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Examining the frequency of *bla*$_{CTX-M}$, *bla*$_{TEM}$, *and bla*$_{SHV}$ genes *in Escherichia coli* isolates from patients in Tabriz hospitals, Iran

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Abstract

Escherichia coli (*E. coli*) is one of the most common hospital infections in which the emergence of antibiotic resistance and the prevalence of Extended Spectrum Beta-Lactamase (ESBL) are considered major problems in the control of hospital infections. The aim of this study was to investigate the frequency of *bla*$_{CTX-M}$, *bla*$_{TEM}$, *and bla*$_{SHV}$ beta-lactamase genes in *E. coli* isolated from different clinical samples by the combined disk and multiplex PCR methods. *E. coli* isolates from different clinical samples were collected over six months and identified with the help of standard microbiological and biochemical tests. The antibiotic resistance of *E. coli* isolates was determined by the disk diffusion method. The production of beta-lactamases was evaluated by the combined disk method, and the presence of *bla*$_{CTX-M}$, *bla*$_{TEM}$, *and bla*$_{SHV}$ genes was assessed by the multiplex PCR method. From the total of 200 examined samples, 114 *E. coli* isolates (57%) were identified with the highest resistance to ceftazidime, amikacin, cefotaxime, and tetracycline (100%) and the lowest resistance to imipenem (3.5%) and ampicillin (14%). 58 isolates (50.88%) were ESBL positive by phenotypic test, of which 20 isolates (34.48%) contained the *bla*$_{TEM}$ gene and 15 isolates (25.86%) contained the *bla*$_{CTX-M}$ gene. No positive *bla*$_{SHV}$ gene was observed in the samples. The presence of beta-lactamase genes examined in this study along with the occurrence of antibiotic resistance indicates the spread of bacterial resistance in the study area and shows the importance of rational antibiotic treatment.

Keywords: *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *Escherichia coli*, Beta-Lactamase

1. Introduction

One of the major concerns for human health is the increasing antibiotic resistance of opportunistic bacteria (1, 2). One of the opportunistic pathogens that causes hospital infections; It is *Escherichia coli* (*E. coli*). Although this bacterium is common in the gastrointestinal tract, it is one of the most common causes of hospital infections such as bacteremia, sepsis, gastroenteritis, neonatal meningitis, wound infection, and urinary tract infection (3). Indiscriminate and intensive use of antibiotics has increased the prevalence of antibiotic resistance among bacteria (4, 5). Which increases the global financial burden for the treatment of diseases caused by antibiotic-resistant pathogens (6). Therefore, excessive use of antibiotics justifies multiple drug resistance in *E. coli*. Community-acquired *E. coli* resistance to extended-spectrum beta-lactamases (ESBLs) is a particular concern for physicians and public health (7). Beta-lactam antibiotics are common and widely used drugs for the treatment of bacterial infections, they are selective inhibitors of cell wall synthesis and are active against growing bacteria (8). The production of beta-lactamase enzymes by bacteria is considered an important strategy to avoid the harmful effects of beta-lactam antibiotics, which increases the resistance of pathogenic bacteria, especially gram-negative bacteria, to beta-lactam antibiotics. Beta-lactamases are divided into four classes A, B, C and D according to the Ambler classification scheme (9). Enterobacteriaceae with ESBL-positive genes cause more severe infections compared to ESBL-negative enterobacteriaceae (10-13). Common ESBLs include *bla*$_{CTX-M}$, *bla*$_{TEM}$, *and bla*$_{SHV}$ (14). Bacterial cells containing *bla*$_{CTX-M}$ gene hydrolyze cephalothin or cephaloridine. Which are not evolutionarily related to the *bla*$_{TEM}$ and *bla*$_{SHV}$ families; they are transmitted by plasmids and were first reported in Europe in 1980 (15, 16). Bacteria with the *bla*$_{TEM}$ gene are resistant to penicillins and first-generation cephalosporins but are sensitive

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to oxyiminocephalosporins. Plasmid and transposon facilitate the spread of *bla*$_{TEM}$ to other bacterial species (17, 18). The *bla*$_{SHV}$ gene is resistant to broad-spectrum penicillins such as piperacillin and ampicillin, but not to oxyimino-substituted cephalosporins and tigecycline. *bla*$_{SHV}$ gene is the cause of 20% of ampicillin plasmid resistance observed in this strain (19). Therefore, the purpose of this study was to investigate the pattern of antibiotic resistance and the prevalence of ESBLs in *E. coli* isolates with two combined disk and multiplex PCR methods in patients referred to Tabriz hospitals.

