Vol. 21, No. 3, Autumn, 2025, 11-21 *Original Article*
DOI: 10.22055/ivj.2025.512793.2783
Journal Homepage: http://www.ivj.ir/

Presence of certain extended-spectrum beta-lactamase (ESBL) genes in fecal strains of *Escherichia coli* from dogs and the antibiotic sensitivity of the isolates

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Received: 16.03.2025 Accepted: 24.06.2025

Abstract

Escherichia coli (*E. coli*) is a member of the *Enterobacteriaceae* and part of the normal flora in the intestines of both humans and warm-blooded animals. It causes a wide range of infections, both gastrointestinal and extra-intestinal, in humans and animals, including dogs and cats. Dogs and cats are often considered potential reservoirs of *E. coli* strains that can cause intestinal or extra-intestinal infections in humans. Therefore, zoonotic transmission aspects of infection are highly important. The use of certain antibiotics and the selective pressure in the environment contribute to the selection and spread of resistance genes to similar antibiotics, complicating the treatment of many bacterial infections. This study aimed to investigate the presence of certain extended-spectrum beta-lactamase (ESBL) genes in *E. coli* strains isolated from feces of both healthy and diarrheal dogs, and to examine their antibiotic sensitivity. A total of 100 *E. coli* isolates were screened phenotypically for the production of ESBL enzymes using cefotaxime and cefotaxime/clavulanic acid combination disks. The antibiotic sensitivity of the ESBL-producing strains to 12 antibiotics from various classes was evaluated. Furthermore, the presence of the genes *blaTEM*, *blaSHV*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1* in the ESBL-producing isolates was assessed using multiplex PCR. The results showed that 31 out of 100 *E. coli* isolates were phenotypically ESBL producers. The *blaTEM* gene was identified as the predominant ESBL gene in 45.2% of the isolates, while the *blaCTX-M-1* gene was found in 25.8%. The highest antibiotic resistance was observed against erythromycin, while the lowest was against meropenem. Additionally, 20 different antibiotic resistance patterns were identified in the isolates. Given the zoonotic aspects of *E. coli* transmission, further epidemiological studies and pre-treatment antibiotic sensitivity profiling are recommended to ensure successful treatment and prevent the spread of ESBL-producing strains.

Key words: Extended, Spectrum Beta, Lactamase, *E. coli*, Antibiotic Sensitivity, Dogs

Introduction

E. coli is a member of the *Enterobacteriaceae* and a part of the normal intestinal flora in humans and warm-blooded animals. It causes a broad spectrum of infections, both gastrointestinal and extra-intestinal, in humans and animals,

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including dogs and cats (Kaper et al, 2004). Dogs and cats are often considered potential reservoirs of *E. coli* strains, which can cause intestinal or extra-intestinal infections in humans, making the zoonotic transmission of infection a crucial concern. Poor hygienic practices in keeping animals may increase the risk of colonization of these pathogens in humans (Sevilla et al, 2020; Yasugi et al, 2021).

The use of antibiotics within similar classes in both human and veterinary medicine, combined with environmental selective pressure, plays a significant role in the selection and spread of resistance genes to similar antibiotics, complicating the treatment of many bacterial infections. Inappropriate use and incorrect prescription of antibiotics, underdosing, irregular administration of antimicrobial drugs, and the evolving role of dogs in society today are key risk factors for the emergence of antimicrobial resistance and the transmission of bacteria. More than 200 types of ESBLs are found worldwide, mostly within the *Enterobacteriaceae*. The *blaTEM*, *blaSHV*, and *blaCTX-M* groups are examples of common ESBLs (Huang et al, 2020; Yasugi et al, 2021; Pathak et al, 2017). Carrying ESBL genes on plasmids facilitates their transfer to other bacteria through conjugation. These plasmids also carry genes responsible for resistance to various antimicrobial classes, including fluoroquinolones, aminoglycosides, sulfonamides, and trimethoprim, thus limiting the treatment options for infections caused by ESBL-producing bacteria (Pitout and Laupland, 2008).

