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Original Article

Detection of Metallo-β-Lactamases (MBLs) Producing *Pseudomonas aeruginosa* Isolates in Tehran Hospitals, Iran

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Introduction: The strains of *Pseudomonas aeruginosa* are known as an opportunistic pathogen that can cause infections in humans and animals. Metallo-β-lactamases (MBLs) are the most significant factors of resistance to carbapenem antibiotics in these bacteria. This study was designed to identify the MBLs producing *P. aeruginosa* isolates in three hospitals of Tehran, Iran. **Methods:** Totally, we obtained 665 samples from patients hospitalized in three hospitals in Tehran, Iran. Antibiotic-susceptibility test of the *P. aeruginosa* isolates was done based on Kirby-Bauer disk diffusion test. The Minimum Inhibitory Concentration (MIC) of the isolates was performed using agar dilution method, and IPM-EDTA test identified MBL producing isolates. **Results:** Among the examined isolates, 473 (71.1%) were *P. aeruginosa*. Among these, 306 (64.7%) were resistant to imipenem, and 289 (94.5%) were MBL producers. Furthermore, the resistance rate of the isolates to other antibiotics was amikacin (26%), tobramycin (24.95%), ceftazidime (23.05%), gentamicin (22.83%), carbenicillin (21.14%), and ceftizoxime (18.19%). The MICs of imipenem and ceftazidime for the majority of the isolates were 4 μg/ml and >128 μg/ml, respectively. **Conclusion:** This study confirmed previous reports on the increased rate of MBL-mediated resistance in *P. aeruginosa* isolates worldwide. Therefore, detection of resistance patterns for these isolates, particularly MBLs, is necessary for prevention and control of *Pseudomonas* associated infections. *J Med Microbiol Infect Dis, 2017, 5 (3-4): 47-50. DOI: 10.29252/JoMMID.5.3.4.47*

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INTRODUCTION

Nosocomial infections are among the most critical issues in developed and developing countries worldwide [1]. *Pseudomonas aeruginosa* is usually the third significant cause of hospital infections after *Staphylococcus aureus* and *Escherichia coli* [2]. For example, a study in Netherland indicated that *P. aeruginosa* strains were the causative agents of 27-37% of wound infections [3]. The spread of antibiotic resistance especially in healthcare centers has made control of pathogens difficult. Nowadays, the emergence of antibiotic-resistant strains has become a significant challenge in patients' treatment. The isolation rate of Multi-Drug Resistant (MDR) *P. aeruginosa* strains from the hospital environments, and the personnel hands are increasing in some countries [4]. For example, at one burn center in Tehran, Iran, the frequency of *P. aeruginosa* among the patients was 73.9%, and more than 95% of the isolates showed resistance to gentamicin, carbenicillin, trimethoprim/sulfamethoxazole, ceftizoxime and tetracycline [5].

Carbapenems such as imipenem and meropenem are among the essential antimicrobial agents used for the treatment of human infections especially those caused by MDR-*P. aeruginosa* in hospital settings [6, 7]. Recently, resistance to carbapenems particularly in clinical isolates was reported to be related to the reduction of drug penetration or production of carbapenems hydrolyzing enzymes like Metallo-β-lactamases (MBLs) [8]. The first report on MBLs was from Japan, followed by reports from different countries in Asia, Europe, Australia and America [9, 10]. These enzymes have a broad substrates spectrum and can hydrolyze all beta-lactam compounds except monobactams (aztreonam). These enzymes are transmitted by integrons and can merge in plasmids or chromosomes, and hence are transferred to different bacteria such as Enterobacteriaceae family and *Pseudomonas* strains [11-13].

Some reports linked the rise of carbapenem-resistant *P. aeruginosa* isolates to MBLs production as the MBL producing *P. aeruginosa* strains are reported worldwide [14]. MBL producing *P. aeruginosa* strains are considered as a significant threat in healthcare centers, due to the ability of gene transfer and long-term colonization in hospitals. Thus, rapid detection of these strains and reporting them in hospital settings can lead to better control measures in order to prevent their spread and also choose effective antibiotics.

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Therefore, the primary purpose of this investigation was to determine and evaluate the production of MBLs among *P. aeruginosa* isolates obtained from several hospitals in Tehran, Iran.

