Original Article

Characterization of extended spectrum β-lactamase producing *Escherichia coli* strains isolated from urogenital system of dogs in Van province of Turkey

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Abstract

Background: *Escherichia coli* is a bacterial agent that causes urogenital system infection in dogs. Beta-lactam (β-lactam) group antibiotics are frequently used in the treatment of *E. coli* infections. **Aims:** This study aimed to investigate the presence of extended-spectrum β-lactamase (ESBL) and plasmidic AmpC in *E. coli* strains isolated from the urogenital tracts of 125 dogs. **Methods:** Fifty *E. coli* strains were identified by conventional bacteriological and PCR methods. Disk diffusion method was used for the determination of antimicrobial susceptibility of the isolates as well as productions of plasmidic AmpC and ESBL. The presence of *bla*TEM, *bla*SHV, and *bla*CTX-M group genes was determined in the isolates by PCR. ERIC-PCR was also used for genotyping of the isolates. **Results:** Although 22 (44%) of 50 *E. coli* isolates were found to be ESBL positive, no isolate shows plasmidic AmpC β-lactamase production. Among 22 ESBL positive isolates, *bla*TEM, *bla*SHV, and *bla*CTX-M group 1 genes were found in 11 (50%), 1 (4.54%), and 6 (27.27%) isolates, respectively. The highest resistance was observed against tetracycline (28%), followed by streptomycin (24%), trimethoprim-sulfamethoxazole (24%), and chloramphenicol (22%), respectively. In the isolates, 11 different main profiles were also determined by ERIC-PCR. It was shown that ESBL positive isolates were related to G10 profiles. **Conclusion:** The use of extended spectrum β-lactam group antibiotics for the treatment of *E. coli* infections in dogs is critical; nevertheless, they may not be effective due to the high rate of resistance to this antibiotic group in *E. coli*.

Key words: AmpC, Dog, *E. coli*, ERIC-PCR, ESBL

Introduction

Beta lactam group antibiotics inhibit the activity of transpeptidase and carboxypeptidase enzymes which are involved in peptidoglycan synthesis (Essack, 2001). Beta lactamase enzymes disrupt the amide bonds in the β-lactam ring of these substances. These enzymes were first identified in *Escherichia coli* in the 1940s (Gür, 1997). The most important mechanism for β-lactam resistance in Gram-negative bacteria, especially in the members of *Enterobacteriaceae*, is the production of β-lactamases (Pool, 2004).

Extended spectrum beta-lactamases (ESBL) can inhibit the effect of penicillin, the first three generations of cephalosporins, monobactam, aztreonam, and partially cefepimes. On the other hand carbapenem, cephamycin, and β-lactamase inhibitors can block the effect of these enzymes (Sader *et al*., 2007). Generally, ESBLs are originated from TEM, SHV, and OXA enzymes (Bush *et al*., 1995). On the other hand, CTX-M group of ESBL can hydrolyze cefotaxime (Oliver *et al*., 2001), and unlike TEM and SHV groups of ESBL, the *CTX-M* genes are spread by horizontal gene transfer among bacteria via plasmids (Livermore, 2007). ESBLs are reported to be extensively present in *E. coli* and *Klebsiella pneumoniae* isolates (Mulvey *et al*., 2004).

AmpC β-lactamases are active against penicillins, but more active against cephalosporins (Galleni *et al*., 1988). The members of *Enterobacteriaceae* can produce AmpC β-lactamases. These enzymes can make the isolates to be resistant to cephalothin, cefazolin, cefoxitin, most of the penicillin, and β-lactam combinations (Jacoby, 2009).

E. coli, which is classified in the *Enterobacteriaceae* family, is an opportunistic pathogen that can be found in the natural flora of the gastrointestinal tract of humans and animals (Kaper *et al*., 2004). While it causes many diseases in human such as enteritis, urinary tract infection, meningitis, septicemia, community and hospital acquired pneumonia (Vila *et al*., 2016), it is frequently isolated from the infected urinary tract and pyometra cases of cats and dogs (Fransson *et al*., 1997; Hagman and Greko, 2005; Windahl *et al*., 2014;

Moyaert *et al*., 2017; Yu *et al*., 2020).

