ORIGINAL ARTICLE

Antibiotic resistance patterns of STEC and ETEC strains: A study on frozen foods of animal origin and children with acute diarrhea

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ABSTRACT

Objective: Shigatoxin producing *E. coli* (STEC) and Enterotoxigenic *E. coli* (ETEC) are infectious pathogens that have been implicated in food and waterborne diseases in human all the world. The aim of this study was to determine the incidence and evaluate antibiotic resistance patterns of STEC and ETEC strains.

Methods: In total 125 frozen foods of animal origin and 466 rectal swabs from children with acute diarrhea were taken to isolate and identify *E. coli* strains based on standard procedures. Antimicrobial susceptibility tests for *E. coli* strains were performed according to the Clinical Laboratory Standards Institute. Resistance to two or more classes of antimicrobials in STEC and ETEC strains was recorded.

Results: A total of 87 strains of *E. coli* strains were detected from 466 rectal swabs from children with acute diarrhea and 40 strains of *E. coli* strains were detected from the 125 frozen food samples of animal origin.

Test results indicated a 5.0% and 2.5% prevalence Shiga toxin (stx) and enterotoxin (estA) genes among *E. coli* strains isolated from frozen foods of animal origin. Similarly, 5.7% and 4.5% prevalence of Shiga toxin (stx) and enterotoxin (estA) genes among *E. coli* strains isolated from children.

Conclusion: We conclude that *E. coli* stains having Shiga toxin (stx), and enterotoxin (estA) genes considered not to be a potential source of infections for in Sanandaj; perhaps other enteric pathogens are the major cause of food-borne diseases. *J Microbiol Infect Dis 2013; 3(1): 31-35*

Key words: *E. coli*, acute diarrhea, children, frozen food sample, stx and estA gene

STEC ve ETEC suşlarının antibiyotik direnç paternleri: hayvansal kökenli dondurulmuş gıdalar ve akut ishalli çocuklar üzerine bir çalışma

ÖZET

Amaç: Shigatoxin üreten *E. coli* (STEC) ve Enterotoksijenik *E. coli* (ETEC) tüm dünyada gıda ve su kaynaklı insan hastalıklarından sorumlu tutulan enfeksiyöz patojenlerdir. Bu çalışmanın amacı, STEC ve ETEC suşlarının insidansını belirlemek ve antibiyotik direnç paternlerini değerlendirmektir.

Yöntemler: *E. coli* suşlarını izole edilmesi ve standart prosedürler esas alınarak tanımlanması için hayvansal kökenli toplam 125 dondurulmuş gıda ve akut ishali olan çocuklardan 466 rektal sürüntü alındı. *E. coli* suşlarının antimikrobiyal duyarlılık testleri Klinik Laboratuvar Standartları Enstitüsü'ne göre yapıldı. STEC ve ETEC suşlarında iki ya da daha fazla antimikrobiyal gruba karşı direnç varlığı kaydedildi.

Bulgular: Akut ishali olan çocuklardan alınan 466 rektal sürüntü örneğinden toplam 87 *E. coli* suşu ve 125 dondurulmuş hayvansal kökenli gıda örneğinden 40 *E. coli* suşu tespit edildi. Test sonuçları hayvansal kökenli dondurulmuş gıdalardan izole edilen *E. coli* suşlarındaki Shiga toksin (stx) ve enterotoksin (estA) genlerinin prevalansını %5,0 ve %2,5 olarak gösterdi. Benzer şekilde, çocuklardan izole edilen *E. coli* suşlarında Shiga toksin (stx) ve enterotoksin (estA) genlerinin prevalansı ise %5,7 ve %4,5 idi.

Sonuç: Shiga toksin (stx), ve enterotoksin (estA) genleri bulunduran *E. coli* suşlarının, Sanandaj için potansiyel bir enfeksiyon kaynağı olmadığı sonucuna vardık; belki de gıda kaynaklı hastalıkların en önemli nedeni diğer enterik patojenlerdir.

Anahtar kelimeler: *E. coli*, akut ishal, çocuklar, dondurulmuş gıda örneği, stx ve estA geni

INTRODUCTION

Several strains of *E. coli*, such as Shiga toxin- producing *Escherichia coli* (STEC), and enterotoxigenic *Escherichia coli* (ETEC), are recognized as important human pathogens of animal origin that are responsible for variety of diseases and constitute one of the most important causes of food-borne disease worldwide.[1-5] The global incidence of food-borne disease is difficult to estimate, but it has been reported that in 2000 alone, 2.1 million people died from diarrheal diseases.[6]

Except for a few countries such as Japan, surveillance of food-borne disease in Asia is non-existent or not systematic.[7] There is a necessity to establish or strengthen the national food-borne disease surveillance programs in these countries. In addition, diarrheal diseases are major causes of morbidity, with attack rates ranging from 2 to 12 or more episodes per person per year, especially in developing countries. Moreover, diarrheal illnesses account for an estimated 12,600 deaths each day in children under than 5 years of age in Asia, Africa, and Latin America.[5]