Detecting extended-spectrum beta-lactamases (ESBLs) and monitoring antibiotic resistance in *E. coli* as a sentinel bacterium is an essential tool in both preventing the spread of antibiotic resistance and controlling antibiotic usage. The availability and consumption of antibiotics, along with hygiene practices and disease prevalence, can influence the frequency and types of ESBL enzymes in bacterial strains within any given geographic area (Marchetti et al, 2021; Salgado-Caxito et al, 2021). Direct and indirect contact with pets carrying bacteria with genetic elements such as plasmids and integrons is one of the routes for antibiotic resistance transmission from animals to humans, complicating the treatment of infections caused by *E. coli* strains (Vinue et al, 2008). Formenti et al. demonstrated the role of household dogs as carriers of ESBL-producing *E. coli* strains (Formenti et al, 2021). Given reports on the spread of ESBL-producing *E. coli* strains in both animal and human infections, controlling and preventing these infections is vital. Furthermore, considering the importance of *E. coli* infections in small animals and its zoonotic potential, the goal of this study was to determine the antibiotic resistance patterns of *E. coli* isolates from dogs and assess the prevalence of extended-spectrum beta-lactamases (*blaSHV*, *blaTEM*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1*) among the isolates. The results of this study can contribute to identifying antibiotic sensitivity patterns in *E. coli* and the potential resistance in dog populations, thus aiding effective control and preventive measures.

Materials and Methods

E. coli isolates

The *E. coli* isolates used in this study were archived isolates collected from both apparently healthy dogs and dogs with diarrhea. A total of 75 isolates were from apparently healthy dogs, and 25 isolates were from dogs with diarrhea. The isolates were stored in skimmed milk at -70°C. After removal from the freezer and thawing, they were cultured onto nutrient agar plates using a sterile loop and incubated for 18–24 hours at 37°C. All *E. coli* isolates were identified based on Gram staining, catalase and oxidase tests, triple sugar iron (TSI) agar reactions, IMViC tests, urease test, phenylalanine deaminase test, lysine decarboxylase test, and the characteristic

metallic sheen on eosin methylene blue (EMB) agar (Markey et al, 2013).

Initial Screening of Isolates Using Ceftriaxone Disc Diffusion Method

According to the CLSI protocol, *E. coli* isolates were first screened using ceftriaxone (30 µg) discs and the Kirby-Bauer disk diffusion method. Isolates with a zone of inhibition equal to or smaller than 27 mm were considered suspected ESBL producers (CLSI, 2023).

Phenotypic Evaluation and Confirmation of ESBL Production by the Combined Disk Method

After the initial screening, the presence of ESBLs in the isolates was confirmed using the combined disk method. For this, a 5 mL suspension of each isolate, equivalent to a 0.5 McFarland standard, was prepared in physiological saline and plated onto Mueller-Hinton agar. Cefotaxime and cefotaxime with clavulanic acid antibiotic discs were then placed on the agar plates and incubated at 37°C for 18–24 hours. After incubation, the zone of inhibition was measured, and isolates that showed an increase of 5 mm or more in the diameter of the zone around the cefotaxime + clavulanic acid disc compared to the cefotaxime-only disc were confirmed as ESBL-producing *E. coli* isolates (CLSI, 2023).

Phenotypic Evaluation of Antibiotic Resistance Patterns in ESBL-Producing *E. coli* Isolates

After identifying the *E. coli* isolates that produce extended-spectrum beta-lactamases (ESBLs), the resistance or susceptibility of each of the 31 isolates to 12 antibiotics (ciprofloxacin, erythromycin, neomycin, nitrofurantoin, cefoxitin, furazolidone, trimethoprim-sulfamethoxazole, ampicillin, gentamicin, tetracycline, meropenem, and nalidixic acid) was individually tested. These antibiotics belong to different groups, including fluoroquinolones, macrolides, aminoglycosides, nitrofurans, cephalosporins, sulfonamides, beta-lactams, tetracyclines, carbapenems, and quinolones, following standard conditions. For this purpose, a 5 mL suspension of each confirmed ESBL-producing *E. coli* isolate was prepared in sterile physiological saline to match the turbidity of the 0.5 McFarland standard. Using a sterile swab, the suspension was cultured onto Mueller-Hinton agar plates, and antibiotic discs were placed on the surface of the agar. The plates were then incubated at 37°C for 18–24 hours. After incubation, the zone of inhibition around each antibiotic disc was measured, and the susceptibility or resistance of each isolate to the tested antibiotics was evaluated based on standard tables (CLSI, 2023).

Detection of *blaTEM, blaSHV, blaCTX-M-1, blaCTX-M-9,* and *blaOXA-1* Genes in ESBL-Producing *E. coli* Isolates by Multiplex PCR

For PCR testing, the DNA of each isolate was extracted by the boiling method. Then, multiplex PCR and specific primers for the genes *blaTEM*, *blaSHV*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1* were used to identify the types of extended-spectrum beta-lactamases (ESBLs) (Table 1). For each ESBL-producing *E. coli* isolate, the following reaction mixture was prepared: 15 µL of master mix, 5 µL of extracted DNA from each isolate, 0.6 µL of forward primer and 0.6 µL of reverse primer for each of the *blaTEM*, *blaSHV*, *blaCTX-M-1*, and *blaOXA-1* genes, 1.2 µL of forward primer and 1.2 µL of reverse primer for the blaCTX-M-9 gene, and 2.8 µL of distilled water, giving a final volume of 30 µL. The thermal cycling program for amplifying the fragments is shown in Table 2 (Ogutu et al., 2015).

PCR Product Analysis by Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis in a 1.5% agarose gel. Seven microliters of PCR products were loaded into the wells of the agarose gel alongside a molecular weight marker and

electrophoresed at 80 volts. After the electrophoresis was completed, the gel was placed in a transilluminator. By exposing the gel to UV light, the amplified DNA fragments were examined for the correct fragment size by comparing them with the molecular marker.

Statistical Analysis

The data obtained from each step were analyzed using SPSS software and the Chi-square statistical test.

Table 1: Primer sequences used to determine the type of extended-spectrum beta-lactamase (ESBL) enzymes in *E. coli* ESBL-producing isolates (Ogutu et al, 2015)

Target Gene	Primer Sequence (5' to 3')	Product Length (bp)
blaTEM	F: 5'-CATTTCCGTGTCGCCCTTATTC-3' R: 5'-CGTTCATCCATAGTTGCCTGAC-3'	800
blaSHV	F: 5'-AGCCGCTTGAGCAAATTAAAC-3' R: 5'-ATCCCGCAGATAAATCACCAC-3'	713
blaCTX-M-1	F: 5'-TTAGGAAGTGTGCCGCTGTA-3' R: 5'-CGGTTTTATCCCCCACAAC-3'	655
blaOXA-1	F: 5'-GCCCTTTACCAAACCAATAC-3' R: 5'-ACTTGATTGAAGGGTTGGGC-3'	564
blaCTX-M-9	F: 5'-GGTGATGAACGCTTTCCAAT-3' R: 5'-TTATCACCYRCAGTCCACGA-3'	518

Table 2: PCR thermal program for determining the type of extended-spectrum beta-lactamase (ESBL) enzymes in *E. coli* ESBL-producing isolates (Ogutu et al, 2015)

Cycle Step	Temperature (°C)	Time (Seconds)	Number of Cycles
Initial Denaturation	94	300	1
Denaturation	94	30	30
Annealing	61	30	
Extension	72	40	
Final Extension	72	600	1

Results

Initial Screening of *E. coli* Isolates Suspected of Producing Extended-Spectrum Beta-Lactamase (ESBL)

Out of 100 *E. coli* isolates (25 from diarrheic dogs and 75 from clinically healthy dogs), 36 isolates (including 13 diarrheic and 23 clinically healthy isolates) showed a zone of inhibition of 27 mm or less in the initial screening, indicating suspicion of ESBL production.

Phenotypic Confirmation of ESBL Production in Isolates Using the Combined Disk Method

The results obtained using the combined disk method indicated that 31 isolates (including 12 diarrheic and 19 clinically healthy isolates) were capable of producing ESBL enzymes. The frequency and percentage of isolates confirmed to produce ESBL enzymes are summarized in Table 3.

Table 3: Frequency of *E. coli* isolates confirmed to produce ESBL enzymes

Origin of Isolate	ESBL-Producing Isolates	Non-ESBL-Producing Isolates
Healthy Dog (75)	19 (25.3%)	56 (74.6%)
Diarrheic Dog (25)	12 (48%)	13 (52%)
Total (100)	31 (31%)	69 (69%)

Results of Antibiotic Sensitivity and Resistance Patterns of *E. coli* Isolates Producing ESBL Enzymes

The results showed that 91% of the isolates were resistant to the antibiotic erythromycin, while resistance to meropenem was 3% (1 isolate) and to gentamicin was 6% (2 isolates). The number and percentage of resistance and sensitivity of the isolates to each antibiotic are presented in Table 4. The resistance patterns of each isolate are shown in Table 5. A total of 20 resistance patterns were observed, with the most common multidrug resistance pattern being simultaneous resistance to four antibiotics: ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin, and nalidixic acid.

