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Original Article

Detection of Genes Encoding Metallo-beta-lactamases in Carbapenem Resistant *Acinetobacter baumannii*

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ABSTRACT

Background: Drug resistant *Acinetobacter baumannii* have emerged as a major problem in many hospitals and intensive care units. The aim of this study was to determine the antibiotic resistance pattern and the prevalence of metallo-beta-lactamase genes among nosocomial *A. baumannii* isolates from Qom/ Iran.

Methods: For this study, a total of 108 *A. baumannii* isolates were collected from hospitalized patients in four teaching hospitals of Qom/ Iran. Antibiotic susceptibility profile of isolates was tested by Kirby-Bauer disc diffusion method and distribution of MBL genes among carbapenem-resistant isolates was determined by polymerase chain reaction (PCR) method.

Results: According to the results, 97 (89.81%) isolates of 108 *A. baumannii* isolates were resistant to carbapenem. All isolates carried *bla* $_{\text{oxa-51like}}$ gene. Among carbapenem resistant isolates, 79.38% carried *bla* $_{\text{VIM}}$ and 1.03% had *bla* $_{\text{IMP}}$ genes. Among the MBL- producing isolates, 7 isolates were MDR, 73 ones were XDR and 5 isolates were PDR.

Conclusion: This study also revealed that suceptibility to carbapenems in the population of *A. baumannii* isolates reduced and the *bla* $_{\text{VIM}}$ gene was the most prevalent metallo-beta-lactamase genotype among carbapenem resistant *A. baumannii* isolates in this area. MBL-producing *A. baumannii* in recent years has become a serious concern. Rapid identification and good infection control are requiered to reduce their impact.

Keywords: *Acinetobacter baumannii*, Metallo-beta-lactamases, Carbapenem resistance, Polymerase chain reaction

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Introduction

Acinetobacter baumannii is a gram-negative, non-fermentative and aerobic coccobacilli that is involved in a variety of nosocomial infections and is frequent especially in Intensive Care Units (1, 2). These infections include bacteremia, pneumonia, surgical site infections, meningitis and urinary tract infections (3). Nutritional requirements of this bacterium are simple and they easily grow on most environmental conditions (4). *A. baumannii* has multidrug resistant (MDR) phenotypes and therefore treatment of this organism is complicated (5, 6). The terms of pan drug resistance (PDR), extensive drug resistance (XDR) and multidrug resistance (MDR) are used for resistance of an isolate to all agents in all antimicrobial categories, resistance to at least one agent in all but two or fewer antimicrobial categories and resistance to at least one agent in three or more antimicrobial categories, respectively. The antimicrobial categories contained aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, monobactams and polymyxins (7). Carbapenems are among the drugs of choice for the treatment of infections caused by multi resistant gram-negative bacilli, but carbapenem resistance in *A. baumannii* has been increased worldwide during the last decade (8). The acquired carbapenem resistance in *A. baumannii* is associated with various mechanisms, including carbapenemase production, that belong to class B β-lactamases (metallo-beta-lactamases) (9). Metallo-beta-lactamases (MBLs) can hydrolyze all carbapenems and other beta-lactam antibiotics except aztreonam (10). At the present, different types of MBLs have been identified in *A. baumannii*. The most common MBLs include Verona integron-encoded metallo-beta-lactamase (VIM), Imipenem hydrolyzing b-lactamase (IMP), German imipenemase (GIM), Seoul imipenemase (SIM) and Sao Paulo metallo-beta-lactamase (SPM) (9, 11).

In recent years, MBLs have been detected with an increasing frequency in *Pseudomonas aeruginosa* and *A. baumannii* strains from several areas of the world and are increasingly implicated in serious nosocomial infections and outbreaks (12). The aims of this study were to access the antimicrobial susceptibility profile and the rate of MDR, XDR and PDR *A. baumannii* isolated from different hospitals in Qom in the center of Iran and to determine the prevalence of MBL genes among carbapenem non-susceptible isolates.

