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Major Article

Clonal relationships, antimicrobial susceptibilities, and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from urinary tract infections and fecal samples in Southeast Iran

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Abstract

Introduction: Multidrug-resistant (MDR) *Escherichia coli*, a species that is a leading cause of urinary tract infections (UTIs) and is a major global public health concern. This study was designed to detect the differences in antibiotic resistance patterns, the production and type of extended spectrum β-lactamases (ESBLs), and the clonal relationships among *E. coli* isolates from UTIs and fecal samples. **Methods:** Antibacterial resistance was determined by the disk diffusion method. ESBL, carbapenemase, and AmpC-producing isolates were detected phenotypically. Then, the ESBL genes were sequenced to detect the type. Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) was performed on the ESBL-positive isolates. **Results:** The most common effective antibacterial agents were colistin, imipenem, and amikacin. Among the isolates, 204 (56.6%) were MDR. Of the 163 ESBL-positive isolates, 11 (6.7%) produced AmpC, and the frequencies of beta-lactamase-positive genes were as follows: $bla_{\text{CTX-Mgroup1}}$, 76%; bla_{TEM1}, 74.8%; bla_{SHV12}, 1.2%; and bla_{OXA1}, 12.88%. ERIC PCR showed a diverse pattern, suggesting that clonal spread of *E. coli* in this area is uncommon, and that most of the infecting strains are endogenous. **Conclusions:** The high rates of antibacterial-resistant and MDR isolates are quite important since these strains can act as source of resistant bacteria that can be spread in the community. Controlling antibiotic use, against inappropriate use and abuse, in the community and continuous surveillance of emerging resistance traits are critical to controlling the spread of resistance.

Keywords: *Escherichia col*. Antimicrobial resistance. ESBL. AmpC beta-lactamas.

INTRODUCTION

Escherichia coli is found in the normal microbiota of the human intestinal tract, and it has an important role in protecting against pathogenic bacteria[1]. An estimated 70-90% of community-acquired urinary tract infections (UTIs) and 50% of hospital-acquired UTIs are caused by *E. coli*[2]. Most nosocomial UTIs are caused by endogenous strains, although exogenous infections from other patients or other sources have also been documented[3].

Many antibacterial agents, such as beta-lactams and fluoroquinolones, are recommended for the treatment of UTIs[4]. However, inappropriate use and overuse of antibacterial agents have resulted in the emergence of multidrug-resistant (MDR) isolates; therefore, the drugs previously used to treat patients, such as fluoroquinolones, trimethoprim/sulfamethoxazole, and cephalosporin, have become ineffective[4]. Beta-lactams are the most commonly used antimicrobial agents for the treatment of UTIs[5]. The main mechanism of resistance to beta-lactams is the production of beta-lactamase enzymes[5]. Beta-lactamase enzymes, such as extended spectrum beta-lactamases (ESBLs), metallo-beta-lactamases (MBLs), and AmpC beta-lactamase, have been reported in gram-negative bacteria[6]. When these resistant bacterial isolates cause hospital- and community-acquired infections, treatment is difficult since the therapeutic options are limited[5]. Beta-lactamase genes are usually encoded on mobile genetic elements that can be easily transmitted between different bacterial species or strains[7]. Therefore, epidemiological studies are crucial for monitoring drug resistance among bacterial isolates and determining the distribution of drug resistance in the community. In this study, the antibacterial resistance profiles, frequency of ESBL genes, and clonal relationships among isolates from fecal samples and the urinary tracts of inpatients and outpatients with UTIs was determined to find better strategies for the control and management of UTIs caused by *E. coli*.