On the other hand, frozen foods of animal origin constitute a vast reservoir of *E. coli*, and it is not surprising that human infection can frequently be traced to contamination of food or water with animal manure. Food contamination with antibiotic resistant bacteria could be a major threat to public health as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, causing compromise in the treatment of severe infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades.[8-11]

Therefore, this study aimed to detect Shiga toxin (stx) and enterotoxin (estA and elt) genes among *E. coli* strains isolated from frozen foods of animal origin and children with acute diarrhea and evaluation on antibiotic resistance

METHODS

Total of 125 frozen foods of animal origin (chicken, fish, mince, slice kebab meat, and beef burger) were purchased from local malls and transported to the laboratory for isolation and identification of *E. coli* strains based on procedure of Institute of Standards and Industrial Research of Iran.[12] Similarly, a total of 466 rectal swabs were examined for the presence of *E. coli* strains in children between the ages of one month to five years, in this study. The specimens were processed at the Beassat Hospital, Sanandaj, which is a reference center for children in Kurdistan province. The *E. coli* were isolated and identified based on biochemical tests.[13] It is worth to note that this study is based on our previous study.[14]

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing for *E. coli* strains to various antimicrobial agents were determined using disk diffusion assay according to the Clinical Laboratory Standards Institute.[15]

Identification of virulence genes

DNA primers used to screen for the presence of stx1, stx2, estA and elt genes in *E. coli* shown in Table 1. STEC was identified by the presence of stx gene according to Lin procedure and ETEC by the presence of estA and elt genes according to Rappelli procedure.[16,17] In brief, the PCR mixture contained: 2 µL of template DNA, 2 µL of 10X PCR buffer, 1.6 µl of a 1.25 mM of dNTP mix , 1.5 µL of $MgCl_2$ (25 mM), 0.2 µL of AmpliTaq DNA polymerase (5 U/ µl); 1 µL of each primer (10 pmol), 15.7 µL of distilled water. Amplification (GeneAmp PCR System 2400; Perkin Elmer) was carried out by heating for 5 min at 94°C, followed by 35 cycles of 94°C for 60 s, 50°C (for eltB), 52°C, (for estA), 43°C (for stx) for 60 s and 72°C for 60 s, followed by one cycle at 72°C for 7 min.

Table1. Polymerase chain reaction (PCR) primers used in this study

Pathogen	Gene	PCR primers	Size of product (bp)
ETEC	e lt	5'-TCTATgTgCATACggAgC-3' 5'-ATACTgATTgCCgCAAT-3'	322
	est	5'-TAAACAAgTAgAggTCTTCAAAA-3' 5'-CggTACAgAgCAggATTACAACA-3'	147
STEC	stx	5'-ACgAAATAATTTATATgT-3' 5'-TgATTgTTACAgTCAT-3'	900

ETEC (enterotoxigenic *Escherichia coli*); STEC (Shiga toxin- producing *Echerichia coli*), Heat-labile toxin (elt); Heat-stable toxin (est); Shiga toxin (stx)

Gel electrophoresis was carried out on 1.5% w/v agarose gel (Gibco Life Technologies, Paisley, United Kingdom) at 120 mV for 30 min. A molecular marker (100 bp DNA ladder; Fermentas) was run concurrently. The DNA bands were visualized and photographed under UV light after the gel was stained with ethidium bromide.

RESULTS

A total of 87 strains of *E. coli* strains were detected from 466 rectal swabs from children with acute diarrhea. Similarly, 40 strains of *E. coli* strains were detected from the 125 frozen food samples of animal origin. The in vitro antibiotic susceptibility pattern of *E. coli* strains isolated from children showed that 78 (89.7%), 77 (88.6%), 69 (79.4%), and 66 (75.9%) of isolates were found to be resistant to tetracycline, chloramphenicol, ampicillin and cefixime respectively. Similarly, the in vitro antibiotic susceptibility pattern of *E. coli* strains isolated from frozen food samples of animal origin showed that 55% and 50% of isolates were found to be resistant to tetracycline and ampicillin respectively (Table 2).

