KOWSAR
The Brilliant Future of Publishing
www.KowsarPub.com

Enteric Pathogens
www.enterpathog.com

Alborz University of Medical Sciences

# Antibiotic Resistance Pattern of *Escherichia coli* Groups A, B1, B2 and D Isolated from Frozen Foods and Children with Diarrhea in Sanandaj, Iran

Hiva Karimi Darehabi [1], Mohammad Hassan Naseri [2,3], Shahoo Menbari [4], Jafar Mobaleghi [5], Enayatollah Kalantar [6,*]

[1] Department of Food Hygiene, Faculty of Veterinary Science, Islamic Azad University, Sanandaj Branch, Sanandaj, IR Iran
[2] Department of Heart Surgery, Baqiyatollah University of Medical Sciences, Tehran, IR Iran
[3] Department of Heart Surgery, Kamali Hospital, Alborz University of Medical Sciences, Karaj, IR Iran
[4] Department of Microbiology, School of Paramedical Sciences, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[5] Department of Surgery, Beasat hospital, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[6] Department of Bacteriology and Virology, School of Medicine, Alborz University of Medical Sciences, Karaj, IR Iran

*Corresponding author*: Enayatollah Kalantar, Department of Bacteriology and Virology, School of Medicine, Alborz University of Medical Sciences, Karaj, IR Iran. Tel: +98-8716131409, Fax: +98-8716664654, E-mail: ekalantar@hotmail.com

## ABSTRACT

***Background:*** *Escherichia coli* is usually a commensal of the digestive system, particularly intestine of humans and animals. However, there are many reports indicating that some strains are known to cause serious problems causing various infections among children worldwide, particularly in the developing countries. *Escherichia coli* strains can be classified into four phylogenetic groups: A, B1, B2 and D. The commensal strains are usually placed into the phylogenetic groups; A and B1 and the extraintestinal pathogenic strains into a group B2 and, to a lesser extent, group D.
***Objectives:*** The present study aimed to investigate the antibiotic resistance pattern of *Escherichia coli* groups A, B1, B2 and D from frozen foods and children with diarrhea.
***Materials and methods:*** From 125 frozen foods of animal origin and 466 rectal swabs from children with diarrhea, *E. coli* were isolated and identified based on standard procedures. Susceptibility testing to antibiotic was carried out according to the CLSI criteria. The phylogenetic group of each strain was determined by using multiplex PCR.
***Results:*** In the current study, 47 and 99 *E. coli* strains were isolated and allocated into four phylogenetic groups (i.e. A, B1, B2 and D). Among the strains isolated from the frozen foods of animal origin, 6.3% were allocated into phylogenetic group B2, and 23.4%% into D. Similarly, among the strains isolated from children with diarrhea 9% were allocated into group B2 and 19% to group D. The data showed that 55.3% and 53.1% of the frozen food-derived *E. coli* strains were resistant to Tetracycline and Amoxycillin respectively. Similarly, 89.9, 88.9 and 79.8% of isolates were resistant to tetracycline, chloramphenicol, and ampicillin, respectively. By a multiplex PCR procedure different phylogenetic *E. coli* were detected.
***Conclusion:*** Detection of *E. coli* isolates is very important and shows that food of animal origin can be of a reservoir for resistant bacteria that potentially could be transferred to humans through the food chain. In addition, results of the current study also revealed the detection of resistant *E. coli* isolates from children with diarrhea.

*Keywords:* Escherichia coli; Drug Resistance; Frozen Food; Diarrhea



Article type: Research Article; Received: 07 Nov 2012; Revised: 15 Jan 2013; Accepted: 15 Jan 2013; Epub: 19 May 2013; Ppub: 05 Aug 2013

Implication for health policy/practice/research/medical education:
The current study presents the initial results of surveillance, on *E. coli* detection rates and their genotype distribution from foods of animal origin and children with acute diarrhea.

Please cite this paper as:
Karimi Darehabi H, Menbari S, Mobaleghi J, Kalantar E. Antibiotic Resistance Pattern of *Escherichia coli* Groups A, B1, B2 and D Isolated from Children with Diarrhea and Frozen Foods in Sanandaj, Iran.Int J Enterpathog. 2013; 01(01): 1-4. DOI: 10.17795/ijep9037

## 1. Background

*Escherichia coli* are usually a commensal of the digestive system, particularly intestine of humans and animals. However, there are many reports indicating that some strains are known to cause serious problems causing various infections among children worldwide, particularly in the developing countries(1-3). *Escherichia coli* strains can be classified into four phylogenetic groups: A, B1, B2 and D (4). The commensal strains are usually placed into the phylogenetic groups; A and B1 and the extraintestinal pathogenic strains into group B2 and, to a lesser extent, group D (5).