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METHODS

Study population and bacterial isolates

From October 2014 to June 2015, 351 *E. coli* strains (100 from inpatients with UTIs and 151 from outpatients with UTIs) were isolated in Kerman, Iran. The patients were considered inpatients when they stayed in a given hospital ward for treatment for ≥48h and/or within 30 days of hospital discharge, otherwise they were considered outpatients. Outpatients were those who visited the emergency ward with acute symptoms or were referred to local private laboratories for urine culture. All outpatients were not previously hospitalized due to any disease[8]. Fecal samples (n=100) were collected by individual rectal swabs from volunteers without any digestive problems and were transported to the laboratory in Cary-Blair transport medium and then placed overnight in buffered peptone water at 37°C. The samples were then cultivated on MacConkey agar (CONDA, Co., SPAIN). Lactose-positive isolates on MacConkey agar were selected for biochemical confirmatory tests for *E. coli*. The criteria for selection were no history of hospitalization and signs or symptoms of UTI or gastrointestinal symptoms, such as nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. All isolates were confirmed to be *E. coli* by the results of standard biochemical tests, including gram-negative, oxidase negative, catalase positive, lactose positive, MR positive, VP negative, Indole positive, and acid/acid and gas positive on TSI medium[9].

Determination of antibacterial susceptibility and ESBL, AmpC, and carbapenemase production

The standard disk diffusion method was used to determine the antibacterial susceptibility profiles of the isolates to 11 antibacterial agents[10]. The following disks (obtained from MAST Chemical Company, England) were used in this study: amoxicillin/clavulanic acid (AUG, 30/10µg), cefotaxime (CTX, 30µg), ceftazidime (CAZ, 30µg), cefepime (CPM, 30µg), imipenem (IMP, 10µg), ciprofloxacin (CIP, 10µg), nalidixic acid (NA, 30µg), trimethoprim (TM, 5µg), piperacillin/tazobactam (PIP, 100/10µg), amikacin (AK, 30µg), and gentamicin (GM, 10µg). MDR isolates were defined as described by Magiorakos *et al.*[11]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST**)** guidelines were used to test for susceptibility to colistin (CO, 10µg)[12]. However, the MIC by E-test (*HiMedia*, Co., India) was determined for isolates that were not susceptible to colistin in the disk diffusion for confirmation[12,13]. ESBL-producing isolates were identified by the combined disk method with clavulanic acid and extended-spectrum cephalosporins (CPD, CTX, and CAZ; 30µg alone and in combination with 10µg of clavulanic acid)[14]. The AmpC disk test was used to detect AmpC beta-lactamases as described by Black *et al.*[15]. The modified Hodge test (MHT) was used to detect carbapenemase-producing isolates[10]. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains in the antibacterial susceptibility testing, and *Klebsiella pneumoniae* ATCC 700603 was used as the positive control for the ESBL test.

Polymerase chain reaction to detect beta-lactamase genes

The boiling method was used to prepare template DNA for PCR as described by Qi *et al.*[16]. The oligonucleotide primers used to detect $bla_{\text{CTX-M group 1-4}}$, bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{PER}, bla_{KPC}, and bla_{NDM} are listed in **Table 1**[17-20]. PCR amplification was performed in a total volume of 50µL containing 1µL of each primer (10pM), 25µL of DNA Polymerase Master Mix RED (Ampliqon, Co., Denmark), 2µL of DNA, and 21µL of DNase and RNase free water in a FlexCycler PCR Thermal Cycler (Analytik Jena, Germany) under the following cycling conditions: an initial denaturation step at 95°C for 5min followed by 30 cycles of denaturation at 95 °C for 1min, annealing at 55-61°C for 1min (**Table 1**), extension at 72°C for 1min, and a final extension step at 72°C for 5min. The PCR products were separated by electrophoresis in a 1.5% agarose gel in 0.5 Tris, EDTA, Boric acid (TBE) buffer. For each ESBL group, amplicons from randomized selected strains were sequenced (Macrogen, South Korea). The obtained nucleotide sequences were compared with those in the GenBank database for homologous nucleotide sequences by BLAST (www.ncbi.nih.gov/BLAST program).

Molecular typing of ESBL isolates by ERIC-PCR

Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) was used to determine the clonal relationships among the isolates using the ERIC2 primer (5′-AAGTAAGTGACTGGGGTGAGC-3′)[21]. The ERIC-PCR amplifications were carried out in a total volume of 25µL containing 1µL of ERIC2 primer (10pM), 12.5µL of DNA Polymerase Master Mix RED, 1µL of DNA template, and 10µL of DNase and RNase free water (SinaClon BioScience, Co., Iran) in a FlexCycler PCR Thermal Cycler under the following cycling conditions: an initial denaturation step at 95°C for 5min followed by 30 cycles of denaturation at 95°C for 1min, annealing at 59°C for 1min, and extension at 72°C for 2min, and a final extension step at 72°C for 10min. The obtained PCR fragments were separated by electrophoresis in a 1.5% agarose gel, and the gel was analyzed by using Gel Compare software (BioMérieux, Applied Maths, Belgium) using a cut off of 70% to discriminate isolates[22].

