Molecular Identification and Antimicrobial Resistance Profile of Acinetobacter baumannii Isolated From Nosocomial Infections of a Teaching Hospital in Isfahan, Iran

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Background: Multidrug resistant (MDR) and extensively drug resistant (XDR) Acinetobacter baumannii are among important causes of nosocomial infections and cause therapeutic problems worldwide. The emergence of extensively drug-resistant A. baumannii (XDRAB) cause serious threats to hospital acquired infections (HAI) worldwide and further limit the treatment options.

Objectives: The current study aimed to identify and isolate the MDR and XDR Acinetobacter baumannii from different wards of a teaching hospital in Isfahan, Iran, and determine the susceptibility pattern of these bacteria.

Materials and Methods: One hundred and twenty one isolates of A. baumannii collected from a teaching hospital in Isfahan, Iran, within eight months (between September 2013 and April 2014) were included in the current study. The samples were isolated from different wards and different specimens. To confirm the species of A. baumannii, Polymerase chain reaction (PCR) was conducted to identify blaOXA-51 gene. Disk diffusion method was employed to evaluate antimicrobial susceptibility against cefotaxime, ceftriaxone, ampicillin-sulbactam, cephalim, meropenem, tobramycin, amikacin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, and aztreonam.

Results: Among the 121 isolated A. baumannii, 44% and 56% were isolated from female and male, respectively. Samples cultured from the trachea (36%), urine (15%), blood (10%), wound (10%), cerebrospinal fluid (7%), bronchial (4%) and the others (18%). Most of the isolates (50%) were obtained from intensive care unit (ICU). Isolated A. baumannii showed high resistance to the evaluated antibiotics except ampicillin-sulbactam, which showed only 33.9% resistance. Also, 62.8% and 100% of the isolates were identified as XDR and MDR.

Conclusions: The result of the current study showed the growing number of nosocomial infections associated with XDR A. baumannii causing difficulties in antibiotic therapy. Resistant strains increasingly cause public health problems; therefore, their early detection is essential for healthcare centers.

Keywords: Acinetobacter baumannii; Drug Resistance; Multidrug Resistant (MDR); Extensively Drug Resistant (XDR)

1. Background

Acinetobacter baumannii is a Gram-negative, aerobic, and non-glucose fermenting cocccobacillus that nowadays has emerged as an important pathogen causing nosocomial infections including pneumonia, urinary tract infections, sepsis, and wound infections. It is frequently involved in outbreaks. These bacteria have a great tendency to acquire resistance against multiple classes of antibiotics (1).

Acinetobacter baumannii has multidrug-resistant phenotypes. Resistance to broad spectrum β-lactams, aminoglycosides, fluoroquinolones, and carbapenems are observed in this bacteria, which complicate the treatment of this pathogen (2). In the last decade, Acinetobacter baumannii became more prevalent as an opportunistic pathogen, specifically as a multidrug-resistant agent (MDR) using different mechanisms of drug resistance (3, 4). The emergence of extensively drug-resistant A. baumannii (XDRAB) limits the treatment options and causes a serious threat to hospital acquired infections (HAI) control. The prevalence of XDRAB among clinical A. baumannii isolates reached 15% in 2005 and up to 41% in 2010. This highlights an evolving challenge posed by XDRAB, and an essential need for effective prevention and control measures (5). Control of multidrug-resistant and extensively drug-resistant Acinetobacter spp. infections is an important challenge for clinical microbiologists and physicians. Its ability to survive in hospital environment and its capability to persist for long periods of time on surfaces make it a common cause of healthcare-associated infections and multiple outbreaks (6). The prevalence of A. baumannii in healthcare centers has increased around the world (7, 8); therefore, finding moderate molecular typing methods for A. baumannii is necessary for infection control studies and epidemiological investigations. There are many molecular methods to identify A. baumannii like ribotyping (9), repetitive extragenic palin-
dromic sequence-based polymerase chain reaction (rep-PCR), random amplified polymorphic DNA (RAPD) analysis (10), infrequent-restriction-site analysis (11), amplified fragment length polymorphism (AFLP) analysis (12), and multilocus PCR and electro spray ionization mass spectrometry (PCR/ESI-MS). Single locus amplification and sequence-based typing at either rpoB, gyrB or bla_{OXA-51} like genes are used for large scale epidemiological studies (13). Bla_{OXA-51}-like genes are special to A. baumannii and could also be used as markers for identification of this species (14). Some evidence portend that A. baumannii is a naturally occurring carbapenemase gene intrinsic to this species (13). The first report of this gene described bla_{OXA-51}. After that a large number of related variants were found (with OXA numbers 64, 65, 66, 67, 68, 69, 70, 71, 75, 76, 77, 83, 84, 86, 87, 88, 89, 91, 92, 94, and 95), and the study referred to them collectively as bla_{OXA-51}-like genes. Bla_{OXA-51}-like genes are endogenous and specific to A. baumannii. (13, 15).

