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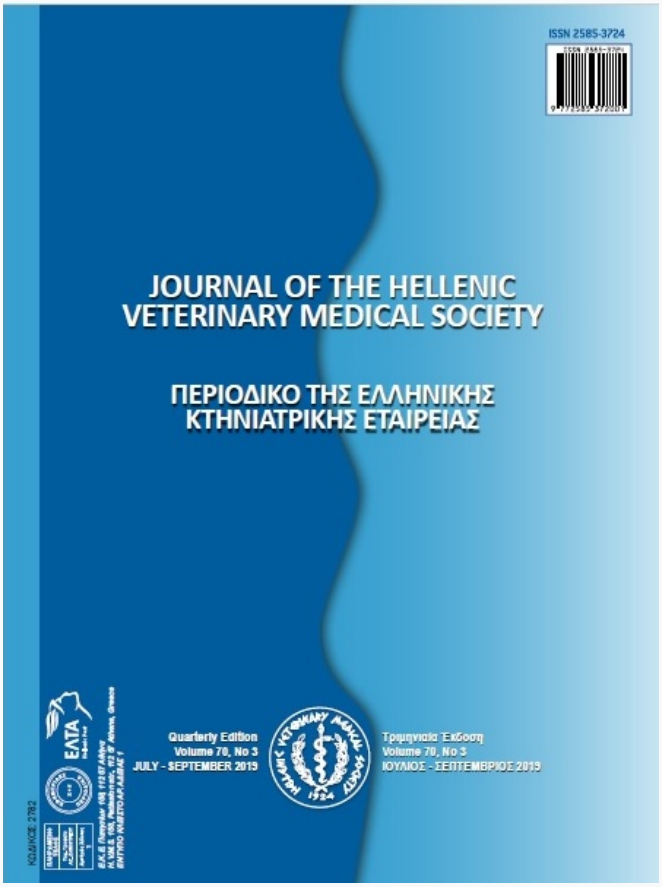

Isolation, genotyping and antimicrobial susceptibility of pathogenic Escherichia coli serotypes in ready to eat foods

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Research article
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Isolation, genotyping and antimicrobial susceptibility of pathogenic *Escherichia coli* serotypes in ready to eat foods

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ABSTRACT. In this study, pathogenic *Escherichia coli* serotypes (*E. coli* O157:H7, O26, O111) and their molecular proximity and antimicrobial susceptibility were investigated in RTE foods. A total of 240 samples; consist of 105 stuffed mussel, 56 meatless cig kofte, 54 Russian salad, 25 cheese halva, were analyzed. The conventional culture and serotyping methods for determination of the organisms were performed and further confirmation by PCR was carried out. Confirmed *E. coli* O157 isolates were genotyped by the enterobacterial repetitive intergenic consensus (ERIC)-PCR. Antibacterial susceptibility testing of the isolates was performed by disc diffusion method. *E. coli* was detected in 7 (2.9 %) of 240 samples, including 3 (5.5%) Russian salad, 3 (2.8%) stuffed mussel, 1 (4 %) cheese halva. Two isolates from Russian salad, 1 from stuffed mussel and 1 from cheese halva were identified as *E. coli* O157 . In addition, stuffed mussel isolate was found to carry *stx*1 ve *hly*A genes whereas one Russian salad isolate carried the *stx*1 gene. *E. coli* isolates were found to be resistant to amoxycillin/clavulonic acid, gentamicin and ciprofloxacin, at the rate of 29%, 14% and 29 %, respectively. Only one (14 %) isolate from stuffed mussel was classified as multidrug resistant to three antimicrobials. Furthermore, the isolates, related to O157 and O157:H7, presented different ribotypes in this study. The results provide useful data for the development of public health policy concerning the potential presence of pathogenic antimicrobial resistant *E. coli* serotypes in RTE foods. Strict surveillance of RTE foods at retail points for emerging pathogens, their antimicrobial resistance patterns and the potential likelihood of cross-contamination is required.

Keywords: Antimicrobial susceptibility, cheese halva, ERIC-PCR, meatless cig kofte, Russian salad, EHEC, stuffed mussel.

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INTRODUCTION

In recent years, ready to eat (RTE) food consumption has increased because of rapid population growth and the modern lifestyle; longer working hours, increasing women's participation in the labour market and the change in cooking and eating habits (Tudoran et al., 2012; Oz et al., 2014). RTE foods do not generally require serious pretreatment process and are shelf-stable, delicious, inexpensive and easily accessible to consumers (Spencer, 2005; Jaroni et al., 2010). However, these types of foods present important microbiological risk since they have been implicated as vehicles of food borne microorganisms including *Escherichia coli* (Ateş et al., 2011; Kochakkhani et al, 2016).

E. coli, a member of *Enterobacteriaceae* family, is the main inhabitant of human and animal guts. They have been accepted as the indicator microorganisms of contamination with fecal and enteric pathogens (Montville et al., 2012). Although most *E. coli* strains are nonpathogenic, some are known to be responsible for serious human gastrointestinal diseases, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Virulence factors such as shiga toxins (*stx*1 and *stx*2), enterohemolysin (*hly*A) and intimin (*eae*A) play an important role in the pathogenesis of these diseases (Bruyand et al., 2018). Three major surface antigens, O (somatic), H (flagellar) and K (capsule) antigens, are used to serologically to differentiate the *E. coli* isolates (Montville et al., 2012). Shiga toxin producing *E. coli* (STEC) strains are the non-O157 strains (O26, O45, O103, O104, O111, O121, O145) and contain O157: H7, the most important serotype (Paton and Paton, 1998; Durso et al., 2005). Although *E. coli* O157: H7 serogroup is responsible for most cases of STECs in humans, it is reported that non O157 STEC strains are increasingly causing diseases (Montville et al., 2012; Bruyand et al., 2018).

Antimicrobial resistance of *E. coli* has been traced world-wide in RTE foods (Musgrove et al., 2006; Zhao et al., 2012; Kochakkhani et al., 2016). Studies on antimicrobial resistant *E. coli* serotypes indicate that increasing antibiotic resistance has become a clinical and public health problem because of complicates treatment of infections caused by *E. coli* (Karlowsky et al, 2002).

Although there are studies focusing on the presence of *E. coli* and other pathogens in RTE foods (Bingol et al., 2008; Ateş et al., 2011; Cokal et al., 2012; Taban, 2012; Delikanli et al., 2014; Secim et al., 2017), to our knowledge, this study is the first report concerning the detection and genotyping of pathogenic *E. coli* serotypes in cheese halva, meatless cig kofte, Russian salad and stuffed mussel in Turkey. Studies on the pathogenic *E. coli* serotypes in RTE foods need to continue in order to complete food safety requires. For this reason, present study aimed to trace the current condition of toxin-producing *E.coli* contamination in RTE foods based on their prevalence, antimicrobial resistance and phylogenetic relationship.

MATERIALS AND METHODS

The samples of the study were purchased, weekly from January to March 2018, from supermarkets of Nigde and Kayseri cities of Central Anatolia /Turkey. A total of 240 RTE samples including 105 stuffed mussel, 56 meatless cig kofte, 54 Russian salad and 25 cheese halva from fishmongers, meatless cig kofte stores, grocery stores, restaurants and supermarkets (Table 1) were randomly collected. All samples were taken under aseptic conditions and transferred to the laboratory within 2 hours under the cold chain. Mix of stuffed mussels were removed from the shells before analysed.

Reference strains

E. coli O 157 NCTC 12900 (National Collection of Type Cultures 12900) reference strain was used as a positive control for isolation, identification and detection of virulence factors of *E. coli* O157: H7.

Table 1. RTE food samples

RTE food samples	N	Ingredients	Obtained from
Stuffed mussel	105	*Mytilus galloprovincialis* meat with mixed of spices, oil, salt and boiled rice in the cockleshells.	Fishmongers, street venders, cig kofte stores
Meatless cig kofte	56	Bulgur (pounded wheat) mixed with salt, tomato paste, onions, garlic and spices.	Cig kofte stores
Russian salad	54	Boiled peas, carrots and potatoes with cucumber pickles mixed in mayonnaise	Restaurants, supermarkets, grocery stores.
Cheese halva	25	Salt-free fresh cheese is melted and mixed with sugar, flour and semolina on the fire.	Restaurants, supermarkets, grocery stores.

