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*$_{\text{OXA-23, 24, 51, and 58}}$ 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* $_{\text{OXA-23, 24, 51, and 58}}$ 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*$_{\text{OXA}}$ gene. The frequency of resistant isolates bearing *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$ and *bla*$_{\text{OXA-58}}$ 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 PBP_1 and PBP_2 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 *bla*$_{OXA\text{-}51\text{-}like}$ 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 *bla*$_{OXA\text{-}23,\ 24,\ 51,\ 58}$ 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 *bla*OXA-51 for proving *A. baumannii* strain; and resistance genes of *bla*OXA-23, *bla*OXA-24 and *bla*OXA-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), $MgCl_2$ (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.

Target gene	Product size	Primer Sequence (5' to 3')
OXA 51-forward	353	5'-TAATGCTTTGATCGGCCTTG-3'
Reverse		5'-TGGATTGCACTTCATCTTGG-3'
OXA24-forward	246	5'-GGTTAGTTGGCC CCC TTA AA-3'
Reverse		5'-AGT TGA GCG AAA AGG GGA TT-3
OXA 58-forward	599	5'–AAG TAT TGG GGC TTG TGC TG-3'
Reverse		5'-CCC CTC TGC GCT CTA CAT AC-3'
OXA 23-forward	501	5'GATCGGATTGGAGAACCAGA-3'
Reverse		5'ATTTCTGACGGCATTTCCAT-3'

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.

Clinical samples	No. CRI*(%)
pulmonary artery catheter sample	11 (61%)
UTI sample	4 (30%)
Wound	5 (55%)
Blood	1 (25%)
CSF	3 (60%)
Sputum	2 (100%)

*CRI: Carbapenem Resistant Isolates.

The PCR results related to *A. baumannii* were shown in Figure 1. All strains carried a *bla*OXA-51 gene. In 24 carbapenem sensitive isolates, the *bla*OXA-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 *bla*OXA-51 gene. In one isolate, 4 *bla* genes (*bla*OXA-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 (*bla*OXA-51, 23, 24) were detected which were related to urine, pulmonary artery catheter and blood samples. In 2 isolates, 3 genes (*bla*OXA-51, 23, 58) were detected that isolated from lung catheter and CSF. The simultaneous presence of two genes (*bla*OXA 51, 58) was detected in one isolate from CSF (Table 3).

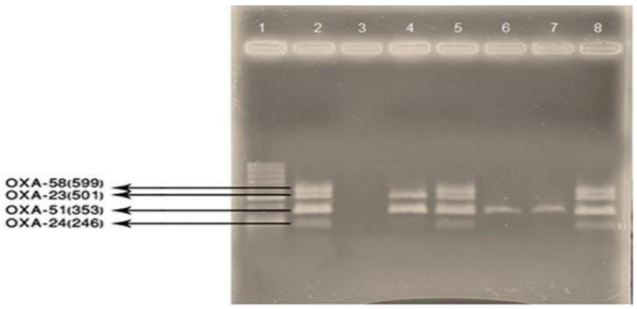

Figure 2. PCR results of *bla*OXA genes in *A. baumannii* isolates. Lane 1: molecular weight marker 50 bp; lane 2, 8: positive control; lane 3: negative control; lane 4, 5, 6 and 7: clinical isolates.

Table 3. Distribution of *bla*OXA genes in the carbapenem resistant *A. baumannii* isolates.

% of Isolates	*bla*OXA-51	*bla*OXA-23	*bla*OXA-24	*bla*OXA-58
38.5	×	×		
7.7	×		×	
3.8	×			×
27	×	×	×	
7.7	×	×		×
3.8	×	×	×	×
11.5	×			

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 *bla*OXA-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 *bla*OXA gene. The *bla*OXA-23 with the frequency of 77% was the most prevalent gene among resistant isolates and the frequency of *bla*OXA-24 and *bla*OXA-58 was 38% and 15%, respectively. The *bla*OXA-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 *bla*OXA-51 which was detected in all isolates, *bla*OXA-23 gene with the frequency of 25% was the most common detected gene and considered as the main cause of resistance; while *bla*OXA-24 and *bla*OXA-58 had the frequency of 17.9% and 9%, respectively. Moreover, simultaneous presence of both *bla*OXA-51 and *bla*OXA-23 genes has been reported in 4.6% of specimens (8). In the present study, the simultaneous presence of both *bla*OXA-51 and *bla*OXA-23 genes was reported more than co-presence of *bla*OXA-51 and *bla*OXA-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 *bla*OXA genes (8).

In another study by Morovat *et al*. *bla*OXA-51 in all isolates was also detected and the frequency of *bla*OXA-23, *bla*OXA-58 and *bla*OXA-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 *bla*OXA-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

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

In many studies around the world, the *bla*$_{OXA-23}$ 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 *bla*$_{OXA-23}$ 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 *bla*$_{OXA-51}$ and *bla*$_{OXA-23}$ with frequency of 85% and 82%, respectively, were the most prevalent among *bla*$_{OXA}$ types (16). Also In a study by Roy *et al*. in 2011, four carbapenem resistant *A. baumannii* isolated from blood culture and *bla*$_{OXA-23}$ 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 *bla*$_{OXA}$ 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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