Statistical analysis

For data analysis, SPSS version 20.0 (IBM, Armonk, USA) was used. Fisher's exact test was used for data analysis and to evaluate the significance of differences. P value less than 0.05 was considered statistically significant.

RESULTS

Antibacterial susceptibility

All the tested isolates were susceptible to colistin. Although 10 isolates showed intermediate susceptibility to colistin by the disk diffusion method, the E test results showed that all isolates were sensitive, with an MIC ≤2µg/ml. The other most effective drugs were imipenem and amikacin (**Table 2**). There were significant differences in resistance to AUG, TM, NA, CTX, CAZ, and PIT among the isolates from inpatient, outpatient,

TABLE 1: PCR Primers used to amplify ESBL genes in *Escherichia coli* strains isolated from urinary tract infections and fecal samples.

Reference	Annealing temperature (°C)	Product size (bp)	Primer sequence (5' to 3')	Target genes
17	55	933	F-AATTTGGGCTTAGGGCAGAA R-ATGAATGTCATTATAAAAGC	bla_{PER}
18	60	392	F-AGGATTGACTGCCTTTTTG R-ATTTGCTGATTTCGCTCG	bla_{SHV}
	60	516	F-ATCAGCAATAAACCAGC R-CCCCGAAGAACGTTTTC	bla_{TEM}
	59	609	F-TCAACTTTCAAGATCGCA R-GTGTGTTTAGAATGGTGA	bla_{OXA}
19	58	232 or 798	F-CGTCTAGTTCTGCTGTCTTG R-CTTGTCATCCTTGTTAGGCG	bla_{KPC}
	58	621	F -GGTTTGGCGATCTGGTTTTC R -CGGAATGGCTCATCACGATC	bla_{NDM}
20	60	499	F-GACGATGTCACTGGCTGAGC R-AGCCGCCGACGCTAATACA	bla_{CTX-M1} group
	61	351	FGCGACCTGGTTAACTACAATCC R-CGGTAGTATTGCCCTTAAGCC	bla_{CTX-M2} group
	60	307	F-CGCTTTGCCATGTGCAGCACC R-GCTCAGTACGATCGAGCC	bla_{CTX-M3} group
	61	474	F-GCTGGAGAAAAGCAGCGGAG R-GTAAGCTGACGCAACGTCTG	bla_{CTX-M4} group

PCR: polymerase chain reaction; **ESBL:** extended spectrum β-lactamases.

TABLE 2: Antibacterial resistant patterns of 351 *Escherichia coli* isolates from in patients and outpatients with urinary tract infections and fecal samples.

Resistant strains (n %)				Antimicrobial agent (µg/disk)
P value	fecal flora (n=100)	outpatients (n=151)	inpatients (n=100)	
0.009	49 49.0	85 56.2	70 70.0	Amoxicillin-clavulanic acid (30/10)
0.05	81 81.0	102 67.5	75 75.0	Trimethoprim (5)
0.03	80 80.0	98 64.9	73 73.0	Nalidixic acid (30)
0.08	49 49.0	63 41.7	56 56.0	Cefotaxime (30)
0.3	47 47.0	65 43.0	53 53.0	Cefepime (30)
0.8	0 0.0	0 0.0	0 0.0	Colistin (10)
0.05	42 42.0	61 40.3	55 55.0	Ceftazidime (30)
0.07	9 9.0	8 5.2	4 4.0	Imipenem (10)
0.2	2 2.0	10 6.6	6 6.0	Amikacin (30)
0.04	13 13.0	33 21.8	27 27.0	Piperacillin/tazobactam (100/10)
0.1	48 48.0	63 41.7	55 55.0	Ciprofloxacin (10)
0.7	16 16.0	24 16.0	19 19.0	Gentamicin (10)

and fecal samples, and the resistance rates were significantly higher in inpatient samples than in outpatient and fecal samples ($p \leq 0.05$). For TM and NA, a significantly higher rate of resistance was observed in fecal samples than in the inpatient and outpatient isolates ($p \leq 0.05$). There was no difference with respect to gender or age.

