Original Article

Antibiotic Susceptibility Assessment of *Escherichia coli* Isolated from Traditional Cheeses in Marand, Iran

Abolfazl Jafari-Sales[1,*], Parisa Hossein-Nezhad[2], Ahmadreza Shahniani[1]

[1]Department of Microbiology, School of Basic Sciences, Kazerun Branch, Islamic Azad University, Kazerun, Iran

[2]Department of Food Science and Technology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

*Corresponding Author E-mail: A.jafari_1392@yahoo.com

ABSTRACT

Background and aim: Dairy products, especially cheese, are a suitable environment for the growth of pathogenic bacteria that cause disease after being transmitted to humans. *Escherichia coli* is one of the microbial agents in traditional cheese that causes disease in humans. The aim of this study was to evaluate the contamination rate of traditional cheeses with *E. coli* and to determine their antibiotic resistance pattern in Marand.

Materials and methods: This cross-sectional study was conducted on 150 cheese samples from different centers of dairy products sales in Marand during the first six months of 2019 and after identification of *E. coli* strains, susceptibility and resistance to 12 antibiotics were studied using Kirby-Bauer test.

Results: Of the 150 samples studied, 60 (80%) were positive for *E. coli* infection. The results of antibiogram test showed the highest resistance to amoxicillin and amoxicillin/clavulanic acid (100%) and Trimethoprim-sulfamethoxazole (83.3%) and the highest sensitivity to ceftriaxone (66.6%) and ciprofloxacin (36.7%), respectively.

Conclusion: Due to the high level of contamination of traditional cheeses in this region with *E.coli*, their production and distribution should be under the control of their respective experts. So, it is necessary to inform them about the dangers of their use.

Key words: *Escherichia coli*, Cheese, Antibiotic

Introduction

Diseases that are transmitted to the body through the consumption of contaminated food are called foodborne illnesses. Most of these factors usually cause gastrointestinal diseases (Fadaei *et al.*, 2008). These diseases, which are classified as intestinal diseases, are on the rise not only in developing countries but also in developed countries with high health standards (De Oliveira *et al.*, 2011). Foodborne pathogens are a threat to public health in these countries, which can be prevented by adhering to health regulations in food

preparation processes (WHO, 2008). Since dairy products are an important source of food, they can be contaminated by various bacteria and cause numerous economic and health problems at the community level, costing millions of dollars annually to millions of people worldwide. Consequently, some die or get hospitalized. These include cheese dairy (Robinson, 1990; Farrokh Eslamlo *et al.*, 2009). **Escherichia coli** belongs to the family Enterobacteriaceae and is an opportunistic pathogen. Its presence in food and water is due to fecal contamination. These bacteria are Gram-negative, spore-free and some contain capsules or microcapsules (Ryan and Ray, 2004). Pyelonephritis, septicemia, meningitis, endocarditis, and epidemic diarrhea in children and adults are other disorders caused by germs (Daini and Adesemowo, 2008). Other research shows that bacteria account for more than 86% of urinary tract infections (Narchi and Al-Hamdani, 2010) and antibiotic resistance is increasing (Kebira *et al.*, 2009). The emergence of antibiotic resistance among the bacteria has become a major public health problem. Therefore, determination of antibiotic resistance patterns in pathogenic bacteria is important for guiding experimental treatments against a particular pathogen (Gangoue *et al.*, 2006). Given the high prevalence of traditional cheese in Marand County, the likelihood of contamination of traditional cheeses in *E. coli* sales centers, and the lack of a specific pattern of resistance to food infections due to consumption of traditional cheeses contaminated with *E. coli*, this study aimed at investigating the prevalence and pattern of antibiotic resistance of *E. coli* strains in traditional cheeses supplied in Marand. Statistical analysis was performed using SPSS software.

Experimental

Materials and methods

This cross-sectional study was conducted in the first six months of 2019. 150 traditional cheese samples were obtained from Marand County sales centers and transferred to the laboratory. Identification and search of *E. coli* were carried out after culturing the specimens in enriched media and selective media by biochemical tests including TSI, SIM and MR-VP. Also, standard strain of *E. coli* ATCC 35218 was used as quality control. To determine the antibiotic susceptibility of the isolates studied by disk diffusion method on Müller Hinton Agar (Merck, Germany), according to the 2014 guidelines, the Institute of Clinical and Laboratory Standards (CLSI) was examined (Wayne, 2011). For this purpose, Muller-Hinton agar medium (Turbidity equivalent to 0.5 McFarland standard) and microbial suspension were prepared and cultured by sterile swabs in three different directions and after 24 hours of incubation, the diameter of the growth inhibition zone was measured. Results were reported based on sensitivity (S), resistance (R), and Intermediate (I) using the standard table. To verify the accuracy of the antibiogram discs, antibiogram were repeated 5 times. The antibiotic discs used were: Ceftazidime (30 µg), Ampicillin/Sulbactam (10 µg), Amoxicillin (25 µg), Amoxicillin/Clavulanic acid (25 µg), Ceftriaxone (30 µg), Clarithromycin (15 µg), Amikacin (30 µg), Cefexime (5 µg), Azithromycin (15 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg) and Trimethoprim/Sulfamethoxazole (5 µg) (Padtan Teb Co). The obtained data were analyzed by SPSS software (version 19).

