Antimicrobial Susceptibility Patterns and Distribution of bla_{kpc} Genes among Acinetobacter baumannii Isolated from Patients at Tehran - Iran Hospitals

Maryam Dehghani¹, Faramaz Masedian², Reza Mirnejad³*, Abbas Ali Imani Fooladi⁴ and Setareh Haghighat¹

¹Department of Cell and Molecular Biology, Islamic Azad University of Pharmaceutical Sciences, Tehran, Iran.
²Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
³Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
⁴Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

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Some strains of Acinetobacter which show multiple-drug resistance have produced therapeutic difficulties in worldwide. This is also a new problem in Iran. The purpose of current study was to define the antimicrobial susceptibility patterns and prevalence of bla_{kpc} gene in Acinetobacter baumannii isolates which had been isolated from patient in Tehran- Iran. This study was performed on 70 isolates of Acinetobacter which were isolated from patients. After identification of isolates in species level using cultural and biochemical methods, the susceptibility tests were carried out on 50 isolates of A. baumannii using disk diffusion method. Then isolates were considered for presence of bla_{kpc} gene by PCR. In this study 50 isolates of A. baumannii and 20 isolates of Acinetobacter lwoffii were isolated from patients. More than 55% of isolates showed multiple-drug resistance and also above 40% resistance to imipenem and meropenem was recorded. The MIC of isolates which were resistant to carbapenemes was above 32 µg/ml. The PCR results showed that 8 cases (16%) of isolates had bla_{kpc} gene which most of them had been isolated from patients who were hospitalized in the ICU. Carbapenem and multiple-drug resistant A. baumannii is expanding in Iran and it is considered as an important hazard for hospitalized patients. Moreover regarding to existence of bla_{kpc} gene in this bacterium and possibility of transformation of these genes to the other bacteria, reconsideration in antibiotics consumption patterns and more attention to nosocomial infections control criteria are inevitable.

Key Words: Acinetobacter baumannii, Nosocomial infections, Multiple-drug resistance, Carbapenem, bla_{kpc}, PCR.

* To whom all correspondence should be addressed.

Reza Mirnejad.
Molecular Biology Research Center, Baqiyatallah, University of Medical Sciences, Tehran, Iran
Hemmat Expway. Tel - Fax: +98(21)86039883,
E-mail: rmirnejadreza@yahoo.com

Acinetobacter spp. are gram-negative, oxidase -negative, nonmotile and strictly aerobic coccobacilli that distributed widely in nature and nowadays are considered as an opportunistic pathogen relating to life-threatening nosocomial infections¹-³. This genus includes several species. A. baumannii is the most common species which have been isolated from patients. Other species such as A. lwoffii, A. johnsonii and A. haemolyticus are rarely isolated from the patients. During the
last few decades A. baumannii is considered as one of the life-threatening microorganisms due to its ability to acquire drug resistance. Various researches show that different strains of A. baumannii have been resisted to broad-spectrum antibiotics and now carbapenems are the selective antibiotic for treating of A. baumannii infections, although resistance to carbapenems is also increasing worldwide. Various studies have illustrated that resistance to carbapenems in Acinetobacter spp. is due to the production of a type of beta lactamase enzyme that is called carbapenemase. Ambler has classified beta lactamases in different classes. Class B (metалo-beta- lactamases) and class D (oxacillinase) are considered as a mechanism of resistance to carbapenems in A. baumannii strains that have been also reported in Iran. 

bla<sub>kpc</sub> family with eight various bla<sub>kpc</sub> variants (bla<sub>kpc</sub>-2 to -9) are also a group of powerful carbapenemases belong to â lactamases class A which identified initially in an isolate of klebsiella in the United States and then in other geographical regions worldwide and in other bacteria. Different studies have demonstrated that variants 2, 3, 4 and 10 are more prevalent in A. baumannii. Regarding to this note that the presence of the bla<sub>kpc</sub> gene has not been described in clinical isolates of A. baumannii strains in Iran, this study was performed for the first time in Iran to determine drug-resistance pattern and existence of bla<sub>kpc</sub> gene in multiple-drug resistant A. baumannii by PCR method.

**METHODOLOGY**

**Bacterial isolates**

500 samples including blood, respiratory secretions, urine, skin ulcer and oral mucosa were obtained from hospitalized patients in Imam Khomeini, Baqyatallah and Milad hospitals between years of 2010 to 2011. 50 isolates of A. baumannii from blood culture (n= 19, 38%), mucosa (n=15, 30%), ulcers swabs (n=6, 12%), urine (n=4, 8%) and unknown origin (n=5, 10%) were obtained by cultivation method. All of the isolates were identified by conventional biochemical and microscopic procedures. The isolates were maintained in nutrient broth medium containing 50% glycerol at -80°C.

