Kafkas Univ Vet Fak Derg
26 (4): 463-468, 2020
DOI: 10.9775/kvfd.2019.23514

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Occurrence and Molecular Characterization of Cephalosporin Resistant *Escherichia coli* Isolates from Chicken Meat

Nebahat BİLGE [1,a] Çiğdem SEZER [1,b] Leyla VATANSEVER [1,c] Sevda PEHLİVANLAR ÖNEN [2,d]

[1] University of Kafkas, Faculty of Veterinary Medicine, Department of Food Safety and Public Health, TR-36100 Kars - TURKEY
[2] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, TR-31040 Antakya, Hatay - TURKEY

ORCIDS: [a] 0000-0002-5229-9847; [b] 0000-0002-9722-3280; [c] 0000-0002-5464-0834; [d] 0000-0002-8582-0598

Article ID: KVFD-2019-23514 Received: 22.10.2019 Accepted: 16.05.2020 Published Online: 19.05.2020

How to Cite This Article

Bilge N, Sezer Ç, Vatansever L, Pehlivanlar Önen S: Occurrence and molecular characterization of cephalosporin resistant *Escherichia coli* isolates from chicken meat. *Kafkas Univ Vet Fak Derg,* 26 (4): 463-468, 2020. DOI: 10.9775/kvfd.2019.23514

Abstract

Contamination of retail meat with extended spectrum beta-lactamase (ESBL) and/or AmpC type beta-lactamase (AmpC) producing *Escherichia coli* may contribute to increased incidences of infections in humans. Regular monitoring of these bacteria is required in the view of one health approach. In this study, 100 chicken meat samples obtained from Kars, Turkey were analysed and 214 isolates recovered from 72/100 samples were identified as *E. coli.* Antibiotic susceptibility tests showed that 15.42% of isolates (33/214) were resistant to at least one cephalosporin antibiotic, 20 (9.34%) were beta-lactamase producer. Among beta-lactamase producing *E. coli* isolates 8 had bla_{TEM}, 7 had $bla_{CTX\text{-}M}$ and 3 had bla_{SHV} genes. Plasmid-mediated AmpC beta-lactamase (bla_{CMY}) gene was present in 13 isolates. Plasmid mediated quinolone resistance genes were also screened by polymerase chain reaction and identified by sequencing of the isolates. As a result, 3 isolates were found to be positive for *qnrB,* whereas the qnrS gene was detected in 4 isolates. Regarding the virulence genes 19 isolates were positive for *fimH* and 2 isolates were carrying *kpsMT* II. Phylo-group D2, A0, A1, B1 and D1 were detected in 13, 2, 2, 2 and 1 isolates, respectively. Our findings indicate that poultry meat could be an important carrier of ESBL positive *E. coli.*

Keywords: *Escherichia coli, Chicken, Cephalosporin, ESBL, AmpC*

Tavuk Etlerinde Sefalosporine Dirençli *Escherichia coli* Varlığı ve Moleküler Karakterizasyonu

Öz

Etlerin geniş spektrumlu beta-laktamaz (GSBL) ve/veya AmpC tipi beta-laktamaz üreten *Escherichia coli* ile kontaminasyonu, insanlarda meydana gelen enfeksiyonların insidensinde artışa neden olabilir. Bu nedenle bu bakterilerin düzenli olarak taranması, halk sağlığının korunması açısından önemlidir. Bu çalışmada Kars ilinden temin edilen100 adet tavuk eti incelenmiş ve bunlardan 72'sinden 214 adet *E. coli* izolatı elde edilmiştir. Yapılan antibiyotik duyarlılık testleri izolatlardan 33'ünün (%15.42) en az bir antibiyotiğe dirençli olduğunu, 20'sinin (%9.34) beta-laktamaz, 5'inin ise (%2.33) GSBL ürettiğini göstermiştir. Moleküler testler, beta-laktamaz üreten *E. coli* izolatlarından 8'inin bla_{TEM}, 3'ünün bla_{SHV}, 7'sinin $bla_{CTX\text{-}M}$ ve 13'ünün plazmid aracılı AmpC beta-laktamaz (bla_{CMY}) genlerine sahip olduğuna işaret etmiştir. Plazmid aracılı kinolon direnç genleri de polimeraz zincir reaksiyonu ile görüntülenip sekanslanarak identifiye edilmiş; 3 izolatın *qnrB*, 4 izolatın da *qnrS* taşıdığı belirlenmiştir. Bunun yanında izolatlardan 19'unun *fimH* ve 2'sinin *kpsMT* II virulens genlerine sahip olduğu görülmüştür. Son olarak 13, 2, 2, 2 ve 1 izolatın sırasıyla filogrup D2, A0, A1, B1 ve D1'e dahil olduğu ortaya konmuştur. Bu çalışmadan elde edilen bulgular, kanatlı etinin GSBL pozitif *E. coli* açısından önemli bir taşıyıcı rolü üstlenebileceğini göstermiştir.