The pathogenic strains have been associated with several diseases including diarrhea, urinary tract infections and meningitis. *E. coli* can easily contaminate food products during animal evisceration at slaughter or during food manipulation (6). Drug resistant *E. coli* can easily spread through water, soil and food and can be transferred from animals to people (7).

Increasing antimicrobial drug resistant flora in animals is a potent public health problem leading to ban the use of several antibiotics in animal food (8, 9). Commensal bacteria such as *E. coli* can serve as reservoirs of resistance genes for potentially pathogenic bacteria (10).

## 2. Objectives

Surveillance of food-borne diseases is of an increasingly high priority in the public health agenda worldwide (11). So, it is important to check the antimicrobial resistance among the bacteria such as *E. coli*, as recommended by the World Health Organization (12), from animals as well as from the biological samples in different geographic areas/countries. In relation to the above-mentioned, the current study aimed to isolate, identify and check the antimicrobial resistance pattern of *Escherichia coli* groups A, B1, B2 and D isolated from frozen foods of animal origin sold in the domestic market and children with diarrhea in Sanandaj, Iran.

## 3. Materials and Methods

### 3.1. Retail Frozen Foods of Animal Origin Marketed in Sanandaj Sampling and E. coli Strains:

*E. coli* isolates were isolated from the samples collected and identified based on Institute of Standards and Industrial Research of Iran guidelines (12). One hundred and twenty five frozen foods of animal origin including 25 samples of chicken, fish, mince, and chicken prepared for barbecue were purchased from different supermarkets located in Sanandaj.

### 3.2. E. coli Strains Isolated from Children with Acute Diarrhea:

Rectal swabs were investigated from a total of 466 children with diarrhea who were one month to five years old and 99 were diagnosed as acute diarrhea caused by *E. coli*. The isolation and identification was carried out based on biochemical tests (13). Susceptibility to various antimicrobial agents was checked in *E. coli* isolates by the disk diffusion method according to the CLSI criteria (14).

### 3.3. Phylogenetic Study of Isolated E. coli

The phylogenetic group of all the isolates was determined by multiplex PCR as described by Clermont et al. (14). The isolated DNA was used in PCR amplifications that were carried out in a PT-100 thermal cycler to identify the chuA (279 bp) and the yjaA (211 bp) genes and the DNA fragment TSPE4.C2 (152 bp).The amplified products were separated by electrophoresis in a 2% agarose gel containing ethidium bromide. After electrophoresis, the gel was photographed and the strains were assigned to phylogenetic group B2 (chuA, yjaA), D (chuA, yjaA 2), B1 (chuA2 , TSPE4.C2), and A (chuA 2 , TSPE4.C2 2).

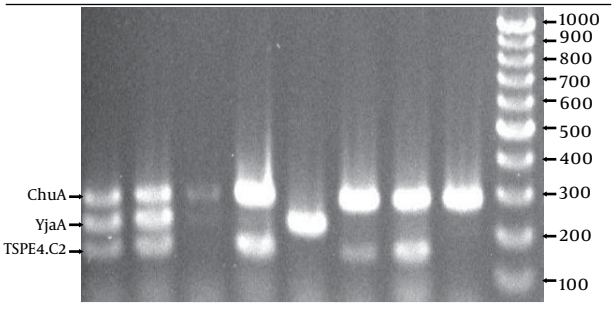
## 4. Results

In the current study, 47 *E. coli* strains were isolated from frozen foods of animal origin, and 99 strains were isolated from children with acute diarrhea, the isolated strains were allocated into four phylogenetic groups (i.e. A, B1, B2 and D) according to the methodology described by Clermont et al.(14).

Among the strains isolated from the frozen foods of animal origin, 30 (63.8%) were allocated into phylogenetic group A, 2 (4.2%) B1, and 3 (6.3%) D (*Table 1*) and (*Figure 1*). Strains isolated from the children with acute diarrhea were allocated into group A (59 strains, 59.5%), group B1 (12 strains, 12.1%), group D (19 strains, 19.1%) and B2 (nine strain, 9%) (*Table 1*).

The data showed that 55.3, 53.1, and 48.9 % of the frozen food-derived *E. coli* strains were resistant to Tetracycline, Amoxycillin and ampicillin respectively (*Table 2*).

