# RESEARCH ARTICLE

# The frequency of multidrug-resistance and extensively drug-resistant *Acinetobacter baumannii* in west of Iran

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**Background and objectives:** *Acinetobacter baumannii* is an opportunistic bacterial pathogen that can cause a variety of hospital-acquired infections, especially in the intensive care units (ICUs)and burn wards. Reportedly, one of the current globally health concerns is the prevalence of multidrug resistant and extensively drug-resistant and *A. baumannii* (MDR-AB, and XDR-AB). Antibiotic resistance and in-hospital survival nature of this bacterium can be attributed to its biofilm formation ability.
The present study aimed to assess the antibiotic resistance pattern and the prevalence of *pga*D and *aba*I genes in *A. baumannii* isolated from the clinical settings in Kermanshah, Iran.
**Material and methods:** Fifty isolates of *A. baumannii* were collected from the hospitals in Kermanshah during April 2016 to September 2017, which were identified according to API-20E system. Then, the antibiotic susceptibility test was performed for 14 antibiotics using disc diffusion method and minimum inhibitory concentration (MIC) based on microplate method for imipenem. The *pga*D and *aba*I genes were analyzed by PCR technique.
**Results:** The study bacterial isolates were from burn wound samples collected from ICU and burn wards of Imam Reza and Imam Khomeini hospitals in Kermanshah. The findings showed that 50 isolates of *A. baumannii* were highly resistant to erythromycin, ofloxacin, ceftazidime, tobramycin, ticarcillin-clavulanate, ceftriaxone and azithromycin (>70%) and cefepime, piperacillin-tazobactam, ampicillin/sulbactam and amikacin (>50%) and sensitive to colistin and tigecycline. In addition, 80% of isolates were resistant to imipenem. The frequency of MDR-AB and MDR-AB strains was 84% and 48% respectively. The *aba*I and pga genes were found respectively in 18% and 58% of isolates.
**Conclusion:** High prevalence of MDR-AB isolates in Kermanshah represents the spread of resistant genes among the bacteria. The antibiotic resistance rate among *A. baumannii* isolates from hospitalized patients of burn and ICU wards is a warning for the region and failure to control appropriately can cause severe problems in the future.
**Key Words:** *Acinetobacter baumannii; MDR-AB; XDR-AB; PCR; pgaD; abaI*

## INTRODUCTION

*Acinetobacter baumannii* is an aerobic, non-fermentative, gram-negative, non-motile, oxidase -negative, *coccobacillus* that can survive for prolonged periods in the environment and cause nosocomial infections, including urinary tract and wound infections (1,2). This opportunistic human pathogenic bacterium develops a wide range of HAIs; for example, bacteremia, urinary tract infectious (UTI), pneumonia and secondary meningitis, but the leading harmful consequences of these bacteria emerge as HAIs especially in the ICUs and burn wards (3-5). It is hard sto distinguish of colonization and infection from *A. baumannii* in the burn sites of patients. In fact, this bacterium plays a severe role in the these infections as it is isolated frequently by increasing the burn patients (6,7). On the other hand, the prevalence of *A. baumannii* is common in the burn wounds. In addition, the affected patients are potential reservoir for *A. baumannii* (8). *A. baumannii* has a severe effect in the colonization and infection of hospitalized patients (9). This bacterial prevalence in healthcare centers has increased around the world (10).

Due to the widespread use of antibiotics during the last two years, multidrug-resistance and especially extensively drug-resistant *A. baumannii* (MDR-AB, and XDR-AB) have accrued therefore cause a critical problem in the world (3,4). The transmission of resistant strains among patients and the application of broad-spectrum antibiotics have been reported between wards within a hospital because of the emergence of MDR-AB (5). The MDR-AB is associated with high mortality in the hospitalized patients (2). In the ICU, the mortality rate associated with *A. baumannii* has been found to be 54% (11). The MDR-AB in the burn wounds of the infected patients is a main risk factor for high mortality rate around the world (12). The prevalence of these resistant strains is the leading cause of infections. Emergence of XDR-AB limits the therapeutic options and causes a serious concern to control the HAIs. For a period of 5 years (2005-2010), the frequency of XDR-AB in the clinical isolates had increased from 15% to over 41%. For the medical communities, the management of MDR-AB and XDR-AB infections is one of essential problems. It is very difficult to treat the infections caused by *A. baumannii* due to many multiple antibiotic resistant strains (11).

