

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 μ g/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*.

INTRODUCTION

Acinetobacter is a genus of Gram-negative, aerobic and non-fermentative coccobacilli. 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 (AS₂₀), ceftriaxone (CRO₃₀), amikacin (AK₃₀), imipenem (IPM₁₀), cefepime (FEP₃₀), gentamicin (GM₁₀), meropenem (MEM₁₀), Cotrimoxazole (BA₂₅), ciprofloxacin (CIP₅), levofloxacin (LVX₅), colistin (CO₁₀), cefpodoxime (CPD₁₀), ceftizoxime (CL₃₀), cefixime (CFM₅), ticarcilin (TC₇₅), aztreonam (ATM₃₀), Ceftazidime (CAZ₃₀) and polymyxin B (PB₃₀₀). 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 light.

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

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

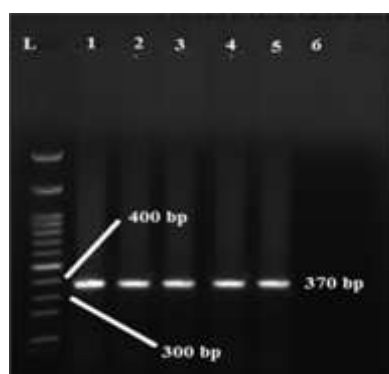


Figure 2- PCR amplification of the *bla*_{VEB} gene. Column L: 100 bp marker; column 1: positive control;



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 $<128 \mu\text{g/ml}$ for ceftazidime, while study of Shahcheraghi et al. (24) reported that 83.1% of isolates had MIC of $>64 \mu\text{g/ml}$. 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

REFERENCES

- Wallace L, Daugherty SC, Nagaraj S, Johnson JK, Harris AD, Rasko DA. *Use of Comparative Genomics To Characterize the Diversity of Acinetobacter baumannii Surveillance Isolates in a Health Care Institution*. Antimicrobial Agents and Chemotherapy. 2016; 60(10): 5933-41. doi: 10.1128/AAC.00477-16.
- Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. *Molecular epidemiology of clinical isolates of carbapenem-resistant Acinetobacter spp. from Chinese hospitals*. Antimicrobial Agents and Chemotherapy. 2007; 51(11): 4022-8.
- Murray PR, Baron EJ, Jorgensen J, Landry M, Pfaller M. *Manual of clinical microbiology*. ASM Press: Washington,DC. 2006(Ed. 9):1-1267.
- Bou G, Oliver A, Martínez-Beltrán J. *OXA-24, a novel class D β -lactamase with carbapenemase activity in an Acinetobacter baumannii clinical strain*. Antimicrobial Agents and Chemotherapy. 2000; 44(6): 1556-61.
- Fagon J-Y, Chastre J, Domart Y, Trouillet J-L, Gibert C. *Mortality due to ventilator-associated pneumonia or colonization with Pseudomonas or Acinetobacter species: assessment by quantitative culture of samples obtained by a protected specimen brush*. Clinical infectious diseases. 1996; 23(3): 538-42.
- Gastmeier P, Schwab F, Bärwolff S, Rüdén H, Grundmann H. *Correlation between the genetic diversity of nosocomial pathogens and their survival time in intensive care units*. Journal of Hospital Infection. 2006;62(2):181-6. DOI:10.1016/j.jhin.2005.08.010.
- Coia J, Duckworth G, Edwards D, Farrington M, Fry C, Humphreys H, et al. *Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities*. Journal of Hospital Infection. 2006; 63 (Suppl 1): S1-44.
- Landman D, Quale JM, Mayorga D, Adedeji A, Vangala K, Ravishankar J, et al. *Citywide clonal outbreak of multiresistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY: the preantibiotic era has returned*. Archives of internal medicine. 2002; 162(13): 1515-20.
- Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. *Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents*. Antimicrobial Agents and Chemotherapy. 1999; 43(6): 1379-82.
- Thirapanmethee K. *Extended spectrum β -lactamases: critical tools of bacterial resistance*. Mahidol Univ J Pharm Sci. 2012; 39(1): 1-8.
- Naas T, Poirel L, Nordmann P. *Minor extended-spectrum β -lactamases*. Clinical microbiology and infection. 2008; 14(1): 42-52.
- Bali EB, Accedil L, Sultan N. *Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-lactamase produced by Escherichia coli, Acinetobacter baumannii and Klebsiella isolates in a Turkish hospital*. African Journal of Microbiology Research. 2010; 4(8): 650-4.
- Baygloo NS, Bouzari M, Rahimi F, Abedini F, Yadegari S, Soroushnia M, et al. *Identification of Genomic Species of Acinetobacter Isolated from Burns of ICU Patients*. Archives of Iranian Medicine (AIM). 2015; 18(10):638-42. doi: 0151810/AIM.005.
- Wayne P. *Performance standards for antimicrobial susceptibility testing: Twenty-second informational supplement. CLSI document M100-S22*. Clinical Laboratory Standards institute. 2012; 32(3): 1-126.
- Swenson JM, Killgore GE, Tenover FC. *Antimicrobial susceptibility testing of Acinetobacter spp. by NCCLS broth microdilution and disk diffusion methods*. Journal of clinical microbiology. 2004; 42(11): 5102-8. DOI:10.1128/JCM.42.11.5102-5108.2004.
- Shakibaie MR, Adeli S, Salehi MH. *Antibiotic resistance patterns and extended-spectrum β -lactamase production among Acinetobacter spp. isolated from an intensive care Unit of a hospital in Kerman, Iran*. Antimicrobial resistance and infection control. 2012;1(1):1. doi: 10.1186/2047-2994-1-1.
- Alikhani MY, Tabar ZK, Mihani F, Kalantar E, Karami P, Sadeghi M, et al. *Antimicrobial resistance patterns and prevalence of blaPER-1 and blaVEB-1 genes among ESBL-producing Pseudomonas aeruginosa isolates in West of Iran*. Jundishapur Journal of Microbiology. 2014; 7(1): e8888. doi: 10.5812/jjm.8888.
- Constantiniu S, Romaniuc A, Chiriac R, Berea C, Kalis O, Rezus E, et al. *Antibacterial antibodies for some enterobacteria in sera of patients with reactive arthritis and other rheumatoid diseases*. Roum Arch Microbiol Immunol. 2008; 67(1-2): 30-5.

