Avicenna J Clin Microb Infec. 2014 May; 1(1): e19216. DOI: 10.17795/ajcmi-19216

Published online 2014 April 28. **Research Article**

Prevalence of Metallo-β-lactamases Encoding Genes Among *Pseudomonas aeruginosa* Strains Isolated From the Bedridden Patients in the Intensive Care Units

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Received: February 14, 2014; **Revised:** March 1, 2014; **Accepted:** March 9, 2014

Background: *Pseudomonas aeruginosa* is one of the most important agents causing nosocomial infections worldwide. Multidrug resistance of isolated bacteria from nosocomial infections makes it difficult and sometimes impossible to treat.
Objectives: The aim of the present study was to investigate the antibiotic resistance and association between *blaIMP* and *blaVIM* genes with resistance to meropenem and imipenem among *P. aeruginosa* strains isolated from Iranian patients in ICU wards of Hamadan, IR Iran.
Patients and Methods: In this cross-sectional study, 100 *P. aeruginosa* strains were isolated from bedridden patients in ICU wards of three university hospitals during five months in 2012. Isolates were confirmed at species level using biochemical tests. Their susceptibility to 18 antibiotics was assessed using Kirby-Bauer disc diffusion method. The minimum inhibitory concentration (MIC) was determined by E-test method. Single PCR was used for detecting *blaVIM*, and *blaIMP* genes.
Results: The highest resistance rates of the isolates to ampicillin/sulbactam, tigecycline, and cefotaxime were 95%, 76%, and 67%, respectively. The lowest resistance rates were observed by colistin sulfate (96%), piperacillin/tazobactam (81%), amikacin (77%), aztreonam (77%), meropenem (76%), imipenem (76%), cefepime (76%), piperacillin (74%), ceftazidime (74%), gentamycin (72%), tobramycin (73%), and levofloxacin (70%) . E-test illustrated that 24% of the isolates were resistant to imipenem (MIC: 32 μg/mL). In contrary to *blaVIM* gene that was not detected in any isolate, *blaIMP* gene was detected in 4% of the isolates. No statistically significant association was observed between resistance rates to imipenem and meropenem with aforementioned genes.
Conclusions: Prevalence of *P. aeruginosa* strains drug resistance in our areas was very high and alerting, but the other antibiotics were effective enough to be used in treatment of the infection. Overall, low resistance rate in this region was probably resulted from low prevalence of metallo-β-lactamases producing strains. Permanent monitoring of changes in *P. aeruginosa* resistance would help to determine national priorities for local intervention efforts.

Keywords:Pseudomonas aeruginosa; Resistance Training; Metallo beta lactamase

1. Background

Infectious diseases have been a prominent cause of mortalities in the whole human history (1). One of the major causes of nosocomial infections is *Pseudomonas aeruginosa*, which is causing almost 9% to 10% of hospital infections. In addition, it causes chronic lung infections in patients with cystic fibrosis. The antibiotic resistance is one of the reasons that makes *P. aeruginosa* an outstanding pathogen (2). Nowadays drug resistance is a general problem in managing infectious diseases; this problem among the gram-positive bacteria includes vancomycin-resistant *Enterococcus* species, methicillin-resistant *Staphylococcus aureus*, and multidrug-resistant *Streptococcus pneumonia*. The most important multidrug-resistant gram-negative bacteria are *P. aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli,* and *Klebsiella pneumonia* (1). The drug resistance mechanisms in *P. aeruginosa* are uptake limitation through the outer membrane, energy-dependent efflux, and β-lactamase (3). Carbapenems (meropenem and imipenem) are effective against *P. aeruginosa* (4). Recent data suggest that the resistance to carbapenems is increasing among isolates of *P. aeruginosa* (5). All carbapenems are hydrolyzing by metallo-β-lactamases (MBLs) that are produced by the bacteria and make it resistant to the meropenem and imipenem. The presence of MBL-producing strains makes treatment difficult and sometimes ineffective (5, 6). The carbapenems-resistant agents are impor-

Implication for health policy/practice/research/medical education:
This research is useful for health policy.

tant not only in treatment failure, but also in horizontal transmission of resistance genes to the other bacteria (5, 7, 8). There are evidences showing that MBL producing *P. aeruginosa* strains casus high mortality (9). MBLs are produced by a group of genes from which *IMP*, *VIM*, *SPM*, *SIM,* and *GIM* have been identified as the source of the resistance to carbapenems (10, 11).

2. Objectives

The present study aimed to investigate the presence of *IMP* and *VIM* genes in the carbapenems-resistant *P. aeruginosa* strains isolated from the bedridden patients in ICU wards of three university hospitals of Hamadan City, IR Iran.