**Figure 1.**




Positive multiplex PCR results for the detection of *E. coli* phylogenetic groups among the frozen food samples and the analyzed rectal swabs of children. Lane 1 denotes the marker (BioRad), and Lane 2 onwards indicates positive results from 2 consecutive trials

**Table 1.** Phylogenetic Groups Detected Among the *E. coli* Strains Recovered From Frozen Foods of Animal Origin and Children with Acute Diarrhea

| Sample source | Phylogenetic group, (%) | | | |
|---|---|---|---|---|
| | A | B1 | B2 | D |
| Children with acute diarrhea | 59 (59.5) | 12 (12.1) | 09 (09) | 19 (19.1) |
| frozen foods of animal origin | 30 (63.8) | 04.2(02) | 03 (06.3) | 11 (23.4) |

**Table 2.** Antimicrobial Resistance of *E. coli* Strains Isolated From Frozen Foods of Animal Origin and Children with Acute Diarrhea.

| Antibiotic | Frozen food, (%) | Children, (%) |
|---|---|---|
| Nalidicxic acid | 36.4 | 10.6 |
| Ciprofloxacin | 30.3 | 10.6 |
| Ceftriaxone | 30.3 | 08.5 |
| Nitrofurantoin | 20.2 | 00.0 |
| Ampicillin | 79.8 | 48.9 |
| Chloramphenicol | 88.9 | 23.4 |
| Amoxycillin | 75.8 | 53.1 |
| Co-trimoxazole | 70.7 | 08.5 |
| Cefixime | 75.8 | 06.3 |
| Tetracycline | 89.9 | 55.3 |
| Gentamicin | ND | 00.0 |
| Cephalotin | 60.0 | ND |

In vitro antibiotic susceptibility pattern of *E. coli* isolates from children with acute diarrhea is shown in *Table 2*. 89.9, 88.9 and 79.8% of isolates were resistant to tetracycline, chloramphenicol, and ampicillin, respectively. Of the 47 *E. coli* strains isolated from the frozen foods of animal origin, 55.3 and 53.1% of isolates were resistant to Tetracycline and Ampicillin respectively.

## 5. Discussion

Food is a main vehicle to transmit enteric pathogens, most notably *E. coli*. Most of the recent data imply the potential role of foods, particularly retail meats, as vehicles to transmit fecal pathogens capable of causing extraintestinal infections. During 2001, Centers for Disease Control reported 12705 cases of food–borne diseases. Of these cases, Salmonella, Campylobacter, and *E. coli* were identified as the causative agents in 5,198, 4,740 and 565 cases, respectively (15). By the Clermont et al. (14) multiplex PCR procedure, *E. coli* were detected concurrently. In the current study, 47 and 99 *E. coli* strains were isolated from five different frozen foods of animal origin and from children with acute diarrhea respectively. These isolates were analyzed for their phylogenetic background and antimicrobial resistance pattern. The *E. coli* strains isolated from the frozen foods of animal origin were assigned to group A (30 strains), B1 (2 strains), B2 (3 strains) and D (11 strains). Similarly, 99 *E. coli* isolated from children with acute diarrhea were assigned to group A (59 strains), B1 (12 strains), B2 (9 strains) and D (19 strains). A similar result was reported in *E. coli* isolated from poultry and meat products in Minnesota and Wisconsin(16, 17). It is interesting to note that the highest percentage of strains belonging to group A was found in the diarrhea cases which has no virulence determinants. On the other hand, 19.1% of strains belonging to group D were found in the diarrhea cases which have fewer virulence determinants than strains from group B2. Previous research by Xia (2011) (18) revealed a different result from that of the current research, identifying a higher prevalence of strains of phylo-group B2 and D among retail meat, which can be usual because of geographic effects in the *E. coli* population among hosts. In the current study, the rate of antimicrobial resistance among *E. coli* strains isolated from children with acute diarrhea was observed for tetracycline, followed by ampicillin and this can be linked to well-known antibiotic-resistant gene. Furthermore, many scientists also reported that these genes can be transferred into the gut flora of humans and animals, where they can play a major role in development, emergence and spread of resistance in both pathogenic and non-pathogenic bacteria. It is worth to note that, they can be transferred into the environment via wastewater, manure and sewage sludge (19).

The current research results indicate that *E. coli* strains isolated from frozen foods were resistant to tetracycline (55.3%), ampicillin (48.9%), and chloramphenicol (23.4%). The multiple antibiotic resistances of *E. coli* demonstrated in the current study is in agreement with other studies from various parts of the world (6, 20, 21).

In summary, the results of the present study revealed that frozen food of animal origin, were contaminated with different phylogenetic *E. coli* that resembled clinical *E. coli* strains. Isolation of *E. coli* strains from the frozen-food samples analyzed in the current study was very important, indicating that food of animal origin can be as a gut flora of resistant bacteria like *E. coli*, which may be transferred to humans through the food chain. In addition, like other studies, results of the current study also indicated the relevance to the detection of resistant *E. coli* isolates from children with diarrhea. More studies should be performed to analyze the flow and evolution of resistant *E. coli* isolates in different ecosystems in order to assess its implication in human health.

