

## Extended-spectrum $\beta$ -lactamases among cloacal *Escherichia coli* isolates in healthy broilers in Turkey

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**Abstract:** The aim of this study was to determine the prevalence and clonal typing of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in healthy broilers in Turkey. Three hundred broiler cloacal samples were collected from various broiler slaughterhouses and inoculated on Levine agar plates supplemented with 2 µg/mL cefotaxime. Suspected strains were identified using a BBL Crystal Enteric/Nonfermenter ID Kit (Becton Dickinson, USA) and ESBL production was confirmed using an ESBL phenotypic confirmatory test. ESBL types were analyzed using PCR and sequencing. Pulsed field gel electrophoresis (PFGE) was performed with XbaI for the clonal typing of ESBL-producing *E. coli* isolates. While 33 phenotypic ESBL-producing *E. coli* isolates were identified, eight of them had only the blaTEM-1. Twenty-five ESBL-producing isolates were detected. This research is the first on the investigation and detection of ESBL-producing *E. coli* isolates from broilers in Turkey. In this study, 8.3% ESBL-producing *E. coli* were isolated from the cloacal samples of broilers collected from slaughterhouses in Turkey. CTX-M-15 (80%) was the most frequently isolated ESBL type. Using PFGE analysis, it was determined that these isolates had clonal similarity.

**Key words:** Antimicrobial resistance, broilers, *Escherichia coli*, extended spectrum beta-lactamase, pulsed-field gel electrophoresis, CTX-M-15

### 1. Introduction

*Escherichia coli* strains are important pathogens in animals and humans, and are normal commensals in intestinal tracts. The  $\beta$ -lactam group of antibiotics has been intensely used in human and veterinary medicine as infectious disease treatments (1). Extended-spectrum beta-lactamases (ESBLs) hydrolyze oxyimino-cephalosporins and give resistance to bacteria against the penicillins, cephalosporins (first, second, and third generations), and aztreonam. They are repressed by  $\beta$ -lactamase inhibitors. ESBLs are classified as  $\beta$ -lactamases containing three main families: TEM (named after the patient Temoneria), SHV (sulfhydryl reagent variable), and CTX-M (active on cefotaxime, first isolated in Munich) (2). The prevalence of CTX-M-type  $\beta$ -lactamase-producing *E. coli* has increased in poultry over the last decade (3).

Increasing prevalence of ESBL-producing *E. coli* in poultry has become a major zoonotic risk because of possible transmission from broilers to humans (3). In

addition, ESBL-producing *E. coli* strains that may have multidrug resistance (MDR) and cause therapeutic problems have been reported in human and veterinary practice (4). The alarming spread of ESBL-producing *Enterobacteriaceae* poses a serious public health threat and has attracted the attention of scientists, politicians, and the general public globally. There was no previous study about the presence, prevalence, and molecular epidemiology of ESBL-producing *E. coli* isolates from broilers in Turkey. The aim of the present study was to investigate the resistance phenotype and genotyping characteristics of ESBL-carrying *E. coli* strains in healthy broilers in Turkey.

### 2. Materials and methods

#### 2.1. Samples, isolations, and identification

From January to April 2012, 300 samples were collected from cloacal samples of healthy slaughterhouse broilers in two regions of Turkey. Broiler cloacal samples were obtained from a total of 30 different poultry houses

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from three integrated firms (two firms in the Aegean region: 200 samples, one firm in the Black Sea region: 100 samples). Swab samples in Cary Blair transport medium was brought to the laboratory under cold chain. Each cloacal swab sample was inoculated on Levine agar plates supplemented with 2 µg/mL cefotaxime (Sigma, USA) and incubated for 24 h at 37 °C. One typical colony showing *E.coli* morphology per plate was selected and identified by conventional methods (Gram staining, morphology, catalase, oxidase, indole, urease, methyl red, Voges-Proskauer, and citrate) (5) and confirmed using a BBL Crystal Enteric/Nonfermenter ID Kit (Becton Dickinson, USA).