The rate of MDR, defined as resistance to at least three classes of antimicrobial agents, was high, and the MDR phenotype was detected in 61%, 55.6%, and 57% of the inpatient, outpatient, and fecal isolates, respectively (**Table 3**). In the MDR isolates, the most frequent resistance pattern was to TM, NA, CPM, CAZ, CTX, and CIP (23 isolates, 11.3%),

TABLE 3: Antibiotic resistance patterns of 206 multidrug-resistant *Escheriria coli* isolates from UTIs and fecal samples.

Pattern of antibiotic resistance	Total number of isolates	Isolates according to sample source (n)		
		fecal flora	outpatient	inpatient
AUG,TM,NA,CTX,CPM,CAZ,PIT,CIP,AK	4	-	2	2
AUG,TM,NA,CTX,CPM,CAZ,PIT,CIP,GM	10	2	3	5
AUG,TM,NA,CTX,CPM,CAZ,PIT,CIP	16	4	6	6
AUG,TM,NA,CTX,CPM,CAZ,CIP,GM	14	4	5	5
AUG,TM,NA,CTX,PIT,CIP,GM,AK	11	1	-	-
AUG,TM,NA,CTX,CPM,CAZ,CIP,AK	1	1	-	-
TM,NA,CTX,CPM,CAZ,PIT,CIP,GM	1	-	-	1
AUG,NA,CTX,CAZ,PIT,CIP,GM,AK	1	-	-	1
AUG,TM,NA,CTX,CPM,CAZ,CIP	29	8	12	9
TM,NA,CTX,CPM,CAZ,PIT,CIP	2	-	1	1
AUG,TM,NA,CTX,CPM,CAZ,PIT	2	-	1	1
AUG,TM,NA,CTX,CPM,PIT,CIP	1	-	1	-
AUG,TM,NA,CTX,PIT,CIP,GM	1	-	1	-
AUG,TM,NA,PIT,CIP,GM,AK	1	1	-	-
TM,NA,CTX,CPM,CAZ,CIP	15	4	6	5
AUG,TM,NA,CTX,CPM,CAZ	11	5	4	2
AUG,TM,NA,CTX,CIP,GM	6	2	2	2
AUG,TM,CTX,CPM,CAZ,CIP	4	-	2	2
AUG,TM,NA,CPM,PIT,CIP	3	-	1	2
AUG,TM,NA,CTX,CAZ,CIP	2	-	-	2
TM,NA,CTX,PIT,CIP,AK	1	-	-	1
TM,CTX,CPM,CAZ,PIT,CIP	1	-	1	-
TM,NA,CTX,CPM,CAZ	10	3	4	3
TM,NA,CTX,CPM,CIP	5	1	2	2
AUG,TM,NA,CPM,CIP	4	1	2	1
AUG,TM,NA,PIT,CIP	4	1	2	1
AUG,TM,NA,CIP,GM	2	-	1	1
AUG,TM,NA,CTX,CAZ	2	2	-	-
AUG,TM,NA,CPM,CAZ	1	-	1	-
AUG,TM,NA,CTX,GM	1	1	-	-
AUG,NA,CTX,CPM,CAZ	1	-	1	-
TM,NA,CTX,CAZ,CIP	1	-	-	1
AUG,TM,CAZ,PIT,CIP	1	-	1	-
AUG,TM,NA,CIP	10	-	5	5
TM,NA,CTX,CIP	2	-	1	1
TM,NA,PIT,CIP	2	-	1	1
AUG,NA,CIP,GM	2	-	1	1
NA,PIT,CIP,AK	1	-	1	-
TM,NA,CTX,GM	1	1	-	-
TM,NA,CTX,CAZ	1	-	1	-
NA,CTX,CPM,GM	1	-	1	-
TM,NA,CIP,GM	1	-	1	-
AUG,TM,NA	6	2	4	-
TM,NA,GM	3	-	2	1
AUG,TM,NA	3	-	3	-
TM,PIT,AK	1	-	1	-
AUG,TM,CIP	3	-	1	-

