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Antimicrobial Resistance Patterns and Frequency of Extended-Spectrum Beta-Lactamase Genes among Acinetobacter Baumannii

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ABSTRACT

BACKGROUND AND OBJECTIVE: Acinetobacter baumannii is an opportunistic pathogen that is resistant to many antibiotics including beta-lactams. Production of β-lactamases is the main mechanism of β-lactam resistance in A. baumannii. The aim of the present study was to determine the antimicrobial susceptibility profile and frequency of ESBL genes in clinical isolates of A. baumannii in hospitals in Kerman, Iran.

METHODS: In this cross-sectional study 102 isolates of Acinetobacter species were collected from clinical specimens, including, respiratory secretions, urine, blood culture and body fluid. For confirming A. baumannii Polymerase chain reaction (PCR) was used to identify blaOXA-51 gene. Antimicrobial susceptibility test was performed using the disk diffusion method. PCR technique was carried out for the detection of blaCTX-M, blaSHV, blaTEM, blaPER and blaVEB genes.

FINDINGS: blaOXA-51 gene was detected in 95 (93/1%) isolates. All of the isolates were resistant to Cefepime and Cefotaxime. Almost all of them were resistant to Imipenem, Meropenem, Gentamicin, Amikacin, Aztreonam, Ciprofloxacin, Ceftazidim, Levofloxacin, Tetracycline and Piperacillin-Tazobactam. The rates of susceptibility to Polymyxin-B and Tigecycline were 61/1% and 23/2%, respectively. The frequency of, blaSHV, blaTEM, blaCTX-M and blaVEB genes were 44 (46.3%), 30 (31.6%), 30 (31.6%), and 13 (13.7%), respectively. None of the isolates were carried blaPER gene.

CONCLUSION: Because of the high rate of ESBLs producing A. baumannii isolates detected in this study, effective infection control strategy should be performed.

KEY WORDS: *Acinetbacter baumanii, Antimicrobial resistance, Extended-spectrum β-lactamases.*

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Introduction

Acinetobacter baumannii is one of the most important pathogens in health centers that causes many infections including bacteremia, pneumonia, meningitis, urinary tract infections and ulcers. The ability to survive in a wide range of environmental conditions has transformed this pathogen into one of the most common causes of infection in health centers (1). This bacterium is the third pathogen isolated from patients with pneumonia admitted to the hospital and is mainly separated from those admitted to the intensive care unit (2). During the last decade, the isolates of Acinetobacter baumannii resistant to multi-drug (MDR) have increased in the world, especially in Asian countries. MDR strains are resistant to more than three antibiotic classes (3). A class of antibiotics that is important in treating infections caused by Acinetobacter baumannii is antibiotics of the β-lactam family. The bacterium is resistant to beta-lactam antibiotics in several ways, which includes the production of beta-lactamase enzymes, changes in outer membrane proteins, production of penicillin-binding proteins, and increasing activity of the efflux pump. The most important mechanism of resistance to broad-spectrum cephalosporin is via β-lactamase enzymes (4). The most important mechanism of resistance to broad-spectrum cephalosporin is through β-lactamase enzymes (4). According to Ambler, β-lactamases are classified into four classes A, B, C, and D (5). In class A, a large number of β-lactamase enzymes that have resistance to broad-spectrum cephalosporin have been identified in Acinetobacter species. Most of these ESBL genes are blaVEB or blaPER. However, the blaTEM, blaSHV and blaCTX-M genes have also been seen in these species (6).

The presence of beta-lactams that can affect a wide range of antibiotics in the β-lactam family has been associated with many challenges in treating by antibiotics in this family. On the other hand, ESBLs produced by isolates are also resistant to antibiotics of other classes, such as Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol, and Trimethoprim-Sulfamethoxazole (7).

