Original Article

PER-1-type extended-spectrum ß-lactamase producing *Acinetobacter baumannii*

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ABSTRACT

Objective: To determine the prevalence of PER-1 gene type ESBLs producing isolates of *A. baumannii* in clinical specimens.

Methodology: During March 2008 to June 2009, a total of 100 *A. baumannii* isolates were recovered from clinical specimens of hospitalized patients in Imam Reza hospital in Tabriz. These isolates were subjected to susceptibility tests using five selected cephalosporins according to CLSI guidelines. Screening for ESBL production was carried out by confirmatory tests including DDST and CDM. The prevalence of PER-1 gene in these isolates was also determined by using PCR.

Results: All of *A. baumannii* isolates in this study were resistant at least to one of the selected cephalosporins. Sixty four percent of isolates exhibited a >5 mm zone size enhancement in the CDM test, whereas 53% of them gave positive results by DDST as ESBL producer. Collectively CD method and DDST showed 70% of *A. baumannii* as ESBLs producer, of which 51% had PER-1 gene.

Conclusion: This research shows high prevalence of PER-1 gene type ESBLs producing *A. baumannii* isolates in Tabriz which is strong evidence for their resistance to cephalosporins.

KEY WORDS: *A. baumannii*, ESBL, PCR, DDST, CDM.

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INTRODUCTION

Acinetobacters spp are isolated as commensals from skin, throat and various secretions of healthy people, as well as causing human infections such as bacteremia, pneumonia, secondary meningitis, urinary tract infection, burns and nosocomial infections.[1] The most important problem associated with *A. baumannii* has been its intrinsic resistance to multiple antibiotics and its ability to rapidly acquire antibiotic resistance from other bacteria with consequent resistance to majority of antibiotic classes.[2] In recent years, these bacteria have acquired resistance to major ß-lactam antibiotics like penicillins, first, second and third-generation cephalosporins and monobactams because of their ability to produce ß – lactamase enzymes which are inhibited by clavulanic

acid.[3] Different mechanisms are involved in conferring ß-lactam antibiotics resistance to *A. baumannii* strains, but Extended Spectrum ß- Lactamase (ESBLs) is of the principal one.[4]

PER-1 gene is found on plasmid or chromosome and was first detected in 1993 in a *Pseudomonas aeruginosa* isolate from a Turkish patient in France[5] and latter this gene was also detected in other bacteria such as *Salmonella enterica* serovar *Typhimurium* and *A. baumannii*.[6] Increasing resistance among *A. baumannii* strains to multiple antibiotics was related to genetic transfer of PER-1 gene.[7] PER-1 gene containing *A. baumannii* strains were reported from various geographic areas, but the highest prevalence was seen in Turkey.[8] This study was performed to find out the prevalence of PER-1 gene in *A. baumannii* isolates from clinical specimens.

METHODOLOGY

Identification of strains: In a cross sectional study from March 2008 to June 2009, a total of 100 *A. baumannii* isolates were recovered from clinical specimens of hospitalized patients in Imam Reza hospital in Tabriz, an eastern Azarbaijan province with wide communication and close proximity to Turkey. Isolates were identified to the genus and species level based on the standard biochemical and microbiological methods such as: morphologic appearance on Gram-stain (gram negative coccobacilli), oxidase negative, catalase and citrate positive, nonmotile, negative esculin hydrolysis, growth at 41°C and negative lysine decarboxylase.[9]

Susceptibility Test: In order to detect ESBL producing *A. baumannii*, test organisms were inoculated onto Muller Hinton agar against five antibiotic discs, including Ceftriaxone (30µg) Ceftazidime (30µg), Cefotaxime (30µg), Aztreonam (30µg) and Cefepime (30µg) (MAST House, Merseyside, UK). Based on CLSI recommendation, each of isolates which showed the inhibition zone of <17 mm for Cefotaxime, <27 mm for Ceftazidime, Ceftriaxone and Aztreonam and <25 mm for Cefepime after 18 hours of incubation at 37°C were suspected for ESBL production. Resistance to one or more of these antibiotics was an indicator for screening of ESBL producing *A. baumannii* strains.[10]

ESBL detection:

Double Disc Synergy Test (DDST)[11]: After inoculation of test organisms onto Mueller Hinton agars, four discs including Ceftazidime, Cefotaxime, Cefepime and Aztreonam were placed around the centrally placed Augmentin disc (Amoxicillin 20µg plus Clavulanic acid 10µg, MAST, UK) with a center to center distance of 20 mm from central disc and were incubated for 18 hours at 37°C. The isolate showing enhancement of the zone and synergy towards to centrally placed Augmentin disc for one or more of the tested discs was considered as ESBL producer (Fig-1).

