

## **Antimicrobial Resistance Pattern of *Acinetobacter baumannii* Strains Isolated from Intensive Care Unit Patients**

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**ABSTRACT**

**Background and Objectives:** *Acinetobacter baumannii* is an opportunistic pathogen that affects different groups of people, especially intensive care unit (ICU) patients. The prevalence of infections caused by this bacterium is very high. Today, prevalence of infections caused by multidrug-resistant (MDR) and extreme-drug resistant (XDR) strains is increasing. This study aimed to determine the antibiotic susceptibility pattern of *A. baumannii* isolates from ICU patients.

**Methods:** This cross-sectional study was conducted from October 2014 to March 2015 on patients admitted to ICU of Imam Khomeini hospital in Tehran, Iran. Clinical samples of various sources were collected from patients. Isolates were detected and identified via microbiological and biochemical tests as well as PCR amplification of the *bla<sub>bla51</sub>* gene. Then, susceptibility testing was performed using the Kirby-Bauer disk diffusion test. Statistical analysis was performed with SPSS (version 22, Chicago, IL, USA) using Chi-square and Fisher's exact tests.

**Results:** Of the total of 62 clinical samples, 24 (39%) were respiratory samples and only three (6%) were cerebrospinal fluid samples. Most MDR and XDR strains were isolated from respiratory samples. The highest resistance rate was against ceftriaxone, ticarcillin and erythromycin (100%), while the lowest resistance rate was against minocycline (20%).

**Conclusion:** Owing to detection of high multi-drug resistance isolates in the present study, and importance of multi-drug resistance in *A. baumannii*, the identification of multi-drug resistance genes and their reporting to health care/treatment centers is important. Thus, it is recommended to perform susceptibility testing to help determine the most effective antibiotic(s) for the treatment of infections in ICU patients.

**Keywords:** *Acinetobacter baumannii*, MDR, XDR, ICU.

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## INTRODUCTION

According to the Infectious Diseases Society of America, *Acinetobacter baumannii* is among the six top priority dangerous drug-resistant organisms (1). *A. baumannii* is an opportunistic gram-negative pathogen that affects different groups of people, especially intensive care unit (ICU) patients (2). The bacterium can cause various infections including urinary tract infection, wound infection, meningitis, endocarditis, peritonitis and skin and soft tissue infections (3). Prolonged length of hospital stay, immunodeficiency, surgery, burns, aging, anti-bacterial agents and invasive devices are among the predisposing risk factors for development of infections caused by this bacterium (2). The infections are typically treated with beta-lactams and fluoroquinolones. In recent years, the increased use of antibiotics has led to the emergence of resistant strains (4). Antibiotic resistance in *A. baumannii* may be acquired through intrinsic mechanisms, such as enzymes, mutations in target genes, permeability of the outer membrane and increased expression of efflux pumps (4, 5). This has caused difficulties in treatment of infections caused by *A. baumannii*, which may lead to increased length of stay, increased health care costs, an unfavorable prognosis and increased risk of mortality (3, 6). Carbapenems, a class of beta-lactam antibiotics with broad antimicrobial activity, are used as antibiotic of choice for treatment of infections caused by resistant strains (6, 7). Considering the rising prevalence of multidrug-resistant (MDR) *A. baumannii* strains that are also resistant to carbapenems, colistin and tigecycline have been recommended (6). Studies on different *Acinetobacter* species have shown that antibiotic resistance is more common among *A. baumannii* compared to other *Acinetobacter* species (8, 9). Identification of antibiotic resistance patterns in different regions is necessary for selecting the treatment of choice and determining appropriate policies to prevent spread of antibiotic resistance. Therefore, this study aimed to determine antibiotic susceptibility pattern of *A. baumannii* strains isolated from ICU patients.

## MATERIAL AND METHODS

This cross-sectional study was conducted over a period of 10 months (January 2015 to October 2016) on patients in ICU of Imam Khomeini hospital in Tehran, Iran. Clinical samples (blood, urine, cerebrospinal fluid, wound and respiratory) were sent to a medical laboratory, and isolates were identified by morphological and biochemical tests, including oxidase, citrate, urease, TSI and growth at 42 °C. PCR amplification of *bla*<sub>OXA51</sub> gene was performed to confirm the presence of *A. baumannii* in the clinical samples (10). For this purpose, specific primers were designed using the Genrunner software and then synthesized by Takapouzist Co., Iran. Sequence of the primers used in the polymerase chain reaction (PCR) process is shown in table 1. Cycling conditions were as follows: initial denaturation at 94 °C for 3 min (1 cycle), 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method (11) according to the Clinical and Laboratory Standards Institute guidelines (No: M2-A9). First, bacterial suspensions with turbidity equivalent to 0.5 McFarland standard (approximately  $1-2 \times 10^8$  CFU/mL) were prepared and spread onto Mueller Hinton agar. Antibiotic disks were placed on the medium and the plate was incubated overnight. After assessing bacterial growth around each disk, growth inhibition zone was measured and compared with the CLSI table (12). The antibiotic disks (purchased from MAST Company) used in this study included: polymyxin B, cefepime, erythromycin, piperacillin-tazobactam, ampicillin-sulbactam, ticarcillin-clavulanic acid, minocycline, doxycycline, tigecycline, rifampicin, netilmicin, kanamycin, colistin, ceftazidime-clavulanic acid, doripenem, imipenem, tobramycin, ceftriaxone, ceftazidime, amikacin, tetracycline, ciprofloxacin, gentamicin and imipenem. Results of the antibiotic susceptibility testing were reported as susceptible, resistant and intermediate. *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as the

negative control and the positive control, respectively (13). The isolates were categorized into three groups of MDR, non-MDR and extensively drug-resistant (XDR) strains. *A. baumannii* strains resistant to three current classes of antibiotics were identified as

