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 Microbiology, TR-58140 Sivas - TURKEY
2 Firat University, Faculty of Veterinary, Department of Microbiology, TR- 23119 Elazig - TURKEY

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

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 [6]. 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.
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, and stx2), 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, stx1, stx2, 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 stx1, stx2, fliCh7 and eae virulence genes by PCR assays. The stx1 gene was detected in only one and stx2 was in three of isolates. One of the isolates possessed both stx1 and
Neither stx₁ nor stx₂ 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 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% [13]. 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 stx₁, stx₂, and intimin are frequently associated with HC and HUS in humans [16]. In this study, multiplex PCR results showed that 16.6% and 50% of E. coli O157:H7 isolates were found to be positive for stx₁ and stx₂ respectively and pose a severe health risk to humans. Studies conducted on cattle and cattle meats were declared high amount of stx₂ 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) 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

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<th>Numbers of Positive Isolates</th>
<th>Number of Samples (n)</th>
<th>Number of Samples Positive by PCR</th>
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It has been reported that the virulence factors such as...
Examination of Escherichia coli ... least one of the stx₁ or stx₂, 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