Bacterial isolation

A 25 g of each sample was transferred aseptically to 225 mL Trypticase Soy Broth (mTSB, CM129 Oxoid, UK) containing novobiocin (20 g/ml, SR0181E' Oxoid, UK) and incubated at 37 °C for 18-24 h. Then, one loopful of enrichment cultures was inoculated onto Chromocult agar (CHROM agar O157, EE222, DRG International, Paris, France) and sorbitol MacConkey Agar (SMAC Agar-109202; Merck KGaA, Darmstadt, Germany) supplemented with 0.05 mg of cefixime and 2.5 mg of tellurite (CT Supplement 109202, Merck KGaA, Darmstadt, Germany). Plates were incubated at 37 °C for 24 h. After incubation, five suspected *E. coli* and *E. coli* O157 colonies were subcultured to blood agar (Oxoid, CM0271) for conducting confirmatory biochemical tests (indole, methyl red, Voges-Proskauer, citrate, urease, sorbitol fermentation and carbohydrate fermentation tests). Subsequently, they were further processed for serological identification (Chapman and Siddons, 1996; Dontorou et al., 2003).

Serological analysis

All suspected isolates were tested with *E. coli* O157, *E. coli* H7 antisera (221591, Difco), and *E. coli* O157 latex agglutination kit (DR0620M, Oxoid) according to the manufacturer's recommendations.

DNA exraction

Total genomic DNA extraction from the isolates was performed using a commercial DNA extraction kit (Axygen Bioscience, Union City, CA, USA) in accordance with the manufacturer's instructions.

Confirming *E. coli* isolates

The universal forward primer targeting the 3′ portion of *trp*B which, together with non-specific *trp*A reverse primer (trpA2.r, table 2), yields a 489 bp product from all *E. coli* strains was included in the reaction as an internal control as mentioned by Clermont et al. (2008).

PCR analysis for the detection of *fliC*h7, *rfb*O111, *wzx-wzy*O26 and *rfb*O157 genes

The primer pairs for *fli*Ch7,*rbf*O157, *rfb*O111 and *wzx-wzy*O26 genes and the PCR assay conditions were performed in reference to Sarimehmetoglu et al. (2009), (Maurer et al. (1999), Paton and Paton (1998) and Durso et al. (2005), respectively.

Table 2. Primers and PCR amplification products used in this study

PCR Reaction	Target gene	Primer	Sequence (5′-to 3′)	Size of PCR ampl. (bp)	Reference
Internal control	*trpB* *trpA*	trpBA.f	CGGCGATAAAGACATCTTCA	489	Clermont et al. (2008)
		trpA2.r	GCAACGCGGCCTGGCGGAAG		
H7	*fliCh7*	FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	625	Sarimehmetoglu et al (2009)
		FLICH7-R	CAACGGTGACTTTATCGCCATTCC		
LPS O157	*rbf*O157	PF8	CGTGATGATGTTGAGTTG	420	Maurer et al. (1999)
		PR8	AGATTGGTTGGCATTACTG		
O26	*wzx-wzy* O26	wzx-wzyO26F	AAATTAGAAGCGCGTTCATC	596	Durso et al. (2005)
		wzx-wzy O26R	CCCAGCAAGCCAATTATGACT		
O111	*rfb*O111	O111F	TAGAGAAATTATCAAGTTAGTTCC	406	Paton and Paton (1998)
		O111R	ATAGTTATGAACATCTTGTTTAGC		
Shiga-like toxin 1	*stx1*	SLTI-F	TGTAACTGGAAAGGTGGAGTATACA	210	Fratamico et al. (2000)
		SLTI-R	GCTATTCTGAGTCAACGAAAAATAC		
Shiga-like toxin 2	*stx2*	SLTII-F	GTTTTTCTTCGGTATCCTATTCC	484	Fratamico et al. (2000)
		SLTII-R	GATGCATCTCTGGTCATTGTATTAC		
Intimin	*eaeA*	AE22	ATTACCATCCACACAGACGGT	397	Fratamico et al. (2000)
		AE20-2	ACAGCGTGGTTGGATCAACCT		
Enterohemolysin	*hlyA*	MFS1-F	ACGATGTGGTTTATTCTGGA	166	Fratamico et al. (2000)
		MFS1-R	CTTCACGTCACCATACATAT		

Detection of virulence genes (*stx*1, *stx*2, *eae*A and *hlyA*) by Multiplex PCR

Multiplex PCR (mPCR) targeting virulence genes of *E. coli* O157: H7, comprising *stx*1, *stx*2, *eae*A and *hlyA* (Table 2) was carried out in a study conducted by Fratamico et al. (2000).

