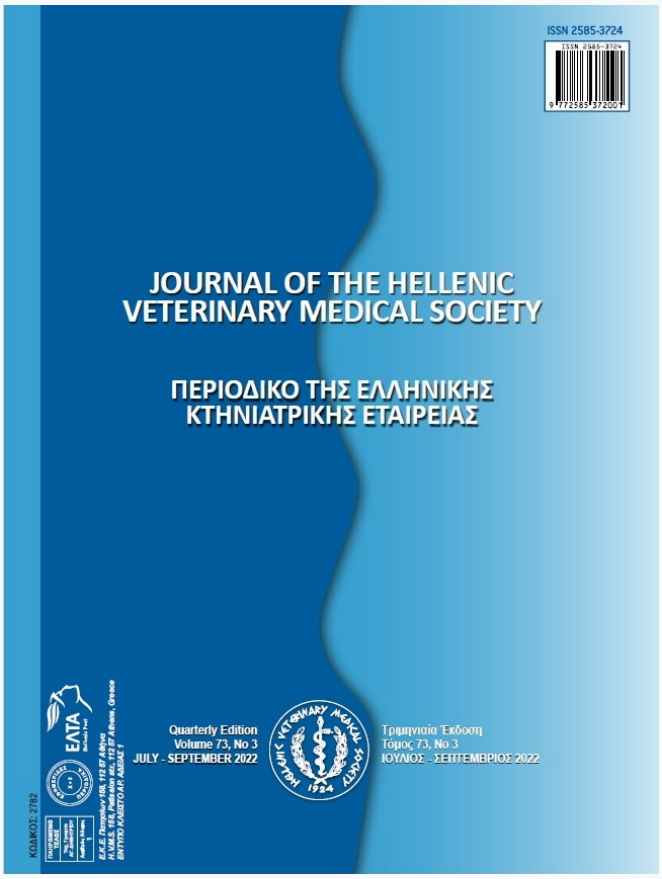
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



Prevalence, Characterization and PFGE profiles of multidrug resistance extended spectrum β-lactamase producing Escherichia coli strains in animal-derived food products from public markets in Eastern Turkey

T Cebeci

doi: 10.12681/jhvms.29251

To cite this article:

Cebeci, T. (2022). Prevalence, Characterization and PFGE profiles of multidrug resistance extended spectrum β-lactamase producing Escherichia coli strains in animal-derived food products from public markets in Eastern Turkey. *Journal of the Hellenic Veterinary Medical Society*, *73*(3), 4633–4644. https://doi.org/10.12681/jhvms.29251

J HELLENIC VET MED SOC 2022, 73 (3):4633-4644
ΠΕΚΕ 2022, 73 (3): 4633-4644

Research article
Ερευνητικό άρθρο

Prevalence, characterization, and PFGE profiles of multidrug-resistant, extended-spectrum β-lactamase-producing *Escherichia coli* strains in animal-derived foods from public markets in eastern Turkey

T. Cebeci*

Department of Medical Services and Techniques, Espiye Vocational School, Giresun University, Giresun,Turkey

ABSTRACT: The prevalence of multidrug-resistant (MDR), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) has increased markedly in recent years, posing a major challenge for antimicrobial treatment and raising concerns regarding the possible transfer of such bacteria through the food chain. The aim of this research was to investigate the prevalence of ESBL-producing *E. coli* strains in milk, cheese, and meat samples and to determine their virulence, pathotype, serotype, antibiotic resistance, and genetic relatedness. A total of 300 food samples were purchased from public markets in different districts of Giresun city. ESBL-producing *E. coli* strains were isolated from five of the samples (1.6%). β-lactamase-encoding genes of the CTX-M (20%), TEM (40%), and SHV (20%) groups were detected singly or in combination. The ESBL-producing *E. coli* isolates were further analyzed for the presence of virulence genes. The following virulence factor genes were detected: *hlyA* (20%), *ehlyA* (20%), *iucD* (40%), *iutA* (60%), *fimH* (100%), *kpsMTII* (100%) and *traT* (100%). Enterohemorrhagic *E. coli* (EHEC) was found as a predominant pathotype, with a frequency of 60%. No enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), diffuse adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAggEC3) were identified. Using polymerase chain reaction (PCR), genes encoding *flicH7* and *stx2* were found in one (20%) and three (60%) ESBL-producing *E. coli* isolates, respectively. In addition, genes encoding Shiga toxins were detected in three (60%) of the five isolates, which encoded the O128 serotype. Antibiotic susceptibility tests of the positive isolates showed resistance to cefuroxime, trimethoprim/sulfamethoxazole, cefazolin, streptomycin, ceftriaxone, tetracycline, ampicillin, and trimethoprim. Most ESBL-producing *E. coli* isolates showed 80% MDR phenotype against at least four classes of antibiotics. Specific PCR detection of antibiotic resistance genes showed the prevalence of the *tet*A gene in most isolates (80%), followed by *dfrA, qnr*, *aadA1,* and *sul1*. Pulsed-field gel electrophoresis (PFGE) results show that the isolates from different districts presented no clonal relatedness. This is the first report of the characteristics of MDR ESBL-producing shigatoxigenic (STEC) *E. coli* in dairy and meat products in a local city in Turkey. Our findings indicate that dairy and meat products could be reservoirs of MDR ESBL-producing STEC *E. coli* strains possessing several virulence factors, which may be a cause of concern for human health.

Keywords: *Escherichia coli*, extended-spectrum β-lactamase, food, MALDI-TOF MS, PFGE

Corresponding Author:
Tugba Cebeci, Department of Medical Services and Techniques, Espiye Vocational School, Giresun University, Giresun,Turkey
E-mail address: tgbcbcdmn@gmail.com

Date of initial submission: 13-01-2022
Date of acceptance: 02-05-2022

INTRODUCTION

Microbial resistance through extended-spectrum β-lactamase (ESBL) was first reported in Europe and the United States in the early 1980s after the introduction of third-generation cephalosporins in clinical practice (Saravanan et al., 2018). In 2019, the Centers for Disease Control and Prevention (CDC) designated ESBL-producing *Enterobacteriaceae* as pathogens that pose a serious threat to health (Frieden, 2013) and are responsible for infections worldwide, posing a challenge to clinicians for infection control (Rupp and Fey, 2003). *Escherichia coli* (*E. coli*) has become increasingly resistant to β-lactam antibiotics due to the production of ESBL (Kim et al., 2018). ESBL-producing Enterobacterales, especially *E. coli*, create a public health problem and present challenges to the healthcare industry. These bacteria are used extensively as key indicators for tracing the evolution of MDR bacteria. Because of the limited treatment options for these pathogens, they pose a serious threat to human health and can presumably pass through the food chain or by close contact to colonize human intestines (Carattoli et al., 2005; Ewbank et al., 2022).

