

## Antimicrobial resistance profiles and virulence factors of *Escherichia coli* O157 collected from a poultry processing plant\*

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**Abstract:** The aims of this study were to investigate the presence of *Escherichia coli* O157 and to determine the antibiotic susceptibility of isolates in different poultry-originated materials. In total 300 samples of chicken carcass swabs (n = 50), chicken feces (n = 50), gizzard (n = 50), liver (n = 50), water in the final scald tank (n = 50), and equipment such as tables, conveyers, and knives (n = 50) were used as materials. Overall, 45 *E. coli* isolates were obtained from the 300 poultry samples. However, 13 (4.3%) of the samples were identified as *E. coli* O157 including 3 liver, 3 carcass, and 7 fecal samples. All 13 isolates were positive for O157 but negative for H7 using PCR. Only intimin (encoded by *eaeA* gene) was detected in 10 *E. coli* O157 isolates while no other virulence factors (enterohemolysin A, shiga toxin 1 and 2) were detected. All *E. coli* O157 isolates were susceptible to gentamicin (100%). In conclusion, the presence of *E. coli* O157 in poultry is of utmost significance (4.3%) and our results demonstrated that poultry carcasses and giblets may be contaminated by *E. coli* O157 with feces during evisceration. To prevent *E. coli* O157 infections, hygiene rules must be tightly observed. In addition, antibiotic use must be controlled for avoiding increased antibiotic resistance.

**Key words:** *Escherichia coli* O157, poultry, virulence, antibiotic resistance

### 1. Introduction

*Escherichia coli* O157 and other enterohemorrhagic *E. coli* (EHEC) are a worldwide threat to public health (1). Mostly in developed countries, *E. coli* O157 has been recognized as a cause of serious clinical symptoms such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) characterized by thrombocytopenia, hemolytic anemia, and nephropathy (2). Cattle are the primary reservoir and asymptomatic carriers of *E. coli* O157. The bacteria were spread via cattle faces (3). Most outbreaks of EHEC infections have been associated with the consumption of uncooked meat and meat products, unpasteurized dairy products, and feces-contaminated vegetables and water (2–4).

*E. coli* O157, a member of EHEC, has important virulence factors such as shiga toxins (*stx1* and *stx2*), enterohemolysin (*hlyA*), and intimin (*eaeA*). Shiga toxin-producing *E. coli* (STEC) can cause HC and HUS (5,6) due to the inhibiting protein synthesis, eukaryotic cell injury, and death (7). Intimin, encoded by the *eaeA* gene, is able

to adhere to intestinal mucosa and cause intestinal lesions (5,7). In addition, enterohemolysin, encoded by *hlyA*, leads to lysis of the erythrocytes, which may contribute to the iron intake of the bacterium to live in the intestine (4).

The frequency of antimicrobial resistance in food-borne pathogens such as *E. coli*, *Salmonella* spp., and *Campylobacter* spp. has recently increased. The misuse of antimicrobial agents for farming and therapeutic purpose in animals and humans is the main cause of transmission of antibiotic-resistant strains, which are very difficult to treat with commonly used antibiotics, to humans via the food supply (8).

The aim of this study was to investigate the potential risk of public health by determining the prevalence of *E. coli* O157:H7 and identifying the virulence genes from The aims of this study were to investigate the prevalence of *E. coli* O157 and to identify virulence genes and also to determine the antibiotic susceptibility of the isolates in different poultry-originated materials.

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## 2. Materials and methods

### 2.1. Collection of samples

During the period from December 2014 to December 2015, a total of 300 poultry and poultry-associated samples including rectal content, giblet (gizzard and liver), carcass, equipment (tables, conveyers, knives), and scald tank water samples were randomly collected monthly at a poultry slaughterhouse in Kayseri, Turkey. The feces samples (n = 50) were aseptically taken from the cloaca immediately after slaughtering. In total 100 giblet samples consisting of gizzard (n = 50) and liver (n = 50) were randomly selected from different broilers. The carcasses (n = 50) were sampled by swabbing after dipping in a scald tank and finally approximately 200 mL of scald tank water samples (n = 50) were taken. Moreover, swab samples were obtained from the equipment used in the processing operations (15 tables, 15 conveyers, 20 knives). The samples were immediately transported to the laboratory in a cool box to be analyzed within 2 h.

