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# Phenotypic and Molecular Detection of Metallo-Beta-Lactamase Genes Among Imipenem Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Strains Isolated From Patients with Burn Injuries

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## Abstract

**Background:** *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the common causes of nosocomial infections especially among patients with burn injuries.
**Objectives:** The current study aimed to determine the frequency of $bla_{IMP}$, $bla_{VIM}$, $bla_{DIM}$, $bla_{AIM}$, $bla_{GIM}$ and $bla_{NDM}$ genes among *P. aeruginosa* and *A. baumannii* strains isolated from patients with burn injuries hospitalized in Shahid Motahari hospital, Tehran, Iran.
**Methods:** The current cross-sectional study evaluated a total of 309 nonduplicate isolates of *P. aeruginosa* and 189 isolates of *A. baumannii* collected from different clinical samples of patients with burn injuries in Shahid Motahari hospital in Tehran, Iran, from 2012 to 2015. Antibiotic susceptibility tests were conducted by Kirby-Bauer disc diffusion and broth microdilution methods according to the clinical and laboratory standards institute (CLSI) guidelines. The frequency of metallo-beta-lactamase (MBL) producers was evaluated by the combination disk diffusion test (CDDT). The $bla_{IMP}$, $bla_{VIM}$, $bla_{DIM}$, $bla_{AIM}$, $bla_{GIM}$ and $bla_{NDM}$ genes were detected by polymerase chain reaction (PCR) and sequencing techniques.
**Results:** The most effective agent against the studied isolates was colistin. By CDDT, it was found that among 278 imipenem resistant *P. aeruginosa* strains, 178(64.02%) were MBL producers. The $bla_{IMP-1}$ and $bla_{VIM-1}$ genes were detected in 30(16.8%) and 52(29.2%) of *P. aeruginosa* isolates, respectively. Result of 187 imipenem resistance *A. baumannii* strains showed that 85(45.4%) were MBL producers. The $bla_{OXA-51}$, $bla_{IMP-1}$ and $bla_{VIM-1}$ genes were detected in 187(100%), 10(5.3%) and 34(18.18%) of *A. baumannii* isolates, respectively.
**Conclusions:** The high prevalence of MBLs-producing *P. aeruginosa* and *A. baumannii* strains in the study were one of the major concerns.

*Keywords:* Metallo-Beta-Lactamase, Antibiotic Resistance, Burn, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*

## 1. Background

Burn infection is one of the most serious and common health problems worldwide, especially in the developing countries. Patients with severe burn injuries need urgent care to diminish complications after severe burns. One of the most notable and crucial complications of burn is wound infections (1). It is estimated that about 75% of the mortality is due to the infections that develop afterwards in burn injuries (2). Burn damages the skin and exposes a large portion of tissue to infectious agents in a long time and as a more sustainable source of infection is a suitable place for opportunistic microorganisms to reside, afterwards colonization and bacterial products increase inflammation and infection (3, 4). Treatment of wound infections is a challenge due to the biofilms formation and resistance to antibiotics (5). Also, inadequate initial therapy is associated with poor clinical outcomes, longer hospital stays and higher costs (6). *Pseudomonas aeruginosa* and *Acinetobacter baumannii* appear as important pathogens particularly in burn wards. Opportunistic pathogens such as *P. aeruginosa* and *A. baumannii*, cause infections such as pneumonia, septicemia, urinary tract infections, endocarditis, skin, ear and eye infections. Antibiotic therapy is considered as the main strategy to treat and manage burn infections. However, increasing antibiotic resistance is a crucial problem for health care systems (7). Recently this problem is hardened by appearance of multidrug resistant

(MDR) strains with more than 40% -50% mortality rates (8). Carbapenems are often used as a last resort to treat infections caused by multidrug-resistant Gram-negative bacilli (9, 10). The increase of carbapenem resistance in these microorganisms is a major concern. The most common mechanism of resistance is the production of carbapenemases, such as Ambler classes A, B and D enzymes. Metallo-beta-lactamases (MBLs) are associated with plasmid genes such as $bla_{IMP}$ and $bla_{VIM}$ that are the major mechanisms to acquire resistance to carbapenems. New Delhi metallo-ß-lactamase (NDM-1) as a new type of MBL was first detected in a Swedish patient admitted to hospital in New Delhi, India (11, 12). MBL genes distribute quickly in many species of Gram-negative bacilli because they exist on mobile gene cassettes (13, 14). Thus, detection of MBL-producing strains is essential to select the best option to treat patients and manage the prevalence of resistance (9).

