Antibiotic resistance patterns and the prevalence of ESBLs among strains of *Acinetobacter baumannii* isolated from clinical specimens

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Abstract

The aim of current study was to define the antibiotic susceptibility patterns and detect the prevalence of producing strains of Extended-Spectrum β-Lactamase (ESBL) in 400 *Acinetobacter baumannii* (*A. baumannii*) which had been isolated from 3 major hospitals in Tehran clinical samples with combined disk test. The majority of isolates were from blood specimens. The susceptibility tests were carried out according to the Clinical and laboratory Standards Institute (CLSI) guidelines using disk diffusion method in order to determine sensitivity of 100 isolates of *A. baumannii* to 11 antibiotics. Isolates of *A. baumannii* revealed the highest resistance to cefepime, ceftriaxone, amikacin, respectively but polymyxin B considered as effective drugs in this study, multidrug resistant in these strains were 70%. The Minimum Inhibitory Concentrations (MIC) were determined for cefepime in 91% and for ceftazidime in 84% of the studied isolates were MIC≥ 128 μg/ml, and finally the phenotypic method of combined disk test demonstrated that 20% of samples were ESBL positive. Regarding to production of ESBL in this bacterium and possibility of coding genes transformation to the other bacteria, reconsideration in antibiotics consumption patterns and as well as more attention to noscomial infections control criteria are inevitable.

Keywords: *Acinetobacter baumannii*, Extended-Spectrum β-Lactamase (ESBL), Combined disk.

1 Introduction

.... *Acinetobacter baumannii* (*A. baumannii*) is a gram-negative cocobacilli bacterium widely available in soil, water and also scattered and remains alive for long time periods in the hospital environment, capable of easily transmitting among patients [1, 2]. Due to the significant clinical activity of this bacterium especially in recent years, and its ability to acquire drug resistance, it is considered as a threatening
microorganism to antimicrobial drugs. Nowadays is one of the problematic opportunistic pathogens, especially in intensive care units, because of the incidence of drug-resistant strains in the world [3]. A.baumannii causes different nosocomial infections, including bacteremia, urinary tract infection and secondary meningitis, but it has a significant role in the development of pneumonia, especially the pneumonia caused by upper respiratory tract of patients in intensive care units. Several studies such as Bassetti et al. (2008) showed that more than 80% of A.baumannii isolated from patients, become resistant against the most of the prescribed antibiotics [4]. The studies of Leung et al. (2006) and Michalopoulos et al. (2010) revealed that the different strains of A.baumannii were resistant to the majority of consumed antibiotics [5, 6].

One of the drugs that have been used extensively throughout the world to treat bacterial infections is β-lactam family [7]. In other hand, these bacteria produce the β-lactamase enzymes and through hydrolysis of the central core of β-lactam antibiotics leading to antibiotics inactivation and also develop resistance to this class of antibiotics [8]. β-lactamase enzymes in bacteria are very diverse, resulting in the emergence of new types of ESBL [9]. El Salabi et al. (2012) showed that the A.baumannii ESBL enzymes have been associated with plasmids and chromosomes, and resistant strains can carry set families of antibiotic resistance genes codes and even transfer this resistance to each other. Hence the spread of this A. baumannii resistant species is not limited to a city hospital and they are important in national scale [10]. Therefore, the information on the prevalence and patterns of antimicrobial enzyme are important in the control, prevention and treatment of infections caused by A. baumannii [1, 2]. The purpose of this research is to determine the antibiotic resistance of A. baumannii producing ESBL strains isolated from clinical specimens using phenotypic methods.

2 Materials and Methods

In this cross - sectional study, 400 clinical samples including urine, blood, skin lesions, chips and samples isolated from the respiratory tract and areas of burn, taken from patients hospitalized in three major hospitals in the Tehran (the capital of Iran) during 2011 - 2012, were prepared in BHI medium and were transferred to the laboratory.

All samples were cultured on BHI or nutrient agar and were incubated at 37 °C in laboratory for 24 hours. The Acinetobacter gram negative cocobacilli were confirmed by microscopic method using direct examination (Gram stain) after 24 hours. The biochemical tests to identify different species of Acinetobacter were catalase, TSI, OF, IMViC, urease, oxidase and growth at 37 and 42 °C. The isolated samplers were kept in - 80 °C on nutrient broth containing 50% glycerol.

