

Full Length Research Paper

Isolation and identification of *Escherichia coli* O157:H7 from ground beef hamburgers in Khuzestan Province, Iran

Ahmad Farajzadeh Sheikh^{1,3}, Soodabeh Rostami^{1,2}, Mansour Amin^{1,3}, Armaghan Abbaspour⁴,
Hamed Goudarzi^{1,2} and Mohammad Hashemzadeh^{1,2,3*}

¹Microbiology Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

²Student Research Committee, Vice-Chancellor for Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

³Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

⁴Expert of laboratory, Golestan Teaching Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Accepted 19 December, 2012

Meat products have been implicated in outbreaks of *E.coli* O157:H7 in most of the world. This bacterium is associated with diseases such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. In this study, we used two Conventional methods and Multiplex PCR for the isolation and identification of *E. coli* O157:H7 from ground beef hamburger samples. In total, 200 fresh ground beef hamburger samples were obtained from different meat factories across Khuzestan Province from March to September 2010. Tryptone- Soya- broth (TSB) supplemented with novobiocine (20 mg/l) was used as enrichment medium and Tellurite cefixime-sorbitol MacConkey agar (TC-SMAC) was used for the detection of Non-Sorbitol Fermenting bacteria. Biochemical tests were performed on the Non-Sorbitol fermenting (NSF) colonies. Colonies confirmed as *E. coli* were selected as templates for Multiplex PCR method and serotyping. Out of the 200 ground beef hamburger samples, 8 samples (4%) had positive results for Non-Sorbitol fermenting colonies (NSF). Out of the 8 samples, three (38%) were confirmed as *E. coli* by biochemical tests. Of the 3 samples, two samples were *E. coli* O157:H7 based on multiplex PCR and serotyping. The results indicate that hamburgers could be a reservoir of *E.coli* O157:H7 in Khuzestan Province. Since this strain is a food-borne pathogen, inspection of meat products for this bacterium is recommended.

Key words: Hamburger, *E. coli* O157:H7, multiplex PCR.

INTRODUCTION

Escherichia coli O157:H7 (*E. coli* O157) is an important food-borne pathogen (You et al., 2006; Bai et al., 2010; Olesen and Jespersen, 2010; Hsu et al., 2005) which was first identified as a human enteric pathogen in 1982

(You et al., 2006; Dontorou et al., 2003; Lee and Choi, 2006). In North America, serotype O157:H7 alone is responsible for a number of *E. coli* outbreaks involving 75,000 human infections per year (Bai et al., 2010). This serotype is a major cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS) (You et al., 2006; Dontorou et al., 2003; Olesen and Jespersen, 2010; Lee and Choi, 2006). The primary reservoir of this strain is the cattle. Cattle feces are a major source of contamination

*Corresponding author. E-mail: Hashemzadeh70@yahoo.com.
Tel: +98-916- 904-3907. Fax: +98-611-3332036.

Table 1. List of target genes, sequence of primers, products size (bp).

Primer		Sequence	Size (bp)	Reference
<i>rfbE</i>	F	5'-AAGATTGCGCTGAAGCCTTTG-3'	497	7
	R	5'-CATTGGCATCGTGTGGACAG-3'		
<i>fliC</i>	F	5'-GCGCTGTCGAGTTCTATCGAGC-3'	625	7
	R	5'-CAACGGTGACTTTATCGCCATTCC-3'		
<i>uidA</i>	F	5'-GCGAAAACGTGGAATTGGG-3'	252	7
	R	5'-TGATGCTCCATAACTTCCTG-3'		

of beef, other food products, and water (You et al., 2006; Dontorou et al., 2003; Bai et al., 2010; Olesen and Jespersen, 2010). The illness is often related to the consumption of contaminated and undercooked ground beef. Also Ground beef hamburger can be a source of *E. coli* O157. Thus surveillance and detection of this bacterium in food and drug laboratories is very important (You et al., 2006; Dontorou et al., 2003; Bai et al., 2010; Olesen and Jespersen, 2010).

This pathogen is identified by classical microbiological diagnostic procedures based on its inability to ferment sorbitol (Lee and Choi, 2006). Several methods from conventional culture methods such as MacConkey agar containing sorbitol instead of lactose (SMAC) to serological assays are used for isolation and identification of *E. coli* O157:H7. Since the conventional methods have low sensitivity and specificity and they will take time, many studies were designed based on molecular techniques such as polymerase chain reaction (PCR) that detect the presence or absence of specific genes (Bai et al., 2010; Al-Ajmi et al., 2006). Many PCR assays have been developed using primers that target specific genes, but combining these primers in a single reaction would be a fast and reliable for determining the presence of the *E. coli* O157:H7.