## 2. Objectives

The current study aimed to determine the prevalence of MBL genes in *P. aeruginosa* and *A. baumanii* species among patients with burn injuries in Shahid Motahari hospital in Tehran, Iran.

## 3. Methods

### 3.1. Bacterial Identification

Samples were collected by sterile swabs from patients with burn injuries referred to the burn unit of Shahid Motahari hospital (level I burn care center) in Tehran; it is the referral state hospital for the patients with burn injuries and also the main center for the burn research in Tehran, Iran. The collected samples were transferred into Stuart media and immediately transported to the department of microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Samples were cultured on MacConkey and blood agar (Merck, Germany) and incubated at 37°C for 24 hours. Identification of bacterial isolates were accomplished by conventional biochemical tests including catalase, oxidase, triple-sugar-iron (TSI) agar, oxidation/fermentation of glucose using OF media and growth at 42°C; *A. baumannii* was further confirmed by $bla_{OXA-51}$ gene using polymerase chain reaction (PCR) and sequencing techniques (15).

### 3.2. Antimicrobial Susceptibility Testing

Resistance to antibiotics was determined by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Merck, Germany) using the following antibiotics: gentamicin (GEN: 10 $\mu$g), amikacin (AK: 30 $\mu$g), imipenem (IPM: 10 $\mu$g), cefotaxime (CTX: 30 $\mu$g), cefepime (FEP: 30 $\mu$g), meropenem (MEM: 10 $\mu$g), doripenem (DPM: 10 $\mu$g), ciprofloxacin (CIP: 5 $\mu$g), ticarcillin (TIC: 75 $\mu$g), piperacillin/tazobactam (PTZ: 100/10 $\mu$g), ceftazidime (CAZ: 30 $\mu$g), aztreonam (AZA: 30 $\mu$g) and piperacillin (PIP, 100 $\mu$g) (Mast, UK) for *P. aeruginosa* strains. Imipenem (IPM: 10 $\mu$g), meropenem (MEM: 10 $\mu$g), ceftazidime (CAZ: 30 $\mu$g), cefotaxime (CTX: 30 $\mu$g), amikacin (AK: 30 $\mu$g), piperacillin/tazobactam (PTZ: 100/10 $\mu$g), piperacillin (PIP, 100 $\mu$g), ceftriaxone (CTX: 10 $\mu$g), tetracycline (TE: 10 $\mu$g), colistin (CT: 10 $\mu$g), ciprofloxacin (CIP: 5 $\mu$g), cefepime (FEP: 30 $\mu$g), trimethoprim-sulfamethoxazole (TS: 2.5 $\mu$g) and gentamicin (GEN: 10 $\mu$g) (Mast, UK) used for *A. baumannii* and *P. aeruginosa* strains. The zones of inhibition were reported according to the clinical and laboratory standards institute (CLSI) (M100-S23) 2013. The quality control strains used for the study were *P. aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 (10, 16).

### 3.3. MBL Detection by Combination Disk Diffusion Test (CDDT)

The frequency of MBLs producers was measured by combination disk diffusion Test (CDDT). The strains resistant to carbapenems (imipenem and meropenem) were screened for MBL production by CDDT using IMP (10 mg) and MER (10 mg) (Mast Group, Merseyside, UK) solely and in combination with ethylenediaminetetraacetic acid (EDTA). An increase of 7 mm or more in the inhibition zone diameter of EDTA containing imipenem disc compared to imipenem disc was considered positive for MBL (17, 18). The positive control strain used for the study was *P. aeruginosa*.