Materials and Methods

Bacterial isolation and identification

In this study, a total of 108 non-duplicated *A. baumannii* isolates were recovered from clinical samples of hospitalized patients in four teaching hospitals in Qom, Iran named Shahid-Beheshti Hospital (a 530-bed referral hospital with three 12-bed ICUs), Nekoei Hospital (a 170-bed referral hospital), Kamkar Hospital (a 150-bed referral hospital) and Valiasr Hospital (a 158-bed referral hospital). The isolates were obtained from clinical specimens, including tracheal aspirate, urine, blood, wounds and cerebrospinal fluid. The samples were immediately inoculated in MacConkey agars. The identification of isolates was carried out using standard microbiological and biochemical methods which include Gram-stain, colony morphology, glucose oxidation, citrate utilization, oxidase test and growth ability at 44 ˚C (13). The identification of *A. baumannii* was confirmed using *bla* $_{\text{oxa-51}}$ PCR (14).

Antibiotic susceptibility testing

To determine antibiotic susceptibility pattern of isolates, the standard Kirby-Bauer disc diffusion testing was carried out using Clinical Laboratory Standard Institute (CLSI) guidelines (15). Antibiotic discs used were ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), Amikacin (30 µg), ciprofloxacin (5 µg), piperacillin (100 µg), piperacillin –tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), Aztreonam (30 µg), cefotaxime (30 µg), cefepime (30 µg), trimethoprime-sulfamethoxazole (25 µg), levofloxacin (5 µg), ampicillin-sulbactam (10/10 µg), ticarcillin-clavulanic acid (75/10 µg), tobramycin (10 µg), tetracycline (30 µg), doxycyclin (30 µg), colistin (10 µg) and polymyxin B (30 unit)(MAST, Merseyside, United Kingdom). The discs were placed on Mueller Hinton agar plates inoculated

with bacterial suspension equal to 0.5 McFarland and incubated at 37 ºC overnight. The diameter of the zone of growth inhibition was measured using the CLSI guidelines (15). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as controls. Intermediate sensitivity was considered as resistance. Minimum Inhibitory Concentrations (MIC) of all imipenem non-susceptible for imipenem was determined by microbroth dilution method according to the recommendations of the standard protocol of CLSI (15).

Identification of metallo-beta-lactamases

The isolates which were intermediate resistant or resistant to imipenem or meropenem were considered to be carbapenem non-susceptible. Carbapenem non-susceptible isolates were evaluated for MBL production by double disk synergy test (DDST) (16). In brief, disks containing 930 µg of EDTA plus 10 µg of imipenem were placed on the inoculated plates containing Muller Hinton agar. An increase of ≥7 mm in zone diameter in the presence of 930 µg of EDTA compared to imipenem tested alone was considered to be positive test for the presence of an MBL. Extraction of genomic DNA from carbapenem non-susceptible isolates was performed by boiling method (16, 17). Detection of MBL-encoding genes was performed by PCR method using *bla* $_{IMP}$, *bla* $_{VIM}$, *bla* $_{SIM}$ and *bla* $_{GIM}$ specific primers (Table 1) (18). The PCR conditions were as follows: Initial denaturation for 5 min at 94 ºC, followed by 35 cycles of denaturation for 1 min at 94 ºC, annealing for 1 min at 52 ºC, and extension for 1 min at 72 ºC, with a final extension for 5 min at 72ºC. Gel electrophoresis was performed in a 1.2 % agarose gel at 85 V. *A. baumannii* reference strain NCTC12516 was used as positive control for *bla* $_{OXA-51}$-like. *P. aeruginosa* ATCC 27853 was used as the negative control and *P. aeruginosa* harboring *bla* $_{IMP}$ and *bla* $_{VIM}$ genes (obtained from Dr. Hashemi, Iran) were used as the positive control.

