Kafkas Univ Vet Fak Derg
20 (6): 957-960, 2014
DOI: 10.9775/kvfd.2014.11310

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

SHORT COMMUNICATION

Examination of *Escherichia coli* O157:H7 and some Virulence Genes in Marketed Minced Meat Samples

Recep KALIN [1] Hasan ÖNGÖR [2]

[1] Cumhuriyet University, Faculty of Veterinary, Department of Microbiyology, TR-58140 Sivas - TURKEY
[2] Firat University, Faculty of Veterinary, Department of Microbiyology, TR- 23119 Elazig - TURKEY

Article Code: KVFD-2014-11310 Received: 01.04.2014 Accepted: 10.06.2014 Published Online: 30.06.2014

Abstract

In this study, the presence of *Escherichia coli (E. coli)* O157:H7 was investigated in minced meat samples. *E. coli* O157:H7 was detected in six (7.5%) of the minced meat samples. Examination of the positive isolates for the presence of virulence genes; *stx1* was determined in one isolate, *stx2* in three isolates and both *stx1* and *stx2* were found in one isolate. Also eae genes were observed in all the positive isolates.

Keywords: *E. coli O157:H7, Minced meat, PCR*

Tüketime Sunulan Kıymalarda *Escherichia coli* O157:H7'nin ve Bazı Virülens Genlerinin Araştırılması

Özet

Bu çalışmada, tüketime sunulan kıyma örneklerinin *Escherichia coli (E. coli)* O157:H7 yönünden incelenmesi amaçlandı. Kıyma örneklerinin 6'sında (%7.5) *E. coli* O157:H7 tespit edildi. Bu kıyma izolatları, virülens genleri bakımından incelendiğinde bir tanesinde *stx1*, üç tanesinde *stx2* ve bir tanesinde hem *stx1* hem de *stx2* geni tespit edildi. Ayrıca izolatların tümünde eae genlerinin varlığı gözlendi.

Anahtar sözcükler: *E. coli O157:H7, Kıyma, PCR*

INTRODUCTION

Shigatoxin producing *Escherichia coli* (STEC) strains are included in the significant foodborne pathogens and O157 is known as the most shigatoxin (stx) releasing serotype [1]. This serotype cause severe diseases in humans such as hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) characterized with hemolytic anemia, thrombo-cytopenia and renal failure [2].

E. coli O157 was firstly reported in the United States in 1982 and gave rise to serious outbreaks in many countries in decades [3]. It has been reported that most of the O157 infections in the US were raised from foods and most of these infections were resulted from consumption of contaminated foods including ground beef [4].

Cattle are considered as the main reservoir of the agent and agents in digestive tract cause contamination of meat and meat products during slaughtering. On the other hand, contamination come up through the environmental route or while dressing [5]. Besides this, the pathogen can enter the production chain in storehouses, butchers, markets, restaurants etc. and pose risk for public health [6].

E. coli O157 has some virulence factors such as flagellar antigen H7, (*fliCh7*) shigatoxins (*stx*), and intimin (*eae*). *Stx* has a similar structure with *Shigella dysenteriae* type 1 toxin and inhibits protein synthesis and cause cell death due to affecting ribosomal RNA [5]. Additionally, the agents adhere to intestinal epithelial cells with *eae* adhesion and cause various diseases in humans [2].

Detection of STEC O157 strains in meat and stool samples by conventional methods is time consuming, owing to the existence of other bacterial species in samples. On the other hand, investigating the *E. coli* O157 and its virulence genes can be carried out in a short time by Polymerase Chain Reaction (PCR) tests.

İletişim (Correspondence)
+90 346 2191010
recep.kalin@gmail.com

The aim of this study was to isolate *E. coli* O157 from minced meat samples obtained from restaurants, grilled meatball restaurants and butchers and to investigate the presence of shigatoxin (*stx*$_1$ and *stx*$_2$), intimin (*eae*), O157 (O157 *rfbE*), and H7 antigen (*fliCh7*) genes by PCR.

MATERIAL and METHODS

Sample Collection

A total of 80 minced meat samples were collected from butchers, restaurants and grilled meatball restaurants in Elazig province between December 2009 and November 2010. Approximately 50 g minced meat sample were put into sterile stomacher bags and transferred to the laboratory within 2 h in cold chain conditions. All the samples were analyzed on same day in the laboratories of Department of Microbiology, Faculty of Veterinary Medicine, University of Fırat.