### 3.4. Molecular Detection of Resistance Genes

Total DNAs of different bacterial isolates were extracted by the DNA extraction kit (Bioneer Company, Korea, Cat. number K-3032-2). The existence of *bla*IMP, $bla_{VIM}$, $bla_{DIM}$, $bla_{AIM}$, $bla_{GIM}$ and $bla_{NDM}$ genes were determined by PCR using the following suitable primers: IMP-F(GGAATAGAGTGGCTTAAYTCTC), IMP-R (GGTTTAAYAAAACAACCACC) (232 bp) for $bla_{IMP}$, VIM-F(GATGGTGTTTGGTCGCATA), VIM-R(CGAATGCGCAGCACCAG) (390 bp) for $bla_{VIM}$, DIM-F(GCTTGTCTTCGCTTGCTAACG), DIM-R(CGTTCGGCTGGATTGATTTG) (699 bp) for $bla_{DIM}$, AIM-F(CTGAAGGTGTACGGAAACAC), AIM-R(GTTCGGCCACCTCGAATTG) (322 bp) for $bla_{AIM}$, GIM-F(TCGACACACCTTGGTCTGAA), GIM-R(AACTTCCAACTTTGCCATGC) (477 bp) for *bla*GIM, NDM-F(GGTTTGGCGATCTGGTTTTC) and NDM-R(CGGAATGGCTCATCACGATC) (621 bp) for $bla_{NDM}$ (15). Reactions were performed on a thermal cycler (Eppendorf, Master Cycler Gradient) and PCR programs used in

this study were as previously described (19). PCR product bands were analyzed after electrophoresis on a 1.5% agarose gel at 95 V for 45 minutes in 0.5x Tris/Borate/EDTA (TBE) containing ethidium bromide, and the result was checked under UV irradiation. *Pseudomonas aeruginosa* PA53 (ACCESSION: KM359726) for IMP-1 and *Pseudomonas aeruginosa* Psa1 (ACCESSION: KT313641) for VIM-1 genes were used as the control strains. DNA sequencing was performed on the purified PCR products by the Bioneer Company (Korea). The nucleotide sequences were analyzed by Finch TV software and compared with sequences in the GenBank using the NCBI basic local alignment search tool (www.ncbi.nlm.nih.gov/BLAST).

### 3.5. Statistical Analysis

To analyze the results, MINITAB16 software was used. P value and confidence intervals (CI) were $< 0.05$ and 95%, respectively.

## 4. Results

This cross-sectional study was performed on hospitalized patients with burn injuries from January 2012 to May 2015. Three hundred and nine nonduplicate isolates of *P. aeruginosa* and one hundred and eighty-nine isolates of *A. baumannii* were collected from inpatients in Shahid Motahari burn hospital, Tehran, Iran.

### 4.1. Antimicrobial susceptibility of P. aeruginosa and A. baumannii isolates

In the current study, the most effective antibiotic against the studied isolates was colistin. The results of disc diffusion test with different antibiotics for *P. aeruginosa* and *A. baumannii* isolates are shown in Tables 1 and 2.

### 4.2. Screening for MBLs Using CDDT

In the current study, among the 309 *P. aeruginosa* clinical isolates, 278 strains were imipenem resistant and 178 (64.02%) were determined as MBL producers by the CDDT test. In addition, it was found that from 189 *A. baumannii* strains, 187 isolates were imipenem resistant and 85(45.4%) were MBL producers.

### 4.3. Detection of MBL Genes

PCR technique showed the existence of $bla_{IMP\text{-}1}$ and $bla_{VIM\text{-}1}$ genes in 30 (16.8%) and 52 (29.2%) isolated strains of *P. aeruginosa*, respectively; while the other gene was not detected. The $bla_{OXA\text{-}51}$, $bla_{IMP\text{-}1}$ and $bla_{VIM\text{-}1}$ genes were detected in 187 (100%), 10 (5.3%) and 34 (18.18%) of *A. baumannii* isolates, respectively; whereas none of them were positive for $bla_{DIM}$, $bla_{AIM}$, $bla_{GIM}$ and $bla_{NDM}$ genes. The nucleotide sequence data reported in this paper was submitted to the GenBank sequence database and assigned accession no. KM359726, KM359725, KT313640, KP780165, KP765726, KP765725, JX648311, KR703251, for $bla_{IMP}$ and KT313641 for $bla_{VIM}$ in *P. aeruginosa* strains and KU372121 , KR424775, KU372120, KU372118, KF723585 for blaIMP in *A. baumannii* strains.