Since they cause hospital infections, and treatment options are limited, there are many studies conducted on the isolation and the identification of extended-spectrum β-lactam resistant members of *Enterobacteriaceae*. Because β-lactam group antibiotics are also frequently used in veterinary medicine, it is important to monitor frequently the resistance profile against these antimicrobials in the strains isolated from animals to acquire up-to-date data (Li *et al*., 2007).

Although the prevalence of ESBL positive *E. coli* isolated from various samples of dogs was revealed different rates in different geographical regions (Carattoli *et al*., 2005; Huber *et al*., 2013; Aslantas *et al*., 2017; Gumus *et al*., 2017; Zogg *et al*., 2018; Paredes *et al*., 2019), it had been reported that the prevalence of plasmidic AmpC β-lactamase producing isolates was generally low (Aslantaş and Yılmaz, 2017; Gumus *et al*., 2017; Shimizu *et al*., 2017; Paredes *et al*., 2019). In these studies, it was reported that *TEM* and *CTX-M* group 1 genes were found at a higher rate than *SHV* genes in *E. coli* isolates.

In this study, the presence of ESBL and plasmid-derived AmpC β-lactamase in *E. coli* strains isolated from the urogenital system of dogs and the susceptibility of these isolates to various antimicrobial agents were investigated.

Materials and Methods

Ethics approval

This study has been approved ethically by Van Yuzuncu Yil University Animal Researches Ethic Committee with the number of 2020/12-11.

Sampling

In this study, urine and/or vaginal swab samples were collected from 125 dogs of different ages and genders, housed in Van/Turkey Metropolitan Municipality Animal Care and Rehabilitation Center between December 2020 and June 2021. A total of 195 clinical samples including urine samples (taken to sterile tubes during physiological urination) from 44 dogs (35.2%), vaginal swab samples from 11 dogs (8.8%), and both urine and vaginal swab samples from 70 dogs (56%) were analyzed.

E. coli isolation

Urine samples were centrifuged at 5000 ×g for 10 min, and the sediment was diluted with 1 ml of physiological saline (FTS). Also, vaginal swabs were transferred to plastic tubes containing 3 ml sterile FTS, and the suspension was centrifuged at 3000 ×g for 10 min. The final sediment was resuspended in 1 ml sterile FTS. Then, 0.1 ml of urine and vaginal swab samples suspension were spread onto 5-7% sheep blood agar base (Oxoid, CM 03331, England), MacConkey agar (MCA) (Merck, 1.05465, Germany), and Eosin Methylene Blue (EMB) agar (Merck, 1.01347, Germany), and incubated at 37°C for 24 h. At the end of the incubation period, the colonies approximately 2 mm in diameter, gray colored, smooth, rounded or mucoid, hemolytic or non-hemolytic on blood agar, pink colored on MCA, and Gram-negative oxidase-negative with metallic shine on EMB were considered as *E. coli* (Papini *et al*., 2006; Quinn *et al*., 2011). A PCR test was used to confirm the identity of suspected colonies.

E. coli identification PCR

The genomic DNA was obtained by boiling method (Pehlivanoglu *et al*., 2016). The specific primers (Table 1) were used for the identification of *E. coli* isolates (Wang *et al*., 2002). The PCR mix consisted of 1 µL of each primer (10 µM), 5 µL of genomic DNA, and 10 µL of A.B.T™ 2X PCR master mix (Turkey). The total volume was completed to 25 µL with nuclease-free water (BP2819, Thermo Fisher Scientific, USA). The mixture was kept at 95°C for 10 min for preliminary denaturation. A total of 35 amplification cycles were performed containing denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min. Final elongation was applied at 72°C for 10 min. *E. coli* ATCC® 25922 reference strain was used as positive control and nuclease-free water was used as no template control (NTC). The amplicons were electrophoresed on 1.5% agarose gel containing gel-red stain at 80 V for 2 h and were examined in a UV transilluminator.

