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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 (121) 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 $bla_{oxa\text{-}51}$ gene. Disk diffusion method was employed to evaluate antimicrobial susceptibility against cefotaxime, ceftriaxone, ampicillin-sulbactam, cefepime, 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 Gramm-negative, aerobic, and non-glucose fermenting coccobacillus that nowadays has emerged as an important pathogen causing nosocomial infections including pneumonia, urinary tract infections, septicemia, 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 $bla_{oxa\text{-}51}$-like genes are used for large scale epidemiological studies (13). $Bla_{oxa\text{-}51}$-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 $bla_{oxa\text{-}51}$. 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 $bla_{OXA\text{-}51}$-like genes. $Bla_{oxa\text{-}51}$-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 intensive care unit (ICU) (50%), surgery (13%) 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 the biochemical and sugar fermentation tests as described by Bouvet 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 Blaoxa-51-like Gene

To confirm the species of *A. baumannii,* PCR was conducted to identify $bla_{oxa\text{-}51}$ 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' TAATGCTTTGATCGGCCTTG-3' and 5'-TGGATTGCACTTCATCTTGG-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 baumanniii* were identified by biochemical methods. Then the isolates were confirmed by PCR for $bla_{OXA\text{-}51}$-like gene. Analysis for the presence of $bla_{OXA\text{-}51}$-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

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

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

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

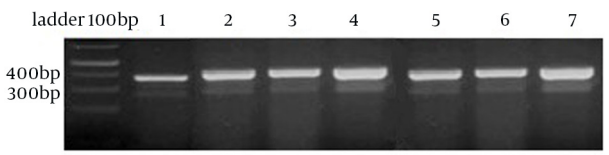

Figure 1. Gel Photographs of PCR Using bla_{oxa51} Gene Primer. Lane 1: *Acinetobacter baumannii* ATCC19606 (Positive Control), Lanes 2-7: Clinical Isolates With bla_{oxa51} Gene (353 bp)

that means the isolates were resistant in all classes of antibiotics except one or two groups. The highest sensitivity of *Acinetobacter* spp. was observed to ampicillin-sulbactam (31.4%). The resistance for pediatrics and adults were 12.5% and 37.1%, respectively. In addition, there was no resistance to ampicillin-sulbactam in pediatric blood specimens. However, in adult blood samples, there were 33.3% resistant to ampicillin-sulbactam.

5. Discussion

Acinetobacter spp. is a Gramm-negative coccobacilus 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 fluroquinolones. 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

were XDR and MDR, respectively. In a similar study conducted in Taipei, Taiwan, 25 XDR *A. baumannii* were found within two years (5). Findings of this study showed that, even in the absence of clonal dissemination, XDRAB can emerge under the selection pressure of broad-spectrum antibiotics therapy, and cause subsequent HAIs in the compromised hosts. The results of the mentioned study in Taiwan confirmed that XDR *Acinetobacter baumannii* is a great challenging Gramm-negative microorganism that should be treated and controlled in the healthcare centers (26, 27). Evidence has accumulated that contaminated surfaces cause the epidemic and endemic transmission of many MDR and XDR bacteria (28). Therefore, proper attention should be paid to provide usable information for physicians when choosing experimental antibiotics, which also helps turn to specific resistant issues within a region to help determine targeted intervention measures.

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