In the present study, we assessed the presence of *E. coli* O157:H7 in ground beef hamburger samples using biochemical tests, serological method and a multiplex PCR that simultaneously detect three genes including: *fliC* (encoding flagellar antigen), *rfbE* (encoding lipopolysaccharide O-antigen synthesis), and *uidA* (encoding beta-glucuronidase).

MATERIALS AND METHODS

Sample collection

In total, 200 fresh ground beef hamburger samples were obtained from different meat factories across Khuzestan Province from March to September 2010. All samples were randomly collected aseptically in sterile disposable bags and immediately transferred to the laboratory on crushed ice (Dontorou et al., 2003; Jamshidi et

al., 2008).

Culture and isolation

After transferring the samples to the laboratory, they were analyzed without delay. For *E. coli* O157:H7 and O157: H- isolation, 25 g of each sample were placed into 225 ml of Tryptone- Soya- broth (Merck Co., Germany) supplemented with 20 mg/l novobiocin (Himedia, India), and were homogenized into a stomacher bag (Model 400 Bags 6041; Seward Ltd, London, UK) for at least 2 min into a stomacher and then they were incubated at 37°C for 16–18 h. The enriched cultures were sub-cultured into Tellurite (2.5 mg/L) - Cefixime (0.05 mg/L) - Sorbitol MacConkey agar (TC-SMAC) (Merck Co., Germany) and incubated at 37°C for 24 h. The non Sorbitol fermenting colonies were selected from TC-SMAC plates, and were confirmed by biochemical tests including reaction in triple sugar iron agar, methyl red, Voges- Proskaur, utilization of citrate, maltose and mannitol, production of urease, phenylalanine deaminase, beta- glucuronidase and indole and serotyping for Non Sorbitol Fermenting (NSF) bacteria as *E. coli* O157:H7 as previously described (You et al., 2006; Lee and Choi, 2006).

Multiplex PCR assay

Presumptive colonies on TC-SMAC confirmed as *E. coli* were employed as templates for the PCR method. Template DNA was extracted from NSF colonies by using the simple boiling as previously described by Bai et al. (2010). One microliters of the supernatant was employed as template for PCR amplification. The DNA templates were subjected to Multiplex PCR method described previously (Al-Ajmi et al., 2006). *E. coli* O157:H7 specific primers were used to determine the presence of *rfbE*, *fliC* and *uidA* gene for somatic antigen O157, flagellar antigen H7 and β -glucuronidase as shown in Table 1. The composition of PCR mixture was 50 mM KCl, 10 mM Tris-HCl (pH, 8.3), 1.5 mM MgCl₂, 200 mM dNTPs, 0.5 μ M of each primer, 2 U of Taq polymerase and 5 μ l of DNA template in a final volume of 50 μ l. Amplification was done on a Techne thermo cycler (UK) using the program as below: An initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 65°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 5 min. The amplified products were analyzed by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining. Results were recorded using the gel documentation system (UVP Systems, UK). Product sizes were identified using a 100 bp DNA ladder. All the reagents were purchased from Sinaclon Company, Tehran, Iran. *E. coli* O157:H7 ATCC43895, reference strain was used as

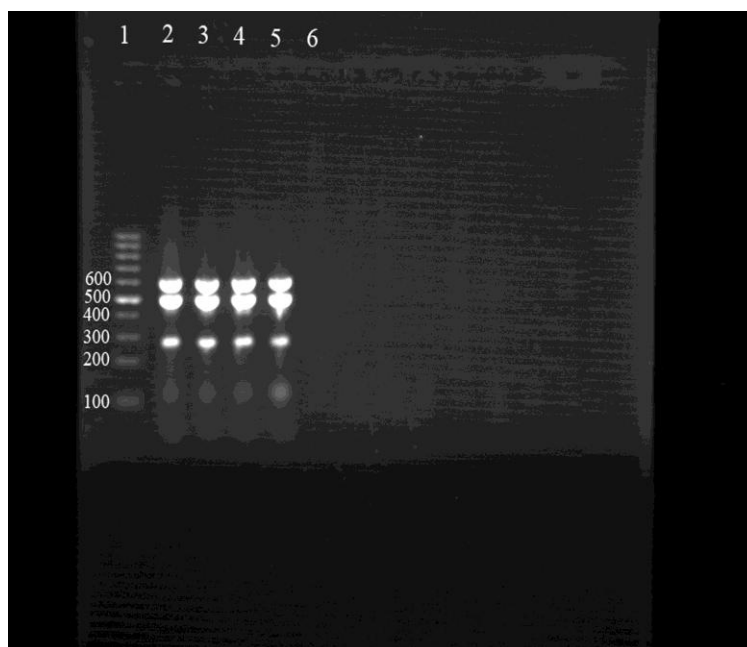


Figure 1. Agarose gel electrophoresis of Multiplex PCR assay for the Identification of *E.coli* O157:H7. Lane1: 100bp DNA marker, lane 2: *E.coli* O157:H7 ATCC 43895 (Control positive), lanes 3 - 5: positive samples, lane 6: control negative.

