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RESEARCH ARTICLE

Prevalence of Antibiotic-Resistant and Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Chicken Meat from Eastern Turkey

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ABSTRACT

This study examined the antimicrobial resistance of 105 *Escherichia coli* isolates from broiler meat obtained from supermarkets in Erzurum, Turkey. Antimicrobial resistance profile of the isolates was done as proposed by the Clinical & Laboratory Standards Institute. Multiplex PCR was used for the presence of extended-spectrum beta-lactamase (ESBL) and determination of the phylogenetic groups in the isolates. The results showed that resistance to penicillin was the highest (97%), while resistance to carbapenems was not observed in any isolates. A high percentage (94.29%) of multidrug-resistant isolates was observed. A total of 43 (52.14%) *E. coli* isolates was determined to be positive for ESBL. ESBL-producing *E. coli* isolates predominantly carried genes of the CTX-M class (28/43, 65.12%), followed by the TEM (26/43, 60.47%) and SHV (1/43, 2.33%) classes. The prevalence of CTX-M class isolates belonging to the CTX-M-1, CTX-M-9, CTX-M-2, and CTX-M-8/25 groups was 41.86%, 16.28%, 9.3%, and 9.3 %, respectively. The phylogenetic groups B1 (37.21%) and A0 (23.26%) were the most frequently detected. These findings show that raw chicken meat sold in Erzurum is highly contaminated with antibiotic-resistant *E. coli*, including ESBL-producing *E. coli*, which poses a serious risk for human health.

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INTRODUCTION

Although viruses, parasites, chemicals, allergens, and toxins are known causes of foodborne diseases, pathogenic bacteria are the most common food contaminant in terms of both major public health problems and economic impact. The factor that makes the dangers caused by pathogenic bacteria even more serious is the possibility of infections that are difficult to treat or control in humans due to the emergence of antibiotic-resistant foodborne pathogens as a result of the excessive use of antibiotics for non-therapeutic purposes (Klimienė *et al*. 2018). Resistance to antimicrobial agents is a natural evolutionary response of bacteria. Different antimicrobial resistance mechanisms are easily spread between a variety of bacterial species (Baylay *et al*., 2019). In particular, the exposure of food animals to antimicrobial agents increases the likelihood of colonization by drug-resistant pathogens in these animals. This increase in the number of pathogens in food animals is transferred to human consumers as a result of the accumulation of resistant bacteria in the environment and the food chain. The development of the aforementioned mechanisms by pathogenic microorganisms found in the human gut may result in the appearance of resistant species. As a result of the increase in the number of pathogens in food animals, antimicrobial resistance may increase the burden of human diseases that do not respond to antimicrobial (Seo and Lee 2018).

Extended-spectrum β-lactamases (ESBLs) are generated by the family Enterobacteriaceae, which is considered to have colonized more than 1.5 billion individuals worldwide, especially in developing countries, but also in developed countries. They are defined as transmissible β-lactamases that can hydrolyse penicillin, cephalosporins (first-, second-, third-, and fourth-generation) and monobactams and are encoded by genes that can be exchanged between species within this group of bacteria. SHV and TEM enzymes are mutants of ESBLs that typically regulate the genes located on transferable plasmids in Gram-negative bacteria (Jacoby, 2018).

In *Escherichia coli* (*E. coli*), genes encoding ESBLs can be transferred to other *E. coli* strains. The source and transmission routes of ESBL-producing E. coli have not yet been fully elucidated. However, in Europe, an increasing number of studies have been conducted on *E. coli*, which produces ESBL in animals and animal foods over the past decade. ESBL genes and plasmids in *E. coli* isolates, particularly in broilers and broiler meat, have been found to be similar to human clinical isolates (Borges *et al*. 2019; Falgenhauer *et al*. 2019; Roer *et al*. 2018). This indicates that broiler meat contaminated with ESBL-producing isolates can cause intestinal colonization in humans. And hence, it suggests that broiler meat can be root of ESBL-producing *E. coli* (Borges *et al*. 2019). Many studies in different countries have investigated these ESBL *E. coli* strains in meat of broiler. However, in addition to the limited number of studies in Turkey, there is no data on the existence of ESBL-producing *E. coli* in raw chicken meat in the east of country. For this reason, this study investigated ESBL-producing *E. coli* strains in raw chicken meat samples obtained from food distribution system in Erzurum, Turkey. Erzurum was chosen due to its large population and numerous supermarkets.

