



# Evaluation of Antibiotic Resistance in *Escherichia coli* Strains Isolated from Pastry Cream in Hamadan, Iran

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## Abstract

**Background:** *Escherichia coli* is one of the most important intestinal pathogens, which is transmitted to humans through food and has significant effects on the quality of food products, including pastry cream. Its presence in food is considered an indicator of fecal contamination.

**Objectives:** This study aimed to determine the prevalence and pattern of antibiotic resistance of *Escherichia coli* in pastry cream prepared in Hamadan, Iran.

**Methods:** The pastry cream samples (n, 350) were randomly purchased from confectionery suppliers, located in different districts of Hamadan. Forty-seven out of 350 samples contained *E. coli*. The bacteria were identified after culturing the samples in a nutrient-broth-enriched medium and solid media, such as violet red bile agar (VRBA), eosin methylene blue (EMB), and nutrient agar (NA), to identify bacteria isolated by the linear method. The samples were then incubated at 37°C for 24 to 48 hours.

**Results:** The results of antibiotic tests showed the highest resistance to tetracycline, vancomycin, oxacillin (100%), and penicillin (72.34%) and the highest sensitivity to chloramphenicol (78.23%), ciprofloxacin (76.59%), and nalidixic acid (61.70%).

**Conclusions:** According to the present findings and similar research from Iran, the rate of *E. coli* resistance to antibiotics is high. To avoid the contamination of pastry cream by these bacteria, some techniques, including cold chain management, employee personal hygiene, use of healthy raw materials, and proper thermal processes, should be adopted.

**Keywords:** *Escherichia coli*, Pastry Cream, Antibiotic Resistance

## 1. Background

Food contamination may emerge at different stages of preparation, transportation, and supply through unnecessary hand exposures, raw materials (such as milk), or contaminated equipment. Developing countries, given their cultural and economic constraints, face a wide range of food-borne diseases. Also, in developed countries, despite technological advances and use of advanced methods, 5% - 10% of people are affected by food-borne diseases, causing health and economic problems in communities (1).

According to the World Health Organization (WHO), food-borne diseases are described as infectious diseases, induced by the consumption of water or food. Food poisoning and spread of infectious foodborne diseases have always been one of the important medical challenges around the world, and many people experience food poisoning annually (2). Generally, milk and pastry products are major causes of food poisoning (3). With regard to

their constituents and preparation and preservation conditions, pastries have a great potential to be contaminated by pathogenic and corrosive microorganisms.

*Escherichia coli*, as a normal flora of the intestine, colonizes the gastrointestinal tract of human and mammalian infants within a few hours after birth and plays a vital role in the physiological performance of digestive system. It should be noted that some *E. coli* strains become pathogenic by exploiting virulence factors through transferable genetic factors, such as plasmids, bacteriophages, and pathogenicity locusts. Generally, different *E. coli* serotypes are a major cause of diarrhea in developing countries or countries with poor health conditions (4).

The complications of *E. coli* infection include bloody and watery diarrhea and hemorrhagic colitis. Hemolytic uremic syndrome is also reported following the progression of pathogenicity and infection, especially by *E. coli* O157:H7 (5). Generally, *E. coli* is one of the important

pathogens, which has shown increased resistance to most antibiotics (6). Some bacteria causing diarrhea in children include *Campylobacter*, *Salmonella*, *Shigella*, and *E. coli* (7). *E. coli* is also known as one of the causes of gastroenteritis in infants and children below five years, accounting for more than 60% of diarrhea cases in high-risk regions (8).

With regard to the frequent consumption of pastry cream in our community, microbial control is necessary from both health and industrial perspectives in order to preserve and increase the shelf-life and quality of food products. The occurrence of cream contamination in pastries is often similar to or even higher than that of pastry itself. Microbial contamination is one of the most common causes of transmission of digestive diseases to humans. The associated diseases also depend on cream preparation and preservation methods, community's dietary habits, observation of hygiene standards by suppliers, and primary and secondary contamination of pastry cream (9).

Considering the high incidence of microbial contamination in pastry cream samples, consumers' health may be at risk, and contamination of products can result in significant economic losses. With regard to the importance of this issue and control of microbial contamination in food products, development of contamination control strategies, observation of basic hygiene principles, proper training of workers, and more accurate monitoring by organizations responsible for production and supply seem essential.