Various genetic mechanisms result in antibiotic resistance from natural or acquired processes (Aibuedefe Osagie, 2019). Extended-spectrum β-lactamases (ESBLs) are generally encoded by genes that are found on large plasmids and they also convey genes related to resistance to other antimicrobial agents. These agents include aminoglycosides, trimethoprim, sulphonamides, tetracyclines, and chloramphenicol (Rawat and Nair, 2010). Due to increased mutations in the ESBL gene, as well as the industrial processing of animal-derived foods, infections have emerged that have impacted human health (Zurfluh et al., 2013).

Virulence characteristics and the possibility of producing certain illnesses are linked to the phylogenetic categories of pathogens. *E. coli* strains are divided into three categories: commensal, intestinal pathogenic (enteric or diarrheagenic), and extraintestinal pathogenic (ExPEC). Pathogenic *E. coli* clones have acquired particular virulence factors that enhance their capacity to adapt to different habitats and cause a wide variety of illnesses (Ombarak et al., 2016).

Among the *E. coli* groups isolated in foods and animals, those producing Shiga toxin and the Shiga-like verotoxin comprise the most important pathotype (Destro and Ribeiro, 2014). Resistant bacteria can carry other virulence-related genes, and it should be noted that strains producing Shiga toxin, found naturally in ruminants and considered foodborne pathogens, have been demonstrated as ESBL producers. This indicates the possibility that the ESBL plasmid can be transferred from commensals to foodborne pathogenic strains (Alegría et al., 2020). Ruminants are a major source of STEC, which is transmitted mainly by consumption of contaminated food, although it can also be transmitted through direct or indirect contact with contaminated people or animals (Valat et al., 2012). Foods that present a high risk for transmission include meat products such as minced meat, sausage, hamburger, and deli lunch meats, as well as dairy products. Serious foodborne outbreaks, most commonly associated with the consumption of uncooked meat or unpasteurized milk products, are often due to enterohemorrhagic serogroup O157 or other, non-O157 serogroups such as O26, O111, O103, and O145 (Nagy et al., 2015).

The presence of ESBL-producing *E. coli* (ESBL-EC) in dairy and meat products represents a significant public health issue. There is a little data with respect to the prevalence of ESBL-EC in Turkey. The aim of the study was to characterize ESBL-EC strains isolated from milk, cheese, and meat samples. Species identification (via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-MALDI-TOF MS) and genetic similarity analysis (pulsed-field gel electrophoresis, PFGE) of the isolates were performed. The ESBL-EC strains' phenotype and genotype for antibiotic resistance, as well as the pathotype and serotype of the isolated ESBL- EC strains, were determined.

MATERIALS AND METHOD

From May 2018 to May 2020, a total of 300 food samples of animal origin (100 samples of raw cow's milk, 100 samples of traditional tulum cheese, and 100 samples of ground beef) were randomly sampled from various public bazaars and butchers in the coastal districts of Giresun province. All samples were transported in an icebox to Espiye Vocational Laboratory, Giresun University, under refrigeration and within 2 hours for conventional microbiological analysis.

Microbiological analysis

Ten milliliters of raw milk was mixed with 90 mL of tryptic soy broth (TSB, Merck), and 25-gram samples of traditional tulum cheese and ground beef were respectively added to 225 mL TSB. The samples were mixed in a blender (Waring, New Hartford, Conn.)

and then incubated at 37 °C for 16 hours with shaking. Sample aliquots were streaked onto MacConkey agar (Merck) and eosin methylene blue (EMB) agar (Merck) plates, which were incubated at 37 °C for 18-24 h. The suspected isolates were biochemically identified (Ombarak et al., 2016).

Matrix-assisted laser desorption ionization time-of-flight confirmation of isolates

Bacterial isolates were identified by MALDI-TOF MS (bioMérieux, France) according to the manufacturer's recommendations (El Garch et al., 2018).

Phenotypic detection of ESBL production

ESBL-producing isolates were characterized phenotypically for ESBL production using the modified double disc synergy test (mDDST), using a disc of amoxicillin-clavulanate (20/10 μg) along with four cephalosporins, 3GC-cefotaxime, ceftriaxone, and 4GC-cefepime, as recommended by the Clinical and Laboratory Standards Institute (CLSI) performance standards. ESBL presence was confirmed by the combination disc test. CTX (30 μg), cefotaxime/clavulanic acid (CTL, 30 μg/10 μg), CAZ (30 μg) and ceftazidime/clavulanic acid (CAL, 30 μg/10 μg) discs were used for the phenotypic confirmatory test. For quality control purposes, *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were utilized (CLSI, 2017; Bardoň et al., 2013).

Antimicrobial susceptibility testing

To determine antibiotic resistance in the isolates; amikacin (AK, 30 μg), ampicillin (AM, 10 μg), cefazolin (CZ, 30 μg), ceftriaxone (CRO, 30 μg), cefuroxime (CXM, 30 μg), ciprofloxacin (CIP, 5 μg), ertapenem (ETP, 10 μg), fosfomycin (FOS, 200 μg), gentamicin (CN, 10 μg), imipenem (IPM, 10 μg), levofloxacin (LEV, 5 μg), meropenem (MEM, 10 μg), piperacillin/tazobactam (TPZ, 100/10 μg), polymixin B (PB, 300 U), streptomycin (S, 10 μg), tetracycline (TE, 30 μg), trimethoprim (TMP, 5 μg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg) antibiotic discs were used. Antibiotic resistance of *E. coli* isolates was investigated using the disc diffusion method according to the rules of the CLSI. After incubation, the diameters of the inhibition zones formed around the discs were measured and evaluated according to the zone table described in CLSI (CLSI, 2020). *E. coli* ATCC 25922 was used as the control strain.

DNA isolation protocols

Templates for PCR were prepared by the boiling method using the procedure described by Martínez-Vázquez et al. (2018).

PCR detection of genes for antimicrobial resistance and β-lactamase

In the present study, various PCR assays were used for the detection of antibiotic resistance genes (AGRs) and beta-lactamase genes of ESBL-EC isolates. All isolates were tested for the presence of β-lactamase (bla_{CTX-M}, bla_{OXA-2}, bla_{SHV}, bla_{TEM}), as well as the resistance genes against streptomycin (*aadA1*), tetracycline (*tetA*, *tetB*), trimethoprim (*dfrA1*), quinolones (*qnr*), gentamicin (*aac3-IV*), sulfonamide (*sul1*), ampicillin (*CITM*), and chloramphenicol (*cat1*, *cmlA*), by PCR using specific primers as described by a previous study (Ranjbar et al., 2018), with some modifications (Table 1).

Genotypic characterization of virulence, pathotype, and serotype genes

All ESBL isolates were screened for the presence of virulence genes, which include α-hemolysin (*hlyA*), specific uropathogenic protein (*usp*), autotransporter toxin (*sat*), *Yersinia* siderophore receptor (*fyuA*), salmochelin (*IroN*), aerobactin receptor (*iutA*), aerobactin (*iucD*), type 1 fimbriae (*fimH*), necrotizing cytotoxic factor type 1 (*cnf1*), serum resistance (*traT*), group II capsule synthesis (*kpsMTII*), p fimbria (*papGII*), enterohemolysin (*eae*); the pathotype genes intimin (*eae*), type IV bundle-forming pili (*bfp*), heat-labile toxin 1 (*Lt*), heat-stable toxin (*stII*), regulatory protein (*virF*), invasion plasmid antigen (*ipaH*), Dr adhesin family protein (*daaE*), aggregative adherence factor II (aafII), Shiga-like toxin genes (*stx1, stx1c, stx1d, stx2, stx2a, stx2c, stx2d, stx2e, stx2f, stx2g*), and the serotype genes (*O157, 045, 0103,026, 0111, 091, 0128, 0121, 0113, 045*) by PCR using the primers listed in Table 2 and Table 3. The methods of Su et al. (2016) and Ranjbar et al. (2018) were modified for genotyping the *E. coli* isolates.

Pulsed-field gel electrophoresis analysis

PFGE of XbaI (Takara Bio Inc., Shiga, Japan)-digested genomic DNA samples of the five ESBL-EC isolates was carried out in a CHEF DR-III electrophoresis chamber (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the instruction manual, and band profile analyses were performed by the Public Health Institution of Turkey (Ankara), as described

Table 1. Oligonucleotide primers for antimicrobial resistance and β-lactamase genes

Antimicrobial agent	Target gene	Primer sequence (5'-3')	Fragment size (pb)	$T_{annealing}$ (°C)
Streptomycin	aadA1	F: TAT CCA GCT AAG CGC GAA CT R: ATT TGC CGA CTA CCT TGG TC	1177	56
Tetracycline	tetA	F: GGT TCA CTC GAA CGA CGT CA R: CTG TCC GAC AAG TTG CAT GA	878	56
Tetracycline	tetB	F: CCT CAG CTT CTC AAC GCG TG R: GCA CCT TGC TGA TGA CTC TT	815	53
Trimethoprim	dfrA1	F: GGA GTG CCA AAG GTG AAC AGC R: GAG GCG AAG TCT TGG GTA AAA AC	992	60
Quinolones	qnr	F: GGG TAT GGA TAT TAT TGA TAA AG R: CTA ATC CGG CAG CAC TAT TTA	931	53
Gentamicin	aac3-IV	F: CTT CAG GAT GGC AAG TTG GT R: TCA TCT CGT TCT CCG CTC AT	688	56
Sulfonamide	sul1	F: TTC GGC ATT CTG AAT CTC AC R: ATG ATC TAA CCC TCG GTC TC	584	54
Chloramphenicol	cat1	AGT TGC TCA ATG TAC CTA TAA CC TTG TAA TTC ATT AAG CAT TCT GCC	270	55
Chloramphenicol	cmlA	CCG CCA CGG TGT TGT TGT TAT C CAC CTT GCC TGC CCA TCA TTA G	188	61
Ampicillin	CITM	F: TGG CCA GAA CTG ACA GGC AAA R: TTT CTC CTG AAC GTG GCT GGC	407	59
β-lactamase genes	blaTEM	F: TCCGCTCATGAGACAATAACC R: TTGGTCTGACAGTTACCAATGC	931	57
β-lactamase genes	blaSHV	F: TCG CCT GTG TAT TAT CTC CC R: CGC AGA TAA ATC ACC ACA ATG	768	55
β-lactamase genes	blaCTX-M	F: TCTTCCAGAATAAGGAATCCC R: CCGTTTCCGCTATTACAAAC	909	57
β-lactamase genes	blaOXA-2 group	F: AAGAAACGCTACTCGCCTGC R: CCACTCAACCCATCCTACCC	478	57

Table 2. Oligonucleotide primers for virulence factors and pathotype genes

PCR sets	Function of gene product	Target gene	Primer sequence (5'-3')	Fragment size (pb)	$T_{annealing}$ (°C)
Multiplex I	α-Hemolysin	*hlyA*	F: AACAA CGATA AGCAC TGTTC TGG R: CCATA TAAGC GGTCA TTCCC G	1177	60
	Yersinia siderophore receptor	*fyuA*	F: GGCTT TATCC TCTGG CCTT R: GAAAA CCCAG TCATC GGTGG	878	60
	Salmochelin	*iroN*	F: CTCTG GTGGT GGAAG CC R: TGTCG GTACA GGCGG TTC	815	60
	Specific uropathogenic protein	*usp*	F: GGAAA ATGGT CGCTC AGTGG R: CTGTA GTGAA TCTCA TCGTG TAGTC	992	60
	Autotransporter toxin	*sat*	F: TCAGA AGCTC AGCGA ATCAT TG R: CATTA TCACC AGTAA AACGC ACC	931	60
	Aerobactin receptor	*iutA*	F: CACTC CGGTA CTCCA GTCA R: CCTCC AACCA GATGT TCTTC G	688	60
	Aerobactin	*iucD*	F: CCGGA GAAGC CTGAA ATATA TTCA R: CCGGA TTGTC ATATG CAGAC C	584	60
Simplex	Enterohemolysin	*ehly*	F: CAA TGC AGA TGC AGA TAC CG R: CAG AGA TGT CGT TGC AGC AG	432	52

