Original Article

Detection of *bla*$_{NDM}$, *bla*$_{DIM}$, *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{CTX-M-15}$ beta-lactamase Genes among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Strains Isolated from Two Hospitals of Tehran, Iran

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Abstract

Background: In this study, we evaluated the existence of bla$_{NDM}$, bla$_{DIM}$, bla$_{IMP}$, bla$_{VIM}$, bla$_{CTX-M-15}$ beta-lactamase genes among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from hospitalized patients.

Materials and Methods: From June 2013 to May 2014, thirty-four nonduplicate nonconsecutive isolates of *A. baumannii* and *P. aeruginosa* were isolated from blood, respiratory tract, wound, sputum and urine samples of patients from hospitalized in two hospitals in Tehran, Iran. Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method according to CLSI guidelines. In this study, the frequency of MBL (metallo-beta-lactamase) producers was evaluated by CDDT (Combined disk diffusion test) and prevalence of bla$_{NDM}$, bla$_{DIM}$, bla$_{IMP}$, bla$_{VIM}$ and bla$_{CTX-M-15}$ genes were evaluated by PCR and sequencing methods among *P. aeruginosa* and *A. baumannii* strains isolated from hospitalized patient of Tehran during 2013 -2014 years.

Results: Of thirty-four non-fermenter isolates, 24 (70.58%) *P. aeruginosa* and 10 (29.41%) as *A. baumannii* were isolated and identified. High rate of resistance to common antibiotics were detected specially among *A. baumannii* isolates that showed 100% resistance to 4 of tested antibiotics. The CDDT results reveal that 4 (16.66%) of the *P. aeruginosa* isolates and 1 (10%) of the *A.baumannii* were positive for production of MBLs. The prevalence of bla$_{CTX-M-15}$ gene among 10 *A. baumannii* isolates was 4 (40%), and for IMP-1, 2 (20%). The *bla*$_{OXA-51}$ has been investigated and was detected in all *A. baumannii* isolates. Also the prevalence of bla$_{CTX-M-15}$ gene among 24 *P.aeruginosa* isolates was 11 (45.83%), and for IMP-1, 3(12.5%). Fortunately, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{DIM}$ gene was not detected in all isolates.

Conclusion: The detection of MBL-producing *A. baumannii* and *P. aeruginosa* strains detected in this research is of great concern and highlights the need of infection control measures, including antimicrobial management and prompt detection of beta-lactamase-producing isolates.

Keywords: *Pseudomonas aeruginosa, Acinetobacter baumannii*, Beta-lactamases

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Introduction

Multidrug-resistant microorganisms have emerged as the causes of nosocomial infections worldwide. In recent years, pandrug-resistant (PDR) bacterial strains, which are resistant to all antimicrobial agents except for the tigecycline, colistin, and extensively drug-resistant (XDR) bacterial strains that are resistant to all antibiotics, have been isolated from patients with nosocomial infections. These bacterial strains have become a great global public health threat[1].

A. Bunny and *P. aeruginosa* are common opportunistic gram-negative bacteria related to hospital-acquired infections with high morbidity and mortality, particularly among patients with immunodeficiency disorders[2]. Currently, increasing antimicrobial resistance among *A. baumannii* and *P. aeruginosa* strains have been considered to be a major concern worldwide (3). Resistance to beta-lactam antibiotics is more prevalent among opportunistic non-fermentative bacteria such as *A. baumannii* and *P. aeruginosa*. The most common mechanism of resistance to beta-lactam antibiotics is the production of beta-lactamase enzymes, including enzymes of Ambler classes A, D and B, with the corresponding genes often been associated with mobile genetic elements such as plasmid[4]. Extended-Spectrum-beta-Lactamases (ESBLs) are among the Ambler Class A beta-lactamases, which can degrade monobactams and cephalosporins but not carbapenems or cephamycins. Metallo-beta-lactamases (MBLs) that are in Ambler class B and metal ion associated enzymes and can hydrolyze a wide range of beta-lactam antibiotics even carbapenems[5]. Furthermore, the MBL-encoding genes located on integrons can be disseminated easily from one bacterium to another. Many MBLs have been found in *P. aeruginosa* and *A. baumannii*, including Verona integron-encoded metallo-beta-lactamases (VIM), Imipenemase (IMP), Seoul imipenemase (SIM), Japan, Kyorin University Hospital Imipenemase (KHM), German imipenemase (GIM), New-Delhi metallo-beta-lactamase-(NDM-1) and Australian Imipenemase (AIM)[6,7]. Also, there are various genotypes of ESBLs such as bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ types (8). Therefore the aim of this study was to determine the frequency of *bla*DIM, NDM, IMP, VIM and CTX-M-15 genes among *P. aeruginosa* and *A. baumannii* isolates from hospitalized patients in two Hospitals, Tehran, Iran during the years 2013 and 2014.