Anahtar sözcükler: *Escherichia coli, Tavuk, Sefalosporin, GSBL, AmpC*

INTRODUCTION

The *Enterobacteriaceae* are considered as a hygiene indicator in food industry [1]. This family possesses various bacterial species that can be pathogenic to humans and animals [2]. Among these species, *E. coli* strains in particular can cause postsurgical, urinary tract, blood-stream and central nervous system infections [3]. The successful treatment of bacterial infections commonly requires a careful usage of antimicrobials [4]. However, because of the several reasons including the use of antimicrobial agents in animal production, the resistance in this family has increased.

Correspondence
+90 5330301882 (GSM)
nebahatbilgeoral@hotmail.com

Addition of antimicrobial agents into animal feeds and veterinary use of cephalosporins and fluoroquinolones played a significant role in selecting resistant clones. The most important mechanism of that resistance is production of β-lactamases that hydrolyses the beta lactam ring and inactivates the β-lactam group antibiotics [1,5]. Among them, extended spectrum β-lactamases (ESBL) and AmpC are the most common enzymes around the world. ESBL producing microorganisms are resistant to 3rd and 4th generation cephalosporins but they are sensitive to carbapenems and cephamycins (cefoxitin), and are inactivated by clavulanic acid. On the other hand, the AmpC enzymes confer resistance to third generation cephalosporins and cephamycins but microorganisms having these enzymes are not inhibited by clavulanic acids and other β-lactamase inhibitors [6].

Extended spectrum β-lactamases genes are commonly located on plasmids that can be easily transferred between and within bacterial species. Some ESBL genes are mutant derivatives of established plasmid-mediated β-lactamases (e.g., $bla_{TEM/SHV}$), and others are mobilized from environmental bacteria (e.g., bla_{CTX-M}) [7].

Prevalence of ESBL producing *E. coli* infections are rapidly increasing in European countries causing longer hospital stays, increased costs and mortality [8]. They are considered as a serious threat causing therapy failure in human medicine [9].

Studies indicated that food producing animals carry increasing numbers of ESBL/AmpC producing *E. coli* isolates leading to the hypothesis that animals might become infection sources or even reservoirs contributing to the spread of these bacteria [10,11]. They also showed that ESBL producers could spread along the broiler production chain [12]. Other researchers reported that human and broiler ESBL *E. coli* were possibly associated and proposed broilers as a potential reservoir of foodborne resistant bacteria that infect human [13,14]. High levels poultry meat contamination with ESBL producers serving as a possible reservoir for human colonization has also been declared by other researchers [15]. According to The European Food Safety Authority (EFSA) the presence of ESBL producing *E. coli* in poultry meat is a significant threat to public health [16].

Regular monitoring of these bacteria is required in the view of one health approach. This study was conducted to understand the current situation of resistance to cephalosporins and its molecular mechanisms in *E. coli* isolated from raw chicken meat samples hoping to quantify the impact of the broiler meat production chain on consumer exposure.

MATERIAL and METHODS

Collection of Samples

One hundred retail packaged raw poultry meat samples were purchased from 47 markets in Kars Province, Turkey during the period from May to August 2017. The samples were transported to the laboratory on ice and processed within 24 h.

Bacterial Isolation

Two hundred and twenty-five millilitres of buffered peptone broth (Oxoid, UK) were added to 25 g of each sample and homogenized by stomacher blender. The homogenate was incubated at 37°C for 24 h. The next day 100 µL of enriched broth were inoculated on Violet Red Bile Lactose Agar (Oxoid, UK) and incubated at 37°C for 24 h [17].

Three colonies per plate exhibiting typical *E. coli* morphological appearance (pink to red, entire-edged colonies that are surrounded by a reddish zone of precipitated bile) were selected and phenotypically confirmed by evaluating catalase activity (positive), motility (positive), indole production from tryptophan amino acid (positive or negative), H_2S production (negative), urea hydrolyzation (negative) and utilization of citrate (negative). Voges-Proskauer (negative), triple sugar iron (glucose and lactose fermentation and CO_2 production positive) and methyl red (positive) tests were also applied to the isolates [18]. *E. coli* isolates were grouped phylogenetically by polymerase chain reaction (PCR) assay targeting the *chuA, yjaA* and *TESPE*4 genes [19,20] *(Table 1)*.

