

Prevalence of Extended Spectrum β -Lactamase-Producing *Enterobacteriaceae* by Phenotypic and Genotypic Methods in Intensive Care Units in Tehran, Iran

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Received 2 Nov 2008; Revised 30 Jun 2008; Accepted 25 Jun 2008

ABSTRACT

Background and the purpose of the study: The occurrence of Extended Spectrum β -Lactamase (ESBL)-producing *Enterobacteriaceae* has been steadily increased in recent years, resulting in limitation of therapeutic options. The purpose of this study was to determine prevalence of ESBL-producing *Enterobacteriaceae* isolated from Intensive Care Units (ICUs) and to investigate their phenotypic and genotypic characteristics.

Methods: A total of one hundred fifty isolates were collected from urine and urinary catheter, sputum, blood, wound and other clinical samples from patient admitted in ICUs. All isolates were identified by biochemical tests and then were screened for ESBL production by Disk Agar Diffusion (DAD) according to the Clinical and Laboratory Standards Institute (CLSI) guideline. The species that met screening criteria were further tested for the effects of clavulanic acid by confirmatory method. ESBL-positive species were tested for *bla*_{TEM} and *bla*_{SHV} genes by PCR assay.

Results: Of total of 150 bacterial isolates, 133(89.3%) isolates were positive in the resistance to all tested cephalosporin indicators; and 89(59.3%) isolates were confirmed as ESBL producer. *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter spp.* were the most ESBL-producing species. All isolates were sensitive to imipenem. The *bla*_{TEM} (55.5%) was the most common gene detected in ESBL phenotypic-positive isolates using PCR method.

Conclusion: The present study shows high prevalence of ESBL-producing *Enterobacteriaceae* from ICU patients. The increased rate of these species is mainly due to the inadequate and unnecessary antimicrobial therapy. Rational administration of β -lactams and appropriate infection control policies may reduce prevalence of ESBL-producing bacteria in ICUs.

Key words: Extended Spectrum β -Lactamases (ESBLs), *Enterobacteriaceae*, Intensive Care Unit (ICU)

INTRODUCTION

The emergence of bacterial resistance to β -lactam antibiotics is one of the serious problems in Intensive Care Units (ICUs). In 1983, Extended spectrum β -lactamases (ESBLs), a heterogeneous group of plasmid-encoded β -lactamases, were first detected in Germany and consequently spread to other countries of the world (1).

Today, more than 150 natural ESBL variants are known. Most ESBLs are derivatives of *TEM* and *SHV* – type β -lactamases by one or more amino acid substitutions that are capable to hydrolyse a wide range of β -lactam antibiotic especially oxyimino cephalosporins and Monobactams but

they have no activity against carbapenems. These enzymes are inhibited by β -lactamase inhibitors such as clavulanic acid (2).

The ESBLs are categorized in class A or D β -lactamases (3). They are mainly found in members of the *Enterobacteriaceae* family and their presences have been reported to be associated with increased mortality and morbidity in ICUs (4).

The CLSI guideline has recommended that ESBL producer should be reported as resistant to all penicillins, cephalosporins and aztreonam, even when they are susceptible to these agents by conventional tests (5). The best methods to detect ESBL-producing bacteria is an initial screening of

reduced susceptibility to the CLSI recommended cephalosporins indicators, then performing phenotypic confirmatory tests which demonstrate a synergistic effect between an cephalosporin indicator and clavulanic acid(6).

The aim of this study was to determine the prevalence of ESBL-producing Entrobacteriaceae isolated from ICU patients and to evaluate the distribution of *TEM* and *SHV* genes among the organisms.

MATERIAL AND METHODS

Bacterial strains

During an 8 month period from April to November 2006, 150 bacterial isolates of the Entrobacteriaceae family were collected from clinical consecutive samples of ICU patients admitted to two Tehran University teaching hospitals (Imam Khomeini and Shariatei Hospitals). All of the isolates were identified by standard biochemical reaction, and stored at -70°C.

ESBL detection tests

The bacterial isolates were screened for the presence of ESBLs by Disk Agar Diffusion method on Muller-Hinton Agar (Difco Laboratories, Detroit, MI) using Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefpodoxime (30µg) and Aztreonam (Mast Diagnostics Ltd., Bootle, UK). The isolates that met screening criteria were confirmed by the phenotypic confirmatory tests according to the CLSI guideline. In this method ESBL production was confirmed with ≥ 5 mm increase in the zone of the diameter of inhibition for Ceftazidime/Clavulanate (30/10µg) and Cefotaxime/Clavulanate (30/10µg) (Mast Diagnostics Ltd., Bootle, UK) compared to the zone diameter of inhibition in the absence of Clavulanate. *K.pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively(7).