**2.2. Antimicrobial susceptibility testing**

Antimicrobial susceptibilities were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck, Germany), and the results were interpreted according to the Clinical Laboratory Standards Institute Guidelines (CLSI) (6). *E. coli* isolates were tested for resistance agents. The 16 antimicrobial agents were ampicillin (AM, 10 µg), amoxicillin/clavulanic acid (AMC, 20 + 10 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX, 30 µg), ceftaxitin (FOX, 30 µg), aztreonam (ATM, 30 µg), imipenem (IPM, 10 µg), gentamicin (GM, 10 µg), tobramycin (NN, 10 µg), amikacin (AN, 30 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), trimethoprim/sulfamethoxazole (SXT, 12.5 + 23.75 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), and chloramphenicol (C, 30 µg). *E. coli* ATCC 25922 was used as an internal quality control strain for antimicrobial susceptibility testing.

**2.3. ESBL phenotypic confirmatory tests**

A ≥5 mm increase in the inhibition zone diameter for either cefotaxime/clavulanic acid or ceftazidime/clavulanic acid tested versus its zone when tested alone was considered as the phenotypic confirmation test for ESBL production (6).

**2.4. Characterization of ESBL genes**

Randomly choosing a colony from each cloacal sample, *bla*<sub>CTX-M</sub> (CTX-M groups 1, 8, 9, and 10), *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>FOX</sub>, *bla*<sub>CMY</sub>, *bla*<sub>SHV</sub>, and *ampC* beta lactamase genes were studied using PCR (7,8). All obtained amplicons were sequenced on both strands, and sequences were compared with those included in the GenBank database and on the website <http://www.lahey.org/Studies/> to identify the beta-lactamase genes.

**2.5. PFGE patterns of *E. coli* isolates producing ESBL**

The clonal relationship between the strains was studied by PFGE, using *XbaI* as a restriction enzyme (9). PFGE patterns were analyzed with BioNumerics software. The results were discussed according to the criteria given by Tenover et al. (10).

**3. Results**

The results demonstrated that decreasing susceptibility for cefotaxime to *E. coli* isolates was detected in 101 of the 300 healthy broiler cloacal samples of our study, representing 33.6% of the total samples. A phenotypic confirmation test for detection of ESBL-producing isolates was carried out and 33 (11%) of all samples were determined to produce ESBL. Thirty-three phenotypic ESBL-producing *E. coli* isolates were further analyzed to examine the presence of beta-lactamase genes *bla*<sub>CTX-M</sub> (CTX-M groups 1, 8, 9, and 10), *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>FOX</sub>, *bla*<sub>CMY</sub>, *bla*<sub>SHV</sub>, and *ampC* beta-lactamases and sequencing. Eight of the 33 *E. coli* isolates were designated as only harboring the *bla*<sub>TEM-1</sub> gene. However, the *bla*<sub>CMY</sub> gene was not detected by PCR in these isolates. TEM-1, SHV-1, and OXA-type β-lactamases have been frequently described as broad-spectrum β-lactamases. It was observed that 25 isolates had ESBL genes. The 25 ESBL-producing isolates were detected as 23 CTX-M (92%) (20 CTX-M-15, 1 CTX-M-1, 1 CTX-M-9, 1 CTX-M-16), 4 OXA-1 (16%), and 2 SHV-12 (8%) (Table 1).

**Table 1.** ESBL genes of *E. coli* in this study.

Detected ESBL gene(s)	Number of <i>E. coli</i> isolates (%)
CTX-M-15, OXA-1	4 (16)
CTX-M-15	6 (24)
TEM-1, CTX-M-15	10 (40)
TEM-1, SHV-12	1(4)
SHV-12	1 (4)
TEM-1, CTX-M-1	1 (4)
TEM-1, CTX-M-16	1 (4)
TEM-1, CTX-M-9	1 (4)

