Original Research Article

Frequency and antibiotic resistance pattern of the clinical isolates of extended spectrum beta Lactamase producing *Escherichia coli* from the blood specimens in hospitals and medical centers in Tabriz, Iran

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DOI: 10.22034/HBB.2019.24

Received: September 3, 2019; **Accepted:** October 13, 2019

ABSTRACT

Production of Extended Spectrum Beta Lactamase (ESBL) enzymes is one of the main reasons for drug resistance in *Escherichia coli* (*E. coli*) isolates. The aim of this study was to determine the ESBL producing *E. coli* isolates in the blood culture of patients in hospitals and medical centers in Tabriz, Iran. In this descriptive cross-sectional study, 200 isolates of *E. coli* from blood specimens in hospitals and medical centers were investigated from December 2018 to May 2019. The production of ESBLs was confirmed using the combined disc method and antimicrobial susceptibility test. Out of 200 isolates, 100 isolates (50 %) were able to produce ESBL. The highest antibiotic resistance of isolates (100 %) was to ceftriaxone, cefotaxime, cefixime and cefuroxime. The highest antimicrobial susceptibility was also observed to amikacin (12 %), meropenem (6 %) and imipenem (2 %), respectively. The results of this study showed the high resistance of *E. coli* isolates especially ESBL producing strains to different antibiotics. Given the relatively high prevalence of the ESBL producing *E. coli* strains and their resistance to common antibiotics, effective control strategies such as the restricted use of broad spectrum cephalosporins should be implemented.

***Keywords*:** *Escherichia coli*, antibiotic resistance pattern, extended spectrum beta Lactamases

INTRODUCTION

Escherichia coli (*E. coli*), as a Gram-negative bacillus from the Enterobacteriaceae family [1] is one of the common pathogens responsible for nosocomial infections [2-3]. Among the different types of nosocomial infections, bloodstream infections are very serious health problems worldwide [4]. One of the most important mechanisms used by gram-negative bacteria against beta-lactam antibiotics is the production of beta-lactamase enzymes [5]. These enzymes hydrolyze a wide range of beta-Lactam antibiotics including penicillins, cephalosporins and monobactams. Production of beta-Lactamase enzymes has emerged as an important mechanism for the resistance against beta-Lactam drugs, which include approximately 50% of used common antibiotics [6-8]. Since the identification of these enzymes in 1989 until now, more than 150 types of ESBLs have been identified, which have been mostly detected in the Enterobacteriaceae family [9]. Extended-spectrum beta-Lactamases (ESBLs) are mainly produced by Klebsiella and *Escherichia coli* and other members of Enterobacteriaceae, respectively [10].

The prevalence of ESBL-producing strains in clinical isolates varies in different cities and counties. In the United States, the production of these enzymes by the members of Enterobacteriaceae family has increased by 0-25 %. The frequency of ESBL-producing bacteria in other countries varied from 3-8 % in Sweden, 34 % in Portugal, 4.8 % in Korea, 8.5 % in Taiwan, 12 % in Hong Kong to 58 % in Turkey [11-12]. Nowadays, numerous reports indicated that the prevalence of these strains is a worldwide problem. In these studies, the multidrug resistance bacteria have been identified as one of the major medical problems, and the need for investigation and control measures have been emphasized [13]. The administration of broad-spectrum antibiotics is necessary for the treatment of infections caused by these strains. This is particularly complicated in patients with weakened immune systems and those admitted to intensive care units. Therefore, microbiology laboratories play an important role in identifying and reporting the ESBL- producing bacteria and facilitating effective treatment for the patients [14]. Due to the importance of ESBL-producing bacteria, their prevalence needs to be determined in order to take appropriate measures to treat the infections caused by them. Since the prevalence of these strains is increasing in hospitals and medical centers, isolation of ESBL-producing strains of *E. coli* from clinical specimens and determination of their antibiotic resistance

pattern were the main objectives of this study.

