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Full Length Research Paper

Isolation and genomic characterization of *Escherichia coli* O157:NM and *Escherichia coli* O157:H7 in minced meat and some traditional dairy products in Iran

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Human diseases caused by *Escherichia coli* O157:NM and *E. coli* O157:H7 strains have been reported throughout the world. In developed countries, serotype O157:H7 represents the major cause of human diseases; however, there have been increasing reports of non-O157 Shiga toxin (Stx)-producing *E. coli* strains associated with gastrointestinal infections. The present study was conducted to investigate the presence of *E. coli* O157 and *E. coli* O157: H7 strains and the presence of virulence genes *stx1*, *stx2*, *eaeA* and *ehlyA* insolates derived from some traditional dairy products and minced beef meat. A total number of 201 samples including 50 samples from traditional butter, 50 samples from traditional cream, 35 samples from kashk, 30 samples from doogh and 36 samples from minced beef meat were purchased in different supermarkets and retailer shops in Isfahan, Chaharmahal, Bakhtyari and Khuzestan provinces in Iran, over a period of 11-month from August 2010 to May 2011. *E. coli* non-O157, *E. coli* O157:NM and *E. coli* O157:H7 were isolated from 14 samples (7%), 3 samples (1.5%) and 1 sample (out of 50 samples of traditional butter) (0.5%) of the 201 dairy products, respectively, in this study. All the *E. coli* O157:H7/NM isolates were positive for *eaeA* and *stx1* and/or *stx2*, and one *E. coli* O157:H7 isolate was positive for *EhlyA*. Of the 3 *stx* positive isolates, 1 and 2 isolates had *stx1* and *stx2*, respectively. To our knowledge, the present study is the first report on isolation and identification of *E. coli* O157 from traditional butter and cream samples in Iran.

Key words: *Escherichia coli* O157, butter, cream, traditional dairy products, minced beef meat, Iran.

INTRODUCTION

Escherichia coli live commensally in the gastrointestinal tract of most mammals, including humans, without causing any disease. However, a small fraction of *E. coli* is human pathogens and has been implicated in foodborne illnesses with increasing frequency over the last 2 decades. *Escherichia coli* O157 is the most common member of a group of pathogenic *E. coli* strains known variously as enterohaemorrhagic, verocytotoxin-producing or Shiga-toxin-producing organisms (Chapman et al., 1997; Abongo and Momba, 2009). The first outbreaks caused by *E. coli* O157 occurred in Oregon

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and Michigan, USA in 1982, when it was isolated from individuals who developed bloody diarrhoea and severe abdominal cramps (CDC, 1982) after eating hamburgers in a restaurant chain. Infection can cause gastroenteritis that may be complicated by hemorrhagic colitis or the hemolytic-uremic syndrome (HUS), which is the main cause of acute renal failure in children. Shiga toxin (Stx)-producing *E. coli* (STEC) strains causing human infections belong to a large, still-increasing number of O:H serotypes. Most outbreaks and sporadic cases of hemorrhagic colitis and HUS have been attributed to the STEC O157 strains (Tarr et al., 2005; Lockary et al., 2007; Werber et al., 2007). However, infections caused by some non-O157 serotypes have also been frequently associated with severe illness in humans. In some geographic areas, STEC non-O157 strains are more commonly isolated from persons with diarrhea or HUS than STEC O157 strains (Pradel et al., 2000).

Domestic and wild animals are the sources of *E. coli* O157, but ruminants are regarded as the main natural reservoirs (Beutin et al., 1993). Sporadic cases and outbreaks of human diseases caused by STEC have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water (Griffin and Tauxe, 1991; Battu and Reddy, 2009). Infections can also be acquired by direct contact with animals and by person-to-person spread (Caprioli et al., 2005; Cho et al., 2006).

The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk (Öksüz et al., 2004). The processing conditions for different milk products are very important from the standpoint of the organism's infection risk. It can grow in trypticase soy broth (TSB) acidified with lactic acid at pH = 4.6 but not at pH = 4.5 (Glass et al., 1992).

Limited studies of the ecology of *E. coli* O157 have been reported, particularly from developing countries. In Iran, no study of the occurrence of STEC in food had been carried out. Therefore, the primary objective of the present study was to determine the prevalence of *E. coli* O157 and *E. coli* O157: H7 strains and the presence of virulence genes *stx1*, *stx2*, *eaeA* and *ehlyA* isolates derived from some traditional dairy products and minced beef meat collected from three provinces of Iran.

