Detection of OXA-Type Carbapenemase Genes in *Acinetobacter baumannii* Isolates from Nosocomial Infections in Isfahan Hospitals, Iran

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**ABSTRACT**

**Background:** *Acinetobacter baumannii* as one of the causes of nosocomial infections has become resistant to almost all antimicrobial agents. The emergence of resistance to carbapenems, one of the last drugs on the shelf, is the major concern about *A. baumannii* antimicrobial resistance. Resistance to carbapenems is mediated by production of class B and D carbapenemases. The aim of this study was to detect the resistance genes including *bla*<sub>OXA</sub>-23, 24, 51, and 58 in *A. baumannii* isolates from nosocomial infections in Isfahan hospitals.

**Methods:** A total number of 456 clinical specimens were collected from nosocomial infections and evaluated in order to isolate *A. baumannii* strains. After identification of the isolates, the antibiotic sensitivity to carbapenems was assessed using disk diffusion method. The resistance genes of *bla*<sub>OXA</sub>-23, 24, 51, and 58 were detected by multiplex PCR method.

**Results:** Fifty *A. baumannii* isolates were isolated from clinical specimens. Fifty two percent of the isolates showed phenotypic resistance to the carbapenems (imipenem and meropenem). According to PCR results, 88% of resistant isolates had ≥1 *bla*<sub>OXA</sub> gene. The frequency of resistant isolates bearing *bla*<sub>OXA</sub>-23, *bla*<sub>OXA</sub>-24, and *bla*<sub>OXA</sub>-58 were 77%, 38% and 15% respectively.

**Conclusion:** This study showed the high frequency of carbapenem resistance genes among *A. baumannii* isolates. Therefore, adopting an appropriate strategy to confine the spreading of these strains and also implementing new treatment regimens are necessary.

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Introduction

Transmission of antibiotic resistance determinants by mobile genetic elements such as plasmids, transposons and integrons play important role in acquisition and distribution of resistance among Gram negative bacteria, especially Acinetobacter baumannii strains. A. baumannii is one of the important causes of nosocomial infections. Resistance of the isolates to all available antibiotics is considered as a major risk to healthcare systems. Currently, carbapenems are used as drug of choice to treat multidrug resistant A. baumannii infections (1, 2).

Imipenem is among the most extended-spectrum beta-lactam antibiotics. It strongly binds to PBP1 and PBP2 and prevents transpeptidation. Imipenem is resistant to many beta-lactamases such as penicillinase and cephalosporinase and is used for the treatment of infections caused by A. baumannii, Listeria monocytogenes, Neisseria meningitides, Pseudomonas aeruginosa, Gram negative enteric bacteria and a number of obligate anaerobic bacteria. Meropenem is another carbapenem with similar antimicrobial features (3).

In recent years, nosocomial infection epidemics caused by carbapenem resistant A. baumannii strains have been increasing. The major mechanism of resistance to carbapenems in A. baumannii is the production of beta-lactamase enzymes including metalobetalactamase (class B) and oxacillinase (class D). These enzymes are encoded by different genes which the most remarkable ones are oxacillinase producing genes (bla OXA) (4). Today, 5 phylogenetic subgroups of carbapenemas related to OXA type have been identified in A. baumannii including OXA-23-like, OXA-24-like, OXA-51-like, OXA-58-like and OXA-143-like (5, 6). The genes are located on both chromosome and plasmid; except for blaOXA-51-like which is chromosomal. Consequently, the transmission of these genes among Gram negative bacteria and especially A. baumannii strains is mediated by these mobile genetic elements (1).

Detection of these resistance genes and their frequency in clinical samples of each geographical region can provide invaluable information for choosing effective therapeutic regimens and also preventing their dissemination in that region. The aim of this study was to detect resistance genes including blaOXA-23, 24, 51, 58 among A. baumannii isolates from nosocomial infections in Isfahan hospitals.

Materials and Methods

Specimens and strains

During a 12-month period (February 2014 - January 2015), 456 clinical specimens (blood, sputum, wound, catheter and CSF) were collected from patients admitted to intensive care units (ICUs) in Isfahan city. The samples were inoculated into the blood agar (Merck, Germany) and MacConky agar (Merck). After incubation of cultures at 37 °C, the colonies were evaluated macroscopically and microscopically. All isolates were identified by routine biochemical tests including oxidase, catalase, motility, glucose oxidation, citrate and malonate. The antibiotic susceptibility of the isolates to carbapenems was done by disk diffusion method. The antibiotic disks (Mast, UK) of imipenem (10 μg) and meropenem (10 μg) were used. The resistance or sensitivity of bacteria to the antibiotics was determined after comparing the results with reference tables of Clinical and Laboratory Standard Institute (CLSI) (7). Escherichia coli ATCC 35218 was used as a quality control strain. DNA extraction of the isolates was done using phenol-chloroform method. The sequence of the primers used for detection of resistance genes were presented in Table 1.