2. Materials and methods

2.1. Collection and identification of samples

In this descriptive cross-sectional study, from August 2022 to January 2023, a total of 200 clinical samples, including blood, urine, wound secretions, and respiratory secretions, were collected from inpatients and outpatients by random sampling method. Demographic information including age, gender, and type of infection was obtained from medical records. The samples were evaluated in blood agar, McConkey agar, and EMB culture media (Merck, Germany) along with gram staining and other biochemical tests to determine phenotypic identity. All samples were stored in tryptic soy broth (TSB) at -70 °C for further tests (20, 21).

2.2. Antimicrobial sensitivity test

The antibiotic resistance pattern of all *E. coli* isolates was determined according to the 2018 clinical laboratory standard guidelines (CLSI 2018) by the Kirby-Bauer disk diffusion method at a 0.5 McFarland concentration of the bacteria and in Mueller Hinton agar culture medium (Merck, Germany). Antibiotic discs used in this study include: tetracycline (TE 30 µg), chloramphenicol (C 30 µg), cotrimoxazole (SXT 25 µg), ciprofloxacin (CIP 5 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), nalidixic acid (NA 30 µg), ceftriaxone (CRO 30 µg), gentamicin (GM 10 µg), amikacin (AMK 30 µg), ampicillin (AMP 10 µg), azetronam (ATM 30 µg), and imipenem (IPM 10 µg) (Padtan Teb, Iran). The standard bacteria *E. coli* ATCC 25922 was used as a positive control.

2.3. Phenotypic detection of ESBL strains

To investigate ESBL bacteria using the combined disc method with discs containing ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime/clavulanic acid (30 µg /10 µg), and cefotaxime/clavulanic acid (30 µg /10 µg). A five-mm no-growth zone difference around ceftazidime/clavulanic acid discs with ceftazidime disc, and cefotaxime/clavulanic acid with cefotaxime disc indicates ESBL enzyme production by bacteria (CLSI2018).

2.4. Identification of *bla*$_{CTX-M}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ genes

In order to evaluate the frequency of the target genes, multiplex PCR method was performed using the SinaClon kit (Iran). In order to identify the desired genes, the specific primers mentioned in Table 1 were used. multiplex PCR with a thermocycler (Applied Biosystems, USA) in a final volume of 25 microliters, including 12.5 µl of master mix, 1 µl of forward primer (10 pmol), 1 µl of reverse primer (10 pmol), 3 µl of DNA and 7.5 µl of nuclease-free water with a thermal profile of initial denaturation at 94 °C for 10 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 57°C for 35 s and extension at 72 °C for 1 min; and final extension at 72 °C for 9 min.

Table 1. Primers used in this research

Genes	Primers	Sequences (5′to3′)	Amplicon Size (bp)	References
bla$_{CTX-M}$	GES-F	ATGTGCAGYACCAGTAARGTKATGGC	592	[22]
	GES-R	TGGGTRAARTARGTSACCAGAAYCAGGG		
bla$_{TEM}$	TEM-F	GAGTATTCAACATTTCCGTGTC	861	[23]
	TEM-R	TAATCAGTGAGGCACCTATCTC		
bla$_{SHV}$	OXA-F	ATGCGTTATATTCGCCTGTG	747	[24]
	OXA-R	TGCTTTGTTATTCGGGCCAA		

2.5. Detection of multiplex PCR products by agarose gel electrophoresis method

The multiplex PCR product was evaluated on a 1.5 % agarose gel by electrophoresis, and the gel containing the PCR products was placed in a tank containing DNA Safe Stain (V2) (SinaClon Company) for 15-20 minutes after the end of the electrophoresis period, and then under UV light. Bands were observed by the GelDoc device (UVtec, UK) and finally photographed and printed.

2.6. Statistical test of data

To check the relationship between the research data, SPSS software version 23 of IBM Company (USA) and Chi-square test were used. A p-value less than 0.05 was considered statistically significant.