MATERIALS AND METHODS

In this descriptive cross-sectional study, 200 isolates of *E. coli* were provided from blood cultures of patients who were suspected to bacteremia in hospitals and health centers of Tabriz during a 6 months period (December 2018 to May 2019). A questionnaire was designed and coded for each patient. The sampling was performed after specifying the purpose of the study for each patient under study and the written consent was obtained from them. The isolates were confirmed using at least 14 biochemical tests including IMViC, amino acid decarboxylation, oxidase, urea and malonate utilization tests. Antibiotic resistance pattern of the isolates was determined using the Disc Diffusion Agar (DAD) method according to the guidelines of Clinical & Laboratory Standards Institute (CLSI). The used antibiotic discs were included: ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), aztreonam (15 µg), imipenem (10 µg), meropenem (10 µg), ampicillin (10 µg), gentamicin (10 µg), amikacin (30 µg), cotrimoxazole (25 µg), cefalexin (30 µg), cefixime (5 µg), ciprofloxacin (5 µg) and cefuroxime (30 µg). In the disc diffusion test, the diameter of inhibitory zones around each disc was measured and the results were reported according to CLSI standards. Combined disc method was used for phenotypical evaluation of ESBL-producing strains. In this method, the discs of ceftazidime plus cefotaxime (30+10 µg), ceftazidime plus clavulanic acid (30+10 µg) and cefotaxime plus clavulanic acid (30+10 µg) were placed on Mueller-Hinton agar medium (Oxoid, England) with a distance of 25 mm from each other (center to center). The plates were incubated at 35 °C for 18-24 h. If the inhibitory zone around clavulanic acid-containing discs was at least 5 mm higher than clavulanic acid-free discs, it would be considered as positive for ESBLs [15]. Standard strains of *Klebsiella pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were used as positive and negative controls, respectively.

RESULTS

In the present study, *E. coli* were isolated from 200 blood specimens of patients including 126 hospitalized (63 %) and 74 ambulatory patients (37 %). Out of total isolates, 119 (59.5 %) and 81 (40.5 %) isolates were belonged to female and male patients, respectively. In this study, antibiotic resistance pattern of the isolates was identified using the DAD method and 16 types of antibiotics. The highest resistance of

isolates (100 %) was observed to ceftriaxone, ceftriaxone, cefotaxime, cefixime, cefuroxime. Also, the lowest antibiotic resistance was detected to amikacin (12 %), meropenem (6 %) and imipenem (2 %), respectively (Figure 1).

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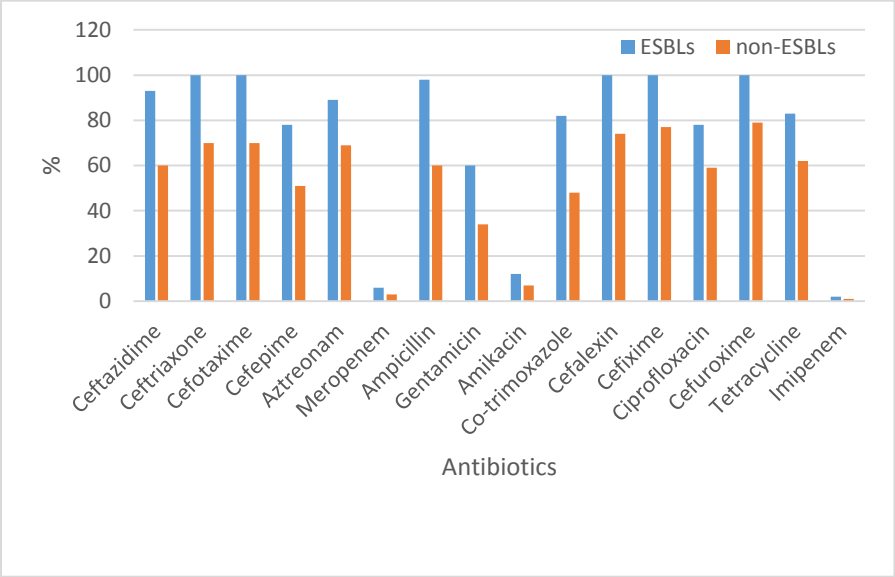

Figure 1. The Antibiotic resistance frequency of ESBL and non-ESBL-producing isolates of *Escherichia coli* against common antibiotics using disc diffusion agar method.

Of the total 200 isolates, 100 isolates (50 %) were identified as ESBL-producing strains which had a higher percentage of resistance to different antibiotics than others. Seventy percent (70 %) of ESBL-producing strains were isolated from females while 30 % of them were detected in males. The mean age of patients with ESBL-producing *E. coli* was 50 ± 24.4 years. The resistance of isolates was significantly more than 50 % for about 13 types of antibiotics ($p<0.05$).

