

Distribution of Aminoglycoside Resistance Genes Among *Acinetobacter baumannii* Strains Isolated From Burn Patients in Tehran, Iran

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Abstract

Background: *Acinetobacter baumannii* is an important cause of nosocomial infections, particularly in burn patients. Hospital Infections caused by these bacteria are difficult to treat.

Objectives: The present study aimed at determining the frequency of genes encoding aminoglycoside-modifying enzymes in *A. baumannii* strains isolated from burn patients in Shahid Motahari hospital.

Methods: This study was performed on 100 *A. baumannii* strains collected from Shahid Motahari hospital in Tehran during 2013 and 2014. The bacteria were cultured and identified. Antibiotic susceptibility testing was performed by disk diffusion method according to the CLSI guidelines. PCR assay was done to find the genes encoding aminoglycoside-modifying enzymes.

Results: In this study, highest resistance to antibiotic was reported for ceftriaxone, ciprofloxacin, and ceftizoxime (100%), whereas the highest susceptibility was observed for colistin (100%), followed by gentamicin, amikacin, and tobramycin with (93%), (90%), and (87%), respectively. In the total of 100 strains studied, *aphA6*, *aadB*, *aacC1* and *aadA1* genes were found in 65/72/21 and 37 of *A. baumannii* isolates, respectively; 8 isolates had *aadB* and *aphA6* genes and 3 had *aadB*, *aadA1*, *aacC1*, and *aphA6* genes.

Conclusions: This study showed the high frequency aminoglycoside-resistance genes among *A. baumannii* strains. Thus, the implementation of appropriate programs to prevent the spread of the bacteria seems necessary in the Shahid Motahari hospital.

Keywords: Aminoglycoside Resistance Genes, Multidrug Resistance, Burn, *Acinetobacter baumannii*

1. Background

Burns are one of the most common forms of trauma. Burn patients have an urgent need of specialized services to minimize the effects of mortality; 75% of all deaths in patients with severe burns over 40% are due to infection caused by burns. In burn patients, normal function of the skin and immune response is impaired (1). Burn wounds are a suitable environment for the growth of different species of bacteria and factors such as age, depth and extent of the burn can affect these infections (2). *Acinetobacter baumannii* is a gram-negative coccobacilli bacterium broadly accessible in water and soil and stays alive for long periods in hospitals and is easily passed from person to person.

Due to the drug-resistant strains in the world, *Acinetobacter baumannii* is now one of the nosocomial pathogens, particularly in burn patients and in those in intensive care units. *A. baumannii* causes various infections such as endo-

carditis, peritonitis, respiratory tract infections, meningitis, burns, and sepsis in different parts of the hospital (1, 2). Nowadays, due to the indiscriminate and unnecessary use of broad-spectrum antibiotics, *A. baumannii* has become resistant to a wide range of antibiotics.

Over the past decade, this bacterium has been considered as one of the most troubling pathogens and its treatment has been limited to a few antibiotics. Studies have shown that *A. baumannii* has innate and adaptive resistance to many antibiotics including beta-lactams, aminoglycosides, fluoroquinolones, and carbapenems (3-6). The fast development and worldwide dissemination of *A. baumannii* as a major nosocomial pathogen is significant and shows its successful adaptation to the 21st century hospital wards (7). The 2 main mechanisms of resistance to aminoglycosides are as follow: first, changes in the structure of the ribosome as a result of the structural protein mutant ribosomes, rRNA or enzymatically; and the second, changes in the structure of antibiotics by enzymes (8). The

most common mechanism of resistance to aminoglycosides in this bacterium is the modification of hydroxyl or amino groups of the antibiotic by modifying aminoglycoside (9, 10), albeit other mechanisms such as reduced permeability and change of the binding sites have been suggested (11). Aminoglycosides have long been used for the therapy of infection in hospitalized patients and still are an essential option for treatment of diseases created by MDR strains. MDR *A. baumannii* was characterized as the strain having acquired nonsusceptibility to at least 1 agent in 3 or more antibiotic agents. Extensively drug-resistant (XDR) *A. baumannii* was characterized as demonstrating nonsusceptibility to at least 1 agent in all but 2 or fewer antimicrobial classes. The genes encoding aminoglycoside modifying enzymes may be located on transposons, plasmids or class 1 integrons in MDR *A. baumannii* strains in Europe (12-14). These genes include nucleotidyltransferases (*aadB*), ANT (3")-Ia (*aadA1*), phosphotransferases (*aphA6*), and acetyltransferases (*aacC1*), which were detected by PCR and sequencing.

