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Method Article

Antimicrobial resistance patterns and their encoding genes among clinical isolates of *Acinetobacter baumannii* in Ahvaz, Southwest Iran

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A B S T R A C T

Acinetobacter baumannii is one of the most important organisms in nosocomial infections. Antibiotic resistance in this bacterium causes many problems in treating patients. This study aimed to investigate antibiotic resistance patterns and resistance-related, genes in clinical isolates of *Acinetobacter baumannii.* This descriptive study was conducted on 124 isolates of *Acinetobacter baumannii* collected from clinical samples in two teaching hospitals in Ahvaz. The antibiotic resistance pattern was determined by disk diffusion. The presence of genes coding for antibiotic resistance was determined using the polymerase chain reaction method. Out of 124 isolates, the highest rate of resistance was observed for rifampin (96.8%). The resistance rate for imipenem, meropenem, colistin, and polymyxin-B were 78.2%, 73.4%, 0.8% and 0.8%, respectively. The distribution of *qnrA, qnrB, qnrS, Tet A, TetB,* and *Sul1*genes were 52.6%, 0%, 3.2%, 93.5% 69.2%, and 6.42%, respectively. High prevalence of *tetA, tetB,* and *qnrA* genes among *Acinetobacter baumannii* isolated strains in this study indicate the important role of these genes in multidrug resistance in this bacteria.

- *Acinetobacter baumannii* is an important human pathogen that has attracted the attention of many researchers Antibiotic resistance in this bacterium causes many problems in treating patients.
- The resistance rate for imipenem, meropenem, colistin, and polymyxin-B were 78.2%, 73.4%, 0.8% and 0.8%, respectively. The distribution of *qnrA, qnrB, qnrS, Tet A, TetB,* and *Sul1*genes were 52.6%, 0%, 3.2%, 93.5% 69.2%, and 6.42%, respectively.

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Specifications table

Subject Area	Biochemistry, Genetics and Molecular Biology
More specific subject area	Microbiology
Method name	Standard biochemical and microbiological tests
Name and reference of original method	Standard biochemical and microbiological tests such as OF, IMVIC, TSI, SIM, MRVP, and catalase. Kirby-Bauer disk diffusion method and PCR was also used.
Resource availability	No Applicable

Introduction

Bacterial nosocomial infections cause many problems in the treatment and mortality of patients, due to their antibiotic resistance. Among these bacteria, *Acinetobacter baumannii* is an important human pathogen that has attracted the attention of many researchers [1]. *A. baumannii* also causes various diseases such as septicemia, ventilator-associated pneumonia, meningitis, urinary tract infections, endocarditis and wound infections [2]. The amount of colonization of *A.baumannii* is increasing in hospitalized patients, especially in patients who have been hospitalized for a long time or have received broad-spectrum antibiotics or anticancer drugs [3]. Today, the spread of antibiotic resistance genes by creating multiple drug resistance (MDR) has become an important problem in the treatment of Acinetobacter infection [4]. Different previous studies have shown that *A. baumannii* is resistant to the majority of antibiotics including fluoroquinolones, cephalosporins, carbapenems, tetracycline, and aminoglycosides [5]. Antimicrobial resistance of *A.baumannii* is mediated by acquired and inherent mechanisms, which include enzymatic changes, a mutation in the target genes, changes in the permeability of the outer membrane, and increased expression of the efflux pumps [6]. Pumping mediation out of the bacteria, due to the mechanisms of the efflux, is one of the reasons for MDR. There are several types of *Tet* genes encoding the efflux pump, which causes resistance to tetracycline and among them, *tetA* and *tetB* genes are the most common [7].

Quinolones are a bunch of antimicrobial compounds that are commonly used to treat infections caused by *A. baumannii.* Several mechanisms make these bacteria resistant to fluoroquinolones. One of these mechanisms is the presence of the *qnr* gene. The plasmid-mediated quinolone resistance (PMQR) genes, such as *qnrA, qnrB, and qnrS*, are responsible for quinolone resistance in *A. baumannii* isolates [8,9]. These *qnr* genes encode proteins of the pentapeptide repeat family that protects DNA from quinolone by binding to DNA gyrase and topoisomerase IV and causes resistance to quinolones [10,11]. Since *A. baumannii* infectious have caused severe complications of treatment for hospitalized patients in Iran and other countries, information on the prevalence of antibiotic resistance genes and the pattern of resistance of these infections is very important. There are not any data available to describe the prevalence of *qnrA, qnrB, qnrS* genes of *A. baumannii* in Ahvaz, southwest Iran, therefore, our study aimed to investigate antibiotic resistance pattern and resistance-related genes such as *qnrA, qnrB, qnrS, Tet A, TetB,* and *Sul1*, in clinical isolates of *A. baumannii* isolated from patients admitted into diverse wards of Golestan and Imam Khomeini hospital in Ahvaz by PCR.