Results

Of the 150 samples tested, 60 cases were positive for *E. coli*. The results of antibiogram showed the highest resistance to amoxicillin and amoxicillin/clavulanic acid (100%) and trimethoprim/sulfamethoxazole (83.3%) and the highest sensitivity to ceftriaxone (66.6%) and ciprofloxacin (36.7%), respectively. The results of the evaluation of the susceptibility of the isolated *E.coli* to other antibiotics are presented in Table 1.

Table 1. Results of antibiogram test of *E. coli* isolated from traditional cheeses on antibiotics used (%)

Antibiotic	Sensitive (S)	Intermediate (I)	Resistant (R)
Ceftazidime	31.6	38.4	30
Ampicillin/Sulbactam	28.4	71.6	0
Amoxicillin	0	0	100
Amoxicillin/Clavulanic acid	0	0	100
Ceftriaxone	66.6	33.4	0
Clarithromycin	26.7	25	48.3
Amikacin	31.6	28.4	40
Cefexime	31.6	38.3	30
Azithromycin	25	35	40
Ofloxacin	16.7	56.6	26.7
Ciprofloxacin	36.7	25	38.3
Trimethoprim/Sulfamethoxazole	0	16.7	83.3

Discussion

The increasing number of foodborne illnesses and poisonings resulting in the economic and social problems have inspired the development of various studies on healthy food production. Since cheese is one of the main foodstuffs used in the country, its microbial contamination should be considered by food industry professionals all seasons long. Cheeses that are traditionally produced in the countryside without regard to hygiene can be an important source of a variety of pathogenic bacteria, including *E. coli* (Farrokh Eslamlo *et al.*, 2009; Rezaei *et al.*, 2014). *E. coli* contamination of dairy products such as cheese was present in most cases. In the study of (Nooroozi *et al.*, 2012; Pourali *et al.*, 2011) 50% and 88% of the samples were contaminated with *E. coli*, respectively. The overall results of this study showed that the status of traditional cheeses produced in this city was in poor condition so that 60 samples (40%) were positive for *E. coli*. The highest resistance was observed in amoxicillin and amoxicillin/clavonic acid (100%) and trimethoprin/sulfamethoxazole (83.3%) and the highest sensitivity to ceftriaxone (66.6%), respectively. (Hamzeh Pour *et al.*, 2019) studying traditional cheese contamination in Mahabad, reported 54 percent of the samples were infected with *E. coli*. All *E. coli* isolates were resistant to amoxicillin and 66.6% of the isolates were susceptible to ceftriaxone which was in line with the findings of the present study. In a study by (Voidarou *et al.*, 2009) in Greece on *E.coli* strains isolated from cheese, tetracycline resistance was 50% and ciprofloxacin sensitivity was 80%. In 2015, (Moezi *et al.*, 2015) found that 7% of traditional foods were contaminated with *E. coli*. The antibiogram results showed that 100% of the isolates were resistant to ampicillin and vancomycin and then the highest resistance to antibiotics cefexime (75%), tetracycline (62.5%), cefalexin (50%), there were ceftriaxone, gentamicin and co-trimoxazole (25%), ciprofloxacin and chloramphenicol (12.50%).

Examining the antibiotic pattern of *E. coli* isolated from traditional Ilam cheese, Wherham and Aziznia (2012) showed that 42.6% (41 samples) were infected with *E. coli*. All of them were 100% resistant to vancomycin, erythromycin and ampicillin. In a study by (Rezai *et al.*, 2014) on traditional cheeses, 34% of the samples were contaminated with *E. coli*. (Giammanco *et al.*, 2011) reported a 44 percent infection rate of *E. coli* by examining the microbial status of traditional cheese. (Tahiri *et al.*, 2005) reported 100 times the rate of coliform contamination in traditional cheeses than industrial cheeses. Differences in primary infection (livestock health, milking and milk containers) and secondary (cheese distribution and storage) can be attributed to different reports of *E. coli* infection rates (Salek moghadam *et al.*, 2001). The high concentration of cheese salt and low pH are two other factors that can be considered in justifying the differences in the results of different cheeses so cheeses with lower percentages of *E. coli* contamination were more favorable for these two factors (Aygun *et al.*, 2005).

Conclusions

The results of this study showed that the intensity and rate of contamination of local cheese produced in Marand with *E. coli* by traditional method was higher than that of standard one. The reason for the high contamination of these microorganisms can be attributed to the use of raw milk, the whole process of manual production, and the maintenance of traditional cheeses, especially at the distribution and sale stage at ambient temperature. Given that there are very few and limited studies nationwide in this area, there is no comprehensive statistics and data on this issue, so it is recommended that more detailed studies be carried out in research centers with more oversight by the Ministry of Health on food.