UTIs: urinary tract infections; **AUG:** amoxicillin-clavulanic acid**; TM:** trimethoprim; **NA:** nalidixic acid; **CTX:** cefotaxime; **CPM:** cefepime; **CO**: colistin; **CAZ:** ceftazidime; **IMP:** imipenem; **AM:** amikacin; **PIT:** piperacillin/tazobactam; **CIP:** ciprofloxacin; **GM:** gentamicin.

followed by AUG, TM, NA, CTX, CPM, CAZ, and CIP (20 isolates, 9.8%; **Table 3**). The antimicrobial resistance patterns of 24 isolates were unique.

Detection of ESBLs, AmpC, carbapenemase, and blagenes

The frequency of ESBL-positive isolates, as determined by the combined disk test, was 46.4% (163) for all isolates, and was 53% (53), 41% (62), and 48% (48) for inpatient, outpatient, and fecal isolates, respectively. Isolates from inpatients had a significantly higher rate of ESBLs compared to outpatient and fecal samples (P=0.000). Among the 163 ESBL-positive isolates, 11 (6.74%) isolates were AmpC producers. The MHT was negative for all imipenem-resistant isolates. The rates of detection for the following ESBL genes by PCR: bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{CTX\text{-}Mgroup1}$ were 74.8% (122), 1.2% (2), 12.88% (21), and 76% (124), respectively (**Figure 1**). The other tested beta-lactamase genes, bla_{PER}, $bla_{CTX\text{-}Mgroup2,3,4}$, $bla_{KPC,}$ and bla_{NDM}, were not detected in any of the tested isolates. The rates of AmpC and bla_{TEM} were significantly higher in isolates from inpatients than in isolates from outpatients (P=0.003; **Figure 1**). Sequencing of the $bla_{CTX\text{-}Mgroup1}$, bla_{TEM}, bla_{OXA}, and bla_{SHV} ESBL genes confirmed that they were $bla_{CTX\text{-}Mgroup1}$, bla_{TEM1}, bla_{OXA1}, and bla_{SHV12}, respectively.

Genetic comparison of the ESBL-producing *Escherichia coli* isolates by ERIC-PCR

The ERIC-PCR amplified sequences produced a well-separated, sharp band in agarose electrophoresis in the range of 100-3000bp as estimated with the Gene Ruler 100bp Plus DNA Ladder (Thermo Scientific, Co. Canada). The amplified sequences were analyzed by the UPGMA program (UPGMA clustering UN weighted pair-group method with arithmetic averages) using Gel Compare software (version 4.0). In the data analysis, a cutoff of 70% was used due to the high diversity of the isolates. With this cutoff, 31 clusters and 17 singletons were detected. The dendrogram showed one major cluster comprising 19 isolates, with 14 from inpatients and 5 from outpatients. The second major cluster included 8 UTI isolates, which were mostly from inpatients. The other clusters contained a combination of isolates from inpatient and outpatient UTIs and fecal samples (**Figure 2**).

DISCUSSION

UTIs are a major public health problem, both in hospitals and in the community. Drug resistance in *E. coli*, due to horizontal gene transfer, has resulted in the emergence of MDR isolates, which are difficult to treat. The bacteria that cause most UTIs are of an endogenous origin; therefore, the fecal flora is important, and is likely a source of resistance genes. In this study, the antimicrobial resistance of fecal and UTI isolates were compared. *E. coli* is an important cause of uncomplicated UTIs in women, and it is also one of the most common causes of septicemia and meningitis in newborn infants, diarrhea in children less than five years of age, pneumonia and other nosocomial infections, especially ESBL-producing isolates,

