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

Jamshidi, A.[1*]; Bassami, M. R.[2] and Rasooli, M.[3]

[1]Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [3]Graduated from Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

[*]**Correspondence:** A. Jamshidi, Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: ajamshid@ferdowsi.um.ac.ir

(Received 9 Aug 2006; revised version 17 Feb 2007; accepted 4 Mar 2007)

Summary

Escherichia coli O157:H7 is an important human pathogen causing haemorrhagic colitis, haemolytic-uraemic syndrom and thrombotic thrombocytopenic purpura. In this study, 100 ground beef samples were collected randomly from beef markets in June 2004. For isolation of the bacteria, samples were firstly enriched in modified trypticase soy broth, followed by plating onto sorbitol MacConkey agar supplemented with cefixime and potassium tellurite. Consequently, the suspected non-sorbitol fermenting (NSF) colonies were confirmed by biochemical tests and employed for polymerase chain reaction (PCR) assay, using primers specific for O157 and H7 antigens gene. In this study, 7 NSF *E. coli* colonies were isolated; in PCR assay only one of them confirmed as *E. coli* O157:H7. The PCR assay employed in this study may be a possible alternative to immunological assays which detects somatic and flagellar antigens.

Keywords: *Escherichia coli* O157:H7, Culture method, Polymerase chain reaction

Introduction

Escherichia coli O157:H7 is an important human pathogen causing haemorrhagic colitis, haemolytic-uraemic syndrom and thrombotic thrombocytopenic purpura (Nataro and Kaper, 1998; Zhao *et al*., 1998). *E. coli* O157:H7 serotypes are identified as enterohaemorrhagic *E. coli* and categorized as verotoxin-producing *E. coli* (Oksuz *et al*., 2004). Verotoxin is also known as shiga-like toxin (Molina *et al*., 2003). Cattle, especially the young ones, have been implicated as a principal reservoir of *E. coli* O157:H7 (Whipp *et al*., 1994; Zhao *et al*., 1995; Trevena *et al*., 1996). Cattle frequently excrete this bacteria in their faeces (Van Donkersgoed *et al*., 1999; Molina *et al*., 2003). The illness is often linked to the consumption of contaminated and undercooked ground beef and unpasteurized fruit juices. Although other means of transmission such as person to person transmission in child care centres and by swimming pools contaminated with faeces (McDonough *et al*., 2000) have been reported. By the way, undercooked ground beef is the major vehicle of food-borne outbreaks (Zhao *et al*., 1998; Oldfild, 2001).

Different culture methods for the screening of food specimens for *E. coli* O157:H7 are available. Among them, MacConkey agar containing sorbitol instead of lactose (SMAC), is most commonly used for isolation of *E. coli* O157:H7 (March and Ratman, 1986). Due to the fact that this bacteria are unable to ferment sorbitol, non-sorbitol-fermenting (NSF) colonies are potentially considered as *E. coli* O157:H7 (March and Ratman, 1986; McDonough *et al*., 2000). Sorbitol-MacConkey supplemented with cefixime and tellurite (CT-

SMAC) increases the sensitivity of this media. In comparison, broth enrichment methods are superior to direct plating (Sanderson *et al*., 1995).

Furthermore, other than the culture methods which are based on biochemical characteristics of the bacteria, many other assays have been developed, including serological techniques, which uses both polyclonal and monoclonal antibodies specific for the O and H antigens (De Boer and Heuvelink, 2000; Kimura *et al*., 2000). Molecular approaches have also been practiced. In this regards, polymerase chain reaction (PCR) assays based on the presence or absence of specific genes such as the *stx*, *eaeA* and *hlyA* have been described (Kimura *et al*., 2000; Pilpott and Ebel, 2003). Serogroup-specific PCR assays targeting the genes encoding O-antigen in *E. coli* serogroups have also been introduced (Pilpott and Ebel, 2003). Sequence data for the *flic*H7 gene which encodes the H7 antigen is also available (Gannon *et al*., 1997).

The objective of the present study was to isolate *E. coli* O157:H7 from ground beef samples by conventional culture method and to confirm it by a serogroup-specific PCR assay in Mashhad, northeastern Iran.

