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MOLECULAR DETECTION OF AMINOGLYCOSIDE-MODIFYING ENZYME GENES IN *ACINETOBACTER BAUMANNII* CLINICAL ISOLATES

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Acinetobacter baumannii is a major opportunistic pathogen in healthcare settings worldwide. In Iran, there are only few reports on the prevalence of aminoglycoside resistance genes among *A. baumannii* isolates. The aim of this study was to investigate the existence of aminoglycoside-modifying enzyme (AME) genes from *A. baumannii* strains collected at a university teaching hospital in Iran. One hundred *A. baumannii* strains were collected between 2014 and 2015 from hospitalized patients at Loghman Hakim Hospital, Tehran, Iran. Antimicrobial susceptibility was determined by disk diffusion method according to the Clinical and Laboratory Standards Institute recommendations. The DNA was extracted using a kit obtained from Bioneer Co. (Korea) and was used as a template for polymerase chain reaction. The most active antimicrobial agent against these strains was colistin. The rate of extended-spectrum cephalosporin resistance was 97%. The *aadA1*, *aadB*, *aac(6)-Ib*, and *aac(3)-IIa* genes were found in 85%, 77%, 72%, and 68% of *A. baumannii* isolates, respectively. This study showed a high prevalence rate of AME genes in *A. baumannii*. This prevalence rate has explained that further aminoglycoside resistance genes may have role in the resistance of clinical isolates of *A. baumannii*. Therefore, control and treatment of serious infections caused by this opportunistic pathogen should be given more consideration.

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Introduction

Acinetobacter baumannii is a major opportunistic, non-fermenting, Gram-negative pathogen in healthcare settings worldwide, and it is now being isolated at an increasing rate from wound infections [1]. The organism's ability to spread among hospitalized patients and to persist for long periods along with multidrug resistance are the major driving forces behind the frequent large outbreaks in different countries [2]. Three decades ago, *A. baumannii* infections were treated with traditional antibiotics, but during the past two decades, *A. baumannii* resistant to a wide range of antibiotics including aminoglycosides, fluoroquinolones, broad-spectrum β -lactams, and carbapenems has emerged [3]. They can cause a variety of nosocomial infections including pneumonia, meningitis, endocarditis, urinary tract infections, and wound infections especially in hospitalized patients in the intensive care units (ICUs). As infections with drug-resistant *A. baumannii* in patients remain difficult to treat, it is becoming a worldwide threat. Aminoglycosides have long been used for the treatment of *A. baumannii* infections and still are an important alternative for therapy. However, resistance to aminoglycosides has increased in recent years in these bacteria [4–6]. Aminoglycoside-modifying enzymes (AMEs) are the most important mechanism of providing aminoglycoside resistance in *A. baumannii* clinical strains. Recent studies have shown several resistance mechanisms to aminoglycosides in *A. baumannii* including enzymatic inactivation by acetyltransferases (AACs) and adenylyltransferases (AADs), usually found on transposons and plasmids, which facilitate acquisition of resistance [7–11]. In Iran, to the best of our knowledge, there are only few reports on the prevalence rate of AME genes among clinical strains. The aim of this study was to evaluate the antimicrobial susceptibility of *A. baumannii* strains and to investigate the existence of AME genes from the strains collected at the university teaching hospital in Iran.

Materials and Methods

Bacterial isolates

A total of 100 *A. baumannii* strains were collected between January 2014 and May 2015 from hospitalized patients at Loghman Hakim Hospital, Tehran, Iran. The isolates obtained were tested by conventional, well-recognized biochemical tests including oxidase, motility, and citrate tests as well as their capability to grow

at 42–44 °C [12]. Polymerase chain reaction (PCR) was performed based on the method of Hujer et al. [13] for identifying the *A. baumannii* species.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method (Mast Group, Merseyside, UK) according to the Clinical and Laboratory Standards Institute recommendations [14]. Antimicrobial agents tested were ciprofloxacin (CIP, 30 µg), imipenem (IPM, 10 µg), gentamicin (GEN, 10 µg), amikacin (AK, 30 µg), tetracycline (TET, 10 µg), colistin (CO, 10 µg), cefepime (CPM, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), and meropenem (MRP, 10 µg).

DNA extraction, PCR amplification, and DNA sequencing

DNA was extracted using a kit obtained from Bioneer Co. (Korea) and was used as a template for PCR. Amplification was achieved under the following thermal cycling conditions: 5 min at 94 °C for the initial denaturation followed by 36 cycles of amplification consisting of 45 s at 94 °C, 45 s at 52–58 °C, and 45 s at 72 °C, with 5 min at 72 °C for the final extension. Target genes, temperatures of annealing, amplicons size, and primers used for PCR amplification are shown in Table I. Results of the PCR were compared with positive controls. The PCR products were analyzed by electrophoresis in a 1%–1.5% vw^{-1} agarose gel. One of the PCR products was purified, and direct sequencing was done. The PCR amplification was performed for AME genes including *aadA1*, *aadB*, *aac(3)-IIa*, and *aac(6′)-Ib*.

Statistical analysis

This was a descriptive application research study. Statistical analyses were done by MINITAB16 software. The *P* value and confidence intervals were ≤ 0.05 and 95%, respectively.

