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Surveillance for enterotoxigenic & enteropathogenic *Escherichia coli* isolates from animal source foods in Northwest Iran

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***Background & objectives*: Diarrhoeagenic *Escherichia coli* strains are common agents of diarrhoea particularly in developing countries. Food products of animal origin are considered as common carriers of *E. coli.* This study was undertaken to identify enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *E. coli* (EPEC) pathotypes in animal-source foods (ASF).**

***Methods*: A total of 222 ASF samples were investigated. Based on the culture and biochemical tests, 109 *E. coli* isolates were identified. Duplex-polymerase chain reaction assay was used to detect ETEC and EPEC. The target genes selected for each category were the *lt* and *st* for the ETEC, and *eae* and *bfp* for the EPEC isolates.**

***Results*: The occurrence of *E. coli* in dairy and meat products was 45 and 52.5 per cent, respectively. Among the *E. coli* isolates, two ETEC, one typical EPEC and three atypical EPEC were detected in meat samples, whereas only one typical EPEC and one atypical EPEC were detected in dairy samples.**

***Interpretation & conclusions*: Our results showed presence of ETEC and EPEC strains in ASFs. The milk without pasteurization and traditional dairy products produced in unhygienic conditions are most likely the main sources of *E. coli* pathotypes and other zoonotic pathogens and thus can be considered a potential hazard to the health of the community.**

Key words Animal source foods - enteropathogenic *Escherichia coli* - enterotoxigenic *Escherichia coli* - food borne diseases - food hygiene

Diarrhoeal disease is a global problem, particularly in developing countries. Various infectious agents cause diarrhoea, such as rotaviruses, coronaviruses, *Campylobacter* spp., *Clostridium perfringens*, *Escherichia coli* and *Salmonella* species[1,2]. Among the bacterial pathogens, diarrhoeagenic *E. coli* (DEC)

strains are the most common agents of diarrhoea, especially in developing countries[3]. Based on the virulence factors, DEC is classified into six groups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC)[1,4]. ETEC is a major cause of childhood diarrhoea in developing countries and in people who travel from industrialized countries to native regions[5-7]. The virulence factors of these bacteria are heat-stable and heat-labile enterotoxins that are encoded by the *st* and *lt* genes[6]. Another important pathotype of the species is EPEC, which is the primary cause of diarrhoea in infants, especially in developing countries. EPEC strains exist in two forms: typical EPEC or tEPEC (having *eae* and *bfp* genes) and atypical EPEC or aEPEC (lacking *bfp* gene)[5,8].

Because the isolation of *E. coli* was easier than that of other enteric pathogens, it could be used as an indicator of faecal contamination and other faecal-origin microorganisms in food materials[9]. Ruminants, especially calves, are identified as a major source of pathogenic *E. coli*[10]. Food products of animal origin, such as fresh meat and raw milk, are considered as common carriers of *E. coli*. Meat and meat products may be contaminated in several ways, such as through direct contact with faeces or hides during slaughter[11]. Moreover, raw milk and milk products could be rich nutritious medium for many microorganisms. Poor sanitation during collection or storage can also cause contamination. Pathogenic *E. coli* has been identified as a major cause of foodborne diseases, because animal source foods (ASFs) are constituents of human diet[12]. Hence, it becomes necessary to determine the occurrence of *E. coli* in ASFs through a reliable and quick test. The aim of this study was to identify ETEC and EPEC as two common pathotypes of *E. coli* in raw meat and dairy products through duplex-polymerase chain reaction (PCR).

Material & Methods

This study was conducted from May to September 2016 in the laboratory of Microbiology, Food and Drug Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. A total of 120 samples of meat products (fresh beef, ground beef and hamburger) and 102 dairy products (raw milk, traditional cheese, *Doogh* and yoghurt) were collected from markets in different localities of Northwest Iran. All specimens were immediately transferred to the laboratory under cold chain and sterile conditions.