Materials and Methods

Sample collection

In June 2004, 100 ground beef samples were randomly collected aseptically in sterile disposable bags from beef markets across Mashhad city, Khorasan province, northeastern Iran.

Culture conditions

The samples were brougt to the laboratory on crashed ice. Immediately in the laboratory, 25 g of each ground beef sample were aseptically transfered to 225 ml of modified trypticase soy broth (mTSB) containing 0.5 mg/ml novobiocin, followed by incubation at 37°C for 24 hrs. The enriched culture were plated onto sorbitol MacConkey agar supplemented with cefixime (0.05 mg/ml) and potassium tellurite (2.5 mg/L) (CT-SMAC). The inoculated CT-SMAC plates were then incubated at 37°C for 24 hrs. Then, NSF colonies were selected from CT-SMAC plates and streaked onto plates containing eosin methylene blue agar (EMB) and were incubated at 37°C for 24 hrs. These isolates, with typical *E. coli* metallic shine on EMB, were characterized by biochemical tests, including conventional indol, methyl red, voges proskauer, citrate and lysine decarboxylase tests.

PCR assay

NSF colonies on CT-SMAC that had been confirmed as *E. coli* employed as templates for PCR assay. *E. coli* O157:H7 (ATCC-35150) were used as the positive control and sterile distilled water as the negative control. A whole-cell suspention was prepared by suspending a NSF bacterial colony from CT-SMAC in sterile distilled water. The cell lysate was made by heating the suspention for 10 min in a boiling water bath. The lysate were spun for five min at 6000 rpm to pellet the cellular debris. Two µl of the supernatant was used as template for amplification by PCR. The presence or the absence of *flic*H7 gene encoding the flagellar antigen H7 and *rfb*O157 gene which encodes the somatic antigen O157 (Desmarchier *et al.*, 1998; Pilpott and Ebel, 2003) were examined. Table 1 describes oligonucleotide sequence of primers used in the PCR reaction mixture. The PCR reaction was performed in a 25 µl amplification mixture consisting of 2.5 µl 10 × PCR buffer (500 mM KCl, 200 mM Tris-HCl), 0.5 µl dNTPs (10 mM), 1 µl $MgCl_2$ (50 mM), 1.25 µl of each primer (0.5 µM), 0.2 µl of Taq DNA polymerase (5 Unit/µl) and 2 µl of template. The thermocycler (Bio Rad) program was started with an initial incubation at 94°C for five min, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 56°C for 30 sec and elongation at 72°C for 60 sec, and a final extention at 72°C for 10 min. The PCR products were separated by electrophoresis on 1.5% agarose gel at 100 V for 40 min in Tris-acetate buffer, visualized by ethidium bromide staining, illuminated by UV-transil-luminator and documented by a gel documentation apparatus. One-hundred bp DNA ladder was used as a size reference for PCR assay. The expected size of PCR products for *rfb*O157 and *flic*H7 genes

Table 1: Primers and thermocycling condition for *E. coli* O157:H7

Target gene	Primer sequence (name)	Predicted product size (bp)	Thermocycling programme
*rfb*O157	F: 5′- CGG ACA TCC ATG TGA TAT GG -3′ R: 5′- TTG CCT ATG TAC AGC TAA TCC -3′	259	94 ºC for 5 min (94 ºC for 60 s-56 ºC for 30 s-72 ºC for 60 s) *35 cycles-72 ºC for 10 min
*flic*H7	F: 5′- GCG CTG TCG AGT TCT ATC GAG-3′ R: 5′- CAA CGG TGA CTT TAT CGC CAT TCC-3′	625	

amplification were 259 and 625 bp, respectively.

Results

From 100 ground beef samples studied, seven NSF colonies were isolated, which confirmed as *E. coli* by biochemical tests. In PCR assay, using specific primers for *rfb*O157 and *flic*H7 genes, only one sample confirmed to be contaminated with *E. coli* O157: H7 (Fig. 1).

