ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2018;4(4):123-130

Investigation of Antimicrobial Resistant Patterns and Prevalence of Carbapenamase Genes (*imp-1*, *vim-2*, and *kpc)* in MDR *Pseudomonas aeruginosa* Strains Isolated from Patients in Mottahari Hospital in Tehran, Iran

ARTICLE INFO

Article Type
Original Research

Authors
Abednezhad A.[1] *MSc,*
Nasirmoghadas P.[2] *MSc,*
Asghari Moghaddam N.*[1] *PhD*

How to cite this article
Abednezhad A, Nasirmoghadas P, Asghari Moghaddam N. Investigation of Antimicrobial Resistant Patterns and Prevalence of Carbapenamase Genes (*imp-1*, *vim-2*, and *kpc*) in MDR *Pseudomonas aeruginosa* Strains Isolated from Patients in Mottahari Hospital in Tehran, Iran. Infection Epidemiology and Microbiology. 2018;4(4):123-130.

[1]Department of Biology, Faculty of Basic Science, Central Tehran Branch, Islamic Azad University, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence
Address: Central Tehran Branch, Islamic Azad University, Ashrafi Isfahani Boulevard, Pounak Square, Tehran, Iran. Postal Code: 1469669191
Phone: +98 (21) 44600184
Fax: +98 (21) 44600184
nas.asgharimoghaddam@iauctb.ac.ir

Article History
Received: June 24, 2018
Accepted: October 05, 2018
ePublished: December 20, 2018

ABSTRACT

Aims The aim of this study was to identify antibiotic resistant patterns and the prevalence rate of carbapenem resistant genes (*imp-1*, *vim-2*, *kpc*) in *P. aeruginosa* strains isolated from burn patients in Shahid Motahari Hospital of Tehran.

Materials & Methods In this study, 63 *P. aeruginosa* strains were collected from infected patients. Isolates were identified by biochemical tests and specific 16SrDNA PCR. Antibiotic susceptibility test was performed by standard Kirby-Bauer method according to the CLSI guidelines. The prevalence of *imp-1*, *vim-2*, and *kpc* genes were assessed by PCR.

Findings All of the isolates were confirmed as *P. aeruginosa* by phenotypic tests and specific 16SrDNA PCR. Totally, 14 antibiotypes were identified. The highest resistance was observed against to tobramycin, gentamicin, amoxi-clavulanic acid, and cefoxitin (100%) and the most sensitivity was shown against colistin (100%). All of the isolates were multidrug resistant (MDR), 100 and 46% were positive for Extended Spectrum β-Lactamases (ESBL) and Metallo-β-Lactamases (MBLs) respectively. The *imp-1* and *kpc* genes were not detected (0%), while *vim-2* gene was present in all of the isolates.

Conclusion In the current study, the high resistance rate to antibiotics might be due to their overuse for burn patients as a prophylactic or therapeutic agents. Colistin is considered a drug of choice for the treatment of wounds infected by *P. aeruginosa* in burn patients. In this study, the majority of P. aeruginosa isolates belonged to Antibiotype 1 and possess carbapenemase *vim-2.* Therefore, to stop this resistance transmission, the prevention and control are apparently essential.

Keywords *P. aeruginosa*; Burns; KPC; VIM; IMP

CITATION LINKS

[1] Mutations and expression of PmrAB and ... [2] Incidence of multidrug resistant ... [3] Characterization of Pseudomonas ... [4] Biofilm formation and virulence ... [5] Multiple mechanisms of antimicrobial ... [6] Mechanisms responsible for the emergence ... [7] Pseudomonas aeruginosa - a phenomenon of bacterial ... [8] Genetic characterization of Pseudomonas ... [9] Metallo-beta-lactamases: The quiet before ... [10] First identification of Pseudomonas ... [11] Interplay of efflux system, ampC, ... [12] OXA (beta)-lactamases in Acinetobacter ... [13] Methods for in vitro evaluating ... [14] Increase of imipenem resistance among ... [15] Detection of Pseudomonas aeruginosa ... [16] Imipenem-EDTA disk method for ... [17] Prevalence of extended-spectrum ... [18] Molecular characterization of integrons in epidemiologically ... [19] Emerging carbapenemases in Gram ... [20] Molecular characterization of carbapenem ... [21] National Nosocomial Infections Surveillance ... [22] Pseudomonas aeruginosa bacteremia ... [23] Antimicrobial susceptibility pattern ... [24] Bacterial etiology and antibiotic ... [25] White blood cell response to ... [26] Beta lactamases mediated resistance ... [27] Reduced susceptibility to carbapenems ... [28] Detection of KPC carbapenemase in Pseudomonas ... [29] Evaluation of phenotypic methods for detection ... [30] Class A and D extended-spectrum ... [31] Detection of metallo-β-lactamase-encoding ... [32] Outbreak of extended-spectrum beta ... [33] Pseudomonas infections in Tohid Burn ... [34] Prevalence of metallo-β-lactamase producing ... [35] The prevalence of extended spectrum ... [36] High prevalence of metallo-beta ... [37] Evaluation of Vitek 2 performance for ... [38] Nationwide investigation of extended ... [39] Antimicrobial resistance profiles of community ... [40] Evaluation of Etest MBL for detection of blaIMP ... [41] Detection and genetic characterization ... [42] Antibiotic resistance pattern and ... [43] Study on the resistance mechanism via ... [44] Dispersal of carbapenemase blaVIM-1 ... [45] Identification of class-1 integron and ... [46] PCR-based assay for differentiation of Pseudomonas ...