Phenotypic determination of the extended spectrum β-lactamase

The presence of ESBL in *E. coli* isolates was determined according to the criteria reported in Clinical

Table 1: Primer sequences used to identify the *E. coli* isolates and to determine genes associated with ESBL

Genes	Primer sequences (5´-3´)	Amplicon size (bp)	Reference
E. coli			
16S rRNA	F: CCCCCTGGACGAAGACTGAC	401	Wang *et al*. (2002)
	R: ACCGCTGGCAACAAAGGATA		
ESBL			
*bla*TEM	F: ATAAAATTCTTGAAGACGAAA	1079	Kapoda *et al*. (2018)
	R: GACAGTTACCAATGCTTAATCA		
*bla*SHV	F: ATTTGTCGCTTCTTTACTCGC	1051	Kapoda *et al*. (2018)
	R: TTTATGGCGTTACCTTTGACC		
*bla*CTX-M group 1	F: GGTTAAAAAATCACTGCGTC	863	Kapoda *et al*. (2018)
	R: TTGGTGACGATTTTAGCCGC		

and Laboratory Standards Institute (CLSI) (2018). For this purpose, the susceptibility of isolates to cefpodoxime (CPD, 10 µg, Himedia), ceftazidime (CAZ, 30 µg, Himedia), aztreonam (AT, 30 µg, Himedia), cefotaxime (CTX, 30 µg, Himedia), and ceftriaxone (CI, 30 µg, Himedia) antibiotic disks were determined by disk diffusion method. Resistance of *E. coli* isolates (according to the cut-off inhibition diameter) to at least one of the following antibiotics was defined as possible ESBL production; cefpodoxime (≤17 mm), ceftazidime (≤22 mm), aztreonam (≤27 mm), cefotaxime (≤27 mm), and ceftriaxone (≤25 mm). A combined disk method was used for the phenotypic confirmation of positive ESBL isolates. For this purpose, the susceptibility of isolates to antibiotic disk combinations of ceftazidime-clavulanate (CAC, 30/10 µg, Himedia) and cefotaxime-clavulanate (CEC, 30/10 µg, Himedia) was determined by disk diffusion test method and a difference of ≥5 mm between the zone diameters of with and without clavulanate was considered ESBL positive. The inhibition zone of the disk with clavulanate should be larger than the zone of a disk without clavulanate.

Phenotypic determination of plasmidic AmpC β-lactamase

The isolates were considered as plasmidic AmpC β-lactamase producers if the inhibition zone diameter of cefoxitin (CX, 30 µg, Himedia) was found to be <19 mm in *E. coli* isolates by disk diffusion test method (EUCAST, 2019). The combined disk method reported by Tan *et al*. (2009) was used for the phenotypic confirmation of the presence of plasmidic AmpC β-lactamases. Therefore, the inhibition zone diameters of cefoxitin and cefoxitin-cloxacillin (CXX, 30/200 µg, Himedia) disks in the isolates were examined separately, and a difference of ≥4 mm was considered positive for plasmidic AmpC β-lactamases. The inhibition zone of the disc with cloxacillin should be larger than the zone of a disk without cloxacillin.

Genotypic determination of extended spectrum β-lactamase

For the genotypic determination of ESBL, *bla*TEM, *bla*SHV, *bla*CTX-M group 1 genes were examined by PCR method using specific primers (Table 1). PCR mix was prepared as described in the identification of the *E. coli* isolates by PCR. The mix was held at 94°C for 10 min for initial denaturation. The amplification process consisted of 35 cycles containing denaturation at 94°C for 1 min, annealing at 52°C, 57°C, and 55°C for *bla*TEM, *bla*SHV, and *bla*CTX-M group 1, respectively, for 1 min and extension at 72°C for 1 min. The final extension was applied at 72°C for 10 min.

