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ORIGINAL ARTICLE

Evaluation of bulk tank raw milk and raw chicken meat samples as source of ESBL producing *Escherichia coli* in Turkey: Recent insights

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Abstract

Extended spectrum β-lactamase producing Escherichia coli (ESBL-EC) was detected in 86.6% of chicken and 22.6% of bulk tank milk (BTM) samples. Pulsed-field gel electrophoresis analysis revealed 49 distinct restriction profiles among 66 isolates, and 62.3% of the isolates carried the $bla_{CTX\text{-}M}$ gene, among which CTX-M-1 was found to be the predominant ESBL types in chicken isolates, whereas CTX-M-15 was the commonest among BTM samples. Additionally, of 52 ESBL-EC isolates from chicken meat samples, 36.5%, 9.6%, and 7.7% harbored the bla_{TEM}, $bla_{CMY\text{-}2}$, and $bla_{SHV\text{-}12}$ genes, respectively, compared with 28.5% of the bla_{TEM} and 7.1% of the $bla_{SHV\text{-}12}$ markers among BTM isolates. The fimH gene was present in 51 isolates of chicken and in 14 isolates of BTM samples, while other virulence genes iutA (n = 31), iroN (n = 26), kpsMT II (n = 5), papC (n = 2), papG allele II (n = 2), papG allele II-III (n = 2), and papEF (n = 2) were only present in chicken meat isolates. Overall, it can be said that contaminated chicken meat and BTM might serve as vehicles for playing potential role in zoonotic transmission of ESBL-EC to humans in Turkey.

Practical applications

Extended spectrum β-lactamase producing *E. coli* (ESBL-EC) have been considered to be one of the major worldwide clinical problems and have been frequently isolated from the foods of animal origins such as chicken meat, fish, and raw milk. The current study aimed to isolate ESBL-EC from chicken and bulk tank milk samples and further characterize the strains by PFGE, identifying resistance genes and as well as virulence genes. Given the high prevalence of CTX-M-15/55 type ESBL-EC strains possessing important virulence genes in chicken and milk samples, it can be said that foods of animal origins might be an important risk factor for extraintestinal ESBL-EC infections for humans.

1 | INTRODUCTION

β-Lactamase producing bacteria have been considered to be one of the major worldwide clinical problems and particular subjects of research studies for about 70 years. In addition, discovery of new β-lactamases able to hydrolize broad spectrum cephalosporins in strains of *Enterobacteriaceae* (Knothe, Shah, Krcmery, Antal, & Mitsuhashi, 1983) has attracted substantial attention for the last two decades (D'Andrea, Arena, Pallecchi, & Rossolini, 2013). These enzymes have been referred to as extended spectrum β-lactamases (ESBL), for which there have been over 300 subtypes identified so far (Bonnet, 2004). Among these ESBL groups, CTX-M type has been the most common one in *Enterobacteriaceae* that initially appeared in human infections, and later on emerged in foods of animal origin and environmental samples (Leverstein van Hall et al., 2011; Zurfluh, Abgottspon, Hächler, Nüesch-Inderbinen, & Stephan, 2014). Of these widespread ESBL Enterobactericeae strains, ESBL producing *Escherichia coli* (ESBL-EC) is of the most impactful to human health in its own right as being the commonest cause of urinary

tract infections (Djuikoue et al., 2017). These illnesses caused by ESBL-EC can be challenging-to-treat due to phenomenon of high level resistance to several other antibiotic groups including fluoroquinolones (Jiang et al., 2014), which have been resulted in immense public health burden including significant rate of mortality (Bonnet, 2004; Ewers, Bethe, Semmler, Guenther, & Wieler, 2012).

ESBL-EC have been frequently isolated from the foods of animal origins such as chicken meat, fish and raw milk (Brahmi et al., 2018; Odenthal, Akineden, & Usleber, 2016; Pehlivanlar Önen, Aslantaş, Yılmaz, & Kürekci, 2015), which resulted in great attention to study the molecular epidemiology of ESBL-EC strains for human illnesses during the last decade. Even though the exact source of ESBL-EC strains for human infections has not been clearly understood yet, a series of experiments have presented convincing evidence implicating contaminated foods of animal origin as possible route of transmission to humans (Leverstein van Hall et al., 2011; Zurfluh et al., 2014).

