

Original Article



Evaluation of Antimicrobial Susceptibility in Extended-Spectrum β -Lactamase Producing *Escherichia coli* Isolates From Patients in Northwest Iran

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Article history:

Received: July 12, 2023

Revised: January 14, 2024

Accepted: January 31, 2024

ePublished: March 30, 2024

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Abstract

Background: *Escherichia coli* is one of the main causes of various diseases worldwide, whose multidrug-resistant strains have caused many public health problems by producing extended-spectrum β -lactamases (ESBLs). The resistance rate varies in different regions. Thus, it is necessary to identify ESBL-producing strains in each region and their antibiotic sensitivity in order to find appropriate treatment options. Hence, the present study aimed to detect the ESBL-producing *E. coli* strains and their antimicrobial susceptibility pattern in Tabriz, Iran.

Methods: This study was conducted at the Imam Reza Hospital in Tabriz from November 20, 2022, to April 20, 2023. A total of 400 *E. coli* isolates were collected from different clinical specimens. Antimicrobial susceptibility testing was performed by the disk diffusion method. ESBL-producing isolates were detected by the double-disc synergy test method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of 400 *E. coli* isolates, 211 (52.75%) were obtained from females, and 189 (47.25%) belonged to males. The mean age of patients was 52.1 ± 27.9 years. Overall, 279 (69.75%) were confirmed as ESBL producers. These producers were mainly recovered from outpatients. The highest antibiotic resistance was observed to ceftriaxone (86.25%) and tetracycline (80.75%), and the least antibiotic resistance was related to imipenem (8%) and amikacin (16.25%), respectively. The rate of antibiotic resistance among ESBL producers was higher than among non-ESBLs.

Conclusion: The present study reported a high prevalence of ESBL-producing *E. coli* among patients referring to Imam Reza hospital in Tabriz. Carbapenems, aminoglycosides, and nitrofurantoin were confirmed as the most efficient drugs for these bacteria, whereas cephalosporins, fluoroquinolones, and sulfonamides were the least effective agents.

Keywords: *Escherichia coli*, Antibiotics, Antimicrobial Susceptibility, Extended-spectrum β -lactamase



Please cite this article as follows: Bahloli M, Samadi Kafil H, Roshani Asl P, Rashidi N, Moaddab SR. Evaluation of Antimicrobial Susceptibility in Extended-Spectrum β -Lactamase Producing *Escherichia coli* Isolates From Patients in Northwest Iran. Avicenna J Clin Microbiol Infect. 2024; 11(1):22-27. doi:10.34172/ajcmi.3474

Introduction

Today, despite numerous advances in healthcare, the treatment of infections caused by drug-resistant bacteria remains a significant threat to public health (1). The indiscriminate use of antibiotics is identified as a primary factor in the development and spread of antimicrobial resistance among bacterial species (2). Infections with *Escherichia coli* are characterized by the production of virulence factors, including toxins that disrupt the intestinal mucosa, leading to symptoms such as diarrhea, abdominal pain, and fever. The spectrum of *E. coli* infections ranges from mild gastroenteritis to more severe conditions such

as urinary tract infections, bacteremia, and potentially fatal kidney failure (3). Currently, β -lactam antibiotics are the most commonly prescribed class of antibacterial agents in clinical practice due to their excellent safety and broad antimicrobial spectrum. Unfortunately, resistance to β -lactams is rapidly increasing (4). The production of β -lactamase enzymes is the most common mechanism of resistance to β -lactam antibiotics, catalyzing the hydrolysis of the amide bond in the β -lactam ring, rendering the antibiotics inactive by no longer binding to the transpeptidase target enzymes involved in cell wall biosynthesis (5). Extended-spectrum beta-lactamases



(ESBLs) constitute a critical category of β -lactamase enzymes, encoded either chromosomally or on plasmids, conferring resistance to penicillins, cephalosporins, and monobactams (6).

