INTRODUCTION

Acinetobacter baumannii (a well-known Gram-negative opportunistic nosocomial pathogen), is one of the most important bacteria with multi-drug resistance (MDR) phenotype. This organism is related with a variety of infections, such as septicemia, pneumonia, urinary tract infection, meningitis, wound infection etc [1, 2]. Carbapenems are one the members of the beta-lactam class of antibiotics that are used as a main choice for treatment of MDR A. baumannii infections, but recently the rise of carbapenem resistance strains have led to limited efficiency of these antibiotics [2]. Several mechanisms can be responsible of resistance of A. baumannii to carbapenems. One of the major mechanisms is the production of bacterial carbapenemases, which are enzymes that hydrolyze carbapenems. In A. baumannii three types of these enzymes have been reported belonging to class A, B and
D of Ambler classification: class A including beta-lactamases inhibited by clavulanic acid such as KPC and GES; class B including metallo-beta-lactamases such as VIM, IMP, SIM, and NDM; and class D including oxacillinases [3, 4]. Six subclasses of oxacillinases (OXA-23, OXA-40, OXA-51, OXA-143, and OXA-235) have been known in A. baumannii. OXA-51 as an intrinsic subclass and five other subclasses are acquired [5]. Dissemination of OXA-type carbapenemase-producing A. baumannii strains have been reported from different geographical areas around the world [6-10]. In Iran, previous studies have described the presence of OXA-type carbapenemase-encoding genes among clinical isolates of A. baumannii from diverse areas; but no information is available from Isfahan, the most important central city of Iran [11-18]. So, the aim of this study was to evaluate the prevalence of OXA-type carbapenemases among clinical isolates of A. baumannii in three major hospitals of Isfahan.

# MATERIALS AND METHODS

**Bacterial isolates**

In this cross-sectional study, conducted from October 2015 to October 2016, one hundred fifty-three non-repeatedly A. baumannii strains were isolated from various clinical sources. All samples were collected from inpatients in three major hospitals (Al-Zahra, Imam Mousa Kazem, and Sharriati) of Isfahan. The samples were cultured on standard laboratory media including blood agar and MacConkey agar (Merck, Germany) and incubated overnight at 37°C. The identification of A. baumannii strains were performed with Gram staining and routine biochemical tests such as oxidative or fermentative metabolism, catalase, oxidase, motility, production of acid from different sugars, urease, etc. [19]. The bacterial isolates were transferred in brain heart infusion broth medium containing 20% glycerol and stored at -20°C.

**Ethical considerations**

This study was evaluated and approved by the Ethics Committee of Isfahan University of Medical Sciences (No-395081).

**Antimicrobial susceptibility testing**

The antimicrobial susceptibility testing was performed based on Kirby-Bauer disk diffusion method using imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), ceftepime (30 µg), ceftazidime (30 µg), ceftriaxone (30µg), piperacillin-tazobactam (100/10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and tetracycline (30 µg) disks (MAST, Merseyside, UK), and results were interpreted based on clinical and laboratory standards institute (CLSI) criteria. Escherichia coli ATCC 25922 was used as the control strain for antibiotic disks in susceptibility testing [20].