Introduction

Pseudomonas aeruginosa is an aerobic gram-negative and non-fermentative bacterium and one of the most etiological agents of nosocomial and opportunistic infections in lungs, urinary tract, surgical site, and sepsis [1, 2]. This bacterium is an important pathogen in burn patients, so that multidrug resistant (MDR) *P. aeruginosa* is a major pathogen, which is present in the moist parts of wounds and leads to mortality [2, 3]. This organism is characterized by innate and acquired resistance to different classes of antimicrobial agents, including beta-lactams (penicillins, cephalosporins, monobactams, carbapenems), aminoglycosides, fluoroquinolones, and lipopeptides [4].

The mechanisms involved in *P. aeruginosa* resistance to β-lactam antibiotics include genetic mutations, transmission of lactamase genes, increase in the expression of efflux pump genes, and reduced penetration to cell membrane [5].

According to Ambler classification (1980), the β-lactamases is comprised of 4 classes: A- Extended Spectrum β-Lactamases (ESBL), B- Metallo- β-Lactamases (MBL), C– Cephalosporinases (AmpC), and D– Oxacillinases (OXA) [6].

MBLs are one of the most important families of β-lactamases, mainly regarding their transmission by mobile genetic elements. Instead of serine in the active site of β- lactamases, MBLs carry Zn^{2+}. Serine enzymes eliminate amide bonds in the beta-lactam ring to inactivate the antibiotics. MBLs also catalyze the same chemical reaction using Zn^{2+}[6].

The acquired MBLs include VIM, IMP, SPM-1, GIM-1, SIM-1, AIM-1, KHM-1, NDM-1, and DIM-1. Generally, *imp* and *vim* type enzymes, which are encoded by integron, contain several types [7, 8]. The carbapenemases are an important group of β-lactamases deactivating carbapenems, which are one of the most effective β-lactams against MDR gram-positive and gram-negative bacteria such as *P. aeruginosa* [6].

Most types of transferable carbapenemases have been detected in *P. aeruginosa* isolates worldwide, among which MBLs are of great clinical significance [9]. KPC (*Klebsiella pneumoniae* carbapenemase), which belongs to Ambler Class A carbapenemase and its presence in *P. aeruginosa* isolates was first reported from Colombia [10], creates mighty resistance due to high rates of carbapenem hydrolysis; consequently, there is no need for other mechanisms like efflux pumps or impermeability.

The Class C and D beta-lactamases have rarely been found in *P. aeruginosa*, therefore, do not have the same clinical importance [10-12].

Detecting the presence of carbapenemase activity in pathogenic bacteria is a critical issue for infection control because it is often associated with extensive resistance to different classes of antibiotics, treatment inefficiency, and mortality.

Objectives: Therefore, the aim of this study was to investigate and determine the antibiotic resistant pattern or antibiotyp: type of isolates based on resistant patterns against different classes of antibiotics) and prevalence of carbapenem resistant genes including *imp-1*, *vim-2*, and *kpc* in MDR *P. aeruginosa* strains isolated from burn patients in Mottahari Hospital in Tehran.

Materials and Methods

Bacterial isolates: In this study, 63 *P. aeruginosa* isolates were collected from wound, blood, and catheter of infected patients hospitalized in Mottahari Hospital in Tehran from June to October, 2016. The demographic information of patients including gender, age, and unit of hospitalization were collected. The isolates were identified by biochemical tests, including Gram staining, citrate, catalase, oxidase, the growth on MacConkey agar, Triple sugar Iron agar (TSI), oxidative-fermentative test (OF), growth at 42°C, and Methyl Red Voges Proskauer (MRVP). All culture media were purchased from Merck company distributor (Merck; Germany). The PCR assay was performed by 16srRNA specific primer pairs of *P. aeruginosa* to confirm the identification. The genomic DNA of isolates was extracted by boiling and subjected to PCR assay for confirmation.

Antimicrobial susceptibility testing: Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA; Merck; Germany) according to the Clinical Laboratory Standard Institute (CLSI) guidelines [13-14]. The antibiotic disks (Padtan teb, IRAN) used in this research include ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), colistin (10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), aztreonam (30 µg), amoxi-clavulanic acid (30 µg), imipenem (10 µg), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), cefoxitin (30 µg), ticarcillin (75 µg), piperacillin (100 µg), and piperacillin/tazobactam (100/10 µg).