Electrophoresis of all amplified products was carried out in 1.5% agarose gel containing 0.06% ethidium bromide for 50 minutes at 100 V (EC250-90, Thermo, Pittsburgh, Pa., USA) and visualized on a U.V transilluminator (Vilber Lourmat, Marne La Vallee, France).

ERIC-PCR

The ERIC-PCR was carried out on four isolates identified as EHEC. The total 50 µL of PCR mixture prepared including of 1xPCR buffer (Vivantis, Chino, CA, USA), 0.2 Mm dNTP mix (Vivantis), 4 mM $MgCl_2$(Vivantis), 5 U Taq polymerase (Vivantis), 25 pmol each primer and 1and 1µL target DNA. ERIC-PCR was performed under the following conditions: initial denaturation at 94 °C for 5 min, 94 °C for 1 min, 25 °C for 1 min, and 72 °C for 2 min (Techne TC-512, Keison Products, Chelmsford, Essex, UK).. The amplified product were subjected to electrophoresis at 100 V for 1h on 2 % agarose gel and was monitored by visual inspection under UV light for distinct DNA profiles (Houf et al., 2002). Banding patterns were photographed and analysed by scoring presence (1) or absence (0) of bands for prediction of similarity. Dendrogram was made by construction of a phylogenetic tree using the online software dendrogram construction utility, DendroUPGMA (http://genomes.urv.cat/UPGMA) (Garcia-Vallvé and Puigbo, 2002).

Antimicrobial susceptibility

Antimicrobial susceptibility of all *E. coli* isolates were tested using disk diffusion methods for Amoxicillin/Clavulanic acid (AMC) (30 µg), Ciprofloxacin (CIP) (5 µg), Gentamicin (GEN) (10 µg), Meropenem (MER) (10 µg) and Trimethoprim/ sulfamethoxazole (STX) (25 µg) according to EUCAST guidelines (European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoint tables v. 8.1; http://www.eucast.org v.8.1, accessed: 12.08.2018).

Table 3. Results for pathogenic *E. coli* serotypes, their virulence genes from RTE foods

RTE food samples	N	n(%)	*rfb*O157, *fliC*h7, *rfb*O111 and *wzx-wzy*O26 and virulence genes							
			*rfb*O157	*fliC*h7	*rfb*O111	*wzx-wzy*O26	*stx*1	*stx*2	*hly*A	*eae*A
Stuffed mussel	105	3 (2.8%)	1(0.95%)	1(0.95%)	-	-	1(0.95%)		1 (0.95%)	-
Cig kofte	56	-	-	-	-	-	-	-	-	-
Russian salad	54	3 (5.5%)	2 (3.7%)	-	-	-	1 (1.85%)	-	-	-
Cheese halva	25	1 (4%)	1 (4%)	1 (4%)	-	-	-	-	-	-

n*:* Detected *E. coli* by *trp*A gene

Table 4. Antimicrobial susceptibility profiles of *E. coli* isolates

Antibiotics	Diameter of the inhibition zones *of E. coli* according to EUCAST, 2018 (mm)		Zone of inhibition (mm) in this study	
	S≥	R<	S/ (%)	R/(%)
Amoxicillin/clavulanic acid (AMC)	19	16	16±0.00 (71 %)	29 %
Ciprofloxacin (CIP)	26	24	26±0.05 (71 %)	29 %
Gentamicin (GEN)	17	14	18±0.00 (86 %)	14 %
Meropenem (MER)	22	16	28±0.00 (100 %)	-
Trimethoprim/ sulfamethoxazole (STX)	14	11	19±0.00 (100 %)	-

S:Susceptible, R: Resistant

RESULTS

Seven (2.9%) out of 240 RTE samples were found positive as a result of conventional culture methods and were confirmed by PCR. Furthermore, of the 7 *E. coli* isolates, 2 (3.7%) from Russian salad were identified as *E. coli* O157 based on PCR and serotyping and 1(1.85%) of them found to carry *stx*1 gene. *E. coli* O157:H7 was detected in 2 (0.83 %) out of 240 samples including 1 (0,95%) stuffed mussel and 1 (4%) cheese halva. One isolate from stuffed mussel were found to harbour the *stx*1 and *hly*A genes (As shown in Table 3). However, *E. coli* O111 and O26 were not detected in any sample.