Table 1. Primers used in PCR reaction for detection of MBL-encoding genes

Primers	Sequence (5´- 3´)	Product size (bp)
OXA-51 F	AACAAGCGCTATTTTTATTTAG	641
OXA-51 R	CCCATCCCCAACCACTTTT	
IMP F	GGAATAGAGTGGCTTAACTCT	188
IMP R	CCAAACCACTAGGTTATCT	
VIM F	GATGGTGTTTGGTCGCATA	390
VIM R	CGAATGCGCAGCACCAG	
GIM F	TCGACACACCTTGGTCTGAA	477
GIM R	AACTTCCAACTTTGCCATGC	
SIM F	TACAAGGGATTCGGCATCG	570
SIM R	TAATGGCCTGTTCCCATGTG	

Results

A total of 108 *A. baumannii* isolates were recovered from different clinical specimens including tracheal aspirate (81 isolates, 75%), urine (17 isolates, 15.74%), blood (7 isolates, 6.48%), wounds (2 isolates, 1.85%) and cerebrospinal fluid (1 isolates, 0.92%). Seventy-five (69.45%) patients were male and thirty-three (30.55%) were female. Their ages ranged from 20 to 90 years with a median of 52 years. *A. baumannii* isolates were obtained from different hospital wards as follows; ICU wards (92 isolates, 85.18%), burn wards (2 isolates, 1.85%), trauma and emergency wards (7 isolates, 6.48%), general surgery wards (2 isolates, 1.85%) and internal wards (5 isolates, 4.62%). Screening for *bla* $_{OXA-51}$-like gene revealed that all isolates were positive for this gene and confirmed them as *A. baumannii* (Figure 1). Of the total 108 *A. baumannii* collected in this study, 97 (89.81%) isolates were found non-susceptible to imipenem and meropenem. The most of the isolates (97.93%) showed MIC ≥32 mg/L for imipenem. Results of antimicrobial susceptible testing in this study (Table 2) showed that the lowest rate of resistance was seen against colistin (41.7%) and polymixin B (12.1%). In addition, among 108 *A. baumannii* isolates screened (18 isolates) 16.66% of isolates were multidrug resistant

(MDR), 84 isolates (77.77%) were extensive drug resistant (XDR) and 5 isolates (4.62%) were pan drug resistant (PDR). The antibiotic resistance rates were higher for carbapenem resistance isolates than for carbapenem susceptible isolates (Table 3). MBL screening by DDS test showed that among 97 carbapenem non-susceptible isolates of *A. baumannii*, 85 isolates (87.62%) were MBL positive. MBL-producing isolates were mainly obtained from tracheal aspirate (76.4%), urine (15.2%), blood (4.7%), burn wound (2.3%) and cerebrospinal fluid (1.1%). The results of PCR assay for 85 isolates (87.62%) that were MBL positive showed that 77 (79.38%) isolates carried *bla* $_{VIM}$ gene and 1 isolate (1.03%) carried *bla* $_{IMP}$ gene (Figure 2-3). The *bla* $_{SIM}$ and *bla* $_{GIM}$ genes were not detected among the studied isolates. In addition, among 85 MBL-producing isolates, 7 isolates (8.23%) were MDR, 73 isolates (85.88%) were XDR and 5 isolates (5.88%) were PDR.

Table 2. Antimicrobial susceptibility patterns of *Acinetobacter baumannii* isolated from 4 university hospitals in Qom, Iran

Antimicrobial	Susceptible (%)	Resistant (%)
Ceftriaxone	2.78	97.22
Ceftazidime	5.56	94.44
Ciprofloxacin	6.49	93.51
Cefepime	6.49	93.51
Cefotaxim	1.9	98.1
Imipenem	10.19	89.81
Meropenem	10.19	89.81
Azotreonam	0	100
Gentamicin	18.52	81.48
Amikacin	6.49	93.51
Tobramycine	52.8	47.2
Piperacillin	2.78	97.22
Piperacillin-tazobactam	5.56	94.44
Levofloxacin	8.34	91.66
Ampicillin-sulbactam	7.40	92.6
Ticarcillin – clavulanic acid	0	100
Tetracycline	10.10	89.90
Doxycycline	17.96	82.04
Trimethoprim- sulfamethoxazole	4.63	95.37
Colistin	58.3	41.7
Polymyxin B	87.9	12.1

Table 3. Antibiotic resistance percentage of carbapenem resistance isolates and carbapenem susceptible isolates of *Acinetobacter baumannii*