The suspension of bacteria (0.5 McFarland) was prepared and streaked on MHA (Merck; Germany) by sterile swabs, and, then, antibiotic discs were placed on with 20 mm distance from each other. Then, the plates were incubated at 37°C for 18-24 hours.

The double-disk synergy test was used for the detection of MBLs production. In this assay, two IPM disks were used, one of which contains EDTA (0.5M), the inhibition zone more than 7 mm was considered as positive. Ethylene- Diamine- Tetra-Acetic acid (EDTA) as a poly amino carboxylic can act as a chelating agent and attach to metal ions such as Zn^{+2} and disable them [15, 16].

The Extended Spectrum Beta- Lactamase (ESBL) producing isolates were detected by the double disc

synergy test (DDST), using ceftazidime (30 µg) and cefotaxime discs (30 µg) with and without clavulanic acid (10 µg). The inhibition regions difference≥5 mm between discs with and without clavulanic acid was considered as ESBL producer [17]. MDR isolates were determined by WHONET 2017 software (O'Brien and Stelling Co).

PCR amplification assay: DNA extraction was performed by boiling method. One loopful of overnight grown bacteria on Brain Heart Infusion (BHI) agar plates (Merck; Germany) was picked up and mixed in 200 µL sterile deionized water and boiled for 10 min. After centrifugation in 12000 rpm, the supernatant was subjected for PCR by *imp-1*, *vim-2,* and *kpc* primers; their sequences are presented in Table 1.

Table 1) Primers used in this study

Primer designation	Primer sequence (5' to 3')	Product size	Reference
Imp_1	F : CTACCGCAGCAGAGTCTTTG R : AACCAGTTTTGCCTTACCAT	587bp	[43]
Vim-2	F : ATGGTGTTTGGTCGCATATC R : TGGGCCATTCAGCCAGATC	510bp	[44]
kpc	F : AGTTCTGCTGTCTTGTCTC R : CTGTGCTTGTCATCCTTG	798bp	[45]
16srRNA	F: GGGGGATCTTCGGACCTCA R: TCCTTAGAGTGCCCACCCG	956bp	[46]

The amplification assay was performed, using a sensoquest LabCycler (Germany) in a volume of 12.5 µL containing 1 µL of template DNA (500 ng.µL^{-1}), PCR Master-mix (6.5 µL; Ampliqon; Denmark), and 0.5 µL of each primer (10 pmol).

The PCR conditions were as follow: an initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 20s; annealing at 57, 59, and 58°C regarding *imp*, *vim* and *kpc*, respectively for 20s; and an extension at 72°C for 40s, followed by a final extension at 72°C for 2 min. PCR products were run on electrophoresis by 1.5 and 1% agarose gel for *imp-1* and *vim-2*, and for *kpc*, respectively, and visualized by UV documentation (Uvitec Cambridge; France).

Acinetobacter baumannii AC54/97 [18], *P. aeruginosa* COL-1 [19], and *K. pneumoniae* AO8053 [20] were used as positive control for *imp, vim, and kpc* genes, respectively.

Statistical analysis: Pearson's Chi-square test was performed to evaluate the correlation between antibiotic resistance and presence of carbapenemase gene, MBLs, and, ESBLs production. A significant level of 0.05 was considered for this test.

Findings

Bacterial isolate: Totally, 63 isolates were obtained from burn patients with wound infection in Motahhari Hospital of Tehran from June to October, 2016. These isolates were collected from wound (84.1%), blood (6.3%), catheters (6.3%), and tissue (1.5%). 79% of the infected patients were men and 21% were women. The patients aged between 1-81 years old and 90% were between 20-55 years old. 73, 16, and 11% were hospitalized in men, women, and pediatric units, respectively. All isolates (100%) were identified and confirmed as *P. aeruginosa* through microbiologic and biochemical standard tests and PCR analysis for 16srRNA.

Antimicrobial susceptibility testing: The antibiotic resistance and antibiotyping pattern of *P. aeruginosa* isolates are displayed in Table 2 and 3, respectively. As shown in Table 2, the most resistance was observed against to gentamycin (100%), tobramycin (100%), amoxi-clavulanic acid (100%), cefoxitin (100%), ciprofloxacin (98.5%), amikacin (98.5%), and imipenem (98.5%), whereas the most sensitivity was observed to colistin (100%) and ceftazidime (23.9%; Table 2).

