Detection of Carbapenemases Emerging in Acinetobacter baumannii Clinical Isolates by Modified Hodge Test

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INTRODUCTION

Acinetobacter baumannii is recognized as an increasingly important opportunistic non-fermentative Gram-negative bacilli. Carbapenem-resistant Acinetobacter isolates are increasingly reported worldwide. Carbapenem is mostly due to the production of enzymes that hydrolyse carbapenems and other β-lactams. Carbapenemase enzymes fall into Ambler classification A, B and D [4]. Unfortunately, resistance to carbapenems in A. baumannii is hard to detect by the routine disc diffusion method used by many microbiology laboratories.

Modified Hodge Test (MHT) is a phenotypic method for detection of carbapenemases. This approach is relatively easy and straightforward and is widely used as a screening test for detection of carbapenemases in Gram-negative bacteria [5].

In this study, we assessed the frequency of drug resistance in A. baumannii isolates originating from different clinical specimens in two teaching hospitals in Ahvaz, southwest of Iran. Also, carbapenemase production was assessed in carbapenem-resistant isolates by MHT.

MATERIAL AND METHODS

A total of 120 isolates of Acinetobacter were collected from patients hospitalized in Golestan and Emam Khomeini hospitals, Ahvaz city, Iran from December 2014 to October 2015. A. baumannii was the most prevalent species (83.3%) isolates. The reminder of them (16.7%) identified as Acinetobacter spp.

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The isolates were obtained from different specimens, including tracheal aspirate, wound, blood, urine, abscess, and CSF. All isolates were identified by biochemical tests including Gram stain, oxidase test, fermentative and oxidative properties, growth at 42°C, and citrate and malonate tests [6].

The susceptibility test of bacteria was performed by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines. Pseudomonas aeruginosa ATCC27853 was used as the quality control strain. The isolates with resistance to imipenem were examined for the production of carbapenemases by the MHT. This test was performed according to CLSI guidelines [7].

Antimicrobial susceptibility test to imipenem (IPM: 10 µg), meropenem (MEM:10 µg), ceftazidime (CAZ: 30 µg), ceftoxime (CTX: 30 µg), cefepime (FEP: 30 µg), ciprofloxacin (CIP: 5 µg), piperacillin (PIP, 100 µg), amikacin (AK: 30 µg), gentamicin (GEN: 30 µg), tetracycline (TE: 30 µg) and colistin (CO, 10 µg) (MAST, UK) was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) (Merck, Germany) based on CLSI Guidelines 2014 [7].

The carbapenem-resistant isolates were evaluated for carbapenemase production by MHT as CLSI recommended. Escherichia coli ATCC25922 at a turbidity equivalent to 0.5 McFarland standard was inoculated onto the Mueller-Hinton agar (MHA) (Merck, Germany) plate. After brief drying for five min, ertapenem (10 µg) disc was placed at the center of the plate. The test strain was heavily streaked from the edge of the ertapenem disc to the periphery of the plate. Four isolates were inoculated in one plate at 90° to each other, and the plates were incubated at 35°C for 18-24 h. Klebsiella pneumoniae ATCCBAA -1705 was used in this study as a quality control.

The presence of a clover leaf type of zone of inhibition near the test organism was interpreted as positive for carbapenem-hydrolyzing enzymes, as per the CLSI guidelines. MHT negative test did not show clover leaf pattern near to the test organism [7-9].

RESULTS

In this study we examined the clinical specimens which were collected from 120 patients, out of 120 clinical isolates of the genus Acinetobacter, 100 were A. baumannii including 58 (58%) from men and 42 (42%) from women. The majority of A. baumannii isolates were obtained from tracheal aspirate (46%) followed by blood (17%), lung discharge (13%), wound (9%), CSF (7%), urinary tract (5%) and sputum (3%) specimen. The isolates showed the highest resistance to ciprofloxacin (98%). The resistance rates to other antibiotics were as follows: ceftoxime, ceftazidime and piperacillin (97%); gentamicin, amikacin and meropenem (96%); imipenem (95%); cefepime (93%); and tetracycline (60%). Most of the isolates (99%) were sensitive to colistin (Table 1).

Among imipenem resistant A. baumannii isolates, 53 (53%) showed carbapenemase production by MHT (Table 2 and Figure 1).

Fig. 1. MHT with ertapenem disks.
1. Quality control (MHT Positive K. pneumoniae ATCCBAA -1705); 2. Positive result, A positive MHT indicates that this isolate is producing a carbapenemase; 3. MHT negative result for carbapenemase detection by MHT.

Table 1. Result for antimicrobial susceptibility test against A. baumannii isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant NO. (%)</th>
<th>Intermediate NO. (%)</th>
<th>Sensitive NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>95 (95)</td>
<td>1 (1)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>96 (96)</td>
<td>0 (0)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>97 (97)</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>97 (97)</td>
<td>3 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>93 (93)</td>
<td>1 (1)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>98 (98)</td>
<td>0 (0)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>96 (96)</td>
<td>0 (0)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>97 (97)</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>96 (96)</td>
<td>0 (0)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60 (60)</td>
<td>12 (12)</td>
<td>28 (28)</td>
</tr>
<tr>
<td>Colistin</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>99 (99)</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity and resistance of clinical isolates of A. baumannii to ertapenem.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant NO. (%)</th>
<th>Intermediate NO. (%)</th>
<th>Sensitive NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertapenem</td>
<td>53 (53)</td>
<td>22 (22)</td>
<td>25 (25)</td>
</tr>
</tbody>
</table>
A. baumannii is one of the most common known agents in nosocomial infections in hospitals. Management of A. baumannii infections has become a great challenge for physicians due to its survival for an extended period of time on various surfaces and development of resistance extends to multiple drug agents [10]. Acinetobacter spp. can survive in hospitals milieu for long periods and this bacteria could cause serious infections, especially in immune suppressed hosts, patients in the intensive care unit and those with critical disorders [11, 12]. In our study, most of (46%) A. baumannii isolates were obtained from tracheal secretions followed by blood (17%), discharge (13%). Other isolates of A. baumannii were derived from the wound infection (9%), CSF (7%), urinary tract (5%) and sputum (3%). In a similar study, by Begum et al. (2013) most of the A. baumannii isolates were obtained from endotracheal tube specimens followed by tracheal secretions and pus [13]. Shanthi et al. (2009) have described that the highest prevalence of A. baumannii was found from the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%) [14].

Acinetobacter infections require complex treatment because isolates of this bacteria resistant to most of the available antibiotics. Carbapenems such as imipenem and meropenem have a broad spectrum of activity against some of the bacteria, and they resist to hydrolysis by many of β-lactamases [15]. The reports show the mechanism resistance to carbapenem mostly is due to the production of enzymes carbapenemases that hydrolysed carbapenems and other β-lactams [16]. However, development of resistant to these drug agents in Acinetobacter have been rising during recent years, and the isolates of these bacteria are often multiple drug resistance (MDR) [17].

Carbapenemase production is determined by the MHT which originally has been recommended by the Center for Disease Control and Prevention (CDC), and it has revealed appropriate efficiency for carbapenemase detection [9, 15].

In the present study, most of the A. baumannii isolates were resistant to cephalosporins, carbapenems, aminoglycosides, ciprofloxacin and tetracycline, and susceptible to colistin.

Vahdani and colleagues (2011) reported a high frequency of cephalosporin (more than 90%) and fluoroquinolone (80%) resistance in A. baumannii isolates in Tehran [18]. Also, Asadollahi and colleagues (2012) showed that the resistance rate to β-lactam antibiotics, ciprofloxacin, and tetracycline was more than 50% and to imipenem 47.8% in Tehran. Unlike our results, antibiotic resistant was reported on the lower level in this study [19].

Fallah et al. (2013) recorded that resistance rate of A. baumannii isolates were 95.4%, 91.7%, 91.7%, 80.6%, and 92.6% to ceftazidime, meropenem, imipenem, amikacin, and ciprofloxacin in Tehran [20]. Also Shoja et al. (2013) reported the resistant rate of A. baumannii isolates to ciprofloxacin, pipercillin 96.6%, to imipenem, meropenem, ceftazidime and cefepime 96.1%, amikacin 88.8%, tetracycline 85% and gentamicin 83% [21].

Our result in the present study agrees with other studies [18, 20, 21] which have all revealed that resistance to most antimicrobial drugs, especially carbapenems have increased during recent years and so it can lead to failure in the clinical treatment of A. baumannii infections.

Carbapenemase production detected in this survey by MHT was 53%. Hence, this test could determine about half of positive carbapenemase isolates.

This study shows that MHT could be used in clinical laboratories for monitoring emergence carbapenemase in A. baumannii, especially MDR isolates.

In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming rampant, complicating treatment and increasing both human morbidity and financial costs. This necessitates the need for detecting the resistant bacteria so that unnecessary use of broad-spectrum antimicrobials can be avoided [5].

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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