MATERIAL AND METHODS

Collection and isolation of *P. aeruginosa.* From March 2015 to February 2016, 665 clinical samples including urine, sputum, wound, blood, and CSF were collected from the hospitalized patients in three hospitals in Tehran, Iran. Informed consent was obtained from all human adult participants and the parents or legal guardians of minors. For identification of *P. aeruginosa* isolates, the samples were cultured in blood agar and EMB medium (Merck, Germany) and then identified by biochemical standard tests such as growth in citrate, reaction in TSI, Oxidization Fermentation (OF) test and pyocyanin pigment production on Muller Hinton agar (MHA) (Merck, Germany) and growth in 42°C. The confirmed isolates were stored at 70°C in a liquid medium of Luria-Bertani (LB) (Merck, Germany) with 20% glycerol for subsequent steps.

Antibiotic susceptibility patterns. Antibiotic resistance patterns of the *P. aeruginosa* isolates were determined by disk diffusion method based on Clinical and Laboratory Standards Institute (CLSI) recommendation (2008) [15] using the antibiotics, imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), carbenicillin (100 µg), tobramycin (10 µg), amikacin (30 µg) ceftizoxime (30 µg), and gentamicin (10 µg) (Mast Co., UK). Inhibition zone diameter (mm) of each antibiotic disc was measured, and the isolates were classified as resistant, intermediate or susceptible. *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain.

Determination of Minimum Inhibitory Concentration (MIC). MIC of imipenem and ceftazidime was determined by agar dilution method as recommended by CLSI [15]. *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain.

Detection of MBL isolates by Ethylenediaminetetraacetic-imipenem test (IPM-EDTA). MBL producing isolates were identified by IPM-EDTA phenotypic method according to the NCCLS recommendation. Briefly, sterilized EDTA 0.5 M (186.1 gr of EDTA in 1000 ml DDW, pH 8) (pH 8) was prepared. Suspension of the isolates equal to 0.5 McFarland was cultured, and 750 µl of 0.5 M EDTA inoculated on imipenem disk (10 µg). Imipenem (IMP) and IPM-EDTA disks were placed on the plates. The isolates were considered as MBL producers if the growth inhibition zone around the EDTA-IPM disk was more than the inhibition zone around the IMP disk [16].

Data Analysis. Statistical analysis were performed using SPSS software version 19.0 for Windows (IBM, Chicago, USA). Chi-square and Fisher's exact tests were used to compare proportions. Furthermore, Pairwise correlations were assessed using Phi coefficients. The *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Participants' demographic characteristics. Among the 473 *P. aeruginosa* isolates from three hospitals, 58.8%, and 41.2% belonged to males and females, respectively. The majority of the isolates were obtained from urine (45.7%), and the minority from the CSF (5.5%) (Table 1). The patients' age ranged from 1 to 60 years old, most within the range of 41-60 years old (mean: 58.55), and a few (10%) were under 10 years old.

Antibiotic susceptibility patterns. The Antibiotic susceptibility pattern of *P. aeruginosa* isolates to different antibiotics is shown in Table 2. The highest resistance rate was observed to ceftizoxime and carbenicillin, and the lowest rate to imipenem and meropenem antibiotics (Table 2).

MIC results. The results of MIC for imipenem and ceftazidime are reflected in Table 3. The MIC of imipenem for all the isolates was between 4 to 64 µg/ml, and the majority of the isolates had a MIC=4 µg/ml. Whereas the MIC of ceftazidime for the isolates was between 2 to >128 µg/ml, and the majority of the isolates showed a MIC >128 µg/ml.

Table 1. The prevalence of MBL-producing *P. aeruginosa* isolated from various samples

Specimen type (n= 665)	No. (%) of *P. aeruginosa* isolates (n= 473)	No. (%) of MBL positive *P. aeruginosa* isolates (n= 289)
Urine	216 (45.7)	142 (49.1)
Sputum	38 (8)	17 (5.9)
Wound	125 (26.4)	69 (23.9)
Blood	68 (14.4)	45 (15.6)
CSF	26 (5.5)	16 (5.5)

Table 2. Antibiotic susceptibility pattern of *P. aeruginosa* isolates

Antibiotics	Results					
	Sensitive		Intermediate		Resistant	
	Number	Percentage	Number	Percentage	Number	Percentage
Imipenem (10 µg)	160	33.82	7	1.48	306	64.7
Meropenem (10 µg)	153	32.34	5	1.06	315	66.6
Ceftazidime (30 µg)	109	23.05	0	0	364	76.95
Carbenicillin (100 µg)	100	21.14	7	1.48	366	77.38
Tobramycin (10 µg)	118	24.95	5	1.05	350	74
Amikacin (30 µg)	123	26	14	2.96	336	71.04
Ceftizoxime (30 µg)	86	18.19	0	0	387	81.81
Gentamicin (10 µg)	108	22.83	6	1.27	359	75.9