DISCUSSION

Bacteremia is one of the leading causes of death among patients. Drug-resistant Gram-

negative bacilli, particularly the members of the Enterobacteriaceae, are the most common organisms responsible for bacteremia in hospitals, and the infections caused by them are associated with high mortality [16-17]. Production of beta-Lactamases is the main resistance mechanism of gram-negative bacteria against beta-Lactams. As beta-Lactam antibiotics are used clinically, beta-Lactamases evolved along with these drugs and played a major role in the therapeutic failures in antibiotic therapy [18]. Since the fifteen years ago, there have been numerous outbreaks of infections caused by beta-Lactamase-producing organisms throughout the world. This phenomenon is a major threat to the use of cephalosporins. It also has been well established that treatment for such cephalosporin-resistant infections will not be led to satisfactory results. The mortality rate caused by ESBL-producing strains is significantly higher than the others. Another issue is whether cephalosporins treatment is appropriate for ESBL-positive organisms whose MIC is in the sensitive range [19]. The emergence and distribution of ESBL-producing bacteria occur due to the widespread use of broad-spectrum beta-lactams, and the prevalence of these strains are increasing in the hospitals in recent years. In this study, the prevalence rate of ESBL-producing *E. coli* strains in clinical samples was 50 %. In similar studies, the prevalence rate of these strains was reported as 17 % in Turkey [20], 3.3–7.7 % in Colombia [21], 29.1 % in India [22], 4.7 % in the USA [23] and 16.2 % in France [24]. On the other hand, in the study of Zhou [25] in Shanghai, it was shown that 47.4 % of *E. coli* isolates from the patients were positive for ESBL. In another study conducted by Wu. [26] at Taiwan hospitals, ESBL-producing *E. coli* was 18.18 %. However, this prevalence was reported as 28.1 % in Beirut, Lebanon [27]. In the study of Fazeli [28] on the clinical specimens of a hospital in Isfahan, 150 (53.9 %) of 278 *E. coli* isolates were positive for the production of ESBL. Mehrgan [29] also reported a prevalence rate of 16 % for beta-Lactamase-producing *E. coli* in a tertiary care hospital in Tehran. Comparison of the above results with the findings of present study shows that the prevalence of ESBL-producing isolates varies between different countries as well as from one hospital to the another one in a country, depending on the infection control system and the treatment level of patients [30]. In the study of Mardaneh [31], 58 % of *E. coli* isolates from blood cultures, were positive for beta-lactamase production, and the most effective antibiotics against these strains were colistin, polymyxin B, imipenem, meropenem, amikacin and piperacillin-tazobactam. Jafari Sales and

Mobin [32] reported that 100 (51.02 %) of 196 *E. coli* isolates from medical centers in Marand city (Iran) were positive for ESBL production. Eighty-two percent (82 %) of the isolates were ampicillin-resistant while 7 % of them were sensitive to imipenem. In the study of Haghighi [33], 33.5 % of isolates were able to produce ESBLs. The highest resistance of isolates (71.35 %) was against amoxicillin and all strains (100 %) were sensitive to imipenem. In the study of Mohajeri [34] studied the frequency of ESBL-producing *E. coli* isolates from urinary tract infections in Kermanshah, Iran. They found that the highest susceptibility and resistance of isolates was to imipenem (100 %) ampicillin (77 %), respectively. Rahbar [35] also reported the highest rate of resistance in *E. coli* isolates to ampicillin (18 strains, 100 %). In the study of Mshana in Tanzania [36], the resistance rate of isolates to ampicillin was 92.7 %, whereas in the present study it was 98 %. In this study, 98 % of isolates were susceptible to imipenem, whereas in other studies this rate was varied from 91.7 % to 100 % [37-38]. In the present study, 100 % of ESBL-positive strains were resistant to cefalexin, ceftriaxone, cefotaxime, cefixime and cefuroxime. This result indicates the increased resistance to these drugs in the country. According to the above results, adequate precautions should be taken to prevent the increasing resistance to these antibiotics. The phenomenon of antibiotic resistance annually imposes huge costs on the healthcare systems of the countries. In addition to the misuse of antibiotics, aggressive therapeutic measures, increasing the number of immunocompromised patients and the lack of practical advices in infection control also play an important role in the increasing of the antibiotic resistance.

CONCLUSION

According to the results of this study, the high prevalence of resistance to ESBL-producing *E. coli* strains is an important health hazard for hospitalized and ambulatory patients. Also, producing ESBLs by these strains is a major threat to the use of extended spectrum cephalosporins. Therefore, in order to treat infections potentially caused by ESBL-producing strains, the appropriate antibiotic should be selected based on the results of antibiogram test. To reduce the prevalence of beta-lactamase-producing organisms, further studies in this area, the strict controlling measures as well as the reduced use of antibiotic are necessary.

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