MATERIALS AND METHODS

All samples were obtained from different supermarkets and retailer shops in Isfahan, Chaharmahal, Bakhtyari and Khuzestan provinces in Iran, over a 11-month period (August 2010 to May 2011). Meat products and traditional dairy products were composed of minced beef meat (n = 36), traditional butter (n= 50), traditional cream (n = 50), Kashk (n = 30) and doogh (n = 35). Kashk and doogh are two popular dairy products in Iran that are available both as traditional and commercial products. Kashk is prepared by prolonged boiling of yogurt and doogh which is also called yogurt soda is prepared by beating unflavored yogurt until it is smooth, and then diluting with water to a consistency similar to whole milk. Samples (0.5 kg each, in sterile glass containers) were transported to the laboratory at 4°C within a maximum of 6 to 12 h after sampling.

Microbiological analyses

Twenty-five grams (25 g) of each sample were homogenized in 225 ml trypton soya broth supplemented with novobiocin (20 mg/L) and incubated at 37°C for 18 to 24 h. Cultures were streaked onto MacConkey sorbitol agar and the plates were incubated as above. From each plate (one plate for each fecal sample), 5 to 10 suspected *E. coli* colonies (sorbitol negative and positive) were selected and sub-cultured onto presumptive diagnostic medium (Stampi et al., 2004) and incubated overnight at 37°C. All sorbitol-negative *E. coli* were screened with O157 antiserum by the slide agglutination test. A total of 14 *E. coli* colonies were recovered and kept on slant agar at room temperature.

The 14 *E. coli* isolates were screened for the presence of *stx1* (encoding for Shiga toxin 1), *stx2* (encoding for Shiga toxin 2), *eae* (encoding for intimin) and *ehlyA* (encoding for enterohemolysin) genes using colony hybridization assays and specific DNA probes as described elsewhere (Rey et al., 2003; Blanco et al., 2003; Schmidt et al., 1995). Base sequences and predicted sizes of amplified products for the specific oligonucleotide primers used only in this study are shown in Table 1. All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). Purification of DNA was achieved using a Genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instruction and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001).

DNA amplification was performed in a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany). The amplification conditions and reagents for the PCR assays were those described by Rey et al. (2006). PCR products were analyzed by agarose gel electrophoresis and the specific DNA bands were visualized using ethidium bromide staining under UV illumination.

Statistical analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a Pearson chi-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

RESULTS AND DISCUSSION

Analysis results of some traditional dairy products and minced beef meat samples are given in Table 2. *E. coli* non-O157, *E. coli* O157:NM and *E. coli* O157:H7 were isolated from *E. coli* non-O157, *E. coli* O157:NM and *E. coli* O157:H7 were isolated from 14 samples (7%), 3 samples (1.5%) and 1 sample (out of 50 samples of traditional butter) (0.5%) of the 201 dairy products, respectively, in this study. All the *E. coli* O157:H7/NM isolates were positive for *eaeA*, and *stx1* and/or *stx2*, and

Table 1. Primer sequences and predicted lengths of PCR amplifications products.

Gene	Primer	Oligonucleotide sequence (5-3)	Fragment size (bp)	Annealing temperature
stx1	VT1-A VT1-B	CGCTGAATGTCGCTCTGC CGTGGTATAGCTACTGTCACC	302	55
stx2	VT2-A VT2-B	CTTCGGTATCCTATTCCCGG CTGCTGTGACAGTGACAAAACGC	516	55
ehlyA	HlyAl HlyA4	GGTGCAGCAGAAAAAGTTGTAG TCTCGCCTGATAGTGTTTGGTA	1551	60
eaeA	EAE-1 EAE-2	GAGAATGAAATAGAAGTCGT GCGGTATCTTTCGCGTAATCGCC	775	55

Table 2. Prevalence of *E. coli* O_{157} from minced beef meat, traditional butter, traditional cream, Kashk and doogh in Iran.

Sample	Number of sample examined	Number of positive sample (%)	Virulence gene			
			Stx₁	***Stx₂***	***eaeA***	***ehlyA***
Butter	50	1 (2.0)	1	0	1	1
Cream	50	1 (2.0)	0	1	1	0
Kashk	30	0 (0.0)	0	0	0	0
Doogh	35	0 (0.0)	0	0	0	0
Minced beef meat	36	1 (2.8)	0	1	1	0
Total	201	3 (1.5)	1	2	3	1

[a]A dairy product prepared by beating unflavored yogurt until smooth, and then diluted with water to a consistency similar to whole milk; it is also called yogurt soda. [b]A dairy product prepared by prolonged boiling yogurt.

one *E. coli* O157:H7 isolate was positive for *ehlyA* (Table 2). Of the 3 *stx* positive isolates, 1 and 2 isolates had *stx1* and *stx2*, respectively.

