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Full Length Research Paper

Prevalence and antimicrobial resistance of *ESCHERICHIA COLI* O157 isolated from traditional cheese, ice cream and yoghurt in Iran

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Verotoxin-producing of *ESCHERICHIA COLI* O157 is an increasingly common cause of severe gastrointestinal illness, enlisted among the most important emerging pathogens. The present study was conducted to investigate the presence of *E. COLI* O157 and *E. COLI* O157: H7 strains and to detect the presence of the *STX1*, *STX2*, *EAE* and *EHXA* insolates derived from 290 samples (120 samples from traditional fresh cheese, 120 samples from traditional ice cream and 50 samples from yoghurt). The samples were purchased from the Isfahan, Chaharmahal, Bakhtyari and Khuzestan provinces in Iran, over a period 6-month from August 2010 to February 2011. Standard cultural method and polymerase chain reaction were applied for these analyses. *E. COLI* O157 was detected in nine of the 290 (3.1%) samples tested (five isolated from traditional cheese and 4 isolated from traditional ice cream samples), whereas *E. COLI* O157: H7 was not detected in any samples. The genes *STX1* and *STX2* were detected in three *E. COLI* isolated obtained from traditional cheese samples none of the *STX1*, *STX2*, *EAE* and *EHXA* was detected in the *E. COLI* isolates obtained from traditional ice cream samples. Susceptibilities of nine *E. COLI* O157 isolates were determined for ten antimicrobial drugs using the disk diffusion assay. Resistance to ampicillin and gentamycin was the most common finding (44.4%), followed by resistance to erythromycin (33.3%), amoxicillin (11.1%), tetracycline (11.1%) and nalidixic acid (11.1%). All *E. COLI* O157 isolates were susceptible to chloramphenicol, cefuroxime, and streptomycin. Thus, traditional cheese and ice cream manufactured from unpasteurized milk have appositional risk as a result of *E. COLI* O157 existence.

Key words: *Escherichia coli* O157, cheese, ice cream, yoghurt.

INTRODUCTION

Shiga toxin (*Stx*)-producing *Escherichia coli* (STEC) were first recognized as a human pathogen in 1982, in the USA, when strains of the serotype O157: H7 caused two outbreaks of hemorrhagic colitis (Riley et al., 1983; Wells et al., 1983). In 1983, the association of *E. coli* O157: H7 and of several other STEC serotypes with sporadic cases of the classical hemolytic uremic syndrome (HUS) was first de-HUS was first described and subsequently confirmed in a prospective study (Karmali et al., 1985). Since then, epidemiological studies from diriment parts of the world established STEC as the major cause of bloody diarrhea and HUS in temperate climates and as an important cause of uncomplicated watery diarrhea in some geographic areas (Spika 1998).

Cattle are the reservoir of the pathogen (Chaman et al.,

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Abbreviations: STEC, Shiga toxin (*Stx*)-producing *Escherichia coli*; **HUS,** hemolytic uremic syndrome; **DNA,** deoxyribonucleic acid; **GMP,** good manufacturing practices.

1993) and consumption of undercooked meat (Riley et al., 1983; Dontorou et al., 2003) and raw milk (Chapman et al., 1993; Oksuz et al., 2004; Solomakos et al., 2009) of bovine origin are considered to be the main cause of several outbreaks of *E. coli* O157: H7. Nevertheless, a variety of other foods have also been implicated in causing outbreaks, such as unpasteurized goat's milk and cheese (Bielaszewska et al., 1997; Solomakos et al., 2009), deer's meat (Keene et al., 1997), meat sandwiches (McDonnell et al., 1997), lettuce (Merimin et al., 1997) and unpasteurized apple cider and apple juice (Besser et al., 1993).

Ruminants seem to constitute a reservoir of *E. coli* O157 in nature (Rey et al., 2003; Oporto et al., 2008). Contaminated unpasteurized dairy products such as raw milk and raw-milk cheese have been incriminated in recent foodborne STEC outbreaks (Deschenes et al., 1996; Honish et al., 2005; CDC, 2007). Fermented dairy products manufactured using raw milk contaminated with E. coli O157:H7 can pose a threat to human health, as it has been shown that, if present in raw milk, the pathogen can survive during the manufacturing and ripening stages of selected fermented dairy products that do not undergo a sufficient heating step or are contaminated after the heat treatment. The ability of the pathogen to survive in raw goat milk lactic (soft) cheeses (Veronzy-Rozand et al., 2005), aged cheddar cheese made from unpasteurized milk (Schlesser et al., 2006), Feta cheese (Govaris et al., 2002a) and even yogurt (Morgan et al., 1993; Govaris et al., 2002b) has been well documented.