## 2. Methods

### 2.1. Samples

In this cross-sectional study conducted in 2015 - 2016, a total of 350 pastry cream samples were randomly selected from confectionery suppliers in different districts of Hamadan and transferred in ice packs (4°C) to the food hygiene laboratory under sterile conditions.

### 2.2. Isolation and Identification of *E. coli*

The samples were homogenized, and 10 g of each sample was diluted in 90 mL of 0.1% sterile peptone water. *E. coli* was identified after culturing the samples in a nutrient-broth-enriched medium and solid media, such as nutrient agar (NA; Merck), violet red bile agar (VRBA; Merck), and eosin methylene blue agar (EMB; Scharlau, Spain), to identify bacteria isolated by the linear method; the samples were then incubated at 37°C for 24 to 48 hours. Finally, the antibiotic resistance pattern of isolated *E. coli* was determined, using standard disk diffusion method for 10 different antibiotics.

The Mueller-Hinton agar (MHA) plate was swabbed with nutrient broth (NB), inoculated with *E. coli*, and incubated to a turbidity of 0.5 McFarland standard (10). The antibiotic-sensitivity discs were placed on MHA agar (Merck), and the agar plates were incubated for 18 ± 2 hours at 36°C. The employed antibiotic disks included gentamicin, streptomycin, nalidixic acid, cefixime, chloramphenicol, ciprofloxacin, tetracycline, oxacillin, vancomycin, and penicillin. According to the findings, three sensitive, semi-sensitive, and resistant groups were classified with respect to the size of inhibited growth around the disk.

## 3. Results

Based on the findings, 47 out of 350 samples contained *E. coli*. The results of antibiotic tests showed the highest rate of resistance to tetracycline, vancomycin, oxacillin (100%), and penicillin (72.34%) and the highest rate of sensitivity to chloramphenicol (87.23%), ciprofloxacin (76.59%), and nalidixic acid (61.70%). Reactions to other antibiotics are presented in Table 1 and Figure 1.

**Table 1.** Antibiotic Resistance of *Escherichia coli* Isolated from Pastry Cream<sup>a</sup>

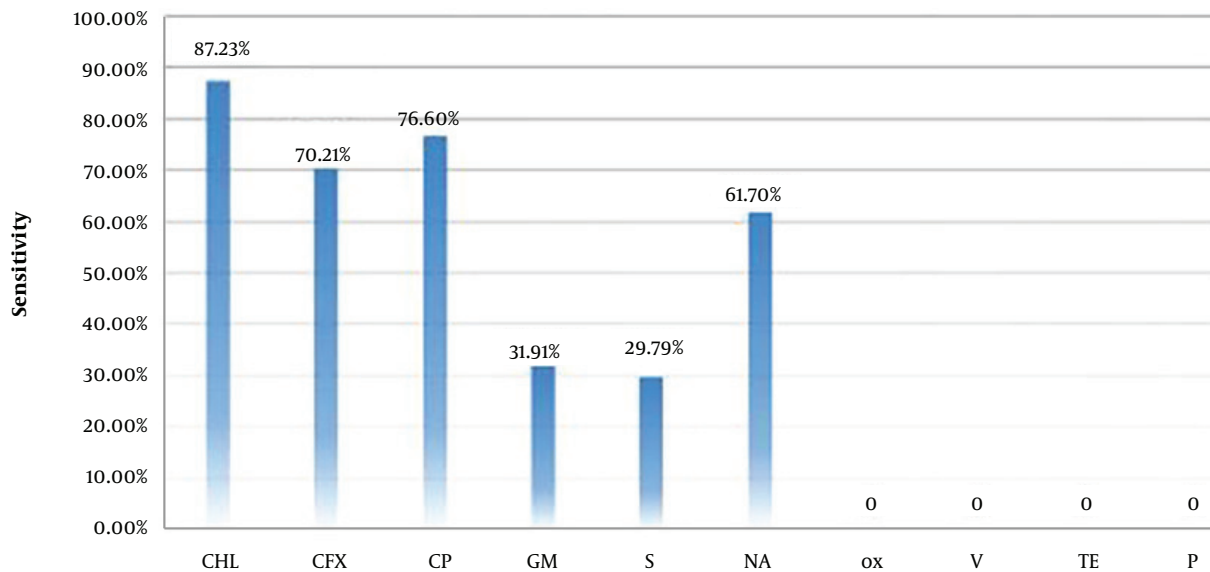
Antibiotics	Sensitive	Semi-Sensitive	Resistant
Chloramphenicol	41 (87.23)	5 (10.64)	1 (2.13)
Cefixime	33 (70.21)	0 (0)	14 (29.79)
Ciprofloxacin	36 (76.60)	4 (8.51)	7 (14.89)
Gentamicin	15 (31.91)	23 (48.94)	9 (19.15)
Streptomycin	14 (29.80)	28 (59.57)	5 (10.63)
Nalidixic acid	29 (61.70)	12 (25.53)	6 (12.77)
Oxacillin	0 (0)	0 (0)	47 (100)
Vancomycin	0 (0)	0 (0)	47 (100)
Tetracycline	0 (0)	0 (0)	47 (100)
Penicillin	0 (0)	13 (27.66)	34 (72.34)

<sup>a</sup>Values are expressed as number of isolates tested (%).

## 4. Discussion

Due to population growth, there is an urgent need for food control around the world. The prevalence of diseases, caused by the consumption of contaminated food, has always been one of the major problems in different communities, as elimination of these diseases, especially in countries with a poor health status, is costly (11).

Confectionery products (especially pastry cream) as nutritious foods are susceptible to the growth and multiplication of different microorganisms and transmission of



**Figure 1.** Antibiotic sensitivity of *Escherichia coli* isolated from pastry cream. Gentamicin (GM), streptomycin (S), nalidixic acid (NA), cefixime (CFX), chloramphenicol (CHL), ciprofloxacin (CP), tetracycline (TE), oxacillin (OX), vancomycin (V) and penicillin (P).

microbial agents, causing food poisoning in consumers. The present findings and similar studies conducted in different parts of Iran and the world confirm the high risk of microbial contamination in such products, besides contamination transmission to consumers (12-14).

Overall, sources of contamination in pastry, especially pastry cream and sweet rolls, are as follows: Primary contamination of milk; use of local and non-pasteurized cream; non-compliance with the freezing cycle during storage; contamination of equipment, such as knives and cutting tools; improper manipulation by individuals working at confectionery workshops; poor personal hygiene of workers; and bacteria transmission from the staff hands and noses during processing and transportation phases (15, 16). Regarding the type of constituents and preparation and design methods, pastries are more likely to be contaminated by pathogenic agents, such as Enterobacteriaceae (17).

The results of the present study showed that 47 out of 350 pastry cream samples contained *E. coli*, which is consistent with the results of previous studies conducted in other cities of Iran on the contamination of pastry cream with *E. coli*. In this regard, Nikniaz et al. in a study from Tabriz, Iran, reported the rate of pastry cream contamination with coliform bacteria and *E. coli* to be 38.8% and 48.8%, respectively (3). In another study by Soltan Dallal et al. in Tehran, Iran, it was shown that the rate of *E. coli* contamination was 4.2% in pastry cream (18).

*E. coli* is one of the pathogens resistant to the majority of antibiotics, causing health problems in different countries (19). Few studies in Iran have investigated the antibiotic resistance of *E. coli* in food products. The research carried out by Saenz et al. indicated the highest antibiotic resistance of *E. coli* to nalidixic acid (53%) (20). This finding is not consistent with the findings of our study, as the rate of resistance was estimated at 12.27% in our study, which can be explained by geographical differences of these studies.

Moreover, in a study by Molaabaszadeh et al. on *E. coli* isolates from traditional ice cream in Khoy, Iran, the highest and lowest resistance rates were 82.98% and 12.76% to oxacillin and ciprofloxacin, respectively. Similarly, in the present study, the highest resistance rate was 100% to oxacillin, and the lowest resistance was 89% to ciprofloxacin. In addition, Molaabaszadeh et al. reported antibiotic sensitivities of 40.43% and 61.7% to tetracycline and gentamicin, respectively, while resistance to these antibiotics was 55.31% and 25.54%, respectively (21).

On the contrary, in the present study, the rates of sensitivity to tetracycline and gentamicin were 0% and 31.91%, respectively, while the rates of resistance to tetracycline and gentamicin were 100% and 19.15%, respectively. In another study by Mahdavi et al. on *E. coli* isolated from traditional cheese in Maragheh, Iran, the rates of sensitivity to chloramphenicol and streptomycin were estimated at 84.35% and 31.2%, respectively (22). These results are in agreement with the findings of the current study, as the correspond-

ing rates were 23% and 29.8% in our study, respectively.

