Journal of the Hellenic Veterinary Medical Society

Vol 70, No 3 (2019)

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

F. KARADAL, N. ERTAS ONMAZ, H. HIZLISOY, S. AL, N. TELLİ, Y. YILDIRIM, Z. GONULALAN

doi: [10.12681/jhvms.21790](https://doi.org/10.12681/jhvms.21790)

Copyright © 2019, F. KARADAL, N. ERTAS ONMAZ, H. HIZLISOY, S. AL, N. TELLİ, Y. YILDIRIM, Z. GONULALAN

This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

KARADAL, F., ERTAS ONMAZ, N., HIZLISOY, H., AL, S., TELLİ, N., YILDIRIM, Y., & GONULALAN, Z. (2019). Isolation, genotyping and antimicrobial susceptibility of pathogenic *Escherichia coli* serotypes in ready to eat foods. *Journal of the Hellenic Veterinary Medical Society*, 70(3), 1661–1668. <https://doi.org/10.12681/jhvms.21790>

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

F. Karadal^{*1}, N. Ertas Onmaz², H. Hizlisoy³, S. Al², N. Telli⁴, Y. Yildirim², Z. Gonulalan²

¹ Department of Food Processing, Bor Vocational School, Nigde Omer Halisdemir University, Nigde, Turkey

² Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

³ Department of Veterinary Public Health, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

⁴ University of Selcuk, Vocational School of Technical Sciences, Konya, Turkey

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 *stx1* ve *hlyA* genes whereas one Russian salad isolate carried the *stx1* 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.

Corresponding Author:

Fulden Karadal, Nigde Omer Halisdemir University, Bor Vocational School,
Department of Food Processing, 51700, Nigde - Turkey
E-mail address: fkaradal@ohu.edu.tr

Date of initial submission: 13-11-2018
Date of revised submission: 19-05-2019
Date of acceptance: 15-06-2019

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 (*stx1* and *stx2*), enterohemolysin (*hlyA*) and intimin (*eaeA*) 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	<i>Mytilus galloprovincialis</i> meat with mixed of spices, oil, salt and boiled rice in the cockleshells.	Fishmongers, street vendors, 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 extraction

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 *trpB* which, together with non-specific *trpA* 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 *fliCh7*, *rfbO111*, *wzx-wzyO26* and *rfbO157* genes

The primer pairs for *fliCh7*, *rfbO157*, *rfbO111* and *wzx-wzyO26* 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	<i>trpB</i> <i>trpA</i>	<i>trpBA.f</i>	CGGCGATAAAGACATCTTCA	489	Clermont et al. (2008)
		<i>trpA2.r</i>	GCAACGCGGCCTGGCGGAAG		
H7	<i>fliCh7</i>	FLICH7-F	GCGCTGTGCGAGTTCTATCGAGC	625	Sarimehmetoglu et al (2009)
		FLICH7-R	CAACGGTGACTTTATCGCCATTCC		
LPS O157	<i>rfbO157</i>	PF8	CGTGATGATGTTGAGTTG	420	Maurer et al. (1999)
		PR8	AGATTGTTGGCATTACTG		
O26	<i>wzx-wzyO26</i>	<i>wzx-wzyO26F</i>	AAATTAGAAGCGCGTTCATC	596	Durso et al. (2005)
		<i>wzx-wzyO26R</i>	CCCAGCAAGCCAATTATGACT		
O111	<i>rfbO111</i>	O111F	TAGAGAAATTATCAAGTTAGTTCC	406	Paton and Paton (1998)
		O111R	ATAGTTATGAACATCTTGTTTAGC		
Shiga-like toxin 1	<i>stx1</i>	SLTI-F	TGTAAGTGGAAAGGTGGAGTATACA	210	Fratamico et al. (2000)
		SLTI-R	GCTATTCTGAGTCAACGAAAAATAC		
Shiga-like toxin 2	<i>stx2</i>	SLTII-F	GTTTTTCTTCGGTATCCTATTCC	484	Fratamico et al. (2000)
		SLTII-R	GATGCATCTCTGGTCATTGTATTAC		
Intimin	<i>eaeA</i>	AE22	ATTACCATCCACACAGACGGT	397	Fratamico et al. (2000)
		AE20-2	ACAGCGTGGTTGGATCAACCT		
Enterohemolysin	<i>hlyA</i>	MFS1-F	ACGATGTGGTTTATTCTGGA	166	Fratamico et al. (2000)
		MFS1-R	CTTCACGTCACCATACATAT		

