

The Frequency of *imp* and *vim* Genes Among *Pseudomonas aeruginosa* Isolates From Children's Medical Center of Tehran

Fatemeh Bagheri Bejestani¹; Mojdeh Hakemi-Vala^{2,*}; Raheleh Momtaheni¹; Ozra Bagheri Bejestani³; Mehrdad Gholami²

¹Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, IR Iran

²Department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

³Molecular Biology Research Center, Baghiyatallah University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Mojdeh Hakemi-Vala, Department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences, Velenjak, Tehran, IR Iran. Tel: + 98-2123872556, Fax: + 98-2122439964, E-mail: m.haklemi@sbmu.ac.ir

Received: June 6, 2014; Revised: December 30, 2014; Accepted: January 13, 2015

Background: The first report of *Pseudomonas aeruginosa* (*P. aeruginosa*) carbapenem resistant strains, and especially the Metallo-Beta-Lactam (MBL) producers was from Japan, however, further reports were global.

Objectives: The current study aimed to determine the frequency of *vim* and *imp* genes among *P. aeruginosa* MBL producer isolated from Children Medicinal Center during from 2011 to 2012.

Materials and Methods: In the current descriptive study, 90 *P. aeruginosa* strains were collected from different clinical samples of children referring to Children's Medical Center of Tehran, Iran. All the isolates were identified and confirmed by the standard tests. Their resistance against common antibiotics was determined by disk diffusion method based on Clinical and Laboratory Standards Institute (CLSI) 2010 protocol. The MBL producers were screened by Combined Disk Diffusion test (CDDT) and using *imp-imp*/Ethylen Ediamine Tetra Acetic Acid (EDTA), and increasing of 7 mm \leq the diameter of inhibiting zone. The frequency of *vim* and *imp* genes was determined by Polymerase Chain Reaction (PCR).

Results: Based on standard tests including Triple Sugar Iron Agar and Oxidation-Fermentation (OF) media and disability of the bacteria for glucose fermentation, 90 *P. aeruginosa* strains were confirmed. Their resistance against the following antibiotics was evaluated by disk diffusion method: 36.6% to cefotaxim, cefpodoxim and ticarcillin 33.3%, meropenem and aztreonam 32.2%, amikacin 28.8%, ciprofloxacin and ceftriaxon 27.7%, ceftazidime 22.3% and imipenem 15.5%. By PCR the frequency of *imp* gene with its 328 base pair band was (3.3%). The *vim* gene was not detected among the tested strains.

Conclusions: Despite of high resistance of *P. aeruginosa*, the resistance rate of *P. aeruginosa* strains isolated from children in the current study was not high. Then accurate prescription of antibiotics can decrease the speed of resistance creation.

Keywords: *Pseudomonas aeruginosa*; Beta-Lactamase; Imipenem

1. Background

Pseudomonas aeruginosa (*P. aeruginosa*) is the major cause of the life threatening agent in nosocomial infections. Although *P. aeruginosa* strains are intrinsically resistant against beta lactams, they are usually sensitive to carbapenems (1). Therefore, carbapenems are usually the last chance in multidrug resistant strains (2). World-wide emergence of new resistant strains, due to change in their intrinsic and acquired resistant genes urge more studies to identify resistance profile of local colonies (3). After several worldwide reports on the emergence of *P. aeruginosa* resistant strains, it was found that being armed with different resistant mechanisms like enzyme production, changes in poring permeability, and efflux pumps were presumptive resistance reasons of the bacteria. Metallo-Beta-Lactamase (MBL) production, which is related to plasmidic genes like *imp* and *vim* is a common mechanism of the acquired resistance to carbapenems.

MBL is related to class B Ambler which can hydrolyze Beta-Lactams, cephalosporins, and carbapenems. Their function is inhibited by Ethylenediaminetetraacetic Acid (EDTA) and thiol compounds (4-7). There are many reports on *P. aeruginosa* MBL producers from Asia, South Europe, and Brazil (8-10).

2. Objectives

The current study aimed to determine the frequency of *vim* and *imp* distribution among *P. aeruginosa* MBL producer strains isolated from patients of Children Medicinal Center during from 2011 to 2012.

3. Materials and Methods

Bacterial isolates: In this descriptive study, 90 *Pseudomonas aeruginosa* (*P. aeruginosa*) strains were isolated from different clinical samples of children under 18 years old referring to Children Medicinal Center of Tehran, Iran

during from September 2011 to March 2012. The sample size was determined based on the frequency of MBL producer *P. aeruginosa* isolates (4.8%), with 95% confidence interval and distance 0.1 in the standard formula ($N = (p)(q)(1.96)^2/d^2$) (11).

