Emergence of Multidrug Resistance and Metallo-beta-lactamase Producing *Acinetobacter baumannii* Isolated from Patients in Shiraz, Iran

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Abstract

**Background:** Metallo-beta-lactamase (MβL) enzymes production is one of the most important resistance mechanisms against carbapenems in some bacteria including *Acinetobacter baumannii*.

**Aims:** This study was aimed to determine the antimicrobial susceptibility and the prevalence of MβL among carbapenem-resistant isolates of *A. baumannii*. **Materials and Methods:** In this cross-sectional study from October 2012 to April 2013, 98 isolates were identified as *A. baumannii* using Microgen™ kits and confirmed by molecular method. These isolates were tested for antimicrobial susceptibilities by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines. Carbapenem-resistant isolates were further detected phenotypically by MβL minimal inhibitory concentration (MIC)-test strips, and subsequently positive MβL isolates were confirmed by polymerase chain reaction (PCR). **Results:** Overall, 98% (96/98) of *A. baumannii* isolates were detected as carbapenem-resistant by MIC test. Highest sensitivity to the tested antibiotic with 42.9% (42/98) was observed to colistin. Of 96 carbapenem-resistant isolates, 43 were phenotypically positive for MβL; out of 43 isolates, 37 were confirmed for the presence of MβL genes by PCR. **Conclusion:** The frequency of drug resistance among the clinical samples of *A. baumannii* isolated in our study against most of the antibiotics was very high. Moreover, all MβL producing isolates were multidrug resistance. Therefore, systematic surveillance to detect MβL producing bacteria and rational prescription and use of carbapenems could be helpful to prevent the spread of carbapenem resistance.

**Keywords:** *Acinetobacter baumannii*, Antibiotic resistance, Carbapenem, Iran, Metallo-beta-lactamase

Introduction

*Acinetobacter baumannii* is a nonmotile, Gram-negative, nonfermentative, oxidase-negative, and aerobic Bacilli, which is one of the most opportunistic pathogens against human.[1,2] The bacteria are widespread in the environment and are considerably resistant to most antibiotics, low nutrient, and arid condition.[3] *Acinetobacter* spp. cause a variety of nosocomial infections, but *A. baumannii* is the prevalent species with high morbidity and mortality, including pneumonia, bacteremia, urinary tract, and skin and soft tissue infections, especially in patients with severe illness.[1,3,4] At the recent years, the continuously increasing prevalence rates...
of nosocomial infections by multidrug-resistant (MDR) *A. baumannii* in the Intensive Care Units (ICUs) have led to rise mortalities at the hospitals.\(^{5-7}\)

*A. baumannii* has propensity to acquire resistance; a mechanism of this resistance is being characterized by the production of a specific enzyme called metallo-beta-lactamases (MβLs).\(^{4,7}\) These enzymes belong to Ambler class B beta-lactamases based on their amino acid sequence homology and to Group 3 according to the Bush classification based on their substrate profiles (imipenem hydrolysis).\(^{7}\) These enzymes are inhibited by ethylenediaminetetraacetic acid (EDTA).\(^{8}\)

In Iran, several studies have previously been carried out on drug resistance of *A. baumannii*, revealing a high resistance rate to most of the antibiotics.\(^{9-11}\) However, a few studies have been conducted in Iran, especially in our region, aiming to find the prevalence of MβL producing genes among clinical isolates of *A. baumannii*. The aims of the present study were to investigate MDR and determine the prevalence of MβL genes among carbapenem-resistant isolates of *A. baumannii* from clinical specimens in Shiraz, Southwest of Iran.

**Materials and Methods**

**Sampling and bacterial isolates**

In this cross-sectional study from October 2012 to April 2013, 120 *Acinetobacter* isolates were recovered from clinical specimens. The specimens were randomly collected from patients in different wards of two hospitals, Nemazee and Faghihi in Shiraz, a major city in Southwest of Iran. Nemazee and Faghihi hospitals are the two major tertiary care hospitals with 1000 beds, affiliated to Shiraz University of Medical Science, Shiraz, Iran. Sample size was calculated as about 100 using the formula\(^{12}\):

\[
 n = \left( \frac{z^2 \cdot \hat{p} \cdot (1 - \hat{p})}{d^2} \right) \cdot pg 
\]

The study was in accordance with declaration of Helsinki; however, because we only used laboratory clinical specimens and did not harm any of the patients, the local ethics committee waived the need for informed consent.