Resistance to Cephalosporin Antibiotics and Phenotypic ESBL Detection

Antimicrobial susceptibility testing for cephalosporins was performed using disc diffusion assay. Discs containing Cefpodoxime (10 µg), Ceftaximide (30 µg), Aztreonam (30 µg), Cefotaxime (30 µg) and Ceftriaxone (30 µg) were used alone and in combination with Clavulanate (10 µg). The phenotypic ESBL confirmation was carried out with a disc combination test, in which the isolates having ≥5 mm larger inhibition zone around the disc containing ceftaximide/clavulanate and cefotaxime/clavulanate than the one without clavulanate were considered ESBL producer. The results interpreted according to the Clinical Laboratory Standards Institute guidelines [21].

Molecular Detection of Antimicrobial Resistance Genes

PCR assay was conducted to determine whether the isolates harboured β-lactamase *(bla_{AmpC}, bla_{TEM}, bla_{SHV}* and *bla_{CTX-M})* [22-24] and plasmid-mediated quinolone resistance (PMQR) *(qnrA, qnrB, qnrC, qnrS* and *aac*[6′]-*Ib)* genes [25-29]. Specific ESBL and PMQR gene types were identified by amplicon sequencing, and then by comparing the obtained sequences with those submitted the online database *(http://blast.ncbi.nlm.nih.gov)*.

Determination of Virulence Genes

A total of 12 *E. coli* virulence genes including *papAH, papC, papEF, papG* allele II, *papG* allele III, *papG* allele II-III, *kpsMT*

Table 1. *PCR conditions and primers used in this study*

Target Gene	Primer Sequence	Amp. Size (bp)	PCR Conditions	Ref.
chuA	F: 5′-ATGGTACCGGACGAACCAAC-3′	288	Initial denaturation at 94°C for 4 min, followed by 30 cycles at 94°C for 5 s and at 59°C 20 s and final extension at 72°C for 5 min	[19,20]
	R: 5′-TGCCGCCAGTACCAAAGACA-3′			
yjaA	F: 5′-CAAACGTGAAGTGTCAGGAG-3′	211		
	R: 5′-AATGCGTTCCTCAACCTGTG-3′			
TESPE4	F: 5′-CACTATTCGTAAGGTCATCC-3′	152		
	R: 5′-AGTTTATCGCTGCGGGTCGC-3′			
bla_{AmpC}	F: 5′-GCACTTAGCCACCTATACGGCAG-3′	758	Initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, at 60°C for 30 s and at 72°C for 30 s and final extension at 72°C for 10 min	[22]
	R: 5′-GCTTTTCAAGAATGCGCCAGG-3′			
$bla_{CTX\text{-}M}$	F: 5′- ATGTGCAGYACCAGTAARGTKATGGC-3′	593	Initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, at 60°C for 30 s and at 72°C for 30 s and final extension at 72°C for 10 min	[24]
	R: 5′-TGGGTRAARTARGTSACCAGAAYCAGCGG-3′			
bla_{TEM}	F. 5′-TGAGTATTCAACATTTCCGTGT-3′	861	Initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, at 53°C for 30 s and at 72°C for 30 s and final extension at 72°C for 10 min	[23]
	R: 5′-TTACCAATGCTTAATCAGTGA-3′			
bla_{SHV}	F: 5′-CAAAACGCCGGGTTATTC-3′	937		
	R: 5′-TTAGCGTTGCCAGTGCT-3′			
qnrB	F: 5′-GG**M**AT**H**GAAATTCGCCACTG-3′	262	Initial denaturation at 95°C for 5 min, followed by 32 cycles at 94°C for 45 s, at 53°C for 45 s and at 72°C for 60 s and final extension at 72°C for 10 min	[25]
	R: 5′-TTTGC**Y**G**YY**CGCCAGTCGAA-3′			
qnrS	F: 5′-TCGACGTGCTAACTTGCG-3′	466	Initial denaturation at 95°C for 5 min, followed by 32 cycles at 94°C for 45 s, at 53°C for 45 s and at 72°C for 60 s and final extension at 72°C for 10 min	[26]
	R: 5′-GATCTAAACCGTCGAGTTCGG-3′			
aac[6′]-Ib	F: 5′-TTGCGATGCTCTATGAGTGGCTA-3′	482	Initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, at 56°C for 30 s and at 72°C for 30 s and final extension at 72°C for 10 min	[29]
	R: 5′-CTCGAATGCCTGGCGTGTTT-3′			
papAH	F: 5′-ATGGCAGTGGTGTCTTTTGGTG-3′	720	Initial denaturation at 95°C for 15 min, followed by 25 cycles at 94°C for 30 s, at 63°C for 30 s and at 68°C for 3 min and final extension at 72°C for 10 min	[30]
	R: 5′-CGTCCCACCATACGTGCTCTTC-3′			
fimH	F: 5′-TGCAGAACGGATAAGCCGTGG-3′	508		
	R: 5′-GCAGTCACCTGCCCTCCGGTA-3′			
papEF	F: 5′-GCGCATTTGCTGATACTGTTG -3′	336		
	R: 5′-CATCCAGACGATAAGCATGAGCA-3′			
iutA	F: 5′-GGCTGGACATCATGGGAACTGG-3′	300		
	R: 5′-CGTCGGGAACGGGTAGAATCG-3′			
papG allele III	F: 5′-GGCCTGCAATGGATTTACCTGG-3′	258		
	R: 5′-CCACCAAATGACCATGCCAGAC-3′			
kpsMT K1	F: 5′-TAGCAAACGTTCTATATTGGTGC-3′	153		
	R: 5′-CATCCAGACGATAAGCATGAGCA-3′			
hlyA	F: 5′-AACAAGGATAAGCACTGTTCTGGCT-3′	1.177		
	R: 5′-ACCATATAAGCGGTCATTCCCGTCA-3′			
papC	F: 5′-GTGGCAGTATGAGTAATGACCGTTA-3′	200		
	R: 5′-ATATCCTTTCTGCAGGGATGCAATA-3′			
kpsMT II	F: 5′-GCGCATTTGCTGATACTGTTG-3′	272		
	R: 5′-CATCCAGACGATAAGCATGAGC-3′			
papG allele II	F: 5′-GGGATGAGCGGGCCTTTGAT-3′	190		
	R: 5′-CGGGCCCCCAAGTAACTCG-3′			
papG allele II-III	F: 5′-CTGTAATTACGGAAGTGATTTCTG-3′	1070		
	R: 5′-ACTATCCGGCTCCGGATAAACCAT-3′			
univenf	F: 5′-ATCTTATACTGGATGGGATCATCTTGG-3′	1.105		
	R: 5′-GCAGAACGACGTTCTTCATAAGTATC-3′			
ireA	F: 5′-AAGTCAAAGCAGGGGTTGCCCG-3′	665		
	R: 5′-GACGCCGACATTAAGACGCAG-3′			

