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RESEARCH ARTICLE

Molecular Evaluation and Antimicrobial Susceptibility Testing of *Escherichia coli* Isolates from Food Products in Turkey

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Abstract Some strains of *Escherichia coli* can be important food borne pathogens. Characterization and antimicrobial resistance testing of 28 *E. coli* isolates from random food samples obtained in Van, Turkey were performed. Primers for 6 indicator genes (*fliC*, *stx1*, *stx2*, *eae*, *hlyA,* and *rfbE*) for shiga toxin-producing *E. coli* and 5 indicator genes for each pathogroup (*bfpA*, *aggR*, *ipaH*, *daaD*, *st*, and *lt*) were used. *E. coli* isolates were also typed using pulsed field gel electrophoresis with the *Xba*I restriction enzyme. Antimicrobial susceptibility of *E. coli* isolates was determined using the disk diffusion method for 17 antimicrobials. *E. coli* isolates were non-pathogenic strains represented by 25 distinguishable PFGE patterns. Antimicrobial susceptibility testing revealed that more than 40% of the *E. coli* isolates showed resistance to ampicillin, sulphafurazole, and tetracycline. Antimicrobial susceptibility of commensal *E. coli* should be monitored because these bacteria are becoming reservoirs of antimicrobial resistance genes.

Keywords: *E. coli*, pulsed field gel electrophoresis, antimicrobials

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Introduction

Escherichia coli (*E. coli*) is a Gram-negative bacterium that can be found in the intestinal tract of healthy humans and warm-blooded animals (1). In a mutually beneficial association, *E. coli* helps the body in production of vitamin K, processing of waste, and absorption of monomers from food (2). Moreover, *E. coli* is used as an indicator microorganism for fecal contamination and its presence suggests the possibility of a microbial hazard (1). Several strains of *E. coli* can become pathogenic by acquiring virulence factors. Pathogenic *E. coli* strains that affect the intestines of humans have been grouped into the 6 main pathotypes of 1) Shiga-toxin-producing *E. coli* (STEC; also called verocytotoxin-producing *E. coli* or VTEC), of which enterohaemorrhagic *E. coli* (EHEC) is a pathogenic subgroup; 2) enteropathogenic *E. coli* (EPEC); 3) enterotoxigenic *E. coli* (ETEC); 4) enteroaggregative *E. coli* (EAEC); 5) enteroinvasive *E. coli* (EIEC); and 6) diffusely adherent *E. coli* (DAEC) (1).

Treatment with antimicrobials is one of the most important ways to treat infections, but antimicrobial resistance in microorganisms, including *E. coli,* is of great concern. Commensal bacteria, which are not pathogenic and are mostly found in the intestines, are becoming reservoirs of antimicrobial resistance genes (3) because of their constant exposure to antimicrobials in the bodies of humans and animals. When these bacteria are excreted into the environment in feces they can enter into humans again due to poor hygienic practices. Sometimes, they can acquire pathogenic genes from other bacteria via horizontal gene transfer. These bacteria can then enter the body while harboring both pathogenic and antimicrobial resistance genes (4). It is, therefore, of importance to monitor antimicrobial resistance of both pathogenic and commensal *E. coli*.

Assessment of the prevalence of both pathogenic and non-pathogenic *E. coli* in foods using the most common subtypes and antimicrobial resistance is important for the export, tourism, and health sectors of an economy. Turkey exports hazelnuts, pistachios, figs, pulses, citrus, melons, vegetables, tomato products, poultry, and cereals to many parts of the world, especially to Europe. Turkey is also a major cheese exporter, particularly of white goat and sheep cheeses preserved with salt or brine (5). Contamination of these products by foodborne microorganisms can inadvertently affect the business partnership between Turkey and its trading partners. In Turkey, the true incidence of pathogenic *E. coli* infections is not known. There are no meaningful data regarding the presence of *E. coli* infection, but foreign researchers have concluded that *E. coli* O104 circulates in Turkey (6). In this regard, the prevalence and the rate of occurrence of pathogenic microorganisms, including *E. coli*, must be known throughout the country for all stakeholders and efforts should be made together for control. Because of the great diversity of *E. coli*, characterization is important to know pathogenicity.

The genetic diversity of clinical *E. coli* isolates has been widely characterized (7). The majority of diversity studies involving commensal *E. coli* have relied on fecal isolation, whereas data concerning the diversity of commensal *E. coli* from food samples are rare (7). Recently, pulsed field gel electrophoresis (PFGE) has become a widely used genetic characterization method for *E. coli* diversity (7). Other molecular methods, such as PCR screening for virulence genes and BOX-PCR patterns, have also been used (8). For phenotypic characterization of *E. coli* diversity, serotyping and antimicrobial susceptibility testing are primarily used. The aim of this study was to determine the prevalence of pathogenic *E. coli*, especially *E. coli* O157:H7, in Turkish food products in a pilot region in the city of Van in the eastern part of Turkey. The most common subtypes of *E. coli* isolates found in food were determined using and PFGE (7) and the antimicrobial susceptibility of the *E. coli* isolates was determined using the disc diffusion method (9). Information obtained regarding *E. coli* isolates collected from Turkey was provided to a publicly available database (http://pathogendetector-metu.rhcloud.com).

Materials and Methods

Sample collection and isolation of *E. coli* Between the 1st of February and the 31st of May, 2011, 37 food samples including raw chicken products (N=17), raw milk (N=6), cheese (N=6), pistachio (N=1), raw patty meat (N=3), red pepper (N=1), minced meat (N=2), and lahmacun (N=1), a traditional prepared Turkish food that looks like a thin piece of dough topped with minced meat and minced vegetables were randomly collected in the market center in the city of Van for *E. coli* isolation (Table 1). Food samples were transferred to the laboratory at the Food Engineering Department of Yuzuncu Yil University in Van, Turkey where sub-sampling and isolation were performed following the *E. coli* isolation method of the Food and Drug Administration (FDA) (10). Twenty five g of each food sample was aseptically weighed. Each food sample was transferred to 225 mL of buffered peptone water (Oxoid Ltd., Basingstoke, UK) for enrichment of *E. coli*, followed by homogenization using a stomacher (BagMixer 400; Interscience, St. Nom., France). Homogenates were incubated at 36°C for 18 h for cell enrichment, then 20 μL of each homogenate was sub-cultured on Endo Agar (CM 0479; Oxoid Ltd.). After 18-24 h of incubation at 36°C in an incubator (Infors AG, Bottmingen, Switzerland), 28 out of the 37 food samples exhibited green colonies with a metallic sheen indicating the presence of *E. coli*. Suspected *E. coli* isolates were stored at −20°C (Thermo Scientific, Waltham, MA, USA) in brain heart infusion (BHI) broth with 15% (v/v) glycerol prior to transport to the Food Engineering Department at Middle East Technical University (METU), Ankara, Turkey.

Table 1. Distribution of *E. coli* among different food samples

Food samples	Number of food samples collected	Number of *E. coli* positive samples
Raw chicken drumsticks	15	15
Raw chicken wing	1	1
Raw turkey wing	1	1
Raw milk	6	6
Herby cheese	4	3
Salted cheese	2	1
Minced meat	2	0
Raw patty meat (cig kofte)	3	1
Lahmacun	1	0
Pistachio	1	0
Red pepper	1	0
Total	37	28

Biochemical and molecular confirmation of suspected *E. coli* isolates The 28 presumptive *E. coli* strains from food samples were transported to the Food Safety Laboratory at METU for further confirmation of *E. coli* and subtyping. The presumptive *E. coli* isolates in BHI broth were vortexed at 20 Hertz (Velp Scientifica, Usmate, Italy) for approximately 5 s, then 5 μL of each isolate was dispensed onto eosin methylene blue (EMB) agar (CM 69; Oxoid Ltd.) and streaked gently using an inoculation loop. Plates were incubated in an incubator (Infors AG), at 37°C for 24 h.

Table 2. Primers for detection of *E. coli* pathogenic subgroups[1)]

Pathogenic Subgroup	Gene	Orientation	Primer sequence (5' to 3')	Final conc. (μM)	Amplicon size (bp)	Annealing temperature (°C)	Reference
ETEC	st	F	ATTTTTATTTCTGTATTATCTT	0.4	190	50	(12)
	st	R	CACCCGGTACAAGCAGGATT	0.4			
	lt	F	GGCGACAGATTATACCGTGC	0.4	450	60	
	lt	R	CGGTCTCTATATTCCCTGTT	0.4			
EAEC	aggR	F	CGAAAAAGAGATTATAAAAATTAAC	0.44	100	60	(13)
	aggR	R	GCTTCCTTCTTTTGTGTAT	0.44			
EIEC	ipaH	F	GTTCCTTGACCGCCTTTCCGATACCGTC	0.04	619	60	(13)
	ipaH	R	GCCGGTCAGCCACCCTCTGAGAGTAC	0.04			
EPEC	bfpA	F	AATGGTGCTTGCGCTTGCTGC	0.2	326	59	(14)
	bfpA	R	GCCGCTTTATCCAACCTGGTA	0.2			
DAEC	daaD	F	TGAACGGGAGTATAAGGAAGATG	0.50	444	60	(13)
	daaD	R	GTCCGCCATCACATCAAAA	0.50			

[1)]ETEC, strains are *st* and/or *lt* positive; EAEC, strains are *aggR* positive; EIEC, strains are *ipaH* positive; EPEC, strains are *bfpA* ; DAEC, strains are *daaD* positive

For molecular confirmation of *E. coli*, PCR confirmation of the *rpoB* gene region was performed (11). Suspected isolates were cultured on BHI agar (Lab M, Lancashire, UK) at 37°C for 24 h, after which a sterile inoculating loop was used to obtain a single colony of each *E. coli* isolate. The colony was put into a tube containing 95 μL of sterile water and gently mixed to ensure uniform mixing. This cell suspension was microwaved at 600 Watt (Arçelik, Istanbul, Turkey) for about 30 s to lyse cells. Lysates were then used as templates for PCR.

The PCR reagents used were 4 μL of 10× buffer, 3 μL of 7.5 mM $MgCl_2$, 1.2 μL of 0.6 mM dNTPs (Thermo Scientific), 0.8 μL of 0.4 μM forward and reverse *rpoB* primers (Iontek, Istanbul, Turkey), and 0.1 μL of 2.5 units of native *Taq* polymerase (Thermo Scientific) in a total volume of 20 μL. PCR assays were conducted using a T100 Thermocycler (Bio-Rad, Nom, France) with denaturation at 95°C for 10 min, 50 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s (11). After amplification, products were visualized using 1.5% agarose gel electrophoresis (Bio-Rad). Ten μL of each PCR product was mixed with 1.5 μL of 6× DNA loading dye (Thermo Scientific) and run at 120 V for 45 min. After electrophoresis, gels were stained using 0.5 μg/mL of ethidium bromide before photography under UV light in a universal hood II (SN 76 S; Bio-Rad). Confirmed *E. coli* isolates were labeled according to the labeling standards of the METU Food Safety Laboratory. The first isolate was labeled as MET K1-001. Afterwards, isolates were frozen in a −80°C in brain heart infusion (BHI) broth with 15% (v/v) glycerol for storage in a −80°C freezer (Thermo Scientific). Detailed information of isolates was uploaded to the METU Pathogen Detector website (http://pathogendetector-metu.rhcloud.com/index.php) for public access. (Contact the website administrator).

Molecular detection of pathogenic subgroups Different gene regions (Table 2) (12-14) were used for molecular detection of pathogenic subgroups. Final primer concentrations and expected amplicon sizes for each PCR assay are shown in Table 2. Each PCR assay was performed at a final volume of 20 μL, including 1 μL of boiled bacterial cell suspension (DNA template), 4 μL of 10× buffer, 3 μL of 7.5 mM $MgCl_2$, 1.2 μL of 0.6 mM dNTPs (Thermo Scientific), and 0.1 μL of 2.5 units of native *Taq* polymerase (Genoks, Ankara, Turkey). Amplified products were then visualized as described above.

Reference strains for the pathogenic subgroups of *E. coli*, provided by the Turkish Ministry of Health Laboratory (Ankara, Turkey), represented ETEC, including *est* (the original number for this isolate was A1851), EIEC, including *ipaH* (the original number for this isolate was 583), EAEC, including *aggR* (the original number for this isolate was 2059), and EPEC, including *eae* (the original number for this isolate was 8064). Strains were labeled as MET K1-042, MET K1-045, MET K1-051, and MET K1-054, respectively. Amplified products were then analyzed and screened as described above.

