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Disinfectant and heavy metal resistance profiles in extended spectrum β-lactamase (ESBL) producing *Escherichia coli* isolates from chicken meat samples

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ABSTRACT

Biocidal compounds are frequently used as disinfectants in poultry industry and their widespread usage has risen concern due to the co-selection and persistence of antimicrobial resistance among bacteria. In this study, extended spectrum β-lactamase producing (ESBL) *Escherichia coli* isolates (n = 60) obtained from chicken meat were characterized by Pulsed Field Gel Electrophoresis (PFGE) and further tested for disinfectant and heavy metal resistance phenotypically and genotypically. Plasmid replicon types of these isolates were also determined. ESBL producing *E. coli* isolates were found to be resistant to ciprofloxacin (48.3 %) and gentamicin (15 %). The majority of these isolates (46.5 %) carried $bla_{\text{CTX-M-55}}$ gene. The isolates showed higher minimal inhibitory concentrations to cetylpyridinium chloride (90 %), cetyltrimethylammonium bromide (50 %), hexadecyltrimethylammonium bromide (46.7 %), triclosan (38.3 %), benzalkonium chloride (28.3 %), chlorhexidine (21.7 %), acriflavine (3.3 %), benzethonium chloride (1.7 %) and N-alkyl dimethyl benzyl ammonium chloride (1.7 %), but 18.3 % of the isolates were resistant to triclosan. Of the quaternary ammonium compounds (QACs) tolerance genes, *mdfA*, *sugE*(*c*), *ydgE* and *ydgF* were most present in all isolates, but the *qacE*, *qacG*, *oqxA* and *oqxB* genes were not detected. Of genes mediating the heavy metal resistance, the *zitB* gene was detected in all isolates, whereas the *copA* and *cueO* genes were detected in 96.67 % and 95 % of isolates, respectively. The IncFIB plasmid was commonly present (93.3 %) in ESBL producing *E. coli* isolates. Consequently, given the detection of genes mediating disinfectant and heavy metal resistance commonly in ESBL producing *E. coli* isolates as well as high rate of MICs against disinfectant compounds, the use of QACs for decontamination of the facilities may not be as effective as expected in poultry sector in Turkey.

1. Introduction

In recent years, the spread of extended spectrum β-lactamase (ESBL) producing *E. coli* has been recognized as one of the major public health issues worldwide. ESBL producing *E. coli* has been isolated from patients with a variety of infections, particularly from community-acquired urinary tract infections (Den Heijer et al., 2010). ESBL producing *E. coli* has also been isolated from a variety of foods including raw chicken meat for human consumption (Kaesbohrer et al., 2019; Kürekci et al., 2019; Rao et al., 2014). Therefore, human exposure to ESBL producing *E. coli* or their resistance genes might occur via the food chain, direct contact and environmental sources (Ewers et al., 2012; Overdevest et al., 2011). The ESBLs are a group of enzymes that have the ability to hydrolyse broad spectrum cephalosporins (such as cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime). The majority of ESBLs belong to Ambler class A and include the SHV or TEM and CTX-M types (Peirano and Pitout, 2019). Various studies have reported that *E. coli* strains containing CTX-M type enzymes are common in human, poultry and chicken meat samples (Daehre et al., 2018; Kürekci et al., 2019; Leverstein van Hall et al., 2011; Overdevest et al., 2011).

Biocides are being utilized with an increasing rate to control the growth of microorganisms on animal husbandry, slaughterhouses and food processing facilities (Long et al., 2016). Disinfectants as biocides, consist of specific formulations containing one or more active ingredients. For this purpose, quaternary ammonium compounds (QACs), benzalkonium chloride (BKC), aldehydes, chlorine and chlorine-based

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derivatives, organic acids, hydrogen peroxide, biguanides (chlorhexidine-CHX), bisphenols (triclosan-TCL), alcohol, isopropyl alcohol and cresols are widely used to inhibit microbial growth (Donaghy et al., 2019; SCENIHR, 2009). QACs are used commonly in both clinical and industrial areas owing to their low toxicity, high antimicrobial activity, lack of corrosivity and irritability (Buffet-Bataillon et al., 2012a). Furthermore, some heavy metal [copper (Cu), zinc (Zn) and silver] compounds are used as antibacterial agents in animal husbandry (McDonnell and Russell, 1999; Silver, 2003). The biocide market size was reported to be approximately 10–11 billion € in the European Union with market growth rate of 4–5 % per annum (Pan Europa, 2012) and biocide usage rate was noted to be rising exponentially to protect public health during the New Coronavirus Disease 2019 (Covid-19) pandemic. As a matter of fact, at least 2000 tons of disinfectant were announced to be used until March 2020 in the city of Wuhan alone (Chen et al., 2021).

Development of antimicrobial resistance is one of the major threats to public health of the 21st century, making it difficult for the treatment and prevention of infectious diseases worldwide (Meade et al., 2021). A concern has been raised that continued exposure to biocides in industrial settings, including food production environments, may trigger or enhance both biocide and antimicrobial resistance and select for antimicrobial resistant strains (Gnanadhas et al., 2013; Roedel et al., 2021). According to the Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) report, biocides are likely to contribute to maintaining selective pressure allowing the presence of mobile genetic elements harboring specific genes involved in the resistance to biocides and antibiotics.

While previous studies focused on the prevalence, antimicrobial resistance and the distribution of resistance genes in foodborne ESBL producing *E. coli* (Ghodousi et al., 2015; Husan and Çadırcı, 2019; Kaesbohrer et al., 2019; Kürekci et al., 2016; Kürekci et al., 2019; Odenthal et al., 2016; Pehlivanlar Önen et al., 2015; Uyanik et al., 2021), limited number of studies are available about disinfectant and heavy metal resistance for foodborne ESBL producing *E. coli* in chicken meat (Deus et al., 2017; Zou et al., 2014). To the best of our knowledge, no data have been reported on the disinfectant and heavy metal resistance, and molecular mechanisms underlying these resistances in ESBL producing *E. coli* isolates in Turkey. Among 60 raw chicken meat samples, *E. coli* isolates ($n = 60$) were detected and they were confirmed as ESBL producers by phenotypic tests. We characterized these isolates for their disinfectant and heavy metal susceptibility profile as well as resistance genes mediating disinfectant and heavy metal. We also evaluated antimicrobial susceptibility profiles and plasmid replicon types of the isolates.