PCR for amplification of bla OXA genes

The sequence of the primers used for detection of resistance genes were presented in Table 1. The primers (T.A.G Company, Denmark) were selected according to the similar articles (8) and their...
specificity for related genes was evaluated by Primer Blast software (8). In order to detect oxacillinase genes, multiplex PCR was done for all (sensitive and resistant) isolates. Four genes, including blaOXA-51 for proving A. baumannii strain; and resistance genes of blaOXA-23, blaOXA-24 and blaOXA-58 were evaluated. A. baumannii NCTC 13302 and E. coli ATCC 25922 were used as the positive and negative controls, respectively. The materials and amounts utilized for PCR were as follow: template DNA (1 μl), Taq polymerase (0.6 μl), primer (1 μl), MgCl2 (0.6 μl), dNTP (1 μl), buffer 10X (3 μl) and nuclease free distilled water for the rest of the volume up to 30 μl. The PCR thermal cycles were as follow: initiation denaturing: 10 min, 95 °C; denaturing: 25 seconds 95 °C; annealing: 40 seconds, 52 °C; extension: 50 seconds, 72 °C; final extension: 5 minutes, 72 °C (the number of cycles was selected as 75 cycles). PCR products were analyzed by agarose gel electrophoresis.

Table 1. The sequence of selected primers for evaluated genes.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Product size</th>
<th>Primer sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-51-forward</td>
<td>353 bp</td>
<td>5’-TAATCTTTGATCGCTTG-F</td>
</tr>
<tr>
<td>E. coli forward</td>
<td>248 bp</td>
<td>5’TGATCTTTGACCTTACCTT-C</td>
</tr>
<tr>
<td>OXA-23-forward</td>
<td>500 bp</td>
<td>5’-AGTAAGACGCTTCATC-T</td>
</tr>
<tr>
<td>OXA-24-forward</td>
<td>501 bp</td>
<td>5’-AGATATTGGAGTTCAGC-T</td>
</tr>
<tr>
<td>OXA-58-forward</td>
<td>500 bp</td>
<td>5’TGATCTTTGACCTTACCTT-C</td>
</tr>
</tbody>
</table>

Results

From the total number of 456 evaluated clinical specimen, 50 bacterial isolates (11%) were identified as A. baumannii. Twenty six specimen (52%) of A. baumannii isolates were resistant to carbapenems (imipenem and meropenem). It should be noted that the sensitivity and resistance to both imipenem and meropenem were the same in all isolates. The resistant isolates from lung catheters, CSF and wound were isolated in higher frequency (Table 2).

Table 2. The frequency of resistant isolates in clinical specimen.

<table>
<thead>
<tr>
<th>Clinical samples</th>
<th>No. CRI* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary artery catheter sample</td>
<td>11 (61%)</td>
</tr>
<tr>
<td>UTI sample</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Wound</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Blood</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>CSF</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>

*CRI: Carbapenem Resistant Isolates.

The PCR results related to A. baumannii were shown in Figure 1. All strains carried a blaOXA-51 gene. In 24 carbapenem sensitive isolates, the blaOXA-23, 24, 58 genes were not detected. Among the 26 isolates that were carbapenem resistant phenotypically, 23 strains (88%) had one or more than 1 resistance gene in addition to blaOXA-51 gene. In one isolate, 4 bla genes (blaOXA-51, 23, 24, 58) were detected that isolated from the pulmonary artery catheter of a 43 years old patient at ICU. In 7 isolates, 3 bla genes (blaOXA-51, 23, 24) were detected which were related to urine, pulmonary artery catheter and blood samples. In 2 isolates, 3 genes (blaOXA-51, 23, 58) were detected that isolated from lung catheter and CSF. The simultaneous presence of two genes (blaOXA 51, 58) was detected in one isolate from CSF (Table 3).

Figure 2. PCR results of blaOXA genes in A. baumannii isolates. Lane 1: molecular weight marker 50 bp; lane 2, 8: positive control; lane 3: negative control; lane 4, 5, 6 and 7: clinical isolates.
The frequency of resistant isolates was long, in Description of OXA-Type

Discussion

In recent years, A. baumannii has been recognized as an emerging opportunistic infectious agent which is responsible for different nosocomial infections (9). This pathogen shows particular capability for fast development of antibiotic resistance in the ICUs which is followed by multiple drug resistance.

In this study, 52% of A. baumannii isolates showed carbapenem resistance. The frequency of carbapenem resistant A. baumannii isolates was greater in comparison with other studies in other locations in Iran (8, 10-12). In the study by Jafari et al., the frequency of resistant isolates was reported as 40.9% (13).