*K*1, *kpsMT* II, *hlyA*, *fimH*, *iutA*, *ireA* and *univenf* were detected with multiplex PCR [30].

RESULTS

In this study coliform bacteria were detected in 99 of 100 chicken samples and 214 isolates recovered from 72/100 samples were identified as *E. coli*. Antibiotic susceptibility tests showed that 33 isolates recovered from 20/100 samples (15.42%) were resistant to at least one cephalosporin antibiotic, 20 isolates recovered from 15/100 (9.34%) were β-lactamase producing and 5 isolates recovered from 4/100 (2.33%) were ESBL producing *E. coli* phenotypically *(Table 2)*. Based on the phylogenetic PCR analysis developed by Clermont et al.[20], phylo-group D_2, A0, A1, B1 and D1 were detected in 13, 2, 2, 2 and 1 isolates, respectively.

PCR was used to determine the ESBL and PMQR genes among the β-lactamase producing *E. coli* isolates (n=20); *(Table 2)*. The bla_{TEM} and plasmid-mediated bla_{CMY} genes were detected in eight (%40) and 13 (65%) of the isolates, respectively. Additionally, ten isolates (50%) harboured ESBLs encoding genes, of which the $bla_{CTX\text{-}M}$ genes were detected in seven isolates, whereas the $bla_{SHV\text{-}12}$ gene was present in three isolates. CTX-M type enzymes were further identified as CTX-M-1 (n=3), CTX-M-8 (n=2) and CTX-M-55 (n=2).

Among the PMQR genes tested, the *qnrS* and *qnrB19* genes were detected in four (20%) and three isolates (15%), respectively *(Table 2)*. However, none of the isolates were found to possess the other PMQR genes *(qnrA, qnrC,* and *aac*[6']*-Ib)*.

Of the virulence genes 19 isolates were positive for *fimH* and 2 isolates were carrying *kpsMT* II *(Table 2)*. Other virulence genes tested were not found among the *E. coli* isolates.

DISCUSSION

Because of plasmid-mediated acquisition of ESBL producing genes by *Enterobacteriaceae* family, especially *E. coli*, there has been increased resistance to beta-lactam antibiotics, lately. Although majority of the ESBL producers related studies are confined within the hospital premises, recent reports indicating ESBL positive microorganisms' presence in chicken meat and raw milk shows that focus should also be given on their occurrence and dissemination of in food producing animals [31].