In a study by Alyamani et al., Which was performed on Acinetobacter baumannii isolates, 81% of isolates had blaCTX-M gene and 71% had blaTEM gene. None of the isolates had blaSHV gene (8). In another study by Fallah et al., 78% of Acinetobacter baumannii isolates with blaPER gene and 39.5% had blaVEB gene (9). The results of a study by Safari et al. showed that blaSHV with 58% had the highest prevalence among Acinetobacter baumannii isolates, and then the blaTEM gene was 20%. None of the isolates had blaCTX-M gene (10). Because of the presence of MDR strains, the treatment of Acinetobacter baumannii strains is associated with unconventional antibiotics such as Polymyxins, Rifampin, and Tetracyclines, especially for resistant Carbapenem isolates (11). Polymyxins have severe side effects, but due to their proper effect on Acinetobacter baumannii isolates are used to treat severe infections caused by MDR strains (12).

Tigecycline is a very good anti-Acinetobacter baumannii strain, and resistance to it is rare. Major isolates resistant to Tetracycline are sensitive to Tigecycline. Most Carbapenem -resistant Acinetobacter isolates are sensitive to Polymyxins and Tigecycline, which is why resistance to these antibiotics is importance (11).

In recent years resistance to antibiotics has increased in Acinetobacter baumannii isolates, which has led to failure in the treatment process. Transmission and release of organisms that are capable of producing enzymes involved in antibiotic resistance, such as broad-spectrum beta-lactamases, have increased the number of hospital infections. As a result, this study was conducted to investigate the antibiotic resistance pattern and frequency of beta-lactamase genes blaPER, blaVEB, blaSHV, blaTEM and blaCTX-M.

Methods

Isolation and identification of bacteria: In this cross-sectional study, 102 isolates of Acinetobacter were collected from hospitals in Kerman from September 2015 to April 2016. Bacterial isolates were from respiratory tract (82 isolates), wound (8 isolates), burn (4 isolates), urine (8 isolates) and body fluids (2 isolates) from patients admitted to ICU, surgery ward, infections ward, orthopedics ward and burns ward. These isolates were transferred to the Microbiology Laboratory of Kerman University of Medical Sciences. Acinetobacter bacterium was confirmed according to standard diagnostic and biochemical methods such as oxidase test, TSI, SIM, Simon citrate, urea and Lysine decarboxylase (13). To detect the Acinetobacter Bumannii species, PCR was used for the blaOXA-51 gene.

Antibiotic resistance test: Antibiotic resistance pattern of isolated bacteria was done by diffusion

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method and Clinical and laboratory standard institute (CLSI) (14). Discs used include: Imipenem (µg10), Meropenem (10µg), Ceftazidime (30µg), Cefotaxime (30µg), Piperacillin/Tazobactam (10µg/µg), Amikacin (30µg), Gentamicin (10µg), Cefepime (30µg) Tetracycline (30µg), Tigecycline (15µg), Aztreonam (30µg), Polymyxin B (300µg), Levofloxacin (10µg), and Ciprofloxacin (5µg) were purchased from Mast UK. The strain of E. coli ATCC 25922 was used as control for antibiogram.

Phenotypic identification of broad-spectrum beta-lactamases (ESBLs): A phenotypic confirmatory test was used to identify isolates producing ESBLs. Cefotaxime/clavulanic acid combination disks compared to Cefotaxime, Ceftazidime / clavulanic acid compared to Ceftazidime and Cefepime/clavulanic acid were used in comparison with Cefepime. After incubation at 37°C for 24 hours, ESBLs producing isolates had an inhibition zone diameter of 5 mm or more around the Ceftazidime/clavulanic acid disks as compared to Ceftazidime or Cefotaxime/clavulanic acid in comparison with Cefotaxime or Cefepime/Clavulanic acid compared to Cefepime (15). Klebsiella pneumoniae strain ATCC 700603 was used as positive control for ESBLs testing.

PCR to detect blaVEB, blaPER, blaTEM, blaSHV and blaCTX-M genes: All isolates resistant to broad-spectrum cephalosporin were investigated for ESBL producing genes. Initially, these isolates were isolated using the DNA boiling method (16).

The Mastermix Kit (Amplicon Corporation of Denmark) was used, for PCR. The primer sequence used and the size of the product bands on the electrophoresis gel are based on the reference (table 1) (15, 17).