Antimicrobial	Carbapenem-susceptible isolates		Carbapenem-resistant isolates	
	Susceptible (%)	Resistant (%)	Susceptible (%)	Resistant (%)
Ceftriaxone	18.19	81.81	12.69	87.31
Ceftazidime	36.37	63.63	15.17	84.83
Ciprofloxacin	36.37	63.63	16.02	83.98
Cefepime	54.55	45.45	16.02	83.98
Cefotaxim	9.1	90.90	11.89	88.11
Imipenem	100	0	19.34	80.66
Meropenem	100	0	19.34	80.66
Azotreonam	0	100	0	100
Gentamicin	45.46	54.54	26.82	73.18
Amikacin	45.46	54.54	16.02	83.98
Tobramycine	63.64	36.36	57.61	42.39
Piperacillin	18.19	81.81	12.69	87.31
Piperacillin-tazobactam	45.46	54.54	15.19	84.81
Levofloxacin	72.73	27.27	17.67	82.32
Ampicillin-sulbactam	45.46	54.54	16.84	83.16
Ticarcillin-clavulanicacid	0	100	0	100
Tetracycline	36.37	63.63	18.58	81.42
Doxycycline	63.64	36.36	26.32	73.68
Colistin	90.91	9.09	62.55	37.45
Polymyxin B	100	0	89.14	10.86
Trimethoprim-sulfamethoxazole	18.19	81.81	14.35	85.65

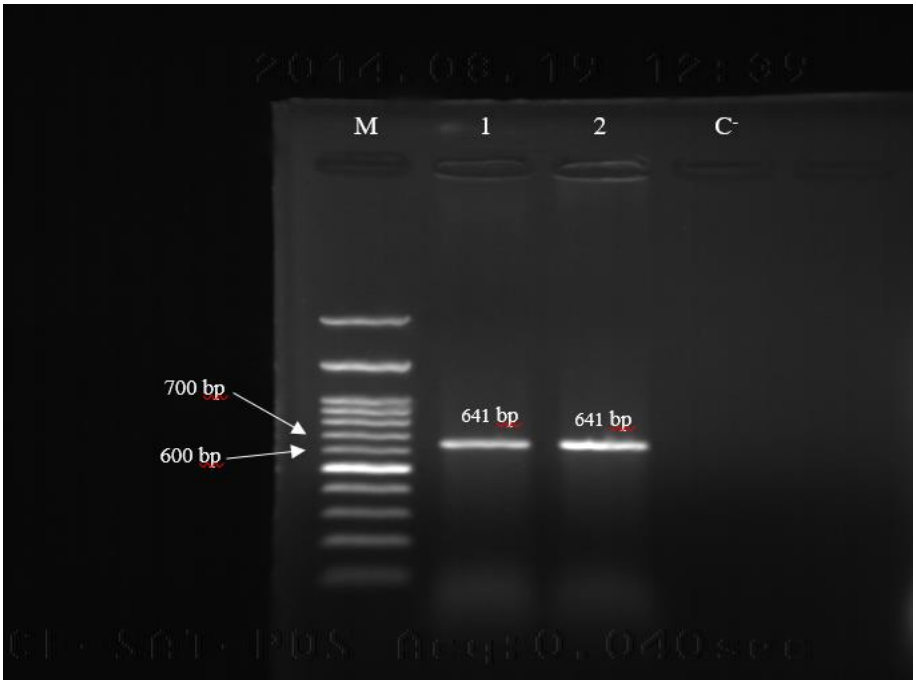

Figure 1. PCR amplification for the detection of *bla*$_{\text{OXA-51-like}}$ gene among *A. baumannii* isolates. Lane M: DNA size marker (100 bp plus), Lane 1: OXA-51 (641 bp) positive control, Lane 2: OXA-51 (641 bp) positive isolate, Lane C$^-$: negative control