Methods

Bacterial Identificationc From June 2013 to May 2014, thirty-four nonduplicate nonconsecutive isolates of *A. baumannii* and *P.aeruginosa* were isolated from blood, respiratory tract, wound, sputum and urine samples of patients from hospitalized in two hospitals in Tehran, Iran. From 10 isolates of *A.baumannii* 1 (10%) were isolated from blood, 1 (10%) from sputum, 2 (20%) from trachea, 2 (20%) from bronsh, 2 (20%) from wound, 2 (20%) from urine. 34 isolates of *P. aeruginosa* were collected from blood 6 (25%), from sputum1 (4.16%), from trachea 4 (16.66%), from bronsh 4 (16.66%), from wound 9 (37.5%). The isolates were identified by conventional biochemical methods[9] and also *A. baumannii* isolates were confirmed by blaOXA-51 gene PCR.

Antimicrobial susceptibility testing: The Kirby-Bauer disk diffusion method on Mueller Hinton agar (Merck, Germany) was performed based on Clinical and Laboratory Standards Institute (CLSI) guidelines 2012[9] Antimicrobial susceptibility tests was accomplished on imipenem (IPM: 10µg), meropenem (MEM: 10µg), ceftazidime (CAZ: 30µg), cefotaxime (CTX: 30µg), amikacin (AK: 30µg), piperacillin/Tazobactam (PTZ: 100/10 µg), ciprofloxacin (CIP: 5 µg), cefepime (FEP: 30 µg), ceftriaxone (CRO: 30µg), aztreonam (ATM: 30µg) , gentamicin (GEN: 10µg), colistin sulphate (CT,10 μg), tetracycline (TE, 10 μg), piperacillin (PIP, 100 μg) and ciprofloxacin (CIP: 5 μg). All purchased from Mast Group, Merseyside, UK. *P. aeruginosa* ATCC27853 and *E. coli* ATCC25922 were used as control strains.

Minimum Inhibitory Concentration (MIC): Strains found resistant to IPM and CAZ by disk diffusion test were re-checked using broth microdilution broth method according to the guidelines of CLSI 2012[10]. MIC for Meropenem (Jaberebne Hayyan Co), ceftazidime (Jaberebne Hayyan Co) and ciprofloxacin

(GLAXO England Co) ranging from 0.5 µg/ml to 256 µg/ml was tested. Also, *P. aeruginosa* ATCC 27853 was used as the control strain.

Phenotypic Detection of MBL: Combined disk diffusion test (CDDT) was performed for identification of MBLs by imipenem and meropenem (Mast Group, Merseyside, UK) alone and in combination with EDTA. An increase in zone diameter of ≥7mm around the Imipenem+EDTA and Meropenem+EDTA disks compared to that of Imipenem and Meropenem disks alone, respectively, was considered positive for MBL production[11].

PCR and Sequencing: Total DNA was extracted by phenol-chloroform method (2). PCR method was used for screening of the *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{DIM}$ and *bla*$_{NDM}$ genes. The primers and PCR programs used in this study were as previously described (10). The predictive PCR product sizes were: 232 bp for (IMP-F and IMP-R), 390bp for (VIM-F and R), 621bp for (NDM-F and R), 699bp for (DIM-F and R), respectively[12]. Amplification for bla$_{CTX-M-15}$ gene was performed with primers CTX-M-15-F (5′-GCGATGGGCAGTACCAGTAA-3′) and CTX-M-15-R (5′-TTACCCAGCGTCAGATTCCG -3′). Amplification was carried out with the following thermal cycling conditions: 5 min at 94°C and 36 cycles of amplification consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with 5 min at 72°C for the final extension. PCR product bands were analyzed after electrophoresis in a 1% agarose gel at 95 V for 45 min in 1X TBE containing Ethidium Bromide under UV irradiation. The PCR purification kit (Bioneer Co., Korea) was used to purify PCR products and sequencing was performed by the Bioneer Company (Korea). The nucleotide sequences were analyzed with Chromas 1.45 software and BLAST in NCBI.