Table 2) Antibiotic resistant and susceptibility

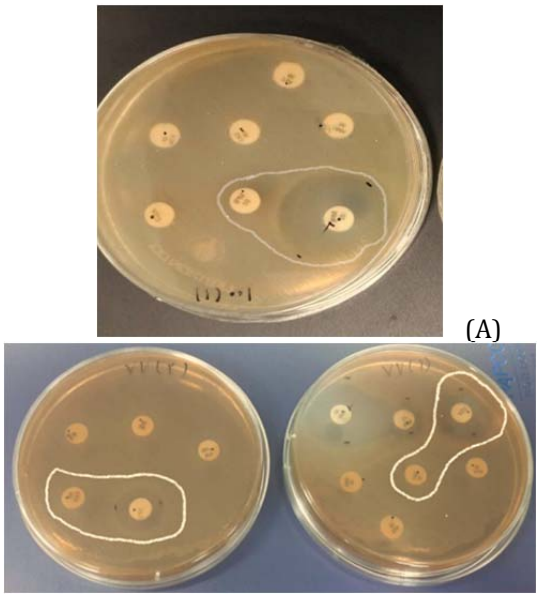
Antibiotic Disk	Resistant (%)	Susceptibility (%)	Intermediate (%)
Gentamycin (10µg)	100	0	0
Tobramycin (10µg)	100	0	0
Amoxi_clavulanic acid (30µg)	100	0	0
Cefoxitin (30µg)	100	0	0
Imipenem (10µg)	98.5	0	1.5
Ciprofloxacin (5µg)	98.5	1.5	0
Amikacin (30µg)	98.5	1.5	0
Ticarcillin (75µg)	92.5	0	7.5
Piperacilin-tazobactam (100/10µg)	90.5	1.5	8
Piperacillin (100µg)	87.5	0	12.5
Ceftazidime (30µg)	76.1	23.9	0
Azteronam (30µg)	65.5	3	31.7
Colistin (10µg)	0	100	0

Table 3) Antibiotyping of isolates

Antibiotypes	Antibiogram Pattern											Percent (%)
	PIP	PTZ	CAZ	AZT	IMP	GEN	TOB	AK	CIP	CST	TIC	
Antibiotype 1	R	R	R	R	R	R	R	R	R	S	R	52.3
Antibiotype 2	R	R	R	I	R	R	R	R	R	S	R	12.6
Antibiotype 3	R	R	S	I	R	R	R	R	R	S	R	9.5
Antibiotype 4	R	R	S	R	R	R	R	R	R	S	R	7.9
Antibiotype 5	I	R	S	R	R	R	R	R	R	S	R	3.1
Antibiotype 6	I	R	R	R	R	R	R	R	S	S	R	1.5
Antibiotype 7	I	S	S	I	R	R	R	R	R	S	R	1.5
Antibiotype 8	R	R	R	R	I	R	R	S	R	S	R	1.5
Antibiotype 9	I	I	S	I	R	R	R	R	R	S	R	1.5
Antibiotype 10	I	R	R	I	R	R	R	R	R	S	I	1.5
Antibiotype 11	I	I	R	I	R	R	R	R	R	S	I	1.5
Antibiotype 12	I	I	R	S	R	R	R	R	R	S	I	1.5
Antibiotype 13	R	I	S	S	R	R	R	R	R	S	I	1.5
Antibiotype 14	R	R	R	I	R	R	R	R	R	S	I	1.5

As shown in Table 3, 14 antibiotypes were determined, in which 52.3% of the isolates belonged to Pattern 1. All of the isolates (100%) were positive in double-disk synergy assay for MBL production (Figure 1A). Overall, 29 isolates (46%) were positive in double disc synergy assay for ESBL production (Figure 1B). MDR observed in all isolates was determined by WHONET application software (2017). The frequency comparison of the two groups (antibiotic resistant and ESBLs or MBLs production) was displayed ($p<0.05$).