Screening for STEC For screening of *E. coli* isolates for subgroup STEC, the indicator STEC genes *stx1*, *stx2*, *eae*, *hly A*, *rfbE,* and *fliC* (Table 3) were used (15). Each PCR assay was performed at a final volume of 20 μL, including 1 μL of boiled bacterial cell suspension (DNA template) and final concentrations of 0.4 μM for each primer, 0.6 mM dNTP, 7.5 mM $MgCl_2$, 2× buffer, and 2.5 units of *Taq*

Table 3. Nucleotide sequences and primers for target genes used to detect STEC (15)

Target gene	Orientation	Primer sequence (5' to 3')	Final conc. (μM)	Amplicon size (bp)
fliC	F	AGCTGCAACGGTAAGTGATTTGGC	0.4	949
fliC	R	AGCAAGCGGGTTGGTC	0.4	
stx1	F	TGTCGCATAGTGGAACCTCA	0.4	655
stx1	R	TGCGCACTGAGAAGAAGAGA	0.4	
stx2	F	CCATGACAACGCACAGCAGTT	0.4	477
stx2	R	TGTCGCCAGTTATCTGACATTC	0.4	
eae	F	CATTATGGAACGGCAGAGGT	0.4	375
eae	R	ACGGATATCGAAGCCATTTG	0.4	
rfbE	F	CAGGTGAAGGTGGAATGGTTGTC	0.4	269
rfbE	R	TTAGAATTGAGACCATCCAATAAG	0.4	
hlyA	F	GCGAGCTAAGCAGCTTGAAT	0.4	199

Table 4. Antimicrobial Recommended Breakpoints (9)

Antimicrobials	Reference range (ATCC 25922)	Interpretative criteria		
	Zone diameter in mm	Susceptible	Intermediate	Resistant
Ampicillin	16-22	≥17	14-16	≤13
Amoxycillin	18-24	≥18	14-17	≤13
Ceftiofur	26-31	≥21	18-20	≤17
Cefoxitin	23-29	≥18	15-17	≤14
Cephalothin	15-21	≥18	15-17	≤14
Ceftriazone	29-35	≥23	20-22	≤19
Sulphafurazole	15-23	≥17	13-16	≤12
Sulphamethoxazole/Trimethoprim	23-29	≥16	11-15	≤10
Nalidixic Acid	22-28	≥19	14-18	≤13
Tetracycline	18-25	≥15	12-14	≤13
Amikacin	19-26	≥17	15-16	≤14
Gentamicin	19-26	≥15	13-14	≤12
Streptomycin	12-20	≥15	12-14	≤11
Kanamycin	17-25	≥18	14-17	≤13
Imipenem	26-32	≥23	20-22	≤19
Chloramphenicol	21-27	≥18	13-17	≤12
Ertapenem	29-36	≥22	19-21	≤18

DNA polymerase (Genoks) (15) under conditions of denaturation at 94°C for 5 min, 25 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 68°C for 75 s, and a final step of extension at 68°C for 7 min (15). Amplified products were then visualized as described above.

The reference strain *E. coli* O157:H7 was provided by Prof. Dr. Kadir Halkman (Food Engineering Department, Ankara University), and labeled as MET K1-029. This strain possessed all of the 6 virulence genes and was used as a positive control. Amplified products were analyzed and screened as described above.

Pulsed-field gel electrophoresis Subtyping of 28 *E. coli* isolates was performed using pulsed field gel electrophoresis (PFGE) (16). Prepared genomic DNA was digested using the restriction enzyme *Xba*I and DNA fragments were separated in a CHEF-DR III system (Bio-Rad) in 0.5× tris-borate-EDTA buffer (Bioshop, Burlington, Canada) at 120 V for 19 h with pulse times ranging from 2.16 to 63 s. Banding patterns were analyzed using BioNumerics software (Applied Maths, Austin, TX, USA).

Antimicrobial susceptibility testing Antimicrobial susceptibility testing was carried out using the disk diffusion method on Mueller-Hinton Agar (Merck, Damstadt, Germany) according to Clinical Laboratory Standards Institute (CLSI) guidelines (9). Antimicrobials used were ampicillin (10 μg), tetracycline (30 μg), cefoxitin (30 μg), cephalothin (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), amoxycillin/clavulanic acid (30 μg), ceftiofur (30 μg), ertapenem (10 μg), ceftriaxone (30 μg), sulphafurazole (300 μg), sulphamethoxazole/trimethoprim (25 μg), nalidixic acid (30 μg), streptomycin (10 μg),

kanamycin (30 μg), and chloramphenicol (30 μg). Susceptibility of the isolates was determined according to the breakpoints recommended by CLSI, 2007 and designated as susceptible, intermediate, or resistant (Table 4). Intermediate strains were grouped with sensitive isolates. *E. coli* ATCC 25922 was used as a quality control strain.

Results and Discussion

Isolation of *E. coli* A total of 28 samples contained *E. coli* varieties from 37 analyzed food samples. The highest prevalence values were in raw chicken drumsticks (100%), chicken wings (100%), raw turkey wings (100%), raw milk (100%), and Herby cheese (100%). No *E. coli* was found in minced meat, lahmacun, pistachio, or red pepper (Table 1). The prevalence of *E. coli* in salted cheese was 50%, and 33% in raw patty meat (Table 1). Evidently, foods were prepared under non-hygienic conditions open to contamination. Raw milk, poultry products, and soft cheeses can be contaminated and can transmit *E. coli* to humans (17). *E. coli* was isolated from all raw chicken drumsticks, raw chicken wings, and raw turkey wings. The prevalence of *E. coli* in fruits, vegetables, and nuts (pistachio) is generally low, compared to foodstuffs of animal origin (18).

Screening of Shiga-toxin producing E. coli (STEC) and pathogenic subgroups genes using PCR STEC indicator genes (*stx1*, *stx2*, *eae*, *hly A*, *rfbE*, and *fliC*) were used for screening of 28 *E. coli* isolates. None of these genes, except *fliC,* was detected among the isolate set. The *fliC* gene was present in 3 samples (2 of raw milk and 1 of raw patty meat), representing 10.71% of the total number of food samples (Table 5). The prevalence and true incidence of *E. coli* O157:H7 and other pathogenic *E. coli* strains in humans and in food products from Turkey are not well-known. Cases are likely to be under estimated owing to improper laboratory diagnostic methods and a lack of awareness of the epidemiologic significance (6). Individual case reports and a few case studies of people travelling to

Table 5. Phenotypic and molecular characteristics of E. coli isolates used in this study

METU IDs	Source	Virulence gene	PFGE pattern	Antimicrobials[1)]
MET K1-001	Raw milk	fliC	Pattern 1	NR
MET K1-002	Herby Cheese	None	Pattern 2	NR
MET K1-003	Raw milk	None	Pattern 3	Gentamycin
MET K1-004	Raw milk	None	Pattern 3	NR
MET K1-005	Raw milk	None	Pattern 4	NR
MET K1-006	Raw patty meat	*fliC*	Pattern 5	NR
MET K1-007	Chicken wings	None	Pattern 6	AMP, FOX, NA
MET K1-008	Salted cheese	None	Pattern 7	NR
MET K1-009	Chicken drumstick	None	Pattern 8	AMP, AMC, SF, SXT, TE, CN, S
MET K1-010	Chicken drumstick	None	Pattern 9	AMP, AMC, SF, SXT, TE, CN, S
MET K1-011	Chicken drumstick	None	Pattern 10	AMP, SF, SXT, NA, TE, CN, S
MET K1-012	Turkey wings	None	Pattern 11	AMP, TE
MET K1-013	Chicken drumstick	None	Pattern 12	NR
MET K1-014	Chicken drumstick	None	Pattern 13	AMC, SF, SXT, NA, TE, S, K.
MET K1-015	Chicken drumstick	None	Pattern 14	AMP, AMC, SF, SXT, NA, TE, S
MET K1-016	Chicken drumstick	None	Pattern 15	AMP, AMC, SF, SXT, NA, TE, S
MET K1-017	Chicken drumstick	None	Pattern 16	AMP, AMC, SF, SXT, NA, TE.
MET K1-018	Chicken drumstick	None	Pattern 17	AMP, SF, SXT, NA, TE, S, K, C
MET K1-019	Chicken drumstick	None	Pattern 18	AMP, KF, SF, SXT, NA, TE, CN, C
MET K1-020	Chicken drumstick	None	Pattern 19	NR
MET K1-021	Chicken drumstick	None	Pattern 20	AMP, SF, SXT, TE, S.
MET K1-022	Chicken drumstick	None	Pattern 21	AMP, KF, SF, SXT, NA, TE, S, K, C
MET K1-023	Raw milk	*fliC*	Pattern 22	NR
MET K1-024	Herby cheese	None	Pattern 23	AMP, AMC, SF, NA, TE, S, C
MET K1-025	Chicken drumstick	None	Pattern 24	AMP
MET K1-026	Herby cheese	None	Pattern 2	NR
MET K1-027	Raw milk	None	Pattern 3	NR
MET K1-028	Chicken drumstick	None	Pattern 25	AMP, SF, S, C

[1)]AMP, ampicillin; AMC, amoxycilllin/clavulanic acid; SF, sulpafurazole; SXT, sulphamethoxazole/trimethoprim; TE, tetracycline; CN, gentamicin; S, streptomycin; NA, nalidixic acid; K, kanamycin; C, chloramphenicol; KF, cephalothin; NR, not resistant

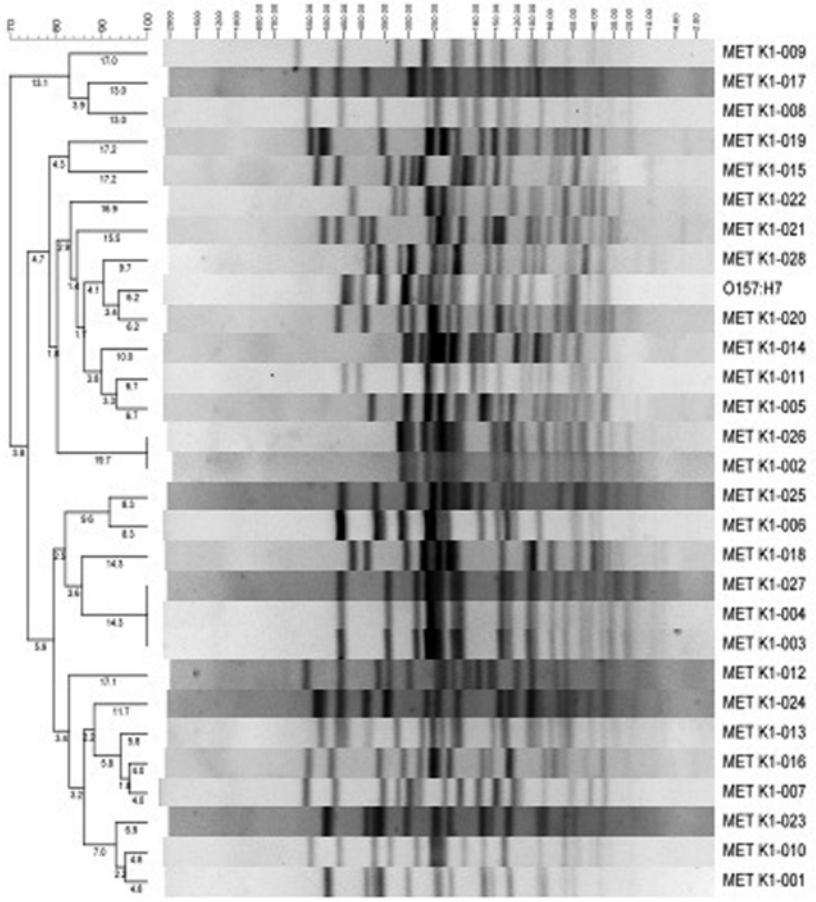

Fig. 1. A dendrogram showing different PFGE band patterns and clusters of *E. coli* isolates.

Turkey have been reported (19). The incidence of *E. coli* O157:H7 in humans in Turkey has been reported as varying from 0% to 4% of total *E. coli* isolates (20), but no report on isolation of *E. coli* O157:H7 from food has been reported for Turkey (20).

The H antigen of *E. coli* is specified by a single structural subunit (flagellin) encoded by the *fliC* gene (21). In this study, 3 isolates (MET K1-001, MET K1-006, and MET K1-023) from the DNA region of *fliC* and encoding flagella proteins were amplified. Flagella aid movement and binding to epithelial tissues (21). However, the presence of flagella may not be important for STEC pathogenesis because there are STEC strains without flagella that have been associated with diseases in Germany (22).