Results

A. baumannii strains were isolated from 100 hospitalized patients. The patients included 75 (75%) men and 25 (25%) women. The age range of the study population was 5–80 years [mean age 42.5 (± 10) years]. The patterns of antibiotic resistance found in *A. baumannii* isolates showed that resistance to tested

Table I. PCR primers and annealing temperatures used in this study

Target genes	Forward	Reverse	Annealing (°C)	Amplicon size (bp)	References
<i>aac(3)-IIa</i>	CGGAAGGCAATAACGGAG	TCGAACAGGTAGCAGCTGAG	58	740	This study
<i>aac(6)-Ib</i>	TTGCGATGCTCTATGAGTGGCTA	CTCGAATGCCGTGGCGTGT	55	611	[15]
<i>aadA1</i>	ATGAGGGAAGCGGTGATCG	TTATTTGCCGACTACCTTGGTG	52	624	This study
<i>aadB</i>	ATGGACACAACGCAGGTCGC	TTAGGCCGCATATCGCGACC	55	495	This study

Table II. Antibiotic susceptibility testing results

Antibiotics	Resistant No. (%)	Sensitive No. (%)	Intermediate No. (%)
Imipenem	70 (70%)	22 (22%)	8 (8%)
Meropenem	70 (70%)	22 (22%)	8 (8%)
Gentamicin	80 (80%)	15 (15%)	5 (5%)
Ciprofloxacin	37 (37%)	53 (53%)	10 (10%)
Amikacin	85 (85%)	12 (12%)	3 (3%)
Ceftriaxone	97 (97%)	0 (0%)	3 (3%)
Ceftazidime	97 (97%)	0 (0%)	3 (3%)
Tetracycline	58 (58%)	30 (30%)	12 (12%)
Cefepime	97 (97%)	0 (0%)	3 (3%)
Colistin	0 (0%)	100 (100%)	0 (0%)

antibiotics was 70 (70%) to IPM, 70 (70%) to MRP, 80 (80%) to GEN, 37 (37%) to CIP, 85 (85%) to AK, 97 (97%) to CRO, 58 (58%) to TET, 97 (97%) to CPM, 97 (97%) to CAZ, and 0 (0%) to CO (Table II). The most active antimicrobial agent against these strains was CO with 100% susceptibility. More than 80% of the isolates resistant against AK and GEN harbored AME genes. The prevalence rate of AAC genes including *aac(6')-Ib* and *aac(3)-IIa* was 72% and 68%, and the prevalence rate of AAD genes including *aadA1* and *aadB* was reported to be 85% and 77%, respectively. Sequencing of PCR products for AAC and AAD genes was confirmed by BLAST in NCBI.

Discussion

A. baumannii has emerged as an etiologic threat for diverse nosocomial infections. The organism's ability to spread among hospitalized patients and to persist for long periods along with multidrug resistance are the major driving forces behind the frequent large outbreaks in different countries [16–18]. The increase of *A. baumannii* strains during recent years, along with rapid progress of antibacterial resistance, led to this study [1, 19]. Therefore, control, prevention, and treatment of serious infections caused by this bacteria should be considered, and there is a need for revising the treatment protocols to decrease the spread of resistant genes among the clinical isolates. The *aadA1*, *aadB*, *aac(6')-Ib*, and *aac(3)-IIa* genes were found in 85 (85%), 77 (77%), (72) 72%, and (68) 68% of *A. baumannii* isolates, respectively. In 2006, a US-based study showed the prevalence rates of *aadA1* and *aadB* genes were 39% and 48%, respectively, for multidrug-resistant *A. baumannii* strains [13]. In 2014, a study from Iran showed *aadA1* and *aadB* genes in 27.9% and 18.6% of *A. baumannii* strains, respectively [4]. Most of the aminoglycoside resistance in *A. baumannii* isolates involves the production of AMEs. Previous studies have shown that genes encoding these enzymes can be present on plasmids, transposons, or within integron-type structures. The increase in the prevalence rate of aminoglycoside-encoding genes during past decades emphasizes the probable importance of these structures in the distribution of aminoglycoside resistance in *A. baumannii* isolates as nosocomial pathogens. In a study from the USA, among 313 Enterobacteriaceae, *aac(6')-Ib* was present in 50.5% of isolates [20]. The *aac(6')-Ib-cr* gene encodes an aminoglycoside AAC(6')-Ib variant marked by Trp102Arg and Asp179Tyr substitutions [15, 21]. These changes afford the new enzyme with the ability to acetylate fluoroquinolones harboring an unsubstituted piperazinyl group, such as CIP and norfloxacin [22]. Therefore, this gene confers the reduction of susceptibility to some fluoroquinolones in addition to tobramycin, kanamycin, and

AK. The results of this study indicated an increase in the prevalence rate of *A. baumannii* strains isolated from a university teaching hospital in Iran. The detection of different resistance genes in this study confirmed a wide distribution of these genes among *A. baumannii* strains. The prevalence rate of drug-resistant *A. baumannii* strains in hospitalized patients implies that care should be taken while identifying these isolates, preventing their infections, and selecting for a correct drug treatment against associated infections. The continuous evaluations on the decrease or increase of antibiotic resistance among drug-resistant *A. baumannii* strains could provide the physicians with new therapeutic choices, so that treatment with ineffectual drugs would be stopped and replaced by effectual antibiotics. This fact leads to a diminish in the circulation of drug-resistant *A. baumannii* isolates among ICU patients. Resistance among *A. baumannii* isolates is continuously increasing, so the best decision on the control of nosocomial infections caused by *A. baumannii* isolates is the use of antibiogram tests in each geographic area.

Conflict of Interest

None.

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