Table 1. Primer sequence, annealing temperature and PCR product size on agarose gel (15-17)

Band size(bp)	Annealing temperature	Primer sequence	Gene
550	59	F- CGCTTTGCGATGTGCAG R- ACCGCGATATCGTTGGT	CTX-M
861	59	F-GAGTATTCAACATTTCCGTGTC R-TAATCAGTGAGGCACCTATCTC	TEM
471	56	F- TCAGCGAAAAACACCTTG R- TCCCGCAGATAAATCACC	SHV
933	55	F- AATTTGGGCTTAGGGCAGAA R- ATGAATGTCATTATAAAAGC	PER
642	55	F- CGACTTCCATTTCCCGATGC R-GGACTCTGCAACAAATACGC	VEB

For PCR, the final volume was 20µl containing, 10µL of master mixes, 2µl of bacterial DNA, 10picomole of primer and sterile distilled water. After performing PCR, the PCR products were electrophoresed in a 1% agarose gel in TBE buffer for 60 minutes at 100 volts, and then the results were observed by the Gel document machine.

Results

Of the 102 Acinetobacter isolates, 95 isolates (93.1%) were recognized as Acinetobacter baumannii. Based on the results of the antibiogram, all isolates were resistant to Ceftazidime and Cefepime. Resistance to antibiotics such as Imipenem, Meropenem, Ceftazidime, Piperacillin/Tazobactam, Amikacin, Gentamicin, Tetracycline, Aztreonam, Levofloxacin and Ciprofloxacin were very high (table 2).

Table 2. Resistance percentage of Acinetobacter baumannii isolate to different antibiotics

Antibiotics	Sensitive N(%)	Semi sensitive N(%)	Resistant N(%)
Cefotaxime	0(0)	0(0)	95(100)
Ceftazidime	1(1.1)	0(0)	94(98.9)
Cefepime	0(0)	0(0)	95(100)
Aztreonam	191.1)	0(0)	94(98.9)
Imipenem	2(2.1)	0(0)	93(97.9)
Meropenem	2(2.1)	0(0)	93(97.9)
Gentamicin	0(0)	3(3.2)	92(96.8)
Amikacin	0(0)	1(1.1)	94(98.9)
Ciprofloxacin	2(2.1)	0(0)	93(97.9)
Levofloxacin	4(4.2)	20(21.1)	71(74.7)
Tetracycline	3(3.2)	6(6.3)	86(90.5)
Tigecycline	22(23.2)	39(41.1)	34(35.8)
Piperacillin/Tazobactam	1(1.1)	1(1.1)	93(97.9)
Polymyxin B	58(61.1)	0(0)	37(38.9)

The most effective antibiotics against the Acinetobacter baumannii isolates was Polymyxin B, 61.1% of isolates were sensitive to this antibiotic. Of 95 isolates of Acinetobacter Bumannii, 94 isolates (98.9%) were resistant to more than three antibiotic classes and were known as MDR. The results of the combined disk test showed that only three isolates (1.3%) phenotypically produce ESBL. However, after performing PCR for resistant to broad-spectrum cephalosporin isolates, which included all isolates, it was found that 73.7% of isolates had at least one of the ESBL producing genes. Images of the gel

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electrophoresis of the products of these genes are visible in Figures (1-5). The highest ESBL gene was blaSHV, of which 44 isolates (46.3%) had this gene. After that, blaTEM and blaCTX-M genes were observed in 30 isolates (31.6%).While 13 isolates (13.7%) contained the blaVEB gene, there were no isolates containing the blaPER gene.

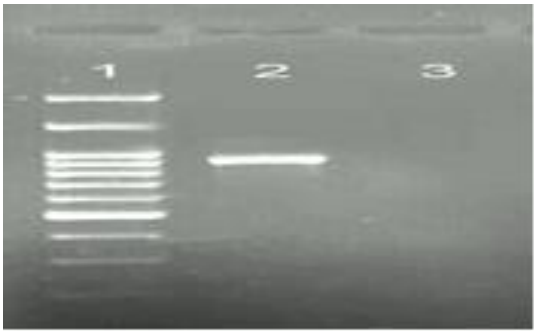


Figure 1. Image of gel electrophoresis of PER gene PCR of Acinetobacter baumannii isolates. Column 1 is the size marker of 100bp, column 2 positive control, column 3 negative control