Phenotypic determination of antimicrobial susceptibility

The susceptibility of isolates against gentamicin (GEN, 10 µg, Himedia), streptomycin (S, 10 µg, Himedia), piperacillin-tazobactam (PIT, 100/10 µg, Himedia), enrofloxacin (EX, 5 µg, Himedia), ciprofloxacin (CIP, 5 µg, Himedia), trimethoprim-sulfamethoxazole (COT, 1.25/23.7 µg, Himedia), chloramphenicol (C, 30 µg, Himedia), tetracycline (TE, 30 µg, Himedia), ertapenem (ERT, 10 µg, Himedia), and imipenem (IMP, 10 µg, Himedia) was determined by disk diffusion test method according to CLSI (2018) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2019) criteria. The isolates were classified as susceptible (S), intermediate (I), and resistant (R) according to inhibition zone diameters. Isolates that showed resistance to at least one or more antibiotics of three different groups were considered as multi-drug resistant (Magiorakos *et al*., 2012). *E. coli* ATCC® 25922 reference strain was used as the test control.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

The genotype profiles of the isolates were determined by ERIC-PCR using 5´-AAG TAA GTG ACT GGG GTG AGC G-3´ ERIC2 primer (Versalovic *et al*., 1991). ERIC-PCR profiles of the isolates were grouped by using cluster analysis with 80% similarity. PCR mix was prepared as described in the identification of the isolates by PCR. The mixture was kept at 94°C for 10 min for preliminary denaturation. 35 amplification cycles were performed with the protocol of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 3 min. Final elongation was applied at 72°C for 15 min.

The PCR products were electrophoresed on 1.5% agarose gel (80 V, 180 min) and photographed by a gel documentation system (Gen-Box imagER Fx, ERBiyotek, Turkey). The ERIC-PCR patterns were analyzed using the PyElph software (version 1.4). The clustering was performed by the unweighted pair group algorithm (UPGMA), and the Dice correlation coefficient.

Statistical analysis

The relationship between urinary and vaginal isolates, multiple-drug resistance, and ESBL positivity, and the genotype profiles and ESBL positivity were analyzed by the Z ratio (two-sample proportion test) and Fisher's Exact test methods using Minitab Statistical Software 20.

Results

E. coli isolation and identification

In this study, 50 *E. coli* (25.64%) strains were isolated from a total of 195 samples collected from 125 dogs by the bacteriological method. All *E. coli* isolates were confirmed by PCR. Forty two isolates (84%) were obtained from urine samples and 8 (16%) from vaginal swab samples. When *E. coli* was isolated from both urine and vaginal swab samples from the same dog, only the isolates obtained from the urine samples were used in the disk diffusion tests.

Phenotypic determination of the extended-spectrum β-lactamase

Twenty nine isolates (58%) were found to be ESBL positive by the disk diffusion method. On the other hand, 22 of 29 isolates (75.86%) were confirmed as ESBL positive by the combined disk method. It was determined that 20 (90.91%) and 2 (9.09%) of the ESBL positive strains were isolated from urine and vaginal swab samples, respectively. It was found that there was not a significant association between ESBL positivity and the sampling site (P=0.439).

Phenotypic determination of the plasmidic AmpC β-lactamase

Plasmid mediated AmpC resistance was not detected in the strains. So, the presence of related genes was not investigated.

Genotypic determination of extended-spectrum β-lactamase

*Bla*TEM, *bla*SHV, *bla*CTX-M group 1 genes were detected in 11 (50%), 1 (4.54%), and 6 (27.27%) ESBL positive isolates, respectively. Both of the *bla*TEM and *bla*CTX-M group 1 genes were found only in 5 (22.72%) isolates. In addition, *bla*TEM, *bla*SHV, *bla*CTX-M group 1 genes were found in 10 (50%), 1 (5%), and 5 (25%) ESBL positive isolates obtained from urine samples, respectively. It was also determined that only 1 ESBL positive isolate obtained from vaginal swab samples carried *bla*TEM, and *bla*CTX-M group 1 genes.

