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Retracted Article: Determination of antibiotic susceptibility, ESBL genes and pulsed-field gel electrophoresis profiles of extended-spectrum β-lactamase-containing *Escherichia coli* isolates

Hidayet TUTUN[1,a,✉], Alper KARAGÖZ[2,b], Levent ALTINTAŞ[3,c], Nadir KOÇAK[4,d]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Burdur; [2]Uşak University, Department of Molecular Biology and Genetics, Uşak; [3]Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara; [4]Selçuk University, Faculty of Medicine, Medical Genetics, Konya, Turkey.
[a]ORCID: 0000-0001-9512-8637; [b]ORCID: 0000-0002-8178-223X; [c]ORCID: 0000-0002-5148-723X; [d]ORCID: 0000-0002-1727-1582.

✉Corresponding author: hidayettutun@mehmetakif.edu.tr
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Abstract: The purpose of this study was to determine the phenotypic antibiotic susceptibility patterns, extended-spectrum β-lactamase (ESBL) genes and genotypic profiles of ESBL-positive *Escherichia coli* strains isolated from urine samples obtained from outpatients with urinary tract infection in Turkey. A total of 120 *E. coli* strains during 2017, 2018, and 2019 (40 patients per year) were examined for antibiotic susceptibility patterns by disc diffusion method, for ESBL genes using PCR and sequencing and for molecular typing by pulsed-field gel electrophoresis (PFGE) method. The isolates were evaluated for their sensitivity to 21 different antibiotics. Four different antimicrobial resistance patterns were determined according to antibiotic susceptibility status of the isolates. The β-lactamase genes detected in the isolates were CTX-M-15 + OXA-1 (n= 14), CTX-M-15 (n= 24), TEM-1 + CTX-M-15 (n= 52), TEM-1 + SHV-12 (n=6), SHV-12 1 (n= 6), TEM-1 + CTX-M-1 (n= 6), TEM-1 + CTX-M-16 (n= 6) and TEM-1 + CTX-M-9 (n= 6). The CTX-M-15 was the most prevalent ESBL enzyme in the isolates. As a result of PFGE analysis performed by XbaI enzyme restriction process, one major PFGE profile and three main groups (Group I-II-III) were observed. While antibiotic resistance profiles of the strains showed four groups (RI-RII-RIII-RIV), PFGE band profiles showed a major group (90% similarity ratio). High ESBL production and decreased susceptibility to broad-spectrum cephalosporins were observed in *E. coli* strains. In addition, PFGE analysis showed high clonal similarity among *E. coli* isolates.

Keywords: Antimicrobial drug resistance, ESBL genes, *Escherichia coli*, molecular subtyping, pulsed-field gel electrophoresis.

Genişlemiş spektrumlu ß-laktamaz üreten *Escherichia coli* izolatlarının antibiyotik duyarlılıklarının, GSBL genlerinin ve pulsed-field jel elektroforez yöntemiyle genotipik profillerinin belirlenmesi

Özet: Bu çalışmada Türkiye'de idrar yolu enfeksiyonu bulunan hastalardan alınan idrar örneklerinden izole edilmiş olan genişlemiş spektrumlu β-laktamaz (GSBL) pozitif *E. coli* suşlarının antibiyotik duyarlılık paternlerinin (fenotipik), GSBL genlerinin ve genotipik profillerinin belirlenmesi amaçlandı. Çalışmada 2017, 2018 ve 2019 yıllarında ve her yıl 40 hastadan olmak üzere, toplam 120 adet *E. coli* suşunun disk difüzyon yöntemi ile antibiyotik duyarlılıkları, PCR ve sekanslama ile GSBL genleri, pulsed-field jel elektroforez (PFGE) yöntemi ile moleküler tipleri belirlendi. Numunelerden elde edilen izolatların 21 farklı antibiyotiğe karşı duyarlılığı değerlendirildi. İzolatların antibiyotik duyarlılık durumlarına göre, değerlendirmede dört farklı antimikrobiyal direnç paterni tespit edildi. İzolatlarda β-laktamaz genleri olarak CTX-M-15 + OXA-1 (n= 14), CTX-M-15 (n= 24), TEM-1 + CTX-M-15 (n= 52), TEM-1 + SHV-12 (n= 6), SHV-12 1 (n= 6), TEM-1 + CTX-M-1 (n= 6), TEM-1 + CTX-M-16 (n= 6) ve TEM-1 + CTX-M-9 (n= 6) tespit edildi. CTX-M-15 izolatlarda en yaygın görülen GSBL enzim tipi olarak belirlendi. PFGE analizi sonucunda, bir majör PFGE profili ve üç ana grup (Grup I-II-III) gözlendi. Suşların antibiyotik direnç profilleri, dört grupta (RI-RII-RIII-RIV) bulunurken, PFGE bant profilleri ise bir majör grup içinde bulunduğu (% 90 benzerlik oranı) belirlendi. Numunelerde *E. coli* yüksek GSBL üretimi ve geniş spektrumlu sefalosporinlere karşı azalan bir duyarlılık gözlendi. Ayrıca, PFGE analizi ile bu izolatların yüksek klonal benzerliğe sahip olduğu da gösterildi.