## Acknowledgements

This work was supported by a grant from Kurdistan University of Medical Sciences, Sanandaj, Iran.

## Authors Contribution

All the authors participated in the manuscript preparation and experiment procedures equally.

## Financial Disclosure

There is no conflict of interest.

## Funding Support

Financial support for the present study is provided by Department of Food Hygiene, Faculty of Veterinary Science, Azad University, Sanandaj Branch, Sanandaj, Iran.

## References

1. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. *J Infect Dis.* 2000;**181**(5):1753-4.
2. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis.* 2000;**181**(1):261-72.
3. Schmidt H, Henkel B, Karch H. A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. *FEMS Microbiol Lett.* 1997;**148**(2):265-72.
4. Herzer PJ, Inouye S, Inouye M, Whittam TS. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of Escherichia coli. *J Bacteriol.* 1990;**172**(11):6175-81.
5. Johnson JR, O'Bryan TT, Kuskowski M, Maslow JN. Ongoing horizontal and vertical transmission of virulence genes and papA alleles among *Escherichia coli* blood isolates from patients with diverse-source bacteremia. *Infect Immun.* 2001;**69**(9):5363-74.
6. Ben Slama K, Jouini A, Ben Sallem R, Somalo S, Saenz Y, Estepa V, et al. Prevalence of broad-spectrum cephalosporin-resistant *Escherichia coli* isolates in food samples in Tunisia, and characterization of integrons and antimicrobial resistance mechanisms implicated. *Int J Food Microbiol.* 2010;**137**(2-3):281-6.
7. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother.* 2004;**54**(2):321-32.
8. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother.* 2003;**52**(2):159-61.
9. Turnidge J. Antibiotic use in animals–prejudices, perceptions and realities. *J Antimicrob Chemother.* 2004;**53**(1):26-7.
10. van den Bogaard Anthony E, Stobberingh Ellen E. Epidemiology of resistance to antibiotics: Links between animals and humans. *International journal of antimicrobial agents.* 2000;**14**(4):327-335.
11. Moldovan AM, Idomir M, Taus N. Determination of the level of microbial food contamination in Brasov. *Bull. Transilvania. Uni. Brasov.* 2009;**2**:65-68.
12. World Health Organization , editor.Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. 2000; Geneva, Switzerland. .
13. Bailey WR, Finegold SM, Martin WJ, Scott EG. Bailey and Scott's Diagnostic microbiology: a textbook for the isolation and identification of pathogenic microorganisms. C. V. Mosby; 1978.
14. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol.* 2000;**66**(10):4555-8.
15. Preliminary FoodNet data on the incidence of foodborne illnesses–selected sites, United States, 2001. *MMWR Morb Mortal Wkly Rep.* 2002;**51**(15):325-9.
16. Johnson JR, McCabe JS, White DG, Johnston B, Kuskowski MA, McDermott P. Molecular Analysis of *Escherichia coli* from retail meats (2002-2004) from the United States National Antimicrobial Resistance Monitoring System. *Clin Infect Dis.* 2009;**49**(2):195-201.
17. Johnson TJ, Logue CM, Wannemuehler Y, Kariyawasam S, Doetkott C, DebRoy C, et al. Examination of the source and extended virulence genotypes of *Escherichia coli* contaminating retail poultry meat. *Foodborne Pathog Dis.* 2009;**6**(6):657-67.
18. Xia X, Meng J, Zhao S, Bodeis-Jones S, Gaines SA, Ayers SL, et al. Identification and antimicrobial resistance of extraintestinal pathogenic *Escherichia coli* from retail meats. *J Food Prot.* 2011;**74**(1):38-44.
19. Fitzpatrick C, Musser A, Mosqueda L, Boker J, Prislin M. Student Senior Partnership Program: University of California Irvine School of Medicine. *Gerontol Geriatr Educ.* 2006;**27**(2):25-35.
20. Blanc V, Mesa R, Saco M, Lavilla S, Prats G, Miro E, et al. ESBL- and plasmidic class C beta-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol.* 2006;**118**(3-4):299-304.
21. Liu JH, Wei SY, Ma JY, Zeng ZL, Lu DH, Yang GX, et al. Detection and characterisation of CTX-M and CMY-2 beta-lactamases among *Escherichia coli* isolates from farm animals in Guangdong Province of China. *Int J Antimicrob Agents.* 2007;**29**(5):576-81.