Various mechanisms of antibiotic resistance have been recognized in these microorganism, such as enzymatic degradation and modification of antibiotics, enhanced expression of efflux pump genes, reduced permeability, ability of forming the biofilm formation and altered drug targets (13), and combination therapy is usually required for effectual treatment of the nosocomial infections from *A. baumannii* (14). It is ubiquitous in the hospital environment and can survive for longsome periods on dry inert surfaces (15). The infected patients are one of the important reservoirs of *A. baumannii*, who might make contamination in their hands, resulting in cross transmission of the bacteria. The specified risk factors for *A. baumannii* colonization and infection are the protracted hospitalization, long stay in ICU, mechanical ventilation, central venous catheterization, urinary catheterization, previous exposure to antimicrobials (broad-

One of the key factors in these infectious diseases is the ability of the bacteria to form biofilm, antibiotic resistance and in-hospital survival causes of this organism have been shown to be due to its biofilm formation ability (17), due to virulence factors, such as the autoinducer synthase *aba*I as a part of the quorum sensing complex, and the *pga*ABCD operon responsible for producing poly-b-1,6-N-acetylglucosamine (PNAG) (18). A direct relationship has been found between the biofilm formation and drug resistance in abaI (19) and pga (20) genes in the A. *baumannii* (21).

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# The frequency of multidrug-resistance and extensively drug-resistant Acinetobacter baumannii in west of Iran

## Objectives

Given the limited data available in the molecular characterizations of *A. baumannii* in Kermanshah, the current study was designed to determine the resistance pattern of different antibiotics and the presence of *aba*I and *pga*D genes in the clinical isolates of *A. baumannii* in this region.

## MATERIALS AND METHODS

## Bacterial isolates

A total of fifty clinic isolates of *A. baumannii* were collected during April 2016 to September 2017 from patients admitted in Imam Reza and Imam Khomeini hospitals of Kermanshah. This isolates obtained from wounds burn were identified using biochemical tests such as oxidase test, and TSI medium and Kit API 20 NE to verify the *A. baumannii* strains that are grown at 42°C.

## Antimicrobial susceptibility test

The sensitivity of these isolates were tested using 14 antibiotics (MAST, Merseyside, UK), including ampicillin/sulbactam (10 μg/10 μg), piperacillin-tazobactam (100/10 μg), imipenem (10 μg), ceftazidime (30 μg), amikacin (30 μg), cefepime (30 μg), ceftriaxon (30 μg), ofloxacin (5 μg), tobramycin (10 μg), colistin (10 μg), azithromycin (1 μg), erythromycin (15 μg), ticarcillin-clavulanate (75 μg/10 μg) and tigecycline (15 μg) according to the CLSI table (2015) and disc diffusion method.

The resistance of bacteria strains against minimum three classes of antimicrobial agents, all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones and aminoglycosides indicates the MDR-AB in this study; and resistance to the three cases of antimicrobials described above (MDR) and carbapenems refers to XDR-AB (22).

