Medical Laboratory Journal, Jan-Feb , 2018; Vol 12: No 1

Original Article

Antibiotic Resistance Patterns and Prevalence of *PER* and *VEB* Resistance Genes among Clinical Isolates of ESBL-Producing *Acinetobacter Baumannii*

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Received : 24 Jun 2017
Revised: 10 Nov 2017
Accepted: 20 Nov 2017

ABSTRACT

Background and Objectives: *Acinetobacter* species are responsible for a wide range of clinical complications in hospitalized patients. Antimicrobial treatment of clinical strains of *Acinetobacter baumannii* may be compromised due to multiple-drug resistance to β-lactams. Aim of this study was to determine antibiotic resistance patterns and frequency of *PER* and *VEB* genes in *A. baumannii* isolates from hospitalized patients.

Methods: In this cross-sectional study, 100 clinical strains of *A. baumannii* were isolated from patients hospitalized in Qom (Iran) using specific culture media and biochemical tests. The disk diffusion method was performed to determine resistance to some antibiotics. Minimum inhibitory concentration (MIC) for cefepime and ceftazidime was evaluated. Identification of ESBL-producing strains and presence of the *PER* and *VEB* genes were determined by combined disk test and polymerase chain reaction, respectively.

Results: The isolates were highly resistant against cefixime, ceftriaxone and cefepime. Lowest level of resistance was against polymyxin B. In addition, 70% of the isolates were multi-drug resistant. MIC<128 µg/ml to ceftazidime and cefepime was observed in 84% and 91% of the strains, respectively. Moreover, 21% of the strains were ESBL-positive and frequency of the *PER* and *VEB* genes was 47% and 32%, respectively.

Conclusion: Majority of *A. baumannii* isolates are highly resistant to the tested antibiotics. Due to presence of the *PER* and *VEB* genes in the isolated strains, there is the possibility of resistance spread to other bacteria. Therefore, it is recommended to modify the consumption pattern for antibiotics and pay more attention to standards of nosocomial infection control.

Keywords: *Acinetobacter baumannii*, Drug resistance, PER, VEB.

This paper should be cited as: Vahidi Emami H, Khalilian M, Yadollahi Movahhed N[Antibiotic Resistance Patterns and Prevalence of *PER* and *VEB* Resistance Genes among Clinical Isolates of ESBL-Producing *Acinetobacter Baumannii*]. mljgoums. 2018; 12(1):21-26

INTRODUCTION

Acinetobacter is a genus of Gram-negative, aerobic and non-fermentative cocobacilli. These bacteria are important opportunistic pathogens responsible for nosocomial infections. *Acinetobacter baumannii* is one of the main causes of hospital infections (1, 2). Infections caused by this bacterium could be life threatening, particularly in patients who are hospitalized in the intensive care unit of hospitals such as patients with cystic fibrosis, neutropenia, immunodeficiency (3). *A. baumannii* infections in hospitals frequently affects respiratory tract and can cause urinary tract infection, wound infection and septicemia (4).

A. baumannii colonization is increasing in hospitalized patients, especially in long-term hospitalized patients or those receiving extensive antimicrobial treatment or anti-cancer therapy (3, 5). In recent years, the prevalence of bacterial resistance to common antibiotics has increased worldwide. Antibiotic resistance is considered as a major problem in the treatment and control of infections (6). In addition, numerous studies have reported that the high rate of antibiotic resistance and subsequent increase in prevalence of hospital infections are due to indiscriminate use of antibiotics (7).

The high resistance of *A. baumannii* against antimicrobial agents may be intrinsic or due to genetic exchange of resistance factors. Majority of *A. baumannii* strains are resistant to ampicillin, amoxicillin-clavulanic acid, antistaphylococcal penicillin, expanded-spectrum cephalosporins (except ceftazidime and cefepime), tetracycline, macrolides, rifampin and chloramphenicol. The resistance to non-carbapenem β-lactamases is widely accompanied by overproduction of cephalosporinases. The resistance to antimicrobial agents among clinical isolates may complicate the infection treatment process and have detrimental effects on disease outcomes and healthcare costs (8, 9).

Different types of β-lactamases have been identified based on their characteristics and activities (10). Extended-spectrum beta-lactamases (ESBLs) are a group of enzymes that are able to hydrolyze β-lactam antibiotics, including penicillin, expanded-spectrum cephalosporins, fourth-generation cephalosporins (cefepime) and aztreonam (11). Most enzymes initially identified in 1980 were SHV and TEM. In addition to the main families, new families of ESBLs including PER, VEB, BEL, TLA, GES and BES have emerged globally (12). β-lactamase production is known as the most important cause of resistance to β-lactam antibiotics (10). In fact, the rapid transmission and dissemination of these enzymes have increased the prevalence of nosocomial infections. This study aimed at phenotypic investigation of ESBLs and molecular identification of *PER* and *VEB* resistance genes in *A. baumannii* isolates.