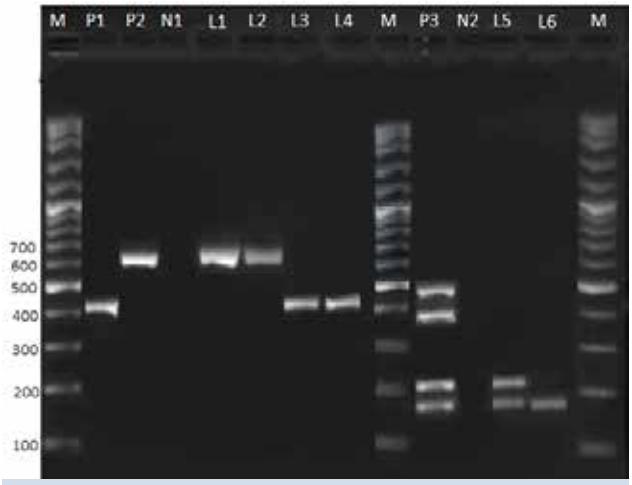

Figure 1. Agarose gel electrophoresis of PCR products of pathogenic *E. coli* isolates and their virulence genes. Lane M: molecular weight marker (Gene RulerTM 100 bp DNALadder Plus, Fermentas); lane P1: Positive control for *rfb*O157 (420 bp),P2 positive control for *fli*Ch7 gene (625 bp), P3: Positive control for toxin genes (for *stx*2 484 bp, for *eae*A 397 bp, for *stx*1 210 bp, for *hly*A 166 bp) N1-2: Negative Control (Sterile H_2O); Line 1-2: *E. coli* O157:H7 isolates; Line 3-4: *E. coli* O157 isolates; Line 5 *stx*1, *hly*A genes positive isolates. Line 6: *stx*1 gene positive isolate.

The results of antibiotic susceptibility test have been summarized in Table 4. All isolates of *E. coli* were highly sensitive to MER and STX. Resistance to AMC occured in 2 (29%) *E. coli* isolates from stuffed mussel, one of which was multidrug resistant to three antibiotics (AMC, CIP and GEN). Furthermore, *stx*1 gene carrying *E. coli* O157 isolate obtained from Russian salad was found to be resistant to CIP.

Figure 2 resumes the ERIC-PCR profiles of pathogenic *E. coli* serotypes. ERIC-PCR genotyping revealed 7-18 fragments resolved per isolate. All of 4 pathogenic *E. coli* isolates under analysis produced 3-7 amplicons ranging from 150 to 1500 bp. Phylogenetic tree (Fig. 2) showed that highly polymorphic DNA fragments among the 4 pathogenic *E. coli* isolates. The Jaccard similarity coefficient of the genotypes was ranging from 0.143 [(A (O157 serotype, carried *stx*1 gene, from Russian salad) and B (O157 serotype from Russian salad)] to 0.125 [B and C (O157 H7 serotype from stuffed mussel)].

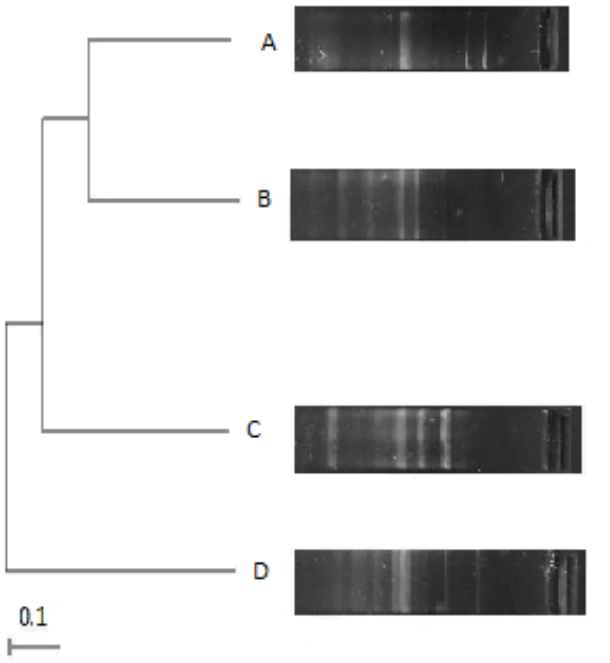

Figure 2. Phylogenetic tree of pathogenic *E. coli* isolates from Russian salad (A and B), stuffed mussel (C), cheese halva (D).