Multiplex II	Type 1 fimbriae	*fimH*	F: GTTTA TAATT CGAGA ACGGA TAAGC C R: GTGCA TAATT TGCCG TTAAT CCC	494	60
	Necrotizing cytotoxic factor type 1	*cnf1*	F: TTCTT CTGTA CTTCC CCCAG R: TGAGC GGCAT CTACT ATGAA GT	407	60
	Group II capsule synthesis	*kpMTII*	F: CATCA GACGA TAAGC ATGAG CA R: TGCGC ATTTG CTGAT ACTGT	270	60
	P fimbria	*papGII*	F: GGGCC CCCAA GTAAC TC R: GGATG AGCGG GCCTT TG	188	60
	Serum resistance	*traT*	F: CATAA CCACG GTTCA GCCAT C R: TTGCA CTGGT CAGTT CCAC	328	60
Specific PCR sets for EPEC	Intimin	*eaeA*	F: ATTACTGAGATTAAGGCTGAT R: ATTTATTTGCAGCCCCCCAT	482	53
	Type IV bundle-forming pili	*bpf*	F: GGAAG TCAAA TTCAT GGGGG TAT R: GGAAT CAGAC GCAGA CTGGT AGT	300	58
Specific PCR sets for ETEC	Heat-labile toxin 1	*Lt*	FGCACA CGGAG CTCCT CAGTC R: TCCTT CATCC TTTCA ATGGC TTT	218	56
	Heat-stable toxin 1	*stII*	F: AAAGG AGAGC TTCGT CACAT TTT R: AATGT CCGTC TTGCG TTAGG AC	129	56
Specific PCR sets for EIEC	Regulatory protein	*virF*	F: AGCTC AGGCA ATGAA ACTTT GAC R: TGGGC TTGAT ATTCC GATAA GTC	618	60
	Invasion plasmid antigen	*ipaH*	F:CTCGG CACGT TTTAA TAGTC TGG R: GTGGA GAGCT GAAGT TTCTC TGC	933	60
Specific PCR sets for DAEC	Dr. adhesin family protein	*daaE*	F: GAACG TTGGT TAATG TGGGG TAA R: TATTC ACCGG TCGGT TATCA GT	542	57
Specific PCR sets for EAggEC3	Aggregative adherence factor II	*aafII*	F: CACAG GCAAC TGAAA TAAGT CTGG R: ATTCC CATGA TGTCA AGCAC TTC	378	58
Specific PCR sets for EHEC	Shiga-like toxin genes	*stx1*	F:AAA TCG CCA TTC GTT GAC TAC TTC T R: TGC CAT TCT GGC AAC TCG CGA TGC A	366	58
		stx2	F: CGA TCG TCA CTC ACT GGT TTC ATC A R: GGA TAT TCT CCC CAC TCT GAC ACC	282	58
		stc1c	F: TTTTCACATGTTACCTTTCCT R: CATAGAAGGAAACTCATTAGG	498	56
		stx1d	F: CTTTTCAGTTAATGCGATTGCT R: AACCCCATGATATCGACTGC	192	56
		stx2a	F: GCGATACTGRGBACTGTGGCC R: CCGKCAACCTTCACTGTAAATGTG	349	65
		stx2c	F: GCGGTTTTATTTGCATTAGT R: AGTACTCTTTTCCGGCCACT	124	56
		stx2d	F: GGTAAAATTGAGTTCTCTAAGTAT R: CAGCAAATCCTGAACCTGACG	175	56
		stx2e	F: ATGAAGAAGATGTTTATAGCG R: TCAGTTAAACTTCACCTGGGC	267	56
		stx2f	F: AGATTGGGCGTCATTCACTGGTTG R: TACTTTAATGGCCGCCCTGTCTCC	428	56
		stx2g	F: GTTATATTTCTGTGGATATC R: GAATAACCGCTACAGTA	573	56

DAEC: diffuse adherent *E. coli*; EAggEC3: enteroaggregative *E. coli*; EHEC: enterohemorrhagic *E. coli*; EIEC: enteroinvasive *E. coli*; EPEC: enteropathogenic *E. coli*; ETEC: enterotoxigenic *E. coli*.

Table 3. Oligonucleotide primers for serotype genes

Target gene	Primer sequence (5'-3')	Fragment size (bp)	$T_{annealing}$ (°C)
0145	F: CCA TCA ACA GAT TTA GGA GTG R: TTT CTA CCG CGA ATC TAT C	609	51
0103	F: TTG GAG CGT TAA CTG GAC CT R: GCT CCC GAG CAC GTA TAA G	321	56
026	F: CAG AAT GGT TAT GCT ACT GT R: CAG AAT GGT TAT GCT ACT GT	423	52
0111	F: TAG AGA AAT TAT CAA GTT AGT TCC R: ATA GTT ATG AAC ATC TTG TTT AGC	406	53
091	F: GCT GAC CTT CAT GAT CTG TTG A R: TAA TTT AAC CCG TAG AAT CGC TGC	291	57
0128	F: GCT TTC TGC CGA TAT TTG GC R: CCG ACG GAC TGA TGC CGG TGA TT	289	61
0121	F: TGG CTA GTG GCA TTC TGA TG R: TGA TAC TTT AGC CGC CCT TG	322	56
0113	F: GGG TTA GAT GGA GCG CTA TTG AGA R: AGG TCA CCC TCT GAA TTA TGG CAG	771	62
045	F: CCG GGT TTC GAT TTG TGA AGG TTG R: CAC AAC AGC CAC TAC TAG GCA GAA	527	62
O157	F:CGG ACA TCC ATG TGA TAT GG R: TTG CCT ATG TAC AGC TAA TCC	259	55
fliCh7	F: GCG CTG TCG AGT TCT ATC GAG C R: CAA CGG TGA CTT TAT CGC CAT TCC	625	62

previously (Durmaz et al., 2009). The TIFF images obtained from PFGE were analyzed with the BioNumerics version 7.5 software package (Applied Maths, Sint-Martens Latem, Belgium). Cluster analysis of Dice similarity indices based on the unweighted pair-group method with arithmetic mean (UPGMA) was utilized to generate the dendrogram illustrating the relationship between PFGE profiles. Isolates with Dice similarity index of ≥80% were classified into the same PFGE cluster. PFGE was done using *Salmonell*a Braenderup H9812 as the molecular weight marker.

RESULTS

ESBL-EC isolates in samples

The overall prevalence of *E. coli* in the commercially available milk, cheese, and meat samples from eight coastal districts in Giresun was found to be 43.3% (130/300). Of the 130 *E. coli* isolates, five (3.8%) were found to be ESBL producing; therefore, the overall ESBL-EC prevalence was 5/300 (1.6%).

E. coli was most common in cheese (53/100 samples, 53%) and meat (52/100 samples, 52%). A much lower prevalence was found in milk (25/100 samples, 25%). ESBL-EC was found in cheese (2/100, 2% of cheese samples), meat (2/100, 2% of meat samples), and milk (1/100, 1% of milk samples) (Table 4).

Phenotypic antibiotic resistance

In this study, ESBL-EC isolates indicated resistance to multiple antimicrobial agents. Five (3.8%) of the *E. coli* isolates were identified as ESBL-EC by antimicrobial susceptibility testing (AMC, CAZ, CTX, ATM, and FEP resistance). The resistance patterns of *E. coli* towards the tested antimicrobial agents are presented in Table 5. All isolates were resistant to CZ, CRO, and CXM. The phenotypic resistance profiles of the ESBL-EC isolates are as follows: AM, 80%; S, 80%; TE, 60%; TMP, 80%; SXT, 80%. No resistance to AK, CIP, ETP, FOS, CN, IPM, LEV, MEM, TPZ, and PB was observed in the *E. coli* isolates.

