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## Spread of Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* Clinical Isolates in Sanandaj Hospitals

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**Abstract:** The aim of this study was to determine the prevalence of Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* and antimicrobial susceptibility pattern of ESBL-producing and non producing strains. We evaluated 158 *E. coli* strains isolated from various clinical specimens. The double-disk synergy test was performed on the isolates for the detection of ESBL. These genes were confirmed by PCR methods. The prevalence of ESBL-producing *E. coli* was found as 16.8%. The ESBL-producing isolate rates were 22.2% (6/27) in intensive care units, 22.2% (6/27) in wards and 44.6% (15/27) in outpatients. This study, present the existence of ESBL-producing isolates and high rate of resistance to antibiotics. Clinicians should be familiar with the clinical importance of these enzymes and potential strategies for dealing with them. The results of the study suggest that community acquired control of ESBL-producing *E. coli* has great importance.

**Key words:** ESBL, *Escherichia coli*, antibiotic resistance, PCR

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### INTRODUCTION

Extended-Spectrum Beta-Lactamases (ESBLs) are enzymes conferring broad resistance to beta-lactam antibiotics, including third-generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime (Apisamthanarak *et al.*, 2007, 2008; Paterson, 2006). More than 150 type of ESBLs have been described and the majority of this enzymes belonging to the TEM and SHV family (Moreno *et al.*, 2007; Mendelson *et al.*, 2005).

The original TEM was first discovered in *E. coli* in a patient named Temoniera in Greece, but it spread rapidly to other bacteria. The SHV enzymes, named after the Sulf-Hydryl variable active site, are commonly associated with *K. pneumoniae* (Samaha-Kfoury and Araj, 2003).

The beta-lactamase genes are located on the large plasmids that confer resistance to other classes of antimicrobial agents and are readily transmissible from strain to strain and between different species of enteric gram-negative bacilli (Paterson, 2006). Extended Spectrum Beta-Lactamases (ESBLs) are found in a variety of members of the family Enterobacteriaceae (Mendelson *et al.*, 2005). ESBLs are detected most commonly in *Klebsiella pneumoniae* and *Escherichia coli* (Fang *et al.*, 2007). Infections due to these organisms often occur in outbreaks and ESBL have therefore become

a serious problem in hospitalized patients (Moreno *et al.*, 2007; Carattoli *et al.*, 2008).

Since, *E. coli* is one of the most common producers of ESBL, we carried out a survey of ESBL-producing clinical isolates of those bacteria in the two hospital in the Kurdistan Province in west of Iran. However, there is no documented report as yet on the incidence of ESBL-positive *E. coli* from these regions. The main objective of the survey was to determine the prevalence of ESBL production among *E. coli* isolated from patients as well as their susceptibility patterns.

### MATERIALS AND METHODS

**Study population and specimen types:** This study was conducted at Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj, Iran. From January 2007 to January 2008, consecutive, non-duplicate isolates of *E. coli* were collected from various specimens of patients who were referred for Toohid and Beasat Hospitals. Specimens included urine, wound, respiratory tract, blood, cerebrospinal fluid, skin and soft tissues.

**Microbiological methods:** All samples were routinely cultured on MacConkey and blood agar plates. Blood samples were cultured in Blood culture bottles. Isolates