Results

Totally, 34 non-fermenter gram-negative bacteria were isolated from two hospitals in Tehran. Of the 34 non-fermenter isolates, 24 (70.58%) were identified as *P. aeruginosa* and 10 (29.41%) as *A. baumannii*. from 10 isolates of *A. baumannii* 1 (10%) were isolated from blood, 1 (10%) from sputum, 2 (20%) from trachea, 2 (20%) from bronsh, 2 (20%) from wound, 2 (20) from urine also 34 of *P. aeruginosa* isolates were collected from blood 6 (25%), from sputum 1 (4.16%), from trachea 4 (16.66%), from bronsh 4 (16.66%), from wound 9 (37.5%). The CDDT results reveal that 4 (16.66%) of the *P. aeruginosa* isolates and 1 (10%) of the *A. baumannii* were positive for production of MBLs. The antimicrobial susceptibility test results for *A. baumannii* are presented in Table 1 and antimicrobial susceptibility results for *P. aeruginosa* are presented in Table 2. The MICs of CAZ, IMP, CIP for *P. aeruginosa* and *A. baumannii* are presented in

Table 1: Antimicrobial susceptibility test results of 10 isolated *A. baumannii*.

Antibiotic	Resistant, number (%)	Intermediate, number (%)	Sensitive, number (%)
Piperacillin	10 (100)	0 (0.0)	0 (0.0)
Piperacillin/tazobactam	9 (90)	1 (10)	0 (0.0)
Ceftazidime	10 (100)	0 (0.0)	0 (0.0)
Cefepime	9 (90)	0 (0.0)	1 (10)
Cefotaxime	10 (100)	0 (0.0)	0 (0.0)
Ceftriaxone	9 (90)	1 (10)	0 (0.0)
Imipenem	6 (60)	1 (10)	3 (30)
Meropenem	5 (50)	1 (10)	4 (40)
Colistin	0 (0.0)	0 (0.0)	10 (100)
Gentamicin	5 (50)	1 (10)	4 (40)
Amikacin	5 (50)	2 (20)	3 (30)
Ciprofloxacin	10 (100)	0 (0.0)	0 (0.0)
Tetracycline	9 (90)	0 (0.0)	1 (10)

Table 2: Antimicrobial susceptibility test results of 24 isolated *P.aeruginosa*.

Antibiotic	Resistant, number (%)	Intermediate, number (%)	Sensitive, number (%)
Piperacillin	10 (41.66)	4 (16.66)	10 (41.66)
Piperacillin/tazobactam	7 (29.16)	5 (20.83)	11 (45.83)
Ceftazidime	16 (66.66)	2 (8.33)	6 (25)
Cefepime	13 (54.16)	5 (20.83)	6 (25)
Aztreonam	17 (70.83)	2 (8.33)	5 (20.83)
Imipenem	8 (33.33)	1 (4.16)	15 (62.5)
Meropenem	9 (37.5)	0 (0.0)	15 (62.5)
Colistin	4 (16.66)	0 (0.0)	20 (83.33)
Gentamicin	15 (62.5)	1 (4.16)	8 (33.33)
Amikacin	14 (58.33)	0 (0.0)	10 (41.66)
Ciprofloxacin	12 (50)	4 (16.66)	8 (33.33)

Table 3: Minimum Inhibitory Concentration (MIC) of ceftazidime, imipenem and Ciprofloxacin for *P. aeruginosa* and *A. baumannii*.

Antibiotic	*Pseudomonas aeruginosa*			*Acinetobacter baumannii*		
	MIC50 (µg/mL)	MIC90 (µg/mL)	MIC range (µg/mL)	MIC50 (µg/mL)	MIC90 (µg/mL)	MIC range (µg/mL)
Ceftazidime	32	256	2-256	128	256	64-256
Meropenem	2	32	2-256	32	128	2-256
Ciprofloxacin	4	32	2-64	64	128	32-256

Table 3.