2. Materials and methods

2.1. Background of ESBL *E. coli* isolates

A total of 60 ESBL producing *E. coli* isolates from raw chicken meat samples were included in the study. These were collected during a period of eight months (December 2018 to July 2019) from the retail supermarkets and butchers from seven different companies in Sivas province, Turkey. All ESBL producing *E. coli* strains were isolated as previously described protocols (Kürekci et al., 2019). Pulsed Field Gel Electrophoresis (PFGE) procedure was performed in Public Health Institution of Turkey (Ankara) as described previously with *Xba*I restriction of DNA (Durmaz et al., 2009). In addition, the phylogenetic analyses (A, B1, B2, C, D, E and F) of isolates were carried out by multiplex PCR (Clermont et al., 2013).

2.2. Molecular and phenotypic resistance profiles

In this study, antibiotic susceptibility of ESBL producing *E. coli* isolates was determined by broth microdilution using the Sensititre™ system with EUVSEC plates (ESB1F; Thermo Fisher Scientific, England, UK) in concordance with the decision 2013/652/EU of the European Union and according to manufacturer's guidelines. The following antimicrobial substances were used: Ceftazidime, TAZ; Cefazolin, FAZ; Cefepime, FEP; Cefoxitin, FOX; Cephalothin, CEP; Cefpodoxime, POD; Cefotaxime, FOT; Ceftriaxone, AXO; Imipenem, IMI; Meropenem, MERO; Gentamicin, GEN; Ampicillin, AMP; Ciprofloxacin, CIP; Piperacillin/tazobactam constant 4, P/T4; Ceftazidime/Clavulanic acid, T/C and Cefotaxime/Clavulanic acid, F/C. Results were interpreted according to the Clinical and Laboratory Standards Institute criteria (CLSI, 2018). The reference strains of ESBL producing *E. coli* NCTC 14477 and ESBL producing *Klebsiella pneumoniae* NCTC 13440 were used as positive control and *E. coli* ATCC 25922 was used as negative control in MIC test in order to compare the obtained results. The antibiotic MIC profiles of the ESBL producing *E. coli* isolates were evaluated as susceptible, or resistant (CLSI, 2018). Isolates detected intermediate on MIC results were regarded as susceptible in this study.

To determine whether ESBL producing *E. coli* isolates harboured β-lactamase genes of the bla_{TEM}, bla_{OXA}, bla_{SHV} and $bla_{\mathrm{CTX\text{-}M}}$, polymerase chain reaction (PCR) assay as described previously were performed (Ahmed et al., 2007). PCR products were sequenced (Sanger sequencing, Macrogen, Korea) and the sequencing data were analyzed with BLAST software and compared to those submitted to the GenBank (http://blast.ncbi.nlm.nih.gov/).

2.3. Determination of minimum inhibitory concentrations (MICs) of disinfectants and heavy metals

The MICs of disinfectants and heavy metals for ESBL producing *E. coli* isolates were determined using the broth microdilution method as recommended by the CLSI (2018). The disinfectants and heavy metals used were QACs N-alkyl dimethyl benzyl ammonium chloride (ADBAC, Merck 814363, Germany), alkyl diaminoethyl glycin hydrochloride (ADH, Sigma Aldrich G2879, USA), benzalkonium chloride (BKC, Sigma Aldrich 12060), benzethonium chloride (BEN, Sigma Aldrich B8879, USA), cetylpyridinium chloride (CTPC, Sigma Aldrich C0732, USA), cetyltrimethylammonium bromide (CTAB, Sigma Aldrich 219374, USA), hexadecyltrimethylammonium bromide (HAB, Sigma Aldrich H5882, USA) and the guanidine compound chlorhexidine (CHX, Sigma Aldrich 282227, USA), the acridine compound acriflavine (ACR, Sigma Aldrich A8126, USA), bis-biguanide compound triclosan (TCL, Sigma Aldrich 93453, USA) the heavy metal salts copper sulfate (COP, Sigma Aldrich 61230,USA), silver nitrate (SIL, Merck 101510, Germany) and zinc chloride (ZKC, Merck 108816, Germany).

The range of concentrations used to define the MICs of the disinfectants and heavy metals were determined based on the previously used reference values (Deus et al., 2017; Zou et al., 2014). Bacterial suspensions were prepared by selecting three to five well-separated overnight colonies from Tryptone Soya Agar (TSA, Oxoid CM0131, UK) and transferred into a tube containing 3 mL of sterile saline solution 0.9 %. The suspension was adjusted to the turbidity standard of 0.5 McFarland and diluted 1:200 with Mueller-Hinton broth (MHB, Oxoid CM0405, UK). In 96-well microtiter plates (LP Italiana, Italy), 20 μL of the biocide solution was added to 180 μL of the bacterial suspension. The final inoculum was adjusted to 5.0×10^5 cfu/mL in wells. The plates were incubated for 16 to 20 h at 35 °C. Optical density of wells at 600 nm (OD_{600}) was measured Multiscan FC Microplate Photometer (Thermo Scientific, Canada). Bacterial growth was compared to a negative control (microtiter well containing disinfectant solution and Mueller-Hinton broth). The MIC was defined as the lowest concentration of a disinfectants and heavy metals that completely inhibited bacterial growth in microtiter plates. The reference strains including ESBL *E. coli* NCTC 14477 and ESBL *K. pneumoniae* NCTC 13440; *E. coli* ATCC 25922 and *S. aureus* NCTC 10788 were used as internal quality control in MIC tests. MIC range of isolates against disinfectant and heavy metal compounds were compared with reference strains (Table 2).