**Table 1: Primers and conditions of polymerase chain reaction used in present study**

Primers	PCR primers (5'-3')	Expected size (bp)	PCR conditions	PCR product
SHV-F SHV-R	GGGTTATTCTTATTTGTCGC TTAGCGTTGCCAGTGCTC	928	94°C, 5 min 35 cycles of 94°C, 1 min, 58°C, 1 min, 72°C, 1 min	SHV-1, -2, -5, -7, -11, -12, -18, -26, -32, -33, -38, -44, -46, -49
TEM-F TEM-R	ATAAAATTCCTGAAGACGAAA GACAGTTACCAATGCTTAATCA	1080	94°C, 5 min; 35 cycles of 94°C, 1 min, 58°C, 1 min, 72°C, 1 min	TEM-1, -52, -71, -104, -105, -138, -151, -152
CTX-M-F CTX-M-R	ACGCTGTTGTAGGAAGTG TTGAGGCTGGGTGAAGT	759	94°C, 5 min; 35 cycles of 94°C, 45 sec, 58°C, 45 sec, 72°C, 1 min	CTX-M-1, -3, -12, -15, -22, -30, -32, -33, -38, -52, -57, -58, -60, -61
OXA-1-F OXA-1-R	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	813	94°C, 5 min; 35 cycles of 94°C, 1 min, 58°C, 1 min, 72°C, 1 min	OXA-1, -4, -30, -31, -47
OXA-2-F OXA-2-R	TTCAAGCCAAAGGCACGATAG TCCGAGTTGACTGCCGGTTG	814	94°C, 5 min; 35 cycles of 94°C, 45 sec, 61°C, 45 sec, 72°C, 1 min	OXA-2, -3, -15, -21, -32

were identified at the species level using standard biochemical tests and microbiological methods. Only one isolate per patient was included in the study.

**Antibiotic susceptibility testing:** Disk-diffusion tests were carried out with antibiotic-containing disks on Mueller-Hinton agar plate (Merck). The results were expressed as susceptible or resistant according to the interpretative zone diameters recommended by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2006). The following antimicrobial agents were tested: amikacin (30 µg), ampicillin (10 µg), cefalotin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), gentamicin (10 µg), tetracycline (30 µg), ceftizoxime (30 µg) and norfloxacin (10 µg). Quality control strain including: *E. coli* ATCC25922 was used to make sure the antibiotic disks accuracy and to validate the procedures.

**Detection of ESBL production:** ESBL production was detected using the Double-Disk Synergy (DDS) test (Jarlier *et al.*, 1988). ESBL presence was assayed using the following antibiotic disks (MAST, UK): cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg). According to CLSI criteria for ESBL detection, each isolate with inhibition zone diameter ≤22 mm for ceftazidime or ≤27 mm for cefotaxime was considered as a potential ESBL-producer or screen positive. A 5 mm or more increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was considered for an ESBL-producing organism. *K. pneumoniae* ATCC700603

(positive control) and *E. coli* ATCC25922 (negative control) were used for quality control of ESBL tests.

**ESBL- PCR:** Template DNA was prepared as follows: a cell pellet from 1.5 mL of overnight culture was resuspended in 500 µL of TE (10 mM Tris, 1 mM EDTA, pH 8.0) after centrifugation and boiling for 10 min. After centrifugation, the supernatant was used for Polymerase Chain Reaction (PCR). The primers and conditions for PCR are listed in (Table 1) (Yao *et al.*, 2007).

**Statistical analysis:** Data were entered into a database using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). Differences between proportions were analyzed using the  $\chi^2$ -test. All differences in which the probability of the null hypothesis was  $p < 0.05$  were considered significant.

## RESULTS

During the study period, 301 consecutive clinical isolates of gram-negative were isolated. Of these, 160 (53%) were identified as *E. coli*. All isolates of *E. coli* were examined for ESBL production as shown in Fig. 1 and 2. Of the 160 *E. coli* isolates, 27 (16.8%) were positive for ESBL.

The sources of the ESBL-producing *E. coli* isolates tested are shown in Table 2. Urinary tract infections were the most abundant source of ESBL-producing *E. coli* strains (13.67%;  $p < 0.001$ ).