The prevalence of $bla_{CTX\text{-}M\text{-}15}$ gene among 10 *A. baumannii* isolates was 4 (40%), and for IMP-1, 2 (20%). The $bla_{OXA\text{-}51}$ has been investigated and was detected in all *A. baumannii* isolates. Also the prevalence of $bla_{CTX\text{-}M\text{-}15}$ gene among 24 *P. aeruginosa* isolates was 11 (45.83%), and for IMP-1, 3 (12.5%). Fortunately, bla_{NDM}, bla_{VIM}, bla_{DIM} gene was not detected in all isolates.

Discussion

A. baumannii and *P. aeruginosa* is responsible for nosocomial infections[13] and reported by different global studies including Iran. At present, carbapenems such as imipenem or meropenem commonly represents main resources for treatment of infections caused by Gram-negative opportunistic bacteria such as *P. aeruginosa* and *A. baumannii* strains[14]. Existence of both intrinsic and acquired resistant against antibiotics among these bacteria especially *P. aeruginosa* and *A. baumannii* strains make it a general challenge.

Based on different studies, it is clear that emergence of resistant *P. aeruginosa* and *A. baumannii* strains is increasing worldwide. In this research, the resistance rates of *P. aeruginosa* strains to most experienced antibiotics were high and the resistance of *A. baumannii* strains to 4 out of 10 tested antibiotics was above 100%. These results are in accordance with those of other studies conducted by Fallah et al, Shahcheraghi et al., Hashemi et al., and Hakemi vala et al. in Tehran[2,11,15]. The most mechanism of resistance to beta-lactam-antibiotics is the production of β-lactamases. Due to the wide extending spreading of the MBLs, potent to Decomposition carbapenems activity and resistance to inhibitors, these enzymes can confer resistance to almost all beta-lactams[15]. The CDDT results reveal that 4 (16.66%) of the *P. aeruginosa* strains and 1 (10%) of the *A. baumannii* were positive for production of MBLs.

Usually, restriction in phenotypic methods urges

researchers to confirm by molecular methods. In the other hand, there are different genes which are coded beta-lactamses[7]. Among MBL genes, IMP gene is more important, specially in Iran[11], however, its first report was from Japan country at 1980.The other gene is bla$_{VIM}$, which was detected before from Ahwaz another city of Iran[7]. In our study, IMP gene was identificated only in 3 (12.5%) of *P. aeruginosa* strain by PCR and sequencing methods. In the other hand, this gene was identificated among 2 (20%) of *A. baumannii* strains in this study. Also, bla$_{VIM}$ gene was not detected among neither *P. aeruginosa* strains nor *A. baumannii* strains. These results are in contrast to other studies from different cities of Iran which reported 11.43% of Tehran isolated *P. aeruginosa* strains and 19.51% of Ahwaz *P. aeruginosa* isolates had VIM gene[11]. Also Saderi et al. reported 94% of *P. aeruginosa* isolates from Tehran were identified as MBL producer and carried the bla$_{VIM-2}$ gene[16]. While Fallah et al. at 2012 showed that *P. aeruginosa* isolates from burn patients similar to our study-only had bla$_{IMP}$ gene[11]. Fortunately, *bla*$_{ND}$, *bla*$_{VIM}$, *bla*$_{DIM}$ gene was not detected in all isolates. The high rate of ESBL prevalence in Iran and its widespread dissemination is a cause worry. However, in this study the existence of bla$_{CTX-M-15}$ was detected in 11 (45.83%) of *P. aeruginosa* and 4 (40%) of MBL-producing *A. baumanii* isolates, respectively, this is worrisome, especially in Iran where the ESBL prevalence is very high.

Conclusion

The existence of MBL-producing isolates and their isolation from life-threatening infections such as hospitalized patients, is increasing at an alarming rate worldwide. Intense pressure for usage of antimicrobial antibiotics in hospitals, resulting in eradication of normal flora and may be a situation of multidrug resistance isolates substitution. It was shown in this research that MBL-producing *P. aeruginosa* and *A. baummanni* strains are emerging threat in hospitals and should be supervised by implementation of timely detection and strict isolation methods that will help to reduce their severe outcomes and mortality rate in these patients.

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Conflict of interest

The authors declare no conflicts of interest.

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