MATERIAL AND METHODS

In this study, 100 clinical samples from blood, urine, skin, wound, tracheal secretion and the respiratory tract were collected from hospitals in Qom province (Iran) between 2014 and 2015. MacConkey agar and Blood agar (Merck, Germany) were used for the primary isolation of bacteria. Bacteria were identified based on morphology (colony shape and Gram-reaction) and conventional biochemical tests including TSI, urease, SIM, MR/VP, OF, oxidase, catalase and etc. (Merck, Germany) (13).

Antimicrobial susceptibility test was done on Mueller–Hinton agar (Merck, Germany) using the disc-diffusion method, according to the manufacturer's instructions and Clinical and Laboratory Standards Institute (CLSI) guidelines. Eighteen antibiotics discs (MAST Diagnostic Co., UK) were used in the study including ampicillin-sulbactam (AS_{20}), ceftriaxone (CRO_{30}), amikacin (AK_{30}), imipenem (IPM_{10}), cefepime (FEP_{30}), gentamicin (GM_{10}), meropenem (MEM_{10}), Co-trimixazole (BA_{25}), ciprofloxacin (CIP_5), levofloxacin (LVX_5), colisitin (CO_{10}), cefpodoxime (CPD_{10}), ceftizoxime (CL_{30}), cefixime (CFM_5), ticarcilin (TC_{75}), aztreonam (ATM_{30}), Ceftazidime (CAZ_{30}) and polymyxin B (PB_{300}). Standard strains of *E. coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606 were used as negative control and positive control, respectively (14). Minimal inhibitory concentrations (MICs) for cefepime and ceftazidime were determined using the serial dilution method according to CLSI guidelines (15). Combined disk

test (CDT) was performed on Mueller–Hinton agar plates (Merck, Germany) to evaluate production of ESBLs. Isolated strains were screened for susceptibility to a panel of four antibiotic discs viz: ceftazidime, cefotaxime, ceftazidime + clavulanic acid and cefotaxime + clavulanic acid. After 24 hours of incubation at 37 °C, growth inhibition zone around the disk containing a combination of ceftazidime/cefotaxime and clavulanic acid was compared with the zone around the disk containing ceftazidime/cefotaxime alone. Inhibition zone diameter of > 5 mm or 50% (according to the manufacturer's guidelines) indicated ESBL production (15, 16).

Genomic DNA of the isolates was extracted by boiling method. The *PER* and *VEB* ESBL genes were amplified using universal primers (CinnaGene, Iran) (Table 1) (17).

The PCR reaction was performed in a final volume of 25 µL containing 9 µL Master Mix (2x) (Amplicon III of Denmark), 15-20 ng/µL of DNA template, 10 pmol of each primer and double-distilled water to volume the solution. Thermal programs used for amplification of the *PER* gene was as follows: initial denaturation at 94 °C for 4 minutes, denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute and final extension at 72 °C for 5 minutes. Thermal program for amplification of the *VEB* gene was as follows: initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, extension at 72 °C for 1 minute and final extension at 72 °C for 5 minutes. The PCR products were subjected to direct double-stranded DNA sequencing (PCR Thermocycler, America).

A 100 bp DNA ladder (PR901644, CinnaGene, Iran) was used as molecular marker for size of the amplicons. The amplified bands were visualized and photographed under UV ligh.

Table 1- The sequence of primers, annealing temperatures and expected PCR product sizes

Primer	Primer sequences	Annealing temperature	Product size (bp)
VEB-F	5'-GAAACAACTTTGACGATTGA-3'	52 °C	370
VEB-R	5'-CCCTGTTTTATGAGCAACAA-3'		
PER-F	5'-ATGAATGTCATTATAAAAGC-3'	48 °C	925
PER-R	5'-AATTTGGGCTTAGGGCAGAA-3'		

RESULTS

Among the 100 clinical samples, 50 isolates were identified as *Acinetobacter* species. In addition, 36 samples (72%) were identified as *A. baumannii*, nine samples (18%) as *A. lwoffii* and five samples (10%) as other *Acinetobacter* species. All *A. baumannii* strains were resistant to cefixime, ceftriaxone and cefepime. The lowest rate of resistance was observed against polymyxin B (11%).

Moreover, 18% of *A. baumannii* isolates were only resistant to one antibiotic. Furthermore, 12% of the *A. baumannii* isolates were resistant to two antibiotics, while 70% of the isolates were resistant to three or more (Table 2). The MIC against ceftazidime and cefepime was <128 µg/ml in 84% and 91% of strains, respectively. According to the results of CDT, 21% of the strains produced ESBL (Figure 1).