Anahtar sözcükler: Antimikrobiyal direnç, GSBL genleri, *Escherichia coli*, moleküler alt tiplendirme, pulsed-field jel elektroforez.

Introduction

Escherichia coli is a commensal bacteria of the digestive tract microflora of humans and animals, some of them cause intestinal and extraintestinal pathologies (25). Animals are defined as important zoonotic reservoirs for human intestinal pathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) causing diseases in farm and pet animals (4, 53). Extraintestinal pathogenic *E. coli* is an important cause of diverse infections, which includes urinary tract infections (UTI) in human and animals (26, 32, 53). UTI is a significant bacterial infection that causes serious complications including emphysematous cystitis and pyelonephritis when infection is inadequately managed. Although most of the patients face a single or rare episode of the disease, many patients experience recurrent UTIs (19, 50). Approximately 80 % of patients who suffer from UTI is caused by *E. coli* and *Staphylococcus saprophyticus* (15).

Antibiotic resistance leads to failure in the treatment of both community- and hospital-acquired infections and appears to be a growing problem worldwide. The application of antibacterial drugs in clinical therapy results in the emergence of microorganisms that are resistant to these drugs. One of the most common bacterial-resistance mechanism against antimicrobial drugs is the inactivation of the drug by the enzymes they synthesize (8). β-lactamases are enzymes that are produced by bacteria providing multiple resistance to β-lactam compounds by hydrolyzing the β-lactam ring in these antibiotic groups. The prolonged exposure of bacterial strains to a large number of β-lactam antibiotics has increased their activities by inducing mutation of the β-lactamases, which are known as extended-spectrum β-lactamases (ESBLs), against the third-generation cephalosporins (42). Types of ESBL generated by mutations in genes coding the narrow-spectrum β-lactamases (TEM-1, TEM-2, or SHV-1) are TEM, SHV, CTX-M, OXA and Amp C. The most frequently identified ESBL genes produced by *E. coli* and *Klebsiella* spp. are TEM and SHV (34). The emergence of ESBL-producing bacteria has been commonly reported in veterinary medicine since β-lactam antibiotics have been used mostly for therapeutic and prophylactic reasons in livestock (35, 47). Several studies showed that similar ESBL isolates were found in human and livestock, suggesting a zoonotic transfer (11, 22, 28).

The emergence of antibiotic resistance is accelerated by the overuse and misuse of antibiotics and the lack of development of new antimicrobial drugs (48). Antimicrobial agents are widely used for therapeutic or nontherapeutic purposes in animal husbandry. Use of these drugs results in selection for antimicrobial resistant *E. coli* in the microflora of these animals. Subsequently, antimicrobial-resistant *E. coli* can be transferred from animals to humans through cross-contamination or consumption of raw or insufficiently cooked meat contaminated with antimicrobial resistant bacteria (4, 38).

Pulsed-field gel electrophoresis is a molecular fingerprinting method considered the “gold standard” among molecular typing methods to classify bacteria. The method is based on the determination and interpretation of the profiles formed by the appropriate restriction endonuclease enzyme of genomic DNA isolated from the bacterial cell embedded in low melting agarose without deterioration of the structural integrity. PFGE technique has been used safely in the typing of many bacteria such as *Salmonella typhimurium*, *Neisseria gonorrhoeae*, methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, and *E. coli* (12, 14, 16, 20, 49).

The purpose of the present study was to determine the antibiotic susceptibility patterns and genotypic profiles of ESBL positive *E. coli* strains isolated from urine samples collected between 2017 and 2019 and also determine the prevalence of ESBL genes among the isolates. The most effective antibiotic selection was provided for the empirical treatment of *E. coli*-induced UTI by forming an antibiotic susceptibility pattern of *E. coli*. In addition, by determining the possible clonal relationship between isolates via PFGE analysis, the similarity of antibiotic susceptibilities of strains with common band profiles was investigated.

Material and Methods

***Sample collection*:** A total of 120 *E. coli* isolates obtained from UTI patient’s urine samples collected from outpatients (n= 120) in Public hospital in Konya province of Turkey between 2017-2019 were evaluated in the present study. Patient data anonymized in this study. These samples were collected with collection containers and transported to the microbiology laboratory.