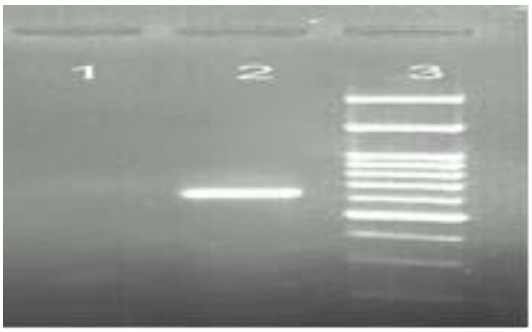


Figure 2. Image of gel electrophoresis of VEB gene PCR of Acinetobacter baumannii isolates. Column 1 is negative control, column 2 positive control, column 3 the size marker of 100bp

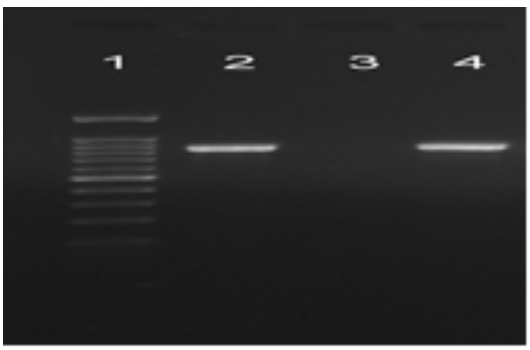


Figure 3. Image of gel electrophoresis of TEM gene PCR of Acinetobacter baumannii isolates. Column 1 is the size marker of 100bp, column 2 positive control, column 3 negative control, Column 4 positive isolate

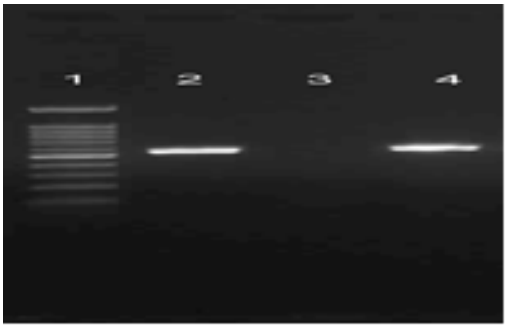


Figure 4. Image of gel electrophoresis of CTX-M gene PCR of Acinetobacter baumannii isolates. Column 1 is the size marker of 100bp, column 2 positive control, column 3 negative control, Column 4 positive isolate

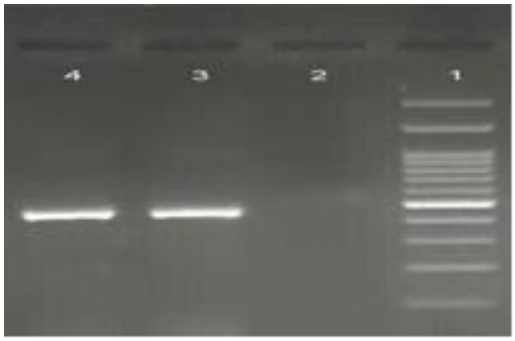


Figure 5. Image of gel electrophoresis of SHV gene PCR of Acinetobacter baumannii isolates. Column 1 is the size marker of 100bp, column 2 positive control, column 3 negative control, Column 4 positive isolate

Discussion

In the present study, from 102 isolates of Acinetobacter 95 isolates (93%) had blaOXA-51 genes and were therefore recognized as Acinetobacter Bumannii. Because of the importance of Acinetobacter Bumannii, its recognition from other members of the genus is valuable. Because blaOXA-51 is fixedly unique in Acinetobacter Bumanni, this gene can be used to detect Aynetobacter Bumannii isolates, which, in addition to simplicity, has a higher accuracy than biochemical tests (11).

In a study conducted in Turkey based on the blaOXA-51 gene, 77.8% of Acinetobacter isolates were found to have blaOXA-51 genes and were recognized as Acinetobacter Bumannii (18). In another study in England, all 144 strains of Acinetobacter Bumannii had the blaOXA-51 gene, while 22 strains of Acinetobacter non- Bumannii lacked this gene (19). Antibiotic resistance in Acinetobacter Bumannii has become a global problem. The appearance of MDR isolates of Acinetobacter Bumannii has caused numerous problems in the treatment of these isolates.

