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

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**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

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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 TSPE4C2 (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).

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 strains, 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, Amoxyclillin and ampicillin respectively (Table 2).

![Figure 1](image)

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.
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The highest percentage of strains belonging to group A was found in Minnesota and Wisconsin (16, 17). It is interesting to note that 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 phylogenic 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.

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