ESBL and antibiotic resistance genes

All ESBL-EC isolates in this study carried more than one of the tested antimicrobial resistance genes. Table 5 displays the number of isolates carrying the various resistance gene combinations. Five different antimicrobial resistance gene patterns were identified. One isolate additionally carried the β-lactamase genes bla_{TEM}, bla_{CTX-M}, and bla_{SHV}. The gene *dfrA1*, conferring resistance to trimethoprim, was observed in four isolates. Resistance genes against tetracyclines were found in four isolates. Among those, two carried *qnr* and one carried *aadA1*; neither carried both. Only one isolate carried *sul1*, conferring resistance to sulfonamide.

Table 4. Presence of *E. coli* and ESBL producing *E. coli* in food samples

Food samples	*E. coli*	ESBL-producing *E. coli*								
		Districts								
		Total	Piraziz n=24	Bulancak n=58	Central n=57	Keşap n=27	Espiye n=45	Tirebolu n=32	Görele n=33	Eynesil n=24
raw milk n=100	25	1	0	0	0	0	0	0	0	1
cheese n=100	53	2	0	1	0	0	1	0	0	0
ground beef n=100	52	2	0	0	1	0	0	1	0	0
Total N=300	130	5	0	1	1	0	1	1	0	1

Table 5. Phenotypic and genotypic resistance patterns of ESBL-producing *E.coli* isolated from food samples

Strain	Food sample	Multidrug resistance profile for antibiotics	Beta-lactam genes	Non-beta-lactam genes
M-1	Milk	CXM, SXT, CZ, S, CRO, TE, AM, TMP	bla_{TEM}	*qnr*, *tetA*, *dfrA1*
C-1	Cheese	CXM, SXT, CZ, S, CRO, TE, AM, TMP	bla_{TEM}, bla_{CTX-M}, bla_{SHV}	*qnr*, *tetA*
C-2	Cheese	CXM, SXT, CZ, S, CRO, TE, AM, TMP	-	*tetA*, *aadA1*
G-1	Ground beef	CXM, SXT, CZ, S, CRO, AM, TMP	-	*sul1*, *tetA*, *dfrA1*
G-2	Ground beef	CXM, CZ, CRO	-	*dfrA1*

AM: ampicillin; CRO: ceftriaxone; CXM: cefuroxime; CZ: cefazolin; S: streptomycin; SXT: trimethoprim/ sulfamethoxazole; TE: tetracycline; TMP: trimethoprim

Table 6. Prevalence of virulence, pathotype and serotype genes in ESBL-producing *E. coli* isolates

Strain	Pathotype	Positive isolates									
		Virulence genes								Serotype	
		hlyA	*ehlyA*	*iutA*	*iucD*	*fimH*	*kpsMTII*	*traT*	*stx2*	*flicH7*	*0128*
M-1	EHEC	-	-	-	-	+	+	+	+	-	+
C-1	EHEC	+	+	+	+	+	+	+	+	-	+
C-2	-	-	-	+	+	+	+	+	-	-	ONT
G-1	EHEC	-	-	-	-	+	+	+	+	-	+
G-2	-	-	-	+	-	+	+	+	-	+	ONT

ONT = O serogroup non-typable, EHEC = enterohemorrhagic *E. coli*

Virulence genes

The presence of virulence factor genes in the five isolates is shown in Table 6. The most common virulence factor profiles, *fimH*, *kpsMTII,* and *traT,* were found in five isolates (100%). Siderophore genes *iutA* and *iucD* were detected in three (60%) and two isolates (40%), respectively. One isolate (20%) was shown to contain the *hlyA*-encoding gene. No isolates harbored *fyuA*, *iroN*, *papGII, usp*, *sat*, *cnf1,* and *eaeA* genes.

Pathotype genes

A total of five ESBL-EC strains were evaluated by PCR to detect 18 virulence genes and to classify the pathotypes (Table 2). Only EHEC strains were found to have the respective virulence genes. The incidence of each virulence gene among ESBL-EC isolates from milk, cheese, and meat is shown in Table 6. EHEC was detected in three isolates (60%) by the presence of the gene for Shiga-like toxin 2 (*stx2*), while the pathotype of the other two ESBL-EC isolates was not determined.

Serotype genes

The specific genotypes of the ESBL-EC strains, with respect to the virulence genes tested, namely, Shiga-like toxin genes (*stx1, stx1c, stx1d, stx2, stx2a, stx2c, stx2d, stx2e, stx2f, stx2g*) and flagellar antigen (*flicH7*) are shown in Table 6. By applying specific oligonucleotide primers for the detection of STEC

serogroups in food samples (Table 3), it was found that three (60%) samples were positive for O128 serogroup, while none of the other serogroups tested (O26, O103, O111 and O145) was detected among the strains. Three of the five isolates (60%) were shown to carry the *stx2* gene. O128 strains were isolated from two dairy samples, while the remaining strain was isolated from meat samples.

PFGE analysis

XbaI-PFGE (Figure 1) revealed that the five ESBL-producing strains are classified into five genotypes, indicating nonclonal transmission.

DISCUSSION

The current research focuses especially on the variety of antimicrobial resistance, molecular lineages, virulence factors, pathotypes, and serogroups of ESBL-EC isolated in Giresun from raw cow's milk, traditional tulum cheese, and ground beef. To our knowledge, this is the first study of ESBL-EC in the milk, cheese, and meat samples from public markets in Giresun, Turkey. ESBL producers comprise one of the most important groups of bacteria, and their presence in food animals facilitates the transfer of antimicrobial resistance through the food chain to humans (Wasiński et al., 2014). We assessed the potential spread of ESBL-EC through milk, tulum cheese, and meat, which are consumed by a large share of the population in Giresun province. In this study, the incidence of ESBL-EC contamination (3.42-54.54%) was reported for milk, cheese, and meat from retail sales, adding to existing studies from various countries, including Turkey (Pehlivanlar Önen et al., 2015), India (Batabyal et al., 2018), and Brazil (Parussolo et al., 2019). Data available on the prevalence of ESBL-EC in milk, cheese, and meat are diverse. Variations in the prevalence rates of ESBL-EC from milk, cheese, and meat may reflect differences in the production, transport, and hygiene conditions during food handling. On the other hand, various factors, including the sample size, detection method, and food origin must be taken into consideration.