Detection of virulence genes (*stx1*, *stx2*, *eaeA* and *hlyA*) by Multiplex PCR

Multiplex PCR (mPCR) targeting virulence genes of *E. coli* O157: H7, comprising *stx1*, *stx2*, *eaeA* and *hlyA* (Table 2) was carried out in a study conducted by Fratafico 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 Vallée, 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 MgCl₂(Vivantis), 5 U Taq polymerase (Vivantis), 25 pmol each primer and 1 and 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 (Technique 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(%)	<i>rfbO157</i> , <i>fliCh7</i> , <i>rfbO111</i> and <i>wzx-wzyO26</i> and virulence genes							
			<i>rfbO157</i>	<i>fliCh7</i>	<i>rfbO111</i>	<i>wzx-wzyO26</i>	<i>stx1</i>	<i>stx2</i>	<i>hlyA</i>	<i>eaeA</i>
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 *trpA* gene

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

Antibiotics	Diameter of the inhibition zones of <i>E. coli</i> 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 *stx1* 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 *stx1* and *hlyA* 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 Ruler™ 100 bp DNALadder Plus, Fermentas); lane P1: Positive control for *rfbO157* (420 bp), P2 positive control for *fliCh7* gene (625 bp), P3: Positive control for toxin genes (for *stx2* 484 bp, for *eaeA* 397 bp, for *stx1* 210 bp, for *hlyA* 166 bp) N1-2: Negative Control (Sterile H₂O); Line 1-2: *E. coli* O157:H7 isolates; Line 3-4: *E. coli* O157 isolates; Line 5 *stx1*, *hlyA* genes positive isolates. Line 6: *stx1* 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 occurred in 2 (29%) *E. coli* isolates from stuffed mussel, one of which was multidrug resistant to three antibiotics (AMC, CIP and GEN). Furthermore, *stx1* 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 *stx1* 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 in terms of *E. coli* and one of them was defined as O157:H7 (0.95%) containing *stx1* and *hlyA* 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 demonstrated 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 contaminated with EHEC isolates which were positive for *eaeA*, *stx* and *hlyA* 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 harmful 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 (Ateş 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 personnel. 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 *stx1* gene and CIP resistance was detected in one of them. Russian salad is a mayonnaise based salad. Although 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 etc) 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 köfte samples. Although 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 köfte 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 *stx1* and one isolate *hlyA* 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 et 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 pathogenicity of *E. coli* serotypes are related to their virulence factors, shiga toxins, enterohemolysin and intimin. Enterohemolysin (encoded by the *hlyA* 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* serotypes which can be defined as the locus enterocyte effacement (LEE) of the adherence system (Obrig 2010). *Stx* lead to inflammatory and thrombotic 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.

REFERENCES

- Ates M, Ozkizilcik A, Tabakoglu C (2011) Microbiological analysis of stuffed mussels sold in the streets. *Indian J Med Microbiol* 51(3):350-354.
- Baloch AB, Yang H, Feng Y, Xi M, Wu Q, Yang Q, Tang J, He X, Xiao Y, Xia X (2017) Presence and Antimicrobial resistance of *Escherichia coli* in Ready-to-Eat Foods in Shaanxi, China. *J Food Prot* 80(3):420-424.
- Bingol EB, Colak H, Hampikyan H, Muratoglu K (2008) The microbiological quality of stuffed mussels (Midye Dolma) sold in Istanbul. *Brit Food J* 110(11):1079-1087.
- Bruyand M, Mariani-Kurkdjian P, Gouali M, de Valk H, King LA, Hello SL, Bonacorsi S, Loirat C (2018) Hemolytic uremic syndrome due to Shiga toxin-producing *Escherichia coli* infection. *Med Mal Infect* 48:167-174.
- Campos J, Mourao J, Pestana N, Peixe L, Novais C, Antunes P (2013). Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. *Int J Food Microbiol*, 166 (3), 464–470.
- Chapman PA, Wright DJ, Siddons CA (1996) A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *J Med Microbiol* 40:424–427.
- Cho JI, Cheung CY, Lee SM, Ko S I, Kim K H, Hwang IS, Kim SH, Cho SY, Lim CJ, Lee KH, Ha SD, Kim KS (2011) Assessment of microbial contamination levels of street- vended foods in Korea. *J. Food Safety*, 31: 41-47
- Clermont O, Lescat M, O'Brien LC, Gordon DM, Tenaillon O, Denamur E (2008) Evidence for a human-specific *Escherichia coli* clone. *Environ Microbiol* 10:1000–1006
- Cody SH, Glynn MK, Farrar JA, Cairns KL, Griffin PM, Kobayashi J, et al. (1999) An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann Intern Med* 130:202-209
- Cokal Y, Dagdelen A, Cenet O, Gunsen U (2012) Presence of *L. monocytogenes* and some bacterial pathogens in two Turkish traditional foods, Mihalic cheese and Hosmerim dessert. *Food Contol* 26:337-340
- Delikanli B, Sonmez B, Ozdemir Y (2014) Microbiological quality of the meat-free cig kofte consumed in Bursa City center. *Harran Univ J Fac Vet Med* 3(1):13-17
- Dontorou A, Papadopoulou C, Filioussis G, Economou V, Apostolou I, Zakkas G, Salamoura A, Kansouzidou A, Levdiotou S (2003) Isolation of *Escherichia coli* O157:H7 from foods in Greece. *Int J Food Microbiol* 82: 273-279.
- Durso LM Bono JL Keen JE (2005) Molecular serotyping of *Escherichia*