3. Materials and Methods

3.1. Sampling and Bacterial Isolation

In a cross-sectional study, 100 *P. aeruginosa* isolates were collected from clinical specimens of patients in ICU wards of three educational hospitals of Hamadan City (Januvary 2012 to May 2012). These isolates were obtained from culture of specimens from tracheal aspirate, blood, urine, sputum, wound, feces, and cornea of the patients. The Isolates were identified by biochemical tests (12). The isolates were identified as *P. aeruginosa* if they had following characteristics: gram-negative bacilli, citrate positive, non-fermentative, TSI Alk/Alk, motile, H_2S negative, urease negative, oxidase positive, and catalase positive. The confirmed isolates kept preserved at -70°C.

3.2. Antibiogram

Susceptibility of the isolates against 18 antibiotics (Mast Co., UK) was assessed by Kirby-Bauer disc diffusion method (12, 13) and the procedure has been done according to the manufacturer's instruction. Briefly, 1.5×10^8 CFU, equivalent to No. 0.5 McFarland Turbidity Standard, was transferred to Muller-Hinton agar (Merck, Germany); antibiogram discs containing imipenem 10 μg, carbenicillin 100 μg, gentamicin 10 μg, aztreonam 30 μg, cefepime 30 μg, ceftazidime 30 μg, amikacin 30 μg, ciprofloxacin 5 μg, tigecycline 15 μg, doxycycline 30 μg, meropenem 10 μg, levofloxacin 5 μg, piperacillin 100 μg, cefotaxime 30 μg, tobramycin 10 μg, colistin sulphate 10 μg, ampicillin/sulbactam 20 μg, and piperacillin/tazobactam 110 μg were placed on the medium. Then it was incubated at 35°C for 18 hours. Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) criteria (14) and reported as resistant, intermediate, and sensitive. The *P. aeruginosa* ATCC 27853 strain was used as quality control strain in susceptibility testing.

3.3. Minimal Inhibitory Concentration

Minimal inhibitory concentration (MIC) was determined using imipenem containing E-test strips (Lioflichem® Italy) according to the manufacturer's instruction of the. In brief, , 1.5×10^8 CFU, equivalent to McFarland Turbidity Standard No. 0.5, were transferred to Muller-Hinton agar (Merck, Germany) by sterile swab. A next, E-test strip was placed on the cultured medium and then was incubated at 35°C for 18 hours. Results were interpreted according to the CLSI criteria. The intermediate level of antibiotic susceptibility was considered resistance (13, 15).

3.4. Detection of VIM and IMP Family Genes by PCR

Single PCR was performed for amplification of *P. aeruginosa VIM* and *IMP* genes, using pair of *blaVIM*, *IMP* primers (Bioneer® Korea); *VIM*-F 5´-GATGGTGTTTGGTCGCATA-3´ and *VIM*-R 5´-CGAATGCGCAGCACCAG-3´; *IMP*-F 5´-GGAATAGAGTGGCTTAAYTCTC-3´and *IMP*-R 5´-CCAAACYACTASGTTATCT-3´. Amplification procedure was performed with 25 μL of master mix containing 2.5 μL of 10X PCR buffer with $MgCl_2$, 2.5 μL of dNTPs MIX (2 Mm), 1 μL of 10 pM from each forward and reveres primers, 0.2 μL of Taq polymerase 5 U/μL, 3 μL of DNA template, and 14.8 μL of DNase/RNase-Free Distilled water.

PCR was performed in the thermal cycler using the following steps: initial DNA denaturation at 94°C for five minutes, then 30 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 40 seconds and extension at 72°C for 50 seconds, followed by final extension at 72°C for five minutes. Agarose gel electrophoresis of the amplified DNA with 100 bp size marker (Fermentas®, Korea) were done for two hours at 80 V in a 2% agarose gel stained with ethidium bromide to detect the specific band.

3.5. Data Analysis

Data were analyzed by SPSS v.16 using Chi-square and t-tests. $P < 0.05$ regarded as significant. The intermediate level of susceptibility was considered as resistance in data analysis.

4. Results

Totally, 100 *P. aeruginosa* isolates were identified by microbiology standard methods. The resistance rates of isolates are shown in Table 1. Highest resistance rates of isolates were observed in ampicillin/sulbactam (95%), tigecycline (76%), and cefotaxime (67%). The highest sensitivity rates were seen in colistin sulphate (96%), imipenem (76%), meropenem (76%), amikacin (77%), aztreonam (77%), and piperacillin/tazobactam (81%). Moreover, 20 and 24 out of 100 isolates were resistant to imipenem in disc agar diffusion and E-test methods, respectively, but the difference was not statistically significant ($P > 0.05$). The MIC to imipenem was ≥ 32 ug/mL (Table 2). *IMP* gene was detected in four isolates, but none of the isolates were carrying *VIM* gene. The frequency of *VIM* gene was higher in imipenem-resistant isolates in DAD method ($P = 0.042$) (Table 3). However, in E-test method, the frequency of *VIM* gene was higher in isolates that were resistant to imipenem ($P = 0.047$).