Isolation

Ten grams of minced meat sample was put into sterile bags and treated with 100 ml enrichment broth that composed of modified tryptone soy broth (mTSB) (CM0989, Oxoid)] containing 20 mg/l novobiocin (SR0181, Oxoid). The samples were homogenized in mTSB broth using a stomacher (Bag mixer, Interscience, France) for 2 min. Liquid part of sample was transferred to erlenmayer flask and left for incubation at 41.5°C for 24 h for preenrichment. After incubation, pre-enriched samples were plated onto CT-SMAC (sorbitol MacConkey's agar [SMAC; CM0981, Oxoid] containing 0.05 mg/L Cefixime and 2.5 mg/L tellurite [SR0172; Oxoid]) both directly and with Immuno Magnetic Separation (IMS) and incubated at 37°C for 24 h.

Samples were subjected to IMS, using dynabeads anti-*E. coli* O157 (Dynal Biotech, Oslo, Norway), as described by the manufacturer. The pellet was resuspended in 50 µL of distilled water and used for cultivation. Forty microliters of the samples from IMS and a loopful (5 mm diameter) from pre-enrichment broths were plated onto CT-SMAC and incubated at 37°C for 24 h.

Non-sorbitol-fermenting β-glucuronidase negative (pale) colonies were selected for the detection of O157 (*rfbE*) and virulence genes by PCR.

DNA Extraction and PCR

A few suspicious colonies grown on CT-SMAC medium were transferred into microcentrifuge tube and homogenized with 300 µl sterile distilled water. DNA extraction method and PCR amplification protocol was performed according to our previous study [7], and the presence of O157 *rfbE*, *fliCh7*, *stx*$_1$, *stx*$_2$, and *eae*, genes were investigated in sorbitol negative isolates by PCR with specific primer pairs *(Table 1)*.

The amplified products were detected by ethidium bromide (0.5 µg/ml) staining after electrophoresis at 80 V for two hours in 1.5% agarose gel. A reference strain of *E. coli* O157:H7 (ATCC 43895) was included as positive control and distilled water was used as a negative control at all steps of the assay *(Fig. 1)*.

RESULTS

Twenty five of minced meat samples cultured on CT-SMAC medium were found to be negative for sorbitol and β-glucuronidase. Of the 25 isolates, 11 were obtained from direct plating and 14 were by IMS method. Isolates determined by direct plating and IMS were different, none of them was detected with both (direct plating or IMS) methods. In the O157-specific PCR analysis *E. coli* O157 was identified in six of IMS isolates but none of the direct plating isolates were found to be positive for *E. coli* O157 *(Fig. 1)*.

All O157 strains were examined for the existence of *stx*$_1$, *stx*$_2$, *fliCh7* and *eae* virulence genes by PCR assays. The *stx*$_1$ gene was detected in only one and *stx*$_2$ was in three of isolates. One of the isolates possessed both *stx*$_1$ and

Table 1. *Primer sequences and lengths of PCR amplification products*
Tablo 1. *PCR amplifikasyon ürünlerinin primer sekansları ve uzunlukları*

Gene	Primer	Oligonucleotide Sequence (5'-3')	Fragment size (bp)	Reference
stx$_1$	VT1-A VT1-B	CGCTGAATGTCATTCGCTCTGC CGTGGTATAGCTACTGTCACC	302	[2]
stx$_2$	VT2-A VT2-B	CTTCGGTATCCTATTCCCGG CTGCTGTGACAGTGACAAAACGC	516	[2]
O157 *rfbE*	O157-AF O157-AR	AAGATTGCGCTGAAGCCTTTG CATTGGCATCGTGTGGACAG	497	[8]
fliCh7	H7-F H7-R	GCGCTGTCGAGTTCTATCGAGC CAACGGTGACTTTATCGCCATTCC	625	[9]
eae	eaeAF eaeAR	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384	[10]