***Isolation and identification of Escherichia coli*:** *E. coli* isolates were incubated in Eosin Methylene-blue (EMB) and Nutrient agar (NA) media overnight at 37ºC by a single colony incubation technique. Colonies which appeared metallic sheen were again fished out into nutrient broths and subcultures were maintained on nutrient agar and used for further identification and antimicrobial sensitivity testing. A single colony picked up and identified as *E. coli* using IMVIC test (citrate, methyl red, Voges-Proskauer, citrate, ornithine, urea, indole, kligler iron agar media). The strains were confirmed as *E. coli* using with gram-negative crystal identification kit (BBL Crystal ID System, Becton Dickinson, Cockeysville). ESBL production was confirmed if the presence of a β-lactamase inhibitor enlarged, the zone size of inhibition by ≥5 mm for all 120 isolates (10).

***Antimicrobial susceptibility testing*:** Disc diffusion method according to Clinical and Laboratory Standards Institute (10) was used for antibiotic susceptibility test.

Tested antibiotics (BBL, Becton Dickinson) were cefoxitin (FOX, 30 µg), cefotaxime (CTX, 30 µg), cefepime (FEP, 30 µg), ceftazidime (CAZ, 30 µg), cefazolin (CFZ, 30 µg), cephalothin (CEF, 30 µg), cefuroxime (CXM, 5 µg), ampicillin (AMP, 10 µg), ampicillin-sulbactam (SAM, 10 µg), amoxicillin-clavulanate (AMC, 30 µg), imipenem (IPM, 10 µg), piperacillin (PIP, 100 µg), trimethoprim-sulfamethoxazole (SXT, 23.75 µg/1.25 µg), ofloxacin (OFX, 5 µg), amikacin (AMK, 30 µg), gentamicin (GEN, 10 µg), sulfisoxazole (SXZ, 0.25 µg), nitrofurantoin (NIT, 100 µg), piperacillin-tazobactam (TZP, 110 µg), ticarcillin-clavulanate (TIM, 85 µg), carbenicillin (CAR, 100 µg). Quality control was performed with *E. coli* ATCC 25922 strain. Isolates were grouped as resistant (R), intermediate-resistant (I), or susceptible (S) according to the CLSI (10).

***Characterization of ESBL genes*:** The presence of genes encoding TEM, SHV, OXA and CTX-M type β-lactamases was examined in this study. PCR screening for the presence of different β-lactamase genes, *bla*TEM-type, *bla*CTX-M-type, *bla*SHV-type and *bla*OXA-1-type, were performed as described previously (6, 7, 24). Amplicons obtained from PCR were sequenced on both strands and sequences were compared to those reported in the Database of the GenBank and on the Lahey Clinic beta-lactamase website (http://www.lahey.org/Studies/) to identify the β-lactamase genes.

***PFGE analysis*:** The isolates, which were identified as *E. coli* by biochemical and molecular methods and confirmed as ESBL-producing *E. coli* by antibiotic susceptibility test, were subjected to PFGE to analyze the genetic diversity of them in order to investigate genetic similarities. A single colony of each isolate was suspended with CSB buffer (cell suspension buffer, 10 mM Tris-HCl, 50 mM EDTA, 20 mM NaCl, pH 7.2). 2 % low-melting agarose (LMA, Sigma-Aldrich) was prepared in the CSB buffer supplemented with Sodium dodecyl sulfate (a final concentration of 1 %) (SDS, Sigma-Aldrich). The agarose-buffer mixture was melted by heating in magnetic stirrer to 45-50°C. Bacteria suspension was added to the agarose tubes by means of a pipette, and the pipette was used for mixing the suspension. This mixture was transferred to plug molds (10mm x 5mm x 1.5mm, Bio-Rad) and after solidification of agarose for the preparation of high-quality DNA, the plugs were incubated overnight at 55°C in lysis buffer (50 mmol Tris-HCI, pH 8.0; 50 mmol EDTA, pH 8.0; 1 % sarcosine; 1 mg of proteinase K/ml). The agarose was washed three times with sterile distilled water, followed by three washes with TE buffer (10 mmol Tris, pH 8.0; 1 mmol EDTA, pH 8.0) Agarose-embedded DNA was transferred to the mixture containing XbaI restriction enzyme (Thermo Scientific), and incubated to digest at 37°C for 2 hours. PFGE was carried out with the CHEF-DR® II system (Bio-Rad Laboratories, Nazareth, Belgium) using a 1 % of pulsed-field certified agarose prepared in standard 0.5xTris-boric acid-EDTA (TBE buffer). The electrophoresis condition was set as follows: Initial switch time, 2 s; final switch time, 35 s, run time, 20 h; gradient, 6V/cm; angle 120°; temperature, 14°C. After electrophoresis, the gel was stained by putting into 400 ml ultrapure water solution containing 5 µg/ml ethidium bromide for 20 minutes and the fingerprinting profile was photographed under ultraviolet light using a Gel Logic 220 imaging system (Kodak Company, USA). The band profiles were analyzed using the Gel Compar II software system (version 3.0, Applied Maths, Sint-Martens-Latem). First of all, three external standard strains (1, 7, 15, carried out in wells, *E. coli* ATCC 25922 strain) in each gel were used to normalize the images. Dendrograms and clustering analysis of PFGE profiles were performed using "the unweighted-pair group method with mathematical averaging" (UPGMA), the Dice coefficient with a 1-1.5 % band position tolerance and optimization. The interpretation of PFGE patterns was categorized as follows: indistinguishable, closely related, possibly related, or different according to the criteria of Tenover et al. (44).

Results

All isolates were tested for antibiotic resistance using Disc diffusion method. Table 1 shows the resistant

Table 1. Antibiotic resistance profiles for *E. coli* isolates.

Resistance phenotype	Resistance pattern	Isolate number	%
RI	SXT, OFX, AMP	1,2,3,5,6,7,8,9,10,13,14,15,17,18,19,20,21,22,25,26,28,34,35,38,39,43,47,51,56,59,63, 69,73,74,78,81,84,88,92,96,97,99,105,106,107,108,109,110,111,112,113,114	43
RII	AMC, TZP, TIM	42,46,50,54,57,60,64,66,67,71,72,77,80,85,87,91,93,94,95,98 100,101,102,103,104,115,116,117,118,119,120	26
RIII	GEN, AMK, CAR, PIP	4,12,16,23,27,29,33,37,41,45,49,52,61,62,65,68,70,75,76,79,82,83,86,89,90	21
RIV	NIT, G25, IMP	11,24,30,31,32,36,40,44,48,53,55,58	10

SXT: Trimethoprim-sulfamethoxazole; OFX: Ofloxacin; AMP: Ampicilin; AMC: Amoxicillin-clavulanate; TZP: Piperacillin-tazobactam; TIM: Ticarcillin-clavulanate; GEN: Gentamicin; AMK: Amicasin; CAR: Carbenicillin; PIP: Piperacillin; NIT: Nitrofurantoin; G25: Gentamicin high resistance; IMP: Imipenem.

phenotypes of *E. coli* isolates. Among the isolates, the predominant resistance profile was RI (SXT-OFX-AMP). Approximately 43 % of all isolates were RI phenotype, whereas isolates with RII phenotype (Combinations of β-lactam-β-lactam inhibitors) accounted for 26 %. 21 % of all isolates were resistant to 4 antibiotics belonging to RIII phenotype (GEN, AMK, CAR, and PIP). Aminoglycoside antibiotics (GEN, AMK) were considered as the most important ones in this group. Isolates (10 %) which are resistant to other antibiotics were classified as RIV phenotype (NIT, G25, and IMP).

In the PFGE study for genotypic typing, after the *E. coli* DNAs were cut with FastDigest XbaI enzyme with restriction endonuclease activity, PFGE gel images in which various band patterns were formed were determined. Dendrogram analysis was performed in the next stage of gel images of *E. coli* strains in which PFGE band profiles were observed. After the band profile analysis using Gel-Compare-II, PFGE profile dendrograms were formed and the relationships between the strains were determined according to the Dice similarity coefficient. When the dendrogram of 120 *E. coli* strains of 2017, 2018 and 2019 were examined; based on Tenover criteria (44), 120 strains were found to be related to each other according to 85% and higher similarity rates. Although there is only one major clone, the strains are divided into three pulsotypes. Group I (90-3/113 strains), Group II (51-81/2 strains), Group III (120-61-67-64-66/5 strains) (Figure 1).

After all the studies, epidemiological data were obtained from the clinical files of the patients in order to correlate all the data with significant results. In the present study that examined patients admitted to the hospital at different times, a data (Table 2) with information about epidemiological information, antimicrobial susceptibility patterns and PFGE types of 120 isolates collected from 2017 to 2019 were obtained.

Group I (113 strains) and Group III (5 strains) were the most common PFGE profile groups in the genotypic investigation of *E. coli* strains. Among the 113 strains in Group I, 50 had RI resistance and had SXT, OFX and AMP resistance. In addition, 27, 24, and 12 strains had RII, RIII and RIV resistances, respectively. The two identical strains in Group II showed the same antibiotic resistance pattern (RI). The 4 strains in Group III show the same antibiotic resistance pattern (RII). Furthermore, there is also a strain with RIII resistance (Table 2).