Bacterial isolation: For the enrichment of the *E. coli* strains, all specimens were cultured in lauryl sulphate broth (Merck, Germany) overnight at 37°C and subsequently were streaked onto MacConkey agar (Merck, Germany) and incubated at 37°C for 24 h. The lactose-fermenting colonies were identified as *E. coli* using Gram staining and conventional biochemical tests as described previously[13]. Finally, *E. coli* isolates were stored at −80°C in trypticase soy broth supplemented with 20 per cent glycerol for further procedures.

DNA extraction: DNA extraction from overnight cultures was done using the Promega DNA extraction kit (A11125, USA), following the instructions given by the manufacturer.

Polymerase chain reaction assays: The DNA templates were examined by two separate duplex PCRs with specific primers (Table I). Duplex-PCR 1 and 2 were for the identification of *lt* and *st* genes (for detection ETEC) and *eae* and *bfp* genes (for detection EPEC), respectively[14-16]. Both duplex-PCR assays were accomplished in a 25 µl reaction mixture, consisting of 2× PCR master mix [2× concentrated solutions of *Taq* DNA polymerase, reaction

Table I. Primers used for the detection of enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *E. coli* (EPEC) pathotypes

Target organism	Target genes	Gene location	Primer sequences (5'→3')	Product size (bp)	Reference
ETEC	*st*	Plasmid	F: ATTTTTMTTTCTGTATTRTCTT R: CACCCGGTACARGCAGGATT	190	15
	lt	Plasmid	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	450	15
EPEC	*eae*	Chromosome	F: AGGCTTCGTCACAGTTG R: CCATCGTCACCAGAGGA	570	14
	bfp	Chromosome	F: AATGGTGCTTGCGCTTGCTGC R: GCCGCTTTATCCAACCTGGTA	326	16

buffer, $MgCl_2$ and dNTPs (CinnaGen Inc., Iran)] with a BioRad T100™ thermal cycler (Bio-Rad Laboratories, Inc., USA). The PCR Master Mix (CinnaGen Inc., Iran) contains all components for PCR, except DNA template and primers. Primers were provided by GeNetBio Inc., Korea. The first duplex-PCR conditions included an initial denaturation at 95°C for five minutes for one cycle followed by 35 cycles of 95°C for 45 sec, 49°C for 45 sec, 72°C for 45 sec and final extension at 72°C for seven minutes. The second duplex-PCR conditions comprised: An initial denaturation at 95°C for three minutes for one cycle followed by 38 cycles of 95°C for one minute, 53°C for one minute, 72°C for one minute and final extension at 72°C for 10 minutes. Amplified PCR products were observed after electrophoresis on one per cent agarose and staining with Safe Dye (CinnaGen Inc., Iran). The PCR products were visualized under ultraviolet UV transilluminator and photographed.

Reference strains: The following standard strains were employed as positive controls: ETEC (H10407) and EPEC (2348/69). These strains were provided by R. Vidal, Microbiology and Mycology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile.

Results

Among the 222 samples (120 meat samples, 102 diary samples) examined in this study, 109 (49%) *E. coli* isolates were obtained [63 (57.7%) from meat products and 46 (42.2%) from dairy products]. The ground beef and hamburger had the highest occurrence of contamination, followed by fresh beef. About dairy products, all of the *E. coli* isolates belonged to raw milk and traditional cheese samples. No *E. coli* isolate was identified in fermented dairy products *i.e., Doogh*, yoghurt.

Occurrence of ETEC and EPEC strains in meat and dairy products: Among *E. coli* isolates, two ETEC and four EPEC (1 typical EPEC and 3 atypical EPEC) were detected in meat samples while no cases of ETEC and only two EPEC (1 typical EPEC and 1 atypical EPEC) were identified in dairy samples. The distribution of virulence target genes is shown in Table II. Fifty per cent (4 of 8 isolates) of the pathogenic *E. coli* isolates harboured both *eae* and *bfp* genes and were considered as tETEC. Furthermore, no *st* and *lt* genes were identified in dairy products (Figs 1 & 2).