DISCUSSION

The RTE foods, frequently preferred by the consumers in recent years, are pre-cooked or prepared and packaged with a suitable material and often require minimal preparation (Spencer, 2005). Wide range of RTE foods, that can be bought from markets, street vendors, restaurants and stores, may contain a variety of microorganisms, while many of which are harmless, some are dangerous (Elobeid et al. 2014; Jaroni et al., 2008). In this study, pathogenic *E. coli* serotypes (*E. coli* O157:H7, O26, O111) was carried out from RTE foods in Central Anatolia region. The content of RTE foods examined in the study are raw and cooked materials, plants, cheese and shellfish with high protein, spices and sauces (Table 1).

Stuffed mussel is a highly consumed traditional shellfish in Turkey. Reported results demonstrated that 3 of 105 (2.85%) stuffed mussel were found to

be positive interms of *E. coli* and one of them was defined as O157 H7 (0.95%) containing *stx*1 and *hly*A genes. It was found that one *E. coli* strain was resistant to three antibiotics (AMC, CIP and CN); other *E. coli* strain was resistant to only AMC. Studies on the microbiological quality of stuffed mussels in Turkey demostrated that they may be contaminated with some foodborne pathogens including *E. coli* however no investigation is available on pathogenic *E. coli* serotypes in stuffed mussels samples (Bingol et al., 2008; Hampikyan et al., 2008; Ateş et al., 2011; Kocatepe et al., 2016). Similar to our results Surendraraj et al. (2010) in India also reported 8.3 % of shrimp samples were contamined with EHEC isolates which were positive for *eae*A, *stx* and *hly*A genes with low incidence of multiple antibiotic resistance. Prakasan et al. (2018) recently reported 33.33% of shellfish samples were contaminated with Shiga toxin-producing *E. coli*. *Mytilus galloprovincialis* is a filter feeder organism which collects pathogenic microorganisms and different harmfull residues including heavy metals and agricultural waste, as well as organic materials from the coastal and estuarine environments. In addition, high amino acid content, high pH (approximately 6.55) and high water activity (0.98) of mussels facilitate to colonization and transmission of *E. coli* and other pathogens (Sengor et al., 2004; Gourmelon et al. 2006). However preparation of the stuffed mussels includes cooking period that is high enough to kill most vegetative cells (Kisla ve Uzgun, 2008). According to Kisla ve Uzgun (2008), stuffed mussels were commonly exposed to unsuitable environmental conditions such as soil, dust, insects, flies etc and high ambient temperatures during retail sale for long times. We also collected stuffed mussel samples from fishmongers which was an outside sale under unsuitable environmental conditions. Furthermore, stuffed mussel mix (spices, oil, salt and boiled rice) is stuffed with hand in the cockleshells (Ates et al., 2011). *E. coli* is classified as faecal coliform and presence of this bacteria in the samples may indicate errors and omissions in handling, lack of sanitary practices by foodhandlers and possible cross-contaminations.

In this study, *E. coli* O157:H7 was isolated from only 1 of 25 (4 %) cheese halva samples. According to literature screening, there is no research related to *E. coli* O157:H7 in cheese halva in Turkey. Nevertheless Secim et al. (2017) investigated presence of *E. coli* in cheese halva samples and reported no contamination. The presence of *E.coli* has been investigated in cheese desserts in some studies; Cokal et al. (2012) and Secim et al (2017) reported that no *E. coli* contamination in Hosmerim desserts. The significance of *E. coli* O157:H7 contamination in milk and cheese samples has previously been reviewed (Zweifel et al., 2010; Lynch et al., 2012). As the cheese halva is a heat-treated dessert, the presence of *E. coli* O157:H7 in cheese halva might have originated from post heating contamination during packaging process or personel. Although *E. coli* is inactivated by some barrier factors like heat treatment in the processed foods, subsequent cross contamination could be of concern (Wahi et al., 2006).

In the present study, 3 Russian salad samples (5.5%) were found positive for *E. coli,* 2 of which (3.7 %) were determined as *E. coli* O157 with *stx*1 gene and CIP resistance was detected in one of them. Russian salad is a mayonnaise based salad. Althought mayonnaise is relatively resistant to microbial spoilage due to its low pH, it is known that *E. coli* and pathogenic *E. coli* serotypes have inducible acid resistance mechanisms. A study by Zhao and Doyle (1993) revealed that *E. coli* O157:H7 can survive at 5°C in mayonnaise for several weeks, in case of unsuitable manufacturing practices or any type of cross-contamination (contaminated vegetables in salad, dirty kitchen equipments, food handlers ect) of mayonnaise. In this study, Russian salad samples were bought from restaurants and grocery stores in which ready to eat foods were sold at retail without package. The contamination may be associated with unhygienic ingredients of salad, food handlers, utensils and contact surfaces.