All the phenotypic ESBL-producing *E. coli* isolates were confirmed by PCR and sequencing for detection of genes encoding TEM, SHV, OXA and CTX-M type β-lactamases. The β-lactamase genes detected in the isolates were CTX-M-15 + OXA-1 (n= 14), CTX-M-15 (n= 24), TEM-1 + CTX-M-15 (n= 52) , TEM-1 + SHV-12 (n= 6),

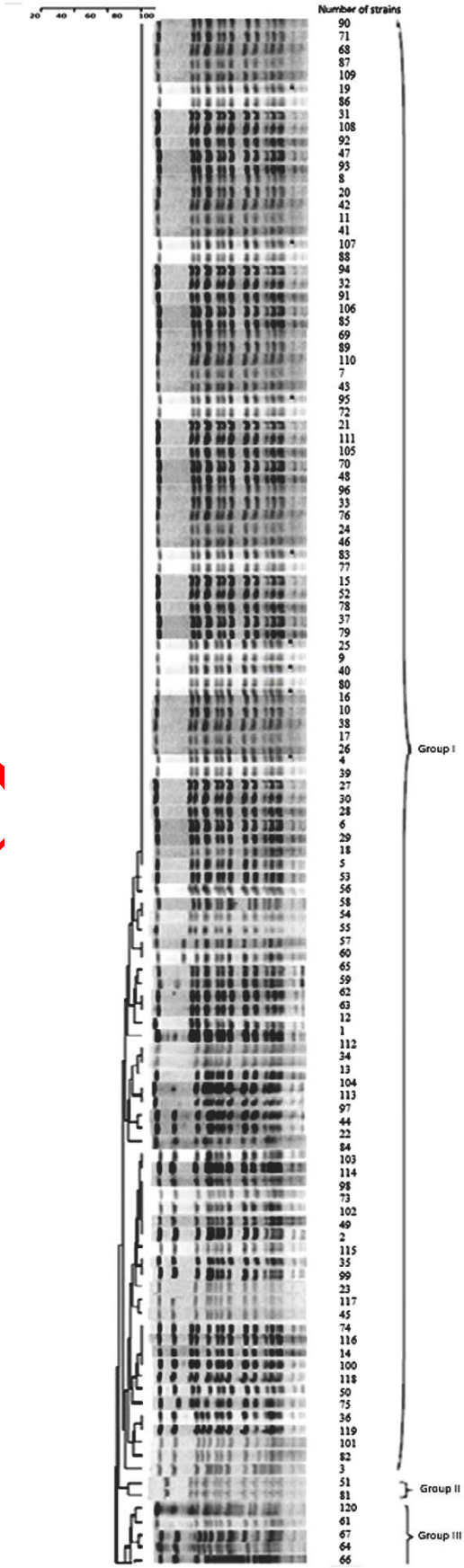

Figure 1. PGFE XbaI digestion patterns and clonal analysis of 120 ESBL-producing *E. coli* isolates (2017-2019) (1-120). The dendrogram using the Dice coefficient and UPGMA clustering methods showing the relationships between *E. coli* strains.

Table 2. Comparison of PFGE and antibiotic resistance profiles of *E. coli* strains.