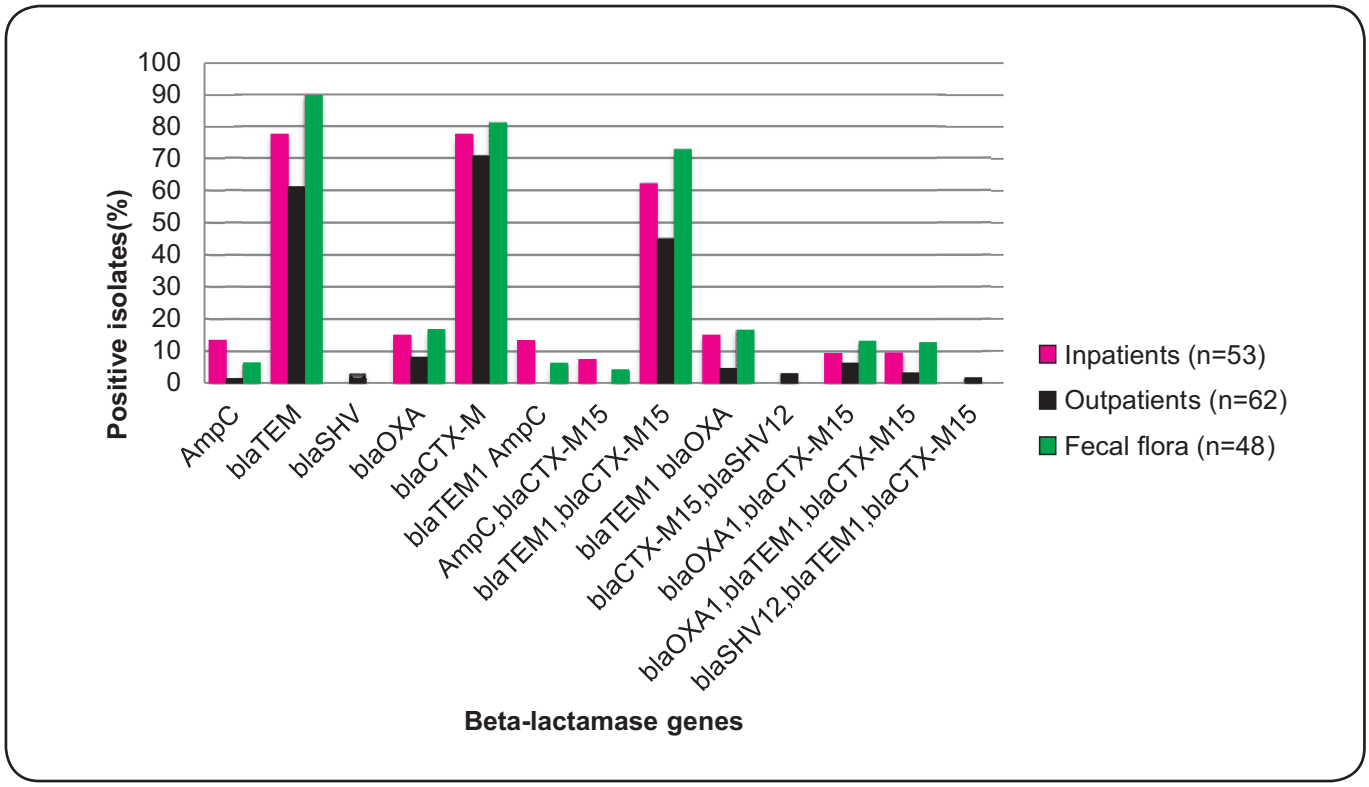

FIGURE 1: Distribution of ESBL genes and AmpC beta-lactamases among 163 ESBL-producing *Escherichia coli* isolates from urinary tract infections and fecal flora. **ESBL:** extended spectrum β-lactamases.

