Antimicrobial Resistance Pattern of Extended-Spectrum β-Lactamases (ESBLs) producing Escherichia coli Isolated from Clinical Samples in Tabriz city, Iran

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ABSTRACT

Background: Extended spectrum β-lactamase (ESBL) producing Escherichia coli has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital-acquired infections. This could be attributed to association of multi drug resistance in ESBL producing isolates. The present study was aimed to determine the antimicrobial resistance profile of ESBL producing Escherichia coli isolates from various clinical samples.

Methods: In this study, 204 cases of Escherichia coli within 7 months from patients referring to the public and private treatment centers Tabriz city were collected and identified by biochemical tests. ESBL screening and confirmation along with antimicrobial resistance test was done according to the Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Of the 204 isolates, 110 (53.92%) were identified as having ESBL producing phenotype. Over 90.2% of ESBL isolates showed resistance to ampicillin. 83.6% of ESBL isolates were imipenem sensitive.

Conclusion: The prevalence of ESBL is increasing day by day in nearly every center of different countries and necessary steps to prevent the spread and emergence of resistance should be taken.

INTRODUCCIÓN

The production of extended-spectrum β-lactamase (ESBL) enzymes is a common mechanism of resistance. ESBLs are enzymes that confer resistance to most beta-lactam antibiotics including penicillins, cephalosporins, and the monobactam aztreonam [7]. These enzymes have been found exclusively in Gram-negative organisms [2]. Although the prevalence of ESBL-producing Escherichia coli (E. coli) can vary from country to country, resistance rates to many commonly used therapies have increased throughout the world [12,4,5,6,7].

Escherichia coli is the most abundant facultative anaerobe of the human intestinal microflora. A limited number of pathogenic E. coli clones have gained specific virulence attributes which enable them to cause urinary tract infections, sepsis, meningitis and diarrheal disease [8]. E.coli is one of the most common organism among ESBL producing microbes. The extended-spectrum β-lactamases (ESBLs), are enzymes that hydrolyze the extended-spectrum cephalosporin, like Ceftazidime and Cefotaxime, and / or the Monobactam Aztreonam [9]. which is one of the most popular ‘virulent enzyme.’ The first isolate of ESBL producing bacteria was reported in Germany in 1983 [10].

Current knowledge of prevalence of ESBL production by commonly isolated organism such as E. coli is necessary to understand the disease burden and to take necessary action to prevent the spread. Therefore the present study was conducted with an objective to find out the prevalence of ESBL producing E. coli and its antimicrobial resistance profile to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.
MATERIALS AND METHODS

This descriptive cross-sectional study, for a period 7 months (from April to October of 2013) on 204 strains of *Escherichia coli* isolated from clinical samples (blood and urine) of patients referred to treatment centers, public and private Tabriz city was made, and after at least 14 test biochemical tests, including IMViC, amino acid decarboxylase, oxidase, Malonate consumption, urease were identified. Antimicrobial susceptibility testing was performed using the disc diffusion method of the Clinical and Laboratory Standards Institute (CLSI) with discs from Padtan Teb - Iran. The results were interpreted according to the current guidelines of the CLSI [11].

The following antibiotics were tested: Ceftazidime (30 micrograms), ceftriaxone (30 micrograms), cefotaxime (30 micrograms), ceftazidine (15 micrograms), imipenem (10 micrograms), ampicillin (15 micrograms), gentamicin (10 micrograms), amikacin (30 micrograms), Co-trimoxazole (25 micrograms), cefpodoxime (30 micrograms). In this method, Mueller-Hinton agar medium (Merck) and 35°C of incubation for a period 24-18 hours. The screening test in order to identify bacteria producing ESBLs in accordance with the instructions NCLS using the disc antibiotics Cefpodoxime, cefotaxime, ceftazidine, ceftriaxone and aztreonam was conducted. Confirmatory test of a series discs of ceftazidime (30 micrograms) and cefotaxime (30 micrograms) distance of 25 mm (center to center) of ceftazidime / clavulanic (30 / 10μg) and cefotaxime / clavulanic acid (30 / 10μg) environment Mueller Hinton agar, from each other was placed. Plate temperature of 35°C, were incubated for a period 24 to 18 hours. If the inhibition zone around the disk containing clavulanic acid, at least 5 (mm) from the disk without clavulanic acid was higher was considered as positive confirmatory tests for ESBLs [12]. The standard strain, *Klebsiella pneumoniae* ATCC 700603 as a positive control and *Escherichia coli* ATCC 25922 was used as a negative control. The collected data were statistically analyzed using SPSS version 18. 