2.4. Detection of disinfectant and heavy metals resistance genes

All ESBL producing *E. coli* isolates were examined for the presence of disinfectant and heavy metal resistance genes [*qacEΔ1*, *qacE*, *qacF/H/I*, *qacG*, *emrE*, *mdfA*, *sugE*(*c*), *sugE*(*p*), *ydgE*, *ydgF*, *oqxA*, *oqxB*, *copA*, *cueO*, *cusC*, *pcoA*, *zntA* and *zitB*] as previously described (Deus et al., 2017; Zou et al., 2014). Primers used for the amplification of genes and amplicon sizes are listed in Supplementary Table S1. PCR amplification was performed in a 50 μL reaction mixture with the use of Taq DNA polymerase under the following thermocycling conditions: 1 cycle at 95 °C for 5 min, 30 cycles of denaturation at 96 °C for 60 s, primer annealing at 50–60 °C for 25–35 s, extension at 72 °C for 25–35 s, and a final extension step at 72 °C for 10 min. The amplified PCR products were analyzed on 1.5 % (w/v) agarose gels.

2.5. Plasmid based replicon typing

In this study, all isolates were examined for the presence of plasmid replicons of different types (n = 30; HI1, HI2, HIB-M, FIB-M, I1α, I1γ, I2, M, L, K, B/O, A/C, N, N2, W, P1, T, U, R, X1, X2, X3, X4, FIA, FIB, FII, FIIS, FIIk, FIB KN and FIB KQ), using the multiplex PCR based replicon typing kit (Diatheva MBK0078 PBRT 2.0 Kit, Hungary) according to the manufacturer's directions.

3. Results

3.1. Molecular characterization of ESBL producing E. coli

PCR based phylogenetic analysis of the ESBL producing *E. coli* isolates (n = 60) revealed the presence of six different phylogroups including B1 (31.7 %; n = 19), E (26.7 %; n = 16), B2 (16.7 %; n = 10), C (11.7 %; n = 7), A (8.3 %; n = 5) and F (5.0 %; n = 3). None of the isolates was found to be the phylogroup of D. Additionally, PFGE analysis generated 58 distinct restriction profiles based on the 85 % similarity, except two clonally related isolates (CU18/008 and CU19/052) (Fig. 1).

Based on the antimicrobial susceptibility testing, 48.3 % of the isolates (n = 29/60) were found to have multi drug resistance pattern (MDR), which defined as displaying resistance to at least two and more antimicrobial classes. As expected, all *E. coli* isolates were resistant to ampicillin, cefazolin, cephalothin and cefpodoxime. However, the resistance rate for ceftriaxone, cefotaxime, ceftazidime, cefoxitin and cefepime were 96.7 %, 95 %, 35 %, 11.7 % and 10 %, respectively. The isolates also showed 48.3 % resistance to ciprofloxacin and 15 % to gentamicin. However, none of the isolates were found to be resistant to imipenem, meropenem and piperacillin/tazobactam (Fig. 2).

In this study, PCR analysis showed the presence of the $bla_{\text{CTX-M}}$ gene among 43 isolates (71.7 %). In addition, 35 % of the isolates had the bla_{TEM} (n = 21) gene and 8.3 % had the bla_{SHV} (n = 5) gene. However, none of the isolates was positive for the bla_{OXA} gene. Sequence analyses revealed the presence of four CTX-M subtypes including CTX-M-55 accounting for 46.5 % (n = 20) of isolates, followed by CTX-M-15 (n = 10; 23.3 %), CTX-M-1 (n = 10; 23.3 %) and CTX-M-3 (n = 3; 6.9 %). In addition, 21 *E. coli* isolates harboured the $bla_{\text{TEM-1}}$ gene, whereas 5 possessed the $bla_{\text{SHV-12}}$ gene.

3.2. MICs of disinfectants and heavy metals in ESBL producing E. coli isolates

The MIC values for different disinfectants and heavy metal compounds were given in Table 1. The MICs ranges of the QACs ADBAC, ADH, BKC, BEN, CTPC, CTAB and HAB were 8–32, >32, 8–32, 16–64, 16–256, 32–128, and 32–256 μg/mL, respectively. The MICs of biguanide compound CHX were between 0.5 and 16 μg/mL, while the MICs for the acridine compound ACR was 16 to 64 μg/mL and for the cationic bis-biguanide TCL ≤ 0.0625 to 16 μg/mL (Table 1).

Because there is no accepted interpretive criteria defined for biocides based on their MIC values, it might be valuable to compare obtained results with those obtained from the reference strain of *E. coli* ATCC 25922. Hence, it can be said that most of the ESBL producing *E. coli* isolates showed higher level of tolerance to all QACs (ADBAC, BKC, BEN, CTPC, HAB and CTAB) except for ADH in the current study. Additionally, the isolates showed higher level of tolerance to CTPC (90 %), CTAB (50 %), HAB (46.7 %), TCL (38.3 %), BKC (28.3 %), CHX (21.7 %), ACR (3.3 %), BEN (1.7 %) and ADBAC (1.7 %) (Table 1).

Results also revealed that the MIC ranges of COP, SIL and ZKC were 512–1024 μg/mL, 16–32 μg/mL and 256–512 μg/mL, respectively. Compared with the reference strain of *E. coli* ATCC 25922, similar MICs for COP (98.3 %; n = 59), SIL (85.0 %; n = 51) and ZKC (56.7 %; n = 34) but lower MICs for COP (1.7 %; n = 1), SIL (15.0 %; n = 9) and ZKC (43.3 %; n = 26) were detected in the isolates (Table 1).