Cephalosporin antibiotics are one of the most important class of antibiotics in human medicine and the development of resistance to this class antibiotics have been identified as the major concern during the last decade. In this study, 214 isolates recovered from 72/100 samples were identified as *E. coli*. Antibiotic susceptibility tests showed that 33 isolates recovered from 20/100 meat samples (15.42%) were resistant to at least one cephalosporin antibiotic. In fact, the findings of this study are not surprising in view of the high frequency of resistance to β-lactam antibiotics

Table 2. *Characteristics of β-lactamase producing E. coli isolates≠*

Isolate No	Phylogenetic Type	Antibiotic Resistance Genes	Virulence Genes
22a*	D2	$bla_{CTX\text{-}M\text{-}1}$ + $qnrB_{19}$	*fim*H
51a*	D2	$bla_{CTX\text{-}M\text{-}55}$	*fim*H
KH51b*	D2	$bla_{CTX\text{-}M\text{-}55}$	*fim*H
93a*	B1	bla_{TEM}	*fim*H
96a*	D2	$bla_{CTX\text{-}M\text{-}1}$ + $qnrB_{19}$	*fim*H
2b	D2	$bla_{CTX\text{-}M\text{-}1}$ + $qnrB_{19}$	*fim*H
6c	A1	bla_{TEM} + bla_{CMY}	*fim*H
8a	A0	bla_{CMY}	nd
11b	D2	bla_{CMY}	*fim*H + kpsMT II
13a	A1	bla_{TEM} + $bla_{SHV\text{-}12}$ + bla_{CMY} + *qnrS*	*fim*H
22b	D2	$bla_{SHV\text{-}12}$ + bla_{CMY} + *qnrS*	*fim*H
KH22c	D2	$bla_{SHV\text{-}12}$ + bla_{CMY} + *qnrS*	*fim*H
33a	A0	bla_{CMY}	*fim*H
36a	D2	bla_{TEM} + $bla_{CTX\text{-}M\text{-}8}$ + bla_{CMY}	*fim*H
K36b	D2	bla_{TEM} + $bla_{CTX\text{-}M\text{-}8}$ + bla_{CMY}	*fim*H
KH36c	D2	bla_{CMY}	*fim*H
39b	D2	bla_{CMY}	*fim*H + kpsMT II
40b	D2	bla_{TEM}	*fim*H
99a	D1	bla_{TEM} + bla_{CMY}	*fim*H
KH99c	B1	bla_{TEM} + bla_{CMY}	*fim*H

*≠ All isolates are phenotypically resistant to Cefpodoxime, Ceftriaxone, Cefotaxime, Ceftaximide and Aztreonam; * indicating the phenotypic ESBL positive isolates,* ***nd:*** *not detected*

as Kürekci et al.[32] already reported that the frequency of ESBL producing *E. coli* isolates in raw chicken meat samples reached 86.6% in Turkey. In addition, cephalosporin resistance has been frankly linked to the heavy field use of these antibiotics in animal husbandry for many years, and the occurrences of resistance frequency was markedly dropped when the usage of ceftiofur in animal production was discontinued in Japan [33]. Therefore, the perpetuation of cephalosporin resistance phenomenon would be expected, even though the use of antibiotics has not been allowed as a growth promoting agent in animal industry in Turkey since 2006.

In this study, based on the phylogenetic PCR analysis developed by Clermont et al.[19], phylo-group D_2, A0, A1, B1 and D1 were detected in 13, 2, 2, 2 and 1 isolates, respectively. Kürekci et al.[32] also indicated that phylo-group D was the most prevalent in isolates (82.7%) followed by A (15.3%), and B_2 (only 1 isolate). However, according to Tansawai et al.[34] phylogenetic grouping of the isolates showed that 51% belonged to group A, 27.5% to group B1 and 17.6% to group D. Only 4 isolates (3.9%) belonged to group B2. In addition, twenty isolates were further identified as β-lactamase producer and tested in details. Among these isolates only 5 isolates were found to be phenotypically ESBL positive, whereas ten isolates possessed ESBLs enzymes. This is not surprising, as the discrepancies between methods have already been reported for determination of ESBL producers [35]. In addition, it has been proven that combination of these methods might produce false negative results among the isolates having inducible AmpC enzyme [36]. The findings of this study in terms of ESBL production among *E. coli* isolates are in agreement with other published results from different countries including Nigeria (0.98%) [37], South Korea (2.43%) [38] Germany (5.4%) [39], and India (5.69%) [31].