The clinical laboratory standards institute (CLSI 2015) guidelines via microplate method were followed to obtain the minimum inhibitory concentration (MIC) value. The TSA-grown bacteria suspension equal to the turbidity of 0.5 McFarland standard was cultured in Muller Hinton broth (Merck, Germany) at a ratio of 1:100 to reach final concentration of $1 \times 106$ CFU/ml. Different dilutions of imipenem (Merck, Germany) were prepared using 0.01 M phosphate buffer. Additionally, various concentrations of 0.25-512 μg/ml from the antibiotic were provided as well. In the next step, 100 μl of different antibiotic dilutions and 100 μl of bacteria suspension were appended into each well of the microplates. The MIC value was defined as the lowest concentration inhibiting visible growth of bacteria after 24 hours incubation at 37°C.

## PCR amplification

DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted only for the *aba*I and *pga*D genes using the primers (SinaClon, Iran) listed in Table 1.

A reaction mixture (25 μl) contained 3 μl of DNA, 1 μl of each primer, 13 μl of Master Mix 2X (SinaClon, Iran), and 7 μl of deionized distilled water.

The experiment was continued according to the following program: initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute and final extension at 72°C for 5 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye, and visualized by Gel Doc apparatus (BioRad, USA).

## Statistical analysis

Data were recorded and inserted in Excel software. Statistical analyses were performed by SPSS version 16 software using chi-square test, and P-value<0.05 was considered as the statistically significance level.

**TABLE 1 Primers used in this study**

| Primer | Sequence (5´-3´) | Expected Amplicon Size (bp) | Reference |
|---|---|---|---|
| *aba*I | F: AAT GCC TAT TCC CTG CTC AC | | |
| | R: AAT GCT TGC AGA ATT GC | 132 | (19) |
| *pga*D | F: TTG ATC AGC CTG AAT ATG TGA | | |
| | R: CAC ACA TAG TCA TAA ATG AGG | 145 | This study |

## RESULTS

The bacteria were mainly isolated from burn wounds from male (62%) and female (38%) patients with the mean age of 39.4 ± 24.2 (min=1, max=73) years. The samples were collected from ICU and burn wards of Imam Reza and Imam Khomeini hospitals of Kermanshah.

A total at 50 isolates of *A. baumannii* were highly resistant to erythromycin, ofloxacin, ceftazidime, tobramycin, ticarcillin-clavulanate, ceftriaxone and azithromycin (>70%) and cefepime, piperacillin-tazobactam, ampicillin/sulbactam and amikacin (>50%) and sensitive to colistin and tigecycline (Table 2).

The MIC limits for imipenem in accordance with CLSI standard table were as follow: resistant (≥8 μg/ml), intermediate (4 μg/ml) and sensitive (≤2 μg/ml). According to the results and this protocol, imipenem-resistant isolates accounted for 80%, intermediate strains were 20% and there was no sensitive isolates.

**TABLE 2 Antimicrobial susceptibility testing of isolates**

| Antimicrobial category | Antimicrobial agent | All isolates (n=50) | | ICU (n=25) | | Burn ward (n=25) | | Imam Reza (n=30) | | Imam Khomeini (n=20) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I% | R% | I% | R% | I% | R% | I% | R% | I% | R % |
| | Ampicilln/ sulbactam | 8 | 60 | 4 | 22 | 4 | 38 | 8 | 26 | - | 34 |
| | Ticarcillin clavulanic acid | 6 | 76 | 4 | 28 | 2 | 48 | 4 | 38 | 2 | 38 |
| Penicillins | Pipracilin-tazobactam | 12 | 60 | 6 | 24 | 6 | 36 | 12 | 24 | - | 36 |
| | Amikacin | 2 | 58 | - | 14 | 2 | 44 | - | 24 | 2 | 34 |
| Aminoglycosides | Tigecycline | 2 | 28 | 2 | 6 | - | 22 | 2 | 6 | - | 22 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Colistin | - | 12 | - | 10 | - | 2 | - | 10 | - | 2 |
| | Tobramicin | 6 | 76 | 6 | 26 | - | 50 | - | 36 | - | 40 |
| Extended-spectrum cephalosporins; 3rd and 4th generation | Ceftazidim | - | 78 | - | 28 | - | 50 | - | 38 | - | 40 |
| | Ceftriaxone | 2 | 76 | 2 | 26 | - | 50 | 2 | 36 | - | 40 |
| Cephalosporins | Cefepime | 10 | 68 | 8 | 20 | 2 | 48 | 8 | 30 | 2 | 38 |
| | Azithromycin | 12 | 70 | 8 | 36 | 4 | 34 | 10 | 40 | 2 | 30 |
| Macrolides | Erythromycin | 6 | 88 | - | 50 | 6 | 38 | 2 | 56 | 4 | 32 |
| Carbapenem | Imipenem | 6 | 48 | 4 | 12 | 2 | 36 | 6 | 14 | - | 34 |
| Second generation Fluoroquinolones | Ofloxacin | - | 80 | - | 30 | - | 50 | - | 40 | - | 40 |

