International Journal of
Enteric Pathogens

Int J Enteric Pathog. 2018 February;6(1):14-17 Original Article

http://enterpathog.abzums.ac.ir

doi 10.15171/ijep.2018.04

Risk of Shiga Toxin-Producing *Escherichia coli* Infection in Humans Due to Consuming Unpasteurized Dairy Products

Naghmeh Moori Bakhtiari[1*], Ali Fazlara[2], Tayebe Hafezizade Jolge[3]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3]Graguated Student of Veterinary Faculty, Shahid Chamran University of Ahvaz, Iran

***Corresponding Author:**
Naghmeh Moori Bakhtiari, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
P. O. Box: 6135714333;
Tel/Fax: +98-61-33360807;
Email: n.moori@scu.ac.ir

Published Online December 3, 2017

Keywords: Shiga-toxigenic *Escherichia coli*, Polymerase chain reaction, Dairy products

Abstract

Background: Cattle transiently harbor Shiga-toxigenic *Escherichia coli* (STEC) in their gastrointestinal tracts, and many human infections result from ingestion of contaminated dairy products. The occurrence of STEC infections in human ranges from mild watery diarrhea to life-threatening conditions such as thrombotic thrombocytopenic purpura, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).

Objective: Isolation of STEC from unpasteurized dairy products as a source of human infections is the aim of this research.

Materials and Methods: In this study, after collecting 150 samples of unpasteurized dairy products from different parts of Ahvaz, primary enrichment, selective enrichment and conventional biochemical tests were done and the suspected DNA isolates were extracted by boiling. Confirmation of being toxigenic isolates was performed by multiplex polymerase chain reaction (mPCR) assay. The stx_1 and stx_2-specific primers were used in m-PCR.

Results: Out of 75 isolates with lactose-fermentation ability, 11 *E. coli* strains were confirmed by biochemical tests. Two isolates (18.18%) were detected as carriers of stx_2 gene by PCR.

Conclusion: Because of low infective dose, the presence of a low percentage of toxigenic *E. coli* in dairy products could be a grand public health risk, while the bacteria other than *E. coli* could be producing Shiga toxin which should not be ignored.

Received June 13, 2017; **Revised** October 30, 2017; **Accepted** November 29, 2017

Background

Pathogenic *Escherichia coli* strains are classified into pathotypes; that is groups of strains with remarkable assortments and common virulence factors.[1] Shiga-toxigenic *E. coli* (STEC) pathotypes are proactive factors for certain severe clinical syndromes in humans such as hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura and Hemolytic Uremic Syndrome (HUS).

The most common aggression factors in this pathotype are 2 phage-encoded SLT_1 and SLT_2 cytotoxins which are encoded by *stx1* and *stx2* genes, respectively.[2] The high fatality rate, low infective dose and severity of the signs make it a harmful threat to food safety.[1,3] It appears that ruminants are a reservoir for STEC in the environment.[4,5] A large variety of foods have been found to be contaminated with STEC, and unpasteurized or traditional dairy products including cheeses and raw milk in particular, could contain these strains.[6-9] Consumption of undercooked hamburgers was associated with the first outbreak of STEC; the next outbreaks have imputed to both plant and foods of animal origin.[10] Raw milk is presumably contaminated with STEC during milking via fecal contamination.[11] However, these organisms have been reported to be directly excreted from infected udders.[12]

Fermented dairy products that are produced from raw milk contaminated with STEC could be considered as a serious threat to public hygiene because of surveillance of this pathogen after insufficient heating in manufacturing fermented products or contamination of products after successful heating step. Survival of this pathotype has been well documented in lactic cheeses made from raw goat milk,[13] aged Cheddar cheese made from raw milk,[14] Feta cheese,[15] and even yogurt.[16] Based on a review of the resources, until now, no study on the prevalence of Shiga-toxin producing *E. coli* in the raw milk supply has been done in Ahvaz, whiles there are several studies regarding the presence of Shiga toxin-producing *E. coli* O157:H7 in raw milk and dairy products in Iran and other countries.[17-20]

Objectives

The purpose of this study was to detect STEC in several dairy products, including raw milk, unpasteurized cheese,

butter, ice cream, "Sarshir" (same as cream but with a different manufacturing procedure) and "Shirberenj" (rice cooked in milk) in Ahvaz.