PCR amplification for TEM and SHV genes

PCR assay was performed to determine the presence of the *bla*_{TEM} and *bla*_{SHV} β-lactamase genes. The specific primers which were used for amplification were TEM-forward (5'-ACA TGG GGG ATC ATG TAA CT-3'), TEM-reverse (5'-GAC AGT TAC AAT GCT TAC T-3'), which amplified a 421 bp fragment and SHV-forward (5'-ATG CGT TAT ATT CGCCT6 TG-3'), SHV-reverse (5'-AGC GTT GCC AGT GCT CGA TG-3')(8), which amplified an 859 bp fragment. PCR condition were as follow: 30 cycles, with 1 cycle consisting of denaturation at 94°C for 30s, annealing at 52°C (TEM) AND 56°C (SHV) for 30s, extension at 72°C for 1 min. each PCR programs pre-

ceded by a denaturation step at 94°C for 5 min and followed by a final extension at 72 °C for 10 min.

In this study *E. coli* ATCC 25922 was used as the negative control and *E. coli* ATCC 35218 (TEM-producing strain) and *K. pneumoniae* ATCC 700603 (SHV-producing strain) were used as positive controls in PCR assay, respectively.

Statistical method

The data were analyzed using Excel program under Microsoft windows.

RESULTS

One hundred fifty bacterial isolates of the Entrobacteriaceae family were detected from ICU patients were detected during 8 months period.

Of the 150 bacterial isolates, 60(40%) were from urine and urinary catheter, 48 (32%) were from sputum, 28 (18.6%) were from blood, 11(7.3 %) from wound and 3(2%) were from other clinical samples (table 1).

As shown in table 1, *K. pneumoniae* was the most common isolated microorganisms from ICUs, followed by *E. coli* and *E. cloacae*.

One hundred thirty-three (89.3%) isolates were positive in the ESBL screening susceptibility test according to CLSI criteria. One hundred and twenty one (80.6 %) of the isolates were resistant to Aztreonam and all 3rd generation cephalosporins of this study.

Eighty-nine (59.3 %) of 150 isolates were ESBL-positive by phenotypic confirmation Disk Diffusion test (i.e the isolates showed a ≥ 5 mm increase in the diameter of the zone of inhibition around the CAZ and CTX disks). *K. pneumoniae* with 76.7 % (33/43) was the most common ESBL-producing isolates, followed by *K. oxytoca* 62.5 % (5/8) and *E. coli* 60.6 % (20/33). The frequency of isolates of each ESBL-producing species is shown in table 2.

The *bla*_{TEM} (55%) was the most frequent gene found in ESBL phenotypic positive isolates using PCR genotyping method. *K. pneumoniae* was the most common isolates carried *bla*_{TEM}, *bla*_{SHV} and both *bla*_{TEM} & *bla*_{SHV} genes whereas non *bla*_{TEM} and non *bla*_{SHV} genes were frequently shown in *E. coli* (table 2). PCR products are shown in fig 1 and fig 2.

DISCUSSION

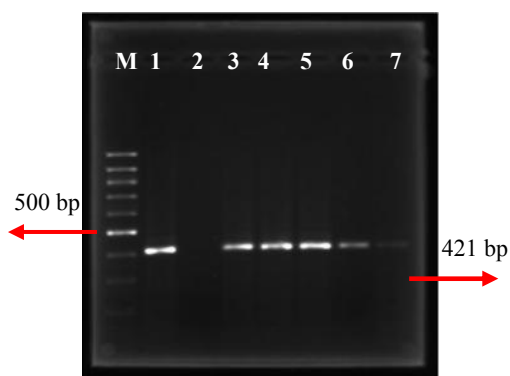
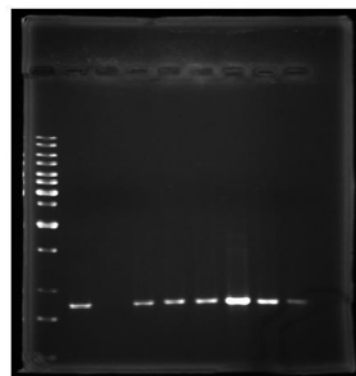
ESBL-producing Entrobacteriaceae are now an increasing problem in ICUs worldwide. The emergence and progressive spread of these bacteria seems to be caused mainly by extensive use of broad-spectrum β-lactamase in empiric therapy and rapid plasmid mediated distribution of resistance genes between bacterial species.