*Escherichia coli* isolates were frequently isolated from out patients 114 cases, in non-ICU wards 32 cases and in ICU wards 14 cases. The prevalence of ESBL-producing isolates differed between hospital wards (Table 3). The prevalence of ESBL-positive *E. coli*

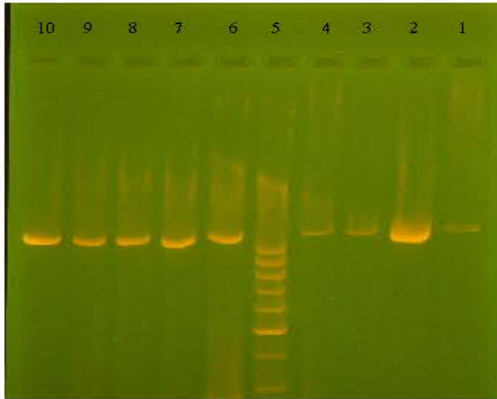


Fig 1: Electrophoresis of TEM gene PCR products (1080 bp), Line 5 load with marker 100 to 1000 bp and other lines load with clinical strains

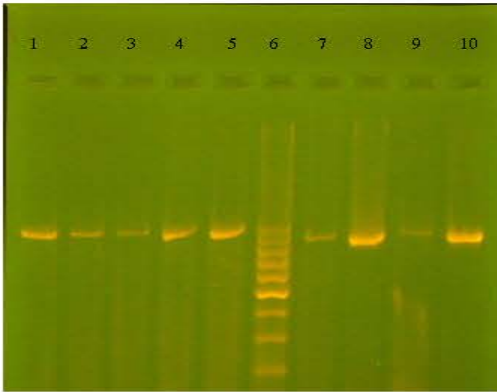


Fig 2: Electrophoresis of SHV gene PCR products (928 bp), Line 6 load with marker 100 to 1000 bp and other lines load with clinical isolates

Table 2: Distribution according to clinical sources of *Escherichia coli* isolates producing Extended-Spectrum  $\beta$ -Lactamases (ESBL)

Source	Number of ESBL-producing isolates	
	Tested	Positive (%)
Urinary tract	139	19 (13.67)
Respiratory tract	8	6 (75.00)
Blood	8	1 (12.50)
Other*	5	1 (20.00)
Total	160	27 (16.80)

\*Skin, soft tissue, pus, etc.  $p < 0.0001$

isolates obtained from Toohid Hospital patients was significantly higher than those Beasat Hospital patients.

ESBL-negative *E. coli* isolates were more sensitive than ESBL-positive isolates (Table 4). In general, susceptibility rates of *E. coli* isolates to ampicillin, gentamicin, trimethoprim/sulfamethoxazole (SXT) and tetracycline were very low. Amikacin, norfloxacin,

Table 3: Frequency of ESBL-producing *Escherichia coli* isolates in the different hospital wards

Hospitals wards	Number of ESBL-producing isolates	
	Tested	Positive (%)
Beasat hospital	86	14 (16.2)
ICU	10	6 (60)
Other wards*	11	0
Present patients	65	8 (12.3)
Toohid hospital	74	13 (17.5)
ICU	4	0
Other wards*	21	6 (28.5)
Present patients	49	7 (14.2)

\*Medicine, neurology, surgery, urology, orthopedics, infectious diseases, etc.  $p < 0.0001$

Table 4: Antibiotic susceptibility rate (%) of extended-spectrum  $\beta$ -lactamase (ESBL)-positive and ESBL-negative *Escherichia coli* isolates

Antibiotics	ESBL-negative (n = 133)	ESBL-positive (n = 27)	Total (n = 160)	p-value <sup>a</sup>
Ceftazidime	74.44	0.00	61.88	<0.001
Cefotaxime	73.68	0.00	61.25	<0.001
Cefalotin	79.70	20.00	66.88	<0.001
Ampicillin	34.59	11.11	30.00	<0.001
Amikacin	94.74	52.26	85.63	<0.001
Ceftriaxone	78.20	10.00	65.00	<0.001
Ciprofloxacin	81.95	22.15	70.63	<0.001
Cotrimoxazole	45.86	19.50	40.63	<0.001
Gentamicin	48.12	23.22	43.75	<0.001
Tetracycline	40.60	35.65	38.75	<0.001
Ceftiozime	82.71	29.11	73.13	<0.001
Norfloxacin	80.45	28.33	70.00	<0.001
Nalidixic acid	65.41	55.20	63.75	<0.001