**Antimicrobial susceptibility testing**

Antibiotic susceptibility tests were performed using disk diffusion method on Mueller Hinton Agar medium as recommended by the Clinical and Laboratory Standards Institute. The standard strain of E.coli ATCC 25922 was used as a negative control and the standard strain of A. baumannii ATCC 19606 was also used as a positive control. The antibiotics (Mast Diagnostics, Mast group Ltd., Merseyside, UK) of ampicillin-sulbactam (10/10 µg), aztreonam (30 µg), amikacin (30 µg), cefepime (30 µg), ceftazidime (30 µg), gentamicin(10 µg), imipenem (10 µg), meropenem (10 µg), norfloxacin (10 µg), ofloxacin (1 µg), ciprofloxacin (5 µg), piperacillin-tazobactam (10/100 µg) and tobramycin (10 µg) were selected for use in antibiotic susceptibility tests. The MICs of piperacillin-tazobactam, imipenem and meropenem were determined by broth microdilution assay. According to the various researches the multiple-drug resistant A. baumannii is referred to those strains of this bacterium which are resistant to three or more than three classes of antibiotics including quinolones (ciprofloxacin), broad-spectrum cephalosporins (cefazidime and cefepime), Beta-lactam-beta-lactamase inhibitor combinations (ampicillin-sulbactam), aminoglycosides (amikacin and tobramycin) and carbapenems (imipenem and meropenem).

**PCR amplification of the bla<sub>kpc</sub> gene**

Total DNA was extracted using high pure PCR template Preparation Kit (Roche Co, Germany). For amplify the gene of bla<sub>kpc</sub>, the primers used were those as previously described by Poirel et al. (Table 1). Each PCR reaction mixture contained 15µl Master mix 1X (Ampliqon III Co, Denmark) that contained 2X PCR buffer, 1.5 mM MgCl2, 1µl template DNA (0.5 µg), 0.15 mM dNTP, 1.25 U Taq DNA polymerase, 20 pmol of each forward and reverse primers and sterile distilled water up to 30 µL. PCR were performed in a GenAmp PCR system.

<table>
<thead>
<tr>
<th>Nucleotide sequence (5’ to 3’)</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGTCTAGTTCTGCTGTCTTG</td>
<td>Kpc-F</td>
</tr>
<tr>
<td>CTTGTCATCCTTGTAGGCCG</td>
<td>Kpc-R</td>
</tr>
</tbody>
</table>
DEHGHANI et al.: DISTRIBUTION OF \( \text{bla}_{\text{kpc}} \) GENES AMONG *Acinetobacter baumannii*

Table 2. Frequency of multi-drug resistance in *Acinetobacter baumannii*

<table>
<thead>
<tr>
<th>Resistance to one or several antibiotics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of antibiotics</td>
<td>1</td>
</tr>
<tr>
<td>Number of isolates resistant <em>A. baumannii</em></td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3. Antibiotic susceptibility patterns of *Acinetobacter baumannii* strains isolated clinical samples

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Resistance No.(%)</th>
<th>Intermediate No.(%)</th>
<th>Susceptibility No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>22 (44)</td>
<td>1(2)</td>
<td>27(54)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>39 (78)</td>
<td>1(2)</td>
<td>10(20)</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>31 (62)</td>
<td>3(6)</td>
<td>16(32)</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>24 (48)</td>
<td>1(2)</td>
<td>25(50)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>49 (98)</td>
<td>0</td>
<td>1(2)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>50 (100)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>50 (100)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32 (64)</td>
<td>3(6)</td>
<td>15(30)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>48 (96)</td>
<td>0</td>
<td>2(4)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>46 (92)</td>
<td>0</td>
<td>4(8)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>46 (92)</td>
<td>0</td>
<td>4(8)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>45 (90)</td>
<td>0</td>
<td>5(10)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>14 (28)</td>
<td>2(4)</td>
<td>34(78)</td>
</tr>
</tbody>
</table>

In the present study, 70 isolates of *Acinetobacter* were isolated from 500 collected samples which 50 (71.4%) samples were identified as *A. baumannii*, 12 (17.1%) samples as *A. lwoffii* and 8 (11.4%) samples were belong to other *Acinetobacter* species. Overall, 82% of the isolates were multidrug-resistant (Table 2). As in the table 1 show, 54% of the isolates were resistant to three or more than three classes of antibiotics and 32% of the isolates were resistant to two classes of antibiotics (Table 1). At least 44% and 78% of the isolates were respectively resistant to meropenem and imipenem and tobramycin. Moreover in this research was not detected any isolates of *A. baumannii* that was resistant to all the tested antibiotics.