PCR analyses of the *E. coli* genes *st, lt*, *aggR*, *ipaH*, *bfpA*, and *daaD* for pathogenic subgroups representing ETEC, EAEC, EIEC, EPEC, and DAEC, respectively, were also conducted. No pathogenic *E. coli* strain was found in the *E. coli* isolates used in this study. Thus, none of the 28 isolates was pathogenic. All were commensal varieties of *E. coli*. The prevalence of pathogenic *E. coli* in food isolates is low, compared to animal isolates and clinical infections (23). The prevalence of commensal *E. coli* isolates from food has been extensively studied in developed countries (24) but little research has been conducted in Turkey (25).

Results of PFGE typing A total of 28 *E. coli* food isolates were characterized using PFGE to provide a better understanding of diversity among isolates. The 28 *E. coli* isolates represented 25 different genotypes. PFGE patterns for all 28 *E. coli* isolates were within the range of 20 kb and 600 kb (Fig. 1). Three isolates (MET K1-003, MET K1-004, and MET K1-027) obtained from raw milk, shared the same PFGE pattern 3 (Fig. 1), and 2 isolates from Herby cheese (MET K1-002 and MET K1-026) also shared the same PFGE pattern 2 (Fig. 1). The rest of the isolates had distinguishable PFGE patterns (Fig. 1). Six *E. coli* strains were isolated from raw milk, 3 of which, MET K1-003, MET K1-004, and MET K1-027, shared the same PFGE pattern, while 3 other isolates from raw milk (MET K1-001, MET K1-005, and MET K1-023), had unique band patterns. Thus, genetic backgrounds of isolates from one food sample may be different from each other. Likewise, 2 isolates from Herby cheese (MET K1-002 and MET K1-026) shared the same PFGE pattern, but a third

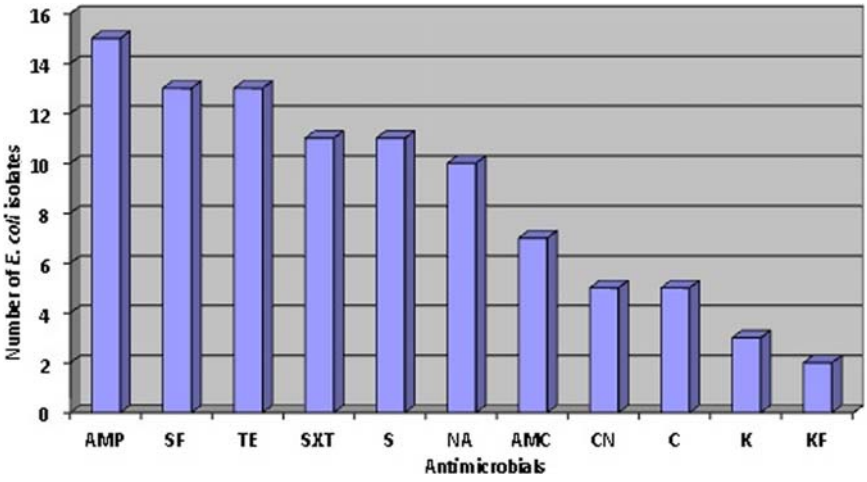

Fig. 2. Number of *E. coli* isolates showing resistance to antimicrobials. AMP, ampicillin; AMC, amoxycilllin/clavulanic acid; SF, sulpafurazole; SXT, sulphamethoxazole/trimethoprim; TE, tetracycline; CN, gentamicin; S, streptomycin; NA, nalidixic acid; K, kanamycin; C, chloramphenicol; KF, cephalothin; NR, not resistant

isolate from Herby cheese (MET K1-024) had a different PFGE pattern (Fig. 1).

PFGE remains the gold standard method for epidemiological studies of *E. coli* since PFGE is more discriminatory than other molecular methods and is reproducible (26). The DNA fingerprinting techniques of PFGE and ribotyping used for microbial source tracking have been used to identify a high genetic diversity of commensal *E. coli* (27,28). The enormous diversity of *E. coli* is manifested by the presence of numerous distinct *E. coli* genotypes (27). Multilocus sequencing techniques (MLST) can be used for revealing genetic relatedness of these isolates in the future. The PFGE profiles of isolates in this study were stored in a public database and can be used for tracing future outbreaks.