In our earlier work, the occurrence and characterization of the molecular traits of ESBL-EC strain in meat samples were investigated in Turkey (Pehlivanlar Önen et al., 2015). However, this study did not evaluate the genetic relatedness of strains and the characterization of virulence genes was neglected. Hence, present study has been undertaken to expand such study for assessing the recent contamination with ESBL-EC in chicken and bulk tank milk samples. Accordingly, we further characterized the isolated strains by pulsed-field gel electrophoresis (PFGE), identifying resistant genes and as well as virulence genes.

2 | MATERIALS AND METHODS

2.1 | Sampling

Cow milk samples were collected from different locations within Hatay province (36.4018° N, and 36.3498° E; Figure 1), in the southern Turkey from September to December 2016. The samples were collected from a total of 62 bulk tanks from 1,252 of farms with a total of about 11,513 cows, data was provided by the Cattle Breeder's Association of Hatay (CBAH). Operated milk tanks ranged in size from 500 to 5,000 L and average daily milk production ranged about 80,000 L in the province (personal communication with CBAH). In addition, 60 raw chicken meat samples were collected from retail supermarkets and butchers from five different provinces (Figure 1). All samples were transported to the laboratory on ice and analyzed immediately.

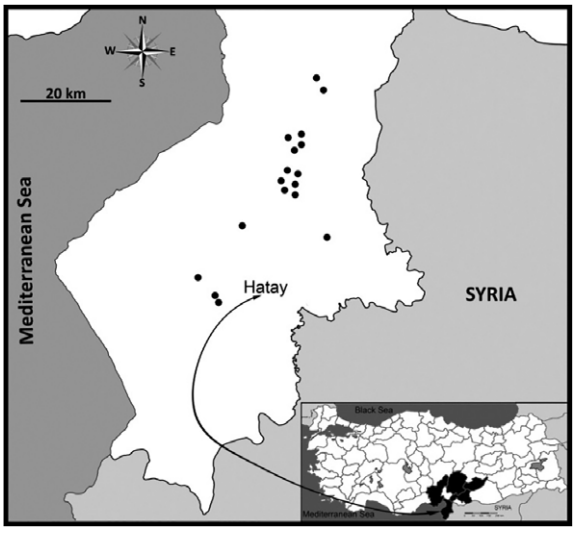


FIGURE 1 Location of the sampling provinces for chicken meat showed dark in the map of Turkey and locations of milk collection points in Hatay province. Dark dots denote tank's locations

2.2 | Isolation and characterization of ESBL producing *E. coli*

Each sample (approximately 25 g for chicken meat and 10 mL for milk) was incubated in buffered peptone water (1:9 ratio) at 37°C overnight. Subsequently, ESBL producing *E. coli* isolation was performed on selective ChromID ESBL plates (bioMerieux, Marcy l'Etoile, France), from which one presumptive colony (pink to burgundy appearance) was collected from agar plate and subcultured on blood agar plates at 37°C for about 20 hr. Species identification was performed using a MALDI-TOF (Bruker Daltonik GmbH, Leipzig, Germany) and then confirmed by the presence of the *usp*A gene highly specific for *E. coli* by a PCR assay as described previously (Chen & Griffiths, 1998).

Screening tests including combination disk and double-disk synergy were performed to determine phenotypic ESBL production and the results were interpreted according to the CLSI criteria (CLSI, 2015). The strains that proved to be ESBL producers by phenotypic methods were grouped phylogenetically following the protocol described by Clermont, Bonacorsi, and Bingen (2000). Besides phenotypic characterization, PFGE procedure, which includes bacterial lysis and digestion of genomic DNA using the restriction enzyme (*Xba*I), outlined in PulseNet was performed to investigate genetic diversity in *E. coli* strains (www.cdc.gov/pulsenet). Strain relatedness dendrogram was built by using the UPGMA algorithm based on Dice similarity coefficient with a 1.2% band position tolerance using BioNumerics Software (Applied Maths, Belgium).

