

Molecular identification of *Acinetobacter baumannii* isolated from intensive care units and their antimicrobial resistance patterns

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Abstract

Background: *Acinetobacter baumannii* is one of the most important pathogens in hospital-acquired infections especially in intensive care units (ICUs). This opportunistic pathogen can be easily isolated from water, soil, and hospital facilities. *A. baumannii* as a nosocomial opportunistic pathogen is resistant to a wide range of antibiotics and responsible for multiple infections, including bacteremia, pneumonia, meningitis, urinary tract infections, and surgical wounds. The aim of this study was to determine frequency and resistance patterns of *A. baumannii* isolated in ICUs of Isfahan Hospitals.

Materials and Methods: During 1 year period (2012-2013), 350 specimens were collected from ICUs of Isfahan hospitals. The isolates were characterized as *A. baumannii* by conventional phenotypic, biochemical tests and confirmed by PCR for *OXA-51*-like gene. Susceptibility of isolates was determined by standard disk diffusion method according to CLSI.

Results: From total of 350 specimens, 43 isolates were *A. baumannii*. The antimicrobial patterns of isolates showed that 53.5% of isolates were resistant to amikacin, 83.7% to tetracyclin, 86% to ceftazidime, 90.7% to Trimethoprim sulfamethoxazol, 93% to imipenem, cefepime, meropenem, ampicillin-sulbactam. All isolates were resistant to ciprofloxacin.

Conclusion: This study showed a high resistance of *A. baumannii* to a wide range of antimicrobial agent. It is necessary to adopt appropriate strategies to control the spread of the bacteria in care unit centers and wards.

Key Words: *A. baumannii*, antibiotic resistance pattern, *blaOXA-51*-like gene, intensive care units, molecular method

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INTRODUCTION

Acinetobacter baumannii, a gram-negative, non-fermenting cocobacilli, is mostly found in soil, different water sources, and many healthcare environments.^[1,2] This bacteria is abundant in normal flora of skin and mucous membranes in humans and able to cause opportunistic infections such as upper respiratory tract infections, pneumonia, meningitis, septicemia, and urinary tract infection, particularly in

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intensive care units (ICUs).^[3] *A. baumannii* is resistant to dehydration, UV, common chemical disinfectants and detergents.^[4] This bacteria can survive in dry environmental conditions and is isolated from various parts of hospital such as curtain, bed, furniture and clinical equipment.^[5] Therefore, it can disseminate from different sources but hands of hospital staff is the most common mode of spread.^[6] Recently, *A. baumannii* appeared significantly in the form of endemic and epidemic infections in hospitals.^[7,8] In the clinical setting *A. baumannii* is extremely dangerous, due to its ability to colonize and infect severely immunocompromised patients in ICUs.^[9,10] Few methods are available to eliminate *A. baumannii* from widely used hospital equipments, especially catheter-related tools.^[11]

The resistance of *A. baumannii* to antimicrobial agents is mediated by all of the known major resistance mechanisms, including modification of target sites, enzymatic inactivation, active efflux and decreased influx of drugs.^[12]

Recently, it has been shown that NaCl, a monovalent cation largely found in our skin, is associated with enhanced multidrug resistance (MDR) of *A. baumannii*.^[12] This bacteria produces metallo-beta-lactamase (IMP-VIM and types of SIM) causing resistance to most beta-lactam.^[13,14] *A. baumannii* harbored enzymes such as Oxacillinase OXA-51 which hydrolyze carbapenems. Also, three groups of irrelevant enzymes of OXA-23, OXA-24, OXA-58 have been defined.^[15] Of 100 isolates of imipenem-resistant *Acinetobacter* spp. collected from Tehran hospitals in 2009 and 2010, 6 isolates produced metallo-beta-lactamases and 94 isolates produced OXA-type carbapenemase.^[10] The mortality rates for *A. baumannii* infections are about 50% for bacteremia and approximately 23-75% for pneumonia.^[16] In the ICU, the mortality rate is 54%.^[17]

MDR phenotype in *A. baumannii* results when integron-borne resistance determinants against different classes of antibiotics coexist, giving rise to MDR gene cassettes.^[18,19]

Most publications defined MDR *A. baumannii* strains as resistant to at least three of the following five drug classes: Cephalosporins, carbapenems, ampicillin-sulbactam, fluoroquinolones, and aminoglycosides.^[20]

Treatment of MDR *A. baumannii* infections is difficult. Currently, carbapenems, as the drug of choice in treating MDR *A. baumannii* infections, are used. However, carbapenem-resistant strains are increasing.^[21]

Due to the high prevalence rate of this infection as well as various patterns of antibiotic resistance in different geographical areas, an investigation on prevalence rate and antibiotic sensitivity pattern in different part of the world is essential. These data would provide useful information on distribution of resistance patterns and the possibility to choose the proper treatment strategy.

The aim of this study was to determine the prevalence and antimicrobial resistance pattern of *A. baumannii* isolated from patients hospitalized in ICUs of Isfahan hospitals.

MATERIALS AND METHODS

During the 1 year period (2012-2013), 350 specimens from different sources including catheter, blood, urine, wound, CSF, sputum, and eye were collected. The specimens were inoculated initially on blood agar (Merck) and MacConkey agar (Merck) medium and incubated for 24 h at 37°C. Conventional biochemical methods such as oxidase, citrate, urea urease, malonate consumption, oxidation and fermentation of sugars, motility and indole production were used to identify *A. baumannii*. The isolates were stored in BHI medium containing 15% glycerol at -20°C.

In order to confirm the strain, isolates were subjected to PCR against the *OXA-51*-like gene. DNA of the isolates was extracted by DNA extraction kit (Fermentas) according to the manufacturer's protocol. DNA concentration and purity was measured using a spectrophotometer.

PCR mixture was done in a total volume of 25 µl including 1 µl MgCl₂ (1.5 mM), 0.3 µl Taq DNA polymerase (500 U), 2.5 µl 10x PCR buffer, 0.5 µl dNTP (200 µM), 1 µl of each primer (10 pmol/ml) and 2 µl of DNA template (5 ng genomic DNA). The primer sets F: 5'-TAA TGC TTT GAT CGG CCT TG and R: 5'-CTT CG TGG ATT CGA CTT CAT was used for amplification of the *OXA-51*-like gene.^[22] *Pseudomonas aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606 strains have been used as negative and positive control, respectively.

Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradient, Eppendorf, Germany) with the following program: One cycle of 5 min at 94°C, 30 cycles as follows: Denaturation at 94°C for 25 s, annealing at 53°C for 40 s, initial extension at 72°C for 50 s, followed by final extension of 6 min at 72°C. PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized by staining with green viewer under UV light.^[23,24]

The antibiotic sensitivity pattern was done according to disk diffusion or Kirby-Bauer method. For this purpose antibiotics including: Amikacin, tetracyclin, ceftazidime, and carbapenem (imipenem and meropenem), trimethoprim sulfamethoxazol, ampicillin–sulbactam, ciprofloxacin (Rosco, Denmark) have been used. A fresh 24 h bacterial lawn of isolates has been used to prepare 0.5 McFarland (1.5×10^8 cfu/ml) bacterial concentration and the diameter of the inhibition of bacterial growth were measured and compared to the reference tables provided by CLSI (Clinical and Laboratory Standard Institute).^[25] Statistical analysis was performed by SPSS software version 14, using Chi-square test (Chi-Square) and kappa coefficient. A $P < 0.05$ was considered as an indicator of significance.

RESULTS

Data presented in Figure 1 demonstrate the frequency of *A. baumannii* from different samples. As it is shown in Figure 1, most of the specimen belongs to catheters (30.27%). 32.6%^[14] of isolates were of women and 67.4% samples^[26] were of men. There was no significant relationship between age, sex and the infection [Table 1 and Figure 2] ($P < 0.05$).

From 350 specimen, 43 isolates were characterized as *A. baumannii* by phenotypic method. All of the isolates were confirmed by PCR. Figure 3 showed the result of PCR for the *blaOXA-51*-like gene. All of the strains harbored the *blaOXA-51*.

Our results showed that 53.5% of the isolates were resistant to amikacin, 83.7% to tetracyclin, 86% to ceftazidime, 90.7% to trimethoprim sulfamethoxazol, 93% to cefepime, imipenem, meropenem, and ampicillin–sulbactam. All of isolates were resistant to ciprofloxacin [Table 2].