Table II. Virulence gene profile of *Escherichia coli* strains screened by duplex polymerase chain reaction

Virulence gene(s)	*E. coli* strains with indicated virulence genes		
	Raw milk products	Raw meat products	Total
st	0	1	1
lt	0	0	0
st+lt	0	1	1
eae	1	1	2
eae+bfp	1	3	4

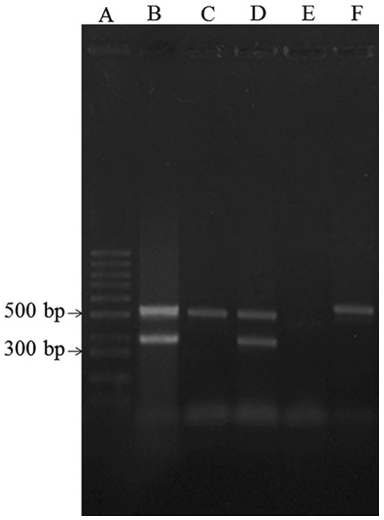

Fig. 1. (A) Ladder 1000 bp, (B) positive control for *eae* and *bfp* genes, (C) positive isolate for *eae* gene, (D) positive isolate for *eae* and *bfp* genes, (E) negative control, (F) positive isolate for *eae* gene.

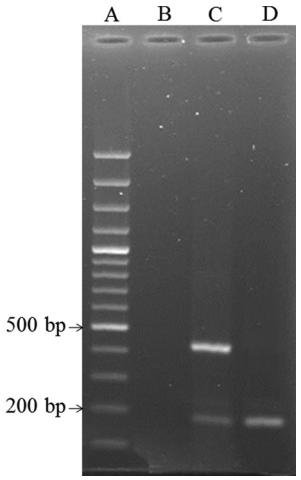

Fig. 2. (A) Ladder 1000 bp, (B) negative control, (C) positive isolate for *lt* and *st* genes, (D) positive isolate for *st* gene.

Discussion

Diarrhoea caused by different intestinal pathogens is one of the major concerns of public health. An important bacterial agent of diarrhoea is *E. coli*, which causes death among under five children and the elderly[17]. Animal food products, especially raw meat and dairy products that are rich in nutritional value are likely to get contaminated with spoilage and pathogenic bacteria, resulting in the transmission of infections to humans[12]. Therefore, use of rapid diagnostic techniques is important in reducing disease development rate and decreasing the financial burden of the disease on the community health[2].

In the present study, the occurrence of *E. coli* was 42.2 and 57.7 per cent in raw milk/raw milk products and raw meat/raw meat products, respectively. In a similar study carried out by Rúgeles *et al*[16], *E. coli* was isolated from 58 per cent of the meat samples. Badri *et al*[18] reported the prevalence of *E. coli* in meat samples as 48 per cent. Several other studies have reported the presence of *E. coli* in raw milk and meat products[12,19-21]. It should be noted that in the studied dairy samples, no *E. coli* isolate was identified in *Doogh* and yoghurt samples. It could be due to acidic *p*H and high temperatures created during their fermentation process[22]. Moreover, majority of *E. coli* identified in meat products were isolated from ground meat and hamburger. It is probable, these products are more contaminated due to poor sanitation of grinder and high manipulation in their production process[23]. According to the results of the duplex-PCR of isolated *E. coli*, only two EPEC were found in raw milk and raw milk products, whereas two ETEC and four EPEC were identified in raw meat products. In similar studies[5,18], no case of ETEC was found in raw meat products, while EPEC (mostly atypical) was the most isolated pathotype, thereby confirming the results of the present study. Mohammed[11] showed that 15.63 per cent *E. coli* isolated from meat products were ETEC, which was higher than that of several previous studies. Holko *et al*[24], reported 2.1 per cent ETEC and 3.09 per cent EPEC in traditional cheese samples made from sheep milk. A study conducted on raw milk materials, found one ETEC and 13 EPEC[5]. In another study on 206 samples of raw milk, 17 EPEC (8.25%) were identified.

In conclusion, our results showed that the use of unpasteurized milk in traditional dairy products and inadequate sanitation during the preparation, transport and/or storage of ASFs and personal hygiene could be a potential factor in the spread of *E. coli* pathotypes and other zoonotic pathogens in the community should be considered and as a threat to public health.

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***Conflicts of Interest*:** None.

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