Isolation year	Isolates number	*bla* gene(s)	Antibiotic resistance phenotype	PFGE profile
2017	1	CTX-M-15, OXA-1	RI	Group I
2017	2	CTX-M-15, OXA-1	RI	Group I
2017	3	CTX-M-15, OXA-1	RI	Group I
2017	4	CTX-M-15	RIII	Group I
2017	5	TEM-1, SHV-12	RI	Group I
2017	6	CTX-M-15, OXA-1	RI	Group I
2017	7	CTX-M-15, OXA-1	RI	Group I
2017	8	CTX-M-15, OXA-1	RI	Group I
2017	9	CTX-M-15, OXA-1	RI	Group I
2017	10	SHV-12 1 (4)	RI	Group I
2017	11	TEM-1, CTX-M-1	RIV	Group I
2017	12	CTX-M-15	RIII	Group I
2017	13	TEM-1, SHV-12	RI	Group I
2017	14	TEM-1, CTX-M-1	RI	Group I
2017	15	TEM-1, CTX-M-15	RI	Group I
2017	16	CTX-M-15	RIII	Group I
2017	17	CTX-M-15, OXA-1	RI	Group I
2017	18	CTX-M-15, OXA-1	RI	Group I
2017	19	CTX-M-15, OXA-1	RI	Group I
2017	20	SHV-12 1 (4)	RI	Group I
2017	21	TEM-1, CTX-M-15	RI	Group I
2017	22	TEM-1, CTX-M-16	RI	Group I
2017	23	CTX-M-15	RIII	Group I
2017	24	TEM-1, CTX-M-15	RIV	Group I
2017	25	CTX-M-15, OXA-1	RI	Group I
2017	26	CTX-M-15, OXA-1	RI	Group I
2017	27	CTX-M-15	RIII	Group I
2017	28	TEM-1, SHV-12	RI	Group I
2017	29	CTX-M-15	RIII	Group I
2017	30	SHV-12 1 (4)	RIV	Group I
2017	31	TEM-1, CTX-M-1	RIV	Group I
2017	32	TEM-1, CTX-M-15	RIV	Group I
2017	33	CTX-M-15	RIII	Group I
2017	34	TEM-1, SHV-12	RI	Group I
2017	35	TEM-1, CTX-M-15	RI	Group I
2017	36	TEM-1, CTX-M-15	RIV	Group I
2017	37	CTX-M-15	RIII	Group I
2017	38	CTX-M-15, OXA-1	RI	Group I
2017	39	CTX-M-15, OXA-1	RI	Group I
2017	40	SHV-12 1 (4)	RIV	Group I
2018	41	CTX-M-15	RIII	Group I
2018	42	TEM-1, CTX-M-15	RII	Group I
2018	43	TEM-1, SHV-12	RI	Group I
2018	44	TEM-1, CTX-M-15	RIV	Group I
2018	45	CTX-M-15	RIII	Group I
2018	46	TEM-1, CTX-M-15	RII	Group I
2018	47	TEM-1, CTX-M-9	RI	Group I
2018	48	TEM-1, CTX-M-15	RIV	Group I
2018	49	CTX-M-15	RIII	Group I
2018	50	TEM-1, SHV-12	RII	Group I
2018	51	SHV-12 1 (4)	RI	Group II
2018	52	TEM-1, CTX-M-15	RIII	Group I
2018	53	CTX-M-15	RIV	Group I
2018	54	TEM-1, CTX-M-15	RII	Group I
2018	55	TEM-1, CTX-M-1	RIV	Group I
2018	56	TEM-1, CTX-M-15	RI	Group I
2018	57	SHV-12 1 (4)	RII	Group I
2018	58	TEM-1, CTX-M-15	RIV	Group I
2018	59	TEM-1, CTX-M-16	RI	Group I
2018	60	TEM-1, CTX-M-15	RII	Group I

2018	61	CTX-M-15	RIII	Group III
2018	62	CTX-M-15	RIII	Group I
2018	63	TEM-1, CTX-M-15	RI	Group I
2018	64	TEM-1, CTX-M-15	RII	Group III
2018	65	CTX-M-15	RIII	Group I
2018	66	TEM-1, CTX-M-9	RII	Group III
2018	67	TEM-1, CTX-M-15	RII	Group III
2018	68	TEM-1, CTX-M-15	RIII	Group I
2018	69	TEM-1, CTX-M-15	RI	Group I
2018	70	CTX-M-15	RIII	Group I
2018	71	TEM-1, CTX-M-15	RII	Group I
2018	72	TEM-1, CTX-M-16	RII	Group I
2018	73	TEM-1, CTX-M-15	RI	Group I
2018	74	TEM-1, CTX-M-15	RI	Group I
2018	75	CTX-M-15	RIII	Group I
2018	76	CTX-M-15	RIII	Group I
2018	77	TEM-1, CTX-M-15	RII	Group I
2018	78	TEM-1, CTX-M-15	RI	Group I
2018	79	TEM-1, CTX-M-1	RIII	Group I
2018	80	TEM-1, CTX-M-15	RII	Group I
2019	81	TEM-1, CTX-M-9	RI	Group II
2019	82	TEM-1, CTX-M-15	RIII	Group I
2019	83	CTX-M-15	RIII	Group I
2019	84	TEM-1, CTX-M-15	RI	Group I
2019	85	TEM-1, CTX-M-16	RII	Group I
2019	86	TEM-1, CTX-M-15	RIII	Group I
2019	87	TEM-1, CTX-M-15	RII	Group I
2019	88	CTX-M-15	RI	Group I
2019	89	TEM-1, CTX-M-15	RIII	Group I
2019	90	TEM-1, CTX-M-15	RIII	Group I
2019	91	TEM-1, CTX-M-15	RII	Group I
2019	92	TEM-1, CTX-M-16	RI	Group I
2019	93	TEM-1, CTX-M-15	RII	Group I
2019	94	TEM-1, CTX-M-9	RII	Group I
2019	95	TEM-1, CTX-M-15	RII	Group I
2019	96	CTX-M-15	RI	Group I
2019	97	TEM-1, CTX-M-15	RI	Group I
2019	98	TEM-1, CTX-M-15	RII	Group I
2019	99	TEM-1, CTX-M-15	RI	Group I
2019	100	TEM-1, CTX-M-15	RII	Group I
2019	101	TEM-1, CTX-M-1	RII	Group I
2019	102	TEM-1, CTX-M-15	RII	Group I
2019	103	TEM-1, CTX-M-15	RII	Group I
2019	104	TEM-1, CTX-M-15	RII	Group I
2019	105	CTX-M-15	RI	Group I
2019	106	TEM-1, CTX-M-15	RI	Group I
2019	107	TEM-1, CTX-M-9	RI	Group I
2019	108	TEM-1, CTX-M-15	RI	Group I
2019	109	TEM-1, CTX-M-15	RI	Group I
2019	110	TEM-1, CTX-M-9	RI	Group I
2019	111	TEM-1, CTX-M-15	RI	Group I
2019	112	CTX-M-15	RI	Group I
2019	113	TEM-1, CTX-M-15	RI	Group I
2019	114	TEM-1, CTX-M-15	RI	Group I
2019	115	TEM-1, CTX-M-16	RII	Group I
2019	116	TEM-1, CTX-M-15	RII	Group I
2019	117	TEM-1, CTX-M-15	RII	Group I
2019	118	TEM-1, CTX-M-15	RII	Group I
2019	119	TEM-1, CTX-M-15	RII	Group I
2019	120	CTX-M-15	RII	Group III