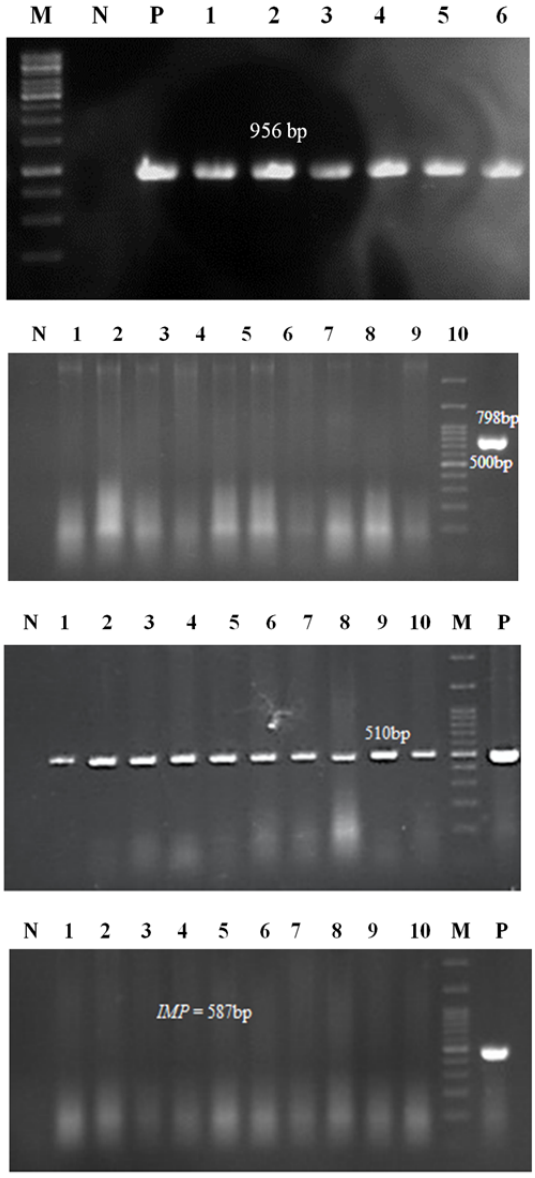
PCR amplification assay: Totally, among 63 isolated samples, 63 isolates (100%) were confirmed as *P. aeruginosa* by 16srDNA PCR (Figure 2 A). The *imp-1* and *kpc* genes were detected in none of the isolates, whereas *vim-2* was positive in all of the isolates (100%; Figure 2B-D). The Pearson's Chi-square analysis showed the comparison of the frequency of the two groups (the resistance of isolates to carbapenems and the presence of *vim-2*) is statistically significant ($p<0.05$).



(A)

(B)

Figure 1) A and B represent the producing isolates of MBLa and ESBL, respectively



(A)

(B)

(C)

(D)

Figure 2) A: PCR amplification of 16srDNA F-R, Lanes N, P representatives of negative and positive controls, Lanes 1-6 *P. aeruginosa* isolates, M: 100bp DNA size marker. 2B-D: PCR amplification of *kpc, vim-2,* and *imp-1* genes, respectively with positive and negative controls

Discussion

Resistance to a wide variety of antimicrobial agents is an increasing global health threat, limiting drug choice, and making it difficult to control and treat infections [21]. MDR *P. aeruginosa* is an important agent in nosocomial infections. This bacterium is one of the most important microorganisms creating several clinical problem as a result of high antibiotic resistance [22].

In this study, 79% of the infected patients were men and 21% were women; given the high risk of men activities, the results seem obvious. The patients aged between 1-81 years old and 90% were between 20-55 years old, which is the age of employment and more probably work with flammable and dangerous equipment. Totally, 73, 16, and 11% were hospitalized in men, women, and pediatric units, respectively.

In present study, the antibiotic sensitivity and prevalence of carbapenem resistant genes (*imp-1*, *vim-2*, and *kpc)* were determined in *P. aeruginosa* strains isolated from burn patients of Motahari Burn Center in Tehran, Iran in 2016. All of the strains were shown to possess MDR features based on the definition explaining, whose isolate is resistant to at least 3 groups of antibiotics. Totally, 14 antibiotypes were identified in the current study, and more than half of the isolates belonged to Antibiotype 1 (52.3%) with the highest resistance level (Table 3). This Antibiotype has been distributed between strains isolated from men, women with different age, and different hospital units. We assume, it is due to the high rate resistant of this type, which affects its circulation in different units and patients with different gender and age range. As was shown in Table 3, the frequency of different isolates decreases from Antibiotype 1 to Antibiotype 14, and it may relate to sensitize the isolates to some antibiotics.

Approximately, all of the isolates were resistant to gentamycin, cefoxitin, tobramycin, imipenem, ciprofloxacin, and amikacin, whereas 23.9, 34.5, and 100% of the isolates were sensitive to ceftazidime, aztreonam, and colistin, respectively (Table 2). The MBLs activity was detected in all of the isolates (100%), whereas 29 isolates (46 %) were positive in double disk synergy test for ESBLs. The production of the enzymes statistically correlates with antibiotic resistant ($p<0.05$). In this cross sectional study, the high antibiotic resistance level in isolates might be due to the overuse of different groups of antibiotics for the prophylaxis and treatment of burn patients.

In a study performed by Golshani *et al*. in Isfahan hospitals, resistance to antibiotics including gentamycin, amikacin, ceftazidime, tobramycin, and imipenem was reported to be 60, 70, 68, 62, and 58%, respectively [23]. In another study performed by Fazeli *et al*. in Isfahan university teaching hospital in Iran, resistance to ceftazidime was 71%, and 11 isolates (31.4%) were positive for ESBL production, 45% of which were resistant to imipenem, and 51% were resistant to meropenem, 9 of which were MBL producers [8]. The lower rate of antibiotic resistance in the mentioned studies compared to the present study may be related to the extent of antibiotic usage and resistance in burn center during 3 years.