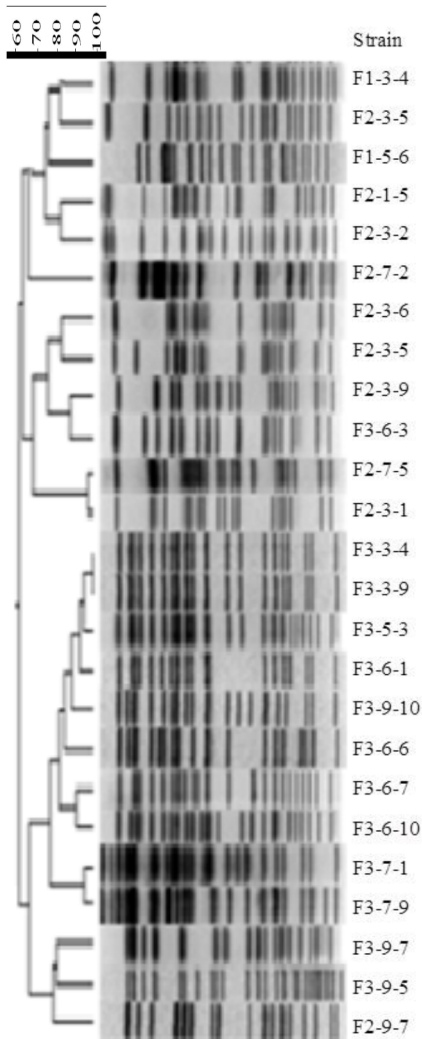
The 25 ESBL-producing *E. coli* isolates were tested against 16 antimicrobial agents using the disk diffusion method. All isolates were determined to be resistant against multiple antibiotics (at least six antibiotic-resistant). The prevalence of antibiotic resistance of ESBL-positive *E. coli* isolates to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, aztreonam, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, tetracycline, and chloramphenicol was 100% (n = 25), 56% (14), 5% (13), 80% (20), 40% (10), 76% (19), 4% (1), 56% (14), 96% (24), 92% (23), 48% (12), 88% (22), 80% (20), and 80% (20), respectively. All of the ESBL *E. coli* isolates were susceptible to ceftazidime and imipenem.

According to a similarity rate of 85% or over, 25 isolates was divided into two main groups, namely A and B, by PFGE analysis. Main group A contained A1 (6 isolates) and A2 (6 isolates), while main group B involved B1 (3 isolates) and B2 (10 isolates) (Figure; Table 1). The PFGE analysis illustrating the distribution of ESBL-producing *E. coli* is presented in Table 2.

**4. Discussion**

This subject has gained importance due to the idiopathic increase in ESBL-producing *E. coli* prevalence among healthy farm animals, especially in poultry. ESBL-producing *E. coli* of poultry origin is a zoonotic risk factor for humans, as it could be directly or indirectly transmitted to humans and the environment by poultry breeding and products.

It has been reported that the prevalence of ESBL-producing *E. coli* in healthy broilers was 3.6% in Britain (11), 10.7% in France (12), 38.2% in Portugal (13), 45% in Belgium (1), and 60.8% in China (14). There has been no research on antibiotic resistance phenotypes and genotyping characteristics of ESBL-carrying *E. coli* strains in healthy broilers in Turkey. In the present study, which was the first report in Turkey (25/300) about ESBL-producing *E. coli* prevalence among healthy broilers, it was detected as 8.3%. Differences in prevalence of ESBL-producing *E. coli* among various countries could result from antimicrobial use policies relating to poultry. In addition, the results reported by Dierikx et al. (15) demonstrated that the prevalence of ESBL-producing *E. coli* in the Netherlands varied between 0% and 100% in the poultry production pyramid during all stages: 0%–24% in 2-day-old chicks, 96%–100% in 18-week-old pullets, and 100% in 31-week-old hens. According to the results of that study (15), there was no correlation between the increasing prevalence of ESBL-producing *E. coli* and the use of antimicrobial agents in poultry; this situation could occur by vertical–horizontal transmission.



**Figure.** Dendrogram from PFGE results.

ESBLs hydrolyze oxyimino-cephalosporins as cefotaxime and ceftazidime (16). ESBL type CTX-M group are the most common currently, although SHV or TEM were common in the past (2). The genes coding CTX-M group  $\beta$ -lactamase are harbored by plasmids as IS *EcpI*. After determining the presence and transmitting by conjugation of ESBL type CTX-M groups in healthy food-producing animals, this issue has become increasingly important (12,17).

The first ESBL-producing *E. coli* (CTX-M-14, SHV-12, and CMY-2) in poultry were isolated from healthy chick feces in Spain in 2000 and 2001 (7). Recently published literature reported that the most common ESBL-type *E. coli* in healthy poultry were CTX-M-1 in France (12) and Britain (11); TEM-1, SHV-12, CTX-M-32, and CTX-M-1 in Italy (18); and CTX-M groups (CTX-M-14, CTX-M-24,