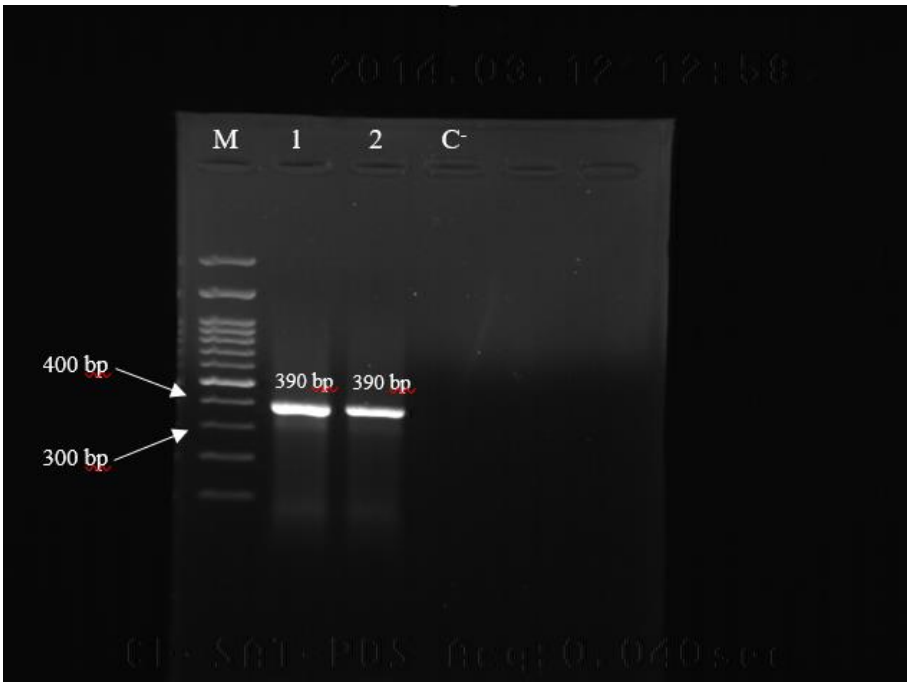

Figure 2. PCR amplification for the detection of *bla* $_{\text{VIM}}$ gene among *A. baumannii* isolates Lane M: DNA size marker (1500 bp), Lane 1: VIM (390 bp) positive control, Lane 2: VIM (390 bp) positive isolate, Lane C$^-$: negative control

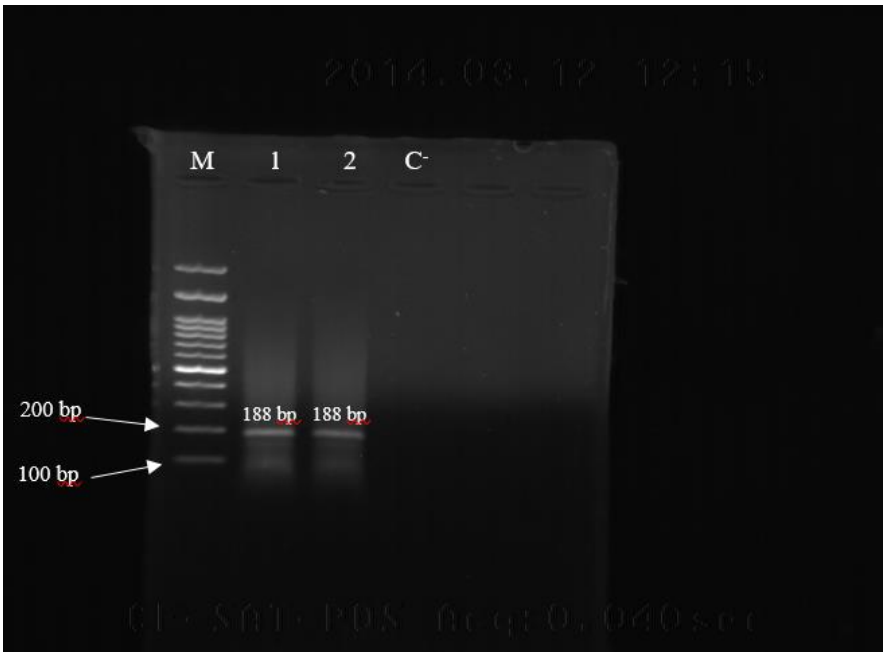

Figure 3. PCR amplification for the detection of *bla* $_{IMP}$ genes among *A. baumannii* isolates Lane M: DNA size marker (1500 bp), Lane 1: IMP (188 bp) positive control, Lane 2: IMP (188 bp) positive isolate, Lane C^-: negative control