2. Methods

2.1. Bacterial Isolation

In this study, from June 2013 to August 2014, 100 strains of *A. baumannii* were collected by sterile swabs from burn patients who referred to Shahid Motahari hospital (level I burn center) in Tehran, Iran. The wound exudates were collected by swabbing and transported to Department of Medical Microbiology. A questionnaire was designed and coded for each patient. The samples were taken from burn patients included skin ulcer, blood, urine, catheter, and isolated samples from the burned area and the respiratory tract. In the laboratory, clinical samples were cultured on blood agar, MacConkey agar, and Nutrient media (Merck, Germany). After 24 hours, the coccobacillus gram-negative *Acinetobacter* was confirmed by Gram stain microscopy method. Isolates were characterized and confirmed in the laboratories of the corresponding hospitals through routine microbiological and biochemical tests such as citrate, moving, oxidase tests, and growth at 42°C. The confirmed samples were kept in 30% glycerol at -70°C.

2.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed according to instructions of CLSI using the following antibiotics: Piperacillin - tazobactam (10/100 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Trimethoprim-sulfamethoxazole (25 µg), Tobramycin (10 µg), Ceftazidime (30 µg), Ampicillin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftriaxone (30

µg), Tetracycline (10 µg), Gentamicin (10 µg), and Colistin (CO, 10 µg) (MAST, UK) (15).

A. baumannii ATCC19606 was used as the control strain, and *Escherichia coli* ATCC 25922 and 352 and *Pseudomonas aeruginosa* ATCC 27853 were used as negative controls, moreover, the confirmed strains were used as positive and negative controls for the PCR detection of AME genes.

2.3. DNA Extraction

Genomic DNA was extracted by standard DNA Extraction Kit (Bioneer, Republic of Korea) according to the previous reports (16).

2.4. PCR and Detection of Aminoglycoside-Resistance Genes

All target genes and corresponding primers used for PCR amplification are listed in Table 1. The PCR mixture contained the forward/reverse primers, DNA template, and master mix (10 Mm of Tris-HCL, 30 Mm of KCL: 10 Mm, 30 Mm, Bioneer Company, Korea, Cat. number K-2016).

Table 1. The Sequences of Reverse and Forward Primers Used in This Study

Primer	Primer Sequences	Product Size, bp	Annealing Temperature, °C
<i>aadB</i> F	ATGGACACAACGCAGGTCGC	495	55
<i>aadB</i> R	TTAGGCCGCATATCGCGACC		
<i>aadA1</i> F	ATGAGGGAAGCGGTGATCG	624	52
<i>aadA1</i> R	TTATTGCGGACTACCTTGGTG		
<i>aphA6</i> F	AGCGAAAATGTTGAGTTGGCT	399	55
<i>aphA6</i> R	TCCGTGATATCGCCATGAGA		
<i>aacC1</i> F	ATGGGCATCATTCGCACATGTAG	465	52
<i>aacC1</i> R	TTAGGTGGCGGTACTTGGGTC		

PCR conditions are illustrated in Table 2 that included 35 cycles of amplification under the following conditions: denaturation at 95°C for 3 minutes; annealing at 52 - 55°C for 1 second; and cycling was followed by 45 seconds for extension at 72°C, with a final extension at 72°C for 5 minutes. The PCR purification kit was used and sequencing was conducted by the Bioneer company. Chromas 1.45 software and BLAST were used to analyze the nucleotide sequences. We investigated genes encoding aminoglycoside resistance including *aacC1* that confers gentamicin resistance; *aadA1* that confers tobramycin, streptomycin and spectinomycin resistance; *aadB* that confers tobramycin, gentamicin and kanamycin resistance; and *aphA6* that confers neomycin, kanamycin, amikacin and gentamicin resistance.