n: number, I: Intermediate, R: Resistance, -: 0%

The frequency of MDR-AB and XDR-AB was 84% and 48%, respectively.

The data were analyzed by chi-square fisher using SPSS software and P-value<0.05. Accordingly, there was no relationship between the incidence of MDR-AB (p-value>0.05) and XDR-AB (P value>0.05) with *aba*I and *pga*D genes.

The ofloxacin, cefepime, ceftazidime, ceftriaxone and azithromycin showed increasingly significant resistant in the ICU.

The *aba*I and pga genes were found respectively in 18% (n=9) and 58% (n=29) of isolates. As well, 6 (12%) isolates contained both *aba*I and *pga*D genes Figure 1.



**Figure 1)** *Polymerase chain reaction (PCR)*
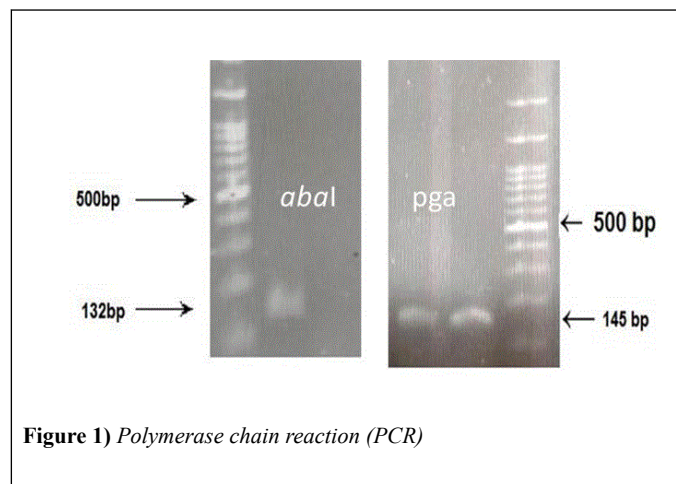
## DISCUSSION

*A. baumannii* is one of the nosocomial pathogens and the cause of burn wound during the last decade (5,23,24). Unfortunately, there is a severe resistance against antimicrobial drugs like penicillins, cephalosporines, macrolides, aminoglycosides, tetracyclines and fluoroquinolones (25).

In this study, all strains of *A. baumannii* were isolated from the ICU and burn wards. These results are in consistent with other studies (26,27). Our study showed that the resistance pattern of all isolates related to burn wound was similar to that reported by Tekin et al. (28).

The present study investigated the susceptibility pattern of 50 clinical strains of *A. baumannii* for 14 commonly used antibiotics, and the results indicated low sensitivity to the variety of antimicrobial agents in treating the infections caused by *A. baumannii*.

Resistance pattern of each antimicrobial agent (except for colisitin and tigecycline) was all above 50% (Table 2). The sensitivity of clinical isolates was 88% for colisitin and 70% for tigecycline. These antibiotics were found to be the most effective agents in vitro. There are only two antimicrobial agents effective in eliminating the hospital-isolated strains of *A. baumannii*, whereas the resistance to colisitin in *A. baumannii* strains is on the rise in the world (29). In this research, the resistance to colisitin was only seen in one of the isolates in burn ward. The last lines of antibiotic therapy against *A. baumannii* infections are just colistin and tigecycline.