Materials and Methods

Collection of Samples

One hundred fifty samples of dairy products including raw milk (39), cheese (24), butter (25), Sarshir (16), ice cream (29) and Shirbrenj (17) were collected in a time span of 6 months from various locations in Ahvaz, and they were examined for the presence of *E. coli*. Samples were transferred to sterile plastic bags under sterile conditions. Transportation of samples to the laboratory was done under cold conditions in short time after sampling. The samples were kept at -20°C until bacteriological analysis.[21]

Bacteriological Analysis

From the center of each sample, a portion (4-5 g or mL) was removed and homogenized aseptically in 40 mL of sterile lactose broth (Merck, Germany) as enrichment broth. Primary enrichment stage was done for 24 hours at 37°C.[21]

Media and Growth Conditions

In order to isolate and identify *E. coli*, a loop full of enriched samples was cultured in MacConkey (Mac) agar (Merck, Germany) as selective medium and incubated for 24 hours at 37°C. Doubtful pinkish colonies (4 colonies per plate) as lactose-positive strains were plated on blood agar for further identification. In addition, suspected isolates were cultured in eosin methylene blue (EMB) agar (Merck, Germany) as selective medium, for producing metallic sheen.[21]

Physiological and Biochemical Examination

From each bacterial plate, 4 to 5 suspected colonies were selected, subcultured, and then confirmed as *E. coli* by different biochemical tests such as gram staining, oxidase test, various sugar fermentation tests, indole, nitrate reduction, methyl red, Voges-Proskauer test, Simmon citrate agar, and urease production (Merck, Germany).[21]

Multiplex Polymerase Chain Reaction

The isolates confirmed as *E. coli* were examined by multiplex polymerase chain reaction (m-PCR) in order to examine the presence of studied genes (*stx1* and *stx2*). Shiga toxin-producing *Escherichia coli* O157:H7 (ATCC-43894) and sterile distilled water were used as the positive and negative control, respectively. A bacterial suspension of each confirmed *E. coli* isolate was prepared in sterile TE (Tris-EDTA) buffer with 2% two-mercaptoethanol. The suspension was heated in a boiling water bath for 10 minutes to make a bacterial lysate. For precipitation of cellular debris, centrifugation was done for 3 minutes at 13000 rpm (Eppendorf, Germany). The supernatant was collected in a new sterile microtube and stored at -20°C as a template in m-PCR. The used primer (SinaGen, Iran) sequence was as follows *stx1* gene encoding the Shiga-like toxin1 (SLT1) and *stx2*, which encodes the Shiga-like toxin 2 (SLT2) (Table 1).[22] The temperature and conditions of amplification in this research was as described before by Brenjchi et al.[17] The total volume of each reaction mixture was 25 µL which contained 0.5 µM of each primer (1 µL), and 5 µL of the template and 12.5 µL 2X Mastermix (SinaGen, Iran). The initial denaturation was done by incubation at 94°C for 5 minutes and followed by 35 cycles consisting of denaturation at 94°C, annealing at 52°C and elongation at 72°C for 60, 30 and 60 seconds, respectively and final extension at 72°C for 10 minutes. The products of PCR were analyzed by electrophoresis in 1.5% agarose gel in TAE (tris-acetate-EDTA) buffer, and they were visualized by safe-staining (SinaGen, Iran), then illuminated by a UV transilluminator (Uvi-tech, Germany) and documented afterward by a gel documentation apparatus. 100 bp DNA ladder was used as a marker for m-PCR assay.[17]

Table 1. Target Genes, Primers Sequences and Expected Sizes for Toxigenic *Escherichia coli*

Target Gene	Primer Sequence	Size (bp)
stx1	F: 5′- ACA CTG GAT GAT CTC AGT GG-3′ R: 5′- CTG AAT CCC CCT CCA TTA TG-3′	614
stx2	F: 5′- CCA TGA CAA CGG ACA GCA GTT-3′ R: 5′- CCT GTC AAC TGA GCA CTT TG-3′	779

Results

Out of 150 dairy product samples, 367 lactose-fermenting colonies were isolated, after enrichment and selective plating. Based on the reaction in EMB medium, 172 colonies were selected. Then, 35 oxidase and gram-negative medium size colonies were isolated.