Table 2. Antimicrobial Resistance Pattern of *A. baumannii* Against Different Antibiotics^a

Antibiotic	Susceptible	Resistant	Intermediate
PTZ	0	99 (99)	1 (1)
CRO	0	100 (100)	0
CAZ	0	100 (100)	0
CTX	0	100 (100)	0
CPM	0	100 (100)	0
CIP	0	100 (100)	0
IMI	0	98 (98)	2 (2)
MEM	1 (1)	98 (98)	1 (1)
GEM	5 (5)	93 (93)	2 (2)
AMK	5 (5)	90 (90)	5 (5)
TET	10 (10)	82 (82)	8 (8)
SXT	4 (4)	94 (94)	2 (2)
PRL	0	100 (100)	0
TOB	7 (7)	87 (87)	6 (6)
COL	100 (100)	0	0

Abbreviations: AMK, Amikacin; CAZ, ceftazidime; CIP, Ciprofloxacin; COL, Colistin; CPM, cefepime; CRO ceftriaxone; DOX, doxycycline; GEM, gentamicin; IPM, imipenem; MEM, meropenem; MIN, minocycline; PRL, piperacillin; PTZ, piperacillin-tazobactam; SAM, ampicillin-sulbactam; SXT, Trimethoprim-sulfamethoxazole; TET, tetracycline; TOB, tobramycin.

^aValues are expressed as No. (%).

2.5. Statistical Analysis

This was a descriptive study. MINITAB16 software was used to analyze the results, with the confidence interval of 95%, and P value less than 0.05.

3. Results

3.1. The Study Population

Of the 100 burn cases, 58 (58%) were male and 42 (42%) female. The age of the burn patients ranged from 2 to 90 years, with the maximum number of cases in the age group of 41 to 60 years (n = 52).

3.2. Antibiotic Susceptibility Testing

From June 2013 to August 2014, 100 *Acinetobacter* strains that were recovered from burn patients at the Shahid Motahari hospital were used for this study. These isolates were collected from different places of the body including the urine (12%), sputum (3%), blood (20%), the catheter (25%), and wound (40%), respectively (Table 2). The

results revealed that all strains were (100%) resistant to ceftriaxone, ciprofloxacin, ceftizoxime, and (0%) colistin (Table 2). In this study, 94 strains collected from Shahid Motahari hospital in Tehran were found to possess multidrug resistance (MDR). Screening of AME genes by PCR technique revealed that frequency of *aphA6*, *aadB*, *aacC1*, and *aadA1* genes were 65%, 72%, 21%, and 37%, respectively. Moreover, 28 strains did not have any aminoglycoside modifying gene. Eight isolates had *aadB* and *aphA6* genes and 3 isolates had *aadB*, *aadA1*, *aacC1*, and *aphA6* genes. The purification of PCR products were done by tge PCR purification kit (Bioneer Co., Korea), and sequencing was done by the Bioneer company. Chi-square test was used for data analysis. A significant correlation was found between resistance to aminoglycosides and studied genes ($P < 0.05$); 65% of the isolates contained the phosphotransferase gene *aphA6*, which confers to amikacin, gentamicin, kanamycin, and neomycin resistance, 21% of isolates contained acetyltransferase genes *aacC1* that confers to gentamicin resistance, and 37% contained adenyltransferase genes *aadA1* as streptomycin, tobramycin, and spectinomycin resistance, and 72% of the isolates contained *aadB* that confers resistance to tobramycin, gentamicin, and kanamycin.

4. Discussion

A. baumannii is an opportunistic pathogen primarily associated with nosocomial infections worldwide (17). In addition to causing broad range of infections (eg, pneumonia, urinary tract, bloodstream, and skin infections), this organism is responsible for 10% of all nosocomial infections. Due to the formation of multi-resistant *A.baumannii* strains and its rapid spread, it is highly difficult to treat the infections caused the bacteria these days. Currently, the bacteria are considered as factors of mortality among hospitalized patients in hospital wards (18). Several sudden outbreaks of the bacteria are reported annually from hospitals around the world, indicating the importance of the bacteria and the need for a suitable plan to prevent infection, particularly by resistant strains. Many studies conducted in hospitals during the sudden outbreaks, revealed that hospital environments had been the source of infection in most cases (19). Various studies showed that *Acinetobacter* could survive for 16 weeks on dry surfaces of an environment; and this is considered as an alarm for its treatment because the isolates of *A. baumannii* could be isolated repeatedly from all various surfaces, indicating its high adaptability to incompatible environmental conditions. Studies have demonstrated that the survival rate of the strains isolated from dry places was higher than the ones isolated from humid places, and thus having a higher potential to cause nosocomial outbreaks (20). The results of