SHV-12 1 (4) (n= 6), TEM-1 + CTX-M-1 (n= 6), TEM-1 + CTX-M-16 (n= 6) and TEM-1 + CTX-M-9 (n= 6) (Table 3). One hundred and eight out of 120 isolates were found to harbor a *bla*CTX-M gene, with the *bla*CTX-M-15 group being the most common type. Most of the *bla*CTX-M-15–containing *E. coli* isolates also harbored different β-lactamase genes, including especially *bla*TEM-1 and *bla*OXA-1. The *bla*TEM-1 was found in 76 isolates, alone and in combination with other genes. Sixteen out of all isolates harbored the *bla*CXT-M-9 gene, 14 isolates harbored a *bla*OXA-1 gene and other genes found in the isolates were *bla*CXT-M-1 (n= 6), *bla*CXT-M-9 (n= 6), *bla*SHV-12 (n= 6) and *bla*SHV-12 1 (4) (n= 6).

Table 3. Detected ESBL genes of *E. coli* isolates.

***bla* gene(s)**	**Number of *E. coli* isolates (%)**
CTX-M-15, OXA-1	14 (12)
CTX-M-15	24 (20)
TEM-1, CTX-M-15	52 (43)
TEM-1, SHV-12	6 (5)
SHV-12 1 (4)	6 (5)
TEM-1, CTX-M-1	6 (5)
TEM-1, CTX-M-16	6 (5)
TEM-1, CTX-M-9	6 (5)

Discussion and Conclusion

In Turkey, as well as in the world, especially outbreaks of infections with ESBL-producing *Enterobacteriaceae* has an increasing frequency. Although ESBLs have been described in almost all enteric bacteria, they are frequently found in *E. coli* and *K. pneumoniae*. The high prevalence of ESBL-positive *E. coli* isolates reported for farm animals, especially poultry, due to misuse and overuse of antimicrobial agents is a zoonotic risk factor for human (5, 35, 47). A study on examining the ESBL prevalence of *E. coli* isolated from urine samples of patients. The results of the study demonstrated that the prevalence of ESBL producers was a significant increase among isolates from inpatients (12.5 % to 44.7 %) and from outpatients (9.6 % to 22.8 %) (41). In 2012, 66 (37.1 %) of a total of 178 patients were ESBL positive-*E. coli* isolated from urine samples (n= 322) collected from Ankara Training and Research Hospital in Ankara province of Turkey (27). In a study conducted in Turkey, Akçam et al. (1) reported that the production of ESBL was found in 7.2 % of *E. coli* and 35 % of *Klebsiella* spp. In another study, although ESBL positivity was observed in 52.2 % of 52 *E. coli* strains and in 58.2 % of 12 *K. pneumoniae* strains, it was not observed in 6 *Proteus* spp. (2). In a similar study, Sahin et al. (40) reported that positivity rates of ESBL for *Enterobacteriaceae* were detected 19.4 % for *E. coli* (n= 108), 15.9 % for *Klebsiella* spp. (n= 44) and 13.6 % for *Proteus* spp. (n= 22). In studies conducted in other countries, the frequency of ESBLs were reported to be 11.0 % to 63.6 % in *E. coli* and 13.0 % to 86.6 % in *Klebsiella* spp. (21, 23, 46).