Finally, 11 strains were confirmed as *E. coli* by gram negative rod shape and catalase positive, oxidase negative, urease and indole negative, and methyl red positive reactions, lactose fermentation, and nitrate reduction. In other words, 7.3% of the samples (11/150) were confirmed as *E. coli*. These strains were isolated from ice cream (1), raw milk (4), Shirbrenj (2), and butter (4). Using *stx1*- and *stx2*-specific primers showed that the 2 isolates (18.18%) were harboring Shiga-like toxin (*stx2*). Toxigenic bacteria detected by PCR were isolated from raw milk and Shirbrenj samples (Figure 1).

There were no significant differences ($P > 0.05$) in the level of contamination with *E. coli* among different types of the samples and the production of *stx2* was dominant.

Discussion

On the whole, the existence of various strains of *E. coli*, as a probable causative agent of food-borne disease, in dairy products is not significant if *E. coli* is considered as a ubiquitous organism.[23] However, in case of the presence of pathogenic strains, they could be harmful to consumers.

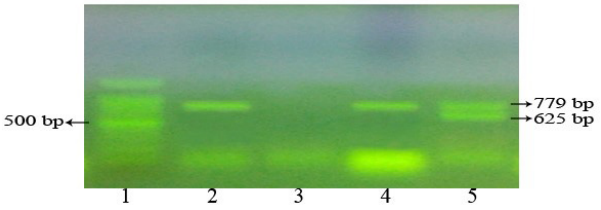

Figure 1. Results of Gel Electrophoresis of Studied Genes PCR Products. Lane1: Marker 100 bp, lane 2: stx2 positive sample (779 bp), lane 3: negative sample, lane 4: stx2 positive sample (779 bp), lane 5: stx1 and stx2 positive control (625 and 779 bp, respectively).

Dairy products such as cheese, butter, ice cream, Shirbrenj and Sarshir as similar as milk are the sources of nutrients and protein as well as casein because of the presence of the 9 essential nutrients. These milk products are of great importance in the Iranian diet, thus, their contamination can bring about different health hazards. Dairy products are prepared as pasteurized or unpasteurized (traditional) and used in different regions of Ahvaz on a daily basis. The production, maintenance and presentation of traditional products were entirely unpasteurized. Traditional methods can create a suitable environment for bacterial contamination.

The most well-known serotype in STEC pathotype is O157:H7 (O157) and other serotypes of STEC called as non-O157. Previous studies have shown that the prevalence of O157. O26, O114, O44, O124, O111, O55, and O119 serotypes are the most common serotypes of *E. coli* isolates.[24] Depending on the geographical location, the most common serotype is different. For instance, O157:H7 has been introduced as the most common serotype in the Unites States, Europe, and Japan.[25] Seasonal distribution of STEC serotypes O157:H7 have been reported before,[26] so that the highest and the lowest occurrence were reported in summer and winter, respectively. Therefore, there is the possibility of a higher percentage of contamination than 18.18% in other seasons; and that is due to the fact that the samples of the present study were collected during the fall and winter months. Up until now, several studies have been conducted in Iran and other countries on the presence of O157:H7 in food.[17,27-29]

Escherichia coli created subclinical mastitis in bovine could reduce milk quality for human consumption. The regulations of inspection and control of milk are of greater importance in cases where milk is consumed raw. The milk of animals with mastitis, unsanitary milk collection and milking machine, methods of processing and milking, and prevention of the contamination of raw milk with extrinsic factors like the staff, dust, and insects, as well as the primary hygiene of milk are important in contamination of milk with STEC strains.[30]

Several disease outbreaks due to *E. coli*[31,32] have shown that review and examination of foodstuff, chiefly those related to animals are important keys to the restriction of contamination risk. In numerous researches, it was shown that these strains are the common sources of poisoning in milk.[8,11,29]

In this study, STEC contamination of different dairy products was evaluated by culture and m-PCR and a relatively significant contamination rate (7.3%) was observed, and 18.18% of isolates were toxigenic (SLT2 producing). Based on previous studies, most strains of this organism produce Stx2, a number of them produce both Stx1 and Stx2, and a few produce solely Stx1.[2] In a study by the present author (unpublished), *E. coli* O157:H7 contamination of raw milk (on the farm) was evaluated in Khuzestan province. In the present research, 13.3% (20/150) of the samples were carriers of O157, some of which were toxigenic. Some studies have indicated that there is a probability of the presence of antibiotic resistance gene along with other virulence genes such as *eae, Ehly, stx2*, and *stx1*, in STEC strains[33,34] and this could be alarming for public health. Based on the results of this research, uncompromising preventive measures are recommended to produce uncontaminated dairy products, which subsequently promote public health.