Phenotypic determination of antimicrobial susceptibility

All isolates in the study were found susceptible to imipenem, whereas it was determined that 1 (2%), 1 (2%), 2 (4%), 4 (8%), 5 (10%), 11 (22%), 12 (24%), 12 (24%), and 14 (28%) isolates were detected to be resistant against ertapenem, piperacillin-tazobactam, gentamicin, enrofloxacin, ciprofloxacin, chloramphenicol, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline, respectively. When the frequencies of antimicrobial resistance in urinary and vaginal isolates shown in Fig. 1 were compared, a significant statistical relationship was not determined among the isolates (P>0.05).

Multi-drug resistance was determined in 12 isolates (24%), and 8 of these isolates (66.66%) were found to be ESBL positive (Table 2). However, 10 out of 12 multi-drug resistant isolates (83.33%) were obtained from urine samples, only 2 isolates from vaginal samples. In the statistical analysis, it was determined that there was no relationship between multi-drug resistance and ESBL positive isolates (P=0.069).

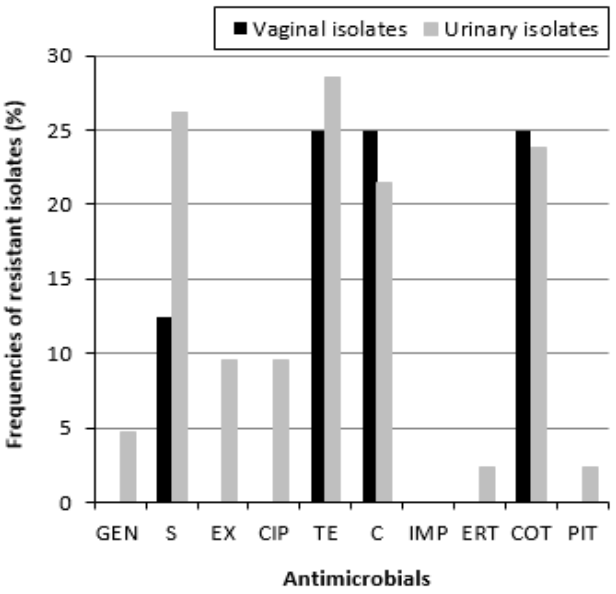

Fig. 1: Frequencies of antimicrobial resistance in vaginal and urinary isolates (%). GEN: Gentamicin, S: Streptomicin, EX: Enrofloxacin, CIP: Ciprofloxacin, TE: Tetracycline, C: Chloramphenicol, IMP: Imipenem, ERT: Ertapenem, COT: Trimethoprim-Sulfamethoxazole, and PIT: Piperacillin-Tazobactam

Genotyping by ERIC-PCR

According to ERIC-PCR results, *E. coli* isolates were divided into 11 different main groups (G). Seven of these groups included subgroups (Fig. 2). It was determined that 2 (4%), 1 (2%), 7 (14%), 2 (4%), 8 (16%), 12 (24%), 7 (14%), 2 (4%), 1 (2%), 7 (14%), and 1 (2%) of the isolates had G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, and G11 profiles, respectively. ESBL positive isolates were distributed in G1, G3, G5, G6, G7, G8, G10, and G11 profiles with different rates (Fig. 2). It was determined that 2 (9.09%), 3 (13.63%), 2 (9.09%), 5 (22.72%), 2 (9.09%), 1 (4.54%), 6 (27.27%), and 1 (4.54%) of 22 ESBL positive isolates had G1, G3, G5, G6, G7, G8, G10, and G11 profiles, respectively (Fig. 2).