ESBLs are predominantly produced by gram-negative bacilli, particularly Enterobacteriaceae (7). Among the various members of the Enterobacteriaceae family, ESBL-producing *E. coli* has been identified as one of the leading multidrug-resistant bacteria implicated in severe hospital and community-acquired infections worldwide. Diseases caused by these bacteria result in substantial medical costs and limit treatment options (8). The aim of this study is to highlight the growing threat of drug-resistant *E. coli* infections, particularly those involving ESBL-producing strains. It also emphasizes the impact of indiscriminate antibiotic use on the development and spread of antimicrobial resistance and the challenges posed by the increasing resistance to β -lactam antibiotics. Additionally, it underscores the significant clinical and economic burden imposed by ESBL-producing *E. coli* infections, particularly in healthcare settings and the community.

Materials and Methods

Study Population and Bacterial Isolates

This study involved 400 *E. coli* isolates obtained from various clinical samples, including urine, blood, wound, cerebrospinal fluid, respiratory fluid such as sputum, and fecal samples. These samples were collected from inpatients and outpatients at Imam Reza hospital in Tabriz, Iran, from November 2022 to April 2023 (Research ethical code: IR. TBZMED. REC.1398. 779). The *E. coli* isolates were identified and isolated using culture methods (MacConkey agar test; this test involves culturing the bacterial sample on MacConkey agar, a selective and differential media that allows for the growth of *E. coli*). The presence of lactose-fermenting colonies indicates the likely presence of *E. coli*. Biochemical tests include the indole test, methyl red test, Voges-Proskauer test, and citrate utilization test (9).

Antimicrobial Susceptibility Testing

The antibiotic sensitivity of the isolates was determined by the standard Kirby-Bauer disk diffusion method on

Mueller-Hinton agar medium (Millipore, Billerica, MA, USA) according to CLSI guidelines. Paper disks containing specific antibiotics are placed on an agar plate inoculated with bacteria. After the 24-hour incubation, the zone of inhibition (where bacteria do not grow) around the disks is measured to determine the susceptibility of the bacteria to the antibiotics. This method provides a qualitative assessment of bacterial susceptibility to different antibiotics (9).

Eleven commercially available antibiotic disks (Mast, UK) were used, including gentamicin (10 μ g), amikacin (30 μ g), ciprofloxacin (5 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), imipenem (10 μ g), tetracycline (30 μ g), ceftriaxone, nitrofurantoin (300 μ g), nalidixic acid (30 μ g), and co-trimoxazole (25 mg). *E. coli* ATCC 25922 and ATCC 35218 MDR strains were used as negative and positive controls, respectively (10).

Detection of Extended-Spectrum Beta-Lactamase-Producing Isolates

All *E. coli* isolates were tested for detecting ESBL production by the double-disc synergy test method as stated by CLSI recommendations (9). In this method, after bacterial culture, antibiotic disks (Mast, UK) in pairs of ceftazidime (30 μ g) with ceftazidime/clavulanic acid (30/10 μ g) and cefotaxime (30 μ g) with cefotaxime/clavulanic acid (30/10 μ g) were placed on the Mueller-Hinton agar medium, at a distance of 20 mm apart from each other. The diameter of the inhibition zone was measured after 24 hours of incubation of the plates at 37 °C (Figure 1). Based on the report of the CLSI, an increase of ≥ 5 mm in the diameter of the inhibition zones around the clavulanic acid combination disks versus the single antibiotic disk confirmed the presence of ESBL-producing isolates. *E. coli* ATCC 25922 and ATCC 35218 were utilized as negative and positive control strains, respectively.

Statistical Analysis

The expression ratio was measured using the Pfaffl formula, calculated manually (11). The data were analyzed by descriptive statistics, the chi-square statistical test, and Fisher's exact test using SPSS, version 26 (IBM SPSS Statistics, New York, USA). The confidence limits for statistical tests were considered below 0.05.