After the identification of Acinetobacter species, the Kirby-Bauer disk diffusion method used to determine the drug resistance phenotype in compliance with the CLSI guidelines [11]. At first, bacteria were cultured in the Mueller-Hinton broth and then incubated at 37° C for one hour, then it was broadcasted on the Mueller - Hinton agar using a sterile swab. The antibiotic discs were placed at the standard distance. The inhibition zone diameters were measured for each antibiotic after 24 hours incubation at 37 °C, then the results were recorded for each antibiotic according to the relevant guidelines as susceptible, intermediate or resistant. In this study, we used 11 different antibiotic discs from MAST (Mast Diagnostics, Mast group Ltd, Merseyside, UK), consisting of cefepime (30 μg), ceftriaxone (30 μg), amikacin (30 μg), imipenem (10 μg), piperacillin – tazobactam (110 μg), meropenem (10 μg), gentamicin (12 μg), tobramycin (10 μg), tetracycline (30 μg), ampicillin – sulbactam (20 μg) and polymyxin B (300 μg). The standard strains of Escherichia coli ATCC 25922 and A.baumannii ATCC 19606 were used for quality control purposes as the negative and positive controls respectively.

By virtue of the previous studies, A.baumannii isolates which showed resistance to three or more antibiotics, including quinolones (ciprofloxacin), broad-spectrum cephalosporins (cefazidime and cefepime), aminoglycosides (amikacin and tobramycin), compound β-lactam / β-lactamase inhibitor
(ampicillin / sulbactam) and carbapenems (imipenem and meropenem), were considered as strains of multi-drug resistant (MDR).

The MIC of drug testing to determine the minimum concentration of antibiotic that prevents bacterial growth was performed by the serial dilution method for the ceftazidime and cefepime antibiotic according to the CLSI guidelines.

The screening combination disc method was used for the production of ESBL, based on the instructions stipulated. Hence four discs of ceftazidime, cefotaxime, ceftazidime - clavulanic acid and cefotaxime – clavulanic acid discs were placed on each other with a distance of 15 mm after the bacterial cultured on the Mueller Hinton agar. The plates were incubated for 24 h at 37 °C, and if the diameter of inhibition zone for each of these antibiotics in combination with clavulanic acid compared to antibiotics alone, increased by more than 5 mm, they reported as the ESBL-producing microorganisms, and in other cases microorganisms were reported as ESBL negative (Figure 1). The statistical data analyzed and interpreted with descriptive statistics by using SPSS13 software.

![Image](image_url)

Figure 1: Inhibition zone of the combination disk in screening method

3 Results

In this study 130 samples of *Acinetobacter* isolates from 400 patients were identified in which 100 samples (76.9%) were identified as *A.baumannii*, 22 (16.9%) samples as *A.lwoffii* and 8 samples (6.2%) were other *Acinetobacter* species. The 40 samples of *A.baumannii* from blood, 27 chip samples, 12 samples from the wound, 8 samples from urine and 13 samples of unknown origin were isolated. The 40 samples from the intensive care units, 30 samples from the infected area, 20 samples from emergency units and 10 samples from other wards were isolated.

The highest antibiotic resistance in 100 isolates of the *A. baumannii* was related to antibiotics namely: cefepime (100%), ceftriaxone (95%), amikacin (95%), imipenem (76%), piperacillin - tazobactam (70%), meropenem (69%), gentamicin (63%), tobramycin (56%), tetracycline (51%), ampicillin - sulbactam (49%) and the lowest resistance to polymyxin B (Table 1). The results of this study showed that 70% of *A. baumannii* isolates are resistant to 3 or more than 3 antibiotics and also no strains of *A.baumannii* isolates is resistant to all of the antibiotics. There are antibiotics that are effective on it. The MIC is greater or equal to 128 μg/ml for ceftazidime in the 84% of studied isolates and in the 91% of cefepime (Table 2). The combined disk test showed that 20% of strains were producer of Extended-spectrum β-lactamase.
Table 1: Distribution of antibiotic resistance patterns among *Acinetobacter baumannii* isolated from three hospitals in Tehran