Table 4: Antibiotic resistance results of *E. coli* isolates producing ESBL enzymes

Antibiotic	Abbreviation	Antibiotic Concentration (µg)	Resistant (Number)	Resistant (%)		Intermediate (Number)	Intermediate (%)	Sensitive (Number)	Sensitive (%)
Ciprofloxacin	CP	5	8	25.8%		1	3.2%	22	71%
Erythromycin	E	15	28	90.3%		2	6.4%	1	3.2%
Neomycin	N	30	3	9.7%		2	6.4%	26	83.9%
Nitrofurantoin	FM	300	3	9.7%		1	3.2%	27	87.1%
Cefoxitin	FOX	30	8	25.8%		0	0%	23	74.2%
Furazolidone	FX	100	2	6.4%		4	12.9%	25	80.7%
Trimethoprim-Sulfamethoxazole	SXT	25/1	21	67.8%		1	3.2%	9	29%
Ampicillin	AM	10	22	71%		3	9.7%	6	19.3%
Gentamicin	GM	10	2	6.4%		0	0%	29	93.5%
Tetracycline	TE	30	20	64.5%		1	3.2%	10	32.2%
Meropenem	MEN	10	1	3.2%		0	0%	30	96.8%
Nalidixic Acid	NA	30	15	48.4%		3	9.7%	13	41.9%

Table 5: Antibiotic resistance patterns and number of *E. coli* strains producing ESBL enzymes with similar antibiotic resistance patterns

Obtained Patterns	Resistant Antibiotics in Each Pattern	Number of Strains with Similar Pattern
1	E	2
2	E and AM	1
3	E and TE	1
4	TE, SXT, and AM	1
5	TE, SXT, and E	3
6	E, AM, and TE	2
7	E, SXT, and AM	3
8	E, AM, GM, and NA	1
9	E, FOX, and AM	1
10	E, SXT, AM, and TE	2
11	E, AM, FM, and NA	1
12	E, SXT, AM, TE, FOX, and NA	1
13	E, SXT, AM, TE, FOX, NA, and MEN	1
14	CP, SXT, E, and NA	4
15	CP, SXT, N, TE, AM, and NA	2
16	CP, E, N, TE, AM, and NA	1
17	CP, SXT, E, FM, and NA	1
18	SXT, AM, TE, FOX, and FM	1
19	CP, E, FOX, SXT, TE, AM, GM, and NA	1
20	E, FM, FOX, SXT, AM, FX, and NA	1

Results of Multiplex PCR Test for Detecting Genes Related to the Production of Extended-Spectrum Beta-Lactamases (ESBLs) in *E. coli* Strains Producing ESBLs

Out of 31 ESBL-producing strains identified by phenotypic methods, 20 strains were found to carry the target genes in the multiplex PCR assay. Fourteen strains (45.2%) had the *blaTEM* gene, and 8 strains (25.8%) had the *blaCTX-M-1* gene. Additionally, 2 strains (6.5%) carried both the *blaCTX-M-1* and *blaTEM* genes. All the strains tested negative for the *blaSHV*, *blaCTX-M-9*, and *blaOXA-1* genes (Figure 1).