- coli* O26:H11. Appl Environ Microbiol 71:4941–4944.
- Elobeid T, Aziz HA, Mousa R, Alzahiri A (2014) Survey on the microbial quality of traditional foods sold by street vendors in Qatar. Austin J NutrMetab 1(2): 4-20.
- Fratamico PM, Bagi LK, Pepe TA (2000) multiplex polymerase chain reaction assay for rapid detection and identification of *Escherichia coli* O157:H7 in foods and bovine feces. J Food Protect 63:1032-1037.
- Gourmelon M, Montet MP, Lozach S, Le Menec C, Pommepuy M, Beutin L, Vernozy-Rozand C (2006) First isolation of Shiga toxin 1d producing *Escherichia coli* variant strains in shellfish from coastal areas in France. J Appl Microbiol 100: 85-97.
- Gupta B, Ghatak S, Gill JPS (2013) Incidence and virulence properties of *E. coli* isolated from fresh fish and ready-to-eat fish products. Vet. World 6(1): 5-9.
- Hampikyan H, Ulusoy B, Bingöl EB, Çolak H, Akhan M (2008) Determination of microbiological quality of some grilled food, salad and appetizers. Türk Mikrobiol Soc 38:87–94 (article in Turkish with an abstract in English).
- Hassanin FS, Reham A, Amin, Shawky NA, Gomaa WM (2014) Incidence of *Escherichia coli* and *Salmonella* in ready to eat foods. Benha Veterinary Medical Journal 27(1): 84-91.
- Houf KL, De Zutter L, Van Hoof J, Vandamme P (2002) Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. Appl Environ Microbiol 68:2172–2178
- Jaroni D, Ravishankar S, Juneya V (2010) Chapter 1: Microbiology of ready-to-eat foods. In: Hwang A. and Huang L (Eds), Ready-to-Eat Foods Microbial Concerns and Control Measures. Boca Raton: CRC Press, pp.1-60.
- Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF (2002) Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrob. Agents Chemother 46: 2540–2545
- Kim JS, Kim Y (2007) The inhibitory effect of natural bioactives on the growth of pathogenic bacteria. Nutr Res Pract 1: 273-278
- Kisla D, Uzun Y (2008) Microbiological evaluation of stuffed mussels. JPF 7:616–620
- Koidis P, Iossifidou E, Abraham A, Ambrosiadis I (2000) The effectiveness of different spices as inhibitors for *Escherichia coli* O157:H7 in nutrient broth stored at 4°C or 12°C. Arch. Lebensmittelhyg 51 (6):129 – 152
- Kocatepe D, Taşkaya G, Turan H, Kaya Y (2016) Microbiological investigation of wild, cultivated mussels (*Mytilus galloprovincialis* L. 1819) and stuffed mussels in Sinop Turkey. Ukrainian Food Journal 5 (2):299-305.
- Kochakkhani H, Dehghan P, Moosavi MH, Sarmadi B (2016) Occurrence, molecular detection and antibiotic resistance profile of *Escherichia coli* O157: H7 isolated from ready-to-eat vegetable salads in Iran. Pharm Sci 22: 195-202.
- Lima CM, Souza IEGL, Dos Santos Alves T, Leite CC, Evangelista-Barreto NS, de Castro Almeida RC (2017) Antimicrobial resistance in diarrheagenic *Escherichia coli* from ready-to-eat foods. J Food Sci Technol 54:3612–3619.
- Lynch MJ, O'Connor L, Fox EM, Jordan K, Murphy M (2012) Verocytotoxigenic *Escherichia coli* O157, O111, O26, O103, O145 in Irish dairy cattle and raw milk: prevalence and epidemiology of emergent strains. Zoonoses Public Hlth 59: 264–271.
- Maurer JJ, Schmidt D, Petrosko P, Sanchez S, Bolton L, Lee MD (1999). Development of primers to O-antigen biosynthesis genes for specific detection of *Escherichia coli* O157 by PCR. Appl Environ Microbiol 65: 2954-2960.