**Amplification of bla\textsubscript{OXA}\textsubscript{genes}**

Detection of bla\textsubscript{OXA} genes was performed with multiplex polymerase chain reaction (PCR) method. Primer sequences used for detection of bla-OXA-51, blaOXA-23, blaOXA-24 and blaOXA-58 gene and their fragment size are presented in Table 1. The amplification was done with an initial denaturation step (5 min at 94°C), followed by 30 cycles (1 min at 94°C as denaturation, 1 min at 50°C as annealing and 72°C at 50 s as extension) and 10 min at 72°C for the final extension [21]. Amplified fragments were separated by 2% agarose gel electrophoresis at 80V for 2h.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Amplicon size (bp)</th>
<th>Sequences</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla\textsubscript{OXA-23}</td>
<td>501</td>
<td>5’-GAT CGG ATT GGA GAA CCA GA-3’ 5’-ATT TCT GAC CGC ATT TCC AT-3’</td>
<td>38</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-24}</td>
<td>246</td>
<td>5’-GTT TAG TTG GCC CCC TTA AA-3’ 5’-AGT TGA GCG AAA AGG GGA TT-3’</td>
<td>38</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-51}</td>
<td>353</td>
<td>5’-CGG CCT TGTAA TGC TTT GAT-3’ 5’-TGG ATT GCA CTT CAT CTT GG-3’</td>
<td>38</td>
</tr>
<tr>
<td>bla\textsubscript{OXA-58}</td>
<td>599</td>
<td>5’-AAG TAT TGG GCC TTG TGC TG-3’ 5’-CCC CTC TGC GCT CTA CAT AC-3’</td>
<td>38</td>
</tr>
</tbody>
</table>
RESULTS

Bacterial isolates
In this study, 153 clinical isolates of A. baumannii were obtained from inpatient in three major hospitals of Isfahan: 126 (82.4%) isolates from Al-Zahra, 14 (9.1%) isolates from Imam Mousa Kazem, and 13 (8.5%) isolates from Shariati. Most isolates were related to the intensive care unit (64%), followed by the internal medicine (13.7%), surgical (12.5%), and emergency unit (9.8%), respectively. Bacterial strains were isolated from trachea (52.9%), wound (11.8%), cerebrospinal fluid (7.8%), sputum (7.2%), urine (5.9%), blood (4.6%), abscess (1.3%), catheter (1.3%), throat (1.3%), synovial fluid (1.3%), eye (1.3%), and other (3.3%) sources.

Antimicrobial susceptibility testing
The highest resistance rates in A. baumannii isolates were observed towards beta-lactam antibiotics including imipenem and ertapenem. Moreover, the highest susceptibility was demonstrated to amikacin. In Table 2 is reported the antibiotic resistance pattern of all A. baumannii isolates.

Amplification of bla_{OXA} genes
PCR assay showed the presence of the bla_{OXA} genes in all isolates. The bla_{OXA-58} gene was recognized in all (100%) isolates. 90.8% and 62.1% of isolates possessed the bla_{OXA-23} and bla_{OXA-24} genes, respectively; but, the bla_{OXA-58} gene was not detected in the studied isolates. Also, 86 (56.2%) of isolates had both the bla_{OXA-23} and bla_{OXA-24} genes, simultaneously. The highest frequency of the bla_{OXA-23} and bla_{OXA-24} genes was related to Al-Zahra hospital isolates with 93% and 63.4%, respectively. While, the lowest prevalence rate of bla_{OXA-23} and bla_{OXA-24} genes was observed among isolates of Imam Mousa Kazem and Shariati hospitals with 71.4% and 53.8%, respectively (Table 3). Figure 1 displays the electrophoretic pattern of the bla_{OXA} genes.

DISCUSSION

Nowadays, resistance to carbapenems in A. baumannii has become a global problem in the field of healthcare. High consumption of antibiotics

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Table 2 - Antimicrobial susceptibilities of the Acinetobacter baumannii isolates (n=153).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible, No. (%)</th>
<th>Intermediate, No. (%)</th>
<th>Resistant, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>153 (100%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1 (0.07%)</td>
<td>0 (0.0%)</td>
<td>152 (99.3%)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>153 (100%)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1 (0.07%)</td>
<td>3 (1.9%)</td>
<td>149 (97.4%)</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>0 (0.0%)</td>
<td>4 (2.6%)</td>
<td>149 (97.4%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4 (2.6%)</td>
<td>1 (0.07%)</td>
<td>148 (96.7%)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>1 (0.07%)</td>
<td>1 (0.07%)</td>
<td>151 (98.6%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9 (5.9%)</td>
<td>28 (18.3%)</td>
<td>116 (75.8%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1 (0.07%)</td>
<td>0 (0.0%)</td>
<td>152 (99.3%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>15 (9.8%)</td>
<td>12 (7.8%)</td>
<td>126 (82.4%)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>7 (4.6%)</td>
<td>2 (1.3%)</td>
<td>144 (94.1%)</td>
</tr>
</tbody>
</table>

Table 3 - Amplification of bla_{OXA} genes in Acinetobacter baumannii isolates.