### 2.2. Reference strains

*E. coli* NCTC 12900 and *E. coli* RHFS 232 reference strains were used as positive controls for the isolation and identification of *E. coli* O157 and determination of their virulence factor. *Escherichia coli* ATCC 25922 was also used for antimicrobial susceptibility testing.

### 2.3. Bacterial isolation

Poultry and poultry-associated samples were homogenized with tryptone soya broth (Oxoid Limited, Basingstoke, Hampshire, UK) with novobiocin (20 mg/L, SR0181E, Oxoid Limited) as described by Dontorou et al. (4) with minor modifications. One loopful of the broth was inoculated onto Sorbitol MacConkey Agar and Chromocult agar (CHROM agar O157, EE222, DRG International, Paris, France) supplemented with 0.05 mg of cefixime and 2.5 mg of tellurite (CT Supplement 109202, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 18–24 h. After that, five different *E. coli* O157 suspicious colonies were subcultured on blood agar (Oxoid, CM0271) for confirmatory testing with biochemical methods (indole, Methyl red, Voges–Proskauer, citrate, urease, and carbohydrate fermentation tests).

### 2.4. Serological analysis

All suspected isolates were tested with *E. coli* O157, *E. coli* H7 antisera (221591, Difco), and *E. coli* O157 latex agglutination kit (DR0620M, Oxoid) according to the manufacturer's recommendations.

### 2.5. PCR analysis for the detection of *E. coli* O157 and virulence factors

Total genomic DNA was extracted from the isolates using a commercial DNA extraction kit (Axygen Bioscience,

Union City, CA, USA) as per the manufacturer's directions. The primer pairs for *fliCh7* and *rbfO157* genes and the PCR assay conditions were according to Sarimehmetoglu et al. (9) and Maurer et al. (10), respectively (Table 1). PCR was carried out in a reaction mixture of 50- $\mu$ L final volume containing 5  $\mu$ L of template DNA, 1x PCR buffer (Vivantis, Chino, CA, USA), 1.5 U of Taq polymerase (Vivantis), 400 mM of dNTP mix (Vivantis), 1.5 mM of  $MgCl_2$  (Vivantis), and 0.5 mM of each primer. For *fliCh7* genes, the reaction mixtures were incubated in a thermal cycler at 94 °C for 2 min for initial denaturation, and the reaction was performed for 35 cycles of 94 °C for 20 s, 54 °C for 1 min, and 72 °C for 1 min. The final extension was 10 min at 72 °C (Techne TC-512, Keison Products, Chelmsford, Essex, UK). DNA amplification was carried out for *rbfO157* including an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min, ending with a final extension at 72 °C for 5 min.

The virulence genes of *E. coli* O157:H7 including *stx1*, *stx2*, *eaeA*, and *hlyA* (Table 1) were detected with multiplex PCR (mPCR) as described by Fratamico et al. (11). The PCR was performed in a reaction mixture of 50  $\mu$ L of final volume containing 5  $\mu$ L of template DNA, 1x PCR buffer (Vivantis), 3.0 mM of  $MgCl_2$  (Vivantis), 400 mM each of dNTPs (Vivantis), 1.5 U of Taq DNA polymerase (Vivantis), 0.50 mM of the primers for *stx1*, *stx2*, and *hlyA* genes, and 0.25 mM of the primer for the *eaeA* gene.

The PCR amplification was performed with an initial denaturation of 94 °C for 5 min and then consisted of 35 cycles of 94 °C for 20 s, 54 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. All amplification products were also visualized (Vilber Lourmat, Marne La Vallee, France) in 1.5% agarose gel with ethidium bromide (0.06%) at 100 V for 50 min (EC250-90, Thermo, Pittsburgh, PA, USA).

### 2.6. Antimicrobial sensitivity test

The antibacterial susceptibility testing of the isolates was performed using disc diffusion. Concentrations and abbreviations for the antimicrobials used in this study were as follows: ampicillin (AMP, 10  $\mu$ g), cefazolin (KZ, 30  $\mu$ g), enrofloxacin (ENR, 5  $\mu$ g), gentamicin (CN, 10  $\mu$ g), neomycin (N, 10  $\mu$ g), tetracycline (T, 30  $\mu$ g), erythromycin (E, 15  $\mu$ g), and amoxicillin clavulanic acid (AMC, 30  $\mu$ g). Briefly, the isolates were grown on blood agar (Oxoid, UK CM0271) at 37 °C for 24 h. Then a suspension of organism was adjusted to McFarland 0.5 using physiological saline. The suspensions were spread onto Mueller-Hinton agar (Merck, GERMANY 1.05437). Antibiotic discs were placed onto the agar and incubated at 37 °C for 24 h aerobically. After 24 h of incubation, the diameters of the inhibition zones were measured with calipers and the results were interpreted according to the CLSI standards (12).