Table 2. Multiplex PCR results of *rfbE*, *fliC* and *uidA* in bacterial strains.

Bacterial strain	Serotypes	Isolates	<i>rfbE</i>	<i>fliC</i>	<i>uidA</i>
<i>Escherichia coli</i>	O157:H7	1	+	+	+
	O157:H	1	+	+	+
	Other serotypes	88	-	-	-
<i>Proteus vulgaris</i>		2	-	-	-
<i>Enterobacter</i>		4	-	-	-
<i>Citrobacter</i>		1	-	-	-

positive control strain (Institute Pasteur, Iran).

RESULTS AND DISCUSSION

From 200 hamburger samples, 8 (4%) Non-Sorbitol fermenting (NSF) colonies were isolated. Out of the 8 samples, three isolates (1%) were confirmed as *E. coli* by biochemical tests. The results of multiplex PCR assay confirmed these strains as *E. coli* O157:H7 (Figure 1) but in one of them, serological tests showed no positive result for the flagellar antigen. The amplicons were sequenced, and the results confirmed with that of Gene bank O157, H7 flagellar antigen and β-glucuronidase sequences (data not shown). Other NSF isolates were

found to belong to other genus of bacteria (Table 2). Also we detected 88 (44%) Sorbitol fermenting *E. coli* strains that none of them harbored these three genes.

Many studies have been conducted around the world to determine the presence of *E. coli* O157:H7 in various foods (Hussein and Sakuma, 2005; Hussein, 2007; Ateba and Bezuidenhout, 2008; McEvoy et al., 2003; Fedio et al., 2011). Beef has been estimated as major reservoirs of *E. coli* O157:H7 and plays a significant role in the epidemiology of human infections. hamburgers made with contaminated meat, if they are not sufficiently heated during the cooking process, can cause infection in consumers (You et al., 2006; Dontorou et al., 2003; Bai et al., 2010; Olesen and Jespersen, 2010; Ateba and Bezuidenhout, 2008; Mora et al., 2005; Cieslak et al.,

1997). As Cieslak et al. (1997) showed contaminated hamburgers can be the cause of large outbreaks of *E. coli* O157:H7 infections. In this study, we found that 1% of the analyzed hamburger samples were contaminated with *E. coli* O157:H7. Jamshidi et al. (2008) reported that 1% of ground beef samples were containing *E. coli* O157:H7, but in another study by Jamshidi et al. (2012) it showed 4% of hamburger samples were contaminated with *E. coli* O157:H7. The results of studies in the past 3 decades have shown that the prevalent rates of *E. coli* O157:H7 ranged from 0.1 to 54.2% in ground beef (Hussein, 2007). But a little study performed in our region about the identification and isolation of *E. coli* O157H7 from food could not provide enough information about the range of prevalence of *E. coli* O157H7 in food products.

Many protocols for isolation and detection of the *E. coli* O157H7 from food, fecal and environmental samples have been suggested. Conventional culture methods can be useful for isolation of *E. coli* O157H7. However, the detection of *E. coli* O157H7 using the current culture methods is time consuming and needs a lot of media and supplement components (Visetsripong et al., 2007). On the other hand, the first isolation of sorbitol-fermenting (SF) O157: H- was reported during an outbreak of HUS in Bavaria, Germany in 1988 and there is a possibility to get false results by the classic microbiological diagnostic procedures based on screening with sorbitol MacConkey agar (Orth et al., 2009). Currently, various molecular procedures such as PCR are available for detection of *E. coli* O157: H7 specific genes (Hsu et al., 2005). In multiplex PCR, the used of combination primers for the detection of several genes eliminates the possibility of false positives, which may arise if, non-O157:H7 strains were to acquire an O157:H7- specific gene. Although in some strains immunoreactive H7 flagella antigen is not expressed, but the encoding gene for H7 flagella antigen is still present within the genome, and may lead to false negative results (Al-Ajmi et al., 2006). We can show the presence of the encoding gene for H7 flagella Antigen by molecular methods. Al-Ajmi et al. (2006) used a three primer set for detection of O157 lipopolysaccharide O-antigen synthesis gene (perosamine synthetase; *rfbE*), β -glucuronidase (*uidA*) and H7 *fliC* gene by multiplex PCR. They reported that these set of primers and multiplex PCR have high sensitivity and specificity to determine the *E. coli* O157:H7 and O157: H- (Al-Ajmi et al., 2006). The same method was used in the present study and two *E. coli* O157:H7 were detected. Although many multiplex PCR methods have been developed and are widely used, further improvement of the detection procedures is possible as more sequence information becomes available. Examination of food for *E. coli* O157:H7 with different methods is effective in reducing infections caused by *E. coli* O157:H7. Further studies to evaluate meat products and to develop fast and economical methods for detection of *E. coli* O157:H7 particularly in

the study region is recommended.