Discussion

A. baumannii is considered as one of the most important nosocomial pathogens. Antibiotic resistance of *A. baumannii* is a major problem in the hospital especially in intensive care units (19). There are increasing reports of co-resistance to antimicrobials in *A. baumannii* isolates in various clinical settings worldwide (20, 21). The current study indicated that, 77.77% and 4.62% of *A. baumannii* isolates were XDR and PDR respectively. Japoninejad *et al*. reported that 89% and 11% of *A. baumannii* isolates in Arak (central part of Iran) were respectively XDR and PDR which was much higher than the rate found in the present study (22). Carbapenems are currently the drugs of choice in the treatment of severe infections caused by *A. baumannii*, however, carbapenem resistant *A. baumannii* is now reported increasingly throughout the world (23, 24). Results of this study revealed that 89.81% of *A. baumannii* isolates were resistant to carbapenems. In other studies, done in Iran, the resistance rate of *A. baumannii* isolates to carbapenems has been reported as 27.7%, 52%, 63% and 90.58% respectively (25-28). These results indicate that the rate of resistance to carbapenems has been significantly increased. In this study, 85.18% of *A. baumannii* isolates were obtained from hospitalized patients in ICU wards suggesting mechanical ventilation is a risk factor for exposure to *A. baumannii* and invasive devices such as tracheal tubes as important reservoir involved in *A. baumannii* transmission. This result is in line with previous reports about the role of *A. baumannii* in ICU infections (25, 29). A study from Taiwan compared DDS, combined disk, and Etest methods for detection of MBLs in Gram-negative bacilli; the DDS was reported as the most sensitive test for all bacterial species (30). In our study, phenotypic detection of MBLs by DDS test showed that 87.62% (85 isolates) of carbapenem non-susceptible *A. baumannii* isolates were MBL positive. This is higher than the rate of MBL positive among *A. baumannii* isolates reported from Iran (49%) (27), (92.5 %) (31) and Egypt (70%) (32). In this study, the MBL producing *A. baumannii* isolates were mainly obtained from tracheal aspirate (76.47%). Our data indicated that 79.38% of the carbapenem non-susceptible isolates of *A. baumannii* contained *bla* $_{VIM}$ gene. The prevalence of VIM type MBL among carbapenem non-susceptible *A. baumannii* isolates varies worldwide. So that, it has been reported to be 29% in Norwest of Iran (27),

60.4% in Tehran, the center of Iran (31), 61% in Korea (33) and 81.66% in United Kingdom (18). Findings of the present study showed that 1.03% of the carbapenem non-susceptible isolates of *A. baumannii* were positive for *bla* IMP gene. In Shahcheraghi *et al.* (34) and Erfani *et al.* (31) studies, *bla* IMP gene was not detected among the studied isolates in the center of Iran. Also, *bla* IMP gene has been reported in 18.33% and 33% of *A. baumannii* isolates in UK (18) and Korea (33) respectively. In this study, the *bla* SIM and *bla* GIM genes were not detected among the *A. baumannii* isolates. Few studies have been carried out about *bla* SIM and *bla* GIM genes in the world. In Amin *et al.* study in Iran, 2.59% of carbapenem resistant *A. baumannii* isolates contained *bla* SIM gene (28). Lee and his colleagues reported *bla* SIM gene in 6% of *A. baumannii* isolates in Korea (33). The *bla* GIM gene was identified in one of *A. baumannii* isolates in the Egypt in 2011 (32). It seems that both *bla* SIM and *bla* GIM genes distribution is restricted to some geographic regions or it has not been studied in other parts of the world. These result showed that *bla* VIM gene is the most common MBL genotype in *A. baumannii* isolated from the center of Iran in recent years.

Conclusion

Outbreaks of MBL-producing A. baumannii isolates are serious problems in hospitals and rapid identification of these isolates is necessary to control further dissemination of MBL resistant genes.

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