antibiotic susceptibility testing of strains show that nosocomial strains have a higher antibiotic resistance, which increases the chances of survival of the bacteria in environments such as ICU, where patients consume broad-spectrum antibiotics such as carbapenems (21). This will lead to an increased risk of colonization and infection of patients. Aminoglycosides have been an essential group of antibacterial agents used in the treatment of genuine bacterial diseases, particularly those with aerobic gram negative bacteria. However, recent studies demonstrated the development of resistance to aminoglycosides in *Acinetobacter* isolates in various parts of the world. Resistance to aminoglycoside in *Acinetobacter* is mainly due to the inactivation of the antimicrobials by particular modifying enzymes such as adenylyltransferases, phosphotransferases, and acetyl transferases (22, 23). In a study done by facile et al in IRAN, colistin resistance rate was 11.6%, and 95% of isolates were considered as MDR isolates. However, in the current study, antibiotic resistance patterns showed that 11.6% of strains were MDR isolates. In this study, the rate of resistance to imipenem and meropenem was 98% and to ciprofloxacin and colistin was 100% (24). Akers et al. in a survey conducted in Texas, determined the susceptibility to aminoglycoside antibiotics among clinical strains of *A. baumannii*. In their investigation, 93% of isolates were resistant to gentamicin and 87% resistant to tobramycin (25). In our study, the resistance to tobramycin and gentamicin was 63% and 86%, respectively. Shakibaei et al. in a study conducted on 50 clinical strains of *A. baumannii* in Kerman, Iran, reported that 73.3% of strains were resistant to imipenem, 66% to ciprofloxacin, and 53.3% to amikacin (26). In another study performed by Aliakbarzafteh et al. in Tabriz, it was found that 94% of *A. baumannii* isolates were resistant to kanamycin, 86% to gentamycin, 81% to amikacin, and 63% to tobramycin (14). The differences in antibiotic resistance patterns in these studies with current investigation might have been due to the variations in the types of clinical samples, as well as the geographical regions. In this study, the prevalence of *aphA6*, *aadB*, *aacC1*, and *aadA1* genes was 65%, 72%, 21%, and 37%, respectively. Nevertheless, Nemec et al. surveyed the distribution of AME genes in *A. baumannii* and reported genes encoding resistance to aminoglycosides in 95% of isolates: *aadB* (n = 31), *aphA6* (n = 55), *aacC1* (n = 68), and *aadA1* (n = 68) (3). Aliakbarzade et al. in their investigation in 2013, similar to the current study, found that the highest rate of resistance was related to colistin (77%). Also, they reported that the frequency of *aadB*, *aphA6*, *aacC1*, and *aadA1* and genes among 103 *A. baumannii* strains was 18.6%, 27.9%, 60.46%, and 65.11%, respectively. In their study, the rate of resistance to aminoglycosides and frequency of AME genes was less than that of our survey (14). According to the current study,

the most effective antimicrobial agent against MDRA *A. baumannii* isolates is colistin. Although it seems that this antibacterial agent has a great influence on this opportunistic pathogen, it has a serious side effect in the host. Therefore, it should only be used as the last choice drug.

In almost all the mentioned studies, *Acinetobacter* resistance to aminoglycosides was less than our study, indicating an increase in resistance to antibiotics in this bacterium. Aminoglycosides resistance in *Acinetobacter* has emerged as an important health problem. Our results revealed that clinical isolates of the bacteria in burn patients carry different types of genes encoding aminoglycoside-modifying enzymes and should be managed by timely detection and exact isolation methods to help diminish their severe sequels and mortality rate of the patients.

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Footnotes

Authors' Contribution: All the authors cooperated in conducting the isolation, performing the experimental work, as well as in writing the manuscript.

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