Results

Demographic Information of the Participants

Out of the 400 *E. coli* isolates, 211 (52.75%) were recovered from females, and 189 (47.25%) were related to males. The isolates were primarily found in the urine ($n=287$), followed by blood ($n=81$) and wounds ($n=11$). The patients' mean age was 52.1 ± 27.9 years, with their ages ranging from 1 month to 80 years. As indicated in Table 1, the patients mostly belonged to the age groups of 60–80 years old ($n=226$, 56%). The percentage of *E. coli* isolation among inpatients was 84%, while it was 15.75% in outpatients.

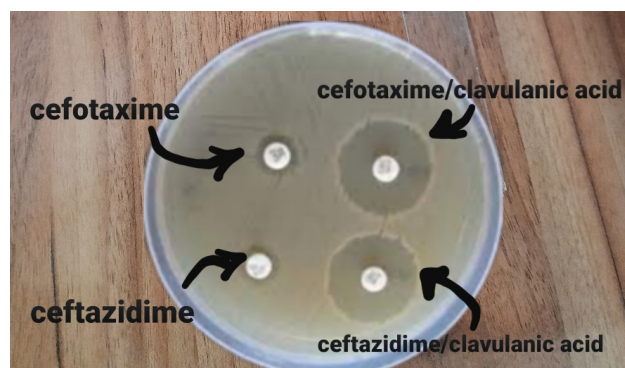


Figure 1. Double Disc Difusion for ESBL Identificaction. Note. ESBL: Extended-spectrum β -lactamase

Antimicrobial Susceptibility Profile and ESBL Production

Table 2 reports the antimicrobial susceptibility pattern of all 400 *E. coli* isolates for tested antibiotics. The highest resistance rate belonged to ceftriaxone (86.25%), tetracycline (80.75%), ciprofloxacin (79.5%), and co-trimoxazole (78.5%), while the least resistance was related to imipenem (8%) and amikacin (16.25%).

Of the 400 *E. coli* isolates, 279 (69.75%) were identified as ESBL producers. The highest percentage of ESBL-producing isolates was detected in children with an age range above 10 (83.3%) (Figure 2). The most and least number of ESBL-positive isolates belonged to the outpatients (80.95%) and special care unit patients (27.2%), respectively. In terms of sample type, most ESBL producers were isolated from blood samples (79%), followed by those

obtained from urine samples (68.9%) (Table 1). According to the information presented in Table 2, the expression of ESBLs resulted in the development of resistance to all antibiotics, especially ceftriaxone, tetracycline, ciprofloxacin, and co-trimoxazole.

Discussion

The emergence and rapid spread of multidrug resistance, particularly in ESBL-producing Enterobacteriaceae, pose a global health problem. These pathogens cause serious nosocomial and community infections, negatively affecting disease management (12). Understanding local epidemiology and the evolution of commonly isolated species, such as *E. coli*, is crucial for selecting effective treatments for each region.

Table 1. Demographic Data About the Source of *Escherichia coli* Isolates and the Rate of ESBL-producing Strains

Variables		Total of 400 <i>E. coli</i> Strains	% Among 400 Strains	ESBLs + Strains n (%)
Gender (Inpatients and outpatients)	Male	189	47.25	121 (64)
	Female	211	52.75	158 (74.8)
Total	Male and Female	400	100	279 (69.75)
Age	<10	6	1.5	5 (83.3)
	10-20	13	3.25	8 (61.5)
	20-40	44	11	32 (72.2)
	40-60	111	27.75	85 (76.5)
	60-80	226	56.5	149 (65)
Total	1-80	400	100	279 (69.75)
Samples type	Urine	287	71.75	198 (69)
	Blood	81	20.25	64 (79.2)
	Wound swab	11	2.75	7 (63.6)
	Others (wound, CSF, saliva, and excrement)	17	4.25	10 (58.8)
Total		400	100	279 (69.75)
Samples form				
Outpatients		63	15.75	51 (82.25)
	Orology	73	18.25	43 (59)
	Infectious	26	6.5	18 (69.2)
	Pulmonary care	22	5.5	19 (86.6)
	Gastro enology	19	4.75	18 (94.7)
	Glandsand rheumatology	18	4.5	12 (66.6)
	General interior	17	4.25	11 (64.7)
	ICU nerves	17	4.25	13 (76.4)
	ICU surgery unite	17	4.25	13 (76.4)
	ICU surgery	15	3.75	14 (93.3)
	ICU lung	14	3.5	11 (57.1)
	Brain	13	3.25	8 (61.5)
	ENT	11	2.75	11 (100)
	Stroke care unit	11	2.75	3 (27.2)
Inpatients	Organ transplants	10	2.5	8 (80)
	Orthopedic	7	1.75	3 (42.8)
	ICU nerves	5	1.25	4 (80)
	Thorax surgery	5	1.25	4 (80)