A high number of commensal *E. coli* isolates showed antimicrobial resistance None of the *E. coli* isolates in this study showed resistance to ceftiofur, ertapenem, ceftriazone, amikacin, or imipenem. However, 15 of 28 isolates were resistant to ampicillin, and 13 isolates were resistant to both sulphafurazole and tetracycline (Fig. 2). Numbers of *E. coli* isolates resistant to the penicillin class (n=11 ampicillin) and the aminoglycosides class (n=11, 5, 3 resistant to streptomycin, gentamycin, and kanamycin, respectively) were comparatively higher than for resistance to other classes (Table 5). This distribution correlates with the European Antimicrobial Resistance Surveillance Report of 2012 (29).

A maximum number 15 isolates were resistant to ampicillin (Fig. 2). In the Enterobacteriaceae, resistance to ampicillin is mainly due to β-lactamases (30). Isolates generally showed lower resistance to the cephems (ceftriazone, cephalothin, and cefoxitin) in this study. The presence and frequency of tetracycline resistance in *E coli* in this study agreed with reports of antimicrobial resistance in *E coli* (31) with 15 multidrug resistant (MDR) *E. coli* isolates (resistant to 2 or more antimicrobials) identified herein. This represents about 53% of the total isolates. Instances of MDR ranged from a minimum of 2 antimicrobial resistance isolates to a maximum of 9 antimicrobial resistance isolates. Resistance of isolates from chicken drumsticks was highest (Table 5). Apart from 3 chicken drumstick isolates (2 susceptible to all antimicrobials and 1 resistant only to ampicillin), all other isolates from chicken drumsticks were resistant to at least 4 antimicrobials. However, almost all of the 6 isolates from milk were susceptible to all of the 17 antimicrobials used. The only exception was MET K1-003, which was resistant only to gentamycin. *E. coli* isolates from 2 out of 3 Herby cheese samples were susceptible to all of the antimicrobials, but one isolate was resistant to 7 antimicrobials. An isolate from chicken wings (MET K1-007) was resistant to 3 antimicrobials and an isolate from turkey wings (MET K1-002) was also resistant to 2 antimicrobials. However, an isolate from salted cheese was susceptible to all the antimicrobials (Table 5).

Long-term use of antimicrobials in animal farms can drive *E. coli* to gain resistance in both pathogenic and non-pathogenic populations (32). Most of the *E. coli* isolates found in Turkish food products in this study exhibited multi-resistance to antimicrobials. Most multidrug resistant *E. coli* strains are acquired in the human community through food and water (33). Use of antimicrobials in the food industry has resulted in the propagation of resistant bacteria. Thus, the levels and patterns of resistance observed in food animals to a wide extent reflect patterns of drug usage (33) in food animals that can increase the

level of multidrug resistance of *E. coli* in animals, and in humans associated with animal products. Therefore, the presence of multidrug resistant *E. coli* in animal products is of great concern. Even non-pathogenic *E. coli* can play a major role in the emergence of antimicrobial resistant pathogenic *E. coli* strains, as well as other pathogens sharing a common ancestor. The massive use of antimicrobial agents in the poultry industry has supported production by facilitating earlier weaning and higher animal densities. However, these gains have come at a great cost (34).

Antimicrobial resistant phenotypes of commensal *E. coli* can act as a reservoir of antimicrobial resistance genes and be a threat to humans. Commensal *E. coli* can be passed or transferred to the environment through feces. Through horizontal gene transfer, opportunistic pathogens can spread resistance genes to other bacteria, including pathogenic species (32), which can, in turn, cause antimicrobial-resistant disease. Therefore, it is a laudable idea to monitor antimicrobial resistance of both pathogenic and non-pathogenic *E. coli*.

The prevalence of *E. coli* in foods in a pilot region conducted in Van, Turkey was high, depending on the food type. Neither STEC nor pathogenic *E. coli* subgroups were identified. However, high numbers of MDR commensal *E. coli* were identified. This should act as an alert for intensive public health education about personal hygiene and reduction of unnecessary usage of antimicrobials to prevent future foodborne disease outbreaks.

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