Of the β-lactamase producing *E. coli* isolates, 50% had ESBLs enzymes with CTX-M type being the predominant, and followed by SHV-12. Overdevest et al.[15] also noted that the predominant ESBL genotype in chicken meat they analysed was $bla_{CTX\text{-}M\text{-}1}$ (58.1%) followed by $bla_{TEM\text{-}52}$ (14%) and $bla_{SHV\text{-}12}$ (14%). Similar results were also reported by Garcia-Graells et al.[6], who reported the frequency of CTX-M (48%), TEM (28%) and SHV (24%) in commensal *E. coli* with an ESBL phenotype. In the current study, the $bla_{CTX\text{-}M\text{-}1}$ gene was found to be predominant, followed by the $bla_{CTX\text{-}M\text{-}8}$ and $bla_{CTX\text{-}M\text{-}55}$ genes. Kürekci et al.[32] also indicated the occurrence of the $bla_{CTX\text{-}M\text{-}55}$ among ESBL producing *E. coli* isolates obtained from raw chicken meat samples. The $bla_{CTX\text{-}M\text{-}8}$ gene has also been identified among raw chicken meat products in Turkey, with low frequency rate (2.8%) [40]. It is a well-known fact that PMQR genes are commonly found on the same mobile elements carrying ESBL resistance genes, in particular $bla_{CTX\text{-}M}$ [32]. In the current study, we also demonstrated the presence of the *qnrB* and *qnrS* genes together with ESBLs enzymes. Other scientists also reported that they detected quinolone resistance gene *(qnrB)* [31], *qnrS* and *qnrB* [32].

In our study, 19 isolates were positive for the *fimH* gene with together the *kpsMT* II gene (n=2). Kürekci et al.[32] also indicated that the *fimH, iutA, iroN, kpsMT II, papC, papG allelle II-III* and *papEF* genes were present among the ESBL producer *E. coli* isolates obtained from the chicken samples. Similarly, Kim et al.[38] found that three isolates they obtained harboured, *fimH, fyuA, iutA, papC, pap EF, papG* allele II*, rfc* and *traT* virulence genes.

Taken together, our results showed poultry meat as an important carrier of ESBL positive *E. coli,* although more studies are needed to understand the dissemination of these clones among the poultry products.

Statement of Author Contributions

NB defined the research theme, designed the experiment, contributed to cultural analyses and wrote the manuscript. ÇS and LV conducted the cultural analyses and have made supervised the analysis of the results. SPÖ conducted the molecular analyses and has made a substantial contribution to interpretation of data. All authors discussed the results and contributed to the final manuscript.

REFERENCES

1. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P: Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. *Antimicrob Agents Chemother*, 52 (4): 1238-1243, 2008. DOI: 10.1128/AAC.01285-07

2. Pitout JDD: Extraintestinal pathogenic *Escherichia coli*: A combination of virulence with antibiotic resistance. *Front Microbiol*, 3:9, 2012. DOI: 10.3389/fmicb.2012.00009

3. Clements A, Young JC, Constantinou N, Frankel G: Infection strategies of enteric pathogenic *Escherichia coli*. *Gut Microbes*, 3 (2): 71-87, 2012. DOI: 10.4161/gmic.19182

4. EFSA Panel on Biological Hazards (BIOHAZ): Scientific opinion on the public health risks of bacterial strains producing extended-spectrum beta-lactamases and/or AmpC β-lactamases in food and food producing animals. *EFSA J*, 9:2322, 2011. DOI: 10.2903/j.efsa.2011.2322

5. Hao H, Cheng G, Iqbal Z, Ai X, Hussain HI, Huang L, Dai M, Wang Y, Liu Z, Yuan Z: Benefits and risks of antimicrobial use in food-producing animals. *Front Microbiol*, 5:288, 2014. DOI: 10.3389/fmicb.2014.00288

6. Garcia-Graells C, Botteldoom N, Dierick K: Microbial surveillance of ESBL *E. coli* in poultry meat, a possible vehicle for transfer of antimicrobial resistance to humans. *WIV-ISP*, 13, 1-6, 2012.