MATERIALS AND METHODS

Sampling: A total of 150 raw chicken meat samples (thigh chops) were taken from April 2017 to January 2018 from various supermarkets. The samples were transported along a cold chain (4±1°C) in sterile bags and analyzed on the same day at Erzurum Vocational School's microbiology laboratory.

Bacteriological examinations: Aseptically twenty-five grams of tissue from each sample were weighted and homogenized in 225 mL of buffered peptone water using a stomacher (Neutec Group Inc., Farmingdale, NY, USA) for 2 min. The homogenized samples were incubated at 37 °C for 24 h. After incubation, a loopful homogenate was streaked onto MacConkey agar (Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 24 h. One colony from each sample exhibiting a typical colony morphology of *E. coli* on MacConkey agar was extracted for further confirmation using a VITEK 2 Compact System (Bio-Rad, Marnes-la-Coquette, France) and Gram-negative identification cards.

Antimicrobial susceptibility testing: Antimicrobial susceptibility tests were performed according to the Clinical & Laboratory Standards Institute (CLSI 2019) guidelines. Antimicrobial susceptibility tests of suspicious *E. coli* isolates by disc diffusion method were performed using commercial discs (Himedia, Bombay, India) on Mueller-Hinton agar (MHA). They included trimethoprim (W), trimethoprim-sulfamethoxazole (SXT), gentamicin (CN), ciprofloxacin (CIP), kanamycin (K), streptomycin (S), ampicillin (AMP), chloramphenicol (C), nalidixic acid (NA), tetracycline (TE), meropenem (MRP), aztreonam (ATM), ceftriaxone (CRO), cefepime (FEP), cefpodoxime (PX), cefoxitin (FOX), ceftazidime (CAZ), and cefotaxime (CTX). CLSI criteria were used for interpretation and reporting of antimicrobial susceptibility of isolates. In this study, positive control strain *E. coli* ATCC 25922 was used. Multidrug resistance (MDR) was defined as acquired nonsusceptibility to at least three or more antimicrobial classes.

Detection of ESBL-producing *E. coli*: The presence of ESBL phenotypes in all identified *E. coli* isolates was determined by the double-disc synergy test using cefotaxime and ceftazidime alone and in combination with clavulanic acid, as recommended by the (CLSI 2019). In the combined synergy method proposed by the CLSI as a phenotypic validation test, cefotaxime (CTX (30 μg)), ceftazidime (CAZ (30 μg)), cefotaxime/clavulanic acid (CTL (30/10 μg)) and ceftazidime/clavulanic acid (CAL (30/10 μg)) discs were placed on MHA, which had previously been incubated at room temperature for 15 min. After 16–18 hours of incubation at 35 °C, a ≥5-mm increase in the zone diameter of the CAL disc and the CAZ disc alone and/or a ≥5-mm increase in the zone diameter of the CTL disc and the CTX disc alone were considered as indicating ESBL-producing *E. coli*.

Genotypic characterization: Genomic DNA from ESBL-positive isolates was extracted by boiling. For this purpose, 100 μL of Tris-EDTA buffer solution (pH 8.00) containing a few colonies was boiled for 10 min. The samples were subsequently cooled on ice and centrifuged at 10,000 xg for 15 sec. The supernatant contains DNA directly used as the PCR template in polymerase chain reaction (PCR). The PCR components were provided by Vivantis Technologies (Shah Alam, Selangor, Malaysia). The primers were obtained from Metabion International AG (Planegg-Martinsried, Germany).

To detect the presence of ESBLs in *E. coli* isolates, multiplex PCR analysis was performed for simultaneous detection using TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8/25, and CTX-M-9 group primers, as described by Le *et al.* (2015). The phylogenetic groups were determined using the genes *chuA*, *yjaA*, and TSPE4.C2 for ESBL-producing *E. coli* isolates, as described by Klimienė *et al.* (2018).

Statistical analysis: Average, percentage and frequency calculations were performed using SPSS Statistics 18.0 (IBM, NY, USA).