**Table 2.** Phenotypic and genotypic profiles of ESBL-producing *E. coli* isolates.

Isolates	Beta-lactamase genes	PFGE	Antibiotic resistance
F1-8**-4	CTX-M-15, OXA-1	A1	AM, AMC, CTX, ATM, GM, NN, NA, CIP, SXT, TE
F1-8-6	CTX-M-15, OXA-1	A1	AM, AMC, CAZ, CTX, ATM, GM, NN, S, NA, CIP, SXT, TE
F2-1-8	TEM-1, CTX-M-15	A1	AM, AMC, CAZ, CTX, S, NA, C
F2-3-1	CTX-M-15	A2	AM, CTX, GM, NN, S, NA, CIP, SXT, TE, C
F2-3-2	TEM-1, CTX-M-15	A1	AM, CTX, CAZ, ATM, GM, NN, S, NA, CIP, SXT, TE, C
F2-3-5	CTX-M-15	A1	AM, CAZ, CTX, GM, NN, S, NA, CIP, SXT, TE, C
F2-3-6	CTX-M-15	A2	AM, AMC, CTX, GM, NN, S, NA, CIP, SXT, TE, C
F2-3-8	CTX-M-15	A2	AM, CTX, GM, NN, S, NA, SXT, TE, C
F2-3-9	CTX-M-15	A2	AM, AMC, CTX, GM, NN, S, NA, CIP, SXT, TE, C
F2-7-2	TEM-1, SHV-12	A1	AM, CAZ, CTX, ATM, AN, S, NA, SXT, C
F2-7-5	TEM-1, CTX-M-1	A2	AM, AMC, CAZ, S, SXT, TE
F2-9-7	TEM-1, CTX-M-15	B2	AM, AMC, CAZ, CTX, ATM, S, NA, CIP, SXT, TE, C
F3-3-4	CTX-M-15, OXA-1	B1	AM, AMC, CAZ, CTX, ATM, GM, NN, S, NA, CIP, SXT, TE
F3-3-9	CTX-M-15, OXA-1	B1	AM, AMC, CAZ, CTX, ATM, GM, NN, S, NA, CIP, SXT, TE
F3-5-3	TEM-1, CTX-M-16	B1	AM, S, NA, CIP, SXT, TE, C
F3-6-1	TEM-1, CTX-M-15	B1	AM, CAZ, CTX, GM, S, NA, SXT, TE, C
F3-6-3	TEM-1, CTX-M-15	A2	AM, CAZ, GM, NN, S, NA, SXT, C
F3-6-6	TEM-1, CTX-M-15	B1	AM, AMC, CTX, GM, S, NA, SXT, TE, C
F3-6-7	CTXM-15	B1	AM, AMC, CAZ, CTX, ATM, GM, NN, S, NA, SXT, TE, C
F3-6-10	TEM-1, CTX-M-15	B1	AM, AMC, CTX, GM, S, NA, SXT, TE, C
F3-7-1	SHV-12	B1	AM, CAZ, ATM, GM, NN, S, TE, C
F3-7-9	TEM-1, CTX-M-9	B1	AM, NN, S, NA, CIP, SXT, TE, C
F3-9-7	TEM-1, CTX-M-15	B2	AM, AMC, CTX, ATM, GM, S, NA, SXT, C
F3-9-8	TEM-1, CTX-M-15	B2	AM, AMC, CTX, GM, S, NA, STX, TE, C
F3-9-10	TEM-1, CTX-M-15	B1	AM, CTX, GM, S, NA, C

\*F: Farm, \*\*: poultry houses, AM: ampicillin, AMC: amoxicillin/clavulanic acid, CAZ: cefotaxime, CTX: ceftazidime, ATM: aztreonam, GM: gentamicin, AN: amikacin, NN: tobramycin, S: streptomycin, NA: nalidixic acid, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethoxazole, TE: tetracycline, C: chloramphenicol.

CTX-M-65) and TEM in China (14). In other studies, however, CTX-M-15 type ESBLs were reported as the most common: Panpan et al. (19) identified that the most common type of ESBL in China was the CTX-M-15 gene, while Maciucă et al. (20) conducted a study in poultry and people in Romania, reporting that the most common type in clinical isolates was also the CTX-M-15 gene. Results from the present study, indicating that the highest prevalence for ESBL types was the CTX-M-15 gene, were consistent with the newly published research (19,20). This result was also similar to findings in the study on human medicine by Gür et al. (21).

In conclusion, from broiler cloacal samples collected from slaughterhouses in Turkey, 8.3% ESBL-producing *E. coli* were isolated. These isolates had multiple antibiotic resistance. CTX-M-15 (80%) was the most frequently isolated ESBL type. Twenty-five isolates were divided into two main groups, namely A and B, by PFGE analysis. It was determined that these isolates had clonal similarity by PFGE analysis.

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