Combined Disc Method (CDM)[12]: Isolates were inoculated onto Mueller Hinton agar and four discs including Cefotaxime (30µg), Cefotaxime/ Clavulanic acid (30µg/10µg), Ceftazidime (30µg) and Ceftazidime /Clavulanic acid (30µg/10µg) were placed at a center to center distance of 30 mm from Cefotaxime/Clavulanic acid and Ceftazidime/ Clavulanic acid discs. All plates were incubated at 37°C for 18 hours and a >5mm increase in inhibition zone of Ceftazidime/Clavulanic acid and Cefotaxime/Clavulanic acid discs in comparison to its zone when tested alone and without Clavulanic acid confirmed ESBL production (Fig-2).

PER-1 gene PCR: All *A. baumannii* isolates were grown for 24 hours at 37°C in blood agar. DNA of isolates was extracted by sodium dodecyl sulphate-protienase K modified with N, N, N-trimethyl ammonium bromide.[13] Oligonucleotide primers, F- (ATGAATGTCATTATAAAAGC) R-(AATTTGGGCTTAGGGCAGAA) were used for amplification of the 925 bp fragment of the *PER-1* gene.[15] The total volume of PCR mix was 25 µl, including sterile redistilled H_2O 17.05µl, 10X PCR buffer 2.5µl, dNTP mix (10mM) 0.5µl, $MgCl_2$ (50mM) 0.75µl, forward primer (25µM) 0.5µl, reverse primer (25µM) 0.5µl, Taq DNA polymerase (5U/µl) 0.2µl,

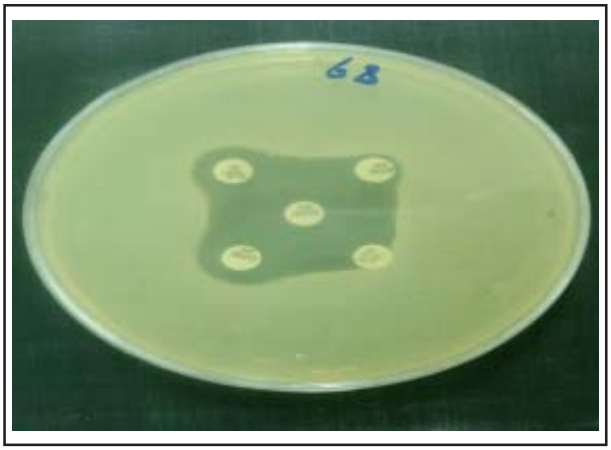

Fig-1: A positive Double-disc synergy test (DDST) using cefepime (CPM 30 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), Aztreonam (AZT 30 µg) and Augmentine (AUG = Amoxicillin 20µg plus Clavulanic acid 10 µg) discs. A representative of *A. baumannii* isolates showing distinct extension of the zone of inhibition towards Augmentine disc indicating ESBL production.

template DNA 3µl. Negative controls contained all components except template DNA. PER-1 gene positive *P. aeruginosa KOAS* was used as positive control. Primers and other reagents were prepared according to the manufacturer's recommendation. PCR reactions were performed with an automated thermal cycler (Eppendorf mastercycler gradient, Germany) with the PCR cycling conditions of initial cycle at 94°C for four minutes, 35 cycles of denaturation at 94°C for one minutes, annealing at 50°C for one minute, extension at 72 °C for one minute, and final cycle extension at 72 °C for 10 minutes. Gel electrophoresis was performed for 60-120 minutes in a 1.2% agarose gel at 75 V. DNA profiles were visualized by ultraviolet (UV) light after ethidium bromide staining on a UV transilluminator. The gels were photographed using a gel documentation system (UVP, USA) for the analysis of bands.[14]

RESULTS

A total of 100 *A. baumannii* were isolated from specimens of respiratory system (54%), urine (21%), blood (7%) and other sources (18%). Infections were observed more frequently in men (72 %). The age of the patients was from 14 to 86 years and whenever *A. baumannii* were isolated, patients sustained trauma (29%). Most of the isolates (37%) were recovered from patients in the intensive care units (ICU). Invasive procedures such as intubation and tracheostomy were used in 63% of the patients.

Results of susceptibility test showed that all of *A. baumannii* isolates were resistant to cefepime, 97% were resistant to two antibiotics, 94% to three antibiotics, 90% to four antibiotics and 88% to all of five selected antibiotics.

Sixty four percent of isolates exhibited a >5 mm zone size enhancement in the CD test, while 53% of them were DDST-positive (Fig-1, 2). Of the100 isolates of *A. baumannii*, 70 (70%) were ESBL positive by both tests (DDST and CDM), and 51 (72.8 %) of them were found to have PER-1 gene (Fig-3).

In 35 cases (66%) synergy was seen between Cefepime and Augmentin while in 11 cases (20%), all of the four discs had an enhanced zone of inhibition towards Augmentin. In 5 cases (9.4%), synergy between Cefepime, Ceftazidime and Augmentine were observed and finally in two cases (3.7%) there was a synergy between Cefepime, Cefotaxime, Ceftazidime and Augmentin.