<sup>a</sup>Exact p-values were determined by  $\chi^2$  test. For statistical analysis, the response of isolates to antibiotics was categorised as susceptible and non-susceptible (Consisting of intermediate and resistant groups).  $p < 0.05$  was considered statistically significant

ciprofloxacin and third-generation cephalosporins were shown to be the most active antibiotics against these isolates *in vitro*. ESBL-positive *E. coli* isolates were resistant to most beta-lactams as well as to non-beta-lactams such as fluoroquinolones, gentamicin, SXT and tetracycline. Their resistance rates to most antibiotics were significantly ( $p < 0.001$ ) higher than those of ESBL-negative isolates. ESBL-producers were more susceptible to amikacin (52.26%) and tetracycline (35.65%). ESBL-negative *E. coli* strains were more susceptible than ESBL-positive *E. coli* isolates to cotrimoxazole (45.86 vs. 19.50%,  $p < 0.0001$ ).

## DISCUSSION

Since, emerging of the first plasmid-mediated beta-lactamase in gram-negatives (Datta and Kontomichalou, 1965), many studies have addressed the emergence of ESBL-positive *E. coli*. The current study is the first report that focusing on the distribution of ESBL-producing *E. coli* in our University Hospitals. The overall rate of ESBL-producing *E. coli* was lower than the other Iranian studies. Mehrgan and Rahbar (2008) reported from Iran

that studied in ICU patients. Although, the high rate of ESBL production (16.8%) by *E. coli* isolates in our University Hospitals in compare with Tonkic *et al.* (2005) and Ozgunes *et al.* (2006) studies indicate the excessive use of broad-spectrum antibiotics in our hospital and a lack of attention to laboratory screening of ESBL production by clinical isolates.

Most of ESBL-positive *E. coli* have been isolated from urinary tract infection (19/27). Although, the high rate of ESBL-positive isolate with respiratory tract infection might be determine the nosocomial spread of this enzyme. This study results concerning the high prevalence of ESBL-positive *E. coli* isolates obtained from Toohid hospital patients than those obtained from Beasat hospital patients are largely in accordance with existence of Infectious ward in Toohid hospital. Inside the Toohid hospital the high rate of ESBL producing *E. coli* was obtained from ICU ward. This is may be indicate the nosocomial spread of isolate, although due to a number of limitations we could not prove this possibility by determining plasmid profiles and pulsed-field gel electrophoresis patterns of the isolates.

In general, susceptibility rates of *E. coli* isolates to ampicillin, gentamicin, trimethoprim/sulfamethoxazole (SXT) and tetracycline in comparison with other studies (Rodriguez-Bano *et al.*, 2006) were very low. Therefore, reduced susceptibilities to gentamicin in *E. coli* isolates as reported from the Norwegian study (Tofteland *et al.*, 2007) may indicate increasing the resistance rate to this antibiotic.

Associated resistance to non-beta-lactams such as fluoroquinolones, gentamicin, SXT and tetracycline was frequently observed among ESBL-positive isolates. Their resistance rates to most antibiotics were significantly ( $p < 0.001$ ) higher than those of ESBL-negative isolates. Overall, as many as 18.05% of *E. coli* isolates producing ESBLs were nonsusceptible to ciprofloxacin. However, in this study, the incidence of fluoroquinolone resistance among ESBL-producing isolates was approximately two times higher than that reported by Russian studies (Edelstein *et al.*, 2003).

Amikacin remained active against 52.26% of *E. coli* isolates, producing various types of ESBLs, however, the susceptibility rate was lower than other studies (Kim *et al.*, 2008; Tonkic *et al.*, 2005; Mendelson *et al.*, 2005). The susceptibility rate to SXT in ESBL-positive isolates was similar to earlier studies in Iran (Mehrgan and Rahbar, 2008). These results shown the reduction of value of SXT in the treatment of infections caused by ESBL-positive isolates.

In conclusion, a high prevalence of antibiotic resistance in ESBL-positive *E. coli* was observed in our

hospital setting. As the available treatment options are limited, antibiotic control policies together with the implementation of infection control measures remain of high importance.

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