Bacterial isolation and identification was done based on standard bacteriologic methods including Oxidase test, Glucose fermentation in Triple Sugar Iron agar (TSI) (Merck, Germany), and Oxidation-Fermentation (OF) test, growing in 42°C, and motility and pigmentation (12). Only the *P. aeruginosa* strains, isolated during six months (from September 2011 to March 2012) were included in this study. Therefore, all replicated isolates were excluded from the study. The ethical approved code was the same as that of grant number 11505. All information regarding the patient's name and gender were reserved in this study.

Antimicrobial sensitivity test: the sensitive or resistance of the isolated bacteria was evaluated against the following antibiotics, based on CLSI 2012: ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (10 µg), and ciprofloxacin (30 µg), ceftriaxon (30 µg), cefotaxim (30 µg), cefpodoxim (10 µg), ticarcillin (75 µg) (all purchased from Mast Ltd, UK) (13).

All plates were incubated at 37°C for 18 hours. Finally, the diameter of zone of inhibition was determined. *Pseudomonas aeruginosa* American ATCC 27853 and *Escherichia coli* 25922 were simultaneously used as control strains. Minimum Inhibitory Concentration (MIC) of imipenem was detected by E-test strips (Lioflichem, Denmark) in the similar conditions of antibiotic sensitivity test based on CLSI 2012 protocol.

3.1. Combination Disk Diffusion Test (CDDT)

Continuously, Metallo-Beta-Lactamase (MBL) producers were screened by imipenem disk alone and in combination with 5 µ EDTA (0.5 M). Any changes in diameter zone of inhibition ≥ 7 mm were considered as MBL positive (13).

3.2. DNA Extraction

To determine the frequency of resistant genes, at first DNA extraction was done by the boiling method. The

concentration of extracted DNA was determined at 260 nm and using nano-drop. Simultaneously, *P. aeruginosa* strain PAO1 was used as the quality control strain.

3.3. Polymerase Chain Reaction Method

Frequency of *imp* and *vim* genes were detected by primers (and Polymerase Chain Reaction (PCR) programs as follow: Initiation denaturation 95°C five minutes, denaturation 95°C one minute, annealing 55°C one minute, extension 72°C one minute, and final extension 72°C five minutes (35 cycles). The primer sequences were obtained from the data bank, National Center for Biotechnology Information (NCBI). A *P. aeruginosa* isolate with mutated *imp* gene submitted with accession No. JX644173 was used as the control strain, simultaneously. The PCR products were visualized after staining by ethidium bromide and under Ultraviolet (UV) irradiation.

3.4. Data Analysis

All data was submitted in an excel sheet and the results were analyzed by SPSS software version 16.0.

4. Results

During six months from September 2011 to March 2012, 90 *P. aeruginosa* strains were isolated from different clinical samples from children referring to Children's Medicinal Center of Tehran, Iran. All patients were under 18 years old. The clinical samples were included: 50 urine, 15 blood, 14 tracheal aspirates, five wound swabs, four biologic fluids, and two throat swabs. Bacteria were identified based on the results of the following biochemical and bacteriologic tests: Gram negative bacilli, oxidase positive, non-fermenter (K/K) on TSI and OF media, and green pigmentation on Muller-Hinton Agar. Resistance of the isolates against the tested antibiotics was as follows, based on disk diffusion method: 36.6% to cefotaxim, cefpodoxim and ticarcillin 33.3%, meropenem and aztreonam 32.2%, amikacin 28.8%, ciprofloxacin and ceftriaxon 27.7%, ceftazidime 22.3%, and imipenem 15.5%. MIC of imipenem resistant isolates was determined by E-test as 64 µg/mL (Table 2). The resistance criteria for the tested antibiotic

Table 1. Sequence of Used Primers

Genes	Primers	PCR Product Size, bp
<i>imp</i>	5' GAAGCGTTTATGTTTCATAC 3'; 5' GTAAGTTTCAAGAGTGATGC 3'	587
<i>vim</i>	5' GTTTGGTTCGCATATCGCAAC 3'; 5' AATGCGCAGCACCCAGGATAG 3'	382

Table 2. The Minimum Inhibitory Concentration Range for *imp* Among *P. aeruginosa* Strains^a

Antibiotic	<i>Pseudomonas aeruginosa</i>		
	MIC50	MIC90	MIC Range
Imipenem	32	64	2 - 128

^a Data is Presented as µg/mL.

disks, MIC for imipenem in E-test, and the difference more than 7 mm between the inhibition zones of imipenem and imipenem/EDTA were determined based on the CLSI 2012; therefore, the frequency of MBL production was (3.3%). The frequency of *imp* gene with its 587 base pair band was detected in three out of 90 *P. aeruginosa* (3.3%) isolates. The *vim* gene was not detected in any of MBL positive isolates (0%).