Table 1. Antibiotic Resistance of *Pseudomonas aeruginosa* Against 18 Studied Antibiotics [a]

Antibiotic	Intermediate	Sensitive	Resistant
Meropenem	2	76	22
Imipenem	4	76	20
Amikacin	4	77	19
Gentamicin	0	72	28
Aztreonam	6	77	17
Cefepime	2	76	22
Ceftazidime	2	74	24
Ciprofloxacin	1	61	38
Tigecycline	13	11	76
Doxycycline	22	41	37
Levofloxacin	2	70	28
Piperacillin	0	74	26
Tobramycin	0	73	27
Colistin sulphate	0	96	4
Ampicillin/sulbactam	1	4	95
Carbenicillin	5	66	29
Piperacillin/tazobactam	0	81	19
Cefotaxime	24	9	67

[a] Data are presented in No. (%).

Table 2. Minimum Inhibitory Concentration of the Studied Isolates to Imipenem by E-Test Method [a]

	Resistant	Intermediate	Sensitive
Break point	≤ 16	4 < X< 16	≥ 4
MIC, μg/mL	32	12	4
Isolates, %	24	1	0
Total, %	25	-	-

[a] Abbreviation; MIC, minimum inhibitory concentration.

Table 3. Presence of *IMP* Gene in Imipenem-Resistant and Sensitive Isolates in DAD Method

	Imipenem		Total	P Value
	Resistant	Sensitive		
***IMP* gene**				0.045
Positive	3	1	4	
Negative	21	75	96	
Total	24	76	100	

Table 4. Presence of *IMP* Gene in Imipenem-Resistant and Sensitive Isolates in E-Test Method

	Imipenem		Total	P Value
	Resistant	Sensitive		
***IMP* gene**				0.047
Positive	3	1	4	
Negative	22	75	96	
Total	24	76	100	

5. Discussion

According to the results of the present study, drug resistance of *P. aeruginosa* against most of the applicable antibiotics, especially meropenem and imipenem, were relatively low in Hamadan, Iran. There are handful of studies in the country that have shown the similar condition in Iran.

Shahcheraghi et al. in Tehran carried out a similar study and reported resistance in 12.4% of 610 isolates. Interestingly, data showed that 4% of the isolates were carrying *IMP* gene, which is exactly equal to our study in Hamadan area (16). If we consider their study and ours, it could be concluded that a portion of imipenem-resistant *P. aeruginosa* carry the *IMP* gene and may be the cause of resistance as our findings supported a significantly higher *IMP* gene frequency in the resistant isolates. In another study conducted by Khosravi et al. (17) in Ahvaz, south west of Iran, the prevalence of piperacillin-, ceftazidime-, imipenem-, and meropenem-resistant *P. aeruginosa* isolates were 68%, 83%, 41%, and 41%, respectively, which showed higher resistance isolates in Ahvaz in comparison to Hamadam. In contrary to what we found about *VIM* and *IMP* gene, 19.5% of their imipenem-resistant isolates were carrying only *VIM* gene rather than *IMP*, which was carried by some of our isolates. If we compare the two results, it would be clear that In Hamadan area the resistance rate was lower than Ahvaz. On the other hand, gene distribution pattern was also different (17). Saderi et al. studied the antibiotic resistance of *P. aeruginosa* in Tehran. They reported higher prevalence of resistant isolates than ours as 74.22%, 67.19%, 64.85%, 38.28%, and 83.6% of resistance *P. aeruginosa* isolates were resistant to ceftazidime, piperacillin, amikacin, imipenem and gentamycin, respectively (18). As it is clear, different areas show different resistance rate even in the same country. Interestingly, Ranjbar et al. reported lower rate of antibiotic-resistant *P. aeruginosa* isolates from Tehran (19) than what we found in Hamadan. If we consider the aforementioned studies and compare them to our studied region, we would come to a conclusion that the resistance rate was varying in different areas and resistance genes were different too.

Continues monitoring of resistance rate of *P. aeruginosa*, and other pathogens is necessary in every region of the glob to have enough data about it. Consequently, it ends in designing better and successful strategies against the problems we face about antibiotic resistance. Prevalence of drug resistance among *P aeruginosa* strains against three antibiotics in Hamadan city was very high and alerting, but the other antibiotics were effective enough to be used in treatment of the infection. Overall, low resistance rate in this region was probably resulted from low prevalence of MBLs producing strains. Permanent monitor of changes in *P. aeruginosa* resistance will help to determine national priorities for local intervention efforts.

Acknowledgements

The authors would like to acknowledge Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences, for approval of this study and those who helped us in this study for their effort.

Authors' Contribution

All authors had equal contribution.

Financial Disclosure

There was no conflict of Interest.

Funding/Support

This study was financially supported by Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences.

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