Table 1. Distribution according to clinical source of the family Enterobacteriaceae isolated from ICUs

	Total	Urine	Sputum	Blood	Wound	Others
<i>K. pneumoniae</i>	43	12	19	7	3	2
<i>E. coli</i>	33	22	4	4	3	0
<i>E. cloacae</i>	17	4	8	4	1	0
<i>E. aerogenes</i>	13	6	4	3	0	0
<i>C. diversus</i>	11	2	4	4	1	0
<i>C. freundii</i>	10	4	4	2	0	0
<i>K. oxytoca</i>	8	1	5	2	0	0
<i>P. mirabilis</i>	8	5	0	1	2	0
<i>S. marcescens</i>	5	4	0	1	0	0
<i>P. vulgaris</i>	2	0	0	0	1	1
Total	150	60	48	28	11	3

Table 2. Distribution of TEM&SHV genes in enterobacteriaceae isolated from ICUs

	Genotype of ESBLs				Total
	TEM	SHV	TEM+SHV	Non TEM & Non SHV	
<i>K. pneumoniae</i>	16	2	11	4	33
<i>E. coli</i>	14	0	1	5	20
<i>E. cloacae</i>	6	0	1	1	8
<i>E. aerogenes</i>	3	0	2	2	7
<i>C. diversus</i>	3	1	0	2	5
<i>C. freundii</i>	3	1	0	0	4
<i>P. mirabilis</i>	3	1	0	0	4
<i>K. oxytoca</i>	1	1	3	0	4
<i>S. marcescens</i>	0	1	0	1	2
Total (%)	49(55.4%)	7(7.7%)	18(20.2%)	15(16.7%)	89(100%)

**Figure 1.** PCR amplification of TEM gene, lane M: 100 bp ladder, lane 1: *E. coli* ATCC35218 positive control, lane 2: *E. coli* ATCC25922 negative control, lane 3-7: clinical isolates.**Figure 2.** PCR amplification of SHV gene, lane M: 1000 bp ladder, lane 1: *K. pneumoniae* ATCC700603 positive control, lane 2: *E. coli* ATCC25922 negative control, lane 3-8: clinical isolates.

ICU isolates mainly showed the highest rate of ESBL-producing bacteria because patients admitted in this ward have predisposing factors such as the length of hospital stay, severity of illness, compromised immunity as well as extensive administration of third generation cephalosporins. Other risk factor such as mechanical ventilation and urinary catheterization, also seem to predispose patients infection with ESBL-producing bacteria (9). ESBL detection is not carried out in hospitals of this study. This is most likely due to lack of awareness of clinical importance of bacteria producing these enzymes in the hospital settings, particularly in ICUs. In the present study, the first characterization of TEM-and SHV-type ESBL variants produced by Enterobacteriaceae which are circulating in Tehran hospitals is reported. Our findings showed that *K. pneumoniae*, *E. coli* and *Enterobacter spp.* are the most frequent species isolated from ICUs which are in agreement with the result other studies(10-11). The highest prevalence of ESBL was in *K. pneumoniae*, followed by *K. oxytoca* and *E. coli*, which are similar to the results of studies performed by Bonfiglio G et al and Nogueira Kda S et al in Brazil (12, 13). In addition it was also found that other members of family of Enterobacteriaceae also produce ESBL. The results show that overall prevalence of ESBL was 59.3%, which is much higher than the findings of other studies (11-14) which is most likely due to extensive use of third generation of cephalosporins and low level of infection control issue in ICUs.

Under this study, all isolates were sensitive to imipenem, which is in agreement with findings of

other studies, and is in contrast to results of another study in which 5.6% of isolates were resistant to imipenem mainly due to high consumption of this drug (15-16). In this study TEM-type enzymes was more frequent than SHV-type enzyme which is in agreement with results of other studies (17, 18). However on the basis of results of other studies, the frequency of SHV-type enzymes were higher than other genotypes of ESBLs (19-20). It is noteworthy that in contrast to another study which was carried out in 2007, isolates having both TEM and SHV were more common in contrast to TEM and SHV alone(21). These discrepancies in Distribution of genotypes of ESBLs may be due to differences in the pattern of the use of third generation cephalosporins in various geographical areas. It should be noted that non-TEM, non SHV genes were also detected in this study.

From the results of this study it may be concluded that in comparison with other parts of the world ESBL – producing Enterobacteriaceae in ICUs of hospital of this study is higher. *Klebsiella species* and *E. coli* are major concerns. In order to circumvent this problem, it is important to emphasize the rational and cyclic use of extended spectrum β -lactam drugs and implication of an appropriate infection control strategies in ICUs of this study. In addition, regular surveillance of resistance to antimicrobial agents is necessary.

ACKNOWLEDGEMENTS

This work was supported by Vice-Chancellor Research from Tehran University of Medical Sciences for the Promotion of Science.

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