Regarding the prevalence of *E. coli* O157 in the raw milk supply in Iran, to our knowledge, there have been only a few published surveys (Mansouri-Najand and Khalili, 2007). In this study, the authors assayed raw milk cheeses for the presence of shiga-like toxigenic *E. coli*. The tested raw milk cheeses samples originated from the province of Kerman (Southern part of Iran). The authors reported the isolation of the pathogen in one sample of raw ovine milk. Besides the study of Mansouri-Najand and Khalili (2007), no other study has looked into the prevalence of *E. coli* O157 in ice cream and yoghurt in Iran. Therefore, the purpose of the present study were to estimate the prevalence of *E. coli* O157 in the traditional cheese, ice cream and yoghurt supply in Iran, to assess the frequency, in the isolated strains, of four genes that encode for known STEC virulence factors, namely *stx1*, *stx2*, *eae* and *ehxA* and to determine the antibiotic resistance of the isolates. The antimicrobial agents tested in this study are widely used to treat infections in people and in food animals in Iran.

MATERIALS AND METHODS

Samples

Traditional sampling cheese (n= 120), ice cream (n= 120) and yogurt (n= 50) samples were obtained from different supermarkets and retailer shops from Isfahan, Chaharmahal and Bakhtyari and Khuzestan provinces in Iran, over a 6-month period (August 2010 to February 2011). Samples (0.5 kg each, in sterile glass containers) were transported to the laboratory at 4°C within a maximum of 6-12 h after sampling.

Microbiological analyses

Twenty-five g of each sample were homogenized in 225 mL trypton soya broth supplemented with novobiocin (20 mg/L) and incubated at 37°C for 18-24h. Then the enrichment samples were streak onto levine eosin methylene blue agar and sorbitol McConkey agar plates supplemented with cefexime (0.5 mg/L) and potassium tellurite (2.5 mg/L) and incubated as above. Suspected colonies were confirmed by TSI agar and indole, methyl red, Voges-Proskauer, citrate (IMViC) tests (Stampi et al., 2004).

Detection of *E. coli* O157:H7 and virulence genes by PCR

Sorbitol negative colonies were confirmed as *E. coli* O157: H7 with PCR assay by using the O-antigen encoding region of O157 gene (Paton and Paton, 1998) and flagellar H7 gene (fli C) generic primers as described previously (Gannon et al., 1997).

The primer sequences of virulence genes used were: VT1-A and VT1-B for gene *stx1* (Rey et al., 2003); VT2-A and VT2-B for gene *stx2* (Rey et al., 2003); Hly A1 and HlyA4 for gene *ehxA* (Schmidt et al., 1995) and EAE-1 andEAE-2 for gene *eae* (Blanco et al., 2004). All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). Purification of deoxyribonucleic acid (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. (2003). PCR products were analyzed by agarose gel electrophoresis and the specific DNA bands were visualized using ethidium bromide staining under UV illumination.

Antimicrobial susceptibility testing

One strain from each *E. coli* O157-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute (CLSI, 2006). The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 µg), cefuroxime (30 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), amoxicillin (30 µg), ampicillin (10 µg), and chloramphenicol (30 µg. After incubation at 42°C for 48 h in a microaerophilic atmosphere, the susceptibility of the Campylobacter spp. to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). Staphylococcus aureus and Escherichia coli were used as quality control organisms in antimicrobial susceptibility determination.

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

Table 1. Prevalence of *E. coli* O_{157} from traditional cheese, ice cream and yoghurt in Iran.

Samples	No. of samples examined	No of positive samples (%)	Virulence genes			
			Stx1	*Stx2*	*EAE*	*EHXA*
Cheese	120	5 (4.2)	1	2	0	0
Ice cream	120	4 (3.3)	0	0	0	0
Yoghurt	50	0 (0.0)	0	0	0	0
Total	290	9 (3.1)	1	2	0	0

Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

RESULTS

Analysis results of the traditional cheese, ice cream and yoghurt samples are given in Table 1. A total of nine *E. coli* O157 strains were isolated from nine (3.1%) traditional cheese and ice cream sources out of 290 samples tested, E. coli O157: H7 was not detected in any sample. Five *E. coli* O157 strain was isolated from the traditional cheese samples and the other four were from the traditional ice cream samples.

Stx1 and *stx2* genes were detected in the three *E. coli* O157 isolates obtained from the three traditional cheese samples. None of these genes were detected in the *E. coli* O157 isolates isolated from the traditional ice cream samples.

Overall, 8 of 9 *E. coli* O157 isolates (88.9%) were resistant to one or more antimicrobial agent. Two strains (22.2%) were resistant to single antibiotic and 5 strains (55.6%) showed resistance to two antimicrobial agents. Multiresistance which was defined as resistance to three or more of drug tested was found in 22.2% of *E. coli* O157 strains. Resistance to ampicillin and gentamycin was the most common finding (44.4%), followed by resistance to erythromycin (33.3%), amoxicillin (11.1%), tetracycline (11.1%) and nalidixic acid (11.1%). All *E. coli* O157 isolates were susceptible to chloramphenicol, cefuroxime, and streptomycin.