Table 2: Multiple antibiotic resistance profiles determined in isolates

Resistance profile	Number of ESBL (+) isolates (%) (n:22)	Number of ESBL (-) isolates (%) (n:28)	Total (%) (n:50)
S+TE+C+COT	4 (18.18)	1 (3.57)	5 (10)
S+TE+COT	0	1 (3.57)	1 (2)
S+EX+CIP+TE+C	0	1 (3.57)	1 (2)
GEN+S+EX+CIP+TE+C+COT	1 (4.54)	0	1 (2)
EX+CIP+TE+ETP+COT+PIT	1 (4.54)	0	1 (2)
TE+C+COT	2 (9.09)	1 (3.57)	3 (6)
Total	8 (36.36)	4 (14.28)	12 (24)

S: Streptomycin, TE: Tetracycline, C: Chloramphenicol, COT: Trimethoprim-Sulfamethoxazole, EX: Enrofloxacin, CIP: Ciprofloxacin, GEN: Gentamicin, ETP: Ertapenem, and PIT: Piperacillin-Tazobactam

In the statistical analysis, ESBL positivity was found to be significantly related to G10 profiles (P=0.040). In addition, it was found that vaginal isolates (n=8) had G6 (n=2), G7 (n=4), G9 (n=1), and G10 (n=1) profiles (Fig. 2). Statistically significant relationship was not determined between genotype profile and isolation site of the strains (P>0.05).

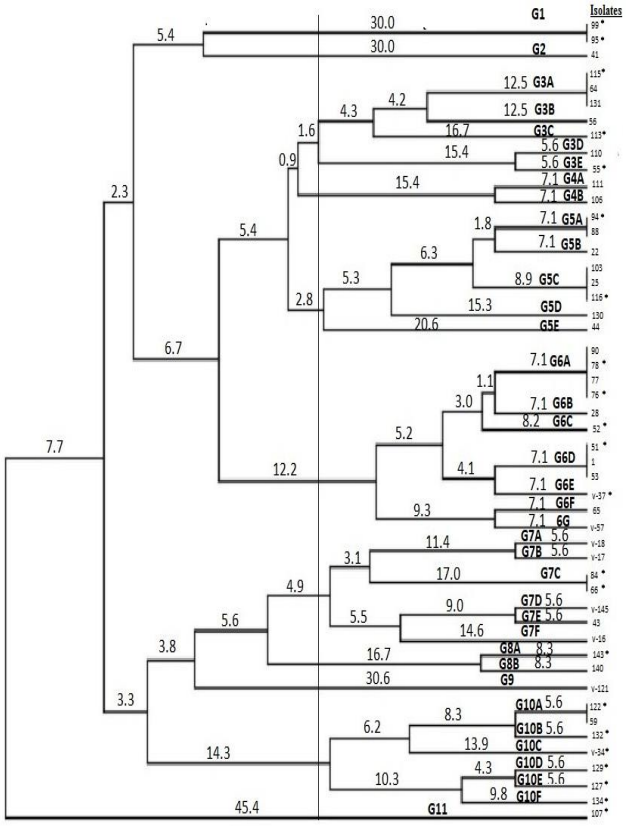

Fig. 2: Cluster analysis and ERIC-PCR genotypes detected in *E. coli* isolates (decimal numbers indicates differences (%) between ERIC-PCR genotype profiles). * ESBL positive isolates, v: Vaginal isolates, and G: ERIC-PCR genotype profile

Discussion

Because *E. coli* is a significant and common pathogen in both humans and animals, the antimicrobial resistance profile of isolates should be monitored continuously to find out the most effective antibiotics against circulating bacteria. Also, dogs are companion animals in close contact with humans. So, the emergence of resistant strains in dogs can adversely affect both animal and human health.

Although there are many studies investigating the presence of ESBL in *E. coli* isolates in the field of human medicine, there is a limited number of studies on animal isolates. Therefore, in this study, ESBL resistance, which is one of the most important resistance mechanisms (Bradford, 2001; Pool, 2004), was investigated in *E. coli* strains isolated from dogs.