3.3. Presence of disinfectant and heavy metal resistance genes in ESBL producing E. coli isolates

In this study, SMR efflux pump genes including *sugE*(*c*) and *ydgE/ydgF*, and the MFS efflux pump gene *mdfA* were most widespread and being found in 100 % of isolates. The *emrE* gene was found in 83.3 % (n = 50) of the isolates, followed by *qacF/H/I* (21.7 %; n = 13), *qacEΔ1* (20.0 %; n = 12) and *sugE*(*p*) (6.7 %; n = 4). However, the *qacE*, *qacG*, *oqxA* and *oqxB* genes were not detected in any of the isolates (Table 3). The top three resistance genotypes were *emrE-mdfA-sugE*(*c*)*-ydgE-ydgF* (51.7 %; n = 31)*; emrE-mdfA-sugE*(*c*)*-ydgE-ydgF-qacF/H/I* (15.0 %; n = 9); *emrE-mdfA-sugE*(*c*)*-qacEΔ1-ydgE-ydgF* (10.0 %; n = 6) and *mdfA-sugE*(*c*)*-ydgE-ydgF* (10.0 %; n = 6) (Fig. 1).

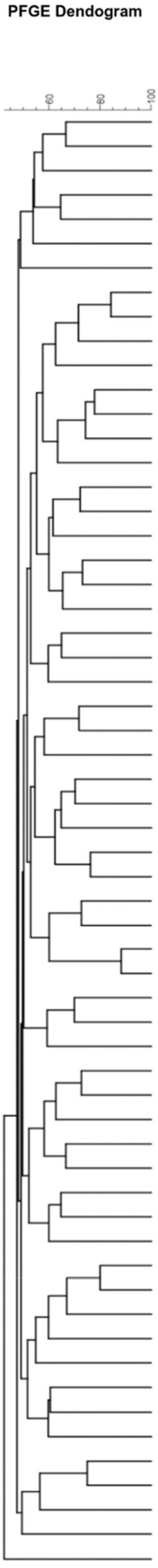
The Zn mediated resistance gene *zitB* was detected in all ESBL *E. coli* isolates, while the other Zn resistance gene *zntA* was found in 41.7 % (n = 25) of isolates. The Cu mediated resistance genes *copA* and *cueO* were commonly found in 96.7 % (n = 58) and 95.0 % (n = 57) of isolates respectively, which were followed by *cusC* (1.67 %; n = 1) and *pcoA* (% 1.67; n = 1). The top three resistance genotypes were *cueO-zitB-copA* (51.7 %; n = 31); *cueO-zitB-copA-zntA* (38.3 %; n = 23); *zitB-copA-zntA* (3.3 %; n = 2) and *cueO-zitB* (3.3 %; n = 2) (Fig. 1).

3.4. Plasmid based replicon typing in ESBL producing E. coli isolates

Among the 30 replicon types examined, ESBL producing *E. coli* isolates harboured a total of 13 different replicon types (I1α, HI2, I2, BO, FIB, FIA, X3, FIIS, A/C, X1, K, X4 and FII (Fig. 1). All the analyzed isolates contained at least one and up to four plasmid replicon types. The most frequently found replicons were FIB (93.3 %; n = 56), FII (78.3 %; n = 47) and I1α (36.7 %; n = 22). Other Inc. groups detected were I2 and BO (10.0 %; n = 6), X3 (8.3 %; n = 5); FIA (6.7; n = 4) HI2 (5.0 %; n = 3), X1 (3.3 %; n = 2), FIIS, A/C and K (1.7 %; n = 1), respectively.

4. Discussion

In the present study, ESBL producing *E. coli* isolates from chicken meat samples were found to be clonally divergent based on PFGE analysis, which had been reported by previous researchers (Alegría et al., 2020; Kürekci et al., 2019). These findings were likely to be attributed to different contamination sources, as it might be caused by the movement of humans, poultry and vectors (Jakobsen et al., 2010; Solà-Ginés et al., 2015). According to the phylogenetic classification by Clermont et al. (2013), the majority of isolates belonged to commensal phylogenetic group of B1 and E, indicating most of the isolates are not pathogenic, whereas ten strains belonged to phylogroup B2, demonstrating extraintestinal pathogenic strains in chicken meats. The occurrence of phylogroup F was reported to be a matter of concern for the spread of β-lactamases in the poultry production chain (Ferraresso et al., 2022), so detection of phylogroup F in three isolates in this study may rise concern for the spread of ESBLs in the poultry production.