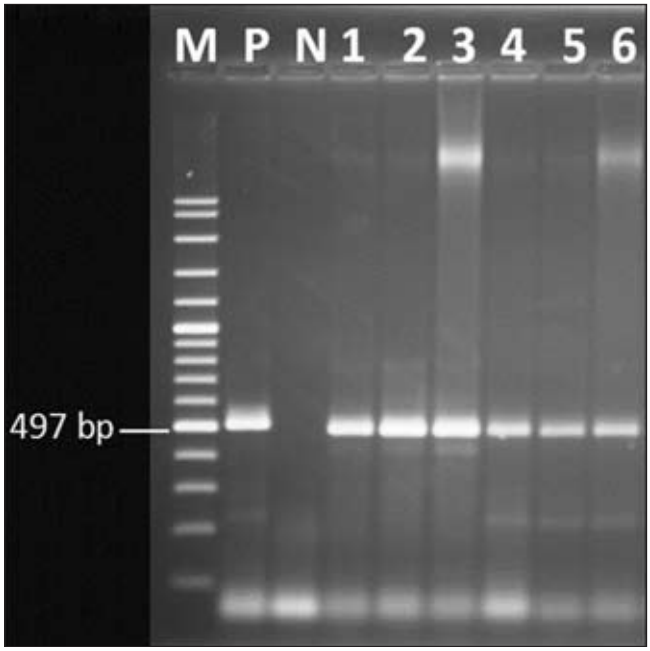

Fig 1. *Escherichia coli* O157 specific polymerase chain reaction products of isolates obtained from minced meat samples. **M:** Marker, **P:** Positive control, **N:** Negative control, 1**, 2, 3, 4, 5, 6:** Positive isolates

Şekil 1. Kıyma örneklerinden elde edilen izolatların *Escherichia coli* O157 spesifik polimeraz zincir reaksiyonu ürünleri. **M:** Marker, **P:** Pozitif kontrol, **N:** Negatif kontrol, **1, 2, 3, 4, 5, 6:** Pozitif izolatlar

products, in Turkey [7,12]. In other studies performed in different countries, the prevalence of *E. coli* O157 was reported to vary from 0.2% to 15% [1,5]. High isolation rate such as 73% has been reported in South Africa [13].

In the present study, *E. coli* O157:H7 was detected in 7.5% (6/80) of minced meat samples by both conventional and molecular methods. This proportion is in parallel with previous studies except the researchers conducted in South Africa. The differences between the results may be due to cultivation method, sample size and resource, geographical region, season and degree of dispersion of *E. coli* O157: H7 infections in the region [14].

Immunomagnetic separation (IMS) is an easy, rapid and reliable assay, which has been widely used for epidemiological studies of *E. coli* O157:H7 and has provided to accomplish efficient recovering microorganisms from heterogeneous samples [15]. In the current study higher *E. coli* O157:H7 isolation rate was obtained by IMS, although all of the direct plated samples were found to be negative. This conclusion suggests that combination of conventional culture method and PCR assay are more advantageous than single cultivation methods for identification of *E. coli* O157:H7 serotype.

It has been reported that the virulence factors such as

Table 2. *Distribution of virulence genes of Escherichia coli O157 isolated from minced meat samples*
Tablo 2. *Kıyma örneklerinden elde edilen Escherichia coli O157 izolatlarının virülens genlerinin dağılımı*

Numbers of Positive Isolates	Number of Samples (n)	Number of Samples Positive by PCR	O157 *rfbE*	*fliCh7*	*eae*	stx_1	stx_2	stx_1 + stx_2
1	80	6 (%7.5)	+	+	+	-	-	-
2			+	+	+	-	-	-
3			+	+	+	-	-	-
4			+	+	+	-	+	-
5			+	+	+	-	+	-
6			+	+	+	+	+	+
Total	80	6	6	6	6	1	3	1

stx_2. Neither stx_1 nor stx_2 genes were determined in three of isolates. All of the *E. coli* O157 isolates were positive for *fliCh7* (H7) and *eae* genes *(Table 2)*.

DISCUSSION

The most important source of *E. coli* O157 originated infections in humans is foods and animal products have a significant role. Raw or undercooked meats may pose the agent. Although *E. coli* O157 has been found in animals such as cattle, sheep, pigs, and goats, studies indicated that poultry may also carry it and pose health risk to humans [11].