RESULTS

Antimicrobial susceptibility patterns: 105 of the total 150 samples (70%) were culture positive for the presence of *E. coli*. Eleven commonly used antibiotics were used to test the antimicrobial susceptibility of 105 isolates. All isolates were resistant to at least one class of antibiotics. As seen in Table 1, most isolates were highly resistant to ampicillin (97.1%), followed by nalidixic acid (77.1%), trimethoprim (70.5%), streptomycin (65.7%), trimethoprim/sulfamethoxazole (63.8%), and chloramphenicol (56.2%). All *E. coli* isolates were sensitive to meropenem. Of those, 99 (94.29%) were multidrug-resistant (MDR), to between three and nine classes of antibiotics. All isolates were determined to be sensitive to carbapenems. The resistance rates of MDR isolates were as follows: penicillins 96/99 (97.0%), quinolones 84/99 (84.8%), cephems 62/99 (62.6%), phenicols 61/99 (61.6%), fluoroquinolones 60/99 (60.6%), folate pathway inhibitors 60/99 (60.6%), aminoglycosides 40/99 (40.4%), and tetracyclines 38/99 (38.4%) (Table 2).

Table 1: Antibiotic susceptibility pattern (Sensitive, Intermediate and Resistance) of *E. coli* isolates.

Antibiotic (µg)	Antibiotic susceptibility pattern		
	Sensitive	Intermediate	Resistance
Ampicillin (10 µg)	3 (2.9)	0	102 (97.1)
Chloramphenicol (30 µg)	44 (41.9)	2 (1.9)	59 (56.2)
Ciprofloxacin (5 µg)	45 (42.9)	15 (14.3)	45 (42.9)
Gentamicin (10 µg)	76 (72.4)	5 (4.8)	24 (22.9)
Kanamycin (30 µg)	63 (60.0)	2 (1.9)	40 (38.1)
Meropenem (10 µg)	105 (100)	0	0
Nalidixic acid (30 µg)	20 (19.0)	4 (3.8)	81 (77.1)
Streptomycin (25 µg)	29 (27.6)	7 (6.7)	69 (65.7)
Tetracycline (30 µg)	67 (63.8)	1 (1.0)	37 (35.2)
Trimethoprim (5 µg)	31 (29.5)	0	74 (70.5)
Trimethoprim/Sulfamethoxazole (25 µg)	37 (35.2)	1 (1.0)	67 (63.8)

Table 2: Antimicrobial resistance class pattern distribution for *E. coli* isolates from chicken meat

No. of classes	Antimicrobial resistance class patterns	Frequency	Prevalence (%)
9	AMGs, FPIs, FQs, PCNs, PHs, TETs, Qs, CEPs, MONs	7	7.07
8	AMGs, FQs, PCNs, PHs, TETs, Qs, CEPs, MONs	4	4.04
	AMGs, FPIs, FQs, PCNs, PHs, Qs, CEPs, MONs	2	2.02
	FPIs, FQs, PCNs, PHs, TETs, Qs, CEPs, MONs	2	2.02
	AMGs, FQs, PCNs, PHs, TETs, Qs, CEPs, MONs	1	1.01
7	FPIs, FQs, PCNs, PHs, Qs, CEPs, MONs	8	8.08
	AMGs, FPIs, FQs, PCNs, PHs, TETs, Qs	3	3.03
	AMGs, FPIs, PCNs, PHs, Qs, CEPs, MONs	3	3.03
	FPIs, FQs, PCNs, PHs, TETs, Qs, CEPs	1	1.01
	AMGs, PCNs, PHs, Qs, CEPs, MONs	1	1.01
	FQs, PCNs, PHs, TETs, Qs, CEPs, MONs	1	1.01
	AMGs, FPIs, FQs, PCNs, TETs, Qs, CEPs	1	1.01
	FPIs, FQs, PCNs, TETs, Qs, CEPs, MONs	1	1.01
	AMGs, FPIs, FQs, PCNs, Qs, CEPs, MONs	1	1.01
6	FQs, PCNs, PHs, Qs, CEPs, MONs	3	3.03
	FPIs, FQs, PCNs, PHs, TETs, Qs	3	3.03
	AMGs, FPIs, PCNs, PHs, TETs, Qs	3	3.03
	AMGs, FQs, PCNs, Qs, CEPs, MONs	3	3.03
	AMGs, FPIs, FQs, PCNs, TETs, Qs	3	3.03
	AMGs, FPIs, PCNs, PHs, CEPs, MONs	2	2.02
	FPIs, FQs, PCNs, Qs, CEPs, MONs	1	1.01
	FPIs, FQs, PCNs, TETs, Qs, CEPs	1	1.01
	AMGs, FPIs, FQs, PCNs, PHs, Qs	1	1.01
	AMGs, PCNs, PHs, Qs, CEPs, MONs	1	1.01
5	FQs, PCNs, Qs, CEPs, MONs	4	4.04
	FQs, PCNs, PHs, Qs, MONs	3	3.03
	PCNs, PHs, Qs, CEPs, MONs	3	3.03
	FPIs, FQs, PCNs, TETs, Qs	2	2.02
	FPIs, PCNs, TETs, Qs, MONs	2	2.02
	FPIs, PCNs, Qs, CEPs, MONs	1	1.01
	FPIs, PHs, TETs, CEPs, MONs	1	1.01
4	FPIs, FQs, PHs, Qs	2	2.02
	FPIs, FQs, PCNs, Qs	2	2.02
	FPIs, PCNs, Qs, MONs	2	2.02
	PCNs, Qs, CEPs, MONs	2	2.02
	AMGs, FPIs, PCNs, PHs	2	2.02
	FPIs, PCNs, PHs, Qs	1	1.01
	PCNs, PHs, CEPs, MONs	1	1.01
	FQs, PCNs, PHs, Qs	1	1.01
	AMGs, FPIs, PCNs, TETs	1	1.01
	PCNs, TETs, CEPs, MONs	1	1.01
	FPIs, PCNs, TETs, Qs	1	1.01
3	PCNs, CEPs, MONs	5	5.05
	PCNs, Qs, MONs	2	2.02
	FPIs, PCNs, PHs	1	1.01
	FPIs, PCNs, Qs	1	1.01
	AMGs, PCNs, TETs	1	1.01
	Total	99	100