**Table 1.** Primer sequences and lengths of PCR amplification products.

Primer	Primer sequence (5'-3')	Target gene	Gene product	Product size (bp)	References
PF8	CGTGATGATGTTGAGTTG	<i>rfb</i> <sub>O157</sub>	O157	420	10
PR8	AGATTGGTTGGCATTACTG				
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	<i>fliC</i> <sub>H7</sub>	H7	625	9
FLICH7-R	CAACGGTGACTTTATCGCCATTCC				
SLT1-F	TGTAAGTGGAAAGGTGGAGTATACA	<i>stx</i> <sub>1</sub>	Shiga-like toxin 1	210	9
SLT1-R	GCTATTCTGAGTCAACGAAAAATAAC				
SLTII-F	GTTTTTCTTCGGTATCCTATTCC	<i>stx</i> <sub>2</sub>	Shiga-like toxin 2	484	9
SLTII-R	GATGCATCTCTGGTCATTGTATTAC				
AE22	ATTACCATCCACACAGACGGT	<i>eaeA</i>	Intimin	397	9
AE20-2	ACAGCGTGGTTGGATCAACCT				
MFS1-F	ACGATGTGGTTTATTCTGGA	<i>hlyA</i>	Enterohemolysin	166	9
MFS1-R	CTTCACGTCACCATACATAT				

### 3. Results

Forty-five *E. coli* isolates obtained from 300 poultry samples were sorbitol negative,  $\beta$ -glucuronidase negative, and indole positive. However, 13 (4.3%) isolates were positive for O157 antiserum and but negative for H7 antigen in the latex agglutination test. All 13 isolates were shown to be positive for *rfb*O157 but negative for *fliC*H7 using PCR (Figure). Overall, 13 (4.3%) of the

samples yielded *E. coli* O157: 3/50 (6%) liver, 3/50 (6%) chicken carcass, and 7/50 (14%) chicken fecal samples, respectively. The positive isolates were obtained from different poultry materials.

In terms of virulence genes, only intimin (*eaeA* gene) was detected in 10 (77%) out of the 13 *E. coli* O157 isolates and other virulence genes were not detected by mPCR (Figure).



**Figure.** The PCR assay for the detection of *E. coli* O157 isolates and their virulence factors.

M: Marker (GeneRuler 50 bp DNA Ladder Plus, Fermentas, Vienna, Austria) P1: Positive control for *rfb*O157 (420 bp, *E. coli* O157 NCTC 12900), P2: Positive control for *fliC*H7 (625 bp, *E. coli* O157 NCTC 12900), P3: Positive control for virulence factors (*E. coli* RHFS 232; for *stx*<sub>2</sub>, 484 bp, for *eaeA*, 397 bp, for *stx*<sub>1</sub>, 210 bp, for *hlyA*, 166 bp), N: Negative control (H<sub>2</sub>O), 1, 2, 3: *rfb*O157 positive isolates (420 bp), line 4–8: Intimin (*eaeA*) gene positive isolates.

In the antibiotic susceptibility testing, all isolates displayed resistance to at least one antibiotic. All isolates of *E. coli* O157 were susceptible to gentamicin (100%), whereas 10 (77%), 7 (54%), 7 (54%), 7 (54%), 5 (38%), 4 (31%), and 4 (31%) of 13 isolates were resistant to erythromycin, amoxicillin clavulanic acid, ampicillin, cefazolin, tetracycline, enrofloxacin, and neomycin, respectively (Table 2). The multidrug resistance profiles of the isolates were determined in this study. All of the isolates were resistant to three or more antibacterial agents (Table 3). As indicated in Table 3, 8 (62%) out of the 13 isolates exhibited multidrug resistance. Resistance to three antimicrobials (AMP, AMC, KZ/AMP, AMC, E) for *E. coli* O157 was found at a rate of 15.3% (2/13) and 7.7% (1/13) in liver samples, respectively. *E. coli* O157 strains were resistant to four antimicrobials (TE, AMP, AMC, KZ) at the rate of 7.7% (1/13) in fecal samples. Resistance to five antimicrobials, namely TE, N, E, KZ, and ENR, was detected at a rate of 7.7% (1/13) in carcass samples. Moreover, resistance to seven antimicrobials (TE, AMC,

N, AMP, E, KZ, ENR) was 23% (3/13) in carcass and fecal samples.