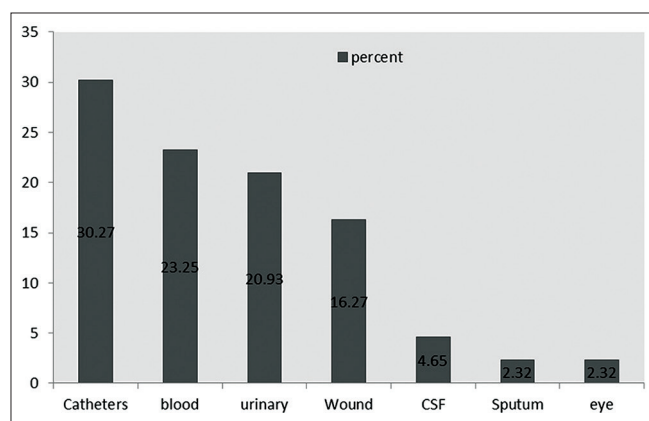


Figure 1: Distribution of *Acinetobacter baumannii* according to type of specimens in ICU

DISCUSSION

In recent years, nosocomial infections of *A. baumannii*, as an opportunistic pathogen, are increasing. Treatment of this bacteria especially MDR and broad-spectrum beta-lactamases strains is of major concern.^[2] In our study from 350 samples collected in ICU, 43 (12.3%) isolates were of *A. baumannii*.

In a study in Tehran, from 100 collected samples in ICU of Rasoul Akram hospital, 21 samples (21%) were *A. baumannii*.^[27] In another study of Rit and his colleagues in 2012 among 4180 clinical isolates 74.02% *A. baumannii* and 25.98% other types of *Acinetobacter* have been diagnosed.^[28]

The present study showed a high percentage (100%) of MDR. The resistance rate to beta-lactam antibiotics,

Table 1: Frequency of patients according to gender

Sex	Frequency	Percent
Female	14	25.5
Male	29	52.7
Total	43	100.0

Table 2: Antibiotics resistant patterns in *Acinetobacter baumannii* isolates

Type of antibiotic	Number (%)		
	Resistance	Semi-resistance	Sensitivity
Amikacin	(23) 53.5	(9) 20.9	(11) 25.6
Tetracyclin	(36) 83.7	(4) 9.3	(3) 7
Ceftazidime	(37) 86	(5) 11.7	(1) 2.3
Trimethoprim sulfamethoxazol	(39) 90.7	(0) 0	(4) 9.3
Cefepime	(40) 93	(2) 4.7	(1) 2.3
Imipenem	(40) 93	(0) 0	(3) 7
Meropenem	(40) 93	(0) 0	(3) 7
Ampicillin–sulbactam	(40) 93	(0) 0	(3) 7
Ciprofloxacin	(43) 100	(0) 0	(0) 0

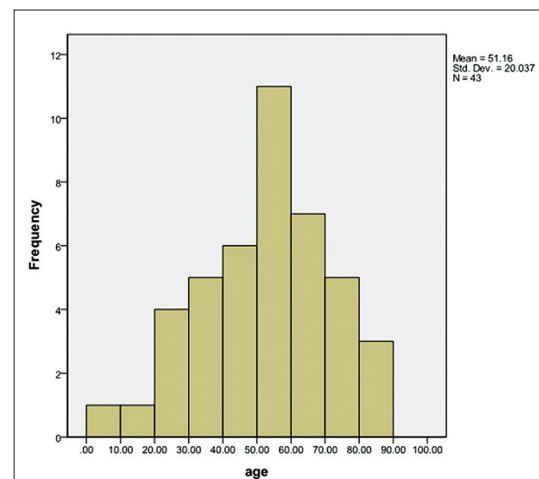


Figure 2: Frequency of patients according to age

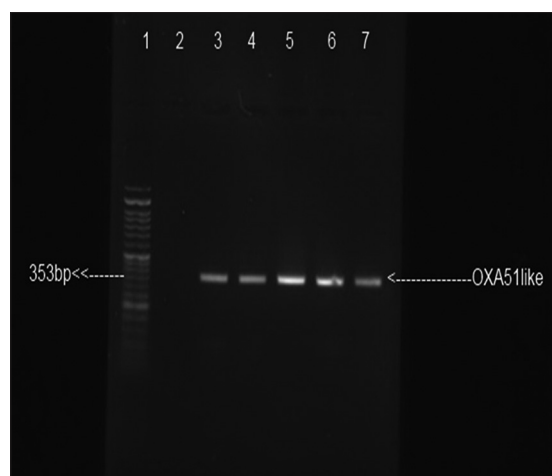


Figure 3: Electrophoresis result of *blaOXA-51* amplification: lane 1: Size marker 50 bp; lane 2: Negative control *P. aeruginosa* ATCC 27853; lane 3: Positive control *A. baumannii* ATCC 19606; lanes 4–7: Clinical isolates

trimethoprim sulfamethoxazol and tetracyclin was more than 80%. Resistance against imipenem and meropenem was more than 90%. The antibiotic resistance observed in this study was relatively similar to reports in other parts of Iran.