2.3 | Phenotypic antimicrobial resistance profile

A total of 66 ESBL-EC isolates were tested using disc diffusion assay for their antimicrobial susceptibilities to the following panel of antimicrobials; amoxicillin/clavulanic acid (AMC; 25 μg), aztreonam (ATM; 30 μg), ampicillin (AM; 10 μg), ceftazidime (CAZ; 30 μg), cefoxitin (FOX; 30 μg), cefuroxime (CXM; 30 μg), cefotaxime (CTX; 30 μg), cefpodoxime (CPD; 10 μg), colistin (CT; 10 μg), imipenem (IPM; 10 μg), chloramphenicol (C; 30 μg), gentamycin (CN; 10 μg), tetracycline (TE; 30 μg), nalidixic acid (NA; 30 μg), ciprofloxacin (CIP; 5 μg), florfenicol (FFC; 10 μg), levofloxacin (LEV; 5 μg), norfloxacin (NOR; 10 μg), and trimethoprim-sulfamethoxazole (STX; 25 μg). The results were interpreted according to the recommendation of the CLSI (2015). MICs of ciprofloxacin were determined by E-test for all strains and results were assessed according to the interpretive criteria of CLSI.

2.4 | Determination of antimicrobial resistance genes

To determine whether the isolates harbored β-lactamase genes (bla_{AmpC}, bla_{TEM}, bla_{SHV}, and bla_{CTX-M}), polymerase chain reaction (PCR) assay was conducted as described (Hasman, Mevius, Veldman, Olesen, & Aarestrup, 2005; Leinberger et al., 2010; Mulvey et al., 2003). ESBL types were confirmed by sequencing the PCR products as described previously (Ahmed et al., 2007). PCR based detection of the plasmid-mediated quinolone resistance (PMQR) markers (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *aac*[*6′*]-*Ib*) was performed (Cattoir, Poirel, Rotimi, Soussy, & Nordmann, 2007; Cavaco et al., 2008; Cavaco, Hasman, Xia, & Aarestrup, 2009; Jacoby, Gacharna, Black, Miller, & Hooper, 2009; Park, Robicsek, Jacoby, Sahm, & Hooper, 2006). In order to identify specific ESBL and PMQR gene types, database searching was carried out by comparing obtained sequences with those sequences submitted to online tool (https://blast.ncbi.nlm.nih.gov).

2.5 | Determination of virulence traits

For the Shiga toxin *stx1* and *stx2* genes, real-time PCR was performed in a multiplex format (ISO, 2012). In addition, a total of 14 *E. coli* virulence genes including *hlyA*, *papC*, *papAH*, *papEF*, *papG* allele II, *papG* allele III, *papG* allele II-III, *cnf1*, *fimH*, *iutA*, *kpsMT* K1, *kpsMT* II, *iroN*, and *univenf* were detected with multiplex conventional PCR according to published protocols (Chapman et al., 2006).

3 | RESULTS

E. coli was detected in 52 of 60 (86.6%) chicken and 14 of 62 (22.6%) tank milk samples. All of these isolates were identified as ESBL producers by phenotypic tests. Based on the phylogenetic PCR analysis developed by Clermont et al. (2000), phylo group D was the most prevalent in 51 strains, 43 from chicken (82.7%) and 8 from milk samples (57.1%). The occurrence of phylo-group A was 15.3% in chicken and 35.7% in milk samples. The phylo-group B_2 was detected in only two isolates; one from chicken meat and one from milk sample. A total of 66 isolates were typed by PFGE analysis, which generated 49 distinct restriction profiles based on the 85% similarity (Figure 2). Thirty eight isolates had unique band profile and the remaining 28 strains grouped into 11 groups, for which 2–6 strains were identical. Strains ME30 and ME52 from milk samples had similar band pattern with chicken strains (Figure 2).

The results of the *in vitro* susceptibility test of ESBL-EC isolates are presented in Table 1. Among the ESBL-EC isolates obtained from chicken meat samples, multiple drug resistance (MDR), which defined as displaying resistance to at least three antibiotic classes, was noted in 46 isolates (88.5%), whereas eight strains (57.1%) displayed MDR among isolates from milk samples. All ESBL-EC isolates were resistant to AM and >80% of isolates presented resistance to CPD, CXM, CTX and KF, while the prevalence of resistance to CAZ was 48.1% and 50% in chicken and milk samples, respectively. The majority of ESBL-EC isolates, irrespective of source, displayed high resistance rates to TE and SXT. Overall, 46.2% (*n* = 24) of the chicken meat isolates were CIP resistant, among which 20 isolates had MIC values for ciprofloxacin of 4 μg/mL or greater (Figure 2). A low level of resistance was seen for AMC and FOX, and all 66 ESBL-EC isolates were found to be susceptible to IMP and CT.

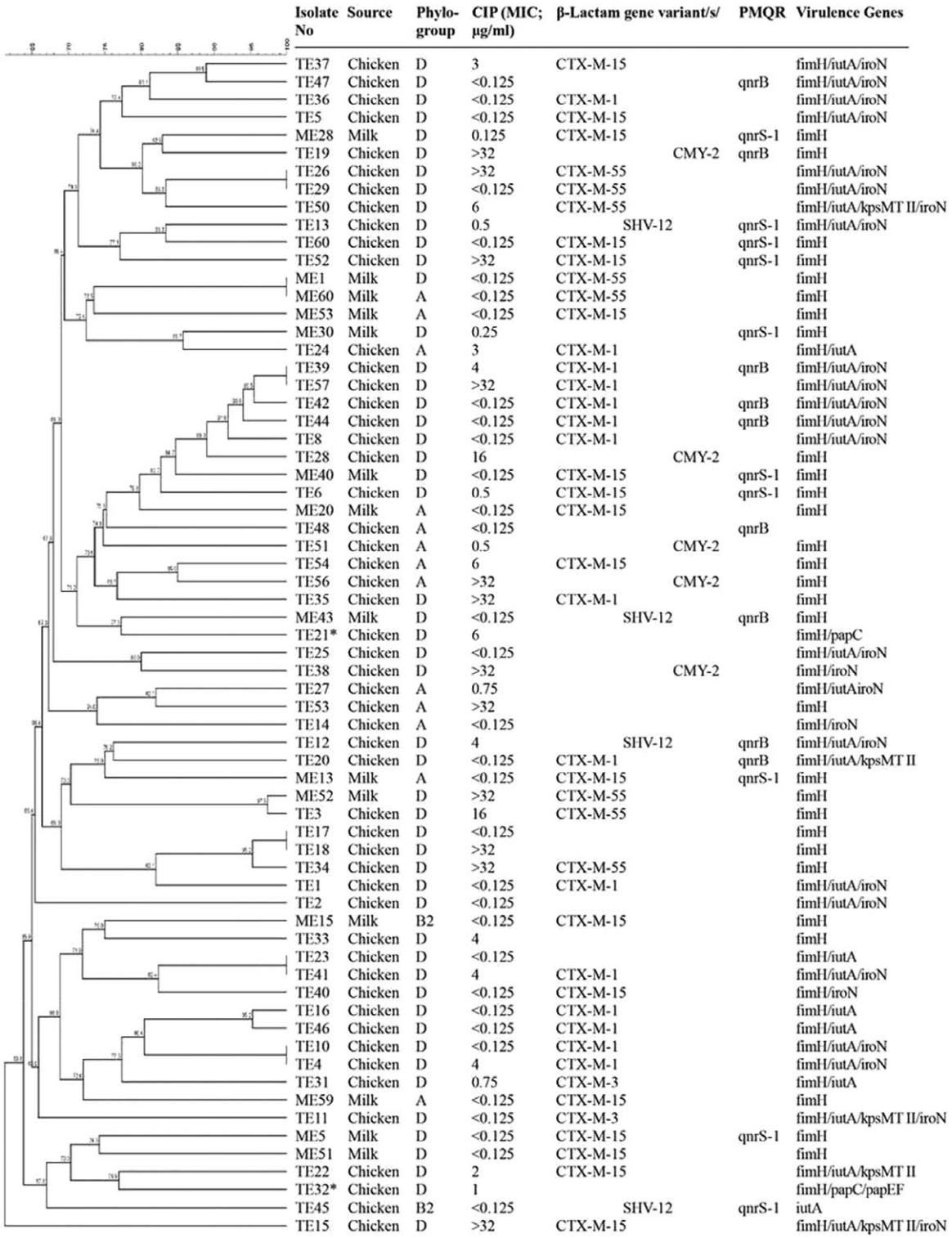
The prevalence of each identified ESBL genes is given in Figure 2. PCR showed that 62.3% of the ESBL producing *E. coli* isolates from chicken meat were positive for the bla_{CTX-M} gene, while 78.6% of isolates from raw milk were bla_{CTX-M} positive. Sequence analyses allowed us to determine the presence of four CTX-M subtypes including that the most abundant ESBL type among chicken isolates was CTX-M-1 accounting for 28.3% (*n* = 15) of isolates, followed by CTX-M-15 (*n* = 9), CTX-M-55 (*n* = 5), and CTX-M-3 (*n* = 2), meanwhile CTX-M-15 was the most frequent ESBL type (n = 9), followed by CTX-M-55 (*n* = 3) among tank milk isolates in the present study. Of the 52 ESBL-EC strains from chicken meat, 19 (36.5%) and 5 (9.6%) had the bla_{TEM} and plasmid mediated *AmpC* β-lactamase (bla_{CMY-2}) genes, while the bla_{TEM} gene was detected in 4 (28.5%) isolates from milk samples. All ESBL producing *E. coli* isolates from milk source were negative for the bla_{CMY} gene. In PCR and sequencing results, four isolates (7.7%) obtained from chicken meat were showing the presence of SHV-12 ESBL type, whereas only one isolate (7.1%) had this type of ESBL from milk samples.