Table 3. The results of MIC for imipenem and ceftazidime against 473 clinical isolates of *P. aeruginosa*

Antibiotics	MIC (µg/ml)									
	0.5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**
Imipenem (No.)	0	0	0	352*	72	25	14	10	0	0
Ceftazidime (No.)	0	0	28*	35*	51*	17	21	43	30	248

*Number of susceptible isolates at MIC breakpoints

Detection of MBLs. According to the results of disk diffusion, 306 (64.7%) isolates were resistant to imipenem, and the phenotype results of MBLs producing *P. aeruginosa* isolates by using IMP-EDTA disk method indicated that 289 (94.5%) imipenem-resistant isolates were MBL producer. The distribution of the MBL producing *P. aeruginosa* isolates among different clinical samples is reflected in Table 1.

DISCUSSION

The *Pseudomonas*-associated infections are as a significant cause of morbidity and mortality in hospitalized patients [13, 17]. Carbapenems such as imipenem and meropenem are the essential class of beta-lactam antibiotics which are used for the treatment of infections caused by the bacteria capable of hydrolyzing penicillin and cephalosporins. Now, MBLs are one of the most important enzymes involved in resistance to carbapenem antibiotics [7]. MBLs producing strains are responsible for chronic and long-term hospital infections [18]. Mortality in severe infections with MBL producing *P. aeruginosa* range from 70% to 95% [19]. In a case-control study in Japan, morbidity and mortality among the patients infected with MBLs producing *P. aeruginosa* were higher than those infected by non-MBLs producing isolates [18]. Consequently, the increasing rate of resistance to carbapenems among the bacteria mainly *P. aeruginosa* should be considered as a substantial threat in the treatment of these infections. Furthermore, the rapid spread of MBL genes among pathogens mainly *P. aeruginosa* is considered as the primary concern worldwide that leads to a limitation on the treatment of bacterial infections in different regions of the world [13, 17].

In our study, the resistance rate of *P. aeruginosa* isolates to imipenem was 64.7% using the disk diffusion method. Whereas, previously in similar studies in Iran, the resistance to this antibiotic among the *P. aeruginosa* isolates from hospitalized patients was 32.9% and 41%, respectively [20, 21]. This result suggests that the imipenem resistance has increased among the *P. aeruginosa* isolates in Iran and it could be an alert for the healthcare system of the country.

Our results also indicated that 289 (94.5%) isolates of 306 imipenem-resistant isolates produced MBLs by IPM-EDTA test. Previously, Van Der Bij and colleagues [22] showed the high accuracy, reproducibility, sensitivity, and specificity of the phenotypic method. In a study by Mihani *et al.* [23] on 100 *P. aeruginosa* isolates from burn wards of hospitals of Iran, 42% isolates showed resistance to imipenem, and 8 (19%) were MBLs producer. In another report by Sadeghi *et al.* [24] in Iran on 108 *P. aeruginosa* isolates, 40 (37%) were resistant to imipenem, and 20 (50%) produced MBLs using IMP-EDTA disk method. In another study in Mashhad, Iran, out of 63 (48.5%) imipenem-resistant isolates, 56 (88.8%) were MBL-producing [25]. These data suggest that the rate of carbapenem resistance linked to MBLs is increasing in Iran. The high prevalence of MBL producing *P. aeruginosa* may be due to the improper administration of carbapenems. There are also reports of the MBL-producing *P. aeruginosa* isolates from other geographic areas of the world. For instance, the high prevalence of MBL-positive *P. aeruginosa* isolates was reported from Brazil, Korea, Italy, Greece [26-28] and Bangladesh [14]. These results show differences in the prevalence of MBLs producing *P. aeruginosa* isolates in different regions of the world. These differences could be due to the application of different methods for detection of MBLs producing strains, type of *Pseudomonas* infection, and geographical regions the isolates were collected, and type of disks used.

Our isolates also showed high resistance to other groups of antibiotics including aminoglycosides, cephalosporins, and penicillins which is a characteristic of the majority of MBLs producing isolates. These findings indicate that the usage of these agents for the treatment of *P. aeruginosa* infections in Iran should be limited because of a high resistance rate to these agents among the clinical isolates.

In conclusion, the high prevalence of MBL producing *P. aeruginosa* is alarming. Since the MBLs producing isolates can become resistant to all the beta-lactam antibiotics, detection of carbapenem-resistant *P. aeruginosa* capable of MBLs production can help physicians to choose proper antibiotics for the treatment of the patients infected with these bacteria. The genetic analysis and typing of the MBL enzymes can elucidate the epidemiology of the MBLs producing *P. aeruginosa* isolates.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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