Subjects and methods

Isolation and characterization of bacteria

In this descriptive cross-sectional study, 124 non-duplicate *A. baumannii* isolates were collected from various infections of patients admitted into diverse wards of Golestan and Imam Khomeini

Table 1
The sequence of primers used in the study.

Primers	Primer sequence (5´-3´)	Product size (bp)	Annealing Temp (°C)	Reference
TetA	F-GCT ACA TCC TGC TTG CCT TC			
	R-CAT AGA TCG CCG TGA AGA GG	210	55	19
TetB	F-TTG GTT AGG GGC AAG TTT TG			
	R-GTA ATG GGC CAA TAA CAC CG	659	55	19
qnrA	F-ATTTCTCACGCCAGGATTTG		53	20
	R-GATCGGCAAAGGTTAGGTCA	516		
qnrB	F-GATCGTGAAAGCCAGAAAGG		53	20
	R-ACGATGCCTGGTAGTTGTCC	469		
qnrS	F-ACGACATTCGTCAACT GCAA		53	20
	R-TAAATTGGCACCCTGTAGGC	417		
sul1	R-CGGCGTGGGCTACCTGAACG	432	55	21
	F-GCCGATCGCGTGAAGTTCCG			
$bla_{\text{OXA-51- like}}$	R- TAATGCTTTGATCGGCCTTG	353	57	18
	F- TGGATTGCACTTCATCTTGG			

hospital in Ahvaz, Iran, from July 2011 to January 2013. 124 strains of *A. baumannii* were obtained from different clinical specimens, including tracheal aspirate, cerebrospinal fluid, wound, urine, discharge, blood, pleura, catheter, and eye infections. At first, *A. baumannii* isolates were identified using standard biochemical and microbiological tests such as OF, IMVIC, TSI, SIM, MRVP, and catalase. *A. baumannii* isolates were saved in Tryptic Soy Broth (TSB) (Merck, Germany), containing glycerol (30%) at -70°C [12].

Antibiotic susceptibility testing

The determination of antibiotic susceptibility was tested by the Kirby-Bauer disk diffusion method based on the Clinical and Laboratory Standard Institute (CLSI, 2016) guidelines [13].

The antibiotics tested in this study included tetracycline (30 µg), meropenem (10 µg), amikacin (30 µg), imipenem (10 µg), ceftriaxone (30 µg), piperacillin/tazobactam (100/10 µg), colistin sulfate (10 µg), piperacillin (100 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), cotrimoxazole (25 µg), aztreonam (30 µg), ampicillin-sulbactam (10/10 µg), tigecycline (15 µg), rifampin (5 µg), tobramycin (10 mg), polymyxin B (300 U), and gentamicin (10 µg) (MAST, Group Ltd, Merseyside, UK).

To conduct the test, a suspension of bacterial colonies equivalent to 0.5 McFarland standard was prepared and plated on Muller-Hinton agar medium (Merck, Germany). Then, the media were incubated for 18-24 hours at 37°C. The results were reported according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2016) [13]. *A. baumannii* ATCC 19606 was used as the positive control strain [14]. The US Food and Drug Administration-approved criteria for Enterobacteriaceae used for tigecycline stop point, respectively [15,16].

DNA extraction

DNA extraction of *A. baumannii* was performed by the boiling method [17].

PCR amplification of tetracycline, sulfonamide, and quinolone resistance genes

The specific primers (Table 1) for the study was confirmed by BLAST, and *Sul1* (sulfonamide resistance), *qnrA, qnrB, qnrS* (quinolone resistance), *tetA* and *tetB* (tetracycline resistance) genes (Bioneer Korea) were detected by PCR.

Detection of bla*OXA-51-like*

To confirm the identity of *A. baumannii, bla*OXA-51-like gene was examined by PCR using specific primers listed in Table 1 [18].To amplify this gene, each reaction was carried out in a final volume of

25 µl containing 10 µl Mastermix (Ampliqon, Denmark), 0.5 µl of each primer (10 pM), 5 µl DNA template. The amplification reaction was programmed by thermal cycler (Eppendorf, Germany) as follows: Initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 45 s, annealing 57°Cfor 45 s, extension 72°C for 1min and final extension 72°C for 5 min. The PCR products were separated on 1.5% agarose gel containing ethidium bromide and finally visualized in the gel documentation system. *A. baumannii* NCTC 12156 (ATCC 19606) was used as a positive control [18].