Totally, 98 isolates were identified as *A. baumannii* isolates using Microgen™ diagnosis Kits. These 98 isolates were recovered from 35 sputum, 15 wound swab, 13 body fluids, 9 blood, 9 urine, 8 endotracheal tube, 5 cerebrospinal fluid, and 4 other samples (included 2 bronchoalveolar lavage, 1 eye swab, and 1 axillary swab). Overall, 53 samples were collected from ICUs and 45 samples from other wards (included gastroenterology, skin, surgery, and transplant) in the two above-mentioned hospitals. Confirmed isolates were kept at −70°C until for long preservation.

**Antimicrobial susceptibility testing**

The susceptibility of the isolates to 16 antibiotics disk (MAST, UK) was investigated using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendation.\(^{11}\) The test was performed on Mueller-Hinton agar (Merck, Germany) with followed antibiotics disks containing 10μg imipenem, 10μg meropenem, 30μg amikacin, 10μg gentamicin, 30μg aztreonam, 5μg cefazidine, 5μg ciprofloxacin, 5μg levofloxacin, 100μg piperacillin, 10μg tobramycin, 25μg colistin, 20μg ampicillin/subbactam, 10μg ampicillin, and 110μg piperacillin/tazobactam. *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain in susceptibility testing.

**Minimal inhibitory concentration**

Minimal inhibitory concentration (MIC)-test strips (Liofilchem, Italy), containing imipenem and meropenem, were used to determine the MIC according to the instruction provided by the company on Muller-Hinton agar (Merck, Germany). Results were interpreted using CLSI criteria.

**Phenotypic metallo-beta-lactamase detection**

MIC-test strip (Liofilchem, Italy), containing imipenem/imipenem + EDTA, was used to determine the phenotypic MβL enzyme production. MIC-test strip was performed according to the manufacturer’s instructions. A reduction in the MIC of imipenem of ≥3 dilutions in the presence of EDTA was interpreted as a positive test. In addition, a strain was considered MβL producer if a phantom zone or deformation of the eclipse was observed.

**DNA extraction and molecular typing**

The isolates which were intermediately resistant or resistant to imipenem or meropenem were considered carbapenem-resistant. Extraction of genomic DNA from *A. baumannii* isolates was performed according to the protocol as previously described.\(^{14}\) Polymerase chain reaction (PCR) assay was performed for the detection of bla_{OXA-51-like} gene, a 353 base-pair (bp) amplicon, as an internal gene for molecular confirmation of *A. baumannii* isolates at the species level (primer sequence: F/5’-TAA TGC TTT GAT CGG CCT TG-3’, R/5’-TGG ATT GCA G TT CAT CTT GG-3’).\(^{15}\) and for amplification of MβL encoding genes, bla_{IMP}, bla_{VIM}, and bla_{SPM} using the following consensus primer sets, bla_{IMP}: F/5’-GAA GGC GTT TTT GAT CGG CCT TG-3’, R/5’-TGG ATT GCA G TT CAT CTT GG-3’,\(^{16}\) and for amplification of MβL encoding genes, bla_{VIM}: F/5’-TGG ATT GCA G TT CAT CTT GG-3’, R/5’-TGG ATT GCA G TT CAT CTT GG-3’,\(^{17}\) and for amplification of MβL encoding genes, bla_{SPM}: F/5’-GAA GGC GTT TTT GAT CGG CCT TG-3’, R/5’-TGG ATT GCA G TT CAT CTT GG-3’.\(^{18}\) A *P. aeruginosa* harboring bla_{IMP}, bla_{VIM}, and bla_{SPM} genes (obtained from Pasteur Institute of Iran) was used as the positive control.\(^{18}\)
Statistical analysis was performed using SPSS™ software version 19.0 (IBM Corp., Armonk, NY, USA). The results for infectious agents and antimicrobial susceptibility presented as descriptive statistics in terms of relative frequency. Chi-square or Fisher’s exact test was used to analyze the results wherever they needed. \( P < 0.05 \) was considered as statistically significant clinical relevance.

**Results**

Of 98 *A. baumannii* isolates, 54.1% (53/98) of isolates were collected from female patients’ samples. Seventy isolates were obtained from Namazee and 28 from Faghihi Hospital. Most of the 98 *A. baumannii* isolates were recovered from sputum \( (n = 35) \), wound swab \( (n = 15) \), and body fluids \( (n = 13) \).

The highest antibiotic resistance rates were observed against aztreonam, ceftazidime, ciprofloxacin, piperaclillin, and cefotaxime since all of the tested isolates showed resistance. In addition, no sensitive isolates were seen against aztreonam and cefotaxime; just a few isolates were in the intermediate level of susceptibility. The results of antibiotic susceptibility patterns for the tested isolates are displayed [Table 1].