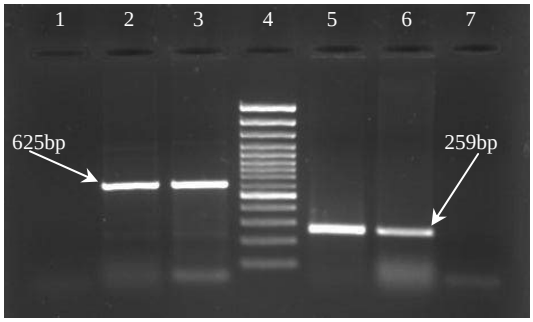

Fig. 1: Results of the PCR assay, amplifying 259-bp segment of *rfb*O157 and 625-bp of *flic*H7 gene of *E. coli* O157:H7. Lanes 3 and 5: positive control; Lane 4: 100-bp marker; Lanes 2 and 6: samples

Discussion

Shiga toxin-producing *E. coli* (STEC) is now a major cause of food-borne disease, mostly in the United States, Canada, Japan and Europe (Griffin and Tauxe, 1991; Nataro and Kaper, 1998). In an earlier study, STEC O157 was isolated from 3.7% of retail beef and 1.5% of pork samples in the United States and Canada (Doyle and Schoeni, 1997). Although most sporadic cases and outbreaks have been reported from developed countries, human infections associated with STEC strains have also been described in Latin American countries, including Argentina, Chile and Brazil (Nataro and Kaper, 1998; Irino *et al*., 2002). It has also been reported from Kenya, Turkey and Iraq (Sang *et al*., 1996; Shebib *et al*., 2003; Ulukanli *et al*., 2006).

We found that 1% of ground beef samples were contaminated with *E. coli* O157:H7. Our results suggested that cattle could be a reservoir of *E. coli* O157:H7 in Iran, like many other countries (Whipp *et al*., 1994; Zhao *et al*., 1995; Trevena *et al*., 1996). Many protocols for isolation of the *E. coli* O157:H7 from food, faecal and environmental samples have been proposed. To date, different PCR assays have been described for detecting the main virulence factors gene such as *stx1* and *stx2* and genes encoding accessory STEC virulence factors, such as *eaeA*, *hlyA* and *flic*H7 (Advienne and Paton, 1998). Flagellar and somatic antigens can be detcted by immunological assays. The main advantage of the employed PCR method is its ability to detect rough isolates or the isolates having a masked O antigen (Desmarchier *et al*., 1998). The method developed in this study can also detect O157 H^- (non-motile) serotype of *E. coli*, although we did not isolate this serotype in this study. Disadvantage of this method is that, it can not discriminate between shiga toxin-producing strains and non-shiga toxin-producing ones, although it is possible to include primers specific for *stx1* and *stx2*, as well.

It has been proposed that the enrichment before plating on selective agar may increase the sensitivity of *E. coli* O157:H7 isolation compared to direct plating of test samples on selective agar (Sanderson *et al*., 1995). It has been described that the CT-SMAC agar medium yields the best results for selective cultivation of *E. coli* O157:H7 (Sanderson *et al*., 1995; De Boer and Heuvelink, 2000). Using this serogroup-specific PCR assays for identification of *E. coli* O157:H7 that have been employed in this study, might be a possible alternative to

immunological assays. Further studies are needed to clarify this possibility.

References

Advienne, WP and Paton, JC (1998). Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assay for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb*O157. J. Clin. Microbiol., 36: 598-602.

De Boer, E and Heuvelink, AE (2000). Methods for isolation of shiga toxin-producing *Escherichia coli.* Symp. Ser. Soc. Appl. Microbiol., 29: 133S-143S.

Desmarchier, PM; Bilge, SS; Fegan, N; Mills, L; Vary, JC and Tarr, PI (1998). A PCR specific for *Escherichia coli* O157:H7 based on *rfb* locus encoding O157 lipopolysaccharide. J. Clin. Microbiol., 36: 1801-1804.

Doyle, MP and Schoeni, JL (1997). Isolation of *Escherichia coli* O157:H7 from retail fresh meat and poultry. Appl. Environ. Microbiol., 53: 2394-2396.