AMGs, aminoglycosides; CARs, carbapenems; CEPs, cephems; FPIs, folate pathway inhibitors; FQs, fluoroquinolones; PCNs, penicillins; PHs, phenicols; Qs, quinolones; TETs, tetracyclines; MONs, monobactams.

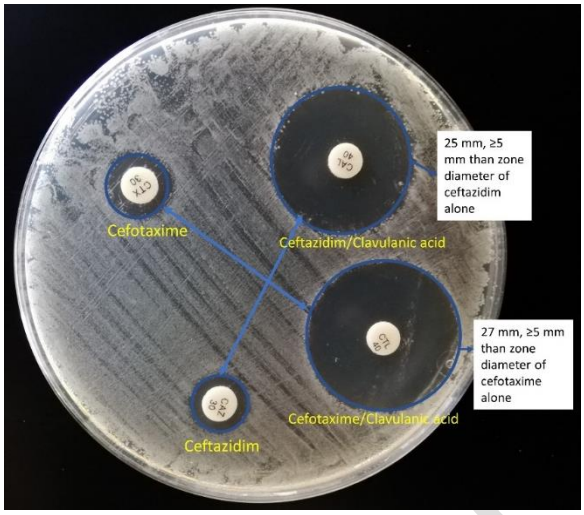

Fig. 1: Double disc synergy test (ESBL positive strain).

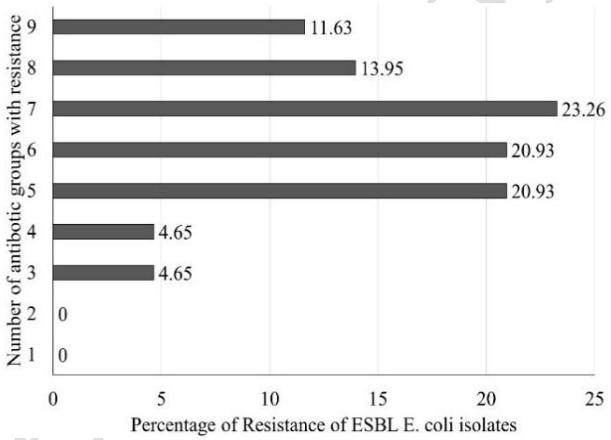

Fig. 2: Antimicrobial resistance class pattern distribution for ESBL E. coli isolates from chicken meat.