DISCUSSION

E. coli O157 and causes severe disease and death in humans (Elder et al., 2000; Su and Brandt, 1995). Human infections of *E. coli* O157 have been mostly attributed or linked to food products from animals (Riley et al., 1983; Kim et al., 1998; Elder et al., 2000).

Since there was no available data regarding the prevalence of *E. coli* O157 in Iran, the aim of this study was to determine the occurrence of *E. coli* O157 in traditional cheese, ice cream and yoghurt produced in Iran. The study showed that nine of the 240 traditional cheese and ice cream (3.75%) from Isfahan, Chaharmahal and Bakhtyari and Khuzestan were contaminated with *E. coli* O157. These *E. coli* were found to be positive for the two target genes *stx1*, and *stx2* genes. No significant differences in the prevalence rates were observed between traditional cheese and ice cream samples isolated in Isfahan, Chaharmahal and Bakhtyari and Khuzestan.

There are a number of studies from different countries concerning the incidence of *E. coli* O157 and *E. coli* O157:H7 isolation on a variety of foods (Abdul-Raouf et al., 1996; Ansay and Kaspar, 1997; Coia et al., 2001; Caro et al., 2006; Cizek et al., 2007; Abong'o and Momba, 2009; Solomakos et al. 2009). Abdul-Raouf et al. (1996) reported that 6% of raw cow's milk samples examined in Egypt were contaminated with *E. coli* O157:H7. Allerberger and Dierich (1997) reported 3% of the milk samples tested in Austria to be positive for *E. coli* O157:H7 and Klie et al. (1997) found that only 0.3% of the milk analyzed in Germany was contaminated with this serotype. Similar studies on raw cow's milk performed in the USA analyzing 42 samples (Ansay and Kaspar, 1997), and in the Netherlands analyzing 1011 samples (Heuvelink et al., 1998) resulted in no *E. coli* O157:H7 isolation. In our study, no *E. coli* O157:H7 strain was isolated from the samples tested. In another study conducted in Egypt, 2% of Kareish cheese samples were positive for *E. coli* O157 by a biochemically and serologically assay (Abd El-Atty and Meshref, 2007). Similar results of cheese samples were reported by Aksu et al. (1999); while Abd El-Hady et al. (1995), El- Kosi (2001), reported higher values. On the other hand Ansay and Kaspar (1997), Ibrahim and Sobeih (2006) failed to isolate *E. coli* O157 from cheese samples.

The presence of *E. coli* O157 in traditional cheese and ice 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.

In the present study, no *E. coli* O157 isolate was detected in yogurt 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 yogurt 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 performed during the processing of these products.

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 (Schmidt et al. 2001), were detected in the three *E. coli* O157 isolates from traditional cheese samples. These findings are supported by several studies (Vivegnis et al., 1999; Pradel et al., 2000; Cagney et al., 2004; Caro et al., 2006; Mansouri-Najand and Khalili, 2007).

The results of antimicrobial susceptibility testing in the present study indicate that there is a high resistance of *E. coli* O157 to ampicillin, gentamycin, and erythromycin. These results are comparable to those reported by other investigators (Lira et al., 2004; Picozzi et al., 2005; Duffy et al., 2006; Caro et al., 2006; Cizek et al., 2007; Solomakos et al., 2009; Abong'o and Momba, 2009; Ngwai et al., 2010). The results of antimicrobial resistance found in this study are correlated to antibiotics that are being used to treat infection in food animals in Iran. Also, high percentage of *E. coli* O157 isolates was found to be resistant to ampicillin, an antibiotic used in human medicine for the treatment of coliform infections. Due to the high number of antimicrobial-resistant isolates, we recommend that in vitro antimicrobial susceptibility testing of *E. coli* be performed and appropriate treatment be instituted especially for those cases of food borne *E. coli* with sever or prolonged symptoms or in immuno-compromised patients.

From the present data it can be concluded that the Traditional cheese and ice cream represents a potential hazard for consumers, due to the potential presence of *E. coli* O157 as well as there is neglected sanitary measures adopted during manufacturing, handling and distribution of such fresh foods. Consequently, food manufacturers and specialists should design comprehensive programs as good manufacturing practices (GMP) and implementtation of HACCP system to ensure the freedom of such foods from these pathogens. In addition, effective heat treatment for foods, provision of information to food handlers and consumers as well as application of strict hygienic measures during manufacturing, storage and selling of these products to improve its quality and safeguard the consumers against infections of such organisms.

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