5. Discussion

In the current study, 90 *P. aeruginosa* strains were isolated from non-duplicated clinical samples of children referring to Children's Medicinal Center of Tehran, Iran during six months from September 2011 to March 2012. The resistance rate of all isolates was determined against common antibiotics by disk diffusion method; MIC was also determined for imipenem by E-test strips. It was cleared that out of 10 tested antibiotics the highest resistance rate was detected against cefotaxim (36.6%), and the least rate was found against imipenem (15.5%). Fazeli et al. indicated that resistance rate of *P. aeruginosa* against ceftazidime was 83.3%. The other study in Tabriz, Iran, declared that, 50% of *P. aeruginosa* strains were resistant against this group of antibiotics (14, 15). In another study conducted by Hakemi-Vala, 62.95% of *P. aeruginosa* strains isolated from burnt wounds showed resistance against ceftazidim (16). These results were in contrast to the obtained results which indicated that, only 23.3% of *P. aeruginosa* strains were resistant against ceftazidim. All aforementioned studies were performed in different parts of Iran in different time; therefore, their origin is not same and, the difference between the results may derive from the group of patients, time of sampling, and the origin. *Pseudomonas aeruginosa* are the responsible for the life threatening conditions in burnt patients with immunodeficiency. Then, isolation of *P. aeruginosa* strains with higher resistance against the antibiotics from such patients is not strange. Also, border cities, may have better conditions for immigration and exchange of resistant bacteria. Indiscriminate use of antibiotic is another reason for this discrepancy. In addition, the bacterial origin in the current study was the children referring to Children's Medicinal Center of Tehran, Iran. Then, proper antibiotic consumption may be related to lower rates of antibiotic resistance.

In Fazeli et al. study, none of the *P. aeruginosa* strains were MBL producer based on CDDT and all the isolates were sensitive to imipenem and meropenem; also, none of *bla*, *imp*, and *vim* genes were detected by PCR (14). Some of these results were in contrast to our study which showed three out of 90 *P. aeruginosa* isolates were MBL producer (3.3%) and all these three isolates carried *imp* gene after PCR analysis. However, no *vim* gene was detected among tested *P. aeruginosa* isolates in both studies. As mentioned before, the difference between time of sampling, change in treatment protocol, and also different primers sequences, which were used, may be the main factors which cause the difference between the two studies.

In Shahcheraghi et al. study of 610 *P. aeruginosa* isolates 68 were *imipenem* resistant with (MIC \geq 4 μ g/mL) and 16 out of 68 were positive for Verona Integron encoded MBL (VIM-1) gene by PCR. The difference in *imipenem* resistant between the two studies (11.14% in Shahcheraghi study vs. to 3.3% in the recent study, respectively) may be related to

the difference in time, hospitals, and also age of patients (adults vs. to children in the recent study), who were from different wards of hospitals, in Shahcheraghi study compare to children who referred to Children's Medicinal Center of Tehran, Iran in the recent study (17).

Children are the most vulnerable part of a population, because of their immature immune system. So, the results of any research about their infectious diseases and microbial agents, including this study are very important. One of the weak points of this study is limitation of the recent results to only 90 bacterial strains which were isolated from children who referred to Children's Medicinal Center of Tehran, Iran. To generalize giving results to all *P. aeruginosa* strains, originated from other children of Tehran, Iran and also Iran, the sample size must be extended and included more hospitals. However, the position of this hospital as the main Children's referral Center in Tehran, Iran and even Iran is very important.

In conclusion, despite of many reports from Iran, or/ even worldwide about high resistance rate to carbapenems among *P. aeruginosa* strains nowadays, hopefully, in this study the resistant rate to carbapenems among *P. aeruginosa* originated from the children of an important Children's Health Center of Tehran, Iran was not high. Also, doing antimicrobial sensitivity test before any prescription is highly recommended. In addition, prudent consuming of the antibiotics under physician surveillance and awareness of patients about its right taking can reduce the emergence of resistant strains.

Acknowledgements

We like to say our thanks to Dr. Bahram Nikmanesh and his team in Children's Medicinal Center of Tehran for their support in sample collection.