PMQR genes were screened by PCR and identified by sequencing for all 66 ESBL-EC isolates. Five isolates from chicken samples and five from milk samples were found to be positive for *qnrS* in the current study (Figure 2). In addition, the *qnrB* gene was present in eight chicken meat isolates and one raw milk isolates (Figure 2). However, the *qnrA*, *qnrC*, *qnrD*, and *aac*(*6′*)-*Ib* genes were not detected in any isolates.

Of the virulence genes that were screened by PCR, 50 (96.1%), 31 (59.6%), and 26 (50%) of ESBL-EC isolates from chicken samples were PCR positive for the *fimH*, *iut*A, and *iroN* genes, respectively (Figure 2). In addition, 9.6% (*n* = 5) of strains harbored the *kpsMT* II gene, whereas *papC*, *papG* allele II, and *papG* allele II-III were found in two isolates. The *papEF* gene was only identified in one ESBL-EC isolate. In contrast to chicken isolates, raw milk isolates only had *fimH* gene. The remaining virulence traits (*hlyA*, *papAH*, *papEF*, *papG* allele III, *cnf1*, *kpsMT* K1, and *univenf*) and the Shiga toxin genes (*stx1* and *stx2*) were not detected.

4 | DISCUSSION

As revealed previously by our group (Pehlivanlar Önen et al., 2015), a very high rate (86.7%) of ESBL-EC in chicken meat samples was found in the current study. This prevalence of ESBL-EC in chicken meat is comparable to these obtained in several countries, for example; recent studies showed that contamination can vary from as low as 65.4% in England (Randall et al., 2017) to as high as 91.7% in France (Casella, Nogueira, Saras, Haenni, & Madec, 2017). It has been previously suggested that the previous use of cephalosporins, aminoglycosides and fluoroquinolones in poultry industry had created selection pressure for the emergence of high rate of ESBL carriage in chickens (Canton et al., 2008). In contrast to chicken meat samples, ESBL-EC was detected in 22.6% of bulk tank milk samples that is not surprising