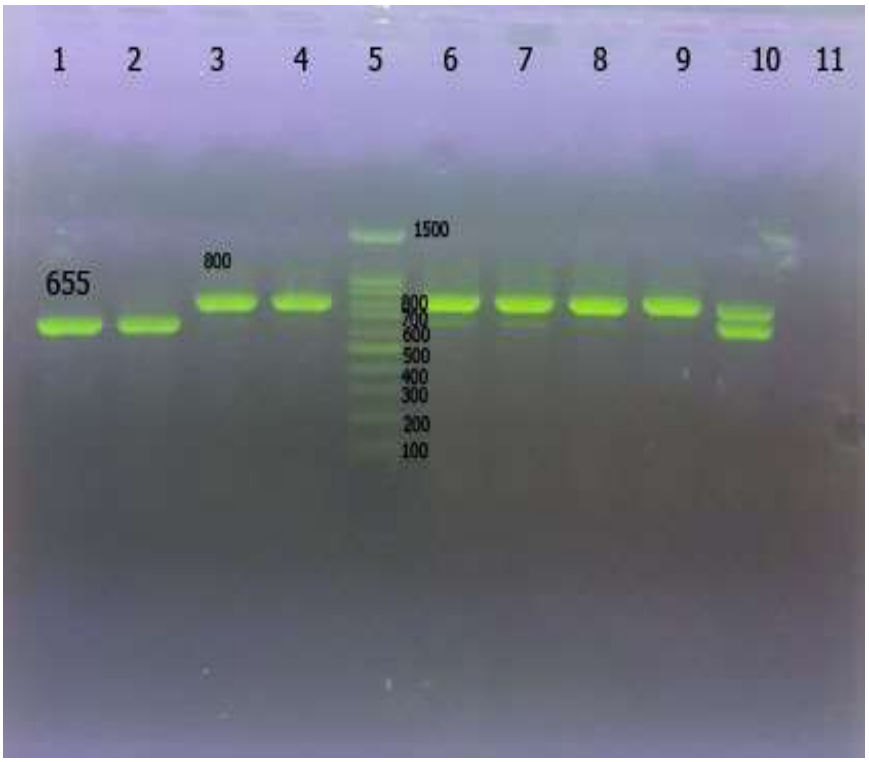

Figure 1 - PCR results for *blaCTX-M-1* and *blaTEM* genes in *E. coli* strains producing ESBLs.1-positive control for the *blaCTX-M-1* gene (655 bp).2-strain containing the *blaCTX-M-1* gene.3-positive control for the *blaTEM* gene (800 bp). 4, 6, 7, 8, 9. strains containing the *blaTEM* gene.5-marker (100 bp).6-strain containing both *blaTEM* and *blaCTX-M-1* genes.11-negative control

Discussion

Although the correct use of antibiotics for the prevention and treatment of infectious diseases leads to the eradication or inhibition of pathogenic microorganisms, inappropriate use can result in unintended consequences. According to available statistics, the per capita drug consumption in Iran is significantly high due to cultural factors and the relatively low cost of medications, which is four times the global average (Hosseinzadeh et al, 2016). In the recent years, there has been a widespread increase in infections caused by antibiotic-resistant bacteria commonly used in treatment. Therefore, monitoring antibiotic resistance patterns is essential for public health surveillance and preventive measures (Miriagou et al, 2003). Like pathogenic bacteria, commensal bacteria are also exposed to antimicrobial agents, and *E. coli* is often used as an indicator for the spread of resistance genes (Bartoloni et al, 2006). The molecular characteristics of antimicrobial resistance can be valuable not only in surveillance studies and tracking multi-drug-resistant strains but also in gaining insights into the relationships between human and animal bacterial strains

(Carvalho et al, 2016). Specifically, attention should be given to bacteria like *E. coli*, which are well adapted for use in both humans and animals, as shared environments create opportunities for the rapid transmission of these strains between hosts (Formenti et al, 2021).

One of the most important mechanisms of resistance in *E. coli* is the production of enzymes, particularly extended-spectrum beta-lactamases (ESBLs), which inactivate beta-lactams by hydrolyzing their beta-lactam rings (Liu et al, 2016). ESBL producers typically exhibit a multidrug-resistant phenotype. Additionally, the genes encoding ESBLs are mostly plasmid-mediated, which facilitates the transfer of antibiotic resistance genes to other bacteria (Huang et al, 2020). Recent studies have raised concerns about the widespread presence of ESBLs and integrons in *E. coli* strains isolated from humans and healthy animals as reservoirs of antibiotic resistance. Dogs and their feces can serve as a source of *E. coli* strains, which may pose a potential threat to humans through virulence factors or multidrug resistance in these bacteria (Sevilla et al, 2020). Since companion animals like dogs and cats are in close contact with humans, they can acquire ESBL-producing microorganisms from humans and potentially transmit them back to humans, which raises a public health concern (Huang et al, 2020).

Various studies have been conducted on ESBL-producing bacteria, especially *E. coli*, in different animals. However, based on the research conducted so far, it appears that no study with this focus has been carried out on household dogs in Khuzestan province.

In the present study, out of 100 *E. coli* isolates, 31 isolates (31%) were phenotypically identified as ESBL producers. Of these, 12 isolates (38.7%) were from diarrheal samples, and 19 isolates (61.3%) were from healthy samples. In other studies, the phenotypic frequency of ESBL-producing *E. coli* isolates ranged from 1.9% to 28.5%. Studies conducted in other parts of the world have also shown variable prevalence of ESBL-producing isolates, ranging from 5% to 28% in both healthy and diarrheal dogs (Marchetti et al, 2021; Aslantas et al, 2017; Formenti et al, 2021; Carvalho et al, 2021; Sevilla et al, 2020; Huang et al, 2020; Carvalho et al, 2016; Liu et al, 2016; Wang et al, 2020; Tamang et al, 2012). Differences in the frequency of ESBL-positive *E. coli* isolates may be due to variations in the antibiotic consumption rates in the studied animals, the origin of the isolates (stray dogs, cats, chickens, pigs, and cows), the prevalence of disease among the animals in the mentioned studies, and the management practices in animal husbandry. The high prevalence of ESBL-producing *E. coli* isolates in the studied area could be attributed to the health status of the animals (both diarrheal and seemingly healthy), the excessive use of beta-lactam antibiotics, and consequently the increased selective pressure on the animal strains in this region. This could potentially raise the risk of the spread and transmission of ESBL-producing strains to the human population in the area.

Another key aspect addressed in this study was the genotypic analysis of *E. coli* isolates producing ESBL enzymes and the genes related to their production. The analyses conducted in this study revealed that, out of 31 *E. coli* isolates producing ESBL enzymes, 20 isolates (64.5%) contained the enzymes of interest. Fourteen isolates (45.2%) harbored the *blaTEM* gene, and eight isolates (25.8%) carried the *blaCTX-M-1* gene. Additionally, two isolates (6.5%) contained both the *blaCTX-M-1* and *blaTEM* genes, and all isolates tested negative for the *blaSHV*, *blaCTX-M-9*, and *blaOXA-1* genes. In studies conducted by other researchers on companion animals, including dogs, the frequency of these genes in *E. coli* isolates varied. In the study by Formenti et al. (2021) in dogs, the *blaCTX-M* gene (79.7%)

was the predominant ESBL gene. *blaTEM* (47.8%), *blaSHV* (5.8%), and *blaCMY* (13%) genes were also identified. In the study by Aslantas et al. (2017), *blaCTX-M-15* (86.1%) was the dominant ESBL gene. Liu et al. (2016) in China found that the predominant ESBL genes were *blaCTX-M* (87.5%) and *blaTEM* (87.5%). Tamang et al. (2012) in South Korea reported that all *E. coli* isolates contained the *blaCTX-M* gene (100%). *blaCTX-M-1* was found in two isolates (16.6%), and *blaCTX-M-9* was present in ten isolates (83.3%). In the study by Carvalho et al. (2016), *blaCTX-M* (75%) was the predominant ESBL gene, with *blaTEM* (66.7%) and *blaSHV* (38.8%) was also identified. Among the 8 ESBL-producing isolates from dog owners, the percentages of *blaCTX-M*, *blaTEM*, and *blaSHV* genes were 50%, 75%, and 12.5%, respectively. Huang et al. (2020) in Taiwan identified *blaCTX-M-1* as the predominant gene (54%) in 65 *E. coli* isolates, followed by *blaCTX-M-9* (32%), *blaTEM* (38%), *blaSHV* (6%), and *blaCTX-M-2* (18.5%). In the study by Carvalho et al. (2021) in Portugal, involving 361 dogs, the *blaCTX-M* gene was found in 95.7% of the isolates, with 21.3% of these related to *blaCTX-M-1*. Four other types of *blaCTX-M* enzymes were identified: *blaCTX-M-15* (55.3%), *blaCTX-M-32* (6.4%), *blaCTX-M-55* (6.4%), and *blaCTX-M-14* (4.2%). As mentioned earlier, it seems that sanitary conditions, the frequency of different infections, and the accessibility and consumption of antibiotics, which affect the level of selective environmental pressure, may be factors contributing to the differences in the prevalence and types of ESBL enzymes in bacterial strains in different geographical regions.

Another aspect investigated in this study was the antibiotic susceptibility and resistance patterns of the ESBL-producing isolates. In the present study, the antibiotic susceptibility of 31 *E. coli* isolates producing ESBL enzymes was investigated. The highest antibiotic resistance was observed against erythromycin (91%), while the lowest resistance was seen with meropenem (3%). Resistance to ciprofloxacin (26%), neomycin (10%), nitrofurantoin (10%), cefoxitin (26%), furazolidone (6%), trimethoprim-sulfamethoxazole (68%), ampicillin (71%), gentamicin (6%), tetracycline (65%), and nalidixic acid (48%) was also measured. Several studies have evaluated the sensitivity and resistance of *E. coli* isolates from dogs, with varying resistance or susceptibility patterns to different antibiotics. In the study by Aslantas et al. (2017), the highest resistance was observed against cephalothin (98.9%), and the lowest against tobramycin (20%). Liu et al. (2016) reported, in their study of 40 *E. coli* isolates producing ESBL enzymes, that the highest resistance was to doxycycline (95%), while the lowest resistance was found for imipenem and meropenem (25%). In Tamang et al.'s study (2012) in South Korea, the highest antibiotic resistance among ESBL-producing *E. coli* isolates was observed against tetracycline (75%), with the lowest resistance against amikacin (3.8%). In the study by Wang et al. (2020) in China, involving 400 *E. coli* isolates from chickens, dogs, pigs, and cows, the highest antibiotic resistance in 100 *E. coli* isolates from dogs was against ampicillin (70%), while the lowest resistance was seen against meropenem (5%). In the study by Marchetti et al. (2021) in Argentina, involving 95 dogs, the highest antibiotic resistance was against tetracycline (50.5%), and the lowest resistance was observed against imipenem, amikacin, and nitrofurantoin (0%). In the study by Formenti et al. (2021) on 266 dogs, the antibiotic susceptibility of 69 *E. coli* isolates producing ESBL enzymes was measured, and all isolates were resistant to at least one antibiotic. The highest resistance was observed against cefotaxime (100%), while the lowest resistance was against imipenem (0%). In the study by Huang et al. (2020) in Taiwan, involving 283 dogs and cats (224 dogs and 59 cats),

