C for 40Sec	56°C for 40 Sec	72°C for 45 Sec	72°C for 4min
		35 cycles			

Conclusion

As a conclusion, rapid identification and monitoring the epidemiological outbreak of the strains which producing these enzymes is essential.

Acknowledgment

Conflict of interest

None declared conflict of interest

References

1. Adel K, Sabiha S. Genetic site determination of antibiotic resistance genes in "*Pseudomonas aeruginosa*" by genetic transformation. *Br J Pharmacol Toxicol* 2010; **1**(2):85-9.
2. Cross A, Allen JR, Burke J, et al. Nosocomial infections due to *Pseudomonas aeruginosa*: review of recent trends. *Rev Infect Dis* 1983; **5**(Supplement_5):S837-S45.
3. Jefferies J, Cooper T, Yam T, et al. *Pseudomonas aeruginosa* outbreaks in the neonatal intensive care unit–a systematic review of risk factors and environmental sources. *J Med Microbiol* 2012; **61**(8):1052-61.
4. Huddleston JR, Zak JC, Jeter RM. Antimicrobial susceptibilities of *Aeromonas* spp. isolated from environmental sources. *Appl Environ Microbiol* 2006; **72**(11):7036-42.
5. Howard C, van Daal A, Kelly G, *et al*. Identification and minisequencing-based discrimination of SHV β-lactamases in nosocomial infection-associated *Klebsiella pneumoniae* in Brisbane, Australia. *Antimicrob Agents Chemother* 2002; **46**(3):659-64.
6. Pitout JD, Gregson DB, Poirel L, et al. Detection of *Pseudomonas aeruginosa* producing metallo-β-lactamases in a large centralized laboratory. *J Clin Microbiol* 2005; **43**(7):3129-35.
7. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; **39**(6):1211.

8. Poirel L, Magalhaes M, Lopes M, et al. Molecular analysis of metallo-β-lactamase gene blaSPM-1-surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil. *Antimicrob Agents Chemother* 2004; **48**(4):1406-9.

9. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob* 2015; **45**(6):568-85.

10. Rodrigues ACS, Chang MR, Nóbrega GD, et al. Metallo-β-lactamase and genetic diversity of *Pseudomonas aeruginosa* in intensive care units in Campo Grande, MS, Brazil. *Braz J Infect Dis* 2011; **15**(3):195-9.

11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. M100-S25. CLSI, Wayne, Pennsylvania 19087 USA, 2016.

12. Moosavian M, Rahimzadeh M. Molecular detection of metallo-β-lactamase genes, blaIMP-1, blaVIM-2 and blaSPM-1 in imipenem resistant *Pseudomonas aeruginosa* isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. *Iran J Microbiol* 2015; **7**(1):2.

13. Lagatolla C, Tonin EA, Monti-Bragadin C, et al. Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired metallo-β-lactamase determinants in European hospital. *Emerg Infect Diseases* 2004; **10**(3):535.

14. Ellington MJ, Kistler J, Livermore DM, et al. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. *J Antimicrob Chemother* 2006; **59**(2):321-2.

15. Wolter DJ, Khalaf N, Robledo IE, et al. Surveillance of carbapenem-resistant *Pseudomonas aeruginosa* isolates from Puerto Rican medical center hospitals: dissemination of KPC and IMP-18 β-lactamases. *Antimicrob Agents Chemother* 2009; **53**(4):1660-4.

16. Mirbagheri SZ, Meshkat Z, Naderinasab M, et al. Study on imipenem resistance and prevalence of blaVIM1 and blaVIM2 metallo-beta lactamases among clinical isolates of *Pseudomonas aeruginosa* from Mashhad, Northeast of Iran. *Iran J Microbiol* 2015; **7**(2):72.

17. Ahmadi A, Soltanpour MM, Imani Fooladi AA. Prevalency of imipenem-resistant bacterial strains isolated from hospital and accuracy of Iranian imipenem disc product. *J Gorgan Univ Med Sci* 2015; **17**(1):61-6.

18. Zafer MM, Al-Agamy MH, El-Mahallawy HA, et al. Antimicrobial resistance pattern and their beta-lactamase encoding genes among *Pseudomonas aeruginosa* strains isolated from cancer patients. *BioMed Res Int* 2014:101635.

19. Rahimzadeh Torabi L, Doudi M, Golshani Z, The frequency of blaIMP and blaVIM carbapenemase genes in clinical Isolates of *Pseudomonas aeruginosa* in Isfahan medical centers. *Medical Journal of Mashhad* Jul-Aug 2016: **59**(3):139-147.

20. Aghamiri S, Amirmozafari N, Fallah Mehrabadi J, et al. Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including bla-IMP and bla-VIM Types in *Pseudomonas aeruginosa* Isolated from Patients in Tehran Hospitals. *ISRN Microbiology* 2014: 941507.