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

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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-born 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...
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 (Hymedia, 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- Proskauer, 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 μM of each primer, 2 U of Taq polymerase and 5 μl of DNA template in a final volume of 50 μl. Amplification was done on a Techene 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 (UVIP 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 a positive control.

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rfbE</td>
<td>F 5'-AAGATTGCGCTGAAGCCTTG-3'</td>
<td>497</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>R 5'-CATTGGCATCGTGTTGACAG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fliC</td>
<td>F 5'-GCCTGCTGAGTTCTGTACGAC-3'</td>
<td>625</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>R 5'-CAACGGTGACTTTATCCGACCCATTC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uidA</td>
<td>F 5'-GCAGAAACTGTGGAAATTGGG-3'</td>
<td>252</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>R 5'-TGATGCTCCATACTTCTCCTG-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Serotypes</th>
<th>Isolates</th>
<th><em>rfbE</em></th>
<th><em>fliC</em></th>
<th><em>uidA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>O157:H7</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>O157:H1</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Other serotypes</td>
<td></td>
<td>88</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 O157:H7 from food could not provide enough information about the range of prevalence of E. coli O157:H7 in food products.

Many protocols for isolation and detection of the E. coli O157:H7 from food, fecal and environmental samples have been suggested. Conventional culture methods can be useful for isolation of E. coli O157:H7. However, the detection of E. coli O157:H7 using the current culture methods is time consuming and needs a lot of media and supplement components (Visessripon 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 use 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.

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REFERENCES