2. Objectives

The current study aimed to identify Acinetobacter baumannii by molecular method and determine its separation among different wards in hospital and determine the antimicrobial patterns of these bacteria.

3. Materials and Methods

3.1. Bacterial Isolates

The current study used 121 non-duplicated clinical isolates of Acinetobacter baumannii collected during a period of eight months (between September 2013 and April 2014) from patients who were hospitalized in a teaching hospital in Isfahan, Iran. Samples were isolated from the trachea (36%), urine (15%), blood (10%), wound (10%), cerebrospinal fluid (7%), pleural fluid (4%), and others (18%). Isolates were obtained from different wards, mostly from ICU (50%) and pediatric wards (12%), followed by internal brain and vessel (6%), infectious disease (4%), general (3%), and other wards (12%). Species identification was done using biochemical and sugar fermentation tests as described by Bovet and Grimont (16). Then the isolates were identified by catalase and oxidase tests, motility, DNase test, acidity or alkalinity in triple sugar iron (TSI) agar slants, growth at 44°C and ability to grow on citrate agar (17).

3.2. PCR of Bla_{OXA-51}-like Gene

To confirm the species of A. baumannii, PCR was conducted to identify bla_{OXA-51} genes, which was endogenous to A. baumannii. Bacterial DNA was extracted by boiling; about 4-5 colonies were dissolved in 500 μl sterile distilled water for 10 minutes. The primer pair 5’TATGCTTTGATCGGCGTTG-3’ and 5’TGGATGCACTCAGCTTTG-3 were used to amplify the gene. The PCR conditions were as follows: initial denaturation at 95°C for five minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds and then 72°C for 10 minutes. Reactions were performed with 2 μl DNA template. Acinetobacter baumannii ATCC 19606 was used as positive control and Pseudomonas aeruginosa ATCC 27853 was used as the negative control. PCR products were analyzed by electrophoresis on 1.2% agarose gel in a tris-borate-EDTA buffer (TBE) buffer at 86 volts alongside DNA ladder. Then the PCR products were visualized under UV light (18).

3.3. Antimicrobial Susceptibility Test

Antimicrobial susceptibility was assessed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar using MAST disks (England), as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2012 guidelines (19). Escherichia coli ATCC 25922 was used as negative control and Klebsiella pneumonia ATCC 700603 was included as the positive control (20). The tested antibiotics were cefotaxime (30 μg), ceftriaxone (30 μg), ampicillin-sulbactam (10/10 μg), cefepime (30 μg), meropenem (10 μg), tobramycin (10 μg), amikacin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and aztreonam (30 μg). The diameters of zones of inhibition were measured. Categories of susceptible, intermediate, or resistant were determined and interpreted using the latest tables published by the CLSI 2012 (21). To analyze susceptibility rates in different wards and different age categories, the WHONET 5.6 software was used.