Table 3: Prevalence of CTX-M genotype among ESBL-Producing *E. coli* isolates from chicken samples

	–[a]	+SHV*	+TEM*	+SHV** +TEM	Total
CTX-M-1 group	5	0	11	0	16 (37.21%)
CTX-M-2 group	6	1	1	0	8 (18.60%)
CTX-M-8/25 group	2	0	2	0	4 (9.30%)
CTX-M-9 group	2	0	2	0	4 (9.30%)
		0	11	0	11 (25.58%)

*Dual positive, **Triple positive [a]Only one group.

ESBL phenotypes and frequency and diversity of ESBL-encoding genes in *E. coli* isolates: Forty-three of the 105 *E. coli* isolates tested positive to ESBL production (52.14%) (Fig. 1). As shown in Table 3, these isolates mostly carried CTX-M class genes (28/43, 65.12%), followed by the TEM (26/43, 60.47%) and SHV (1/43, 2.33%) classes. The prevalence of isolates with CTX-M-1, CTX-M-9, CTX-M-2, and CTX-M-8/25 genes was 41.86%, 16.28%, 9.30% and 9.30%, respectively. ESBL-producing *E. coli* harboring combinations of CTX-M-1 and CTX-M-2 plus TEM genes was also observed a higher incidence (37.21% and 18.60 %, respectively). Eleven of the 43 isolates (25.58%) carried only genes of the TEM group. It was determined that three isolates (6.98%) which were determined to be phenotypic ESBL-producing *E. coli* did not carry the β-lactamase genes.

Phylogenetic analysis: The PCR analysis of the 43 ESBL-positive *E. coli* isolates revealed that the phylogenetic groups B1 and A0 were the most frequent (37.21% and 23.26%, respectively). The prevalence of isolates with the phylogenetic groups A1, D1, and D2 was 13.95%, 11.63%, and 9.30 %, respectively.

Antimicrobial susceptibility patterns of ESBL *E. coli* isolates: The antimicrobial resistance patterns of the ESBL producing *E. coli* isolates are presented in Fig. 2. None were resistant to CARs and only 15 were resistant to TETs. However, all 43 isolates (100%) exhibited multidrug resistance characteristics.

DISCUSSION

Research has shown that prophylactic and/or uncontrolled use of antibiotics may cause to the possession and spreading of resistant *E. coli* strains through direct contact with animals or food consumption (Osman *et al.* 2018). For this reason, it is necessary to conduct systematic monitoring studies of the genetic characteristics, antimicrobial resistance patterns, and specific virulence of these groups of bacteria. Recent studies conducted in various provinces of Turkey have detected antimicrobial residue in chicken meat, indicating an uncontrolled use of antibiotics by the poultry industry (Er *et al.* 2013). This is consistent with our findings showing significant antimicrobial resistance of *E. coli* isolates collected from commercially available chicken meat. It was observed that the isolates had the most resistance against ampicillin followed by nalidixic acid and trimethoprim. Although there is no data on the antibiotic types used by the poultry industry nationally, our field observations suggest that these three antibiotics have been used extensively for prophylactic or therapeutic purposes. Studies have reported similar findings showing resistance to these antibiotics observed in *E. coli* isolates from chickens and chicken meat (AHMAD *et al.* 2018; Sary *et al.* 2019). Importantly, we observed several MDR *E. coli* isolates in our study. This finding is consistent with similar studies (Nahar *et al.* 2018; Sary *et al.* 2019). Sary *et al.* reported that more than 90% of the *E. coli* isolates from chicken carcasses in Vietnam were MDR. All these results suggest that the presence of antimicrobial-resistant *E. coli* in chicken meat poses a serious threat of transmission to humans.

Cephalosporin- and carbapenem-resistant *E. coli*, especially in foods, poses serious risks for public health. In Turkey, studies on the presence of cephalosporin- or carbapenem-resistant (ESBL-producing) *E. coli* in animals and foods have focused on the western region of the country (Buyukunal *et al.* 2019; Husan and Çadirci 2019; Tepeli and Demirel Zorba 2018). Ours is the first study investigating the drug resistance patterns and the β-lactamase prevalence in *E. coli* isolates from chicken meat in the eastern part of Turkey (specifically, Erzurum). Our data shows that more than half of isolates were positive for ESBL-producing *E. coli*. A previous study by Kürekci *et al.* (2019) reported that *E. coli* isolates from domestic chicken meat samples in Hatay, Turkey were found positive for ESBL-producing *E. coli* at a rate of 86.6%, which is considerably higher than the rate in our study.