<table>
<thead>
<tr>
<th>Oxa type</th>
<th>bla_{OXA-23}</th>
<th>bla_{OXA-24}</th>
<th>bla_{OXA-58}</th>
<th>bla_{OXA-58}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Zahra</td>
<td>126 (100%)</td>
<td>117 (93%)</td>
<td>80 (63.4%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Imam Mousa Kazem</td>
<td>14 (100%)</td>
<td>12 (85.7%)</td>
<td>8 (57.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Shariati</td>
<td>13 (100%)</td>
<td>10 (77%)</td>
<td>7 (53.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>153 (100%)</td>
<td>139 (90.8%)</td>
<td>95 (62.1%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

86 (56.2%)
particularly beta-lactams and long-term hospitalization may be two important causes of the isolation of carbapenem-resistant *A. baumannii* strains from inpatients. Infections caused by these strains are associated with high rates of mortality in hospitalized patients as well as the high cost of treatment [22, 23]. Therefore, awareness of the contribution of these strains in infectivity in hospitalized patients as well as continuous tracking of involved genes in each region can significantly contribute to the prevention and control of these infections.

In our study, the rate of resistance to imipenem was 100% that was higher than those reported from others Iranian cities by Shoja et al. (Ahvaz), Safari et al. (Hamadan), Akbari et al., Najjar Peerayeh et al., Bahador et al., and Asadollahi et al. (Tehran) and Alaei et al. (Shiraz); but, it was similar to studies conducted by Zanganeh et al. and Salimizand et al. (Mashhad) [11, 13-15, 24-28]. In the present study, similar to other studies conducted in Iran, OXA-23 was the most prevalent acquired OXA-type carbapenemase among carbapenem-resistant isolates, followed by OXA-24 [18, 26, 29]. Nevertheless, OXA-58 was not detected in any of the isolates that in parallel with the results of above-mentioned studies.

At the regional level, in a study that was done by Zowawi and colleagues, OXA-23 was detected as a dominant type (91.5%) among carbapenem-resistant *A. baumannii* isolates from hospitals of Saudi Arabia, United Arab Emirates, Oman, Qatar, Bahrain, and Kuwait, followed by OXA-24 (4.3%) [20]. Similar to our results, OXA-58 could not be identified by them in any of resistant isolates. Likewise, in the studies from other parts of the world have been reported that OXA-23 as a dominant type in carbapenem-resistant isolates has been associated with outbreaks [31-34].

Contrary to our findings, Kooti et al. and Sohrabi et al. have reported the low prevalence of OXA-58 among carbapenem-resistant isolates in their studies from (Shiraz) (0.5%), (Tehran) (3%), and (Tabriz) (3.2%) [17, 35]. However, two separate Turkish studies, have reported high prevalence of OXA-58 among carbapenem-resistant *A. baumannii* isolates [35, 36]. In addition, other studies from China, Brazil, France and Iran have reported the presence of bla*OXA-58* gene among resistant isolates [37-40]. These findings suggest that since the transfer of bla*OXA-58* gene is plasmid-mediated, the prevalence of OXA-58 among carbapenem-resistant *A. baumannii* isolates is geographically different.

To the best of our knowledge, this is the first documented report of prevalence of bla*OXA* genes among clinical isolates of *A. baumannii* from Isfahan. The results of this study demonstrated high prevalence of OXA-type carbapenemase among imipenem resistant *A. baumannii* in the different wards of hospitals, moreover, correct prescription of antibiotics and effective infection control policies for preventing extent of resistant strains is needed.
CONCLUSIONS

In conclusion, we found that the prevalence of OXA-type carbapenemases among carbapenem-resistant A. baumannii isolates is high in Isfahan and OXA-23 is the major carbapenemase mechanism responsible for the resistance phenotype.

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Conflict of interest

None declared

REFERENCES