MDR, while *A. baumannii* strains resistant to three common classes of antibiotics in addition to imipenem were identified as XDR (7). Statistical analysis was performed with SPSS (version 22, Chicago, IL, USA) using Chi-square and Fisher's exact tests.

Table1- The primers used for amplification of the *bla*<sub>OXA-51</sub>-like gene

Primers	Oligonucleotides sequence	Target gene
Forward	TAA TGC TTT GAT CGG CCT TG-3-5	<i>bla</i> <sub>OXA-51</sub> -like
Reverse	5-TGG ATT GCA CTT CAT CTT GG-3	<i>bla</i> <sub>OXA-51</sub> -like

## RESULTS

Of the total of 62 clinical samples, 24 (39%) were respiratory samples and only three (6%) were cerebrospinal fluid samples. Most MDR and XDR isolates (according to the WHO criteria) were related to respiratory samples (Table 2). The highest resistance rate was observed against ceftriaxone, ticarcillin and erythromycin (100%), and the lowest resistance rate was to minocycline (20%).

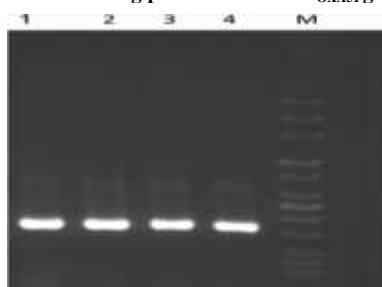
Of 10 imipenem-resistant isolates, only two isolates were resistant to minocycline. Based on the results of the susceptibility testing, 55%, 16% and 29% of *A. baumannii* strains isolated from ICU patients were MDR, XDR and non-MDR, respectively.

The result of PCR amplification of the *bla*<sub>OXA51</sub> gene is shown in figure 1.

Table 2- Frequency distribution of resistant *A. baumannii* isolates based on source of isolation

Type of samples	MDR	XDR	Non-MDR	Total
Respiratory	15	3	6	24
Wound	3	2	5	10
Blood	3	2	3	8
Urine	12	3	2	17
CSF	1	0	2	3

Figure 1- Presence of a 351 bp band indicating presence of the *bla*<sub>OXA51</sub> gene in all samples (M: 100 bp DNA ladder)



## DISCUSSION

*A. baumannii* is one of the main causes of hospital-acquired pneumonia, particularly in ICU patients (14). In our study, most MDR strains of *A. baumannii* were isolated from respiratory samples, and the prevalence of MDR strains was lowest in CSF samples. This finding is in line with findings of Zarifi et al. (15). Emergence and spread of MDR *Acinetobacter* strains is a growing global health problem (2, 4). In the present study, *A. baumannii* was highly resistant to most antibiotics, and 55% and 16% of the isolates

were MDR and XDR, respectively. These findings are in agreement with findings of previous studies in Iran (16, 17).

The highest resistance rate was to ceftriaxone, ticarcillin and erythromycin (100%), while the lowest resistance rate was observed against minocycline (20%) and tigecycline (35%). A previous study also showed resistance of over 95% to imipenem, meropenem, ceftazidime, cefotaxime, cefuroxime, ceftriaxone, cefepime, ertapenem and ampicillin/sulbactam but not to colistin (15).

In the present study, of 10 imipenem-resistant isolates, only two isolates were resistant to colistin and sensitive to minocycline. In present study, the resistance rate to tigecycline was low. In line with this finding, some studies on *Acinetobacter* species reported sensitivity of 86.7-93.3% and resistance of 65.47% to tigecycline (18, 19). A similar study in Turkey also reported tigecycline as the most effective antibiotic against MDR *Acinetobacter* strains (21). Since most tigecycline-resistant strains are also resistant to other antibiotics including carbapenems (22), as shown in our findings, infections caused by tigecycline/imipenem-resistant strains can be treated with tobramycin. However, further studies are required to confirm these findings. We also observed that most MDR and XDR strains were related to respiratory samples,

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elucidating the importance of combination antibiotic therapy for treatment of respiratory infections caused by *A. baumannii*.

#### CONCLUSION

Considering the high prevalence of MDR *A. baumannii* isolates in our study population, it is recommended to perform susceptibility testing to help determine the most effective antibiotic(s) for the treatment of infections in ICU patients.

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#### CONFLICT OF INTEREST

All authors declare that there is no conflict of interest

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