Note. ICU: Intensive care unit; ENT: Ear, nose, and throat; ESBL: Extended-spectrum β -lactamase; CSF: Cerebrospinal fluid.

Table 2. Drug Resistance Rate Among 400 *Escherichia coli* Isolates n (%)

Drugs	Resistance Rate Among 279 ESBL-Producing <i>E. coli</i> Isolates n (%)	Resistance Rate Among 121 Non-ESBL <i>E. coli</i> Isolates n (%)
Ceftriaxone	92	70.2
Tetracycline	84.3	71.4
Ciprofloxacin	82.37	71.89
Co-trimoxazol	80.56	73.17
Ceftazidim	76.61	42.85
Cefotaxime	72	70.37
Nalidixic acid	72	68
Gentamycin	49.27	37.78
Nitrofurantoin	21.13	16.14
Amikacin	18.2	11.89
Imipenem	9.1	6.03

Note. ESBL: Extended-spectrum β -lactamase.

The present study was conducted to determine the prevalence of ESBL-producing *E. coli* and its antimicrobial resistance profile based on the laboratory results of the patients attending the out-patient and inpatient departments of Imam Reza hospital in Tabriz, Iran, from November 2022 to April 2023 and to find an effective antibiotic strategy to prevent the spread of these strains. In this survey, 400 *E. coli* isolates were collected from different samples. The majority of the *E. coli* strains were isolated from urine samples (n=287, 71.75%) of females (n=211, 52.75%). This finding is in line with those of other studies reporting *E. coli* as the most common pathogen isolated from women with urinary tract infections compared to men (13,14). This could be due to women's increased susceptibility to urinary tract infections because of anatomical factors and hormonal changes (15). It was found that the isolates were frequently related to ≥ 60 -year-old patients. This is in conformity with the findings of other studies in which the outbreak of *E. coli* was higher among elderly patients (16,17).

In this study, 69.75% (n=279/400) of the isolates were ESBL producers. In some previous studies conducted in different countries such as Iran, Turkey, and China, the prevalence of ESBL-producing *E. coli* has been reported as 89.8%, 84%, and 68.2%, respectively (18–20), which corroborates the results of our study. On the other hand, some other researchers reported the prevalence rate of ESBL-producing *E. coli* as 35.7% in Iran, 19.3% in Zimbabwe, and only 3% in India (21–23), which contradicts our findings. These differences can be due to the geographical area and the diagnostic methods used in the studies (24).

In the present study, the rate of ESBL-producing *E. coli* in outpatients was higher than in inpatients (80.95% vs. 53.25%). This is consistent with the results of other published studies, which revealed that over the recent decades, ESBL-producing enterobacteria, especially *E. coli*, have emerged as important pathogens in outpatients in many areas of the world (25–28). In determining the

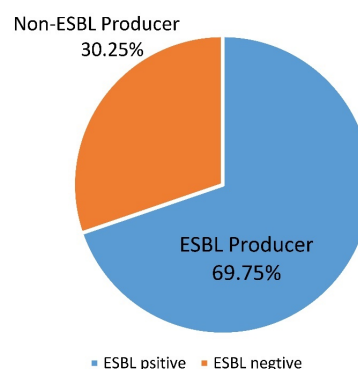


Figure 2. Percentage of ESBL and Non-ESBL-producing Isolates. Note. ESBL: Extended-spectrum β -lactamase

antibiotic susceptibility patterns of *E. coli* by commonly prescribed antibiotics, the highest resistance belonged to ceftriaxone (86.25%), tetracycline (80.75%), ciprofloxacin (79.5%), and co-trimoxazole (78.5%), while the least resistance was related to imipenem (8%), amikacin (16.25%), and nitrofurantoin (19.75%).

