



Microbiological investigation of coliforms and *Listeria* contamination in traditional cheese from Babol City, Iran, during the fall 2020: Short report

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Short report

Abstract

BACKGROUND: Traditional cheese has a special place in the diet of the people. However, its contamination with pathogenic microorganisms can endanger human health and cause significant economic losses. This study aimed to determine the contamination of traditional cheese by coliforms and *Listeria*.

METHODS: In this cross-sectional study in Babol City, Iran, 60 samples of traditional cheeses (Khiki, Tabrizi, and Lactic) were randomly collected in the Fall (October, November, and December) 2020. To separate *Listeria*, *Listeria* selective enrichment broth and PALCAM *Listeria* selective agar were used. MacConkey agar was used for coliform detection. Frequency distribution table and one-way analysis of variance (ANOVA) with SPSS software were used for data analysis.

RESULTS: Sixty-five percent of Khiki cheese, 67% of Lactic cheese, and 55% of Tabrizi cheese samples were contaminated with *Listeria*. Moreover, 35% of Khiki cheese, 28% of Lactic cheese, and 59% of Tabrizi cheese samples were contaminated with coliforms. A significant relationship between microbial contamination and the West region of the city was observed ($P = 0.04$).

CONCLUSION: Contamination of cheese samples taken in this study with coliform and *Listeria* indicates the poor quality and hygienic condition of traditional cheeses offered in Babol City; therefore, the necessary measures should be taken to create appropriate conditions for hygiene.

KEYWORDS: Traditional Cheese; *Listeria*; Coliforms

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Introduction

In the preparation of traditional cheese, unpasteurized milk is used and if the milk contains bacteria, the cheese will also be infected with them. Coliforms are now known as health indicators. These bacteria can cause diarrhea, stomach pain, vomiting, fever, and pneumonia.^{1,2} *Listeria* is also a bacterium that causes listeriosis,

which is a common disease in humans and animals.³ In the study performed by Vaziri and Naghshbandi in Iran, 98% of the traditional cheese samples of Lighvan were contaminated with coliforms, and 50% were contaminated with *Escherichia coli* (*E. coli*).⁴ Khedmati Morasa et al. in Iran reported that out of 128 samples of traditional cheeses, 14 samples (10.9%) were positive for *Listeria monocytogenes*, and the highest contamination was seen in white cheeses (7%) in summer and winter (3.1%).⁵ In the preparation of traditional dairy products, it is

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important to observe hygienic principles in all stages of production. Due to the texture of milk and the presence of many nutrients, this product is a suitable substrate for the growth and multiplication of various pathogens, which are listed as indicators of these microbial agents to *Listeria* and coliforms. The study of local dairy contamination in Babol City, Iran, is very rare, and there is not much published research on the traditional cheese contamination with bacteria in this city. This city is located in the north of Iran in a humid region, and humidity plays a prominent role in the activity of microbes; therefore, informing people in this field is valuable. It is also significant in terms of novelty in the study samples. This study was performed to determine the degree of contamination of traditional cheese to coliform bacteria and *Listeria* species.

Methods

In this cross-sectional study, traditional cheeses were randomly (using a table of random numbers) collected by the researcher from markets in Babol City, by two-stage cluster sampling during the Fall 2020. At first, Babol City was divided into 3 regions – considered a district (North, East, and West). Each of the 3 regions was then divided into several headquarters. Then 20 samples were obtained from each headquarter, randomly, with a one-month interval (60 samples including 20 Khiki, 20 Tabrizi, and 20 Lactic). The samples were aseptically sent to the laboratory in sterile containers at 4 °C and under hygienic conditions, and they were stored in the refrigerator (to keep samples fresh) until the time of testing. First, the samples were placed in a container that was the right size and shape so that it was not pressed and crushed. Then, using sterile metal spoons, the sample cheese was removed from the depths carefully, weighed about 25 grams with a digital scale, and transferred to a sterile porcelain mortar. Then 225 cc of physiological

serum was mixed with the sample as a dilution solution. To separate *Listeria*, 1 cc of the diluent mixture was cultured in *Listeria* selective enrichment broth (Merck, Germany) and incubated for 37 days at 37 °C.

The selected culture medium of PALCAM *Listeria* selective agar (Merck, