Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 22 No. 4 Ser. No. 77 2021

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

The evaluation of *ESBL* genes and antibiotic resistance rate in *Escherichia coli* strains isolated from meat and intestinal contents of turkey in Isfahan, Iran

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10.22099/ijvr.2021.39493.5737

(Received 15 Jan 2021; revised version 28 May 2021; accepted 26 Jul 2021)

Abstract

Background: Extended spectrum beta-lactamase (ESBL) has been described in *Escherichia coli* strains that have been isolated from humans and animals; it has induced a main concern with antibiotic resistance in serious bacterial infections. **Aims:** This study aimed to investigate the frequency of ESBL-producing *E. coli* (EPE) strains in meat and intestinal contents of turkey, and to compare the antibiotic resistance profile between EPE and non-EPE strains. **Methods:** Totally, 70 and 110 *E. coli* strains were isolated from turkey meat and turkey intestinal content samples, respectively. To determine EPE strains, double disc synergy test was applied by that 20 and 22 EPE strains were finally identified in meats and intestinal contents of the turkeys, respectively. Antibiotic susceptibility was exerted using disc diffusion method. *Escherichia coli* isolates were then characterized for virulence genes (*stx-1* and *stx-2*) and *ESBL* genes (*TEM*, *SHV*, and *CTX-M*). **Results:** None of the *E. coli* strains harbored *stx* genes. The EPE strains in comparison with non-EPE strains were significantly more resistant to ciprofloxacin (47.6 *vs* 26.5%), tetracycline (80.9 *vs* 67.3%), ampicillin (47.6 *vs* 22.4%), penicillin (23.8 *vs* 10.2%), ceftazidime (57.1 *vs* 16.3%), ceftriaxone (38.1 *vs* 18.4%), and cefotaxime (47.6 *vs* 8.2%). The majority of EPE strains carried *CTX-M* gene. *SHV* showed the lowest frequency and it was not detected in EPE strains isolated from the intestinal contents. In this study, 75% of *TEM*-producing *E. coli* strains and 33% of *SHV*-producing *E. coli* strains were resistant to ampicillin. In addition, 41.7% of *TEM*-producing *E. coli* strains were resistant to penicillin, and 76.9% of *CTX*-producing *E. coli* were resistant to cefotaxime. Furthermore, 4.7% of EPE strains isolated from turkey meat were imipenem resistant. **Conclusion:** The resistance to cefotaxime and imipenem in EPE strains induces a concern in growing antibiotic resistance against broad spectrum antibiotics in *E. coli* strains.

Key words: *ESBL* genes, ESBL-producing *Escherichia coli*, Turkey meat

Introduction

Escherichia coli is usually a commensal bacterium of humans and animals. Although many *E. coli* strains are non-pathogens, some strains are pathogens. Pathogenic strains cause intestinal and extra-intestinal infections such as urinary tract infection, gastroenteritis, peritonitis, meningitis, and septicemia (Roberts, 1996). Some *E. coli* strains cause bloody diarrhea, anemia, and kidney failure which can lead to death. Most *E. coli* strains can produce shiga toxin that is harmful to the epithelium of the small intestine. This bacterium causes many forms of infections, including colisepticemia, yolk sac infection, coligranuloma, cellulitis, and swollen head syndrome, commonly described as colibacillosis in birds. Colibacillosis often requires antimicrobial therapy (Gholami-Ahangaran and Zia-Jahromi, 2014). Widespread and incorrect use of antibiotics may potentially lead to antibiotic resistance emergence in the bacteria (Gholami-Ahangaran *et al*., 2021a; Sengeløv *et al*., 2003). Antibiotic resistance is a major problem in the treatment and control of infections, and various strategies are used by bacteria to protect themselves from the harmful effects of antibiotics (Chopra and Roberts, 2001).

Increasing antibiotic resistance is a global concern.

Receiving antibiotic-resistant bacteria through food can play a role in developing antibiotic resistance in humans (Rahimi *et al*., 2012). Programs for controlling antibiotic resistance are well defined in most governments (Birgand *et al*., 2018). Resistance in *E. coli* can be an indicator of antibiotic resistance in a country (Chopra and Roberts, 2001). Antibiotic resistance usually occurs more rapidly in the antibiotics more widely used (Teimuri *et al*., 2008).

Beta-lactam antibiotics are one of the most antibiotics widely used in the treatment of bacterial infections in animals and humans; due to their low toxicity and strong antimicrobial activity (Carvalho *et al*., 2020). The production of beta-lactamase enzymes is the main mechanism of bacterial resistance to the beta-lactam family (Badri *et al*., 2017; Kuralayanapalya *et al*., 2019). One of these mechanisms used in Gram-negative bacteria against antibiotics is beta-lactamase production (Mobasseri *et al*., 2019). Broad-spectrum beta-lactamases are mainly produced by *Klebsiella* as well as other *Enterobacteriaceae* bacteria e.g. *E. coli* (Samanta *et al*., 2018). Extensive beta-lactamases (ESBL) are enzymes produced by some bacteria that inactivate the lethality of beta-lactams via breaking the beta-lactam ring (Carvalho *et al*., 2020). These enzymes develop resistance to these antibiotics by hydrolyzing the beta-lactam ring (Haeggman *et al*., 2004). Due to the widespread use of antimicrobial agents to treat animals; the genus *E. coli* is highly resistant to most antibiotics (Gholami-Ahangaran *et al*., 2021b). Production of extensive beta-lactamases in strains isolated from poultry increases the possibility of transferring extensive beta-lactamase-producing genes to other microbial flora, as well as to human microflora through non-compliance with hygiene principles. Due to the importance of detecting bacterial contaminations in animal protein sources, especially meat, this study aimed to investigate the prevalence of ESBL-producing *E. coli* (EPE) strains in meat and intestinal contents of turkey and also to compare the antibiotic resistance profile between EPE and non-EPE strains.

Materials and Methods

Sample collection

Totally, 400 samples were collected from 200 meats and 200 swabs from intestinal contents of turkeys that were prepared from different slaughterhouses in Isfahan province from spring 2019 to 2020. Approximately, 25 g meat sample was collected from neck, thigh, and breast muscles, and homogenized with 225 ml buffered peptone water in a stomacher. The mixture was incubated at 37°C overnight.

Isolation and identification of *E. coli* strains

All samples were firstly cultured in peptone water and then streaked on MacConkey's agar and incubated at 37°C for 24 h. *E. coli* was confirmed after the growth of the isolates on the Eosin methylene blue (EMB) agar medium. The Gram-negative colonies from EMB were subjected to biochemical tests (IMViC test: Indole, methyl red test, Voges Proskauer test, and citrate utilization test) to confirm the colonies as *E. coli*. The isolates with typical IMViC patterns (positive Indol and MR, and negative VP and citrate utilization) were considered as *E. coli* (Gholami-Ahangaran and Zia-Jahromi, 2014).

For extraction of DNA, colonies of overnight growth bacteria were used. The colonies were put in a test tube containing 1 ml of distilled water and boiled for 10 min, and then centrifuged for 5 min at 10000 × g. Five microliters of the supernatant were used for the PCR. Molecular confirmation of the colonies was achieved according to the *16S rRNA* gene of *E. coli* described by Sabat *et al*. (2000).

The primer set of ECP79F (forward, targeting bases 79 to 96, 59-GAA GCT TGC TTC TTT GCT-39) and ECR620R (reverse, targeting bases 602 to 620, 59-GAG CCC GGG GAT TTC ACA T-39) were used for identification of *E. coli* (Sabat *et al*., 2000). *E. coli* ATCC 25922 was used as a reference and quality control organism in the PCR method for *16S rRNA* amplification confirmation.

The PCR mixture (25 µL) contained 2 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl (pH = 9.0), 0.1% Triton X-100, 200 µm of each dNTP (Fermentas, Germany), 1 µm primers, 1.5 IU of *Taq* DNA polymerase (Fermentas, Germany), and 5 µL (200 ng/µL) of DNA.

The PCR reaction was established in a DNA thermocycler (Eppendorf Mastercycler, Eppendorf-Netheler-Hinz GmbH, Biorad, Germany) for 5 min of initial denaturation at 94°C; followed by 35 cycles of 94°C for 50 s; 55°C for 50 s, and 72°C for 90 s. The PCR products were finally analyzed by gel electrophoresis in a 1.5% (w/v) agarose gel.

Detection of *stx-1* and *stx-2* genes

For detection of *stx-1* and *stx-2* genes, 5 µL of the extracted DNA was added to the PCR reaction mixture (50 µL) containing 1 µL of each primer (CinnaGen, Iran) in a 25-pmol concentration, 1 µL of 0.2 mM for each dNTPs (Fermentase, Germany), 5 µL of 10x concentrated PCR buffer, 2 µL of 50 mM $Mgcl_2$, and 0.2 µL of Taq polymerase (1 U/ml) (Fermentase). The DNA-sequencing of oligonucleotide primers, and the size of amplified fragments are listed in Table 1. The amplification products were analyzed by gel electrophoresis on a 1% agarose gel, stained with ethidium bromide, and photographed at UV exposure (Gholami-Ahangaran and Zia-Jahromi, 2014).

Table 1: Oligonucleotide sequences, and sizes of amplified fragments

Gene	Oligonucleotide sequences (5´-3´)	Fragment sizes (bp)
Stx-1	CGCTGAATGTCATTCGCTCTGC CGTGGTATAGCTACTGTCACC	302
Stx-2	CTTCGGTATCCTATTCCCGG CTGCTGTGACAGTGACAAAACGC	516

Antibiotic sensitivity

To evaluate the susceptibility of *E. coli* strains to antibiotics, disc diffusion method (Kirby-Bauer method) was established according to the Clinical & Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). The commercial antibiotic discs (Padtan-Teb, Iran) comprised cefotaxime (30 µg), imipenem (10 µg), ceftazidime (30 µg), gentamicin (10 µg), ampicillin (10 µg), cefexime (5 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), nitrofurantoin (300 µg), furazolidone (100 µg), tetracycline (30 µg), amoxicillin (25 µg), amikacin (30 µg), and nalidixic acid (30 µg). A suspension of pure bacterial culture equivalent to 0.5 McFarland (1.5×10^8 CFU/ml) was firstly prepared. The bacteria were then cultured on Müller-Hinton agar and after disc placement, incubated at 37°C for 24 h. The diameter of the growth inhibition zone was measured and interpreted according to the standard antibiograme protocol.

Phenotypic identification of ESBL-producing *E. coli* (EPE)

To evaluate the ability of bacteria to produce extensive beta-lactamase, a standard microbial suspension was prepared from the isolated *E. coli* strains according to a concentration of 0.5 McFarland and spread on Müller-Hinton agar. After 20 min, the discs of cefotaxime (30 µg), cefotaxime-clavulanic acid (30 plus 10 µg), ceftazidime (30 µg), and ceftazidime clavulanic (30 plus 10 µg) (MAST, UK) were placed at a distance of at least 25 mm from each other. The growth inhibition zone around the disc with clavulanic acid was measured using a ruler, and compared to that without clavulanic acid. So that, if the growth inhibition zone around the discs containing clavulanic acid is greater than or equal to 5 mm in comparison with the same disc alone, it is known as a beta-lactamase-containing strain according to CLSI (CLSI, 2018). *Klebsiella pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were respectively used as a beta-lactamase-producing strain (positive control) and a negative control for quality control of antibiogram.

Genotypic identification of EPE

The purified bacteria were incubated in nutrient broth at 37°C for 1 day and the bacterial DNA was extracted by the boiling method. The extracted DNA was stored at -70°C until the achievement of the experiments. PCR was performed to identify *ESBL* genes comprising *TEM*, *CTX*, and *SHV*. The characteristics of primers for *ESBL* genes are presented in Table 2. The PCR reaction was performed in a final volume of 25 µL, including 20 µL of PCR buffer (50 mmol KCL, 50 mmol Tris-HCL and 50 mmol $Mgcl_2$), 1.5 µL dNTPs (0.2 mmol), 1 µL of each primers (1 µL); 0.5 µL of *Taq* DNA polymerase (Fermentas), and 1 µL of extracted DNA (200 nmol). Primers targeting the *CTX*, *TEM*, and *SHV* genes amplified 214, 847, and 590 bp products (Acikgoz *et al*., 2007; Junyoung *et al*., 2009; Yazdi *et al*., 2010). Amplification of target genes in a thermocycler (Eppendorf, Germany) with a thermal cycle program including the primary denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 60 s, 58°C (for *TEM* gene annealing), 60°C (for *CTX* gene annealing), and 61°C (for *SHV* gene annealing) for 60 s, 72°C for 60 s, and the final extension at 72°C for 5 min. In this study, a 100 bp marker was used to determine the amplified fragment size. The PCR products were analyzed by electrophoresis in 1% agarose gels and stained with ethidium bromide.

In each assay, *K. pneumoniae* ATCC 700603, and *E. coli* ATCC 25922 were used as positive and negative controls for ESBL production, respectively.

Results

The *E. coli* strains were identified by microbiological, biochemical, and molecular (Fig. 1) examinations. 110 and 70 *E. coli* strains were respectively detected from 200 intestinal contents and 200 meat samples of turkeys. In this study, none of the *E. coli* strains carried the *stx* genes (*stx-1* or *stx-2* genes).

Details of the antibiotic resistance in *E. coli* strains isolated from intestinal contents and meat samples are presented in Table 3.

According to cephalosporin plus clavulanic acid sensitivity, 21 and 22 ESBL-producing *E. coli* strains isolated from meats and intestinal contents of turkeys were identified, respectively. The percentages of antibiotic resistance in ESBL and non-ESBL-producing *E. coli* strains isolated from meat samples are presented in Table 4.

The comparison of antibiotic resistance frequencies between ESBL and non-ESBL *E. coli* strains represents that the mean percent of antibiotic resistance in ESBL-producing *E. coli* strains was significantly higher than non-ESBL-producing *E. coli* strains (43.26 ± 20.77 *vs* 28.10 ± 21.56) (P=0.022). This statistical difference is higher about Cefotaxime, Ceftazidime, Ceftriaxone, and

Table 2: The characteristics of primers for *ESBL* genes amplification

Gene name	Sequence of primer	Product size (bp)	Reference
blaCTX-M-F	5-GATGAACGCTTTCCCATGATG-3	214	Acikgoz *et al*. (2007)
blaCTX-M-R	5-CGCTGTTATCGCTCATGGTAA-3		
blaSHV-F	5-GATGAACGCTTTCCCATGATG-3	590	Junyoung *et al*. (2009)
blaSHV-R	5-CGCTGTTATCGCTCATGGTAA-3		
blaTEM-F	5-ATGAGTATTCAACATTTCCG-3	847	Yazdi *et al*. (2010)
blaTEM-R	5-GTCACAGTTACCAATGCTTA-3		

Table 3: The frequency/percentage of antibiotic resistance in *E. coli* strains isolated from intestinal contents and meat samples in turkey

Antibiotics	Meat (70 strains)			Intestinal content (110 strains)		
	Resistance	Intermediate	Sensitive	Resistance	Intermediate	Sensitive
Cefotaxime (30 µg)	14 (20)	13 (18.6)	43 (61.4)	17 (15.5)	35 (31.8)	58 (52.7)
Imipenem (10 µg)	2 (2.8)	3 (4.3)	65 (92.8)	2 (1.8)	5 (4.6)	103 (93.6)
Ceftazidime (30 µg)	20 (28.6)	17 (24.3)	33 (47.1)	22 (20)	45 (40.9)	43 (39.1)
Gentamicin (10 µg)	5 (7.1)	11 (15.7)	54 (77.2)	5 (4.5)	17 (15.5)	88 (80)
Ampicillin (10 µg)	21 (30)	18 (25.7)	31 (44.3)	27 (24.5)	3 (2.7)	80 (72.7)
Ciprofloxacin (5 µg)	23 (32.9)	22 (31.4)	25 (35.7)	32 (29)	35 (31.8)	43 (39.1)
Ceftriaxone (30 µg)	17 (24.3)	16 (22.9)	37 (52.9)	18 (16.4)	35 (31.8)	57 (51.8)
Nitrofurantoin (300 µg)	14 (20)	19 (27.1)	37 (52.9)	16 (14.5)	34 (30.9)	60 (54.6)
Furazolidone (100 µg)	11 (15.7)	12 (17.1)	47 (67.1)	14 (12.7)	24 (21.8)	72 (65.5)
Tetracycline (30 µg)	50 (71.4)	11 (15.7)	9 (18.4)	50 (45.5)	38 (34.5)	22 (20)
Amoxicillin (25 µg)	25 (35.7)	20 (28.6)	25 (35.7)	30 (27.3)	62 (56.4)	18 (16.4)
Amikacin (30 µg)	21 (30)	25 (35.7)	24 (34.3)	22 (20)	58 (52.7)	30 (27.3)
Nalidixic acid (30 µg)	57 (81.4)	5 (7.1)	8 (11.4)	77 (70)	16 (14.5)	17 (15.5)
Cefixime (5 µg)	28 (40)	17 (24.3)	25 (35.7)	25 (22.7)	57 (51.8)	28 (25.5)
Oxytetracycline (30 µg)	26 (37.2)	25 (35.7)	19 (27.1)	33 (30)	31 (28.2)	46 (41.8)
Doxycycline (30 µg)	21 (30)	24 (34.3)	26 (37.1)	22 (20)	40 (36.4)	50 (45.5)
Trimethoprim + sulfamethoxazole	21 (30)	21 (30)	28 (40)	27 (24.5)	44 (40)	39 (35.5)
Erythromycin (15 µg)	56 (80)	11 (15.7)	3 (4.3)	77 (70)	18 (16.4)	15 (13.6)
Penicilline (10 µg)	10 (14.3)	15 (21.4)	45 (64.3)	22 (20)	39 (35.5)	49 (44.5)
Amoxicilline + Clavulanic acid	14 (20)	25 (35.7)	31 (44.3)	17 (15.4)	34 (30.9)	59 (53.6)
Lincomycin (2 µg)	29 (41.4)	16 (22.9)	25 (35.7)	36 (32.7)	48 (43.7)	26 (23.6)
Chloramphenicol (30 µg)	18 (25.7)	24 (34.3)	28 (40)	21 (19.1)	40 (36.4)	49 (44.5)

Table 4: The frequency/percentage of antibiotic resistance in EPE- and non-EPE-producing *E. coli* strains

Antibiotics	EPE isolated from meat (21 strains)			Non-EPE isolated from meat (49 strains)		
	Resistance	Intermediate	Sensitive	Resistance	Intermediate	Sensitive
Cefotaxime (30 µg)	10 (47.6)	7 (33.3)	4 (19.1)	4 (8.2)	6 (12.2)	39 (79.6)
Imipenem (10 µg)	1 (4.75)	1 (4.75)	19 (90.5)	1 (2.0)	2 (4)	46 (94)
Ceftazidime (30 µg)	12 (57.1)	6 (28.6)	3 (14.3)	8 (16.3)	11 (22.5)	30 (61.2)
Gentamicin (10 µg)	3 (14.3)	4 (19.1)	14 (66.6)	2 (4.1)	7 (14.3)	40 (81.6)
Ampicillin (10 µg)	10 (47.6)	5 (23.8)	6 (28.6)	11 (22.4)	13 (26.5)	25 (51)
Ciprofloxacin (5 µg)	10 (47.6)	6 (28.6)	5 (23.8)	13 (26.5)	16 (32.7)	20 (40.8)
Ceftriaxone (30 µg)	8 (38.1)	6 (28.6)	7 (33.3)	9 (18.4)	10 (20.4)	30 (61.2)
Nitrofurantoin (300 µg)	7 (33.3)	7 (33.3)	7 (33.3)	7 (14.3)	12 (24.5)	30 (61.2)
Furazolidone (100 µg)	6 (28.6)	7 (33.3)	8 (38.1)	5 (10.2)	5 (10.2)	39 (79.6)
Tetracycline (30 µg)	17 (81)	1 (4.7)	3 (14.3)	33 (67.3)	10 (20.4)	6 (12.3)
Amoxicillin (25 µg)	10 (47.6)	5 (23.8)	6 (28.6)	15 (30.6)	15 (30.6)	19 (38.8)
Amikacin (30 µg)	7 (33.3)	9 (42.8)	5 (23.8)	14 (28.6)	16 (32.7)	19 (38.8)
Nalidixic acid (30 µg)	17 (81)	2 (9.5)	2 (9.5)	40 (81.6)	3 (6.1)	6 (12.3)
Cefixime (5 µg)	10 (47.6)	6 (28.5)	5 (23.8)	18 (36.7)	11 (25.5)	20 (40.8)
Oxytetracycline (30 µg)	10 (47.6)	7 (33.3)	4 (19.1)	16 (32.7)	18 (36.7)	15 (30.6)
Doxycycline (30 µg)	8 (38.1)	6 (28.6)	7 (33.3)	13 (26.5)	17 (34.7)	19 (38.8)
Trimethoprim + sulfamethoxazole	8 (38.1)	5 (23.8)	8 (38.1)	13 (26.5)	16 (32.7)	20 (40.8)
Erythromycin (15 µg)	19 (90.5)	1 (4.75)	1 (4.75)	37 (75.7)	10 (20.4)	2 (4.1)
Penicilline (10 µg)	5 (23.8)	6 (28.6)	10 (47.6)	5 (10.2)	9 (18.4)	35 (71.4)
Amoxicilline + Clavulanic acid	5 (23.8)	5 (23.8)	11 (52.4)	9 (18.4)	20 (40.8)	20 (40.8)
Lincomycin (2 µg)	10 (47.6)	5 (23.8)	6 (28.6)	19 (38.8)	11 (22.5)	19 (38.8)
Chloramphenicol (30 µg)	7 (33.3)	6 (28.6)	8 (38.1)	11 (22.4)	18 (36.7)	20 (40.8)

Cefixime (47.57 ± 7.57 *vs* 19.10 ± 12.03) (P=0.008).

The detection of *ESBL* genes in ESBL-producing *E. coli* strains showed that *TEM* (Fig. 2), *CTX-M* (Fig. 3), and *SHV* (Fig. 4) detected from 21 out of 70 *E. coli* strains from meats (30%), and 22 out of 110 *E. coli* strains from intestinal contents (20%). The frequency of each *ESBL* genes in *E. coli* strain isolated from meat presented in Table 5. The results represent that *SHV* did

not detect from *E. coli* strains isolated from intestinal content of turkeys.

Discussion

In this study, the frequency of *CTX-M*, *TEM*, and *SHV* genes in *E. coli* strains isolated from the meats and digestive contents of turkeys was evaluated and the relationship between antibiotic resistance and the presence of ESBL-producing genes was studied. As result, 30% of *E. coli* strains isolated from turkey meats, and 20% of *E. coli* strains isolated from the digestive contents of turkeys were ESBL-producing strains that showed high resistance to antibiotics. The average rate of antibiotic resistance in ESBL-producing *E. coli* strains was significantly higher than *E. coli* strains without the ability to produce ESBL (43.26 *vs* 28.1%). Also, the relationship between the presence of *CTX-M* gene and resistance to cefotaxime was investigated; high resistance to cefotaxime was found in EPE strains, which may be due to the presence of *CTX-M* gene in these strains. The comparison among the frequencies of ESBL-producing genes showed that the highest ESBL-producing gene was *CTX-M* in *E. coli* isolates.

There are many reports about the prevalence of EPE in poultry meat in different countries. In Pakistan (46/70%) (Rahman *et al*., 2019), Ghana (29%) (Falgenhauer *et al*., 2019), Turkey (81%) (Pehlivanlar Ennen *et al*., 2015), and the Netherlands (94%) (Leverstein-van Hall *et al*., 2011), the strains isolated from poultry had the ability to produce ESBL. In Iran,

Table 5: The frequency (and percentage) of *ESBL* genes in *E. coli* strains isolated from meats and intestinal contents of turkeys

ESBL genes	*E. coli* strains isolated from meat (70 strains)	*E. coli* strains isolated from intestinal content (110 strains)
TEM	6 (8.57)	6 (5.45)
CTX	7 (10)	13 (11.81)
SHV	2 (2.85)	0 (0)
TEM and *CTX*	5 (7.14)	3 (2.72)
TEM and *SHV*	0 (0)	0 (0)
CTX and *SHV*	0 (0)	0 (0)
TEM; *CTX* and *SHV*	1 (1.42)	0 (0)
Total *ESBL* genes	21 (30)	22 (20)

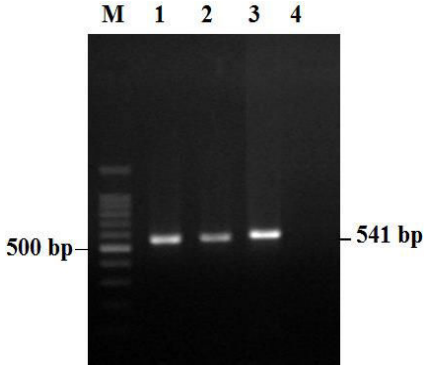

Fig. 1: Electrophoresis of PCR product of *16S rRNA* gene of *E. coli* in the samples. Lane M: Marker with a size of 100 bp DNA, Lanes 1 and 2: Positive samples, Lane 3: Positive control (*E. coli*, ATCC 25922), and Lane 4: Negative control (ddH_2O)

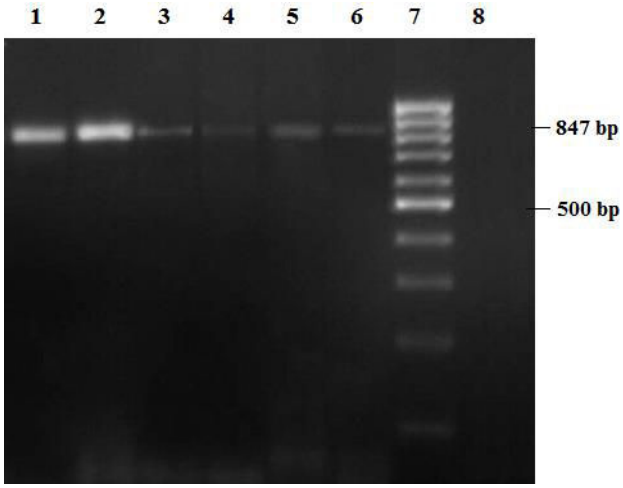

Fig. 2: Electrophoresis of PCR product of *TEM* gene in the *E. coli* strains. Lanes 1-5: Positive samples, Lane 6: Positive control (*K. pneumonia*, ATCC 700603), Lane 7: Marker with a size of 100 bp DNA, and Lane 8: Negative control (*E. coli*, ATCC 25922)

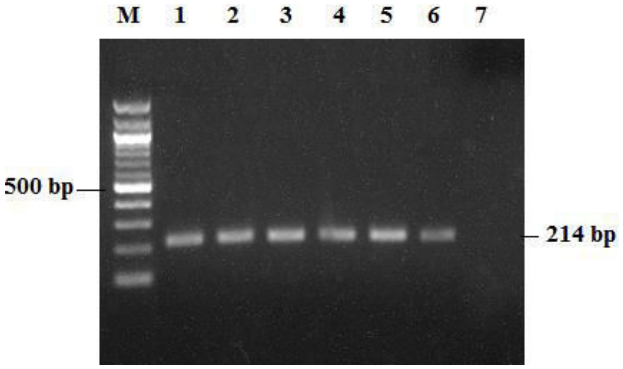

Fig. 3: Electrophoresis of PCR product of *CTX* gene in the *E. coli* strains. Lane M: Marker with a size of 100 bp DNA, Lanes 1-5: Positive samples, Lane 6: Positive control (*K. pneumonia*, ATCC 700603), and Lane 4: Negative control (*E. coli*, ATCC 25922)

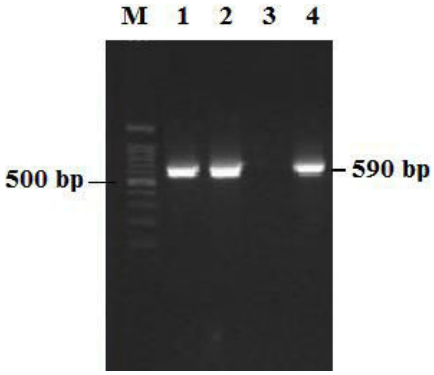

Fig. 4: Electrophoresis of PCR product of *SHV* gene in the *E. coli* strains. Lane M: Marker with a size of 100 bp DNA, Lanes 1 and 2: Positive samples, Lane 3: Negative control (*E. coli*, ATCC 25922), and Lane 4: Positive control (*K. pneumonia*, ATCC 700603)

Jafari *et al*. (2016) detected at least one *ESBL* gene in 48% of *E. coli* strains isolated from broilers. In Belgium, Smet *et al*. (2008) isolated EPE strains in 45% of cloaca swabs collected from five broiler farms. In Tunisia, 7 out of 26 (26.92%) food samples with chicken and turkey origins contained EPE strains (Ben Slama *et al*., 2010). In another study, 22 out of 26 *E. coli* strains isolated from cloaca swabs of broiler chicken were possessed ability to produce ESBL (Dierikx *et al*., 2013). In one study in Germany, 86.60% of carcasses and 72.50% of cecums contained ESBL-producing bacteria that most of them were *E. coli* (Reich *et al*., 2013). Overdevest *et al*. (2011) detected EPE strains in 76.80% of chicken samples collected in the Netherlands. However, EPE strains are rising all over the world, which could raise concerns about the treatment of *E. coli* infection in humans and animals.

Regarding the frequency of ESBL-producing genes, there is always an epidemiological change (Jafari *et al*., 2016). In the 1990s, *E. coli* strains containing the *TEM* and *SHV* genes were recognized as the main EPE, but now *CTX-M* is the most prevalent *ESBL* gene, which has been appeared since 1987 (Li *et al*., 2010). In our study, *CTX-M* was the predominant *ESBL* gene in *E. coli* strains isolated from turkeys, and *SHV* was not detected in EPE strains isolated from gastrointestinal contents. This has been reported in similar studies. Jafari *et al.* (2016) identified 34.67% *E. coli* strains with ESBL production ability in chickens among that the *CTX-M* gene was higher than *TEM* and *SHV* genes in isolates. Pehlivanlar Önen *et al*. (2015) in Turkey, detected *ESBL* genes in 81% of *E. coli* strains isolated from chicken, with the most frequency of *CTX*. There are reports that did not detect *SHV* gene in EPE strains, similar to our study. Momtaz *et al*. (2012) did not find any *E. coli* strain with *SHV* gene in 57 *E. coli* strains isolated from chicken in Shahrekord slaughterhouse. In another study, out of 22 strains with ampicillin-resistant *E. coli* from feces of healthy chickens, 17 isolates had *TEM* gene and no *SHV* gene was detected in these strains (Dierikx *et al*., 2013). In Portugal, 38.20% of *E. coli* strains isolated from broiler fecal samples in a slaughterhouse were positive for *CTX-M* and *TEM* (Costa *et al*., 2009). Recently, there are some reports from Germany and the United States that represented the presence of *CTX-M* in *E. coli* strains isolated from human infections (Xu *et al*., 2011; Bezabihe *et al.*, 2021).

In our study, EPE strains showed high resistance to beta-lactam and other antibiotics. This subject may be caused by the co-transfer of resistance genes located in the same plasmid as *ESBL* genes. EPE in comparison with non-EPE strains were significantly more resistant to ciprofloxacin (47.6 *vs* 26.5), tetracycline (80.9 *vs* 67.3), ampicillin (47/22 *vs* 22.4), penicillin (23.8 *vs* 10.2), ceftazidime (47.6 *vs* 8.2), ceftriaxone (38.1 *vs* 18.4), and cefotaxime (47.6 *vs* 8.2). Previous studies have shown that EPE strains, in addition to resistance to beta-lactam antibiotics, are also highly resistant to aminoglycosides, fluoroquinolones, and tetracyclines (Kar *et al*., 2015). Also, Koga *et al*. (2015) found high resistance to tetracycline (97.4%), and nalidixic acid (79.5%) in EPE strains isolated from chicken carcasses in Brazil. This could be due to the common location of *ESBL* resistance genes and other resistance determinants on the plasmid, which in turn transmits resistance to other antibiotics. However; differences in antibiotic resistance may be due to geographical location. Antibiotic availability, arbitrary consumption of antibiotics, and various antibiotic susceptibility tests play main roles in diversifying the pattern of antibiotic resistance in different geographical areas. EPE strains are reported to be highly resistant to cefotaxime that mainly had *CTX-M* gene. The presence of high resistance, as well as the frequency of *CTX-M* gene, can be related to the presence of the *ESBL* gene and resistance to cefotaxime. Several reports have evaluated the relation between the presence of *CTX-M* gene and the presence of resistance to cefotaxime. Botelho *et al*. (2015) reported 58% resistance to cefotaxime in EPE strains isolated from 16 chicken carcasses. Also, Li *et al*. (2010) reported the frequency of cefotaxime resistance in *CTX*-producing *E. coli* strains isolated from cloacal swabs of broiler chickens on various Chinese farms ranged from 2 to 90%.

In the recent study, 75% of *TEM*-producing *E. coli* strains (9 out of 12) and 33% of *SHV*-producing *E. coli* strains (one out of 3) were resistant to ampicillin. In addition, 41.7% of *TEM*-producing *E. coli* strains (5 out of 12) were resistant to penicillin and 76.9% of *CTX*-producing *E. coli* (9 out of 13) were resistant to cefotaxime. The resistance to cefotaxime in *CTX*-producing *E. coli* strains is more than that of other studies. This indicates the increasing spread of cefotaxime resistance genes among *E. coli* strains. Another concern from our results is the resistance to imipenem, a broad-spectrum antibiotic, observed in 4.7% of EPE strains isolated from turkey meat. Although this is the lowest resistance percentage among the antibiotics, frequent monitoring of this antibiotic resistance is highly recommended because imipenem is the last line of treatment for serious and complicated *E. coli* infections in humans. In India, the rate of imipenem resistance in *E. coli* strains isolated from poultry feces has been reported by 27% (Kar *et al*., 2015), while other studies have not reported any imipenem resistance in bacteria isolated from poultry meat (Pehlivanlar Önen *et al*., 2015). The main reason for this variation among the countries may be due to the widespread use of broad-spectrum antibiotics in health care facilities. Extensive use of broad-spectrum antibiotics, especially third-generation of cephalosporins and penems can contaminate wastewater and even the environment and transfer resistance genes to other bacteria. Although imipenem is not used in livestock and poultry, the occurrence of this resistance may increase the transmission of the antibiotic resistance genes among bacteria; it is necessary to take more care to prevent infectious diseases by replacing safe biological compounds instead of antibiotic consumption in poultry production. These actions can prevent the increase of antibiotic resistance to broad-spectrum antibiotics used against serious infectious diseases in humans.

In conclusion, the appearance of transferable antibiotic resistance and *ESBL* genes in *E. coli* strains isolated from turkey, especially cefotaxime, and imipenem resistance, possesses a high public health threat and induces a concern in the growing of antibiotic resistance against broad spectrum antibiotics in *E. coli* strains.

Acknowledgements

The authors thank all those who helped us in collecting the samples and performing the molecular experiments.

Conflict of interest

The authors declare no conflict of interest.

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