Further, this study indicated that ESBL-producing isolates had higher resistance to tested antibiotics compared to non-ESBL producers. Nevertheless, both groups of isolates were highly sensitive to imipenem and amikacin. Many studies around the world have provided similar results in this regard. For example, the study conducted by Rodriguez-Baño et al showed that resistance to imipenem and amikacin was 5.98% and 5.1%, respectively (29). In the study by Hashemizadeh et al, 96.2%, 85.1%, and 72.6% susceptibility to imipenem, amikacin, and nitrofurantoin were observed, respectively (30). In another study by Khan et al (31), the isolates were highly resistant to tetracycline (95%), ciprofloxacin (85%), ceftriaxone (88%), cefotaxime (78%), ceftazidime (63%), and gentamicin (60%), while they were highly susceptible to imipenem (91%). According to a report by Lautenbach et al, the resistance rates to nalidixic acid, ceftriaxone, gentamicin, amikacin, and nitrofurantoin were 84%, 73%, 36%, 12%, and 8%, respectively (32). In addition, Pourakbari et al indicated that ESBL-positive bacteria were highly resistant to cefotaxime (100%), ceftriaxone (100%), and ceftazidime (70.6%), while both ESBL-producing and non-ESBL-producing isolates demonstrated low resistance to amikacin (9.5%), and no resistance was observed toward imipenem (33). In line with the results of our study and these similar investigations, carbapenems (imipenem) and aminoglycosides (amikacin) are the best options to prescribe against both ESBL-producing *E. coli* and non-ESBL-producing isolates.

Limitations of the Study

The number of samples was limited due to high financial costs.

Conclusion

The findings of the current study demonstrated

a high rate of infection with ESBL-producing *E. coli* isolates. The ESBL producers were primarily outpatients. High resistance to different types of antibiotics was observed in all isolates. The amount of resistance in ESBL producers was higher than in non-ESBLs. Meanwhile, the analysis of antibiogram results revealed that carbapenems and aminoglycosides are suitable options for the treatment of ESBL-producing strains, while cephalosporins, fluoroquinolones, and sulfonamides are not recommended and their prescription should be limited. Finally, due to the spread of these isolates in the community and increasing resistance to most of the common antibiotics, it is necessary to carefully screen the isolates before prescribing the drug to closely monitor antibiotic prescriptions and choose the appropriate and effective treatment options.

Acknowledgments

The authors are grateful to the research staff of the Department of Medical Laboratory Sciences, Faculty of Allied Medical Science, Tabriz University of Medical Sciences, Iran, for their contribution to improving the quality of this research.

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Funding acquisition: Niloufar Rashidi, Seyyed Reza Moaddab.

Investigation: Mohammad Bahloli, Niloufar Rashidi.

Methodology: Mohammad Bahloli, Parisa Roshani Asl, Niloufar Rashidi, Seyyed Reza Moaddab.

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Writing—original draft: Mohammad Bahloli, Niloufar Rashidi, Seyyed Reza Moaddab.

Writing—review & editing: Mohammad Bahloli, Niloufar Rashidi, Seyyed Reza Moaddab, Parisa Roshani Asl.

Competing Interests

The author declared no conflict of interests.

Ethical Approval

All ethical aspects of this research were approved by the Research and Ethics Committee of Tabriz University of Medical Sciences. The patients' demographic data were collected from the medical records database, and their information remained confidential. Informed consent was obtained from all participants or their legal guardians before the study.

Funding

There is no funding or support for the publication of this study.

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