The production of ESBLs constitutes an important resistance mechanism impeding the antimicrobial treatment of infections caused by *Enterobacteriaceae* and poses a significant risk for antibiotics that are currently available (Shaikh et al., 2015). Furthermore, the consumption of, or contact with, animal products increases consumers' exposure to resistant bacteria (Ma et al., 2020). In this study, ESBL-producing strains were tested for susceptibility to 18 antimicrobial drugs of veterinary and human health significance. ESBL-producing strains were examined in terms of the antimicrobial susceptibility testing, revealing that a significant proportion of isolates was resistant to various drugs, particularly the cephalosporins (CXM, CZ, and CRO), sulfonamides (TMP, SXT), β-lactams (AM), aminoglycosides (S), and tetracyclines (TE). Very high resistance to CXM (100%), KF (100%), S (100%), SXT (85.7%), AM (85.7%) and TE (85.7%) was noted by Pehlivanlar Önen et al. (2015) in strains of ESBL-EC isolated from beef meat in Turkey. Parussolo et al. (2019) reported 100% resistance to CRO and 100% resistance to CTX in a group of four strains of ESBL-EC isolated from milk and cheese in Brazil. By contrast, Abayneh et al. (2019) demonstrated no resistance to third-generation cephalosporin in ESBL-EC isolated from meat samples in Ethiopia. The results reveal the high rate of increase in multidrug resistance. These fluctuations can be expected to vary

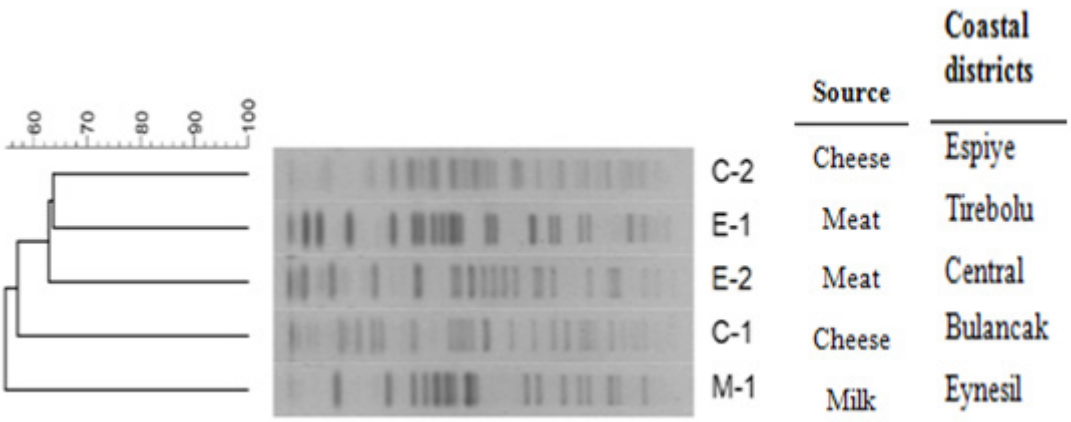


Figure 1. Pulsed-field gel electrophoresis of *Xba*I-digested DNA from the five ESBL-producing *E. coli* isolates

between countries because of the differences in terms of antibiotic use.

Together with the increasing use of antibiotics in agriculture and clinics, the number of ARGs has increased, and the gradually rising number and spread of MDR bacterial strains can be considered as a global health threat (Tóth et al., 2019). Transferability of antimicrobial resistance (AR) between bacteria on mobile genetic elements (MGEs) may result in rapid development of MDR in bacteria from animals, creating a foodborne risk to human health (Aibuedefe Osagie, 2019). The likely primary mechanism of transmission of ARGs among food animal and human populations is by horizontal gene transfer (Tóth et al., 2019). This study found that *tetA* (80%) was the most common resistance gene, followed by *dfrA1* (60%) and *qnr* (40%), whereas *aadA1* (20%) and *sul1* (20%) were the least common resistance genes, which is partially consistent with phenotypic findings. Similarly, Skočková et al. (2015) have tested *E. coli* species for *tetA*, *tetB*, *tetC*, *tetG*, *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')Ib*. The most prevalent genes are the *tet* genes, which were found in 12.5% of isolates.

The places where foods are processed can be seen as important reservoirs and vectors in terms of antimicrobial-resistant bacteria. Furthermore, food handlers also constitute a risk factor regarding the transmission of ESBL-producing bacteria (Lavilla et al., 2008). It is known that ESBLs are encoded by plasmids and MGEs, and they are also transferred to other bacteria (Tekiner and Özpınar, 2016). ESBLs, including SHV, TEM, and CTX-M variants, are among the most widely detected β-lactamases found in *Enterobacteriaceae*, particularly in *E. coli* and *K. pneumoniae* (Ouchar Mahamat et al., 2021). TEM- and SHV-type enzymes previously predominated the ESBL strains worldwide; today, CTX-M-type enzymes are the most commonly found. Of these, the CTX-M-15 variant is predominant worldwide, followed in prevalence by CTX-M-14; an emergent variant is CTX-M-27, observed in some parts of the world (Bevan et al., 2017; Castanheira et al., 2021). In this study, TEM was the dominant genotype, followed by CTX-M and SHV in milk and cheese isolates. The OXA-encoding gene was not detected in all five isolates. Different countries have reported different predominant ESBL genotypes of *E. coli* in milk and cheese samples, including CTX-M genotype in India (Batabyal et al., 2018) and TEM genotype in Brazil (Parussolo et al., 2019). Studies showing the presence of AGR genes and beta-lactamase resistance genes of *E. coli* cultured from food samples, performed in different countries, have varying results, which could be due to different geographical regions or the different practices in using antibiotics or antimicrobial agents, for example, in feed supplements. The selective pressure from such administration causes the evolution of antibiotic resistance in the microbial isolates due to the acquisition of antibiotic-resistance genes. While some of these genes are silent in bacteria in vitro, these silent genes can spread to other bacteria or "turn on" in vivo, especially under antimicrobial pressure.

The relationship between virulence factors and mortality in ESBL-EC bacteremia has been examined only in very few studies (Hung et al., 2019). Virulence factors play an important role in the epidemiology and pathophysiology of *E. coli* infections (Kluytmans et al., 2013). Besides the fact that pathotypes of *E. coli* are known to cause intestinal diseases, their accumulation, disease outbreaks, and clinical outcomes present an increasingly important health issue. Previous studies reported that *E. coli* pathotypes keep transmitting and evolving among populations in ruminants, food, water, and humans (Govindarajan et al., 2020). In the present study, three out of five ESBL-EC isolates (60%) were EHEC, including three O128 isolates. In PCR analysis of the isolates, three *stx2* strains were found. Overall, five isolates tested positive for one or more of the VF genes. Among them, the prevalence of individual VF genes was 100% for *fimH,* 100% for *traT,* 100% for *kpsMTII*, 60% for *iutA*, 60% for *stx2*, 40% for *iucD*, 20% *hlyA*, 20% *ehlyA,* and 20% *flicH7.* Our results are in agreement with a study by Kim et al. (2018) in South Korea, where *fimH, iutA,* and *traT* were observed in 20%, 15%, and 15% of ESBL-producing *E. coli* isolates, respectively, and is also similar to a study reported by Kürekci et al. (2019) in Turkey, where *fimH*, *iutA*, and *kpsMT II* were detected in 96.1%, 59.6% and 9.6% of ESBL-producing *E. coli* isolates, respectively. In contrast, in another study conducted by Ribeiro et al. (2016), *tsh* was detected in one isolate of ESBL-EC. ESBL-EC strains carrying virulence genes encoding toxins similar to those of other pathogenic *E. coli*, such as STEC (which produces ESBL enzymes), are reported to be rare but extremely dangerous (Frank et al., 2011). Regarding the serotyping of the isolated ESBL-EC strains, the five *E. coli* isolates from milk, cheese, and meat samples were subjected to serological identification. Three strains (60%) belonged to one serogroup, and the most predominant serogroup was O128 (60%),