4. Results

One hundred and twenty-one clinical isolates of Acinetobacter baumannii were identified by biochemical methods. Then the isolates were confirmed by PCR for bla_{OXA-51}-like gene. Analysis for the presence of bla_{OXA-51}-like gene showed that all isolates were positive and A. baumannii was confirmed (Figure 1); 56% and 44% of the isolates were obtained from male and female. Acinetobacter spp. infection was more common in patients of aged over 40 years. Most of these patients had respiratory problems like bronchial asthma and respiratory failure. Forty four A. baumannii (36%) were obtained from tracheal samples and eighteen of them (15%) were obtained from blood. Most of the isolates were obtained from ICU (50%) followed by surgery (13%) and pediatric wards (12%) (Table 1). Acinetobacter spp. has a great capacity to develop antibiotic resistance in response to challenge with new antibiotics. Resistance rate between the antibiotics was high: cefotaxime (100%), ceftriaxone (100%), ampicillin-sulbactam (33.9%), cefepime (99.2%), meropenem (100%), tobramycin (86.8%), amikacin (87.6%), tetracycline (92.6%), ciprofloxacin (100%), trimethoprim-sulfamethoxazole (99.2%), and aztreonam (100%) (Table 2). All isolates (100%) were resistant to at least one agent in ≥3 antimicrobial categories, it means they were MDR, and 62.8% were XDR.
Table 1. Number and Rate of Acinetobacter Species Isolated From Different Clinical Specimens and Different Wards

<table>
<thead>
<tr>
<th>Clinical samples</th>
<th>No (%)</th>
<th>Wards</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal</td>
<td>44 (36)</td>
<td>ICU</td>
<td>61 (50)</td>
</tr>
<tr>
<td>Urine</td>
<td>18 (15)</td>
<td>surgery</td>
<td>16 (13)</td>
</tr>
<tr>
<td>Blood</td>
<td>12 (10)</td>
<td>Pediatric</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Wound</td>
<td>12 (10)</td>
<td>Internal brain and vessel</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>8 (7)</td>
<td>Infectious disease</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>5 (4)</td>
<td>general</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Other samples</td>
<td>22 (18)</td>
<td>other</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>121 (100)</td>
<td>total</td>
<td>121 (100)</td>
</tr>
</tbody>
</table>

Table 2. High Rate of Resistance to all Groups of Antibiotics According to Antimicrobial Susceptibility Testing

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant (%)</th>
<th>Intermediate (%)</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>33.9</td>
<td>34.7</td>
<td>31.4</td>
</tr>
<tr>
<td>Cefepime</td>
<td>99.2</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>100</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>86.8</td>
<td>7.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>87.6</td>
<td>5</td>
<td>7.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>92.6</td>
<td>3.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>99.2</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>100</td>
<td>0</td>
<td>0</td>
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</table>

5. Discussion

Acinetobacter spp. is a Gram-negative coccobacillus that deeply contributes to the burden of modern medicine. Acinetobacter spp. is the second most commonly isolated non-fermenter in human specimens (after Pseudomonas aeruginosa). Acinetobacter spp. appears to be an important cause of ICU infections. Multidrug-resistant Acinetobacter spp. is alert pathogens, mostly in ICUs and is related with outbreaks of infection. Their presence in the ICU environment and inadequate infection control practice has continuously raised the incidence of Acinetobacter infections over the last decades. The diagnosis of Acinetobacter infections in the ICU is really important (22).

In the present study, the highest number of isolates was obtained from ICU; A. baumannii was mostly found in trachea samples. Almost similar results were observed in a study by Sana Islahi in India (23). Most of the strains were highly resistant to the antibiotics. Therefore, treatment of these infections are complicated (24). The present study aimed to find MDR and XDR A. baumannii by determining antibiotic resistance rate through disk diffusion method. In the current study, MDR Acinetobacter spp. was defined as the isolates resistant to at least three classes of antimicrobial agents, penicillins, cephalosporins (including inhibitor combinations), aminoglycosides, and fluoroquinolones. XDR Acinetobacter spp. was defined as the isolate resistant to all classes of antibiotics except one or two groups. Finally, PDR Acinetobacter spp. was the XDR Acinetobacter spp. resistant to polymyxins and tigecycline (25). In the current study, 62.8% and 100% of the isolates
References


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