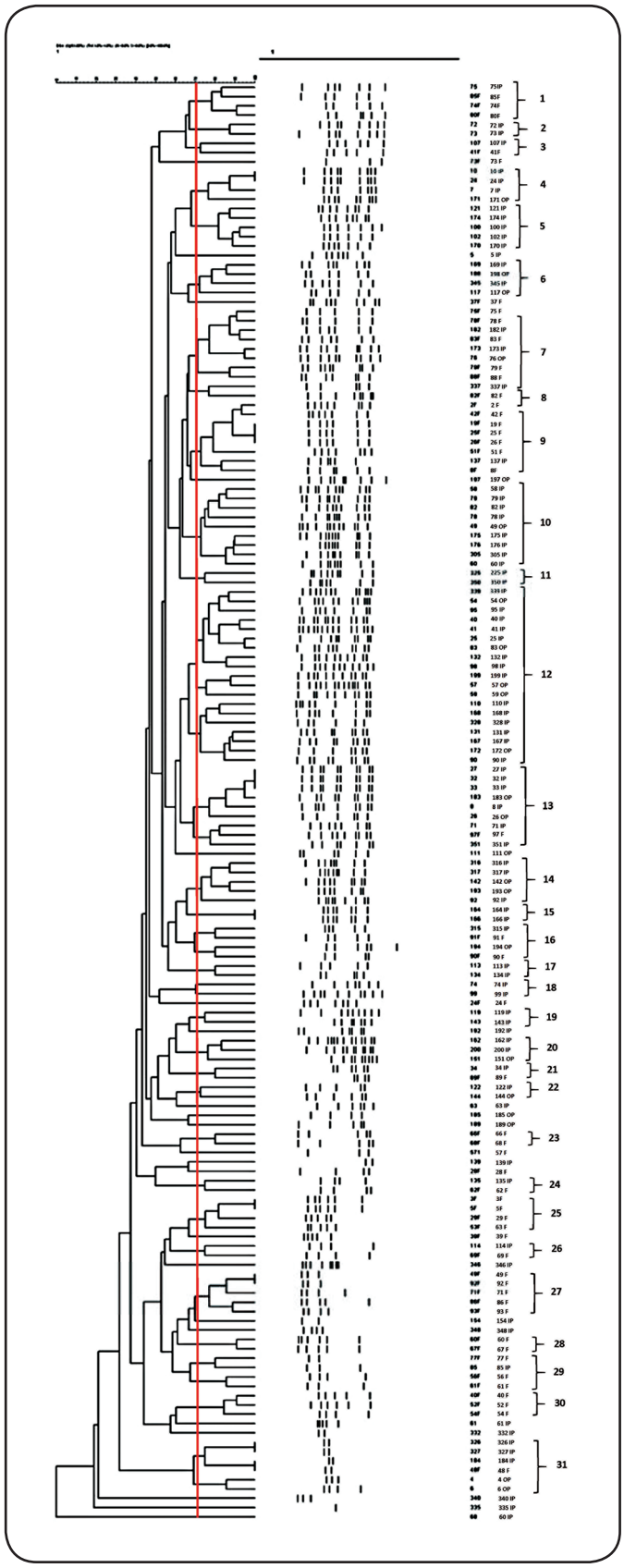

FIGURE 2: Dendrogram generated with ERIC-PCR data for ESBL-positive *Escherichia coli* isolates from UTIs and fecal flora. **ERIC-PCR:** enterobacterial repetitive intergenic consensus-polymerase chain reaction; **ESBL:** extended spectrum β-lactamases; **UTIs:** urinary tract infections; **IP:** inpatient; **OP:** outpatient; **F:** fecal sample.

in immunocompromised and debilitated patients[8]. In recent years, the emergence of antimicrobial resistance has become an increasingly common factor in the management of patients with UTIs. Antibiotic resistance in *E. coli* isolates from women with uncomplicated UTIs has been reported in Germany, Spain, Sweden, and the UK[23], and higher rates of resistance have been reported from non-European countries, such as India, China, and South Korea[24-26]. Our results also showed a high rate of resistance to various antimicrobial agents.