Table 2- Antibiotic resistance patterns of *A. baumannii* isolates

Antibiotic	abbreviation	Resistance (%)	Antibiotic	Abbreviation	Resistance (%)
Cefpodoxime	CPD	98	Co-Trimixazole	BA	75
Ceftizoxime	CL	92	Gentamicin	GM	78
Cefixime	CFM	100	Ciprofloxacin	CIP	80
Ticarcilin	TC	85	Levofloxacin	LVX	84
Aztreonam	ATM	95	Meropenem	MEM	91
Ceftriaxone	CRO	100	Imipenem	IPM	94
Ceftazidime	CAZ	97	Ampicillin-sulbactam	AS	45
Amikacin	AK	92	Colisitin	CO	16
Cefepime	FEP	100	Polymyxin B	PB	11

Results of PCR showed that 32% and 47% of the isolates contained the *VEB* and *PER* resistance genes, respectively (Figures 2 and 3). Results of sequencing for *VEB* and *PER* were reported as Veb:kx349205 and Per:kx349204, respectively.

Figure 1- Inhibition zone diameter of *A. baumannii* isolates in the CDT method

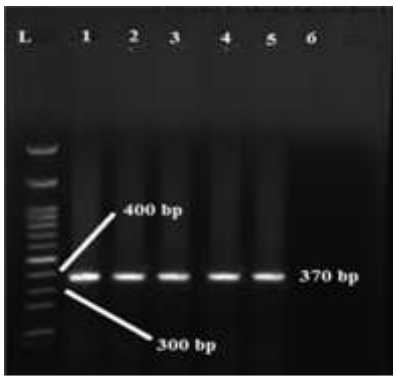
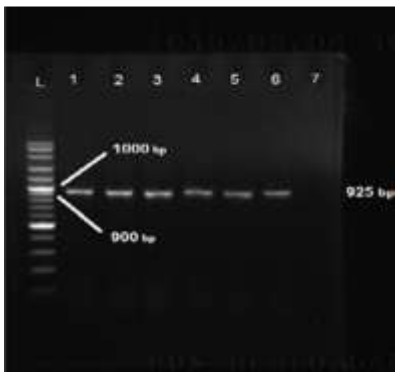


Figure 2- PCR amplification of the *bla_VEB* gene. Column L: 100 bp marker; column 1: positive control;



DISCUSSION

A. baumannii is an opportunistic pathogen and one of the main causes of nosocomial infections. Treatment is difficult, especially in the case of multi-drug resistant and ESBL-producing strains. In the past 30 years, several new β-lactam antibiotics have been produced that are resistant to β-lactamases. Nowadays, the prevalence of drug resistance is increasing due to inactivation of a wide range of β-lactam antibiotics, especially third-generation cephalosporins and aztreonams. This has created numerous problems in the treatment of microbial infections.

In this study, 72% of *Acinetobacter* isolates were identified as *A. baumannii*. The rest were *A. lwoffii* and other *Acinetobacter* species. These results are similar to study of Constantinou et al. which reported that 71.5% and 29% of isolates were *A. baumannii* and *A. lwoffii*, respectively (18). Ritand et al. also reported that among 4180 clinical isolates, 74.02% were *A. baumannii* and the rest were *A. lwoffii* and other *Acinetobacter* species (19).

The rate of antibiotic resistance for most isolates is in agreement with the results obtained by Perez et al. and Begum et al. (20, 21) Similar to studies of Daly et al. (22) and Kamalbeik et al. (23), we found that most isolates were resistant to ceftazidime and cefepime. We also found that 84% of the strains had MIC of <128 µg/ml for ceftazidime, while study of Shahcheraghi et al. (24) reported that 83.1% of isolates had MIC of >64 µg/ml. Regarding the MIC value for ceftazidime, our results are similar to the results of some studies conducted in Korea and Taiwan (25). In this study, 23% of *A. baumannii* strains produced ESBL. This findings is in line with findings of Sinha et al. (26) and Shahcheraghi et al. (24) .

We also found that 47% of *A. baumannii* isolates contained the *PER* gene. However, studies conducted by Kim et al. (27) and Farajnia et al. (28) reported the frequency of *PER* gene as 78.6% and 51%, respectively. In the present study, frequency of the *VEB* gene was 32% in *A. baumannii* isolates. Different

results have been reported in studies conducted by Farajnia et al. (10%) (28), Pasteran et al. (47.61%) (29) and Poirel et al. (66%) (30). This difference could be due to the type of samples and antibiotic disk, concentration of antibiotics, geographical location and test conditions.

CONCLUSION

The increased resistance to expanded-spectrum cephalosporins is of great clinical importance, especially for hospitalized patients. Identification of patients infected with ESBL-producing bacteria, appropriate selection of antibiotics, identification of β-lactamase-producing strains and preventing the spread of resistant bacteria in hospitals could help eliminate this problem.

ACKNOWLEDGMENTS

The authors would like to thank the Islamic Azad University of Qom for supporting this project.

CONFLICT OF INTEREST

There is no conflict of interest.

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