**4. Discussion**

Poultry meat is widely consumed because of its flavor and nutritious characteristics. The contamination of poultry meat by foodborne pathogens such as *E. coli* O157:H7 can occur along the food chain and but may harbor significant *E. coli* O157:H7 (13).

In the present study, 13 (4.3%) isolates were positive for *E. coli* O157 including 3 (6%) chicken carcass, 3 (6%) liver, and 7 (14%) fecal samples, respectively. A lower result was reported in a previous study by Akbar et al. (8), who isolated *E. coli* O157 from 2% of poultry meat samples in Thailand. However, in studies conducted by Jo et al. (14) in Korea and Abay et al. (15) in Turkey, none of the *E. coli* O157 isolates were positive in chicken meat samples. Similar results (4% and 10.3%) were reported by Abdul-Raouf et al. (16) from Egypt and Chinen et al. (17) from Argentina, respectively.

**Table 2.** The antibiotic susceptibility of *E.coli* O157 isolates.

Antibiotics	Diameter of the inhibition zones of <i>E. coli</i> according to CLSI (mm)			Number of isolates (n = 13)		
	Susceptible	Intermediate	Resistant	Susceptible (%)	Intermediate (%)	Resistant (%)
Erythromycin	≥23	14–22	≤13	-	3 (23%)	10 (77%)
Amoxicillin clavulanic acid	≥18	14–17	≤13	1 (8%)	5 (38%)	7 (54%)
Ampicillin	≥17	14–16	≤13	6 (46%)	-	7 (54%)
Enrofloxacin	≥23	17–22	≤16	6 (46%)	3 (23%)	4 (31%)
Cefazolin	≥18	15–17	≤14	2 (15%)	4 (31%)	7(54%)
Gentamicin	≥16	13–15	≤12	13 (100%)	-	-
Neomycin	≥15	13–14	≤12	3 (23%)	6 (46%)	4 (31%)
Tetracycline	≥19	15–18	≤14	8 (62%)	-	5 (38%)

**Table 3.** Multidrug resistances profiles of *E. coli* O157 isolates.

Number of antibiotics	Antibiotic profiles	Number of isolates	Origin of isolates
3	AMP, AMC, KZ	2	Liver
3	AMP, AMC, E	1	Liver
4	TE, AMP, AMC, KZ	1	Feces
5	TE, N, E, KZ, ENR	1	Carcass
7	TE, AMC, N, AMP, E, KZ, ENR	3	Carcass, feces

AMP: Ampicillin, AMC: Amoxicillin/clavulanic acid, KZ: Cefazolin, E: Erythromycin, TE: Tetracycline, N: Neomycin, ENR: Enrofloxacin

In the present study, *E. coli* O157 was detected in 3 (6%) of chicken liver samples in Kayseri, Turkey. Alonso et al. (18) in Argentina found *E. coli* O157:H7 in chicken giblets at rates of 2%. Higher results were reported in Egypt by Darwish et al. (19), who detected *E. coli* O157:H7 in 16% of giblets samples.

In Costa Rica, Arias et al. (20) reported the survival and growth capability of *E. coli* O157 in chicken giblets stored at 0, 6, and 12 °C and stated that low rates of contamination of *E. coli* O157 found in poultry giblets may be hazardous for public health.

In our study, *E. coli* O157 was detected of 7 (14%) chicken fecal samples. Tabatabaei et al. (21) found shiga toxicogenic *E. coli* (STEC) in 14 (4%) of 350 broiler fecal samples from Iran, while Jo et al. (14) and Abay et al. (15) found no *E. coli* O157 in any fecal samples. The results of the present study demonstrated that poultry processing plants might be a reservoir of *E. coli* O157. During the slaughter of poultry, fecal contamination of the carcass and giblets is due to the evisceration process, implying spread of the bacteria present in intestines as contaminants. For this reason, hygiene measures are of utmost importance. The different prevalence ratio of *E. coli* O157 isolation might be due to several factors including age, management practice, season, sampling method, and detection technique (4,18,21,22).