In another study carried out by Ghanbarzadeh *et al*., the resistance was reported to ciprofloxacin (93.7%), amikacin (82%), aztreonam (86.8%), piperacillin (85.4%), ceftazidime (82.6%), and imipenem (79.2%); in addition, 93.1% of the isolates were MDR [4]. The results their study are approximately similar to susceptibility assay results of our study.

In the present study, all of the isolates (100%) were susceptible to colistin, consistent with Akhi *et al*.'s study. It seems that colistin can be considered as a responsive therapiotic agent [24].

There are several studies concluding that *P. aeruginosa* isolates and gram-negative bacilli resistant to carbapenems are increasing rapidly in Asia, Europe, and South America [25-27]. The results of the present study showed that all of the *P. aeruginosa* isolates (100%) lack *kpc* gene; this result is similar to other studies reported from Iran, indicating low prevalence of *kpc* gene [28, 29].

In a study by Pakbaten Toupkanlou *et al*. conducted on infected burn injuries in Tehran, most of the isolates (88%) were MDR, and none of the 50 imipenem resistant isolates possessed *kpc* gene [30]. Their study and the present study revealed that resistance rate is increasing, and fortunately, *kpc* gene has not yet been transmitted to studied isolates [30].

The diversity in the obtained results may be due to different used methods, samples, and variation in bacterial species.

Our results demonstrated that whereas 46% of isolates produced ESBLs, *P. aeruginosa* has the highest ESBL production, followed by other gram-negative bacilli such as *A. baumannii* [31-33]. In addition to intrinsic resistance to cephalosporins and aztreonam, ESBL-producing organisms have shown that co-resistance to many other classes of antibiotics like quinolones and aminoglycosides is mainly due to restriction in therapeutic options. The most remarkable risk factors for infection with ESBL-producing organisms in burn patients include long-term antibiotic usage, lengthy hospitalization, and overuse of third-generation cephalosporin and invasive methods [34, 35]. Even though β-lactamase inhibitors possess significant activity against ESBL *in vitro*, their clinical effects against serious infections caused by ESBL-producing organisms is polemical [36, 37].

In the present study, the phenotypic tests indicated that all of the isolate were MBLs producer, and PCR test indicated that the presence of *vim* gene was

positive in all of the isolates, while *imp-1* gene was present in none of the isolates. This finding is in accordance with the phenotypic tests. Correspondingly, it seems that the high resistance to carbapenem (imipenem 98.5%) in this study is due to the production of VIM. Of course, other mechanisms are involved in carbapenem resistance, highlighting the need for more investigation.

The production of MBL enzymes such as *vim* and *imp* is one of the most important reasons for resistance against carbapenems, which is highly prevalent in *P. aeruginosa*. Several studies have indicated that carbapenems resistance in *P. aeruginosa* isolates has been risen [38-40] that is consistent with our results.

In a study performed by Abiri *et al*. in Kermanshah University of Medical Science, of 76 imipenem resistant *P. aeruginosa* isolates, 34 (75%) isolates carried *imp-1* gene, and 1 (2.2%) isolate carried *vim-2* gene [41]. In another study by Aghamiri *et al*., of 100 imipenem resistant *P. aeruginosa* isolates collected from 9 different hospitals in Tehran, 70 isolates carried *vim* gene, and 20 isolates had *imp* gene [42]. The low prevalence of *imp* gene compared to *vim-2* in the mentioned study is comparable with this study results.

Comparing with the results of other studies, the current study exhibited an obvious rise of antibiotic resistance in *P. aeruginosa* isolates in Iran. This study indicated that most of the MBLs positive *P. aeruginosa* isolates carried β-lactamase genes, and horizontal and vertical transfer between the bacterial species, strains, and integrons leads to the development of antibiotic resistance in the global level. Therefore, the focus on antibiotics resistant patterns either phenotypic or genotypic properties is crucial.

Conclusion

In this study, the high resistance rate against to antibiotics might be due to the excessive use of antibiotics for prophylaxis and treatment of burn patients. Hence, appropriate use of antibiotics is necessary. Colistin is a proper option for the treatment of wounds infected by *P. aeruginosa* in burn patients. The majority of *P. aeruginosa* isolates under study belonged to Antibiotype 1, and the prevention and control of this resistance transmission is apparently essential.

Acknowledgements

The team conducting this study would like to thank the Microbiology Laboratory of Motahari Hospital, Tehran, for their support. They would also like to appreciate the staff of the Laboratory of Central Tehran Branch, Islamic Azad University.

Ethical Permissions: Ethical permission code number is 3.094 from Ethics Committee of Isfahan University of Medical Sciences.

Conflict of Interests: The authors declare no conflict of interest.

Authors' Contribution: Arash Abednezhad (First author), Introduction author/ Original researcher/ (35%); Pourya Nasirmoghadas (Second author), Assistant/ Statistical analyst (35%); Nastaran Asghari Moghaddam (Third author), Methodologist/ Discussion author (30%).

