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# Evaluation of the presence of *AmpC (FOX)* beta-lactamase gene in clinical strains of *Escherichia coli* isolated from hospitalized patients in Tabriz, Iran

**Zahra SADEGHI DEYLAMDEH[1], Abolfazl JAFARI SALES[2,*]**

[1]Department of Biology, Faculty of Sciences, Malayer Branch, Malayer, Iran
[2]Department of Microbiology, School of Basic Sciences, Kazerun Branch, Islamic Azad University, Kazerun, Iran

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**Abstract**

Beta-lactamases are the most common cause of bacterial resistance to beta-lactam antibiotics. *AmpC*-type beta-lactamases hydrolyze cephalosporins, penicillins, and cephamycins. Therefore, the study aims was to determine antibiotic resistance and to investigate the presence of *AmpC* beta-lactamase gene in clinical strains of *Escherichia coli* isolated from hospitalized patients in Tabriz. In this cross-sectional descriptive study, 289 *E. coli* specimens were collected from clinical specimens. Disk diffusion method and combined disk method were used to determine the phenotype of extended spectrum β-Lactamase producing (ESBLs) strains. Then PCR was used to evaluate the presence of *AmpC (FOX)* beta-lactamase gene in the strains confirmed in phenotypic tests. Antibiotic resistance was also determined using disk diffusion by the Kibry-Bauer method. A total of 121 isolates were identified as generators of beta-lactamase genes. 72 (59.5 %) isolates producing ESBL and 49 (40.5 %) isolates were identified as *AmpC* generators. In the PCR test, 31 isolates contained the *FOX* gene. The highest resistance was related to the antibiotics amoxicillin (76.12%), ceftazidime (70.24%) and nalidixic acid (65.05%). The results indicate an increase in the prevalence of beta-lactamase genes and increased resistance to beta-lactam antibiotics, which can be the result of improper use of antibiotics and not using antibiotic susceptibility tests before starting treatment. Also, using phenotypic and molecular diagnostic methods such as PCR together can be very useful.

**Keywords:** extended spectrum β-lactamase producing, *AmpC*, *Escherichia coli, FOX* gene

## 1. Introduction

*Escherichia coli* is a major member of the Enterobacteriaceae family and is known to cause many nosocomial infections such as gastroenteritis, neonatal meningitis, sepsis, and urinary tract infections (Eslami and Najar Peerayeh, 2012). *E. coli* is the most common gram-negative bacillus isolated from clinical cases (Jafari Sales et al., 2014; Jafari-Sales and Rasi-Bonab, 2017; Jafari Sales and Mobaiyen, 2017) and is also the cause of more than 80% of cases of community-acquired urinary tract infections (Yazdi et al., 2010). Beta-lactamase is an enzyme that inactivates beta-lactam antibiotics. Penicillinase was the first beta-lactamase to be identified. This enzyme was first isolated from *E. coli* in 1940 (Agrawal et al., 2008). These enzymes inactivate almost all beta-lactam antibiotics by hydrolyzing the amide bond in the beta-lactam ring (Jafari-Sales, 2018; Jafari-Sales and Shadi-Dizaji, 2018; Jafari-Sales et al., 2019; Jafari-Sales and Khaneshpour, 2020). Excessive use of new antibiotics to treat patients and selective pressure on bacteria may affect the production of new beta-lactamases by bacteria (Peter-Getzlaff et al., 2011). In gram-negative pathogens, the production of beta-lactamases is considered to be the most important cause of resistance to beta-lactam antibiotics (Rahimi et al., 2014; Shebani et al., 2010). *E. coli* strains are resistant to beta-lactams through several mechanisms, including changes in outer membrane proteins, overproduction of cephalosporinase (chromosomal and plasmid), or production of an ESBL (Mohamudha et al., 2012). Classification of beta-lactamases functionally began when cephalosporinases were differentiated from penicillinases (Fleming et al., 1963). *AmpC* beta-lactamases were discovered in the late 1970s. *AmpC* beta-lactamases belong to group C or group 1 cephalosporinases, but to some extent have the ability to hydrolyze other beta-lactams. These enzymes hydrolyze broad-spectrum cephalosporins and monobactams but are not inhibited by conventional inhibitors such as clavulanate (Mohamudha et al., 2012). *AmpC* beta-lactamases include *ACC, CMY, FOX, ACT, MOX* and *MIR*. The *ACT, FOX*, and *MOX* genes are resistant to cephalosporins such as ceftazidime, cefotaxime, and aztronam (Philippon et al., 2002). Beta-lactam antibiotics are widely used and the production of *AmpC*-type beta-lactamases results in significant antimicrobial resistance (Peter-Getzlaff et al., 2011). *AmpC* beta-lactamases are generally chromosomal but are easily propagated between other organisms due to motile genetic elements, especially plasmids, and because they are resistant to beta-lactamase inhibitors, especially clavulanic acid, can lead to disruption as a result of confirmatory test ESBL (False negative). Therefore,