Authors' Contributions

The study concept and design was done by Mojdeh Hakemi-Vala and Fatemeh Bagheri Bejestani; analysis and interpretation was done by Raheleh Momtaheni and Mojdeh Hakemi-Vala, administration of Technical and material supports was done by Raheleh Momtaheni, Ozra Bagheri Bejestani, and Fatemeh Bagheri Bejestani; study supervision was done by Mojdeh Hakemi-Vala. Statistical analysis was done by Raheleh Momtaheni; drafting of the manuscript, critical revision of the manuscript, and intellectual contents was done by Mehrdad Gholami and Mojdeh Hakemi-Vala.

Funding/Support

This study was supported by grant No. 11505 from Pharmaceutical sciences branch, Islamic Azad University, Tehran, Iran.

References

1. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test

- for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol*. 2003;**41**(10):4623-9.
2. Rodriguez-Martinez JM, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2009;**53**(11):4783-8.
 3. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamases in a large centralized laboratory. *J Clin Microbiol*. 2005;**43**(7):3129-35.
 4. Poirel L, Nordmann P. Acquired carbapenem-hydrolyzing beta-lactamases and their genetic support. *Curr Pharm Biotechnol*. 2002;**3**(2):117-27.
 5. Morita Y, Komori Y, Mima T, Kuroda T, Mizushima T, Tsuchiya T. Construction of a series of mutants lacking all of the four major mex operons for multidrug efflux pumps or possessing each one of the operons from *Pseudomonas aeruginosa* PAO1: MexCD-OprJ is an inducible pump. *FEMS Microbiol Lett*. 2001;**202**(1):139-43.
 6. Samuelsen O, Buaro L, Giske CG, Simonsen GS, Aasnaes B, Sundsfjord A. Evaluation of phenotypic tests for the detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in a low prevalence country. *J Antimicrob Chemother*. 2008;**61**(4):827-30.
 7. Jean SS, Hsueh PR, Lee WS, Yu KW, Liao CH, Chang FY, et al. Carbapenem susceptibilities and non-susceptibility concordance to different carbapenems amongst clinically important Gram-negative bacteria isolated from intensive care units in Taiwan: results from the Surveillance of Multicentre Antimicrobial Resistance in Taiwan (SMART) in 2009. *Int J Antimicrob Agents*. 2013;**41**(5):457-62.
 8. Livermore DM. The impact of carbapenemases on antimicrobial development and therapy. *Curr Opin Investig Drugs*. 2002;**3**(2):218-24.
 9. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect*. 2002;**8**(6):321-31.
 10. Polotto M, Casella T, de Lucca Oliveira MG, Rubio FG, Nogueira ML, de Almeida MT, et al. Detection of *P. aeruginosa* harboring bla CTX-M-2, bla GES-1 and bla GES-5, bla IMP-1 and bla SPM-1 causing infections in Brazilian tertiary-care hospital. *BMC Infect Dis*. 2012;**12**:176.
 11. Su SC, Vanechoutte M, Dijkshoorn L, Wei YF, Chen YL, Chang TC. Identification of non-fermenting Gram-negative bacteria of clinical importance by an oligonucleotide array. *J Med Microbiol*. 2009;**58**(Pt 5):596-605.
 12. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. *Manual of clinical microbiology*. 9th ed Washington DC: ASM Press; 2007.
 13. Performance standards for Antimicrobial Susceptibility Testing (CLSI); Twenty-second Informational Supplement. Wayne, PA, USA: Clinical and Laboratory Standards Institute. CLSI Document M100-S22. 2012, **32**(3).
 14. Fazeli, H., Sadighian, H., Nasr Esfahani, B., Pourmand, M.R. Identification of Class-1 Integron and Various β -lactamase Classes among Clinical Isolates of *Pseudomonas aeruginosa* at Children's Medical Center Hospital. *J Med Bacteriol*. 2012;**1**(2):25-36.
 15. Ghorashi Z, Nezami N, Ghotaslou R, Ghorashi S. Pattern of *Pseudomonas aeruginosa* drug resistance in Tabriz Children Hospital. *Pak J Biol Sci*. 2010;**13**(8):400-4.
 16. Hakemi-Vala M, Hallajzadeh M, Fallah F, Hashemi A, Goudarzi H. Characterization of the Extended-Spectrum beta-Lactamase Producers among Non-Fermenting Gram-Negative Bacteria Isolated from Burnt Patients. *Arch Hyg Sci*. 2013;**2**(1):1-6.
 17. Shahcheraghi F, Nikbin VS, Feizabadi MM. Identification and genetic characterization of metallo-beta-lactamase-producing strains of *Pseudomonas aeruginosa* in Tehran, Iran. *New Microbiol*. 2010;**33**(3):243-8.