Several studies have reported the prevalence of *E. coli* O157:H7 on meat products and dairy products. For example, Dontorou et al. (2003) recovered *E. coli* O157:H7 from 0.5% foods including ewes' milk, cow's milk, goat's milk, minced beef meat, uncooked frozen beef hamburgers, sandwiches, kokoretis and fresh sausages. The pathogen was detected in 1 out of 100 (1.0%) samples of ewe's milk, 1 out of 75 (1.3%) fresh sausages and 1 out of 50 (2.0%) swine intestines prepared for kokoresi (Dontorou et al., 2003). In our study, *E. coli* O157:NM was detected in 1 out of 37 (2.7%) samples of beef minced, 1 out of 50 (2.0%) traditional butter and 1 out 50 (2.0%) traditional cream. Only one *E. coli* O157:H7 was isolated from 50 samples of traditional butter. In comparison with other countries, the prevalence reported in this study is similar to studies from Greece (Dontorou et al., 2004), Morocco (Badri et al., 2009) and Egypt (Abd El-Atty and Meshref, 2007). The presence of *E. coli* O157 and *E. coli* O157:H7 in traditional butter and cream samples could be attributed to the fact that it is usually made from raw milk, in addition to the primitive way of processing, handling and selling.

There are no data on the presence of *E. coli* O157:NM and *E. coli* O157:H7 in kashk and doogh and to our knowledge, this is the first reported case from kashk and doogh. In the present study, no *E. coli* O157 isolate was detected in kashk and doogh samples. Survival of *E. coli* O157 in foods depends on the sample acidity; the bacteria disappear when the pH falls to 3.5. Furthermore, the absence of *E. coli* O157 in kashk and doogh samples in this study could possibly be accounted for by the acidity of these products; however, it could also be due to the boiling stage achieved during the processing of these products.

Meat has been linked to consumer health problems, as evidenced by outbreaks and recalls from marketplaces associated with contaminated products. Between 1992 and 1999, 16% of general outbreaks in England and Wales were related to red meat, specifically by beef (34%) and pork (32%) (Smerdon et al., 2001). More than 21 million pounds of ground beef were recorded in 2007 due to contamination of *E. coli* O157:H7 (USDA, 2007). Beef minced meat samples have been examined in several countries for the presence of *E. coli* O157: H7. In

the Republic of Ireland, *E. coli* were detected in 45 (30.2%) of the 149 samples examined, mainly in the hamburger samples mixed with vegetables and in the loose minced beef. *E. coli* O157 was found in one sample of hamburger and two samples of hamburger mixed with vegetables (2%) collected (Stampi et al., 2004). In Denmark, 0.3% of 1584 samples examined were contaminated with *E. coli* O157:H7 (Boel et al., 1997). In Netherlands, 1.1% of 571 samples of raw minced beef were contaminated (Heuvelink et al., 1999) and in Switzerland and Greece, no *E. coli* O157:H7 was isolated from 211 and 64 minced beef samples tested, respectively (Fantelli and Stephan, 2001; Dontorou et al., 2004). In our study, no *E. coli* O157: H7 strain was isolated too. Direct comparison of results is difficult due to differences in the study methodologies, such as sampling over a full year and the sampling of all regions in the country.

The genes encoding for verotoxins (*stx1* and *stx2* genes), that determine the virulence potential of the organism which are essential in the establishment of the disease (Schmid et al., 2001), were detected in the three *E. coli* O157 isolates from traditional butter, traditional cream and beef minced samples. These findings are supported by several studies (Vivegnis et al., 1999; Pradel et al., 2000; Caro et al., 2006; Mansouri-Najand and Khalili, 2007).

In conclusion, the presence of *E. coli* O157 in beef minced and some traditional dairy products indicate the potential risk of infection with *E. coli* O157 in people consuming raw milk, unpasteurized milk or traditional dairy products. Therefore, high-risk groups should avoid previously prepared unpasteurized dairy products. To the best of our knowledge, the present study is the first report on *E. coli* O157 traditional butter and cream samples in Iran.

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