In a study performed in this area in 2002, the rates of resistance to various antimicrobial agents was much lower than those observed in the present study, and the sensitivity rates to GM, CTX, ZOX (ceftizoxime), and FM (nitrofurantoin) for fecal and UTI isolates were greater than 90%[27]. This marked increase in resistance rates in a short time should be taken very seriously, and steps should be taken to reduce the rate of this increase. According to Walker et al.[28] and based on other available literature, the rate of trimethoprim sensitivity remains high for most MDR *E. coli* isolates from UTIs. Commensal *E. coli* in the gastrointestinal tract are regarded as an important source of antibiotic-resistance genes, and these organisms can transfer resistance genes to other bacteria when exposed to antimicrobial agents[29]. In this study, the rates of resistance to amoxicillin-clavulanic acid and piperacillin/tazobactam were significantly higher in UTI isolates than in fecal isolates ($P \geq 0.05$). This demonstrates the important role of the fecal flora as a source of drug resistance in the community. The presence of antimicrobials in animal food for growth promotion and prophylaxis may be responsible for the emergence of antibacterial resistance strains in humans[30,31]. Simultaneous resistance to 5-9 antimicrobial agents was detected in 79.4% of the isolates, while resistance to 3-4 antimicrobial agents was detected in 17.1% of the isolates. These results indicate that there is a shortage of available drugs for the treatment of UTIs.

ESBL-producing bacterial strains have spread worldwide, and nosocomial infections with ESBL-producing *E. coli* isolates are responsible for high rates of morbidity and mortality in infected patients[32]. Therefore, early detection and identification of ESBL-producing isolates are necessary to control the infection and prevent the spread of these organisms in hospitals and in the community. Significant associations have been reported for UTIs caused by ESBL-producing *E. coli* and male sex, residence in a nursing home, diabetes mellitus, and recurrent UTIs[33]. Other risk factors have also been reported, such as indwelling urinary catheters, old age, ventilation, and organ transplants or other surgery, as well as high-dose or long-time treatment with antimicrobials, especially beta-lactams and fluoroquinolones[33]. In the present study, 46.4% of the isolates were ESBL producers. However, isolates from outpatients had a significantly lower rate of ESBL production compared to isolates from inpatients and fecal samples. We did not find any difference in ESBL production according to age or gender. A high rate of ESBL-producing *E. coli* isolates from fecal samples has been reported by other researchers, and Leflon et al.[30] reported an increase in the rate, from 0.6% to 6%, over a five-year period in Paris, France. In the present study, we observed relatively

high numbers of isolates producing $bla_{\text{CTX-Mgroup1}}$ (76%) and bla_{TEM} (74.8%). The presence of $bla_{\text{CTX-Mgroup1}}$ and bla_{TEM} were significantly higher in isolates from fecal samples and UTIs of inpatients (P=0.0004). Recently, a higher rate of $bla_{\text{CTX-M}}$ has been reported in enteric bacteria, especially *E. coli* and *Klebsiella pneumoniae*, likely since the genes encoding these enzymes are located on plasmids or other mobile genetic elements. Because CTX-M family genes are present on plasmids or other mobile genetic elements, these enzymes have become predominant worldwide and are substituting for the more common SHV and TEM[7,32]. The most common type of CTX-M reported in various geographic regions is CTX-M15; these strains are usually MDR and are detected not only in nosocomial infections but also in the community[4,24,32], and this result is in accordance with our finding of a high frequency of CTX-M15-producing *E. coli* isolates. The $bla_{CTX\text{-}M}$-positive isolates in this study were MDR, showing resistance to fluoroquinolones, trimethoprim, tetracycline, and aminoglycosides, as well as non-carbapenem beta-lactamases, as was reported by Woodford & Ward in 2004[34]. It has been suggested that CTX-M15 was disseminated by clonal expansion of the pandemic *E. coli* clone ST131[24], which could also be the source of the genes observed in this study.

The genetic diversity of ESBL-producing *E. coli* isolates, as determined by ERIC-PCR, revealed the presence of diverse clonal groups (**Figure 2**). Similar results were observed according to the antimicrobial resistance patterns, with 42 different combinations of antimicrobial resistance. These results suggested that different strains of *E. coli* are widely distributed in this area, and empiric therapy is not recommended for the treatment of UTIs in this region.

Finally, we suggest that antimicrobial susceptibility testing should be performed to select the best available drug for the treatment of patients with UTIs. Due to high impact of CTX-M on public health, such as the difficultly in treating community-acquired UTIs, the spread of isolates producing ESBLs, especially CTX-M15 beta-lactamase, needs to be closely monitored. It could be concluded that in this area, inappropriate use or misuse of antimicrobial agents may be a cause of the high resistance rates instead of clonal spread of resistant strains.

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Conflict of interest

The authors declare that there is no conflict of interest.

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