Results:

In this study antibiotic resistance patterns 204 *Escherichia coli* strains isolated from patients referred to treatment centers of Tabriz city, compared to 10 antibiotics were determined using DAD (Disk Agar Diffusion). Samples were collected from 111 (54.41%) females and 93 (45.59%) males. Of the 204 *E. coli* isolates, 110 (53.92%) ESBL *E.coli* isolated and 94 (46.08%) non-ESBL producing *E.coli* isolated. The result of ESBL and non-ESBL producing *E.coli* recovered from urine and blood given in Table 1. 72.72% of ESBL-producing isolates were from females and 27.28% isolates from males. In 7 of the antibiotic, resistance of greater than 50% is observed. This difference is meaningful.

### Table 1: Results of ESBL producing *E.coli* in urine and blood specimens.

<table>
<thead>
<tr>
<th><em>E.coli</em> isolated N=204</th>
<th>Urine</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL</td>
<td>90(44.12%)</td>
<td>20(9.8%)</td>
</tr>
<tr>
<td>Non-ESBL</td>
<td>80(39.22%)</td>
<td>14(6.86%)</td>
</tr>
<tr>
<td>Total N</td>
<td>170(83.34%)</td>
<td>34(16.66%)</td>
</tr>
</tbody>
</table>

High-level resistance was for ampicillin (90.2%) and least resistance was found to imipenem (9.9%) (Figure 1).

**Fig. 1:** Antibacterial resistance of ESBL and non-ESBL producing *Escherichia coli*.

**Discussion:**

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. This has increased the hospital stay of the patients, which in turn causes economic burden. In the present study, an attempt was made to understand the prevalence of ESBL producing *E. coli*.

The emergence and spread of ESBL-producing bacteria appear to be mostly caused by the widespread use of broad-spectrum beta-lactam drugs. So that nowadays, we are witnessing the increasing rate of the bacteria in different parts of our treatment centers. The prevalence of ESBL-producing *Escherichia coli* in clinical samples in this study is 53.92% cent. In studies performed throughout the world, the frequency of ESBL positive
E. coli was from 0.2% to 95.4% [13,21]. For example, in a study Tasli [22], in Turkey producing ESBL enzymes in Escherichia coli strains of 17% [22], in the study Villegas, in Colombia 3.3-4.7% [23], in the study Duttaroy [24], in India 29.1 percent [24], in study Lavigne [11], in France 16.2 percent [11], have been reported.

On the other hand, study of Zhou [25], in Shanghai show that 47.4% of E. coli isolated from patients were ESBL producers [25], in another study by the WU, be was conducted in Taiwan hospitals, a rate of 18.18% of ESBL-producing E. coli [26], While in Lebanon the amount of at 28.1 percent, respectively [16].

Comparing these results indicate that the rate of ESBL in strains isolated from different countries and also within a country -from a hospital- with other hospital varies, this issue depending on infection control and management of the hospital, are differrent [18].

In study Stephan in Tanzania, resistance towards ampicillin 92.7 percent reported. (23) whereas in the present study, this ratio is 90.2 percent. In this study, 83.6 percent of strains were sensitive to imipenem, Whereas in other studies the rate of 91.7 to 100 percent have been reported [27,30].

In the present study 55 percent of the samples positive ESBL, were resistant to ceftazidime and this indicates the increase in resistance to these drugs in our country compared to the past, with regard to this results to prevent the increase in resistance in the above consumption antibiotics carefully. In this study, compared the pattern Resistance, Escherichia coli producing ESBL compared to antibiotics is high, with the results achieved by the study Babypadmini and his colleagues compatible [31].

Conclusion:
Finally, the present study showed high prevalence of ESBL-producing E. coli in our region. Appropriate use of antibiotics and following of CLSI guidelines for screening beta-lactam resistance by using DAD test is recommended.

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REFERENCES