Isolate ID	Source	Phylogroup	β-lactam gene variant/s	Plasmid based replicon types	Disinfectant resistance genes												Heavy metal resistance genes					
					emrE	*mdfA*	*sugE(c)*	*qacEΔ1*	*gacG*	*ydgE*	*ydgF*	*qacF/H/I*	*sugE(p)*	*qacE*	*oqxA*	*oqxB*	*cueO*	*pcoA*	*cusC*	*zitB*	*copA*	*zntA*
CU19/051	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	I2	■	■	■			■	■						■			■	■	
CU19/056	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	I1α, FIB, FIA, FII	■	■	■	■		■	■						■			■	■	
CU19/057	Chicken meat	A	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■						■			■	■	
CU19/058	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, X1, FII	■	■	■			■	■						■			■	■	
CU19/059	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$	I1α, FIB, FII		■	■	■		■	■						■			■	■	
CU19/032	Chicken meat	F		FIB, FII	■	■	■			■	■	■					■			■	■	■
CU19/042	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII		■	■			■	■						■			■	■	■
CU19/018	Chicken meat	B2	$\mathit{bla}_{\text{SHV-12}}$	FIB, X3, FII	■	■	■			■	■						■			■	■	■
CU19/030	Chicken meat	B2	$\mathit{bla}_{\text{SHV-12}}$	FIB, X3, FII	■	■	■			■	■						■			■	■	■
CU19/041	Chicken meat	B1	$\mathit{bla}_{\text{CTX-M-55}}$	BO, FIB	■	■	■			■	■						■			■	■	
CU19/060	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	FIB	■	■	■			■	■						■			■	■	
CU18/005	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	I1α, I2, FIB, FII	■	■	■	■		■	■						■			■	■	
CU19/031	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■						■			■	■	
CU18/007	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■						■			■	■	
CU19/025	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$	I1α, FIB, FIA, FII	■	■	■			■	■						■			■	■	
CU18/009	Chicken meat	C		I1α, I2, FIB, FII	■	■	■			■	■						■			■	■	■
CU18/011	Chicken meat	A	$\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FII	■	■	■			■	■						■			■	■	
CU19/033	Chicken meat	E		I1α, FIB, FII	■	■	■			■	■	■								■	■	■
CU19/019	Chicken meat	F		FIB	■	■	■			■	■	■					■			■	■	■
CU19/037	Chicken meat	F		FIB, FII	■	■	■			■	■	■					■			■	■	■
CU18/010	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	I2, FII	■	■	■			■	■		■				■			■	■	
CU19/026	Chicken meat	B1	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII, X4	■	■	■			■	■						■			■	■	
CU18/002	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-15}}$	FIB	■	■	■	■		■	■	■					■			■	■	■
CU19/027	Chicken meat	B1	$\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FII	■	■	■			■	■						■			■	■	
CU19/039	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII		■	■			■	■									■	■	■
CU19/049	Chicken meat	E	$\mathit{bla}_{\text{TEM-1}}$	HI2, BO, FIB, FII		■	■	■		■	■	■	■				■			■	■	
CU19/055	Chicken meat	C	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	I1α, FIB, FII	■	■	■			■	■						■			■	■	
CU18/014	Chicken meat	B2	$\mathit{bla}_{\text{SHV-12}}$	FIB, X3, FII	■	■	■			■	■						■			■	■	■
CU19/016	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	FIB	■	■	■			■	■						■			■	■	■
CU19/024	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-1}}$	FIB, FII	■	■	■			■	■						■			■	■	
CU19/015	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-3}}$	FIB, FII	■	■	■			■	■						■			■	■	■
CU19/020	Chicken meat	E	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-3}}$	FIB, FII	■	■	■			■	■	■					■			■		
CU19/047	Chicken meat	C	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII		■	■	■		■	■	■					■			■	■	■
CU18/004	Chicken meat	B1	$\mathit{bla}_{\text{CTX-M-55}}$	I1α, FIB, FII		■	■			■	■						■			■	■	
CU18/008	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII		■	■			■	■						■			■	■	■
CU19/052	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	BO, FIB, K, FII	■	■	■			■	■						■			■	■	
CU18/006	Chicken meat	B2	$\mathit{bla}_{\text{CTX-M-1}}$, $\mathit{bla}_{\text{SHV-12}}$	I1α, FIB, X3	■	■	■			■	■						■			■	■	■
CU19/054	Chicken meat	E		I1α, FIB, FII	■	■	■			■	■						■			■	■	
CU19/046	Chicken meat	B2	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■						■			■	■	■
CU18/001	Chicken meat	A	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	I2, X4	■	■	■	■		■	■						■			■	■	
CU19/029	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	HI2, I2	■	■	■	■		■	■	■								■	■	
CU18/013	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	I1α, FIB, FII	■	■	■			■	■	■					■			■	■	
CU18/012	Chicken meat	C	$\mathit{bla}_{\text{CTX-M-15}}$	HI2, BO, FIB, FII	■	■	■			■	■	■					■			■	■	
CU19/034	Chicken meat	A	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FII	■	■	■			■	■	■					■			■	■	■
CU19/044	Chicken meat	B2	$\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FII	■	■	■			■	■						■			■	■	■
CU19/045	Chicken meat	B2		I1α, FIB, FII	■	■	■			■	■		■				■			■	■	■
CU19/023	Chicken meat	B2	$\mathit{bla}_{\text{SHV-12}}$	FIB, X3, FII	■	■	■			■	■						■			■	■	■
CU19/035	Chicken meat	C	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	FIB, FII	■	■	■			■	■						■			■	■	
CU19/050	Chicken meat	E		BO, FIB, AC, FII		■	■	■		■	■		■				■			■	■	
CU19/043	Chicken meat	E	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	FIB, X1, FII	■	■	■	■		■	■						■			■	■	■
CU19/017	Chicken meat	B1		I1α, FIB		■	■			■	■						■			■	■	
CU19/038	Chicken meat	B1	$\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FII	■	■	■			■	■						■			■		
CU19/048	Chicken meat	C	$\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FIA	■	■	■			■	■						■			■	■	
CU19/053	Chicken meat	B1	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■	■					■			■	■	
CU18/003	Chicken meat	B1		I1α, FIB, FIA		■	■			■	■						■			■	■	
CU19/022	Chicken meat	B2	$\mathit{bla}_{\text{CTX-M-15}}$	FIB, FII	■	■	■			■	■						■			■	■	■
CU19/036	Chicken meat	B2	$\mathit{bla}_{\text{CTX-M-55}}$	FIB, FII	■	■	■			■	■						■			■	■	■
CU19/021	Chicken meat	E	$\mathit{bla}_{\text{CTX-M-3}}$	FIB, FII	■	■	■	■		■	■						■			■	■	■
CU19/028	Chicken meat	A	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-15}}$	BO, FIB	■	■	■	■		■	■						■	■	■	■	■	
CU19/040	Chicken meat	C	$\mathit{bla}_{\text{TEM-1}}$, $\mathit{bla}_{\text{CTX-M-1}}$	I1α, FIB, FIIS, FII	■	■	■			■	■						■			■	■	

Fig. 1. PFGE analysis of 60 ESBL producing *E. coli* isolates from chicken meats, their genotypic disinfectant and heavy metal resistance as well as the distribution of phylogroup, *bla* gene variants and plasmid replicon types. Isolates (CU18/008 and CU19/052) are clonally related.