Huber *et al*. (2013) reported that ESBL producing *E. coli* isolates were identified from 7.47% of urine samples of cats and dogs, whereas this rate was determined as 54.68% by Zoogg *et al*. (2018) in Zurich, Germany. In other studies, it was reported that extended-spectrum β-lactam resistance varied between 42.69%, and 91.30% in *E. coli* isolates obtained from the rectal swabs, and stool samples of cats, and dogs (Aslantaş and Yılmaz, 2017; Gumus *et al*., 2017; Paredes *et al*., 2019). On the other hand, Rzewuska *et al*. (2015) reported that the ESBL phenotype was detected at a very low rate in the strains which were isolated from nasal, pharyngeal, and ear swab samples of dogs in Poland.

In the present study, extended-spectrum β-lactam resistance was phenotypically determined in 44% of *E. coli* isolates obtained from urine, and vaginal swab samples of dogs. It was observed that the data were compatible with the results of the study conducted by Aslantaş and Yılmaz (2017). However, it is determined that the results achieved were lower than the results reported by Gumus *et al*. (2017) and Paredes *et al*. (2019).

There are few studies that investigated the presence of plasmid related AmpC β-lactamase in *E. coli* isolates of dogs. In these studies, it was reported that the prevalence of plasmid related AmpC β-lactamase positive *E. coli* strains isolated from dogs was generally low (Aslantaş and Yılmaz, 2017; Gumus *et al*., 2017; Shimizu *et al*., 2017; Paredes *et al*., 2019). However, Bortolami *et al*. (2019) reported that 40.85% of *E. coli* strains obtained from various clinical samples of cats, dogs, and horses were plasmidic AmpC producers. In this study, plasmid related AmpC β-lactamase was not detected in *E. coli* isolates of dogs.

In various studies, it was concluded that *bla*TEM group genes were detected with a higher rate (17.07-100%) in ESBL positive *E. coli* isolates, but *bla*SHV genes were reported at a lower rate (2.43-35.29%) (Carattoli *et al*., 2005; Huber *et al*., 2013; Rzewuska *et al*., 2015; Aslantaş and Yılmaz, 2017; Gumus *et al*., 2017; Karkaba *et al*., 2017; Bortolami *et al*., 2019; Garcia *et al*., 2019; Sfaciotte *et al*., 2021; Shin *et al*., 2021). In this study, similar to other studies, the *bla*TEM gene was detected in 11 of 22 ESBL positive isolates (50%), whereas the *bla*SHV gene positivity was lower (4.54%) than *bla*TEM gene positivity in the isolates.

It is known that *bla*CTX-M genes, classified in 1, 2, 8, 9, and 25 groups, unlike TEM and SHV group genes, are transferred among bacteria (Livermore, 2007). In addition, it has been reported that the *bla*CTX-M-*15* gene, which is classified in group 1, has been determined at a higher rate in *E. coli* isolates in the studies. In various research, it was reported that the presence of *bla*CTX-M group 1 genes varied between 2.43-100% (Carattoli *et al*., 2005; Huber *et al*., 2013; Rzewuska *et al*., 2015; Aslantaş and Yılmaz, 2017; Gumus *et al*., 2017; Karkaba *et al*., 2017; Shimizu *et al*., 2017; Zogg *et al*., 2018; Bortolami *et al*., 2019; Garcia *et al*., 2019; Sfaciotte *et al*., 2021; Shin *et al*., 2021). Similarly, in this study, *bla*CTX-M group 1 genes were detected in 27.27% of *E. coli* isolates.

E. coli isolates have resistance mechanisms against β-

lactam group antimicrobial substances and these mechanisms are spread by horizontal gene transfer among isolates. Consequently, the use of antimicrobial substances other than the β-lactam group is mandatory for the elimination of these infections (Panos *et al*., 2006). It is important to follow the resistance profiles of isolates against antimicrobial agents other than penicillin and cephalosporin group antibiotics, which are frequently used in veterinary medicine.