3. Results

From the total of 200 examined samples, 114 *E. coli* isolates (57%) were identified. The average age of the patients was 43.92 ± 13.21, ranging from at least 20 years to 60 years. *E. coli* isolates were isolated from 57 urine culture samples (50%), 51 blood samples (44.74%), 3 wound secretion samples (2.63%) and 3 respiratory secretion samples (2.63%). 85 samples (74.56%) were related to women and 29 samples (25.44%) were related to men. 68.42% (78 samples) of the samples were isolated from the inpatient department and 31.58% (36 samples) from the outpatient department. No statistically significant difference was observed in terms of the distribution of *E. coli* isolates between age groups, male and female groups, and outpatient and inpatient settings ($p>0.05$). Antibiogram results showed that *E. coli* had the highest

resistance to ceftazidime, amikacin, cefotaxime and tetracycline (100%) and the lowest resistance to imipenem (3.5%) and ampicillin (14%) (Fig. 1). Among 114 *E. coli* isolates, 58 isolates (50.88%) were positive for beta-lactamase production by phenotypic test. The number of positive ESBL isolates in the female group (65.52%) was more than the male group (34.48%). The most beta-lactamase-producing isolates belonged to urine samples (53.45%) and from the inpatient department (82.76%). PCR results for the target genes showed that out of 58 beta-lactamase producing isolates, 20 isolates (34.48%) contained the bla_{TEM} gene and 15 isolates (25.86%) contained the bla_{CTX-M} gene. 8 isolates (13.79%) contained both bla_{TEM} and bla_{CTX-M} genes. No positive bla_{SHV} gene was observed in the samples (Table 2). ESBL producing isolates showed the highest resistance to antibiotic discs. A significant relationship was found between resistance to antibiotics and the genes used ($p<0.05$).

Table 2. The frequency of ESBL-producing isolates based on clinical specimens, gender, types of referrals and age average

		N (%)	ESBL		Genes		
			ESBL+ (%)	ESBL- (%)	bla_{CTX-M}	bla_{TEM}	bla_{SHV}
Clinical specimens	Blood	51 (44.74)	25 (43.10)	26 (44.83)	8 (53.33)	8 (40)	0 (0)
	Urine	57 (50)	31 (53.46)	26 (44.83)	5 (33.33)	9 (45)	0 (0)
	Wound secretions	3 (2.63)	1 (1.72)	2 (3.45)	1 (6.67)	2 (10)	0 (0)
	Respiratory secretions	3 (2.63)	1 (1.72)	2 (3.45)	1 (6.67)	1 (5)	0 (0)
Gender	Male	29 (74.56)	20 (34.48)	9 (16.07)	6 (40)	7 (35)	0 (0)
	Female	85 (25.44)	38 (65.52)	47 (83.93)	9 (60)	13 (65)	0 (0)
Types of Referrals	Inpatient	78 (68.42)	48 (82.76)	30 (53.57)	10 (66.67)	11 (55)	0 (0)
	Outpatient	36 (31.58)	10 (17.24)	26 (46.43)	5 (33.33)	9 (45)	0 (0)
Age average		43.92 ± 13.21	42.88 ± 11.74	44.69 ± 14.77	39.27 ± 12.75	37.35 ± 13.77	0