Sadeghifard and colleagues in 2010 showed that all isolates of *A. baumannii* had 100% resistance to aztreonam, cefotaxime, ceftazidime, ceftriaxone, meropenem and ticarcycline-clavulanate.^[29] In another study these researcher showed that the isolated bacteria had 100% resistance to tetracyclin, 95/2% to gentamicin, amikacin, and 90/5% to ceftazidime.^[27]

Ayan and colleagues reported that from 52 isolates, all of strains were resistant to piperacillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, gentamicin, and aztreonam.^[26]

Wang and colleagues (2003) described in an epidemic of drug-resistant strains of *A. baumannii* in the ICU, all the strains were resistant to aztreonam, amikacin, ampicillin–sulbactam, ceftazidime, cefepime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin, tazobactam, and ticarcillin-clavlonic and sensitive to polymixine B.^[30]

Other studies in Asia and the Middle East indicate prevalence of MDR *A. baumannii* in these regions.^[31,32]

The difference in resistance pattern of *A. baumannii* is probably due to diversity in clinical samples, time of doing study and approaches of treatment in each geographical area. Comparing our results with other studies reveals that resistance of the bacteria to current antibiotics is increasing. In our study 32.6% of samples (14 persons)

were of women and 67.4%^[26] samples were of men and the highest age percentage was 50-60 years old [Figure 3] with the mean age of 51 years. Hujer, *et al.* in 2006 reported that from 75 *A. baumannii* isolates recovered from patients; 67 (89%) were of men and 8 (11%) were of women (mean age = 35 years).^[33] Hello, *et al.* (2010) stated that the mean patient age was 57.8 years.^[34] Similarly, Kwon, *et al.* (2007) indicated that the mean age of 80 *A. baumannii*-infected patients was 47 years, 57 (71.25%) of them were males and 23 (28.75%) were females.^[35] In another study, Tsakris, *et al.*, (2008) showed that the age of patients ranged from 22 to 83 years (mean age 68 years) and found the males outnumbered the females.^[36] In our study from 43 clinically isolated *A. baumannii* the highest percentage (30.27%) was catheters followed by blood (23.25%), urine (20.93%), wound (16.27) and other clinical specimens (9.29%).

Amudhan, *et al.* (2011) found the highest isolation percentage (53.49%) was from respiratory secretions followed by 21.55%, 15.17%, 3.45%, 3.45% and 2.59%, respectively, from blood, wounds, cerebrospinal fluid, body fluids and urine.^[37] Furthermore, Martins, *et al.* (2009) found from 53 clinically isolated *A. baumannii* the highest percentage (73.58%) was from respiratory tract followed by blood (16.98%), urine (3.77%) and other clinical specimens (5.66%).^[38] Moreover, Mammina, *et al.* (2012) found that from 36 *A. baumannii* isolates, 26 (72.2%) isolates were from the respiratory tract infections.^[39]

In a survey conducted in 2010 in Saudi Arabia from 1210 isolates collected from different samples such as breathing samples (469), blood (400), injury/tissues (235), urine (56), nasal swab (35), and CSF (15 from patients in section ICU composed, 40.9% *A. baumannii*, 19.4% *Klebsiella pneumoniae* and 16.3 *Pseudomonas aeruginosa*.^[40]

ICU is of major concern of infection due to duration time of hospitalization and intensity of illness. Previous studies have shown *A. baumannii* as the most frequent nosocomial infection in the ICU.^[41,42] Geographical data about resistance pattern of Acinetobacter would provide suitable information for treatment strategies. This study showed a high percentage of resistance to antimicrobial agent in *A. baumannii*. Therefore, it is necessary to adopt appropriate strategies to control the spread of these strains in ICU.

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