the highest resistance was observed against ampicillin (100%), and the lowest resistance was against imipenem (0%). Similarly, in the study by Carvalho et al. (2021) in Portugal, involving 361 dogs, the highest resistance was against ampicillin (100%), and the lowest was against imipenem (0%).

The analysis conducted in this study showed that out of the 31 *E. coli* isolates confirmed to produce ESBL enzymes, only 2 isolates (6%) were resistant to a single antibiotic (erythromycin), 2 isolates (6%) showed resistance to two antibiotics, and 27 other isolates (88%) demonstrated resistance to at least three antibiotics or more, and were identified as multi-drug resistant (MDR) isolates. In this study, no isolate was found to be sensitive to all the antibiotics tested. In the study by Aslantas et al. (2017), 17.9% of isolates were resistant to one antibiotic, 14.7% to two antibiotics, and 67.4% of isolates were resistant to three or more antibiotics, identifying them as MDR strains. Furthermore, in the study by Carvalho et al. (2021) in Portugal, conducted on 361 dogs, it was shown that out of 47 *E. coli* isolates producing ESBLs, all isolates were resistant to at least one antibiotic. Only 1 isolate (2.1%) was resistant to one antibiotic, 3 isolates (6.4%) were resistant to two antibiotics, and 41 isolates (91.5%) were resistant to three or more antibiotics, classifying them as MDR strains. In the study by Liu et al. (2016) in China, 165 *ExPEC* isolates were examined, and it was shown that out of 40 *E. coli* isolates producing ESBLs, only 1 isolate (2.5%) was resistant to two antibiotics, while 39 isolates (97.5%) exhibited an MDR phenotype. In the retrospective study by Tamang et al, (2012) in South Korea, conducted between 2006 and 2007 on 628 dogs, 12 *E. coli* isolates producing ESBLs were analyzed. Of these, 2 isolates (16.6%) were not resistant to any antibiotics, 1 isolate (8.3%) was resistant to two antibiotics, and 9 isolates (75%) showed an MDR phenotype. In the study by Wang et al. (2020) in China, involving 400 *E. coli* isolates from chickens, dogs, pigs, and cows, it was shown that 267 isolates (66.8%) were resistant to three or more antibiotic classes. Additionally, in the study by Marchetti et al. (2021) in Argentina, involving 95 dogs, it was found that 17 isolates were resistant to one antibiotic, 10 isolates were resistant to two antibiotics, and the remaining 41 isolates were resistant to three or more antibiotic classes. The differences in the level of antibiotic resistance observed in the present study compared to other studies can be explained by factors such as the availability and use of antibiotics, hygiene levels, the occurrence of infections in animals, living environments, the presence of specific traits such as broad-spectrum beta-lactamase production by strains, and their origin (apparently healthy dogs, stray dogs, out-of-town dogs, hospital samples, livestock, and poultry). The high prevalence of MDR *E. coli* isolates and the high resistance rates to various antibiotic groups suggest the need for further studies on controlling antibiotic resistance and appropriate antibiotic prescription in treating infections.

Considering the diversity in the characteristics of *E. coli* strains from different geographic regions and animal species, the high prevalence of ESBL-producing isolates, and the presence of multi-drug resistance (MDR) patterns in these isolates in this study, the zoonotic transmission aspects of *E. coli* and the potential transfer of this bacterium from companion animals, such as dogs and cats, to the human population are significant. To ensure successful treatment and prevent the spread of ESBL-producing strains, further epidemiological studies are recommended, along with the determination of antibiotic susceptibility patterns of *E. coli* isolates over various time periods and locations, as well as prior to treatment.