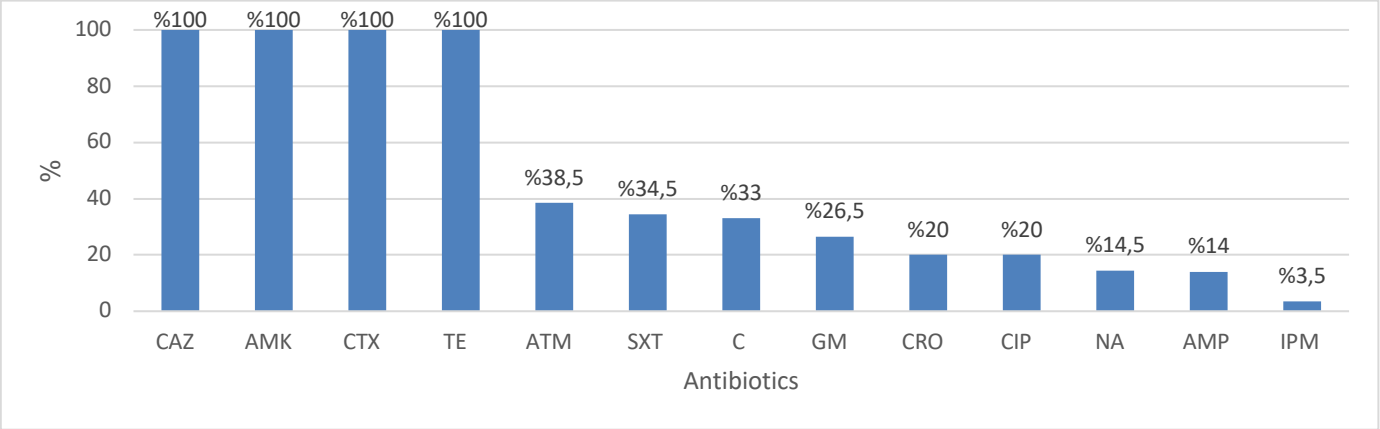

Fig.1. Frequency of antibiotic resistance pattern
Tetracycline: TE, chloramphenicol: C, cotrimoxazole: SXT, ciprofloxacin: CIP, cefotaxime: CTX, ceftazidime: CAZ, nalidixic acid: NA, ceftriaxone: CRO, gentamicin: GM 10, amikacin: AMK, ampicillin: AMP, azetronam: ATM, imipenem: IPM

4. Discussion

Antibiotic resistance caused by the presence of ESBLs in *E. coli* isolates involved in hospital infections and their simultaneous transmission to other strains shows the importance of rational antibiotic treatment and preventing the spread of these strains in hospitals and understanding the clinical consequences caused by these organisms (25). The studies conducted in the last few years indicate the increasing number of ESBL-producing organisms in developing countries, which imposes many concerns on the health of society (26). In the last few years, various studies have shown that ESBL-producing organisms are sensitive to carbapenem. Therefore, the successful treatment of various infections using these agents is reported (27). The present findings showed that *E. coli* isolates had the lowest resistance to imipenem (3.5%) and ampicillin (14%), which can be useful in treating infections caused by this organism. Rezai et al., by investigating the properties of *E. coli* producing beta-lactamase resistant to several drugs, showed that *E. coli* isolates showed high resistance to carbapenem antibiotics (28). These findings are

