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

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ABSTRACT

Background and the purpose of the study: The occurrence of Extended Spectrum β-Lactamase (ESBL)-producing Entrobacteriaceae 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 Entrobacteriaceae 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 *Entrobacter 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 Entrobacteriaceae 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 Entrobacteriaceae 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

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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 bacreial 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 ≥5mm 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 primes which where 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 56ċ (SHV) for 30s, extension at 72°C for 1 min. each PCR programs preceded 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. c*oli 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-tree (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 ≥5mm 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
K. pneumoniae	43	12	19	7	3	2
E. coli	33	22	4	4	3	0
E. cloacae	17	4	8	4	1	0
E. aerogenes	13	6	4	3	0	0
C. diversus	11	2	4	4	1	0
C. freundii	10	4	4	2	0	0
K. oxytoca	8	1	5	2	0	0
P. mirabilis	8	5	0	1	2	0
S. marcescens	5	4	0	1	0	0
P. vulgaris	2	0	0	0	1	1
Total	150	60	48	28	11	3

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

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

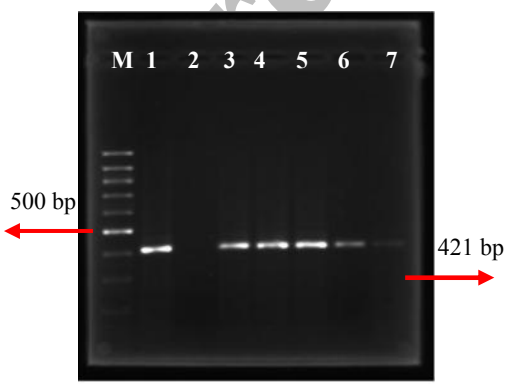

Figure 1. PCR amplification of TEM gene, lane M: 100 bp ladder, lane 1: *E coli* ATCC35218 positive control, lane 2: *E coli* ATCC25922 negative control, lane 3-7: clinical isolates.

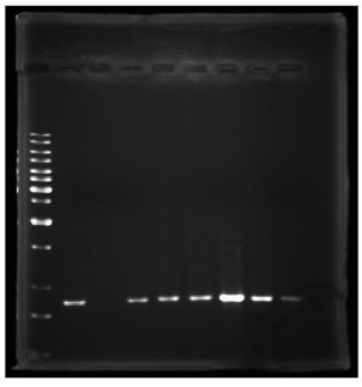

Figure 2. PCR amplification of SHV gene, lane M: 1000 bp ladder, lane 1: K. pneumoniae ATCC700603 positive control, lane 2: E coli ATCC25922 negative control, lane 3-8: clinical isolates.

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 Entrobacteriaceae which are circulating in Tehran hospitals is reported. Our findings showed that *K. pneumoniae*, *E. coli* and *Entrobacter 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 Entrobacteriaceae 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 cocluded 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.

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REFRENCES

1. Jain A, Mondal R. Prevalence & antimicrobial resistance pattern of extended spectrum beta-lactamase producing Klebsiella spp isolated from cases of neonatal septicaemia. Indian J Med Res 2007; 125 : 89-94.
2. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update.Clin Microbiol Rev 2005; 18 : 657-86.
3. Jacoby GA. Beta-lactamase nomenclature. Antimicrob Agents Chemother 2006; 50 : 1123-9.
4. Linkin DR, Fishman NO, Patel JB, Merrill JD, Lautenbach E. Risk factors for extended-spectrum beta-lactamase-producing Enterobacteriaceae in a neonatal Intensive care unit. Infect Control Hosp Epidemiol 2004; 25 : 781-3.
5. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 15th informational supplement.Wayne, Pennsylvania: Clinical Laboratory Standards Institute Document M100-S15; 2005.
6. Stürenburg E, Mack D. Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. J Infect 2003; 47 : 273-95.
7. Antimicrobial susceptibility testing of Escherichia coli strains isolated from urinary tract infections to fluoroquinolones and detection of gyrA mutations in resistant strains. Akbari-Nakhjavani F.,, Mirsalehian A., Hamidian M., Kazemi B., Mirafshar M., Jabal Ameli F., Pajand O., Peymani AAkbari-Nakhjavani F., Mirsalehian A.,& et al. DARU, 2007;15:94-99.
8. Sundsfjord A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmevoll SO, Littauer P, Dahl KH Genetic methods for detection of antimicrobial resistance. APMIS 2004; 112 : 815-37

9. Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Ann Intern Med 2001 20; 134 : 298-314
10. Gaynes R, Edwards JR; National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005 15; 41 : 848-54
11. Kucukates E. Antimicrobial resistance among Gram-negative bacteria isolated from intensive care units in a Cardiology Institute in Istanbul, Turkey. Jpn J Infect Dis 2005;58 : 228-31
12. Bonfiglio G, Perilli M, Stefani S, Amicosante G, Nicoletti G. Prevalence of extended spectrum beta-lactamases among Enterobacteriaceae: an Italian survey. Int J Antimicrob Agents 2002; 19 : 213-7.
13. Nogueira Kda S, Higuti IH, Nascimento AJ, Terasawa LB, Oliveira S, Matos AP, Souza HA, Cogo LL, Dalla Costa LM. Occurrence of extended-spectrum beta-lactamases in Enterobacteriaceae isolated from hospitalized patients in Curitiba, southern Brazil. Braz J Infect Dis 2006; 10 : 390-5.
14. Ndugulile F, Jureen R, Harthug S, Urassa W, Langeland N. Extended spectrum beta-lactamases among Gram-negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. BMC Infect Dis 2005; 5 : 86.
15. Feizabadi MM, Etemadi G, Yadegarinia D, Rahmati M, Shabanpoor S, Bokaei S. Antibiotic-resistance patterns and frequency of extended-spectrum beta-lactamase-producing isolates of Klebsiella pneumoniae in Tehran. Med Sci Monit. 2006; 12: 362-5.
16. Cao V, Lambert T, Nhu DQ, Loan HK, Hoang NK, Arlet G, Courvalin P. Distribution of extended-spectrum beta-lactamases in clinical isolates of Enterobacteriaceae in Vietnam.Antimicrob Agents Chemother 2002; 46 : 3739-43
17. Lavigne JP, Bouziges N, Chanal C, Mahamat A, Michaux-Charachon S, Sotto A. Molecular epidemiology of Enterobacteriaceae isolates producing extended-spectrum beta-lactamases in a French hospital. J Clin Microbiol 2004; 42 : 3805-8.
18. Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G. Occurrence of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae in Italy: implications for resistance to beta-lactams and other antimicrobial drugs. Antimicrob Agents Chemother 2002;46 : 196-202
19. Wu TL, Chia JH, Su LH, Kuo AJ, Chu C, Chiu CH. Dissemination of extended-spectrum beta-lactamase-producing Enterobacteriaceae in pediatric intensive care units. J Clin Microbiol 2003; 41 : 4836-8.
20. Shahcheraghi F, Moezi H, Feizabadi MM. Distribution of TEM and SHV beta-lactamase genes among Klebsiella pneumoniae strains isolated from patients in Tehran. Med Sci Monit 2007;13 : 247-250.
21. Lal P, Kapil A, Das BK, Sood S. Occurrence of TEM & SHV gene in extended spectrum beta-lactamases (ESBLs) producing Klebsiella sp. isolated from a tertiary care hospital. Indian J Med Res 2007; 125 : 173-8