Authors' Contributions

This study was designed by NMB and AF. Sampling was done by AF and THJ. Treatment, isolation, and biochemical and molecular identifications were done by NMB and THJ.

Ethical Approval

We hereby declare that all ethical standards have been respected in the preparation of the article.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Acknowledgments

Authors would like to thank the research council of Shahid Chamran University of Ahvaz for their financial support and Dr. Roya Firuzi and Dr. Katayoun Nofuzi from Shiraz and Tabriz universities of Iran for provision of *E. coli* O157:H7.

References

1. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2004;2(2):123-140. doi:10.1038/nrmicro818
2. Law D. Virulence factors of Escherichia coli O157 and other Shiga toxin-producing *E. coli*. J Appl Microbiol. 2000;88(5):729-745.
3. Meng J, Doyle MP, Zhao T. Enterohemorrhagic *Escherichia coli*. In: Doyle MP, Beuchat LR, Eds. Food Microbiology: Fundamentals and Frontiers. 3rd ed. Washington, DC: ASM Press; 2007.
4. Rey J, Blanco JE, Blanco M, et al. Serotypes, phage types and virulence genes of shiga-producing *Escherichia coli* isolated from sheep in Spain. Vet Microbiol. 2003;94(1):47-56.
5. Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A. *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing E. coli in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Public Health. 2008;55(2):73-81. doi:10.1111/j.1863-2378.2007.01080.x
6. Vernozy-Rozand C, Montet MP, Berardin M, Bavai C, Beutin L. Isolation and characterization of Shiga toxin-producing