<table>
<thead>
<tr>
<th>The name of antibiotic</th>
<th>Resistance</th>
<th>Semi-Sensitive</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>97</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>95</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Amikacin</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>76</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Piperacillin – tazobactam</td>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Meropenem</td>
<td>69</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>63</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>56</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>51</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Ampicillin – sulbactam</td>
<td>49</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>3</td>
<td>0</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 2: MIC of ceftazidime and cefepime antibiotics among *Acinetobacter baumannii* isolated from three hospitals in Tehran

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Ceftazidime</th>
<th>Cefepime</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>64</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>128</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
A. baumannii has been an opportunistic pathogen with high virulence factor during the past 30 years. These bacteria and particularly its multidrug resistant strain and ESBL producing cause severe infections in patients admitted in various hospitals and treatment of such infections is problematic due to their broad resistance to antimicrobial drugs. [1, 2] and [12]. As the environmental factors and different patterns of antimicrobial agents uses, are involved in developing these strains in parts of the world, this research is trying to determine the antibiotic resistance and prevalence of ESBL producing strains in A. baumannii strains isolated from clinical specimens using the phenotypic method. In this study, the 76.9% of isolates were A. baumannii and the rest were A.lwoffii and other species of Acinetobacter. In a similar survey, Constantiniu et al. at 2012 [13] reported that from 24 clinical isolates, 71% were A. baumannii and the rest were A.lwoffii.

In this research, the highest rates of antibiotic resistance belong to cefepime, ceftriaxone, amikacin and the lowest amount denote to polymyxin B. In a similar study, Farahani et al. at 2009 [14] based on 60 strains of the Acinetobacter, concluded that the strain of the A. baumannii isolates were mostly resistant to the amikacin, tobramycin and ceftazidime and had the lowest resistance to the imipenem and ampicillin – sulbactam. These results are consistent with our study to some extent, except for the lower degree of resistance to tobramycin and the higher degree for imipenem. It is quite possible that the discrepancies are due to the differences in the time of the studies [11].

susceptibility rate to meropenem and imipenem was reported above 40% in Feizabadi et al. at 2008 [15], Mirnejad et al. at 2012 [16] study in Iran, which is in contrariety with the findings of Hujer et al. at 2006 [17] who reported a resistance of about 20% to the mentioned antibiotics.

Discrepancies between the results of the studies can be due to type of samples and the kind of antibiotic and the antibiotic sensitivity discs. This difference can be reffered to the excessive use of antibiotics.

This study also has similar results with Wang et al. at 2003 [18] and Smolyakov et al. at 2003 [19] studies, revealing that most strains were resistant to amikacin, ampicillin - sulbactam, ceftazidime, cefepime, gentamicin, imipenem, meropenem, piperacillin – tazobactam and were susceptible to polymyxin B. The present research like the Joshi et al. studies at 2003 [20] showed that antibiotic resistance was seriously increasing so that 70% of the isolated A.baumannii strains would show multiple-drug resistance (MDR) phenotype.

The minimum inhibitory concentration of ceftazidimein the 79 (83.1%) of isolates were 64 ≤μg / ml in a study done by Shahcheraghi et al. at 2011. In our study, the MIC of ceftazidime in 84% of samples was MIC ≥ 128 μg / ml and in 91% of cases was MIC ≥ 128 μg / ml to cefepime. MIC to cefepime was not determined by Shahcheraghi et al [21].

According to the results of the combined disk test for screening of ESBL producing isolates, the 20% of the isolates were ESBL producer which are similar to the results of studies such Deiham et al. at 2012 [22] and Sinha et al. at 2007 [23], while they are different from the results of Hashemizade et al. at 2010 [24]. A.baumannii multidrug resistant and beta-lactamase producing are expanding in Iran and are considered as a major risk for hospitalized patients. Hence the identification and isolation of patients carrying ESBL producing bacteria, the proper use of antibiotics, particularly penicillins and cephalosporins, health and hygienic of the patients rooms in the hospitals and monthly reports of resistant bacteria in hospitals to the physicians are the important items to control and prevent the spread of resistant bacteria in hospitals.

According to this study, only twenty percent of isolates were ESBL producing. Therefore, it seems that the mechanisms other than ESBL bacteria such a secretory pumps and changes in purine are causing resistance the rapid identification and detection of which is important to prevent from their spread.
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