PCR amplification (Fig. 1) of *KPC* gene revealed that 8 (16%) of the isolates contained \( \text{bla}_{\text{kpc}} \). This is also the first report of \( \text{bla}_{\text{kpc}} \) in...
A. baumannii in the Iran. Most of these isolates were multidrug-resistant such as meropenem and imipenem (MIC ≥ 16µg/ml). Meanwhile four cases of these strains had been isolated from blood and two cases from trachea of the hospitalized patients in the intensive care units who were treated with multiple antibiotic courses.

**DISCUSSION**

Acinetobacter baumannii is an opportunistic pathogen and one of the agents of nosocomial infections in the past 30 years. This bacterium especially multidrug-resistant isolates cause serious infectious in hospitalized patients mostly in intensive care units\(^9\). Due to expanded resistance to antimicrobial drugs, treatment of these infections is often difficult and carbapenems are currently the antibiotics of choice. Although resistance to carbapenems is also increasing. One of the ways of resistance to carbapenems is often associated with acquired carbapenemase production that is under control of the bla\(_{kpc}\) gene which is now observed increasingly worldwide\(^9\). As regards the presence of the bla\(_{kpc}\) gene in clinical isolates of A. baumannii in Iran has not been determined, so the present study was performed in Iran for the first time in order to determine the presence of the KPC gene in clinical isolates of A. baumannii in Tehran by PCR method.

In this study, 71.5% of isolates were identified as A. baumannii and 28.5% were A. lwoffii and other species of Acinetobacter. These results are approximately similar to Constantinii and et al. findings at 2001 to 2004 who isolated 24 clinical isolates of Acinetobacter which 71% and 29% of them were A. baumannii and A. lwoffii respectively\(^8\).

The present study like the Bayugo et al. studies at 2002 and Joshi et al. studies at 2003 showed that antibiotic resistance is seriously increasing so that 82% of the isolated A. baumannii strains showed multiple-drug resistance (MDR) phenotype. They in their studies, the rate of isolation of multidrug-resistant A. baumannii strains about 45% to 75% have been reported\(^21,22\).

Like the Feizabadi et al.\(^11\) study in Iran, this study, susceptibility rate to meropenem and imipenem was reported above 40% which is in contrariety with the findings of Hujer et al.\(^23\) who reported a resistance of about 20% to the mentioned antibiotics. In another research which was carried out by Cisneros et al. in Spain susceptibility rate to the imipenem has been reported about 43%.

Anyway according to the various studies like the Zarrilli et al. study resistance rate to the carbapenems is increasing worldwide\(^10,24\).

Although a lot of researches have been performed about determining of amount of the bla\(_{kpc}\) gene in different isolates of Klebsiella, Pseudomonas and E. coli but the performed studies on A. baumannii in this case are low\(^24,25\). However the isolation percentage of this gene in various areas is different. for example in a study which carried out by Robledo et al., in Puertorico the isolation rate of A. baumannii containing the carbapenemase encoding gene has been reported.

![Agarose gel electrophoresis of PCR amplified products generated from DNA samples. Lanes 1 DNA size marker (100bp DNA ladder, SM#333), Lane 2 negative control (Klebsiella pneumoniae ATCC BAA-1706), Lane 3 Positive control (Klebsiella pneumonia ATCC BAA-1705), and Lane 4 show 798 bp bla\(_{kpc}\) amplification product in A. baumannii strains isolated clinical samples.](image-url)
about 3.8% which this is less than 16% that was obtained in the present study. In another study by Zulueta et al., the isolation rate of bla\textsubscript{KPC} gene were reported about 22% which is almost similar to our report in this study. This study like the Poirel et al. study a rapid technique based on PCR by a pair of primers were used for identifying of carbapenemases encoding genes in \textit{A. baumannii}.

As regards this is the first report of multidrug-resistant \textit{A. baumannii} clinical isolates harboring the bla\textsubscript{KPC} gene in Iran and as this genes on the transportable elements, It is possible that be transferred from one bacterium to another and in the community are distributed. Presence of the bla\textsubscript{KPC} gene in \textit{A. baumannii} strains with several acquired and innate mechanisms for resistance can be lead to the emerging of carbapenem resistant \textit{A. baumannii} strains (carbapenems are the selective antibiotic for treating of the infections caused by multiple-drug resistant \textit{A. baumannii} strains). Therefore reasonable consumption of antibiotics in hospitals for controlling of infections especially in ICU departments can has an important preventing role in appearance of these resistant strains and their related infections. This clearly emphasizes the importance of reasonable consumption of antibiotics in hospitals for controlling of infections especially in ICU -and reduce the possibility of nosocomial transmission and potential outbreaks.

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