Isolate No	Source	Phylo-group	CIP (MIC; µg/ml)	β-Lactam gene variant/s/	PMQR	Virulence Genes
TE37	Chicken	D	3	CTX-M-15		fimH/iutA/iroN
TE47	Chicken	D	<0.125		qnrB	fimH/iutA/iroN
TE36	Chicken	D	<0.125	CTX-M-1		fimH/iutA/iroN
TE5	Chicken	D	<0.125	CTX-M-15		fimH/iutA/iroN
ME28	Milk	D	0.125	CTX-M-15	qnrS-1	fimH
TE19	Chicken	D	>32	CMY-2	qnrB	fimH
TE26	Chicken	D	>32	CTX-M-55		fimH/iutA/iroN
TE29	Chicken	D	<0.125	CTX-M-55		fimH/iutA/iroN
TE50	Chicken	D	6	CTX-M-55		fimH/iutA/kpsMT II/iroN
TE13	Chicken	D	0.5	SHV-12	qnrS-1	fimH/iutA/iroN
TE60	Chicken	D	<0.125	CTX-M-15	qnrS-1	fimH
TE52	Chicken	D	>32	CTX-M-15	qnrS-1	fimH
ME1	Milk	D	<0.125	CTX-M-55		fimH
ME60	Milk	A	<0.125	CTX-M-55		fimH
ME53	Milk	A	<0.125	CTX-M-15		fimH
ME30	Milk	D	0.25		qnrS-1	fimH
TE24	Chicken	A	3	CTX-M-1		fimH/iutA
TE39	Chicken	D	4	CTX-M-1	qnrB	fimH/iutA/iroN
TE57	Chicken	D	>32	CTX-M-1		fimH/iutA/iroN
TE42	Chicken	D	<0.125	CTX-M-1	qnrB	fimH/iutA/iroN
TE44	Chicken	D	<0.125	CTX-M-1	qnrB	fimH/iutA/iroN
TE8	Chicken	D	<0.125	CTX-M-1		fimH/iutA/iroN
TE28	Chicken	D	16	CMY-2		fimH
ME40	Milk	D	<0.125	CTX-M-15	qnrS-1	fimH
TE6	Chicken	D	0.5	CTX-M-15	qnrS-1	fimH
ME20	Milk	A	<0.125	CTX-M-15		fimH
TE48	Chicken	A	<0.125		qnrB	
TE51	Chicken	A	0.5	CMY-2		fimH
TE54	Chicken	A	6	CTX-M-15		fimH
TE56	Chicken	A	>32	CMY-2		fimH
TE35	Chicken	D	>32	CTX-M-1		fimH
ME43	Milk	D	<0.125	SHV-12	qnrB	fimH
TE21*	Chicken	D	6			fimH/papC
TE25	Chicken	D	<0.125			fimH/iutA/iroN
TE38	Chicken	D	>32	CMY-2		fimH/iroN
TE27	Chicken	A	0.75			fimH/iutAiroN
TE53	Chicken	A	>32			fimH
TE14	Chicken	A	<0.125			fimH/iroN
TE12	Chicken	D	4	SHV-12	qnrB	fimH/iutA/iroN
TE20	Chicken	D	<0.125	CTX-M-1	qnrB	fimH/iutA/kpsMT II
ME13	Milk	A	<0.125	CTX-M-15	qnrS-1	fimH
ME52	Milk	D	>32	CTX-M-55		fimH
TE3	Chicken	D	16	CTX-M-55		fimH
TE17	Chicken	D	<0.125			fimH
TE18	Chicken	D	>32			fimH
TE34	Chicken	D	>32	CTX-M-55		fimH
TE1	Chicken	D	<0.125	CTX-M-1		fimH/iutA/iroN
TE2	Chicken	D	<0.125			fimH/iutA/iroN
ME15	Milk	B2	<0.125	CTX-M-15		fimH
TE33	Chicken	D	4			fimH
TE23	Chicken	D	<0.125			fimH/iutA
TE41	Chicken	D	4	CTX-M-1		fimH/iutA/iroN
TE40	Chicken	D	<0.125	CTX-M-15		fimH/iroN
TE16	Chicken	D	<0.125	CTX-M-1		fimH/iutA
TE46	Chicken	D	<0.125	CTX-M-1		fimH/iutA
TE10	Chicken	D	<0.125	CTX-M-1		fimH/iutA/iroN
TE4	Chicken	D	4	CTX-M-1		fimH/iutA/iroN
TE31	Chicken	D	0.75	CTX-M-3		fimH/iutA
ME59	Milk	A	<0.125	CTX-M-15		fimH
TE11	Chicken	D	<0.125	CTX-M-3		fimH/iutA/kpsMT II/iroN
ME5	Milk	D	<0.125	CTX-M-15	qnrS-1	fimH
ME51	Milk	D	<0.125	CTX-M-15		fimH
TE22	Chicken	D	2	CTX-M-15		fimH/iutA/kpsMT II
TE32*	Chicken	D	1			fimH/papC/papEF
TE45	Chicken	B2	<0.125	SHV-12	qnrS-1	iutA
TE15	Chicken	D	>32	CTX-M-15		fimH/iutA/kpsMT II/iroN