The prevalence of *E. coli* O157 was reported to vary between 0-6% in studies conducted on meat and meat

stx_1, stx_2 and intimin are frequently associated with HC and HUS in humans [10]. In this study, multiplex PCR results showed that 16.6% and 50% of *E. coli* O157:H7 isolates were found to be positive for stx_1 and stx_2 respectively and pose a severe health risk to humans. Studies conducted on cattle and cattle meats were declared high amount of stx_2 gene presence in *E. coli* O157 serotype [2]. Also all the *E. coli* O157:H7 isolates in the present study were found to possess intimin that leads to diarrhea (and encourage HC) in humans by an attaching and effacing (A/E) ability. This result was in parallel with previous studies [2].

In conclusion, this study revealed that some of the marketed minced meats were contaminated with *E. coli* O157:H7. All the isolates were determined to possess the *eae* gene and 50% of them were found to contain at

least one of the stx_1 or stx_2, which are important in the development of HC and HUS conditions. The consumption of these meats may have potential risk for human health. Taking hygienic precautions and complying with Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practice (GMP) requirements in all steps of processing meat and meat products are important to prevent the *E. coli* O157:H7 infections in humans.

REFERENCES

1. Fantelli K, Stephan R: Prevalence and characteristics of Shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int J Food Microbiol*, 70, 63-69, 2001.

2. Blanco M, **Padola NL**, **Krüger A**, **Sanz ME, Blanco JE**, **González EA, Dahbi G**, **Mora A**, **Bernárdez MI, Etcheverría AI**, **Arroyo GH**, **Lucchesi PMA, Parma AE**, **Blanco J:** Virulence genes and intimin types of Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina. *Int Microbiol*, 7, 269-276, 2004.

3. Centers for Disease Control and Prevention (CDC): Multistate outbreak of Escherichia coli O157:H7 infections from hamburgers Western United States, 1992-1993. http://www.cdc.gov/mmwR/preview/mmwrhtml/00020219.htm, *Accessed*: 13.01.2014.

4. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL: Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002 *Emerg Infect Dis*, 11, 603-609, 2005.

5. Nataro JP, and Kaper JB: Diarrheagenic *Escherichia coli. Clin Microbiol Rev*, 11, 142-201, 1998.

6. Cagneya C, Crowleya H, Duffya G, Sheridana JJ, O'Briena S, Carneya E, Andersonb W, McDowellc DA, Blairc IS, Bishop RH: Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology*, 21, 203-212, 2004.

7. Ongor H, Kalin R, Cetinkaya B: Investigations of *Escherichia coli* O157 and some virulence genes in samples of meat and faeces from clinically healthy cattle in Turkey. *Vet Rec,* 161, 392-394, 2007

8. Desmarchelier PM, Bilge SS, Fegan N, Mills L, Vary JR, Tarr PI: A PCR specific for *Escherichia coli* O157 based on the *Rfb* locus encoding O157 lipopolysaccharide. *J Clin Microbiol*, 36, 1801-1804, 1998.

9. Gannon VP, D'souza S, Graham T, King RK, Rahn K, Read S: Use of the flagellar H7 gene as a target in multiplex pcr assays and improved specificity in identification of Enterohemorrhagic *Escherichia coli* strains. *J Clin Micro*biol, 35, 656-662, 1997.

10. Paton AW, Paton JC: Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. *J Clin Microbiol*, 36, 598-602, 1998.

11. Kalin R, Ongor H, Cetinkaya B: Isolation and molecular characterization of *Escherichia coli* O157 from broiler and human samples. *Foodborne Pathog Dis*, 9, 313-318, 2012.

12. Kalender H. Isolation, virulence genes and antimicrobial susceptibilities of Shiga Toxin-Producing *Escherichia coli* O157 from slaughtered cattle in abattoirs and ground beef sold in Elazığ. *Kafkas Univ Vet Fak Derg*, 19 (3): 461-467, 2013. DOI: 10.9775/kvfd.2012.8040

13. Abong'o BO: Prevalence of *Escherichia coli* O157:H7 in Water and Meat and Meat Products and Vegetables Sold in the Eastern Cape Province of South Africa and its Impact on the Diarrhoeic Conditions of HIV/AIDS Patients. 1-446, University of Fort Hare, 2008.

14. Hussein HS, Sakuma T: Invited review: Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J Dairy Sci*, 88, 450-465, 2005.

15. Wright DJ, Chapman PA, Siddons CA: Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiol Infect*, 113, 31-39, 1994.