7. Hawkey PM, Jones AM: The changing epidemiology of resistance. *J Antimicrob Chemother*, 64 (Suppl. 1): i3-i10, 2009. DOI: 10.1093/jac/dkp256

8. de Kraker MEA, Davey PG, Grundmann H: Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteraemia: Estimating the burden of antibiotic resistance in Europe. *PLoS Med,* 8 (10):e1001104, 2011. DOI: 10.1371/journal.pmed.1001104

9. Livermore DM: Has the era of untreatable infections arrived? *J Antimicrob Chemother,* 64 (Suppl. 1): i29-i36, 2009. DOI: 10.1093/jac/dkp255

10. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L, Haesebrouck F, Butaye P: Broad-spectrum β-lactamases among *Enterobacteriaceae* of animal origin: Molecular aspects, motility and impact on public health. *FEMS Microbiol Rev*, 34 (3): 295-316, 2010. DOI: 10.1111/j.1574-6976.2009.00198.x

11. Aslantaş Ö, Elmacıoğlu S, Yılmaz EŞ: Prevalence and characterization of ESBL- and AmpC-producing *Escherichia coli* from cattle. *Kafkas Univ Vet Fak Derg,* 23, 63-67, 2017. DOI: 10.9775/kvfd.2016.15832

12. Projahn M, von Tippelskirch P, Semmler T, Guenther S, Alter T, Roesler U: Contamination of chicken meat with extended spectrum beta-lactamases producing- *Klebsiella pneumoniae* and *Escherichia coli* during scalding and defeathering of broiler carcasses. *Food Microbiol,* 77, 185-191, 2019. DOI: 10.1016/j.fm.2018.09.010

13. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH: Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: A global perspective. *Clin Microbiol Infect,* 18, 646-655, 2012. DOI: 10.1111/j.1469-0691.2012.03850.x

14. Kluytmans JAJW, Overdevest ITMA, Willemsen I, Kluytmans-van den Bergh MFQ, van der Zwaluw K, Heck M, Rijnsburger M, Vanderbroucke-Grauls CMJE, Savelkoul PHM, Johnston BD, Gordon D, Johnson JR: Extended-spectrum beta-lactamase producing *Escherichia coli* from retail chicken meat and humans: Comparison of strains, plasmids, resistance genes, and virulence factors. *Clin Infect Dis*, 56, 478-487, 2013. DOI: 10.1093/cid/cis929

15. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey PM, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijdens X, Kluytmans J: Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerg Infect Dis*, 17 (7): 1216-1222, 2011. DOI: 10.3201/eid1707.110209

16. EFSA: Scientific opinion on the public health hazards to be covered by inspection of meat (poultry). *EFSA J*, 10 (6): 2741, 2012. DOI: 10.2903/j.efsa.2012.2741

17. Ben Said L, Jouni A, Klibi N, Dziri R, Alonso CA, Boudabous A, Ben Slama K, Torres C: Detection of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in vegetables, soil and water of the farm environment in Tunisia. *Int J Food Microbiol*, 203, 86-92, 2015. DOI: 10.1016/j.ijfoodmicro.2015.02.023

18. Fattahi F, Mirvaghefi A, Farahmand H, Rafiee G, Abdollahi A: Development of 16S rRNA targeted PCR method for detection of *Escherichia coli* in Rainbow Trout *(Onchorynchus mykiss). Iranian J Pathol*, 8 (1): 36-44, 2013.

19. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twenty-fourth informational supplement. M100-S24, 2014.

20. Clermont O, Bonacorsi S, Bingen E: Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol*, 66, 4555-4558, 2000.

21. Escobar-Páramo P, Le Menac'h A, Le Gall T, Amorin C, Gouriou S, Picard B, Skurnik D, Denamur E: Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. *Environ Microbiol*, 8, 1975-1984, 2006. DOI: 10.1111/j.1462-2920.2006.01077.x

22. Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM: β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in the Netherlands. *J Antimicrob Chemother*, 56, 115-121, 2005. DOI: 10.1093/jac/dki190

23. Leinberger DM, Grimm V, Rubtsova M, Weile J, Schröppel K, Wichelhaus TA, Knabbe C, Schmid RD, Bachmann TT: Integrated detection of extended-spectrum-beta-lactam resistance by DNA microarray-based genotyping of TEM, SHV, and CTX-M genes. *J Clin Microbiol,* 48, 460-471, 2010. DOI: 10.1128/JCM.00765-09

24. Mulvey MR, Soule G, Boyd D, Demczuk W, Ahmed R, Multiprovincial *Salmonella* Typhimurium Case Control Study Group: Characterization of the first extended-spectrum beta-lactamase-producing *Salmonella* isolate identified in Canada. *J Clin Microbiol,* 41, 460-462, 2003. DOI: 10.1128/JCM.41.1.460-462.2003

25. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P: Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother*, 60, 394-397, 2007. DOI: 10.1093/jac/dkm204