In our study, no *E. coli* or pathogenic *E. coli* serotypes was detected in meatless cig kofte samples. Althought meatless cig köfte can serve as a vector for the transmission of some human pathogens (Taban, 2012; Delikanli et al. 2014), no reports are available about the examination of *E. coli* O157:H7 in meatless cig köfte samples. Several studies have demonstrated that garlic, spices and onion which are meatless cig kofte ingredients are able to inhibit pathogenic *E. coli* serotype growth, depending on the concentration, storage time and temperature (Koidis et al., 2000; Kim and Kim, 2007; Rounds et al., 2013).

In this study, one isolate found to carry *stx*1 and one isolate *hly*A gene. These results for detection rates of toxin genes were higher than the study conducted by Cho et al. (2010) which showed absence of the *stx* genes of street-vended foods in Korea. However, Gupta el al. (2012) reported from India the preva-

lence of *stx1* and *stx2* genes of RTE fish product were 5.55% and 7.4% respectively, higher than our results. The pathogenity of *E. coli* serotypes are related to their virulence factors, shiga toxins, enterohemolysin and intimin. Enterohemolysin (encoded by the *hly*A gene) causes the lysis of erythrocytes, which provide iron uptake in the intestinal environment (Dontorou 2003). Shiga toxins (*Stx* 1, 1c, 2, 2c, 2d, 2dact, 2e, 2f) are the primary virulence factor of pathogenic *E. coli s*erotypes which can be defined as the locus enterocyte effacement (LEE) of the adherence system (Obrig 2010). *Stx* lead to inflammatory and thrombogenic changes in the endothelial cells causing HUS and thrombotic microangiopathy (TMA), especially effects kidneys and other potential organs (Bruyand et al., 2018). *E. coli* O111 and O26 were not detected in any sample in our study. In contrast, the current results were reported by Hassanin et al. (2014), for RTE meat and chicken products, the rates of O111 and O26 serotypes were between 6.7-33.3%.

Results of this study demonstrated that MER and STX were the most effective agents against *E. coli* with susceptibility rate of 100%. Recent studies have also been describing STX and MER resistant *E. coli* isolates (Campos et al, 2013; Rasheed et al. 2014; Lima et al. 2017; Ye et al., 2018) in RTE foods. Of the 7 *E. coli* isolates examined, we found an overall prevalence of 42% (n=3) isolates showed resistance rate to AMC (29%), CIP (29%) and GEN (14%) (Table 4). AMC, CIP and GEN resistance has been reported in studies performed worldwide, concerning RTE foods (Rasheed et al. 2014; Kochakkhani et al. 2016; Baloch et al. 2017; Ye et al. 2018). Our results were lower than those found by Kochakkhani et al. (2016) (100% for AMC and CIP) and Baloch et al. (2017) (80.9% for AMC and 18% for GEN). Also, the low percentage of resistance to AMC, CIP and GEN was noted by Campos et al. (2013) (5% for CIP), Lima et al (2017) (none for CIP and GEN) and Rasheed et al. (2014) (8.6% for AMC). Moreover 1 (14.2%) multidrug resistant isolate also detected in the study (Table 4). This result is in accordance with those reported by Lima et al. (2017) and Baloch et al. (2017) as 13.3% and 17.6% resistance rate respectively. The existence of multidrug resistant strain could create serious threat to the patients because of transferring antimicrobial resistance genes to other pathogens and to humans through food.

The prevalence of pathogenic *E. coli* serotypes always should be carefully evaluated in RTE foods. To our knowledge, no study concerning the prevalence of pathogenic *E. coli* serotypes in RTE foods, including the detection of virulence genes, genotyping and antimicrobial susceptibility, has been conducted previously in Turkey. Results of the study would be useful for monitoring of pathogenic, antibiotic resistant *E. coli* serotypes and for providing information about possible role of RTE foods acting as a vehicle for this pathogen.

CONFLICT OF INTEREST

None declared by the authors.

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