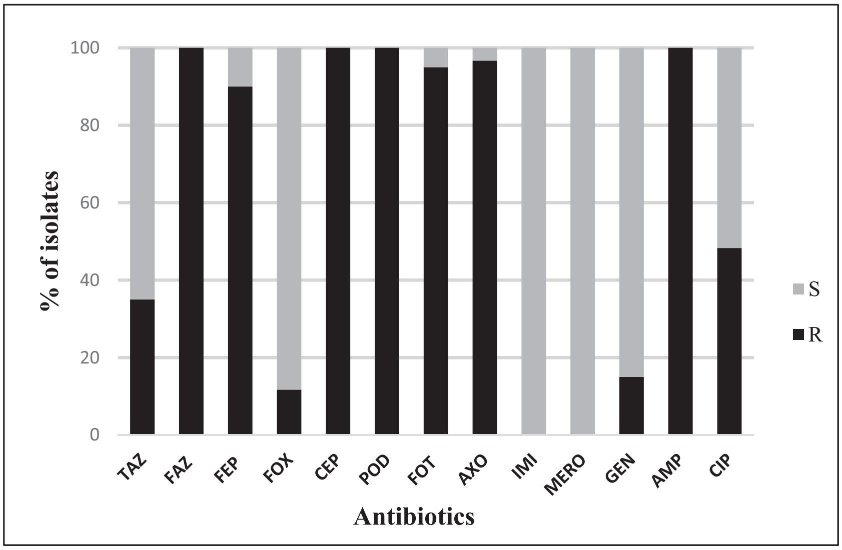


Fig. 2. Antimicrobial susceptibility profile of 60 ESBL producing *E. coli* isolates recovered from chicken meat samples. S, susceptible; R, resistant; TAZ, Ceftazidime; FAZ, Cefazolin; FEP, Cefepime; FOX, Cefoxitin; CEP, Cephalothin; POD, Cefpodoxime; FOT, Cefotaxime; AXO, Ceftriaxone; IMI, Imipenem; MERO, Meropenem; GEN, Gentamicin; AMP, Ampicillin; CIP, Ciprofloxacin.

Table 1
Disinfectant and heavy metal MIC profiles for 60 ESBL producing *Escherichia coli* isolates.

Disinfectant and heavy metal	Distribution of MIC value of isolates (μg/mL) n (%)																	
	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192
ADBAC								14 (23.3)	45 (75)	**1** **(1.7)**								
ADH											**60**** **(100)**							
BKC								1 (1,7)	42 (70)	**17** **(28.3)**								
BEN									7 (11.7)	52 (86.7)	**1** **(1.7)**							
CHX				1 (1.7)	17 (28.3)	29 (48.3)	**9** **(15)**	**1** **(1.7)**	**3** **(5)**									
ACR									3 (5)	55 (91.7)	**2** **(3.3)**							
CTPC									2 (3.3)	4 (6.7)	**48** **(80)**	**5** **(8.3)**	**1** **(1.7)**					
HAB										32 (53.3)	**15** **(25)**	**12** **(20)**	**1** **(1.7)**					
CTAB										30 (50)	**19** **(31.7)**	**11** **(18.3)**						
TCL	22 (36.7) (≤0.0625)	9 (15)	5 (8,3)	1 (1.7)	**2** **(3.3)**	**10** **(16.7)**	**5*** **(8.3)**	**5*** **(8.3)**	**1*** **(1.7)**									
COP														1 (1.7)	59 (98.3)			
SIL									9 (15)	51 (85)								
ZKC													26 (43.3)	34 (56.7)				

ADBAC: N-alkyl dimethyl benzyl ammonium chloride, ADH: alkyl diaminoethyl glycin hydrochloride, BKC: benzalkonium chloride, BEN: benzethonium chloride, CHX: chlorhexidine, ACR: acriflavine, CTPC: cetylpyridinium chloride, HAB: hexadecyltrimethylammonium bromide, CTAB: cetyltrimethylammonium bromide, TCL: triclosan, COP: copper sulfate, SIL: silver nitrate, ZKC: zinc chloride. n = number of isolates, gray areas indicate test concentrations. * = the isolates resistant to compounds compared to Morrissey et al. (2014). ** = isolates indicated higher MICs out of dilution range. The isolates indicating higher MICs compared to *E. coli* ATCC 25922 were printed in bold.

The *bla*$_{\text{CTX-M}}$ genes are commonly found in ESBL producing *E. coli* isolated from chicken meats and their subtypes differ according to different geographical regions. For example, previous studies indicated the prevalence of *bla*$_{\text{CTX-M-1}}$ (69 %) in Netherlands (Kluytmans et al., 2013), *bla*$_{\text{CTX-M-15}}$ (53 %) in Romania (Maciuca et al., 2015), *bla*$_{\text{CTX-M-65}}$ (52.2 %) in South Korea (Park et al., 2019), *bla*$_{\text{CTX-M-55}}$ (25.3 %) in Singapore (Guo et al., 2021), *bla*$_{\text{CTX-M-1}}$ (80.6 %) in Germany (Kaesbohrer et al., 2019). In addition, *bla*$_{\text{CTX-M-1}}$ was the dominant type (28.3–44.3 %) reported in previous studies carried out in Turkey (Kürekci et al., 2019; Pehlivanlar Önen et al., 2015). The most striking result to emerge from the current study is that 46.5 % of ESBL producing *E. coli* isolates carried the *bla*$_{\text{CTX-M-55}}$ variant, which highlights the dramatic shift of the ESBL subtype during the recent years in Turkey poultry production chain and needs to be carefully monitored, because previous studies in Turkey reported the much lower prevalence rate (0–9.6 %) of *bla*$_{\text{CTX-M-55}}$ (Kürekci et al., 2019; Pehlivanlar Önen et al., 2015).

Table 2
MIC ranges of ESBL producing *Escherichia coli* isolates and cut-off values of reference strains for common disinfectants and heavy metals compounds.

	MIC range of disinfectant and heavy metals (µg/mL)												
	ADBAC	ADH	BKC	BEN	CHX	ACR	CTPC	HAB	CTAB	TCL	COP	SIL	ZKC
ESBL *E. coli* isolates (n = 60)	8–32	>32	8–32	16–64	0.5–16	16–64	16–256	32–256	32–128	0.0625–16	512–1024	16–32	256–512
E. coli NCTC 14477	16	>32	16	32	4	32	32	64	32	8	1024	16	512
K. pneumonia NCTC 13440	8	>32	16	16	32	128	32	64	64	16	1024	32	256
S. aureus NCTC 10788	2	>32	2	2	1	16	2	4	4	≤0.0625	512	32	256
E. coli ATCC 25922	16	>32	16	32	2	32	32	32	32	0.5	1024	32	512
E. coli[a]	ND	ND	64	ND	64	ND	ND	ND	ND	2	ND	ND	ND

ND = not determined. n = number of isolates.