References

Aygun, O, Aslantas, O, Oner, S. (2005). A survey on the microbiological quality of Carra, a traditional Turkish cheese. *J. Food Eng.*, 66:401–404.

Daini, OA, Adesemowo, A. (2008). Antimicrobial susceptibility patterns and R-Plasmid of Clinical Strains of *Escherichia coli. Aust. J Basic. Appl. Sci.*, 2(3):397-400.

De Oliveira, MA, De Souza, VM, Bergamini, AMM, De Martinis, ECP. (2011). Microbiological quality of ready to eat minimally processed vegetables consumed in Brazil. *Food Control.*, 22(8):1400-403.

Fadaei, AM, Jamshidi, A, Kheiri, S. (2008). Comparison of bacterial contamination of raw and pasteurized milk used in Shahrekord in 2006. *J. Shahrekord Univ. Med. Sci.*, 10:37-44.

Farrokh Eslamlo, H, Athari, S, Hami, M, Haji Mohammadi, B, Hosseini Jazani, N. (2009). The evaluation of contamination rate with *E. coli*, staphylococcus aeureus, Listeria monocytogenesis and Salmonella sp. In handmade butters in Urmia City. *J. Urmia Nurs. Midwifery Fac.*, 7(3):157-65.

Gangoue, P.J, Koulla, Sh.S, Ngassam, P, Adiogo, D, Ndumbe, P. (2006). Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon. *Afr. J. Health Sci.*, 6(4):232-235.

Giammanco, GM, Pepe, A, Aleo, A, D'Agostino, V, Milone, S, Mammina, C. (2011). Microbiological quality of Pecorino Siciliano "primosale" cheese on retail sale in the street markets of Palermo, Italy. *New Microbiol.*, 34:179-85.

Hamzeh Pour S, Vaziri S, Molaee Aghaee E. (2019). Survey on the contamination rate and determination of antibiotic resistance of Staphylococcus aureus, *Escherichia coli* and Salmonella strains isolated from traditional cheeses distributed in Mahabad, *Iran. J. Health Environ.*, 11(4) :465-476.

Kebira, AN, Ochola, P, Khamadi, SA. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections. *J. Appl. Biosci.*, 22:1320-1325.

Moezi, P, Bahador, N, Baseri Salehi, M. (2015). Serological typing of isolated *Escherichia coli* from traditional food and their evaluation of antibiotic resistant pattern. *Med. Sci.*, 25(4):269-276.

Narchi, H, Al-Hamdani, MAM. (2010). Antibiotic resistance trends in paediatric community acquired first urinary tract infections in the united Arab Emirates. *East. Med. Health J.*, (*EMHJ*), 21(16):45-50.

Nooroozi, M. (2012). Investigation of contamination of traditional cheeses of Lighvan with *E. coli* and in Coliforms Maragheh. *Iran. J. Med. Microbiol.*, 5(4):23-28 .

Pourali, BM, Mirzaei, H. (2011). Study on the contamination rate of traditional white cheese presented in Tabriz markets to coliforms and pathogenic *Escherichia coli*. *J. Food Saf.*, 1(3):71-80.

Rezaei, M, Yahyaei, M, Parviz, M. (2014). A survey of microbial contamination in traditional cheese distributed in Markazi Province in 2010. *Iran. J. Health Environ.* (*IJHE*)., 7(1):115-22.

Robinson, RK. (1990). The microbiology of milk products. Second edition. London and New York: Elsevier applied Science., p 203-89.

Ryan, K. J.; Ray, C. G. (2004). Sherris Medical Microbiology 4th edition McGraw Hill.

Salek moghadam, A, Foruhesh Tehrani, H, Ansar, H, Ravadgar, B, Noorani, vatani A, Ghaseemi, M. (2001). A survey on bacterial contamination on one hundred unpasteurized cheese samples and pasteurized chees as control and stability of commonly contamination bacterial to different salt concentration. *Med. J. Islam. Repub. Iran.* 8(25):175-81.

Tahiri, R. (2005). A Comparison on Microbial Conditions between Traditional Dairy Products Sold in karka and same products produced by modern dairies. *Pakistan J. Nutrit.*, 4(5):345-48.

Voidarou, X, Alexopoulos, A, Plessas, S, Bezirtzoglou, E. (2009). Antibiotic profile of common pathogens related to food safety and health. *J. Ege. Academ. Rev.*, 9(3):961-967.

Wayne, P. A. (2011). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.

Wherham, H, Azania, S. (2012). Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Traditional Cheese in Ilam. *Veterin. Laborator. Res.*, 4(1):62-62.

World Health Organization. Foodborne disease outbreaks: guidelines for investigation and control, 2008. Available from: http://whqlibdoc.who.int/publications/2008/9789241547222_eng.pdf

How to cite this article: Abolfazl Jafari-Sales, Parisa Hossein-Nezhad, Ahmadreza Shahniani, Antibiotic Susceptibility Assessment of *Escherichia coli* Isolated from Traditional Cheeses in Marand, Iran. *International Journal of Advanced Biological and Biomedical Research*, 2020, 8(2), 236-141. Link: http://www.ijabbr.com/article_37332.html