FIGURE 2 PFGE analysis of ESBLEC isolates. *Strains having the *papG* allele II and *papG* allele II-III genes as well

and consistent with the recent report, in which ESBL-EC was isolated from 7% of the investigated cattle rectal swap samples in the same region of Turkey (Aslantaş, Elmacıoğlu, & Yılmaz, 2016). On the other hand, a lower (0–9.5%) prevalence has been reported in the bulk tank milk samples in Switzerland by Geser, Stephan, and Hächler (2012) and in Germany by Odenthal et al. (2016).

In order to assess the variation among ESBL-EC isolates we conducted phlo-typing method as well as PFGE. As consisting with the previous findings (Pehlivanlar Önen et al., 2015), phylo-group B_2, that has been identified as the pathogenic strain to humans, was identified only in few isolates from both samples, whereas the majority of isolates belonged to phylo-group D, which was identified as the low virulent group (Clermont et al., 2000). Our results are in line with other studies that identified the low virulent groups commonly from foods of animal origins (Egea et al., 2012; Mo, Slettemeås, Berg, Norström, & Sunde, 2016; Tansawai, Sanguansermsri, Na-udom, Walsh, &

TABLE 1 Antimicrobial resistance profile of ESBL producing *E. coli* strains by disc diffusion assay

Antimicrobial resistance profile (%)																					
Source	*n*	AM	AMC	CPD	CXM	CTX	KF	CAZ	ATM	FOX	FFC	IPM	CT	C	CN	SXT	TE	LEV	NOR	CIP	NA
Chicken meat	52	100	9.6	92.3	84.6	98.1	98.1	48.1	19.2	7.7	28.8	0	0	30.8	21.2	71.2	63.5	19.2	50	46.2	69.2
Raw Milk	14	100	0	85.7	85.7	92.9	100	50	42.9	7.1	7.1	0	0	14.3	14.3	50	42.9	0	7.1	7.1	14.3

AMC = amoxicillin/clavulanic acid; ATM = aztreonam; AM = ampicillin; CAZ = ceftazidime; FOX = cefoxiti; CXM = cefuroxime; CTX = cefotaxime; CPD = cefpodoxime; CT = colistin; IPM = imipenem; C = chloramphenicol; CN = gentamycin; TE = tetracycline; NA = nalidixic acid; CIP = ciprofloxacin; FFC = florfenicol; LEV = levofloxacin; NOR = norfloxacin; STX = trimethoprim-sulfamethoxazole.

Niumsup, 2018). In addition, the PFGE method has have revealed considerable phylogenetic diversity within ESBL-EC isolates obtained from foods in this study, for which anthropogenic interventions, birds, insects and movement of animals have been identified as the main influences driving these diversity (Afema et al., 2018). Another interesting finding of the present work was that some ESBL-EC strains from chicken meat and milk samples were grouped together, demonstrating the spread of these strains to the different environments.

Several studies have reported that the distribution and prevalence of ESBL types appears to be varied significantly depending on the source of isolates and geographic variations (Afema et al., 2018). In the current study, a significant proportion of ESBL-EC strains harbored the $bla_{CTX\text{-}M}$ gene, for which four CTX-M types were identified; of these, CTX-M-1 was more common in chicken meat samples and CTX-M-15 was more prevalent in milk samples. Previous studies also indicated the high prevalence of CTX-M-1 in chicken meat samples in Thailand (69%) (Tansawai et al., 2018) and in Switzerland (79.4%) (Vogt et al., 2014). In contrast, the CTX-M-2 was found to be the main ESBL type in Brazil, Korea and Japan (dos Santos Iark, Koga, Vespero, Kobayashi, & de Oliveira, 2018; Kawamura, Goto, Nakane, & Arakawa, 2014; Kim et al., 2018), while CTX-M-15 was the predominant type in Romania (Maciuca et al., 2015). Additionally, in some studies (Kawamura et al., 2014; Mo et al., 2016) other β-lactamase genes including the bla_{SHV} and $bla_{CMY\text{-}2}$ exceed CTX-M, and the variance were already attributed to the some factors such as environmental and geographical features, antimicrobial substrates, and mobility of plasmids (Ewers et al., 2012). Surprisingly, the most notable difference between the current findings and our previous study (Pehlivanlar Önen et al., 2015) is the occurrence of CTX-M-55 type ESBL that differs from CTX-M-15 type at a single amino acid (Picard et al., 1999) and, to the best of our knowledge, has not been reported from Turkey so far. On the other hand, this type of ESBL enzyme has been recently reported more commonly from human and foods of animal origins isolates in China (Xu et al., 2014). Besides β-lactamase genes, PMQR genes (*qnrB* and *qnrS*) have been identified among ESBL-EC isolates. The presence of PMQR genes have been reported with a different frequency in some other countries due to the mobile genetic elements that carry both ESBL and PMQR genes together (Brahmi et al., 2018; Maciuca et al., 2015; Xu et al., 2014; Yu et al., 2015).