* Correspondence: A.jafari_1392@yahoo.com

phenotypic diagnosis of *AmpC*-producing microorganisms and ESBLs is difficult (Hanson and Sanders, 1999). The prevalence of *E. coli* strains producing ESBLs in nosocomial infections is increasing and is of concern to the medical community; therefore, this study was performed to evaluate the presence of *AmpC* beta-lactamase gene in clinical strains of *E. coli* isolated from hospitalized patients in Tabriz.

## 2. Materials and methods

### 2.1. Sampling

In this descriptive cross-sectional study, 289 *E. coli* samples were collected from clinical specimens including urine, feces, wounds, and blood during six months from March to August 2020 by random sampling method. Clinical samples were collected weekly from hospitalized patients to different wards of the hospital with positive culture for *E. coli*. Then, after obtaining the consent of each patient and filling in the form of their personal and clinical details and observing the statute of the Ethical Commitment Committee regarding the secrecy of the name and details of each person tested, the samples were transferred to the specialized microbiology laboratory of the Islamic Azad University, Ahar branch, and we proceeded to isolate and identify the bacteria causing the infection.

### 2.2. Isolation and identification

Samples were incubated on Blood agar and McConkey agar for 24-18 hours at 37 °C. The grown colonies were then identified by differential biochemical tests including simon citrate, lysine decarboxylase, urease, TSI, SIM and MR / VP (all media were obtained from Merck, Germany). Then, for further studies, these bacteria were frozen in tryptix medium in broth (Merck, Germany) containing 20% glycerol until further tests at -20 °C (Lin et al., 2010).

### 2.3. Investigation of drug resistance pattern

Antibiotic resistance pattern of all *E. coli* isolates by Kirby–Bauer standard method and through CLSI (Clinical and Laboratory Standards Institute instructions) and preparation of 0.5 McFarland concentration of bacteria and culture on Mueller-Hinton agar medium against imipenem antibiotics (10 µg), Cefotaxime (30 µg), ceftazidime (30 µg), nalidixic acid (30 µg), amoxicillin (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), norfloxacin (10 µg), and cefxime (30 µg) (Padtan Teb, Iran) was performed. Results were reported according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2013). *E. coli* strain ATCC 25922 was used as quality control of strains for antimicrobial susceptibility testing.

### 2.4. Phenotypic confirmation of ESBL and *AmpC* producing strains

Combined disk method was used to confirm ESBL-producing bacteria according to CLSI instructions. To confirm the ESBL producing isolates, they were tested using the combined disks of Cefotaxime + Clavulanic acid and Ceftazidime + Clavulanic acid (Jafari et al., 2013). To confirm the phenotypic nature of *AmpC*-producing strains, Cefoxitin sensitivity and Clavulanic acid insensitivity were used and strains with the growth inhibition zone less than 18 mm were selected as possible bla*AmpC*-producing strains (Rostamzad and Padervand, 2016).