In our study, according to susceptibility test results, a considerable proportion of *A. baumannii* isolates (80%) were resistant to imipenem suggesting that carbapenems are unsuitable for the treatment of *A. baumannii* infections in burn patients hospitalized at Imam Reza and Imam Khomeini hospitals. In the present study, there was a difference between imipenem MIC results and imipenem disc diffusion. This indicated that imipenem disk could not detect 46% of resistant isolates and the use of this disc for antimicrobial susceptibility test could lead to false sensitive results.

Alavi-Moghadam et al. reported that all isolates of this bacterium were resistant to imipenem (30). The resistant pattern of *A. baumannii* isolates to carbapenem investigated in this study is different to compare with other studies, probably due to geographical differences in isolates. Based on a meta-analysis in Iran, imipenem-resistant isolates of *A. baumannii* were 55% (95% confidence interval (CI), 53.0-56.5) and MDR isolates included 74% (95% CI, 6103-83.9) (31).

Based on a study in Iran (2006-2010), there is a similarly enhance in resistance to imipenem and meropenem of *A. baumannii* strains (32). A research in a burns ward of USA showed 87% prevalence for imipenem-resistant strains of *A. baumannii* (33).

*Multidrug-resistant Acinetobacter spp. is potent pathogens often in burn ward and ICU with high outbreak, which needs epidemiologic monitoring as a measure for controlling the nosocomial infections (34).*

*A. baumannii* strains isolated from clinical setting have shown globally increase in MDR and XDR types. The resistance of *A. baumannii* isolates showed that 84% were MDR, which were resistant to three groups of antibiotics including aminoglycosides, fluoroquinolones and cephalosporins. Moreover, 48% were XDR; the high prevalence of MDR and XDR causes epidemic in different geographic regions. The findings of the present study are in line with results reporting prevalent MDR *A. baumannii* throughout the world with 67% to 100% prevalence (35-38).

Most of the XDR isolates belonged to the patients hospitalized in burn ward. In the patients with burn wounds, the infection is the most common reason for death due to the fact that burn patients are susceptible and at high risk of infection (27). Studies carried out in the burn and ICU wards as the main origins of HALs have isolated *A. baumannii* frequently (39-41).

Indiscriminate use of antimicrobial agents in the ICU, long hospital stay, prolonged use of catheters and ventilators, implants and other synthetic instruments lead to spread resistant strains colonized in susceptible patients (42).

There are serious needs to detect the key risk factors of catching HAIs, particularly ICU, to better manage and appropriate strategy to design practical protocols for infection control committees or medical doctors to adapt the best therapeutic decisions, resulting in short hospitalization, improve survival rate, reduced financial burden, low side effects, clinical decreased antibiotic resistance, and control the resistant bacteria.

In this study, the *aba*I and *pga*D genes were seen in 18% and 58% of isolates, respectively. In a study, the genes of biofilm formation, including ompA and csuE, were detected in all samples, while bap and blaPER-1 were detected in 66% and 64% of the isolates, respectively (38). In research of last decades, the PCR method revealed that all 30 clinical isolates of *A. baumannii* have the *pga*D gene (20).

## CONCLUSION

High prevalence of MDR isolates in Kermanshah region represents the spread of resistant genes among bacteria. Based on the results, an approach preventing this trend seems to be the treatment of *A. baumannii* infections according to the antimicrobial susceptibility testing. Our findings indicated that carbapenems are not effective against resistant isolates. Unfortunately, effective drugs such as colistin and tigecycline showed also resistance. The rate of antibiotic resistance among *A. baumannii* isolates from hospitalized patients of ICU and burn wards in Kermanshah is a warning for the region and failure to control appropriately can cause severe problems in the future.

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