Escherichia coli strains from raw milk cheeses in France. Lett Appl Microbiol. 2005;41(3):235-241. doi:10.1111/j.1472-765X.2005.01756.x
7. Caro I, Mateo J, Garcia-Armesto MR. Phenotypical characteristics of Shiga-like toxin *Escherichia coli* isolated from sheep dairy products. Lett Appl Microbiol. 2007;45(3):295-300. doi:10.1111/j.1472-765X.2007.02186.x
8. Stephan R, Schumacher S, Corti S, Krause G, Danuser J, Beutin L. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in Swiss raw milk cheeses collected at producer level. J Dairy Sci. 2008;91(7):2561-2565. doi:10.3168/jds.2008-1055
9. Escherichia coli O157:H7 infection associated with drinking raw milk--Washington and Oregon, November-December 2005. MMWR Morb Mortal Wkly Rep. 2007;56(8):165-167.
10. Erickson MC, Doyle MP. Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. J Food Prot. 2007;70(10):2426-2449.
11. Hussein HS, Sakuma T. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J Dairy Sci. 2005;88(2):450-465.
12. Lira WM, Macedo C, Marin JM. The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. J Appl Microbiol. 2004;97(4):861-866. doi:10.1111/j.1365-2672.2004.02384.x
13. Vernozy-Rozand C, Mazuy-Cruchaudet C, Bavai C, et al. Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. Int J Food Microbiol. 2005;105(1):83-88. doi:10.1016/j.ijfoodmicro.2005.05.005
14. Schlesser JE, Gerdes R, Ravishankar S, Madsen K, Mowbray J, Teo AY. Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of cheddar cheese made from unpasteurized milk. J Food Prot. 2006;69(5):990-998.
15. Govaris A, Papageorgiou DK, Papatheodorou K. Behavior of *Escherichia coli* O157:H7 during the manufacture and ripening of feta and telemes cheeses. J Food Prot. 2002;65(4):609-615.
16. Govaris A, Koidis P, Papatheodorou K. Behaviour of *Escherichia coli* O157:H7 in sour milk, cows' milk yogurt and ewes' milk yogurt. J Dairy Res. 2002;69(4):655-660.
17. Brenjchi M, Jamshidi A, Farzaneh N, Bassami MR. Identification of Shiga toxin producing *Escherichia coli* O157:H7 in raw cow milk samples from dairy farms in Mashhad using multiplex PCR assay. Iran J Vet Res. 2011;12(2):145-149. doi:10.22099/ijvr.2011.56
18. Muehlherr JE, Zweifel C, Corti S, Blanco JE, Stephan R. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. J Dairy Sci. 2003;86(12):3849-3856. doi:10.3168/jds.S0022-0302(03)73992-7
19. Caro I, Garcia-Armesto MR. Occurrence of Shiga toxin-producing *Escherichia coli* in a Spanish raw ewe's milk cheese. Int J Food Microbiol. 2007;116(3):410-413. doi:10.1016/j.ijfoodmicro.2007.02.015
20. 20- Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. St Louis, USA: Mosby; 2004.
21. Rey J, Sanchez S, Blanco JE, et al. Prevalence, serotypes and virulence genes of Shiga toxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. Int J Food Microbiol. 2006;107(2):212-217. doi:10.1016/j.ijfoodmicro.2005.08.025
22. Holland JL, Louie L, Simor AE, Louie M. PCR detection of *Escherichia coli* O157:H7 directly from stools: evaluation of commercial extraction methods for purifying fecal DNA. J Clin Microbiol. 2000;38(11):4108-4113.
23. Hahn G. Pathogenic bacteria in raw milk situation and significance. In: Bacteriological Quality of Raw Milk. Brussels (Belgium): Int. Dairy Federation; 1996:67-83.
24. Salwa MH, Ammar MA, Aisha RA, et al. Molecular and virulence characterization of *Escherichia coli* strains isolated from persistent bovine mastitis. *J Am Sci*. 2011;7:614-624.
25. Fode-Vaughan KA, Maki JS, Benson JA, Collins MLP. Direct PCR detection of *Escherichia coli* O157:H7. Lett Appl Microbiol. 2003;37(3):239-243. doi:10.1046/j.1472-765X.2003.01386.x
26. Cagney C, Crowley H, Duffy G, et al. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. Food Microbiol. 2004;21(2):203-212. doi:10.1016/S0740-0020(03)00052-2
27. Rahimi E, Kazemeini HR, Salajegheh M. *Escherichia coli* O157:H7/NM prevalence in raw beef, camel, sheep, goat, and water buffalo meat in Fars and Khuzestan provinces, Iran. Vet Res Forum. 2012;3(1):15-17.
28. Sami M, Firouzi R, Shekarforoush SS. Prevalence of *Escherichia coli* O157:H7 on dairy farms in Shiraz, Iran by immunomagnetic separation and multiplex PCR. *Iran J Vet Res*. 2007; 8(4):319-324.
29. Solomakos N, Govaris A, Angelidis AS, et al. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. Food Microbiol. 2009;26(8):865-871. doi:10.1016/j.fm.2009.06.002
30. Momtaz H, Safarpoor Dehkordi F, Taktaz T, Rezvani A, Yarali S. Shiga toxin-producing *Escherichia coli* isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. ScientificWorldJournal. 2012;2012:618709. doi:10.1100/2012/618709
31. European Food Safety Authority (EFSA). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. *EFSA J*. 2007;130:1-352.
32. Gillespie IA, O'Brien SJ, Adak GK, Cheasty T, Willshaw G. Foodborne general outbreaks of Shiga toxin-producing *Escherichia coli* O157 in England and Wales 1992-2002: where are the risks? Epidemiol Infect. 2005;133(5):803-808. doi:10.1017/s0950268805004486
33. Wenz JR, Barrington GM, Garry FB, Ellis RP, Magnuson RJ. *Escherichia coli* isolates' serotypes, genotypes, and virulence genes and clinical coliform mastitis severity. J Dairy Sci. 2006;89(9):3408-3412. doi:10.3168/jds.S0022-0302(06)72377-3
34. de Verdier K, Nyman A, Greko C, Bengtsson B. Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. Acta Vet Scand. 2012;54:2. doi:10.1186/1751-0147-54-2