[a] Morrissey et al. (2014).

Table 3
Results from PCR screening of disinfectant and heavy metal tolerance mediating determinants.

Disinfectant and heavy metals	MIC (µg/mL)	Number of isolates	Genes and number of positive isolates (n)											
Quaternary ammonium compounds			*qacEΔ1*	*qacE*	*qacF/H/I*	*qacG*	*emrE*	*mdfA*	*sugE(c)*	*sugE(p)*	*ydgE*	*ydgF*	*oqxA*	*oqxB*
ADBAC	32	1	-	-	-	-	1	1	1	-	1	1	-	-
ADH	>32	60	12	-	13	-	50	60	60	4	60	60	-	-
BKC	32	17	3	-	4	-	12	17	17	-	17	17	-	-
BEN	64	1	-	-	-	-	1	1	1	-	1	1	-	-
CTPC	256	1	-	-	-	-	1	1	1	-	1	1	-	-
HAB	256	1	-	-	-	-	1	1	1	-	1	1	-	-
CTAB	128	11	2	-	4	-	9	11	11	1	11	11	-	-
Acridine compound														
ACR	64	2	-	-	-	-	2	2	2	-	2	2	-	-
Bis-biguanides														
TCL	16	1	-	-	-	-	1	1	1	-	1	1	-	-
CHX	16	3	3	-	2	-	2	3	3	-	3	3	-	-
Heavy metals			*copA*	*cueO*	*cusC*	*pcoA*	*zntA*	*zitB*	*oqxA*	*oqxB*				
COP	1024	59	58	49	1	1			-	-				
SIL	32	51			1				-	-				
ZKC	512	34					12	34	-	-				

n = number of isolates. Grey shading indicates that the genes listed above were not screened in PCR assays. – = respective genes were not detected by PCR.

Similarly, the same trend was also obtained in Brazil, where Gazal et al. (2021) reported that 77.0 % of ESBL producing *E. coli* isolates from poultry production systems contained *bla* genes and isolates carried the $bla_{\text{CTX-M-55}}$ variant (38 %).

In the current study, we revealed that the varying degrees of resistance to ciprofloxacin and gentamicin as well as β-lactam antibiotics. Majority of isolates (48.3 %) showed resistance to two or more classes of critically important antibiotics including quinolones, classified by the World Health Organization (WHO) (2019). That is not surprising as Azargun et al. (2020) also noted that one to two-thirds of ESBL producing *Enterobacteriaceae* were found to be quinolone resistant. A possible explanation for this might be because of the plasmid contents that carries the *bla* gene with other resistance traits (Adenipekun et al., 2019).

ESBL producing *E. coli* isolates in our study showed the relatively higher susceptibility (lower MICs) to TCL (MIK_{TCL} = ≤0.0625–16 µg/mL), reduced susceptibility (higher MICs) to CTAB (MIC_{CTAB} = 32–128 µg/mL) and CTPC (MIC_{CTPC} = 16–256 µg/mL) for most of the isolates compared to the reference strain. Several studies have reported the similar reduced susceptibility for CTAB and CTPC in *E. coli* isolates obtained from meat samples (Sun et al., 2019; Zhang et al., 2016). In contrast to our finding, Sun et al. (2019) reported that *E. coli* isolates from retail meats showed relatively reduced susceptibility (MIC_{TCL} > 2.048 µg/mL) to TCL, which might be related to the disinfectant preference and different concentrations used in different countries. For BKC, another QAC compound, several studies have reported the susceptibility of in *Enterobacteriaceae* isolates from different sources (Deus et al., 2017; Roedel et al., 2021; Zhang et al., 2016). In the study of Zhang et al. (2016), *E. coli* isolated from retail meat showed the MICs of BKC in the range of 16–1024 µg/mL. Deus et al. (2017) have shown that the MICs of BKC for ESBL producing *E. coli* isolates from humans and poultry ranged from 4 to 32 and 4 to 16 µg/mL, respectively. Compared with these results, ESBL producing *E. coli* isolates in our study showed MICs of 8 to 32 µg/mL to BKC. In another study, ESBL *E. coli* isolates from broiler farms exhibited MICs of 20–40 µg/mL for BKC (Roedel et al., 2021). Meanwhile, the user concentrations of BKC in food sector are commonly between 200 and 1000 µg/mL (Møretrø et al., 2017), which are much higher than the MICs of ESBL producing *E. coli* isolates in the current study. Most of QACs such as BKC compounds do not require rinsing with water after application; so, contact between bacteria and QACs may be prolonged. Long term exposure to sub-lethal QACs concentrations may favour the development of resistance (Buffet-Bataillon et al., 2012b). Therefore, it was not surprising that ESBL producing *E. coli* isolates in the current study showed a low-level MICs for BKC. MIC values of biocides are difficult to compare among studies because laboratory conditions have not yet been standardized. Slipski et al. (2019) compared different antimicrobial susceptibility test methods and concluded that the cycles of bacterial growth were substantially affected by QACs tolerant phenotypes. Thus, harmonized susceptibility methods are urgently needed. Compared with the study by Morrissey et al. (2014), one of the interesting findings of our study is that 18.3 % ESBL producing *E. coli* isolates were found to be resistant to TCL. This might be a warning for emergence of TCL resistant *E. coli*. Broadly, phenotypic biocide susceptibility testing results did not evidence tolerance to disinfectants within our study since MIC range values of the biocides tested were well below in-use concentrations.