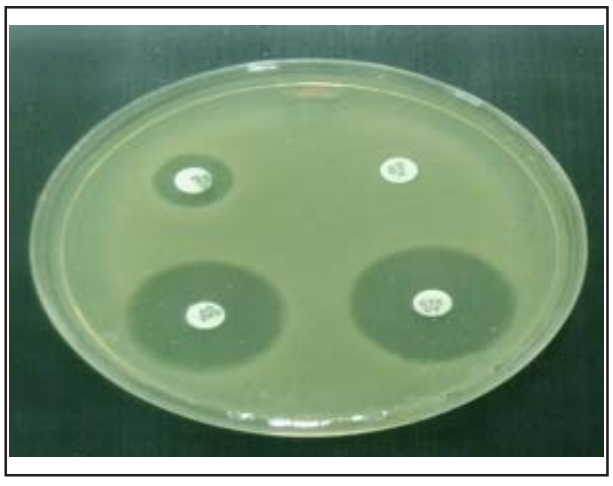

Fig-2: A positive Combined disc (CD) using cefotaxime (CTX 30 µg), Cefotaxime/Clavulanic acid (30µg/10µg) and ceftazidime (CAZ 30 µg), Ceftazidime/Clavulanic acid (30µg/10µg) discs. A representative of *A. baumannii* isolates showing a >5 mm zone size enhancement in the CD test indicating inhibition of ESBL production.

DISCUSSION

The PER-1 gene ESBL is of particular interest in the management of severe nosocomial infections for at least three reasons; (a) it confers resistance to most ß-lactams, (b) plasmid-mediated transfer of PER-1 gene from PER-1 gene positive *A. baumannii* to PER-1 gene negative strains of the same species is possible (c) unlike other class A -lactamases of *A. baumannii*, PER-1 gene appeared to be transmissible among different species.[15]

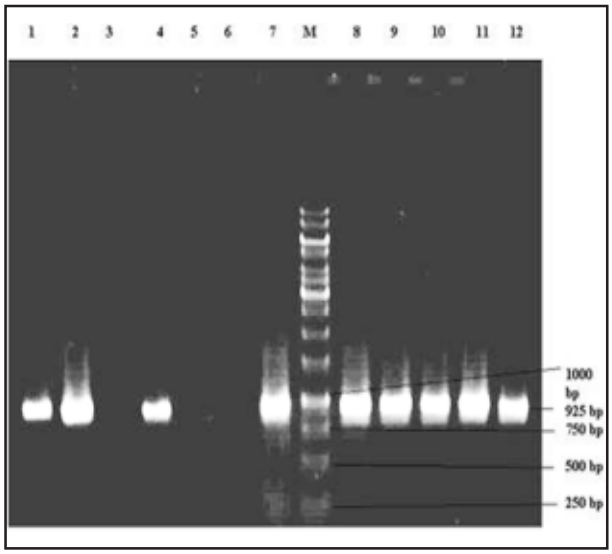

Fig-3: Detection of PER-1 gene in *A. baumannii* isolated from clinical specimens. Lane M, Size marker (1kbp DNA ladder); Lane 6, Blank; Lane 7, positive control (PER-1 gene positive *P. aeruginosa KOAS*); Lane 3 & 5, PER-1 negative isolates of *A. baumannii*; Lane 1,2,4,8,9,10,11,12, PER-1 positive isolates of *A. baumannii*. The main band of positive control and the positive isolates represent the PER-1 specific 925 bp fragment.

The finding of this study showed that the majority of *A. baumannii* isolates (37%) were isolated from patients in the intensive care units (ICU). Further, the highest prevalence was revealed in patients under invasive treatment procedures such as intubation, tracheostomy and catheterization (63%). Other studies have also shown that 20-25% of nosocomial infection develops in ICUs in Europe.[16]

All of the *A. baumannii* isolates obtained in this study were resistant to at least one of the selected cephalosporin, which correlates well with findings of Ranjbar and colleagues (2007) in Iran and Jeong and colleagues (2005) in South Korea.[13,16] Indiscriminate use of antibiotics by patients can be main factor in induction of resistance in these bacteria.

Out of 70 (70%) ESBL producing *A. baumannii*, 51 (72.8%) were found to have PER-1-gene which is similar to the reports were published by investigators from Turkey[8] (46%) and South Korea[17] (54.6%). This is the first report about high prevalence of PER-1 gene in *A. baumannii* isolates in Tabriz that could explain one of the most important possible reasons for the presence of antibiotic resistance to cephalosporins in this region.

The high prevalence of PER-1 gene among *A. baumannii* recovered from nosocomial cases in the present study and Turkey can be correlated with geographical closeness of these two cities and wide communication. Of course, in order to prove this presumption, it requires typing of strains to compare with types which are common in Turkey.

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