while two *E. coli* strains (40%) were untypable. In previous studies, the most frequent serotypes in ESBL-EC from food samples were O128, O157, O26, O103, O111, O145, O45, O91, and O113 (Momtaz et al., 2013); O121, O128, O78, O91, and O26 (Younis et al., 2017); and O2, O9, O20, O68, and O157 (Ntuli et al., 2017). Variation in the prevalence of virulence and serotype genes from ESBL-EC reported in other studies may be the result of different sampling methods employed, types of samples, isolation procedures, the environmental conditions, or geographical locations.

The dendrogram analysis performed using the five ESBL-EC isolates classified the samples into five species-specific groups, with a total of five profiles. Previous studies reported the genetic heterogeneity among strains isolated in food samples. Using PFGE, Ntuli et al. (2017) reported five clusters (A-F) with 75% similarity among 25 ESBL-EC strains isolated from food in South Africa. Cluster group E was dominated by serotype O9 (66.6%). Moreover, the common phenotypes found in clusters C, E, and F were determined to be resistant to CPD-CAZ, ATM-CPD, and ATM-CPD-CAZ, respectively.

CONCLUSION

This study is the first to present a detailed description of the possible contamination of dairy and meat products with ESBL-EC and ESBL-producing STEC in Giresun, Turkey. In this study, a limited number of ESBL-EC were isolated, and no clonal spread of ESBL-producing isolates contaminating food samples was observed. Thus, more comprehensive research using a larger sample will provide further details. Moreover, better understanding of the epidemiology and genetic structure of ESBL-EC will also be useful in analyzing the origin. Another important point is that the antimicrobial resistance surveillance programs examining the ESBL-EC (including STEC) should be conducted in both clinical and agricultural fields. The findings to be obtained from these analyses will provide additional information in developing control strategies.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Barış OTLU (Malatya Inonu University, Turkey) for helping me to analyze PFGE banding pattern on software.

REFERENCES

Abayneh M, Tesfaw G, Woldemichael K, Yohannis M, Abdissa A (2019) Assessment of extended-spectrum β-lactamase (ESBLs) - producing *Escherichia coli* from minced meat of cattle and swab samples and hygienic status of meat retailer shops in Jimma town, Southwest Ethiopia. BMC Infect Dis 19:1-8.

Aibuedefe Osagie E (2019) Multiple Drug Resistance: A Fast-Growing Threat. Biomed J Sci Tech Res 21:2574-1241.

Alegría Á, Arias-Temprano M, Fernández-Natal I, Rodríguez-Calleja JM, García-López M, Santos JA (2020) Molecular diversity of ESBL-producing *Escherichia coli* from foods of animal origin and human patients. Int J Environ Res Public Health 17: 1312.

Bardoň J, Husičková V, Chromá M, Kolář M (2013) Prevalence and characteristics of *Escherichia coli* strains producing extended-spectrum β-lactamases in slaughtered animals in the Czech Republic. J Food Prot 76:1773-1777.

Batabyal K, Banerjee A, Pal S, Dey S, Joardar SN, Samanta I, Isore DP, Singh AD (2018) Detection, characterization, and antibiogram of extended-spectrum beta-lactamase *Escherichia coli* isolated from bovine milk samples in West Bengal, India. Vet world 11:1423-1427.

Bevan ER, Jones AM, Hawkey PM (2017) Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145-2155.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219-228.

Castanheira M, Simner PJ, Bradford PA (2021) Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resist 3:dlab092.

Clinical and Laboratory Standard Institute (2017) Performance standards for antimicrobial susceptibility testing; 27th Informational Supplement CLSI supplement M100-A27. Wayne, PA: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standard Institute (2020) Performance Standards for Antimicrobial Susceptibility Testing. 30th Informational Supplement CLSI supplement M100-A30. Wayne, PA: Clinical and Laboratory Standards Institute.

Destro MT, Ribeiro VB (2014) Foodborne zoonoses. In: Encyclopedia of Meat Sciences. Academic Press, Cambridge, MA, USA: pp 17-21

Durmaz R, Otlu B, Koksal F, Hosoglu S, Ozturk R, Ersoy Y, Aktas E, Gürsoy NC, Calıskan A (2009) The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, Escherichia coli and *Klebsiella* spp. Jpn J Infect Dis 62:372-7.

El Garch F, de Jong A, Bertrand X, et al (2018) mcr-1-like detection in commensal *Escherichia coli* and *Salmonella* spp. from food-producing animals at slaughter in Europe. Vet Microbiol 213:42-46.

Ewbank AC, Fuentes-Castillo D, Sacristán C, Cardoso B, Esposito F, Fuga B, de Macedo EC, Lincopan N, Catão-Dias JL (2022) Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* survey in wild seabirds at a pristine atoll in the southern Atlantic Ocean, Brazil: First report of the O25b-ST131 clone harboring blaCTX-M-8. Sci Total Environ 806:150539.

Frank C, Werber D, Cramer JP, Askar M, Faber M, Heiden M, Bernard H, Fruth A, Prager R, Spode A, Wadl M, Zoufaly A, Jordan S, Kemper MJ, Follin P, Müller L, King LA, Rosner B, Buchholz U, Stark K, Krause G (2011) Epidemic Profile of Shiga-Toxin-Producing *Escherichia coli* O104:H4 Outbreak in Germany . N Engl J Med 365:1771-1780.

Frieden T (2013) CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019. Centers Dis. Control Prev.

Govindarajan DK, Viswalingam N, Meganathan Y, Kandaswamy K (2020) Adherence patterns of *Escherichia coli* in the intestine and its role in pathogenesis. Med. Microecol. 5:100025.

Hung WT, Cheng MF, Tseng FC, Chen YS, Lee SSJ, Chang TH, Lin HH, Hung CH, Wang JL (2019) Bloodstream infection with extended-spectrum beta-lactamase-producing *Escherichia coli*: The role of virulence genes. J Microbiol Immunol Infect 52:947-955.