Funding: None declared by the authors.

References

1- Lee JY, Ko KS. Mutations and expression of PmrAB and PhoPQ related with colistin resistance in Pseudomonas aeruginosa clinical isolates. Diagn Microbiol Infect Dis. 2014;78(3):271-6.

2- Biswal I, Arora BS, Kasana D, Neetushree. Incidence of multidrug resistant pseudomonas aeruginosa isolated from burn patients and environment of teaching institution. J Clin Diagn Res. 2014;8(5):DC26-9.

3- Ranjbar R, Owlia P, Saderi H, Mansouri S, Jonaidi Jafari N, Izadi M, et al. Characterization of Pseudomonas aeruginosa strains isolated from burned patients hospitalized in a major burn center in Tehran, Iran. Acta Medica Iranica. 2011;49(10):675-9.

4- Ghanbarzadeh Corehtash Z, Khorshidi A, Firoozeh F, Akbari H, Mahmoudi Aznaveh A. Biofilm formation and virulence factors among Pseudomonas aeruginosa isolated from burn patients. Jundishapur J Microbiol. 2015;8(10):e22345.

5- Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare?. Clin Infect Dis. 2002;34(5):634-40.

6- Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia. 2012;16(4):303-7.

7- Strateva T, Yordanov D. Pseudomonas aeruginosa - a phenomenon of bacterial resistance. J Med Microbiol. 2009;58(Pt 9):1133-48.

8- Fazeli H, Sadighian H, Esfahani BN, Pourmand MR. Genetic characterization of Pseudomonas aeruginosa-resistant isolates at the university teaching hospital in Iran. Adv Biomed Res. 2015;4:156.

9- Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: The quiet before the storm?. Clin Microbiol Rev. 2005;18(2):306-25.

10- Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP, et al. First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. Antimicrob Agents Chemother. 2007;51(4):1553-5.

11- Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. Antimicrob Agents Chemother. 2006;50(5):1633-41.

12- Brown S, Amyes S. OXA (beta)-lactamases in Acinetobacter: The story so far. J Antimicrob Chemother. 2006;57(1):1-3.

13- Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9.

14- 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.