- Montville TJ, Matthews KR, Kniel KE (2012) Food Microbiology an Introduction, In: Chapter 12, Enterohemorrhagic *Escherichia coli*. Washington, USA: ASM Press, 3rd Edition, p.170-87.
- Obrig TG (2010) *Escherichia coli* shiga toxin mechanisms of action in renal disease. Toxins 2: 2769–2794.
- Oz V, Karadayi S, Cakan H, Karadayi B, Cevik FE (2014) Assessment of microbiological quality of ready-to-eat foods in Istanbul, Turkey J Food Agric Environ 12:56-60
- Paton AW, Paton JC (1998) Detection and characterization of Shiga toxin-producing *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. J Clin Microbiol 36:598–602
- Prakasan S, Prabhakar P, Lekshmi M, Nayak BB, Kumar S (2018) Isolation of Shiga toxin-producing *Escherichia coli* harboring variant Shiga toxin genes from seafood. Vet World 11: 379-385.
- Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K (2014) Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Rev Inst Med Trop Sao Paulo 56(4):341–346.
- Rounds L, Havens CM, Feinstein Y, Friedman M, Ravishankar S (2013) Concentration-dependent inhibition of *Escherichia coli* O157:H7 and heterocyclic amines in heated ground beef patties by apple and olive extracts, onion powder and clove bud oil. Meat Sci 94(4):461-467.
- Sarimehmetoglu B, Aksoy MH, Ayaz ND, Ayaz Y, Kuplulu O, Kaplan YZ (2009). Detection of *Escherichia coli* O157:H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Control 20: 357-361.
- Secim Y, Ucar G (2017) Evaluation of the desserts; which are hosmerim, cheese halva, kunafah produced in Turkish cuisine -in aspect of tourism. IJSSER 3(5): 1478-1484
- Sengor GF, Kalafatoğlu H, Gun H (2004) The determination of microbial flora, water activity and chemical analyses in smoked, canned mussels (*Mytilus galloprovincialis*, L.). Turk J Vet Anim Sci 28: 793-797.
- Spencer KC (2005) Chapter 12: Modified atmosphere packaging of ready-to-eat foods. In: Han J.H. (ed) Innovations in food packaging. Elsevier, USA, pp. 185-203.
- Surendraraj A, Thampuran N, Joseph TC (2010) Molecular screening, isolation, and characterization of enterohemorrhagic *Escherichia coli* O157:H7 from retail shrimp J Food Protec 73 (1): 97–103.
- Taban MB (2012) *Listeria monocytogenes* in cig kofte without meat: A novel bulgur ball product. J Food Agric Environ 10(2): 130-132,
- Tudoran AA, Fischer ARH, van Trijp HCM, Grunert K, Krystallis A, Esbjerg L (2012) Overview of consumer trends in food industry, retailer and consumer acceptance of promising novel technologies and collaborative innovation management, Retailer and Consumer Acceptance of Promising Novel Technologies and Collaborative Innovation Management, Deliverable D2.1 p.8.
- Wahi S, Bansal S, Ghosh M, Ganguli A (2006) Growth and survival of *Escherichia coli* O157:H7 during manufacture and storage of Indian cheese (paneer). Foodborne Pathog Dis 3(2):184-189.
- Ye Q, Wu Q, Zhang S, Zhang J, Yang G, Wang J, Xue L and Chen M (2018). Characterization of Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae from retail food in China. Front Microbiol 9: 1-12
- Zhao T, Doyle MP (1993) Fate of Enterohemorrhagic *Escherichia coli* O157: H7 in commercial mayonnaise. J Food Prot 57: 780-783
- Zweifel C, Giezendanner N, Corti S, Krause G, Beutin L, Danuser J, Stephan R (2010) Characteristics of Shiga toxin-producing *Escherichia coli* isolated from Swiss raw milk cheese within a 3-year monitoring program. J Food Prot 73: 88–91.