Totally, 98% (96/98) of the isolates were detected as carbapenem-resistant by MIC-test strip. Among the carbapenem-resistant *A. baumannii* isolates, 44.8% (43/96) were found to be MβL producers by MβL MIC-test strips. Interestingly, the majority 85.7% (84/98) of the isolates showed an MIC \( \geq 48 \mu g/mL \). MIC ranges for the tested isolates to imipenem are presented in Table 1. In addition, no sensitive isolates were seen against aztreonam and cefotaxime; just a few isolates were in the intermediate level of susceptibility. The results of antibiotic susceptibility patterns for the tested isolates are displayed [Table 1].

Finally from MβL producing isolates, 86% (37/43) were positive for MβL genotypes, of which 53.4% (23/43) isolates carried bla\(_{IMP}\) 32.6% (14/43) carried bla\(_{VIM}\) MβL genes, and none of the isolates carried bla\(_{SPM}\) gene. PCR results for amplification of MβL producing genes are displayed in Table 3.

From the total ICUs recovered isolates, 47.2% (25/53) carried one of the bla\(_{IMP}\) or bla\(_{VIM}\) genes; this rate for isolates from other hospital wards was 26.7% (12/45). Despite the higher proportion for bla\(_{IMP}\) and bla\(_{VIM}\) gene in ICUs compared to other wards, no significant differences were observed \( (P = 0.06) \). The frequencies of bla\(_{IMP}\) and bla\(_{VIM}\) genes for Namazee hospital isolates were 22.9% (16/70) and 12.9% (9/70), respectively. The similar rates for Faghihi Hospital isolates were 25% (7/28) for bla\(_{IMP}\) and 17.9% (5/28) for bla\(_{VIM}\) genes.

**Discussion**

One of the carbapenem resistance mechanisms is production of carbapenem-hydrolyzing \( \beta \)-lactamases, which is called carbapenemase; one of them, MβL is more important in drug resistance against carbapenemase.\(^7\) in Iran. However, in the recent years, it has been reported that there is reduced susceptibility to imipenem.\(^{10,11,20}\)

In the recent years, there have been numerous reports on MDR *A. baumannii* from hospital settings in Iran.\(^{10-11}\) In most of these studies, only characterized isolates were obtained from ICUs while this study attempted to determine the resistance among isolates obtained from various medical wards as well. In particular, 45 clinical samples were isolated from wards outside ICUs. Antibiotic susceptibility testing showed that the majority of the isolates were resistant to three or more antibiotics [Table 1] while all the isolates were resistant to imipenem, cefotaxime, and ciprofloxacin, and 95% and 96% of the isolates were resistant to meropenem and amikacin, respectively.

Carbapenems are the best choice for nosocomial infections of *Acinetobacter* in Iran. However, in the recent years, it has been reported that there is reduced susceptibility to imipenem.\(^{10,11,20}\)

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**Table 1: Susceptibility patterns of Acinetobacter baumannii isolates to 16 tested antibiotics by disc diffusion method**

<table>
<thead>
<tr>
<th>Antibiotic disks</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistance ≥ 16 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (n=98)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>3 (3.1)</td>
<td>0</td>
<td>95 (96.9)</td>
</tr>
<tr>
<td>Imipenem(^a)</td>
<td>4 (4.1)</td>
<td>0</td>
<td>94 (95.9)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>24 (24.5)</td>
<td>6 (6.1)</td>
<td>68 (69.4)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13 (13.3)</td>
<td>11 (11.2)</td>
<td>74 (75.5)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Piperaclillin</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Piperaclillin-tazobactam</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>0</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>5 (5.1)</td>
<td>0</td>
<td>93 (94.9)</td>
</tr>
<tr>
<td>Colistin</td>
<td>42 (42.9)</td>
<td>0</td>
<td>56 (57.1)</td>
</tr>
</tbody>
</table>

\(^a\)Resistance rate against imipenem using MIC was 96%, which is higher compared to 96% detected by disc diffusion method. MIC: Minimal inhibitory concentration

**Table 2: Distribution of resistance rate of the studied Acinetobacter baumannii isolates to imipenem by E-test based on source of isolation**