contrary to the findings of the present study and the studies conducted by Manoharan et al., (2011) in India, Benenson et al., (2009) in Malaysia, and Kader et al., (2004) in Saudi Arabia (29-31). Mortezavi et al., by examining the frequency of *E. coli* strains resistant to beta-lactam antibiotics, showed that *E. coli* isolates have the highest resistance to nalidixic acid (51.21%) and the lowest resistance to imipenem (0%), ceftazidime (17.88%) and ciprofloxacin (22.76%) (32), which is not consistent with the findings of the present study. Shabanpishe and Rezaeian, by examining the frequency of antibiotic resistance of *E. coli* isolated from clinical samples, showed that the highest resistance to antibiotics was cotrimoxazole (46.34%), imipenem (62.12%), cefotaxime (41.42%), gentamicin (13.12), Cefixime (42.22%), and Ciprofloxacin (62.13%) (33). In the present study, among 114 *E. coli* isolates, 58 isolates (50.88%) were positive for beta-lactamase production by phenotypic test, of which 20 isolates (34.48%) contained the bla_{TEM} gene and 15 isolates (25.86 %) contained the $bla_{CTX\text{-}M}$ gene. 8 isolates (13.79%) contained both bla_{TEM} and $bla_{CTX\text{-}M}$ genes. No positive bla_{SHV} gene was observed in the samples. Yazdi et al., investigated the prevalence of $bla_{SHV}/bla_{CTX\text{-}M}/bla_{TEM}$ beta-lactamase (ESBL) resistance genes in *E. coli* isolated from Tehran and showed that 47.1% of *E. coli* isolates were resistant to ceftazidime and 39.2% to cefotaxime. Also, 109 isolates (44.3%) are ESBL positive. $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} genes were found among 95 (87.1%), 75 (68.8%) and 77 (70.6%) ESBL positive isolates, respectively (34). Moradi et al, by examining and molecular identification of $bla_{CTX\text{-}M}$ and bla_{SHV} beta-lactamase genes in clinical isolates of *E. coli* in Isfahan city, showed that the highest antibiotic resistance was related to cefotaxime (45%) and the lowest antibiotic resistance was related to cefepime (39%). Among the 100 *E. coli* confirmed by differential tests, 33 samples (86.8%) were identified as producers of ESBL, 69.7% (23 strains) of them had both $bla_{CTX\text{-}M}$ and bla_{SHV} coding genes, 15.2% (5 strains)) had $bla_{CTX\text{-}M}$ coding gene and 12.1% (4 strains) had bla_{SHV} coding gene (35), which did not match the findings of the present study. Enyinnaya et al., by examining the characteristics of antibiotic sensitivity and detecting ESBL genes in *E. coli* isolates, showed that meropenem, fosfomycin, and tigecycline showed very good activities against all isolates, and in total, 100%, 67.8%, 54.8% and 52.0% of isolates were resistant to ampicillin, amoxicillin-clavulanate, ciprofloxacin and gentamicin, respectively (36). Mortezavi et al., Shabanpishe and Rezaeian identified 26.5% and 42.26% of *E. coli* isolates as ESBL positive respectively, of which 34.85% and 23.17% contained the bla_{CTX} gene, 50.94% and 30.48% contained the bla_{TEM} gene, and 47.16% and 0% contained bla_{SHV} gene (32, 33), which did not match the findings of the present study. Jalilian and Rokh Bakhsh zamin reported the frequency of bla_{CTX}, bla_{TEM} and bla_{SHV} beta-lactamase genes 68.3%, 46.6% and 0%, respectively (37). Ghaddar et al., by examining the phenotypic and genotypic characteristics of ESBL-producing *E. coli* in pregnant women, showed that the sensitivity of the isolates to cotrimoxazole antibiotics was 39%, ciprofloxacin 49.2%, gentamicin 91.5%, aztreonam 18.6%, and cefepime 35.6%. Most isolates were highly sensitive to meropenem and imipenem (93.2%). $bla_{CTX\text{-}M}$ (90.7%), bla_{TEM} (88.4%) and bla_{SHV} (44.2%) were dominant genes (38). Ghafourian et al., by examining the microbial spectrum and antibiotic resistance of *E. coli* isolates in outpatients and inpatients, showed that the most antibiotic resistance was related to cotrimoxazole (55%), cefotaxime (49%), and ceftriaxone (41%). 63% of *E. coli* isolates were ESBL positive (39). In 2008, Mirsalehian et al., reported 59.3% of Enterobacteriaceae isolates in Tehran as ESBL producing isolates (40), which was close to the findings of the present study. . In the study of Tasli et al., in Turkey and Villegas et al., in Colombia, the production of ESBL enzyme in isolates was reported as 17% and 4.7%, respectively (41, 42), which was lower than the findings of the present study. The results of research on beta-lactamase-producing isolates in recent years show the highest percentage of ESBL-producing isolates in Latin American countries, 54.4%, Europe 26.6%, and the Western Pacific region 26.6% (43). The difference in the findings of the present study and other researchers can be caused by the sample size, the study area, excessive and arbitrary use of antibiotics, the length of time spent in the hospital, the use of vascular and urinary catheters, history of surgery and etc, among these things. Also, the occurrence of resistance in a country differs from one hospital to another, which depends on the infection control system and how the patients are treated.

The presence of ESBLs and their antibiotic resistance in *E. coli* isolates and their simultaneous transfer to other strains is considered as a big threat to the use of broad-spectrum cephalosporins in Tabriz city, which indicates the importance of rational treatment and preventing the spread of ESBL-positive isolates and the consequences of it. Therefore, it seems necessary to take appropriate measures in diagnosis and treatment.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical Approval: All procedures performed in studies including human participants were according to the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments or

comparable ethical standards.

Authors' contributions

Concept: A.J.S., M.E., F.M., G.P, Design: M.E., F.M., G.P., Data Collection or Processing: A.J.S., M.E., F.M., Analysis or Interpretation: A.J.S., M.P., Literature Search: A.J.S., M.E., F.M., Writing: M.E., F.M., G.P.

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