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.

19. Rit K, Saha R. *Multidrug-resistant acinetobacter infection and their susceptibility patterns in a tertiary care hospital*. Nigerian Medical Journal. 2012; 53(3): 126-8. doi: 10.4103/0300-1652.104379.
20. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. *Global challenge of multidrug-resistant Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy. 2007;51(10):3471-84. DOI:10.1128/AAC.01464-06.
21. Begum S, Hasan F, Hussain S, Shah AA. *Prevalence of multi drug resistant Acinetobacter baumannii in the clinical samples from Tertiary Care Hospital in Islamabad, Pakistan*. Pakistan journal of medical sciences. 2013; 29(5): 1253-1258.
22. Dally S, Lemuth K, Kaase M, Rupp S, Knabbe C, Weile J. *DNA microarray for genotyping antibiotic resistance determinants in Acinetobacter baumannii clinical isolates*. Antimicrobial Agents and Chemotherapy. 2013; 57(10): 4761-8. doi: 10.1128/AAC.00863-13.
23. Kamalbeik S, Talaie H, Mahdavinnejad A, Karimi A, Salimi A. *Multidrug-resistant Acinetobacter baumannii infection in intensive care unit patients in a hospital with building construction: is there an association?* Korean journal of anesthesiology. 2014; 66(4): 295-9. doi: 10.4097/kjae.2014.66.4.295.
24. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. *Isolation and genetic characterization of metallo- β -lactamase and carbapenamase producing strains of Acinetobacter baumannii from patients at Tehran hospitals*. Iranian journal of microbiology. 2011; 3(2): 68-74.
25. Lee K, Yong D, Jeong SH, Chong Y. *Multidrug-Resistant Acinetobacter spp.: Increasingly Problematic Nosocomial Pathogens*. Yonsei Med J. 2011; 25(6): 879-91. doi: 10.3349/ymj.2011.52.6.879.
26. Sinha M, Srinivasa H, Macaden R. *Antibiotic resistance profile & extended spectrum beta-lactamase (ESBL) production in Acinetobacter species*. Indian journal of medical research. 2007; 126(1): 63-7.
27. Kim J, Heo S, Jin J, Choi C, Lee Y, Jeong Y, et al. *Characterization of Acinetobacter baumannii carrying blaOXA-23, blaPER-1 and armA in a Korean hospital*. Clinical microbiology and infection. 2008; 14(7): 716-8.
28. Farajnia S, Azhari F, Alikhani MY, Hosseini MK, Peymani A, Sohrabi N. *Prevalence of PER and VEB type extended spectrum betalactamases among multidrug resistant Acinetobacter baumannii isolates in North-West of Iran*. Iranian journal of basic medical sciences. 2013; 16(6): 751-5.
29. Pasterán F, Rapoport M, Petroni A, Faccone D, Corso A, Galas M, et al. *Emergence of PER-2 and VEB-1a in Acinetobacter baumannii strains in the Americas*. Antimicrobial Agents and Chemotherapy. 2006; 50(9): 3222-4. doi: 10.1128/AAC.00284-06.
30. Poirel L, Corvec S, Rapoport M, Mugnier P, Petroni A, Pasteran F, et al. *Identification of the novel narrow-spectrum β -lactamase SCO-1 in Acinetobacter spp. from Argentina*. Antimicrobial Agents and Chemotherapy. 2007; 51(6): 2179-84. doi: 10.1128/AAC.01600-06.