Table 2. Antimicrobial resistance of *E. coli* strains isolated from frozen foods of animal origin and children with acute diarrhea

Antibiotic	Number of frozen food (%)	Number of children (%)
Nalidicxic acid	08 (20.0)	32 (36.8)
Ciprofloxacin	08 (20.0)	26 (29.9)
Ceftriaxone	04 (10.0)	26 (29.9)
Nitrofurantoin	08 (20.0)	18 (20.7)
Ampicillin	20 (50.0)	69 (79.4)
Chloramphenicol	16 (40.0)	77(88.6)
Amoxycillin	20 (50.0)	66 (75.9)
Co-trimoxazole	09 (22.5)	62 (71.3)
Cefixime	04 (10.0)	66 (75.9)
Tetracycline	22 (55.0)	78 (89.7)
Clindamicin	07 (17.5)	53 (61.0)

The results of the detection of Shiga toxin (stx) and enterotoxin (estA and elt) genes among *E. coli* strains isolated from frozen foods of animal origin and children with acute diarrhea using PCR method are shown in Table 3. Results indicated a 5% and 2.5% prevalence Shiga toxin (stx) and enterotoxin (estA) genes among *E. coli* strains isolated from frozen foods of animal origin. Similarly, 5.7% and 4.5% prevalence of Shiga toxin (stx) and enterotoxin (estA) genes among *E. coli* strains isolated from children. Enterotoxin gene elt was not detected in both groups.

Table 3. Prevalence of *stx, estA* and *elt* genes (PCR positives) among 146 *E. coli* strains isolated from frozen foods of animal origin and children with acute diarrhea

Gene	Frozen foods strains No (%)	Diarrhea strains No (%)	Negative control	Positive control
stx	2 (5)	5 (5.7)	*E. coli* HB101	*E. coli* EDL933
estA	1 (2.5)	4 (4.5)	*E. coli* HB101	*E.coli* ATCC 35401
elt	0.0	0.0	*E. coli* HB101	*E.coli* ATCC 35401

DISCUSSION

There are a large number of foods that have been shown to harbor food-borne pathogens with many of these foods requiring little or no further processing or preparation. Centers for Disease Control reported that in 2001 there were 13,705 laboratory diagnosed cases of food-borne illnesses from a surveillance of 10 food-borne diseases. Of these

cases, *E. coli* O157 was identified as the causative agents in 565 cases.[18]

In order to determine the prevalence of Shiga toxin (stx) and enterotoxin (estA and elt) genes among *E. coli* strain, we perform several PCRs with different primers specific for these genes. In this study, 40 and 87 *E. coli* isolates recovered from frozen foods of animal origin and children with acute diarrhea and analyzed for detection of Shiga toxin (stx) and enterotoxin (estA and elt) genes and antimicrobial resistance patterns. The present study demonstrated that stx and estA gens were present in both frozen foods of animal origin and children with acute diarrhea (Table 3). Although a high proportion food samples and children with acute diarrhea were positive for *E. coli* by biochemical tests but few isolates were PCR positive for stx gene (5.0% and 5.7% respectively). The finding of only 2.5% and 4.5% of estA gene in food samples and rectal swabs from children was somewhat surprising, but appears to confirm a recent trend that has been observed in Iran.[19-20]

Human infections due to STEC and ETEC are primarily associated with the consumption of fecally contaminated foodstuffs. In our study STEC and ETEC strains were found in the both groups, which are in agreement with studies in other developing countries, in which STEC and ETEC plays important role in childhood diarrhea.[21-23] The low incidence of STEC and ETEC in our study could be attributable to the lack of consumption of raw meat or vegetables in our area; Kurdistan province, west of Iran.

Human infections with STEC strains are usually transmitted from cattle or dairy products particularly undercooked meat or unpasteurized milk and often are linked with rural setting.[23] Resistance to two or more classes of antimicrobials was found in STEC strains. Due to using antimicrobial agents for diseases prevention and growth support of animals for more than four decades, farm animals are often exposed to antimicrobial substances, so resistance phenotype can give a selective advantage to bacteria. As a result, humans became more possible to be exposed to these organisms via food and direct and indirect transmission from animals.[24]

Antimicrobial resistance in ETEC as a cause of diarrhea in children was more common than other STEC. ETEC isolated from children with acute diarrhea were considerably more resistance to Ciprofloxacin and Nalidixic–acid than STEC isolated from frozen foods of animal origin. This group of *E. coli* also showed higher resistance to Ampicillin, Tetracycline, and Co-trimoxazole.

A limitation of this study was that we had no available data on the use of antibiotics in our hospital to correlate antibiotic consumption with resistance rate. Another limitation was that we investigated only stx1, stx2, estA and elt genes because we had limited funding.

We conclude that *E. coli* stains having Shiga toxin (stx), and enterotoxin (estA) genes considered not to be a potential source of infections for human beings in Kurdistan province of Iran; perhaps other enteric pathogens are the major cause of foodborne diseases.

Finally, this data shows that perhaps antimicrobial treatment, usage for diseases prevention and usage of antibiotic for growth encouragement in farming are important in the choice of antimicrobial-resistant phenotypes.

As it is mentioned in our previous study[24] the limitation of this study was the possible presence of entropathoges such as some diarrheagenic viruses in the area that we did not test. Moreover, further studies are needed to investigate the ecological, socio-economical, and epidemiological basis of *E. coli* infections as an emerging pathotype in children in Sanandaj.

Competing interest: The authors declare that they have no competing interests.

Acknowledgements

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