In addition, the percentage of carbapenem resistance in our study was greater than similar studies in Asia and other locations around the world. The frequency of imipenem and meropenem resistant A. baumannii isolates at hospitals in the Middle Asia has been reported as 25% (5). In the study by Valencia et al. in Spain in 2009, the imipenem resistance among A. baumannii isolates was 43% while in the study by Hujer et al. published in 2006, this rate was 20% (14, 15).

Comparing the results of this study with other studies in Iran and the rest of the world it can be indicated that the frequency of carbapenem resistant A. baumannii strains is increasing. Since carbapenem resistance in A. baumannii is mainly caused by the production of class D carbapenemases and their coding genes are often located on plasmid and other mobile genetic elements, the observed increased resistance to these antibiotics was expected (16, 17).

The blaOXA-23, 24, 58 genes were not detected in 3 carbapenem resistant isolates. The resistance to carbapenem in these isolates was probably caused by other mechanisms such as change of outer membrane proteins, increased efflux of drugs and changes in penicillin binding proteins (PBPs) (4). In the present study, 88% of resistant strains had one or more than one blaOXA gene. The blaOXA-23 with the frequency of 77% was the most prevalent gene among resistant isolates and the frequency of blaOXA-24 and blaOXA-58 was 38% and 15%, respectively. The blaOXA-51 is chromosomal gene and was detected in all evaluated strains (4, 5). Although the results of this study was consistent with the results of other studies in other locations in Iran, but the frequency of the mentioned genes was higher. In the study by Feyzabadi et al. in Tehran, in addition to blaOXA-51 which was detected in all isolates, blaOXA-23 gene with the frequency of 25% was the most common detected gene and considered as the main cause of resistance; while blaOXA-24 and blaOXA-58 had the frequency of 17.9% and 9%, respectively. Moreover, simultaneous presence of both blaOXA-51 and blaOXA-23 genes has been reported in 4.6% of specimens (8). In the present study, the simultaneous presence of both blaOXA-51 and blaOXA-23 genes was reported more than co-preservation of blaOXA-51 and blaOXA-24. The findings were consistent with the results of the study by Feyzabadi et al. In which more than 75% of resistant strains had two or more blaOXA genes (8).

In another study by Morovat et al. blaOXA-51 in all isolates was also detected and the frequency of blaOXA-23, blaOXA-58 and blaOXA-24 genes were reported as 25%, 21.2% and 15%, respectively. The simultaneous presence of two, three and four genes was reported in 41.2%, 6.2% and 2.5% of isolates, respectively (18).

The blaOXA-58 has been reported with high frequency in different studies around the world, while in the present study, lower frequency was reported for this gene. This difference may be caused by the difference between the

Table 3. Distribution of blaOXA genes in the carbapenem resistant A. baumannii isolates.

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>blaOXA-23</th>
<th>blaOXA-24</th>
<th>blaOXA-51</th>
<th>blaOXA-58</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.5</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.5</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

implemented molecular methods for detecting the genes or may be due to geographical differences.

In many studies around the world, the blaOXA-23 has been the most frequent carbapenemase gene in carbapenem resistant \( A.\) \( baumannii. \) In the study by Karunasagar and colleagues in 2011 in 62 isolates of \( A.\) \( baumannii,\) the blaOXA-23 was the most frequent gene (19). In the study by Amudhan \( et\) \( al.\) in 2011, the OXA carbapenemases were detected in 91.3% of carbapenem resistant \( A.\) \( baumannii\) strains. The blaOXA-51 and blaOXA-23 with frequency of 85% and 82%, respectively, were the most prevalent among blaOXA types (16). Also In a study by Roy \( et\) \( al.\) in 2011, four carbapenem resistant \( A.\) \( baumannii\) isolated from blood culture and blaOXA-23 gene was reported as the most frequent detected carbapenemase (20). Currently, the emergence of carbapenem resistant \( A.\) \( baumannii\) strains has become a worldwide problem. The main factor that contributes to this problem is the innate and unusual capability of this pathogen for long-term survival at hospital environments (21, 22).

**Conclusion**

Resistance has restricted the successful treatment for the infections caused by \( A.\) \( baumannii.\)

The results of this study and studies by other researchers showed that carbapenem resistance is increasing among \( A.\) \( baumannii\) strains isolated in Iran and is often associated with multiple drug resistance. Moreover, the diversity of blaOXA genes was high among these isolates. Adopting an appropriate strategy to confine the spreading of these strains and providing more precise supervision on the use of antibiotics at medical centres, especially the intensive care units, is necessary.

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**Conflict of interests**

No conflict of interests is declared.

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