Our results indicated that the *mdfA*, *sugE*(*c*), *ydgE* and *ydgF* genes accounting for QACs tolerance were most prevalent in all ESBL producing *E. coli* isolates obtained from chicken meat, but the *qacE*, *qacG*, *oqxA* and *oqxB* genes were not found in any of the isolates. Similar results have been also reported by numerous researchers, which noted the prevalence of *ydgE/ydgF* (83–100 %), *mdfA* (86–100 %), and *qac* (0–18 %) genes in *E. coli* isolates from different sources (Deus et al., 2017; Roedel et al., 2021; Sun et al., 2019; Zhang et al., 2016; Zou et al., 2014). Besides, SMR carriers *emrE*, *qac*F/*H*/*I*, *qacEΔ1* and *sugE*(*p*) were detected with varying frequencies in our study. It was also reported that these genes are located on the mobile genetic element, which is closely related to the antibiotic resistance genes of *sul1* and *bla*$_{\text{CMY-2}}$ (Roedel et al., 2021). In the current study, the presence of *qacEΔ1* was found in 20 % of the isolates. Previous research has pointed out that *qacEΔ1* is common in enteric bacteria and located in the 3′-conserved segment of class 1 integrons carrying *sul1* gene (Paulsen et al., 1993; Zhang et al., 2016). In another study, antibiotic resistance genes and the *qacEΔ1* gene were located on the same plasmids (Pal et al., 2015). Zhang et al. (2016) reported that the use of QACs in the food processing facilities may facilitate the selection of strains that exhibit acquired QACs resistance and carry genes encoding resistance to medically important antibiotics. The findings from this study were consistent with other epidemiological studies conducted in Germany (Roedel et al., 2021; Wieland et al., 2017), United States (Zou et al., 2014), and China (Sun et al., 2019; Zhang et al., 2016) on *E. coli* isolated from poultry meat, meat products and farms. According to these results, the widespread use of QACs in the poultry industry may contribute to the emergence of antimicrobial resistant bacteria and the spread of resistance genes. Therefore, the use of QACs for decontamination of the facilities may not be as effective as expected in poultry sector.

In the current study, high prevalence of heavy metals resistance genes was detected in ESBL producing *E. coli* isolates from chicken meat, suggesting that these genes are ubiquitous. Heavy metals such as Cu, Zn, Ag, Hg and As have been widely used as feed additives in veterinary medicine for years, due to their antimicrobial properties as well as their growth enhancing effects in food animals (Rensing et al., 2018). In the current study, *copA* and *cueO*, which primarily confer resistance to copper, were widespread in ESBL producing *E. coli* isolates. The detection of these genes *copA* and *cueO* in the vast majority of isolates is reasonable because *cueO* enzymes are responsible for the synthesis of multiple copper oxidases that protect copper from induced damage, while *copA* is responsible for the synthesis of a P-type ATPase, which is necessary in copper homeostasis (Argudín et al., 2019; Deus et al., 2017; Rensing and Grass, 2003). Conversely, the *cusC* and *pcoA* genes, which also confer resistance to copper were found in only one ESBL producing *E. coli* isolate in this study. Similarly, Deus et al. (2017) showed that ESBL producing *E. coli* strains isolated from humans and poultry were less frequently obtained for the *cusC* and *pcoA* genes. The plausible explanation for this situation might be that while diverse heavy metal resistance genes located on chromosome more prevalent, genes encoded on plasmids or other mobile genetic elements scarce (Deus et al., 2017). Interestingly, the *zntA/zitB* gene which also confer resistance to zinc were widespread in ESBL producing *E. coli* isolates in our study. The *zitB* gene functions as a first-line defense against high zinc concentrations, and the gene encoding *zntA* must be regulated to reduce the free Zn concentration in *E. coli* (Wang et al., 2012). In addition, the high prevalence of heavy metal resistance genes might be associated with the extensive usage of them in poultry feed as growth promoters. The usage of these metals seems to have risen due to the ban on the use of antibiotics in most of the countries. The potential unfavorable effects of Zn and Cu contamination in the environment are the potential of co-selecting antibiotic-resistance genes and possibly creating more pathogenic strains (Rensing et al., 2018).

In the current study, FIB, FII and I1α replicons were the most frequent in our isolates. In concordance with the results of this study, Gazal et al. (2021) reported that the most common plasmid replicon type in ESBL producing *E. coli* isolates on poultry production systems was IncFIB (76.0 %). In a study conducted in Turkey, CTX-M-15 producing *E. coli* isolates obtained from different sources such as chicken meat, raw milk, Sürk cheese and wastewater were investigated for the presence of plasmid replicon types and the most common replicon type was found to be FII (53.6 %) (Azizoğlu, 2020). In a recent study of Lambrecht et al. (2018), IncFII to IncFIB combination was found to be predominant in commensal MDR *E. coli* from broilers. Besides, IncF and IncI plasmids have been reported in association with MDR *E. coli* strains, mainly ESBL producers from food animals (Xie et al., 2016). Moreover, the researchers noted that chicken meat plays an important role in the transport of the IncI plasmid into the food chain (Zurfluh et al., 2015).

5. Conclusion

Chicken meat could be an important source for cross-contamination of disinfectant and heavy metal resistant ESBL producing *E. coli* isolates. To the best of our knowledge, disinfectant and heavy metal resistance genes were found to be prevalent in ESBL producing *E. coli* isolates obtained from chicken meat for the first time in Turkey. Given the detection of genes mediating disinfectant resistance in isolates commonly and rising of MICs against disinfectant compounds, it can be concluded that disinfectant compounds implemented in slaughterhouse decontamination may not be as effective as expected.

This study provided valuable information on disinfectant and heavy metal resistance profiles of ESBL producing *E. coli* isolated from chicken meats in Turkey, which may help establish guidelines for appropriate practice of disinfectants in the poultry industry. Further research is needed to elucidate the roles of specific genes in disinfectant and heavy metal resistance.

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Ethical approval

Not required.

CRediT authorship contribution statement

Seyda Sahin: Conceptualization, Investigation, Project administration, Writing – original draft, Funding acquisition. **Mahmut Niyazi Mogulkoc:** Investigation, Visualization, Writing – review & editing. **Cemil Kürekci:** Conceptualization, Investigation, Project administration, Writing – review & editing.

Declaration of competing interest

There are no conflicts of interest among authors.

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