The differences in ESBL production rates in both Turkey and other countries are related to the fact that the production in bacteria changes with certain conditions. It is known that the increase in the production is closely related to the use of broad-spectrum β-lactam antibiotics and in parallel with the increase in β-lactam resistance. Ozkan et al. (33) found that *E. coli* and *K. pneumoniae* strains were sensitive to 80-85 % and 60-63 % of third-generation cephalosporins including cefinaxone, ceftazidime, and cefotaxime, respectively. The third-generation cephalosporin resistance of *E. coli* strains in the present study is also proportional to the ESBL production in these bacteria.

In Turkey, SHV-2, SHV-5, SHV-12, OXA-1, CTX-M-2, CTX-M-15, CTX-M-16, and TEM-1 type ESBLs were reported in *E. coli* isolates (17, 18, 47). Sequencing of β-lactamase genes revealed that *bla*CTX-M-15 was the most prevalent (90/120) in the ESBL-producing *E. coli* isolates, followed by *bla*TEM-1(76/120), *bla*CTX-M-9 (16/120), *bla*OXA-1 (14/120), *bla*CXT-M-1 (6/120), *bla*CXT-M-9 (6/120), *bla*SHV-12 (6/120) and *bla*SHV-12 1 (4) (6/120) in this study. A study conducted in Izmir province of Turkey between 2004 and 2005 showed that *E. coli* isolated from patients with UTI produced an ESBL, of which CTX-M-15 was predominant (53 %) (51). Similarly, CTX-M-15 group has been reported to be found in 86.8 % of *E. coli* isolated from inpatients and outpatients at the hospital of İstanbul Faculty of Medicine between 2002 and 2004 (17). Among ESBLs, CTX-M-15 was found as the most prevalent type enzymes as reported in different studies from Turkey and several other countries (9, 17, 18, 29, 30, 31, 51). In several studies conducted in different province of Turkey, it has been reported that ESBL-positive *E. coli* isolates harbored *bla*TEM gene (3, 7). TEM-1 was found to be harbored in 63 % of ESBL-positive *E. coli* isolates in combination with other genes in this study. In animal studies performed in Turkey, it has been reported that CTX-M-15 was the most frequent ESBL enzyme type in ESBL-positive *E. coli* isolates obtained from both healthy broilers (47) and laying hens (35). The results of our study are similar to the results of these studies. CTX-M-15 is the predominant ESBL in both human and poultry. This may be caused by the transmission of ESBL-producing bacteria between humans and animals.

In the present study, the standard criteria of Tenover (13, 43-45) were used for the analysis of *E. coli* strains using PFGE system. If the restriction patterns of isolates according to the Tenover criteria have the same number of

bands and the reciprocal bands are the same size, these isolates are the same. In the present study, 120 strains which constitute the main cluster and evaluated as 3 groups in this cluster may be epidemiologically identical strains. When these strains were examined epidemiologically, it was determined that patients admitted to the hospital from the same region. However, four different groups were observed when resistance profiles were compared. According to XbaI digestion profiles, 120 strains were found to be related to each other epidemiologically.

Studies in different countries show that resistance to β-lactam antibiotics is increasing and this is an emerging threat in today's world. In the last decade, plasmid-encoded ESBL-producing organisms have increased rapidly (13, 36, 37). Currently, most of these strains can be treated with combinations of β-lactam and β-lactamase inhibitor. However, the number of strains that are not treated with these combinations is also increasing (52). In addition, ESBL-producing strains are becoming more and more resistant to other antimicrobial drugs such as aminoglycosides, sulphonamides, and tetracyclines through various mechanisms (39), and it is foreseen that our antibiotic options will be severely restricted if no precautions are taken.

CTX-M-15-positive *E. coli* is more widespread in the isolates and the presence of different types of enzymes in each isolates shows that the epidemiology of ESBLs in the hospital is complex. High ESBL production and decreased susceptibility to broad-spectrum cephalosporins are present in *E. coli* strains in our hospital. If empirically treated with these broad-spectrum antimicrobial agents, treatment of ESBL-producing *E. coli*-associated infections with these agents may result in failure. The resistance problem is still observed for infections with *E. coli*. However, it is known that resistance properties can be transferred to different types of bacteria. In the coming years, resistance can be encountered in different bacteria in our hospital.

Consequently, in order to help slow the increasing antibiotic resistance, it is necessary to develop activities and policies to promote more rational use of antibiotics. The local prevalence and antibiotic susceptibility of the bacterial organism should be considered during the selection of empirical antibiotic therapy. High levels of ofloxacin, trimethoprim-sulfamethoxazole, and ampicillin resistance were found in the *E. coli* strains isolated from the patients who admitted to our hospital. Overuse of these antibiotics may have led to the development of resistance to bacteria.

Conflict of Interest

The authors declared that there is no conflict of interest.

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