Studies have reported a high rate of quinolone-resistant ESBL-producing *E. coli* (Hinenoya *et al.* 2018; Seo and Lee 2018; Wang *et al.* 2019). Similarly, a high resistance rate was found in the quinolone group in our study. This is because quinolone drugs, which can spread among herds without selective pressure, have been used as feed additives for a long time worldwide. All isolates in our study were found to be sensitive to meropenem. A similar finding was reported by Nahar *et al.* (2018) for imipenem. As known, carbapenems are reserved as drugs of last resort in the treatment of infections and are not used in animal food production.

Beta -lactam antibiotics are frequently preferred in the cure of infections in human and also, in veterinary medicine. Many Gram-negative bacteria, especially *E. coli*, have been found to produce β-lactamase. TEM, SHV and CTX-M are some of the β-lactamase genes. The most common is the CTX-M class. The types and rates of genes of ESBL-producing strains may differ from country to country. In our study, PCR analysis of β-lactamase genes showed that many ESBL-producing *E. coli* isolates harboured genes encoding enzymes of family of CTX-M. In Germany, *E. coli* isolates from chicken meats were found to carry predominantly CTX-M-2 genes, followed by CTX-M-8 and CTX-M-1 (Müller *et al.* 2018). Tansawai *et al.* detected CTX-M-1 (69%), CTX-M-9 (13%), TEM (1%), SHV-2 (1%), and SHV-12 (1%) genes in ESBL-producing *E. coli* isolates from chicken meats in Thailand. Studies in other countries have also reported that CTX-M ESBL-encoding genes are the most common (Hayashi *et al.* 2018; Kaesbohrer *et al.* 2019; Nahar *et al.* 2018). However, the CTX-M groups identified in our study were different from those in other studies conducted in Turkey (Aslantaş 2018; Kürekci *et al.* 2019). This finding supports the hypothesis that the CTX-M strain is the dominant group of genes in various geographic regions (Fuzi *et al.* 2020). In our study, CTX-M-1 and CTX-M-2 were identified as the most common CTX-M groups. This finding is similar to those reported by other studies, where the CTX-M-1 ESBL-encoding genes was the most widespread in *E. coli* isolates from chicken meat (Hinenoya *et al.* 2018; Irrgang *et al.* 2018; Kürekci *et al.* 2019; Tansawai *et al.* 2018).

In the current study, the number of CTX-M-1 plus TEM group gene regions was found to be high in ESBL-producing *E. coli* isolates. CTX-M and TEM genes in *E. coli*, isolated from different sources (foods and human), have also been documented in Spain (32.0%) (Alegría *et al.* 2020), Thailand (30.59%) (Tansawai *et al.* 2018), and Turkey (36.5%) (Kürekci *et al.* 2019.

Phylogenetic analysis in a previous study revealed that virulent extra-intestinal strains mostly belonged to the B2 group and to a lesser degree to the D group, whereas most commensal strains belonged to group A (8). The isolates in our study were found to belong to groups A, B1, and D. This finding is in line with previous studies (Park *et al.* 2019; Tansawai *et al.* 2018).

In our study, all ESBL-producing *E. coli* isolates were identified as MDR. Seo and Lee (2019) found that β-lactamase-producing *E. coli* had a higher rate of multidrug resistance than non-producing *E. coli*. This result is compatible with previous studies revealing that β-lactamase genes increase resistance to other antimicrobials and result in multidrug resistance (Kürekci *et al.* 2019; Müller *et al.* 2018).

Conclusions: In this study, 105 (70%) of 150 different retail chicken carcass samples were found to be positive with respect to *E. coli*. The vast majority (94.29%) were MDR. More than half (52.14%) of the 105 *E. coli* isolates were found to be ESBL-positive. The main types of β-lactamase identified were CTX-M-1, CTX-M-9, CTX-M-2, and CTX-M-8/25. The presence of ESBL-producing and MDR *E. coli* in chicken meat poses a serious threat to public health in Turkey.

Authors contribution: AB and MCA designed the project. The sampling, data collection, processing and interpretation of results were made by AB, MCA and MY. The data analysis was made by AB. The manuscript was written by AB, MCA and MY. All the authors read the manuscript and approved the final version.

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