In previous studies, resistance profiles of *E. coli* strains isolated from pet animals were determined at different rates. In this context, the highest resistance was found against tetracycline (45-100%), which was followed by ciprofloxacin (41-100%), chloramphenicol (22-100%), enrofloxacin (15-100%), trimethoprim+ sulfamethoxazole (24-65%), streptomycin (37-54%), and gentamicin resistance (8-54%), respectively (Carattoli *et al*., 2005; Maluta *et al*., 2012; Huber *et al*., 2013; Aslantaş and Yılmaz, 2017; Shimizu *et al*., 2017; Qekwana *et al*., 2018; Zogg *et al*., 2018; Garcia *et al*., 2019; Paredes *et al*., 2019). It was also reported that all *E. coli* isolates were found to be susceptible to imipenem (Huber *et al*., 2013; Aslantaş and Yılmaz, 2017; Garcia *et al*., 2019; Paredes *et al*., 2019).

In the present study, the resistance rates against gentamicin, streptomycin, enrofloxacin, ciprofloxacin, tetracycline, chloramphenicol, and trimethoprim+ sulfamethoxazole in *E. coli* strains were determined as 4%, 24%, 8%, 10%, 28%, 22%, and 24%, respectively. Although the resistance rates in this study were found to be lower than those of other studies, the highest resistance was found against tetracycline (28%), which was consistent with the results of other studies. Similar to data reported in the previous studies, no resistance was detected against imipenem in this study. Also, it was determined that the number of resistant strains to ertapenem and piperacillin-tazobactam was limited (2%). These antibiotics are mostly used in the treatment of infections caused by ESBL positive *E. coli* in human medicine.

The ERIC-PCR provides advantages over PFGE, and MLST methods in the genotyping of *E. coli* isolates because ERIC-PCR is easier and more economical. However, because of different PCR optimizations, different band profiles may result in different studies with the same primer which means that the reproducibility of ERIC-PCR is more limited than the other genotyping methods (Meacham *et al*., 2003). In Brazil, Maluta *et al*. (2012) reported that 5 different genotype profiles were determined in 13 *E. coli* isolates, which were obtained from urine samples of dogs by ERIC-PCR method. In a similar study, Tramuta *et al*. (2011) reported that 36 different profiles were determined in isolates in Italy, whereas Bourne *et al*. (2019) reported that only 6 different ERIC-PCR profiles in *E. coli* isolates were detected. In another study, 14 ESBL positive *E. coli* isolated from fecal samples of cats and dogs, and 4 different band profiles were determined by ERIC-PCR method. It was also reported that the genotype profiles of the isolates, which carried the genes associated with ESBL resistance, show different distributions (Moreno *et al*., 2008). In the present study, 11 different main genotypes were determined in 50 *E. coli* isolates by ERIC-PCR. It was observed that the distribution of genotype profiles in ESBL positive isolates was at different rates, and only G10 profile was statistically associated with the ESBL positivity.

In this study, it was determined that more attention should be paid to extended-spectrum β-lactam resistance in *E. coli* strains that were isolated from dogs in close contact with humans in social life. Also, it was concluded that increasing resistance rates against cephalosporin group antibiotics, which are frequently used in the treatment of infections caused by *E. coli* isolates in both human and veterinary medicine, have critical importance. However, resistance against tetracycline, streptomycin, trimethoprim-sulfamethoxazole, and chloramphenicol should also be considered in *E. coli* isolates obtained from dogs. It has been concluded that the data obtained from this study would contribute to the control of infections due to *E. coli* which cause important infections in pet breeding and to the establishment of antimicrobial surveillance programs in the field of veterinary medicine.

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Conflict of interest

None of the authors have any potential conflict of interest to declare.

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