Additionally, in the study by Molaabasazadeh et al. on *E. coli* isolates from urinary tract infections in Tabriz, Iran, the rates of sensitivity to gentamicin and nalidixic acid were estimated at 37.98% and 52.1%, respectively (21). These results are similar to the findings of the present research. Moreover, in a study by Bonyadian et al. on *E. coli* isolates from raw milk and pasteurized cheese in Shahrekord, Iran, the resistance rates of *E. coli* to streptomycin and ciprofloxacin were reported to be 25.4% and 25.4%, respectively (23). The corresponding rates in the present study were reported to be 10.63% and 14.89%, respectively. Considering multiple reports on the transmission of resistant *E. coli* from dairy products to humans (24) and similarity of antibiotic resistance of *E. coli* in animal products and human infections, antibiotics should not be overprescribed (25).

#### 4.1. Conclusions

According to the present findings and similar research in Iran, *E. coli* is extremely resistant to antibiotics. The major sources of this type of food contamination include microbial contamination of raw materials, such as cream, poor personal hygiene of workers, and microbial contamination of utensils used for pastry production. Therefore, all these factors can influence the final product and cause contamination. To avoid the contamination of pastry cream with bacteria, some techniques, including cold chain management, employee personal hygiene, use of healthy raw materials, and proper thermal processes, should be adopted.

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#### Footnotes

**Authors' Contribution:** Ali Rezaei and Nesa Rezaei, analysis and interpretation of data: Ali Rezaei, Nesa Rezaei, and Saeid Shahmoradi, drafting of the manuscript: Abbas Bashtin.

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#### References

- Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *Int J Food Microbiol.* 2010;**139** Suppl 1:S3-15. doi: [10.1016/j.ijfoodmicro.2010.01.021](https://doi.org/10.1016/j.ijfoodmicro.2010.01.021). [PubMed: 20153070].
- Peles F, Wagner M, Varga L, Hein I, Rieck P, Gutser K, et al. Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *Int J Food Microbiol.* 2007;**118**(2):186-93. doi: [10.1016/j.ijfoodmicro.2007.07.010](https://doi.org/10.1016/j.ijfoodmicro.2007.07.010). [PubMed: 17727995].
- Nikniaz Z, Mahdavi R, Jalilzadeh H, Vahed Jabbari M. [Evaluation of microbial contamination in cream filled pastries distributed in Tabriz confectionaries]. *J Food Technol Nutr.* 2011;**8**(1):66-71. Persian.
- Osek J. Development of a multiplex PCR approach for the identification of Shiga toxin-producing *Escherichia coli* strains and their major virulence factor genes. *J Appl Microbiol.* 2003;**95**(6):1217-25. doi: [10.1046/j.1365-2672.2003.02091.x](https://doi.org/10.1046/j.1365-2672.2003.02091.x). [PubMed: 14632994].
- Tarr PI. *Escherichia coli* O157:H7: Clinical, diagnostic, and epidemiological aspects of human infection. *Clin Infect Dis.* 1995;**20**(1):1-8. quiz 9-10. doi: [10.1093/clinids/20.1.1](https://doi.org/10.1093/clinids/20.1.1). [PubMed: 7727633].
- Akinjogunla OJ, Eghafona NO, Ekoi OH. Diarrheagenic *Escherichia coli* (DEC): Prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agents. *Afr J Bacteriol Res.* 2009;**1**(3):34-8.
- Marcus R. New information about pediatric foodborne infections: The view from FoodNet. *Curr Opin Pediatr.* 2008;**20**(1):79-84. doi: [10.1097/MOP.0b013e3282f43067](https://doi.org/10.1097/MOP.0b013e3282f43067). [PubMed: 18197044].
- Kandakai-Olukemi YT, Mawak JD, Onojo MM. Isolation of enteropathogenic *Escherichia coli* from children with diarrhoea attending the national hospital in Abuja, Nigeria. *Shiraz E-Med J.* 2009;**10**(3):99-19.
- Iranian Standard and Industrial Researches Institute. [(IS: 2395), *Microbiological of pastry and confectionary products - specifications and test method*]. Iran: Iranian Standard and Industrial Researches Institute; 2014. Persian.
- Sayah RS, Kaneene JB, Johnson Y, Miller R. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Appl Environ Microbiol.* 2005;**71**(3):1394-404. doi: [10.1128/AEM.71.3.1394-1404.2005](https://doi.org/10.1128/AEM.71.3.1394-1404.2005). [PubMed: 15746342]. [PubMed Central: PMC1065171].
- World Health Organization. *Tuberculosis and air travel: guidelines for prevention and control*. World Health Organization; 2008.
- Smith JP, Daifas DP, El-Khoury W, Koukoutsis J, El-Khoury A. Shelf life and safety concerns of bakery products—a review. *Crit Rev Food Sci Nutr.* 2004;**44**(1):19-55. doi: [10.1080/10408690490263774](https://doi.org/10.1080/10408690490263774). [PubMed: 15077880].
- Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. *Genet Mol Res.* 2003;**2**(1):63-76. [PubMed: 12917803].
- Asadi S, Rezaei Maram Z, Kooshk F. Evaluation of microbial contamination of pastry cream in Arak city of Iran. *J Food Saf Hyg.* 2015;**1**(1):26-9.
- Jay J, Jay M. *Modern food microbiology*. 7th ed. 2005.
- Snyder OP. Hand washing for retail food operations: A review. *Dairy, food and environmental sanitation: A publication of the international association of milk, food and environmental sanitarians (USA)*. 1998.
- Ertas N, Gonulalan Z, Yildirim Y, Kum E. Detection of *Staphylococcus aureus* enterotoxins in sheep cheese and dairy desserts by multiplex PCR technique. *Int J Food Microbiol.* 2010;**142**(1-2):74-7. doi: [10.1016/j.ijfoodmicro.2010.06.002](https://doi.org/10.1016/j.ijfoodmicro.2010.06.002). [PubMed: 20573416].
- Soltan Dallal MM, Fazelifard PM, Tabatabaei Bafroei A, Rashidi S, Zarrin M. [Determination the rate of microbial contamination of cream pastry from confectionaries in south of Tehran]. *J Microb Biotechnol.* 2010;**2**(6):7-11. Persian.
- Nováková I, Kačániová M, Haščík P, Pavličová S, Hleba L. The resistance to antibiotics in strains of *E. coli* and *Enterococcus* sp. isolated from rectal swabs of lambs and calves. *Sci Pap Anim Sci Biotechnol.* 2009;**42**(2):322-6.