26. Cavaco LM, Frimodt-Møller N, Hasman H, Guardabassi L, Nielsen L, Aarestrup FM: Prevalence of quinolone resistance mechanisms and associations to minimum inhibitory concentrations in quinolone-resistant *Escherichia coli* isolated from humans and swine in Denmark. *Microb Drug Resist*, 14, 163-169, 2008. DOI: 10.1089/mdr.2008.0821

27. Cavaco LM, Hasman H, Xia S, Aarestrup FM: *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* Serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother*, 53, 603-608, 2009. DOI: 10.1128/AAC.00997-08

28. Jacoby GA, Gacharna N, Black TA, Miller GH, Hooper DC: Temporal appearance of plasmid-mediated quinolone resistance genes. *Antimicrob Agents Chemother*, 53, 1665-1666, 2009. DOI: 10.1128/AAC.01447-08

29. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC: Prevalence in the United States of *aac (6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother*, 50, 3953-3955, 2006. DOI: 10.1128/AAC.00915-06

30. Chapman TA, Wu XY, Barchia I, Bettelheim KA, Driesen S, Trott D, Wilson M, Chin JJ: Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. *Appl Environ Microbiol*, 72, 4782-4795, 2006. DOI: 10.1128/AEM.02885-05

31. Kar D, Bandyopadhyay S, Bhattcharyya D, Samanta I, Mahanti A, Nanda PK, Mondal B, Dandapat P, Das AK, Dutta TK, Bandyopadhyay S, Singh RK: Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. *Infect Genet Evol*, 29, 82-90, 2015. DOI: 10.1016/j.meegid.2014.11.003

32. Kürekci C, Osek J, Aydın M, Tekeli İO, Kurpas M, Wieczorek K, Sakin F: Evaluation of bulk tank raw milk and raw chicken meat samples as source of ESBL producing *Escherichia coli* in Turkey: Recent insights. *J Food Saf,* 39:e12605, 2019. DOI: 10.1111/jfs.12605

33. Shigemura H, Matsui M, Sekizuka T, Onozuka D, Noda T, Yamashita A, Kuroda M, Suzuki S, Kimura H, Fujimoto S, Oishi K, Sera N, Inoshima Y, Murakami K: Decrease in the prevalence of extended-spectrum cephalosporin-resistant *Salmonella* following cessation of ceftiofur use by the Japanese poultry industry. *Int J Food Microbiol*, 274, 45-51, 2018. DOI: 10.1016/j.ijfoodmicro.2018.03.011

34. Tansawai U, Sanguansermsri D, Na-udom A, Walsh TR, Niumsup PR: Occurrence of extended spectrum β-lactamase and AmpC genes among multidrug-resistant *Escherichia coli* and emergence of ST131 from poultry meat in Thailand. *Food Control,* 84, 159-164, 2018. DOI: 10.1016/j.foodcont.2017.07.028

35. Linscott AJ, Brown WJ: Evaluation of four commercially available extended-spectrum beta-lactamase phenotypic confirmation tests. *J Clin Microbiol*, 43 (3): 1081-1085, 2005. DOI: 10.1128/JCM.43.3.1081-1085.2005

36. Drieux L, Brossier F, Sougakoff W, Jarlier V: Phenotypic detection of extended-spectrum beta-lactamase production in *Enterobacteriaceae*: Review and bench guide. *Clin Microbiol Infect*, 14 (Suppl. 1): 90-103, 2008. DOI: 10.1111/j.1469-0691.2007.01846.x

37. Ojo OE, Schwarz S, Michael GB: Detection and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* from chicken production chains in Nigeria. *Vet Microbiol,* 194, 62-68, 2016. DOI: 10.1016/j.vetmic.2016.04.022

38. Kim YJ, Moon JS, Oh DH, Chon JW, Song BR, Lim JS, Heo EJ, Park HJ, Wee SH, Sung K: Genotypic characterization of ESBL-producing *E. coli* from imported meat in South Korea. *Food Res Int,* 107, 158-164, 2018. DOI: 10.1016/j.foodres.2017.12.023

39. Müller A, Jansen W, Grabowski NT, Monecke S, Ehricht R, Kehrenberg C: ESBL- and AmpC-producing *Escherichia coli* from legally and illegally imported meat: Characterization of isolates brought into the EU from third countries. *Int J Food Microbiol*, 283, 52-58, 2018. DOI: 10.1016/j.ijfoodmicro.2018.06.009

40. Tekiner İH, Özpınar H: Occurence and characteristics of extended spectrum beta-lactamase-producing *Enterobacteriaceae* from foods of animal origin. *Braz J Microbiol*, 47, 444-451, 2016. DOI: 10.1016/j.bjm.2015.11.034