PCR amplification of Tet *genes*

The sequences of primers used for the detection of *tetA* and *tetB* are shown in Table 1. The reaction volume was set to 25 µl containing 10 µl Mastermix (Ampliqon, Denmark), 1 µl Primer forward (10 pM), 1 µl Primer reverse (10 pM), 5 µl DNA template. Amplification of DNA was performed in a thermal cycler (Eppendorf, Germany) with 5 min of initial denaturation at 95°C, followed by 35 cycles, including denaturation at 95°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 1min, and a final extension at 72°C for 10 min [19]. The PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide and finally visualized in the gel documentation system. *Shigella sonnei* ATCC 9290 was used as a positive control strain.

PCR for the screening of qnr *and* sul1 *genes*

The specific primers of *Sul1, qnrA, qnrB, qnrS* are shown in Table 1. PCR amplification was performed using 10 µl Mastermix (Ampliqon, Denmark), 0.5 µl from each reverse and forward primers (10 pM), 5 µl DNA template. The final volume for each reaction was 25 µL. The amplification reaction was carried out by thermal cycler (Eppendorf, Germany) with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing for 45 s at primer set specific temperatures in Table 1, and extension at 72 C for 1 min, followed by a final extension at 72 C for 10 min [20,21]. PCR products were electrophoresed on ethidium bromide containing 1.5% of agarose gel and finally visualized in the gel documentation system.

The confirmed *Klebsiella pneumoniae* strain containing the *qnr* gene was used as a positive control for *qnr* genes and *E. coli* DH5a was used as a positive control for the *sul1* gene. Also, the strain of *Escherichia coli* ATCC25922 was used as a negative control strain in this study.

Statistical analysis

The results were analyzed using SPSS version 16 to obtain frequencies and comparisons among clones. A nonparametric chi-square test was used. A P value < 0.05 was considered statistically significant.

Results

124 *A. baumannii* isolates were collected. The rate of isolates from each ward and specimen are shown in Table 2. As shown in Table 2, of 124 isolates of *A. baumannii* isolated from the type of clinical specimens, the highest infection was related to the Tracheal aspirate with 57.3% and the lowest level of infection was related to eye infection with 0.8%.

Antimicrobial susceptibility test

Antibiotic susceptibility test results by the disk diffusion method are shown in Table 3.

Among 124 isolates, one isolate (0.8%) was resistant to colistin and 123 isolates (98.2%) were susceptible to this antibiotic.

Table 2
The rate of *Acinetobacter baumannii* species isolated from each ward and specimen.

Ward	Rate of isolates	Specimen	Rate of isolates
ICU	74.2	Tracheal aspirate	57.3
Outpatients	8.1	Cerebrospinal fluid	11.3
Neurosurgery	4	Wound	10.5
Dermatology	4	Urine	8.1
Orthopaedic	2.4	Blood	3.2
Gynecology and Obstetrics	2.4	Pleura	1.6
Surgery	0.8	Catheter	1.6
Neonatal	0.8	Eye infection	0.8

Table 3
The results of antibiogram test for *A.baumannii* isolates.

Antibiotic	Sensitive	Intermediate	Resistant
Imipenem	24.2	1.6	74.2
Meropenem	19.4	0.8	79.8
Ceftazidime	15.3	2.4	82.3
Cefepime	16.1	4	79.8
Ceftriaxone	1.6	12.1	86.3
Colistin	98.2	0	0.8
Piperacillin	12.1	3.2	84.7
Piperacillin-tazobactam	16.9	1.6	81.5
Polymyxin-B	99.2	0	0.8
Gentamicin	28.2	4.8	66.9
Tobramycin	33.9	1.6	64.5
Amikacin	21	12.1	66.9
Tetracycline	21.8	12.1	66.1
Ampicillin-sulbactam	32.3	21.8	46
Ciprofloxacin	13.7	1.6	84.7
Cotrimoxazole	19.4	4.8	75.8
Rifampin	0	3.2	96.8
Aztreonam	0	4.8	95.2
Tigecycline (FDA)	6.5	58.1	35.5
Tigecycline (Jones)	45.2	50.8	4

Determination of frequency of antibiotic-resistant genes

The percentage of *qnrA, qnrB,and qnrS* genes in *A. baumannii* strains were (52.6%), (0%), and (3.2%), respectively. According to PCR results, in the isolates of *A. baumannii, TetA* and *TetB* genes were detected in 93.5% and 69.2% of strains, respectively. PCR results showed that 6.42% of *A. baumannii* species were carried *sul1* gene.

Discussion

Various studies have shown that *A. baumannii* strains are resistant to most antibiotics, and these multi-drug-resistant strains are rapidly expanding among hospitalized patients [4]. These resistances are often mediated by genes that are located on moving genetic elements such as transposons and integrons and are simply distributed among bacteria [22]. In our study, most isolates of *A. baumannii* (74.2%) were isolated from patients in ICU. Other studies worldwide have shown that the rate of infection with *A. baumannii* in ICU patients is high [23,24]. Also, *A. baumannii* can cause infections such as ventilator-associated pneumonia (VAP), particularly in ICU patients. Previously, it has been reported that *A. baumannii* is more common in tracheal aspirate specimens [25,26].In comparison with other studies, most of the *A. baumannii* isolates (57.3%) were obtained from tracheal aspirates.

In the present research, antimicrobial susceptibility pattern showed that polymyxin-B and colistin had the most effect on *A. baumannii* isolates. Colistin is the latest antibiotic for the treatment of multi-

drug resistant *A. baumannii* infections [27,28]. However, clostin-resistant isolates have been reported worldwide. In this study, only 0.8% of the isolates were resistant to this antimicrobial agent. Fallah et al. (2014) reported that the resistance rate of the *A. baumannii* strains to colistin was (1.8%) [29].

The results of this study, according to other studies, showed an increase in resistance to antibiotics β-lactam, ciprofloxacin, cotrimoxazole, Piperacillin, and tetracycline by more than 50%. The results of this research consistent with the previous reports [30-32].

The level of resistance of *A. bumanni* isolates to ciprofloxacin is important because the clinical application of ciprofloxacin is better than carbapenems [33]. One of the main goals of this study was to determine the distribution of antibiotic resistance genes. The *tetA* and *tetB* genes encoding the efflux pump, and they are factors of resistance to tetracycline and minocycline. There was a significant relationship between the presence of these genes, *tetA* (93.5%) and *tet*B (69.2%), and tetracycline resistance in this study. In a study conducted by Asadollahi et al, the prevalence of *tetA* and *tetB* genes, (95.5%) and (65%) was reported [34]. These results were consistent with our findings. But, our results were highest than the Previous study in Ahvaz [30]. Increasing the frequency of *tetA* and *tetB* genes in this research had shown that resistance to *A. baumannii* is increasing, due to the increasing use of tetracycline.

PMQR genes are responsible for resistance to quinolone in *A. baumannii*. The prevalence of quinolone resistance in *A. baumannii* has increased in recent years and has complicated the treatment of these infections [35]. The *qnrA* appears predominant *qnr* gene identified in our study. The prevalence of *qnrB* gene was an agreement with the study carried out by Mirnejad et al [36]. The prevalence of *qnrS* gene in our study was similar to another study in Tehran [37]. Also, due to the excessive consumption of quinolones and fluoroquinolones, the emergence of resistant strains has caused many problems in treating and creating a transferable resistance among bacteria.

In recent years, the use of sulfonamide and trimethoprim for the treatment of infections is increasing in most countries, and excessive use of these drugs has led to resistance and unsuccessful treatment [38]. In this study, we showed that 6.42% of *A. baumannii* isolates had the *sul1* gene. Our results are not consistent with previous studies [30,39,40]. The most reason for this contrast with other studies may be due to differences in clinical specimens, the number of samples, sampling method, type of study, geographical area, and the availability of various antibiotics.

This study showed that the most effective antibiotic against clinical strains of *A. baumannii* was colistin and we recommend clinicians to use this agent in patients infected with MDR *A. baumannii*. The results of this study indicated that *tet A, tet B,* and *qnrA* genes are the most important resistant factors to tetracycline and ciprofloxacin in *A. baumannii* isolates in our region. Due to the high prevalence of these genes, physicians should be careful in prescribing fluoroquinolones and tetracyclines antibiotics in the treatment of infections associated with this bacterium.

Limitations

In this study, the results had certain limitations. The major limitations were the short period of our study, sample size, and not examining more genes of antibiotic resistance.

Declaration of Competing Interest

The authors declare no conflict of interest.

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