15- Pitout JD, Gregson DB, Poirel L, Mc Clure 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.
16- Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798-801.
17- Rafiee R, Eftekhar F, Tabatabaei SA, Minaee Tehrani D. Prevalence of extended-spectrum and metallo β-lactamase production in AmpC β-lactamase producing Pseudomonas aeruginosa isolates from burns. Jundishapur J Microbiol. 2014;7(9):e16436.
18- Gombac F, Riccio ML, Rossolini GM, Lagatolla C, Tonin E, Monti-Bragadin C, et al. Molecular characterization of integrons in epidemiologically unrelated clinical isolates of Acinetobacter baumannii from Italian hospitals reveals a limited diversity of gene cassette arrays. Antimicrob Agents Chemother. 2002;46(11):3665-8.
19- Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect. 2002;8(6):321-31.
20- Nobari S, Shahcheraghi F, Rahmati Ghezelgeh F, Valizadeh B. Molecular characterization of carbapenem-resistant strains of Klebsiella pneumoniae isolated from Iranian patients: first identification of blaKPC gene in Iran. Microb Drug Resist. 2014;20(4):285-93.
21- National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004;32(8):470-85.
22- Vitkauskienė A, Skrodenienė E, Dambrauskienė A, Macas A, Sakalauskas R. Pseudomonas aeruginosa bacteremia: Resistance to antibiotics, risk factors, and patient mortality. Medicina (Kaunas). 2010;46(7):490-5.
23- Golshani Z, Ahadi AM, Sharifzadeh A. Antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from patients referring to hospitals. Arch Hyg Sci. 2012;1(2):48-53.
24- Akhi MT, Ghotaslou R, Asgharzadeh M, Varshochi M, Pirzadeh T, Memar MY, et al. Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran. GMS Hyg Infect Control. 2015;10:Doc02.
25- Griswold JA. White blood cell response to burn injury. Semin Nephrol. 1993;13(4):409-15.
26- Bandekar N, Vinodkumar CS, Basavarajappa KG, Prabhakar PJ, Nagaraj P. Beta lactamases mediated resistance amongst gram negative bacilli in burn infection. Int J Biol Med Res. 2011;2(3):766-70.
27- Wang XD, Cai JC, Zhou HW, Zhang R, Chen GX. Reduced susceptibility to carbapenems in Klebsiella pneumoniae clinical isolates associated with plasmid-mediated beta-lactamase production and OmpK36 porin deficiency. J Med Microbiol. 2009;58(Pt 9):1196-202.
28- Falahat S, Shojapour M, Sadeghi A. Detection of KPC carbapenemase in Pseudomonas aeruginosa isolated from clinical samples using modified hodge test and boronic acid phenotypic methods and their comparison with the polymerase chain reaction. Jundishapur J Microbiol. 2016;9(9):e27249.
29- Azimi L, Rastegar Lari A, Talebi M, Ebrahimzadeh Namvar AM, Soleymanzadeh Moghadam S. Evaluation of phenotypic methods for detection of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae in Tehran. J Med Bacteriol. 2013;2(3-4):26-31.
30- Pakbaten Toupkanlou S, Najar Peerayeh Sh, Pirhajati Mahabadi R. Class A and D extended-spectrum β-lactamases in imipenem resistant Pseudomonas aeruginosa isolated from burn patients in Iran. Jundishapur J Microbiol. 2015;8(8):e18352.
31- Yousefi S, Farajnia S, Nahaei MR, Akhi MT, Ghotaslou R, Soroush MH, et al. Detection of metallo-β-lactamase-encoding genes among clinical isolates of Pseudomonas aeruginosa in Northwest of Iran. Diagn Microbiol Infect Dis. 2010;68(3):322-5.
32- Poirel L, Menuteau O, Agoli N, Cattoen C, Nordmann P. Outbreak of extended-spectrum beta-lactamase VEB-1-producing isolates of Acinetobacter baumannii in a French hospital. J Clin Microbiol. 2003;41(8):3542-7.
33- Rastegar Lari A, Bahrami Honar H, Alaghehbandan R. Pseudomonas infections in Tohid Burn Center, Iran. Burns. 1998;24(7):637-41.
34- De AS, Kumar SH, Baveja SM. Prevalence of metallo-β-lactamase producing Pseudomonas aeruginosa and Acinetobacter species in intensive care areas in a tertiary care hospital. Indian J Crit Care Med. 2010;14(4):217-9.
35- Jazani N, Babazadeh H, Sohrabpour M, Zartoshti M, Ghasemi Rad M. The prevalence of extended spectrum beta-lactamases in Acinetobacter baumannii isolates from burn wounds in Iran. Internet J Microbiol. 2010;9(2).
36- Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing Acinetobacter baumannii in a teaching hospital in Tabriz, Iran. Jpn J Infect Dis. 2011;64(1):69-71.
37- Diamante P, Camporese A. Evaluation of Vitek 2 performance for identifying extended spectrum beta-lactamases in Enterobacteriaceae "other than Escherichia coli, Proteus mirabilis and Klebsiella spp". Le Infezioni in Medicina. 2006;14(4):216-26. [Italian]
38- Hocquet D, Plésiat P, Dehecq B, Mariotte P, Talon D, Bertrand X, et al. Nationwide investigation of extended-spectrum beta-lactamases, metallo-beta-lactamases, and extended-spectrum oxacillinases produced by ceftazidime-resistant Pseudomonas aeruginosa strains in France. Antimicrob Agents Chemother. 2010;54(8):3512-5.
39- Ozyurt M, Haznedaroğlu T, Sahiner F, Oncül O, Ceylan S, Ardiç N, et al. Antimicrobial resistance profiles of community-acquired uropathogenic Escherichia coli isolates during 2004-2006 in a training hospital in Istanbul. Mikrobiyoloji Bülteni. 2008;42(2):231-43. [Turkish]
40- Lee K, Yong D, Yum JH, Lim YS, Bolmström A, Qwärnström A, et al. Evaluation of Etest MBL for detection of blaIMP-1 and blaVIM-2 allele-positive clinical isolates of Pseudomonas spp. and Acinetobacter spp.. J Clin Microbiol. 2005;43(2):942-4.
41- Abiri R, Mohammadi P, Shavani N, Rezaei M. Detection and genetic characterization of metallo-β-lactamase IMP-1 and VIM-2 in Pseudomonas aeruginosa strains from different hospitals in Kermanshah, Iran. Jundishapur J Microbiol. 2015;8(9):e22582.
42- Aghamiri S, Amirmozafari N, Fallah Mehrabadi J, Fouladtan B, Samadi Kafil H. 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 Microbiol. 2014;2014:941507.
43- Cai S, Chen Y, Song D, Kong J, Wu Y, Lu H. Study on the

resistance mechanism via outer membrane protein OprD2 and metal β-lactamase expression in the cell wall of Pseudomonas aeruginosa. Exp Ther Med. 2016;12(5):2869-72.
44- Tato M, Coque TM, Baquero F, Cantón R. Dispersal of carbapenemase blaVIM-1 gene associated with different Tn402 variants, mercury transposons, and conjugative plasmids in Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2010;54(1):320-7.
45- Fazeli H, Sadighian H, Nasr Esfahani B, Pourmand MR. 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(3-4):25-36.
46- Spilker T, Coenye T, Vandamme P, LiPuma JJ. PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. J Clin Microbiol. 2004;42(5):2074-9.