Intimin is required for human and animal intestinal mucosal colonization of enterohemorrhagic *E. coli* (7,22,23). According to our mPCR results, 10 (77%) of the 13 isolates had only the *eaeA* gene (intimin). Comparing our results on the presence of a virulence gene, similar results were found by Kobayashi et al. (22) in Finland, i.e. 29 (15%) out of 199 broiler samples were positive for the *eaeA* gene, and also in Korea by Oh et al. (23), who reported that the *eaeA* gene was found in 30 *E. coli* strains obtained from cloacal samples of 184 chicken flocks.

However, in Turkey, Karadal et al. (24) showed that the *sxt1* gene was defined in one *E. coli* O157 of 100 processed poultry product samples. In another study, conducted by Dipineto et al. (25), *eaeA* and *stx2* genes were detected in all 26 STEC isolated from 720 cloacal samples of layer hens, while Momtaz and Jamshidi (26) found *stx1*, *eaeA*, and *ehlyA* virulence genes in 31 (21.23%) EHEC isolates in Iran. However, according to some other researchers (14,27), the presence of neither *E. coli* O157 isolates nor their virulence genes was found.

This result shows that intimin might be a significant virulence factor for *E. coli* O157 infections because HUS are closely related to the enterohemorrhagic *E. coli*-carrying *eaeA* gene (7,22). Therefore, *E. coli* O157 serotypes-carrying *eaeA* genes might be more pathogenic for humans.

Due to excessive antibiotic use in veterinary medicine, resistance to the most effective antibiotics has emerged. In our study, *E. coli* O157 isolates exhibited resistance to most of the antibiotics (7/8) tested. Dursun and Kaya (13) suggested that *E. coli* O157:H7 isolates from cloacal swabs were resistant to ampicillin (100%), cephalothin (100%), erythromycin (81.25%), and amoxicillin/clavulanic acid (62.5%). Benshaban et al. (28) reported that the highest resistance rate was measured against erythromycin and cephalothin (100%) in chicken liver samples, followed by neomycin (93%), ampicillin (88.7%), sulfamethoxazole/trimethoprim (85.7%), tetracycline (84.96%), doxycycline (78.19%), nitrofurantoin (76.69%), amoxicillin (74.4%), nalidixic acid (71.4%), augmentin (64.66%), and chloramphenicol (58.64%).

Schroeder et al. (29) showed that 27% of *E. coli* O157 isolates were resistant to tetracycline, 26% were resistant to sulfamethoxazole, 17% were resistant to cephalothin, and 13% were resistant to ampicillin. In contrast, Momtaz and Jamshidi (25) and Miles et al. (30) reported high resistance to tetracycline (76.8% and 82.4%, respectively) in terms of *E. coli* isolated from chicken meat.

In another study, conducted by Darwish et al. (19) concerning the antibiotic susceptibilities of *E. coli* serogroups, *E. coli* O86 was highly sensitive to cefotaxime, followed by ciprofloxacin and amoxicillin, whereas *E. coli* O127 was highly susceptible to cefotaxime but resistant to amoxicillin and showed weak sensitivity to other antibiotics tested. In the same study (19), *E. coli* O114 was highly susceptible to danofloxacin and norfloxacin.

In conclusion, the presence of *E. coli* O157 in poultry is significant (4.3%). Our results suggested that poultry might play a role in the epidemiology of human *E. coli* O157 infection and could be a potential source of contamination for people in contact with poultry and chicken carcasses at poultry processing plants. Raw or undercooked poultry-originated foods and foodstuffs may be contaminated through either slaughtering or processing and handling such as cross contamination during processing, and human-to-food contamination via food handlers. A proper heating process is required to be applied to these products before consumption in order to avoid the potential risk for public health. Antimicrobial susceptibility testing of *E. coli* O157 isolates showed a high prevalence of resistance to amoxicillin/clavulanic acid and ampicillin. The multidrug resistant isolates might be jeopardous for public health. It is important to engage in ongoing surveillance and to take some preventive control measures about resistant bacteria, in particular *E. coli* O157, obtained from poultry products. Hence, chicken meat producers have to keep in mind not to use veterinary drugs on their farms.

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