### 2.5. Preparation of samples for molecular analysis

Then, all bacteria were cultured in Loriabertani liquid medium for 24 hours at 37 ° C overnight, and then DNA extraction was performed from the samples using the Invitek Strateg Business kit (made in Canada). The *FOX* gene was then applied together in a PCR reaction based on the specific b-oligonucleotide primers F-AACATGGGGTATCAGGGAGATG and R-CAAAGCGCGTAACCGGATTGG (190 bp) (Mansouri et al., 2014). The final reaction mixture with a volume of 50 µl included 0.1 mmol of each primer, 2.5 µl of 1X PCR buffer, 1.5 mmol of MgCl2, 0.2 mmol of dNTPs, 2 µl of template DNA, 1.5 units of Taq DNA enzyme (Sinagen Iran), which the final volume was increased to 50 µl with sterile deionized water. Thermocycle program (Eppendorf master cycler Germany) 35 cycles as initial denaturation for 3 minutes at 94°C, denaturation stage for 1 minute at 94°C, annealing stage for one minute at 58°C, Extension/elongation stage for one minute at 72 °C, and the final elongation step was performed for 10 minutes at 72°C (Pérez-Pérez and Hanson, 2002). Electrophoresis of PCR products in 0.8% agarose gel was performed in the presence of 100 bp markers and after staining with ethidium bromide, the results were observed with UV. Positive control included (*FOX* gene positive) samples donated by Tabriz University of Medical Sciences. Analyze the data, the twentieth version of SPSS software and Chi-square test was used. $P<0.05$ was considered statistically significant.

## 3. Results

From 289 samples of *E. coli* in this study, 168 samples of men (58.13%) and 121 samples of women (41.87%) were collected. There were 151 urine samples (52.25%), 70 stool samples (24.22%), 40 blood samples (13.84%) and 28 wound samples (9.69%). The mean age of patients was 44.7±28 years ranging from a minimum of one year to a maximum of 68 years. Antibiogram results show that the highest resistance is related to the antibiotics amoxicillin (76.12%), ceftazidime (70.24%) and nalidixic acid (65.05%) (Figure 1). A total of 121 isolates were identified by the combined disk method as generators of beta-lactamase and *AmpC* genes. 72 isolates producing ESBL and 49 isolates were identified as presumptive producing *AmpC*, which were selected for molecular analysis. In the PCR test, 31 isolates contained the *FOX* gene.

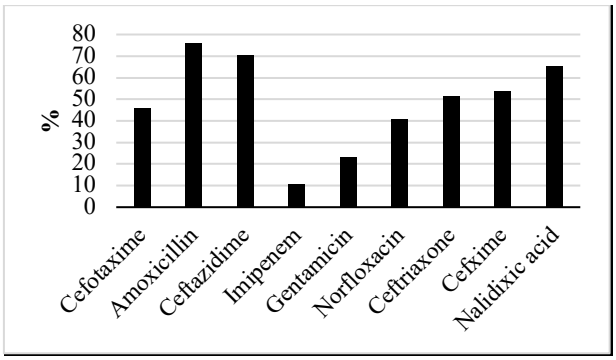


**Fig. 1.** Percentage of frequency of antibiotic resistance in *E. coli*

## 4. Discussion

*AmpC* β-lactamases are dramatically recognized as a growing clinical problem (Den drijver et al., 2018). Detection of *AmpC*-type beta-lactamases in *E.coli* challenges microbiological laboratories. For better treatment, and molecular diagnosis, the use of PCR and other sequencing techniques is necessary, but these techniques are not always available (Kiiru et al., 2012). One of the objectives of this study was to determine the productive strains of beta-lactamase and *AmpC* enzymes.