20. Saenz Y, Zarazaga M, Brinas L, Lantero M, Ruiz-Larrea F, Torres C. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *Int J Antimicrob Agents*. 2001;**18**(4):353-8. doi: [10.1016/S0924-8579\(01\)00422-8](https://doi.org/10.1016/S0924-8579(01)00422-8). [PubMed: [11691568](https://pubmed.ncbi.nlm.nih.gov/11691568/)].
21. Molaabaszadeh H, Hajisheikhzadeh B, Mollazadeh M, Eslami K, Mohammadzadeh Gheshlaghi N. [Study of sensibility and antimicrobial resistance in *Escherichia coli* isolated from urinary tract infection in Tabriz city]. *J Fasa Univ Med Sci*. 2013;**3**(2):149-54. Persian.
22. Mahdavi A, Nahaei MR, Akhi MT, Akhi M, Dibavar Akbari M. [Antibiotic resistance pattern against fluoroquinolones among *Escherichia coli* isolated from ICU and out-patient clinic admitted patients with urinary tract infection]. *Med J Tabriz Univ Med Sci*. 2009;**31**(3):91-6. Persian.
23. Bonyadian M, Salehi TZ, Mahzounieh MR, Taheri FA. [Virulence genes of verotoxigenic *E. coli* isolated from raw milk and unpasteurized cheese]. *J Vet Res*. 2011;**66**(3):223-81. Persian.
24. Gobernado M, Valdes L, Alos JJ, Garcia-Rey C, Dal-Re R, Garcia-de-Lomas J, et al. Antimicrobial susceptibility of clinical *Escherichia coli* isolates from uncomplicated cystitis in women over a 1-year period in Spain. *Rev Esp Quimioter*. 2007;**20**(1):68-76. [PubMed: [17530038](https://pubmed.ncbi.nlm.nih.gov/17530038/)].
25. Hunter PA, Dawson S, French GL, Goossens H, Hawkey PM, Kuijper EJ, et al. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. *J Antimicrob Chemother*. 2010;**65** Suppl 1:i3-17. doi: [10.1093/jac/dkp433](https://doi.org/10.1093/jac/dkp433). [PubMed: [20045808](https://pubmed.ncbi.nlm.nih.gov/20045808/)].