## 5. Discussion

*Pseudomonas aeruginosa* and *Acientobacter baumannii* are responsible for hospital-acquired infections and are recently two of the most important healthcare-associated infections in hospitals. Infection caused by these bacteria often lead to significant mortality and morbidity (15, 20). In the current study, the best coverage against *P. aeruginosa* isolates was obtained with colistin sulfate and gentamicin. Also, the resistance rate of *A. baumannii* isolates against most of the antibiotics was 100%. Therefore, the best coverage against the study *A. baumannii* isolates was obtained with colistin sulfate. Colistin is active against a broad range of Gram-negative bacteria, including most members of *Enterobacteriaceae* (20). In "the lancet infectious diseases", Yi-Yun Liu et al. (21), described mcr-1 a plasmid-mediated gene that confers colistin resistance in *E. coli* and *Klebsiella pneumoniae* strains isolated from animals and patients in China. Transfer of the resistance to multidrug resistant *Enterobacteriaceae* would seriously limit the current treatment options. Keep it in mind that the resistance genes responsible for antimicrobial resistance are found on conjugative plasmids and that carbapenem and colistin-resistant *E. coli* may be found in retail meat, if such strains colonise in the human intestinal tract they can transfer the resistance plasmids to other Gram-negative pathogens such as *P. aeruginosa* and *A. baumannii* and the consequence is untreatable infections (22). Carbapenem resistance mechanisms in Gram-negative bacilli are associated with resistance to other classes of antibiotics such as penicillins, monobactams and cephalosporins possibly because of parallel resistance mechanisms (23, 24). Actually, resistance to carbapenems caused resistance to other valuable antibiotics, which makes the treatment process very difficult. Therefore, identifying carbapenem resistant strains and infection control programs are very useful (25). The most common mechanism of resistance is the production of $\beta$-lactamases, including enzymes of Ambler classes A, D and B, with the corresponding genes often associated with mobile genetic elements such as plasmids (19). Simple and suitable tests are needed to identify MBL-producing isolates that is a crucial step to monitor these emerging resistance. Suppression of enzyme via EDTA is an efficient method used to differentiate MBL mechanisms

**Table 1.** Antimicrobial Susceptibility Testing Results Among *Pseudomonas aeruginosa* Isolates

| Antibiotics | Resistance in 2012, No. (%) | Resistance in 2013, No. (%) | Resistance in 2014, No. (%) | Resistance in 2015, No. (%) |
|---|---|---|---|---|
| **Gentamicin** | 49 (49) | 34 (72.34) | 59 (95.1) | 95 (95) |
| **Amikacin** | 79 (79) | - | 52 (83.8) | 95 (95) |
| **Imipenem** | 83(83) | 37 (78.72) | 58 (93.5) | 96 (96) |
| **Carbenicillin** | 83 (83) | - | - | - |
| **Cefepime** | 87 (87) | 32 (68.08) | 53 (85.5) | 96 (96) |
| **Meropenem** | 83 (83) | 35 (74.46) | 54 (87.1) | 96 (96) |
| **Ciprofloxacin** | 88 (88) | 32 (68.08) | 55 (88.7) | 97 (97) |
| **Piperacillin/tazobactam** | 86 (86) | - | 42 (67.7) | 95 (95) |
| **Ceftazidime** | 83 (83) | 34 (72.34) | 41 (66.1) | 85 (85) |
| **Aztreonam** | 84 (84) | 39 (82.97) | 39 (62.9) | 97 (97) |
| **Piperacillin** | 90 (90) | - | 47 (75.8) | 95 (95) |
| **Tobramycin** | 90 (90) | - | - | - |
| **Colistin** | 0 | - | 0 | 1 (1) |
| **Ticarcillin** | - | - | 59 (95.1) | 99 (99) |
| **Doripenem** | - | - | 55 (88.7) | 95 (95) |

**Table 2.** Antimicrobial Susceptibility Testing Results for *Acinetobacter baumannii* Isolates

| Antibiotics | Resistance in 2012, No. (%) | Resistance in 2013, No. (%) | Resistance in 2014 - 2015, No. (%) |
|---|---|---|---|
| **Gentamicin** | 27 (96.42) | 56(93.33) | 95 (94.05) |
| **Co-trimoxazole** | 28 (100) | 60 (100) | 101 (100) |
| **Amikacin** | 28 (100) | 50 (83.33) | 101 (100) |
| **Imipenem** | 28 (100) | 58 (96.66) | 101 (100) |
| **Cefotaxime** | 28 (100) | 60 (100) | 101 (100) |
| **Cefepime** | 28 (100) | 60(100) | 101 (100) |
| **Meropenem** | 28 (100) | 60(100) | 101 (100) |
| **Ciprofloxacin** | 28 (100) | 60 (100) | 101 (100) |
| **Ceftriaxone** | 28 (100) | 60 (100) | 101 (100) |
| **Piperacillin/tazobactam** | 28 (100) | 60 (100) | 101 (100) |
| **Ceftazidime** | 28 (100) | 60 (100) | 101 (100) |
| **Tetracycline** | 28 (100) | 50 (83.33) | 101 (100) |
| **Piperacillin** | 28 (100) | 60 (100) | 101 (100) |
| **Colistin** | 0 | 0 | 0 |

from other $\beta$-lactamases (9, 17, 26) and also PCR method was valuable to determine MBL-producing isolates (13). In the current study, from 278 imipenem-resistant *P. aeruginosa* and 187 imipenem-resistant *A. baumannii* isolates, 178 and 85 isolates were MBL-producers, respectively. Large outbreaks by MBL-producing *P. aeruginosa* strains were described in hospitals in Greece, Italy and Korea (27). Among MBL genes, IMP is more important, especially in Iran; however, its first report was from Japan in 1980. The other gene is VIM reported from Ahwaz, Iran (15). In the current study, PCR techniques showed the existence of $bla_{IMP-1}$ and $bla_{VIM-1}$ genes in 30 (16.8%) and 52 (29.2%) of *P. aeruginosa* strains, respectively; while the other gene was not detected. The $bla_{OXA-51}$, $blaI_{MP-1}$ and $bla_{VIM-1}$ genes were detected in 187

(100%), 10 (5.3%) and 34 (18.18%) of *A. baumannii* isolates, respectively; whereas none of them were positive for $bla_{DIM}$, $bla_{AIM}$, $bla_{GIM}$ and $bla_{NDM}$ genes. The prevalence of $bla_{IMP}$ and $bla_{VIM}$ types of MBL-producing *A. baumannii* was previously reported in Iran in 2014. It was shown that out of 99 imipenem resistant *A. baumannii* strains, 86 (86.86%) were MBL producers. The frequencies of $bla_{IMP}$ and $bla_{VIM}$ genes in MBL producing *A. baumannii* isolates were 3 (3.48%) and 15 (17.44%), respectively (15). Another study revealed that among 75 Gram-negative isolates from patients with burn injuries, 47(62.67%) were recognized as *P. aeruginosa* and 28 (36.33%) as *A. baumannii*; the CDDT results showed that 13 (17.8%) of the *P. aeruginosa* isolates and 12 (16.4%) of the *A. baumannii* isolates were the MBLs producers. Additionally, this study reported that the mortality rate caused by MBL producing *P. aeruginosa* and *A. baumannii* infection was 5 (20%) among the burn patients (19). Also, it is reported that 94% of *P. aeruginosa* isolates from Tehran were identified as MBL producers and carried the $bla_{VIM-2}$ gene (19). In another study as the first carbapenem resistance report from Libya in 2014, totally 49 isolates (24 *P. aeruginosa* and 25 *A. baumannii*) were collected and imipenem resistance was observed in twenty-one *P. aeruginosa* and twenty-two *A. baumannii* isolates (87.75%); nineteen *P. aeruginosa* isolates had the $bla_{VIM-2}$ gene (28). A study in Poland on MBL-producer *A. baumannii* isolates showed that 10.3% of the isolates carried $bla_{IMP-like}$ gene and $bla_{VIM-4}$ was not detected in the isolates. Also, in a similar study in India, 47% of *A. baumannii* isolates carried $bla_{VIM}$ and 0.9% of them harbored $bla_{IMP}$ (29). The $bla_{IMP}$ and $bla_{VIM}$-producing *P. aeruginosa* strains are reported worldwide (27). The rapid diagnosis of MBL isolates is helpful to select suitable options for antimicrobial therapy and prevent the spread of MBL strains. The clinical microbiology laboratories should consider it important to detect MBL producing *P. aeruginosa* and *A. baumannii* isolates. It is recommended to routinely check that all carbapenem resistant *P. aeruginosa* and *A. baumannii* isolates for the MBL production.

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## Footnotes

**Authors' Contribution:** All authors were involved in: study design, data collection, article approval and statistical analysis.

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