ACKNOWLEDGEMENT

This work was supported by a grant (No. 88S53) from Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

REFERENCES

- Al-Ajmi D, Padmanabha J, Denman SE, Gilbert RA, Al Jassim RMM, Mcsweeney CS (2006). Evaluation of a PCR detection method for *Escherichia coli* O157: H7/h-bovine faecal samples. *Lett. Appl. Microbiol.* 42:386-391.
- Ateba CN, Bezuidenhout CC (2008). Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *Int. J. Food. Microbiol.* 128:181-188.
- Bai J, Shi X, Nagaraja TG (2010). A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. *J. Microbiol. Methods* 82:85-89.
- Cieslak PR, Noble SJ, Maxson DJ, Empey LC, Ravenholt O, Legarza G, Tuttle J, Doyle MP, Barrett TJ, Wells JG (1997). Hamburger-associated *Escherichia coli* O157: H7 infection in Las Vegas: a hidden epidemic. *Am. J. Pub. Health* 87:176-180.
- Dontorou C, Papadopoulou C, Filioussis G, Economou V, Apostolou I, Zakkas G, Salamoura A, Kansouzidou A, Levidiotou S (2003). Isolation of *Escherichia coli* O157: H7 from foods in Greece. *Int. J. Food. Microbiol.* 82:273-279.
- Fedio WM, Jinneman KC, Yoshitomi KJ, Zapata R, Wendakoon CN, Browning P, Weagant SD (2011). Detection of *E. coli* O157: H7 in raw ground beef by Pathatrix (TM) immunomagnetic-separation, real-time PCR and cultural methods. *Int. J. Food Microbiol.* 148(2):87-92.
- Hsu CF, Tsai TY, Pan TM (2005). Use of the duplex TaqMan PCR system for detection of Shiga-like toxin-producing *Escherichia coli* O157. *J. Clin. Microbiol.* 43:2668-2673.
- Hussein HS (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J. Anim. Sci.* 85:E63-72.
- Hussein HS, Sakuma T (2005). Invited review: prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J. Dairy Sci.* 88:450-465.
- Jamshidi AB, Bassami MR, Khanzadi S, Soltaninejad V (2012). Using multiplex-PCR assay in identification of *Escherichia coli* O157:H7 isolated from hamburger samples in Mashhad, Iran. *Iran. J. Nutr. Sci. Food Technol.* 35:101-106.
- Jamshidi AB, Bassami MR, Rasooli M (2008). Isolation of *Escherichia coli* O157: H7 from ground beef samples collected from beef markets, using conventional culture and polymerase chain reaction in Mashhad, northeastern Iran. *Iran. J. Vet. Res.* 9:72-76.
- Lee JH, Choi SJ (2006). Isolation and characteristics of sorbitol-fermenting *Escherichia coli* O157 strains from cattle. *Microbes Infect.* 8:2021-26.
- McEvoy JM, Doherty AM, Sheridan JJ, Thomsona-Carter FM, Garvey P, Mcguire L, Blair IS, McDowell DA (2003). The prevalence and spread of *Escherichia coli* O157: H7 at a commercial beef abattoir. *J. Appl. Microbiol.* 95(2):256-266.
- Mora A, Blanco JE, Blanco M, Alonso MP, Dhahi G, Echeita A, Gonzalez E A, Bernardez MI, Blanco J (2005). Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* o157:H7 and non-o157 strains isolated from humans, cattle, sheep and food in Spain. *Res. Microbiol.* 156(7):793-806.
- Olesen I, Jespersen L (2010). Relative gene transcription and pathogenicity of enterohemorrhagic *Escherichia coli* after long-term adaptation to acid and salt stress. *Int. J. Food. Microbiol.* 141(3):248-253.
- Orth D, Grif K, Zimmerhackl LB, Wurznner R (2009). Sorbitol-fermenting

- Shiga toxin-producing *Escherichia coli* O157 in Austria. *Wien. Klin. Wochenschr.* 121:108-112.
- Visetsripong A, Pattaragulwanit K, Thaniyavarn J, Matsuura R, Kuroda A, Suthaikul O (2007). Detection of *Escherichia coli* O157: H7 vt and *rfb* (O157) by multiplex polymerase chain reaction. *Southeast Asian. J. Trop. Med. Pub. Health* 38(1):82-90.
- You JY, Moon BM, Oh IG, Baek BK, Li LG, Kim BS, Stein BD, Lee JH (2006). Antimicrobial resistance of *Escherichia coli* O157 from cattle in Korea. *Int. J. Food. Microbiol.* 106(1):74-78.