Gannon, VPJ; D'souza, S; Graham, T; King, RK; Rahn, K and Read, S (1997). Use of flagellar H7 gene as a target in multiplex PCR assay and improved specificity and identification of enterohemorrhagic *Escherichia coli* strains. J. Clin. Microbiol., 35: 656-662.

Griffin, P and Tauxe, RV (1991). The epidemiology of infections caused by *Escherichia coli* O157:H7, other entero-hemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev., 13: 60-98.

Irino, K; Vaz, TM; Kato, MA; Naves, ZV; Lara, RR; Marco, ME; Rocha, MM; Moreira, TP; Gomes, TA and Guth, BF (2002). O157:H7 shiga toxin-producing *Escherichia coli* strains associated with sporadic cases of diarrhea in Sao Paulo, Brazil. Emerg. Infect. Dis., 8: 446-447.

Kimura, R; Mandrell, RE; Galland, JC; Hyatt, D and Riley, LW (2000). Restriction-site-specific PCR as a rapid test to detect enterohemorrhagic *Escherichia coli* O157:H7 strains in environmental samples. Appl. Environ. Microbiol., 66: 2513-2519.

March, CB and Ratman, S (1986). Sorbitol-Mac Conkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol., 23: 869-872.

McDonough, PL; Rossiter, CA; Rebhun, RB; Stehman, SM; Lein, DH and Shin, SJ (2000). Prevalence of *Escherichia coli* O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. J. Clin. Microbiol., 38: 318-322.

Molina, PM; Parma, AE and Sanz, ME (2003). Survival in acidic and alcoholic medium of shiga toxin-producing *Escherichia coli* O157:H7 and non-O157:H7 isolates in Argentina. BMC Microbiol., 13: 3-17.

Nataro, JP and Kaper, B (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.

Oksuz, O; Arici, M; Kurultay, S and Gumus, T (2004). Incidence of *Escherichia coli* O157:H7 in raw milk and white pickled cheese manufactured from raw milk in Turkey. Food Control. 15: 453-456.

Oldfild, EC (2001). Emerging foodborne pathogens: keeping your patient and your families safe. Rev. Gastroenterol. Di., 1: 177-186.

Pilpott, D and Ebel, F (2003). *E. coli: shiga toxin methods and protocols*. 1st. Edn., Totowa, Humana Press Inc., PP: 9-45.

Sanderson, MW; Gay, GM; Hancock, DD; Gay, CC; Fox, LK and Bess, TE (1995). Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J. Clin. Microbiol., 33: 2616-2619.

Sang, WK; Saidi, SM; Yamamoto, H; Fzaki, T; Iida, T; Yoh, M and Honda, T (1996). Haemorrhagic colitis due to *Escherichia coli* O157:H7 in Kenya. J. Trop. Pediatrics. 42: 118-119.

Shebib, ZA; Abdul Ghani1, ZG and Mahdi, LK (2003). First report of *Escherichia coli* O157 among Iraqi children. East. Mediterr. Health J., 9: 159-166.

Trevena, WB; Hooper, RS; Wray, C; Wilson, GA; Cheasty, T and Domingue, G (1996). Verocytotoxin-producing *Escherichia coli* O157:H7 associated with companion animals. Vet. Rec., 138: 400.

Ulukanli, Z; Çavli, P and Tuzcu, M (2006). Detection of *Escherichia coli* O157:H7 from beef doner kebabs sold in Kars. G. U. J. Sci., 19: 99-104.

Van Donkersgoed, J; Graham, T and Gannon, V (1999). The prevalence of verotoxins, *Escherichia coli* O157:H7 and *Salmonella* in the feces and rumen of cattle at processing. Canadian Vet. J., 40: 332-338.

Whipp, SC; Rasmussen, MA and Cray, WCJr (1994). Animals as a source of *Escherichia coli* pathogenic for human beings. J. Am. Vet. Med. Assoc., 204: 1168-1175.

Zhao, T; Doyle, MP; Harmon, BG; Brown, CA; Eric Mueller, PO and Parks, AH (1998). Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by

inoculation with probiotic bacteria. J. Clin. Microbiol., 36: 641-647.

Zhao, T; Doyle, MP; Shave, J and Garber, L (1995). Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol., 61: 1290-1293.