Acknowledgments

The authors gratefully acknowledge Shahid Chamran University of Ahvaz (Khuzestan Province, Iran) for providing financial support, laboratory space, and facilities, and covering associated costs..

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research was funded by the research grant number (SCU.VP1401.261) from Shahid Chamran University of Ahvaz.

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Received: 16.03.2025
Accepted: 24.06.2025

مقاله کامل

دوره بیست و یکم ـ شماره ۳ ـ پاییز ۱۴۰۴

DOI: 10.22055/ivj.2025.512793.2783

حضور برخی ژن‌های بتالاکتاماز وسیع‌الطیف (ESBL) در سویه‌های مدفوعی *اشریشیا کلی* جدا شده از سگ‌ها و بررسی حساسیت آنتی‌بیوتیکی این جدایه‌ها

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تاریخ دریافت: ۱۴۰۳/۱۲/۲۶ تاریخ پذیرش: ۱۴۰۴/۴/۳

چکیده

اشرشیا کلی، یکی از اعضای خانواده *انتروباکتریاسه* و بخشی از فلور طبیعی روده در انسان و حیوانات خون‌گرم است. این باکتری می‌تواند طیف وسیعی از عفونت‌های گوارشی و خارج گوارشی را در انسان و حیوانات، از جمله سگ‌ها و گربه‌ها، ایجاد کند. سگ‌ها و گربه‌ها اغلب به عنوان مخازن بالقوه سویه‌های E. coli در نظر گرفته می‌شوند که قادرند عفونت‌های روده‌ای یا خارج‌روده‌ای را در انسان ایجاد کنند؛ از این رو، جنبه‌های زئونوزی انتقال این باکتری اهمیت بالایی دارد. استفاده از برخی آنتی‌بیوتیک‌ها و فشار انتخابی محیط، در انتخاب و گسترش ژن‌های مقاومت نسبت به آنتی‌بیوتیک‌های مشابه نقش داشته و درمان بسیاری از عفونت‌های باکتریایی را دشوار کرده است. هدف از این پژوهش، شناسایی برخی از ژن‌های بتالاکتاماز گسترده‌طیف (ESBL) در سویه‌های E. coli جدا شده از مدفوع سگ‌های سالم و مبتلا به اسهال و بررسی الگوی حساسیت آنتی‌بیوتیکی آن‌ها بود. در مجموع، ۱۰۰ جدایه E. coli به صورت فنوتیپی از نظر تولید آنزیم‌های ESBL با استفاده از دیسک‌های سفوتاکسیم و ترکیب سفوتاکسیم/کلاولانیک اسید بررسی شدند. حساسیت آنتی‌بیوتیکی سویه‌های تولیدکننده ESBL نسبت به ۱۲ آنتی‌بیوتیک از کلاس‌های مختلف ارزیابی گردید. همچنین، وجود ژن‌های blaTEM، blaSHV، blaCTX-M-1، blaCTX-M-9 و blaOXA-1 در جدایه‌های ESBL مثبت با استفاده از واکنش زنجیره‌ای پلیمراز چندگانه (Multiplex PCR) مورد بررسی قرار گرفت. نتایج نشان داد که ۳۱ مورد از ۱۰۰ جدایه E. coli از نظر فنوتیپی تولیدکننده ESBL بودند. ژن blaTEM به عنوان ژن غالب در ۲/۴۵ درصد از جدایه‌ها شناسایی شد، در حالی که ژن blaCTX-M-1 در ۸/۲۵ درصد وجود داشت. بیش‌ترین مقاومت آنتی‌بیوتیکی در برابر اریترومایسین و کم‌ترین مقاومت در برابر مروپنم مشاهده گردید. علاوه بر این، ۲۰ الگوی مقاومت آنتی‌بیوتیکی متفاوت در جدایه‌ها شناسایی شد. با توجه به جنبه‌های زئونوزی انتقال E. coli، انجام مطالعات اپیدمیولوژیک بیش‌تر و بررسی الگوی حساسیت آنتی‌بیوتیکی پیش از درمان، برای اطمینان از موفقیت درمان و جلوگیری از گسترش سویه‌های تولیدکننده ESBL توصیه می‌شود.

کلمات کلیدی: بتالاکتاماز گسترده طیف (ESBL)، *اشریشیا کلی*، حساسیت آنتی‌بیوتیکی، سگ‌ها

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