<table>
<thead>
<tr>
<th>MIC (μg/mL) sample</th>
<th>Sensitive Intermediate Resistance ≥ 16 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Sputum</td>
<td>0</td>
</tr>
<tr>
<td>Wound</td>
<td>1</td>
</tr>
<tr>
<td>Body fluids</td>
<td>0</td>
</tr>
<tr>
<td>Urine</td>
<td>0</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
</tr>
<tr>
<td>ETT(^b)</td>
<td>0</td>
</tr>
<tr>
<td>CSF(^c)</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td>Total number</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\)ETT: Endotracheal tube, \(^b\)CSF: Cerebrospinal fluid. MIC: Minimal inhibitory concentration
Further, resistance to colistin, polymyxin, and tigecycline which are the usual choices for nosocomial infections of carbapenem-resistance Acinetobacter is increased around the world, especially in Iran[10,11,21]. Our results suggest colistin as the best choice for A. baumannii isolates in-vitro; this is in accordance with the finding of Japoni-Nejad et al. from Arak, Central area of Iran, that reported colistin and tigecycline as the most effective antibiotic agents against A. baumannii isolates.[10]

In a relatively similar study, Feizabadi et al. reported the prevalence of susceptibility of A. baumannii to imipenem, meropenem, piperacillin-tazobactam, and amikacin rate of 50.7%, 50%, 42.1%, and 38.2%, respectively.[22] These rates indicated higher sensitive isolates compared to our rates with 4.1%, 3.1%, 0%, and 24.5%, respectively. Our results showed a higher resistance rate of A. baumannii isolates against most of the studied antibiotics which were almost similar compared to some studies carried out throughout Iran and other countries.[23-25]

In this study, of the 96 (98%) carbapenem-resistant A. baumannii, 44.8% were found to be MβL producers by MIC-test strips. Such a high resistance rate to imipenem is not uncommon since two separate studies by Safari et al. and Noori et al. have previously shown 99% resistance among their tested isolated by MIC-test strips and disk diffusion methods, respectively.[11,21] Abdalhamid et al. from Saudi Arabia, in contrast with Iranian studies, reported lower carbapenem-resistant A. baumannii (46/141; 32.6%); however, MβL was much higher than that of Iranian report since 43 of 46 carbapenem-resistant A. baumannii isolates were MβL positive.[26]

All MβL producing isolates in our study were MDR and exhibited high resistance to beta-lactams, aminoglycosides, and fluoroquinolones. This high rate of MDR in our study was in agreement with a study from our neighboring country, Pakistan, which reported the prevalence of MDR 100% among A. baumannii isolates.[27] Three of our MβL-producing isolates were sensitive to ampicillin-sulbactam.

In the present study, from a total of 43 MβL producing isolates, 86% were positive for MβL genotypes and subsequently 53.4% and 32.6% of the isolates carried bla_{IMP} and bla_{VM} genes, respectively.

As it is seen in our results, phenotypic and genotypic results of the detection of MβL producing isolates were not the same. Possible reason of higher positive rates for phenotypic MβL production could be the presence of MβL genes other than those we have screened.[28,29]

In a previous Iranian study conducted in Tabriz, from a total of 63 carbapenem-resistant A. baumannii, 31 (49%) were found to be MβL producers by MIC-test strips, of which 19 isolates carried bla_{IMP} and 9 carried bla_{VM} genes; these rates were remarkably close to our findings.[20] In addition, Noori et al. from the capital of Iran reported a bla_{IMP, VM} gene prevalence of 3 of 86 (3.48%) among MβL A. baumannii isolates.[21]

In the current study, no bla_{SPM} gene was detected among A. baumannii isolates; previously, two similar attempts for detection of bla_{SPM} gene by Shahcheraghi et al. (2009–2010) and Noori et al. (2012–2013) were made in Tehran, Iran; only, Shahcheraghi et al. was able to detect this gene in 6 out of 100 tested A. baumannii isolates.[18,21] Reports about the prevalence of these genes among A. baumannii isolates from other parts of the world have shown different patterns. Abdalhamid et al. from Saudi Arabia and Al-Agamy et al. from Egypt reported negative PCR results for bla_{IMP, VM} genes among all MβL producing isolates.[26,30] However, previously, bla_{IMP} and bla_{VM} prevalences have been reported from Greece, China, Korea, and India.[31-34] The present study has some limitations. First, lack of DNA sequencing for knowing the IMP and VIM variants must be mentioned as a limitation of our study. Moreover, employed a typing method could clear the connection between isolates and source of infections in studied hospitals.

**Conclusion**

The prevalence of drug resistance among the clinical samples of A. baumannii isolated in our study against most of the antibiotics is very high. Hence, early detection and optimization of infection control practices are the best defenses.
against these organisms. Therefore, systematic surveillance to detect MβL producing bacteria and rational prescription and use of carbapenems would be helpful to prevent the spread of carbapenem resistance.

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Conflicts of interest
There are no conflicts of interest.

References


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