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The MDR Acinetobacter Bumannii isolates are reported rapidly from around the world. In this study 98.9% of isolates were known as MDR. In a large number of previous studies, MDR strains have a high percentage of Acinetobacter Bumannii isolated from clinical specimens (20-22).

Until recently, carbapenems were considered as the best option for the treatment of Acetobacter Bumannii MDR isolates. But these antibiotics do not seem to be effective in treating the infections caused by these isolates. Resistance to Imipenem and Meropenem was high in this study, 97.9% of isolates were resistant to these antibiotics. This result is similar to the results obtained from many studies (1,20,23). In a number of previous reports, resistance to Amikacin and Levofloxacin has been reported at a lower level than the present study (2,11).

However, in other studies such as this study, a high percentage of Acinetobacter Bumannii isolates were resistant to these antibiotics (24, 25). Based on this study, in addition to Carbapenems, Aminoglycosides and Fluoroquinolones also have no other efficacy for the treatment of Acinetobacter Bumannii infection. Although the resistance to Tigecycline was lower than most antibiotics, however, due to the importance of this antibiotic in the treatment of MDR strains of Acinetobacter Bumannii, the isolates resistance of these antibiotics is also higher than other studies (11, 21, 24). Resistance to Polymyxin B was also high in Acinetobacter Bumann isolates, so that 38.9% of isolates were resistant to this antibiotic. Polymyxins are the last line of treatment for MDR isolates of Acinetobacter Bumannii, It is very difficult to treat Acinetobacter Bumannii isolates resistant to these antibiotics (26).

Although resistance to Polymyxins in Asian and European countries is higher than in the United States and Latin American countries, the level of resistance in Iran is still alarming (26). In our study, all isolates were resistant to broad-spectrum cephalosporin. This data, according to the results obtained in other studies, was not unexpected (4,21,24,27). In our study, only three isolates of Acinetobacter baumannii were based on production ESBL phenotypic test. In many other studies, the prevalence of ESBL-induced actinobacter isolates has been reported (10,28,29). But in a study by Singla et al., it was shown that ESBL producing isolates are better detected if sulbactam is used instead of clavulanic acid. The reason for this is that clavulanic acid, unlike sulbactam, induces AmpC production, and AmpC causes hydrolysis of beta-lactam antibiotics. Consequently, despite ESBL, the result is false negative (30). In the present study, the blaSHV, blaTEM and blaCTX-M genes were more prevalent. In various studies, the prevalence of ESBL genes is different in Acinetobacter baumannii isolates. In a number of studies, blaPER and blaVEB genes have been identified as the most important ESBL genes in Acinetobacter baumannii isolates (6).

However, this difference in the prevalence of genes may be due to the difference in ecological status, the difference in the antibiotic treatment program, and the difference in the antibiotic resistance pattern in different locations (31). The results of this study showed that blaSHV is the most common ESBL gene in Acinetobacter baumannii isolates in Kerman. This was similar to that of Safari et al. that blaSHV was identified as the most common ESBL with 58%. In this study, blaTEM was found in 20% of isolates, which is largely similar to our study, but unlike our study, the blaCTX-M gene was not seen in any of the isolates (10). In another study by Al-Agamy in Egypt, contrary to the current study, the blaTEM gene was found in 87.5% and the blaPER gene was found in 55% of Acinetobacter baumannii isolates. Also, these isolates were negative for blaSHV and blaCTX-M genes (4). In the present study, blaPER gene was not observed in any of the isolates, this is unlike most studies, that investigated blaPER gene is one of the most common ESBL genes in Acinetobacter baumannii isolates (32, 33). This study showed that Acinetobacter baumannii isolates have a high resistance to various antibiotics and ESBLs play an important role in the development of resistance. It seems that the adoption of a new therapeutic approach and the implementation of appropriate strategies to prevent the spread of infection by MDR isolates of Acinetobacter baumannii are necessary.

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