In the present study, out of 121 isolates, 72 ESBL-producing isolates (59.5%), and 49 *AmpC*-producing isolates (40.49%) were identified. In a study conducted in 2012 in Tehran (Soltan et al., 2013) on clinical specimens including urine, stool, blood and wounds, using a combined disk method, out of 128 samples, 115 isolates (89.8%) were ESBL generators and 13 isolates (10.2%) were *AmpC* generators. The results of this study do not match our study. In a study conducted in 2014 in Kerman (Mansouri et al., 2014) on clinical specimens including urine, blood, and body fluids, using a combination double disk test method, 133 isolates (39.3%) were generators of *AmpC* which is similar to our research and 148 samples (43.76%) were generators of ESBL, which is less than our result. In another study conducted in 2017 in Kerman (Koshesh et al., 2017), out of 105 samples, 42 isolates (40%) were ESBL generators and only 2% of the samples were *AmpC* generators, which does not correspond to our research. As a result of PCR test, 31 isolates contained *AmpC (FOX)* gene. Various studies on the prevalence of the *AmpC* gene, which can give us good information about beta-lactam resistance. In a 2004 study in North America (Deshpande et al., 2006), out of 1429 *E. coli* isolates, 29 samples (2.7%) showed the *AmpC* phenotype and were also found in 3 *FOX* gene isolates, which is much less than our results. In a 2011 study in Pakistan (Hussain et al., 2011), *FOX* gene was detected in 2 samples out of 121 *E. coli* isolates, which is much less than our results. In a study conducted in 2015 in Ilam (Maleki et al., 2015), out of 110 *E.coli* isolates, 28 isolates (40%) were *AmpC*-producing, and finally found in 3 isolates of *FOX* gene. The results of these studies are significantly different from the present study on the prevalence of *AmpC* genes, and it can be concluded that the prevalence of resistance genes in this bacterium is increasing over time, and this issue should be further investigated. It is also estimated from the comparison of the obtained results that the frequency of beta-lactamase enzymes in strain isolated from different countries, and even in hospitals of one country is different, which may be related to differences in infection control and treatment of patients. As long as beta-lactam antibiotics are used in clinical treatments, beta-lactamases play a major role in treatment failure (Maleki et al., 2015). Beta-lactamase-producing bacteria are resistant to various antibiotics, and cause serious problems in the treatment process (Eslami and Najar Peerayeh, 2012). Antibiogram results show that the highest resistance is related to amoxicillin (76.12%), ceftazidime (70.24%), nalidixic acid (65.05%), and the lowest resistance is related to gentamicin (23.18%), and imipenem (10.38%). In a study conducted in 2010 in Tehran (Yazdi et al., 2011) on clinical samples including urine, diarrheal stool, blood, and wounds , using the disk diffusion method, out of 200 samples, the highest resistance was related to amoxicillin (94.5%), cotrimoxazole (80.5%), nalidixic acid (74%) ,which is more than the results of this study, and the lowest resistance is related to imipenem (0.5%) which is less than our study .In a study conducted in 2015 in northwestern Iran (Hasani et al., 2015), among 146 samples, the resistance to cefixime was 94.5%, and nalidixic acid was 80.8% which is more than the results of the present study, and the lowest resistance was to imipenem (5.5%).In another study conducted in 2017 in Kerman (Koshesh et al., 2017), out of 105 *E.coli* isolates, the highest resistance was related to ciprofloxacin (80%), nalidixic acid (71.4%) and the lowest resistance was related to imipenem (3.8%), colistin (3.8%), gentamicin (22.8%), and the results of this study, except for gentamicin resistance, are different from the present study.

Comparing the results of different studies, we find that the prevalence of antibiotic resistance varies in different regions, and this percentage difference can be due to differences in the number of samples, infection control system or history of antibiotic use in each region. One of the limitations of the present study is the time limit in conducting research, and examination of urine, feces, blood, and wound samples of hospitalized patients in all wards of the hospital without classification under study, which can affect the external validity of the results. It is suggested that in future studies, different clinical samples from all wards of the hospital be studied by category, and a pattern of resistance to other antibiotics be determined. Beta-lactamases play an important role in the failure of treatment with beta-lactam antibiotics. In recent years, we have seen an increase in the prevalence of beta-lactamase genes and higher resistance to beta-lactam antibiotics. Therefore, it is better to use antibiotic susceptibility tests before starting treatment to avoid treatment failure and thus the development of resistant strains and it is important to be careful in the use and prescription of antibiotics and to avoid the arbitrary use of antibiotics.

### Conflict of interest

The authors declared that there is no conflict of interests.

### Acknowledgments

None to declare.

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