Kim YJ, Moon JS, Oh DH, et al (2018) Genotypic characterization of ESBL-producing *E. coli* from imported meat in South Korea. Food Res Int 107:158-164.

Kluytmans JAJW, Overdevest ITMA, Willemsen I, Bergh MFQK, Zwaluw K, Heck M, Rijnsburger M, Vandenbroucke-Grauls CMJE, Savelkoul PHM, Johnston BD, Gordon D, Johnson JR (2013) Extended-spectrum β-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.

Kürekci C, Osek J, Aydın M, et al (2019) 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.

Lavilla S, González-López JJ, Miró E, Tekeli İO, Kurpas M, Wieczorek K, Sakin F (2008) Dissemination of extended-spectrum β-lactamase-producing bacteria: The food-borne outbreak lesson. J Antimicrob Chemother 61:1244-1251.

Ma F, Xu S, Tang Z, Li Z, Zhang L (2020) Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. medcentral.net 3:32-38.

Martínez-Vázquez AV, Rivera-Sánchez G, Lira-Méndez K, Reyes-López MA, Bocanegra-García V (2018) Prevalence, antimicrobial resistance and virulence genes of *Escherichia coli* isolated from retail meat in Tamaulipas, Mexico. J Glob Antimicrob Resist 14:266-272.

Momtaz H, Safarpoor Dehkordi F, Rahimi E, Ezadi H, Arab R (2013) Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat. Meat Sci 95:381-388.

Nagy B, Szmolka A, Smole Možina S, Kovač J, Strauss A, Schlager S, Beutlich J, Appel B, Lušicky M, Aprikian P, Pászti J, Tóth I, Kugler R, Wagner M (2015) Virulence and antimicrobial resistance determinants of verotoxigenic *Escherichia coli* (VTEC) and of multidrug-resistant *E. coli* from foods of animal origin illegally imported to the EU by flight passengers. Int J Food Microbiol 209:52-59.

Ntuli V, Njage PMK, Buys EM (2017) Extended-spectrum β-lactamase, shigatoxin and haemolysis capacity of O157 and non-O157 *E. coli* serotypes from producer-distributor bulk milk. Int Dairy J 66:126-134.

Ombarak RA, Hinenoya A, Awasthi SP, Iguchi A, Shima A, Elbagory ARM, Yamasaki S (2016) Prevalence and pathogenic potential of *Escherichia coli* isolates from raw milk and raw milk cheese in Egypt. Int J Food Microbiol 221:69-76.

Ouchar Mahamat O, Kempf M, Lounnas M, Tidjani A, Hide M, Benavides JA, Carrière C, Bañuls AL, Jean-Pierre H, Ouedraogo AS, Dumont Y, Godreuil S (2021) Epidemiology and prevalence of extended-spectrum β-lactamase and carbapenemase-producing *Enterobacteriaceae* in humans, animals and the environment in West and Central Africa. Int. J. Antimicrob. Agents 57:106203.

Parussolo L, Sfaciotte RAP, Dalmina KA, Melo FD, Da Costa UM, Ferraz SM (2019) Detection of virulence genes and antimicrobial resistance profiles of *Escherichia coli* isolates from raw milk and artisanal cheese in Southern Brazil. Semin Agrar 40:163-178.

Pehlivanlar Önen S, Aslantaş Ö, Şebnem Yilmaz E, Kürekci C (2015) Prevalence of β-lactamase Producing *Escherichia coli* from Retail Meat in Turkey. J Food Sci 80:M2023-M2029.

Ranjbar R, Safarpoor Dehkordi F, Sakhaei Shahreza MH, Rahimi E (2018) Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products. Antimicrob Resist Infect Control 7:1-11.

Rawat D, Nair D (2010) Extended-spectrum β-lactamases in Gram Negative Bacteria. J Glob Infect Dis 2:263.

Ribeiro LF, Barbosa MMC, Pinto FDR, Maluta RP, Oliveira MC, Souza V, de Medeiros MIM, Borges LA, do Amaral LA, Fairbrother JM (2016) Antimicrobial resistance and virulence factors of *Escherichia coli* in cheese made from unpasteurized milk in three cities in Brazil. Foodborne Pathog Dis 13:469-476.

Rupp ME, Fey PD (2003) Extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*: Considerations for diagnosis, prevention and drug treatment. Drugs 63:353-365.

Saravanan M, Ramachandran B, Barabadi H (2018) The prevalence and drug resistance pattern of extended-spectrum β-lactamases (ESBLs) producing *Enterobacteriaceae* in Africa. Microb. Pathog. 114:180-192.

Shaikh S, Fatima J, Shakil S, Danish Rizvi SM, Kamal MA (2015) Antibiotic resistance and extended-spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 22:90-101.

Skočková A, Bogdanovičová K, Koláčková I, Karpíšková R (2015) Antimicrobial-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* in raw cow's milk. J Food Prot 78:72-77.

Su Y, Yu CY, Tsai Y, et al (2016) Fluoroquinolone-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* from the milk of cows with clinical mastitis in Southern Taiwan. J Microbiol Immunol Infect 49:892-901.

Tekiner İH, Özpınar H (2016) Occurrence and characteristics of extended-spectrum beta-lactamases-producing *Enterobacteriaceae* from foods of animal origin. Brazilian J Microbiol 47:444-451.

Tóth AG, Csabai I, Krikó E, Tőzsér D, Maróti G, Patai AV, Makrai L, Szita G, Solymosi N (2019) Raw milk for human consumption may carry antimicrobial resistance genes. bioRxiv 853333.

Valat C, Haenni M, Saras E, Auvray F, Forest K, Oswald E, Madec JV (2012) CTX-M-15 extended-spectrum β-lactamase in a shiga toxin-producing *Escherichia coli* isolate of serotype O111:H8. Appl Environ Microbiol 78:1308-1309.

Wasiński B, Osek J, Różańska H (2014) Antimicrobial resistance of ESBL and AmpC-producing *Escherichia coli* isolated from meat. Bull Vet Inst Pulawy 58:567-571.

Younis GA, Elkenany RM, Fouda MA, Mostafa NF (2017) Virulence and extended-spectrum β-lactamase encoding genes in *Escherichia coli* recovered from chicken meat intended for hospitalized human consumption. Vet World 10:1281.

Zurfluh K, Hächler H, Nüesch-Inderbinen M, Stephan R (2013) Characteristics of extended-spectrum β-lactamase- and carbapenemase-producing *Enterobacteriaceae* isolates from rivers and lakes in Switzerland. Appl Environ Microbiol 79:3021-3026.