In this study, phenotypic antibiotic resistance data showed that high levels of prevalence of ESBL-EC strains, regardless of source, exhibited MDR. Frequency of resistance to β-lactam antibiotics except CAZ in ESBL-EC isolates of current study was over 80%. In addition, ESBL-EC isolates from chicken samples displayed higher frequency of resistance to NOR, CIP, and NA, when compared to those isolated from raw milk. Similar results have been also reported by numerous researchers (Abreu et al., 2014; Casella et al., 2017; Maciuca et al., 2015), who indicated that ESBL-EC strains from chicken samples were highly resistant against quinolones, TE and STX. Of particular note is that all our ESBL-EC strains were found to be susceptible to imipenem and colistin, for which latter one renowned attention recently in treatment of various diseases as a last line therapy. However, some resistance to imipenem and colistin among ESBL-EC strains were reported in several regions, for instance; in a recent study of ESBL-EC in human and chicken meat samples in Denmark by Haenni et al. (2016) plasmid mediated colistin resistance has been identified and authors noted that broad spectrum cephalosporins might cause selection pressure for colistin resistance. Moreover, in another study conducted in Thailand by Tansawai et al. (2018), 16.5% of ESBL-EC strains obtained from chicken meat samples were found to be resistant to imipenem. The difference has been attributed to a number of factors including geographical locations and antibiotic usage practice in veterinary medicine (Ewers et al., 2012).

In the current study, the *fimH* gene, known to contribute autoaggregation of *E. coli*, was the most frequently identified gene among virulence markers tested. This is not surprising since this gene previously reported to be present in commensal and clinical *E. coli* isolates as well as all ESBL-EC isolates from foods of animal origins (Brahmi et al., 2018; Delicato, de Brito, Gaziri, & Vidotto, 2003). In addition to the *fimH* gene, plasmid-related virulence genes including *iroN* and *iutA* were found in more than 50% of strains of ESBL-EC obtained from chicken meat samples. In a study conducted in Switzerland, the *iroN* gene was exclusively found in avian pathogenic *E. coli* (APEC) isolates. The *kpstM* II gene associated with group II capsules production was found in 9.6% of chicken isolates in the current study. The studies examining APEC, uropathogenic *E. coli* (UPEC) (Barbieri et al., 2013; Rodriguez-Siek et al., 2005), and neonatal meningitis *E. coli* (NMEC) strains (Bonacorsi et al., 2003) identified the majority of isolates possessing the *kpstM* II gene, suggesting that birds might act as foodborne source for extraintestinal pathogenic *E. coli* strains (Barbieri et al., 2013; Rodriguez-Siek et al., 2005). Interestingly, two isolates had *papC*, *papG* allele II, and *papG* allele II-III, which were identified more significantly among avian colibacillosis *E. coli* strain (Delicato et al., 2003). In contrast to the high frequency of these genes in chicken meat isolates, none of the ESBL-EC strains had these genes, except *fimH*, in raw milk samples.

5 | CONCLUSIONS

Given the high prevalence of CTX-M-15/55 type ESBL-EC strains possessing important virulence genes in chicken and milk samples, it

can be said that foods of animal origins might be an important risk factor for extraintestinal ESBL-EC infections for humans. However, analyses of well-defined clinical ESBL-EC isolates together with those obtained from the foods of animal origins will help to understand the potential reservoirs in Turkey.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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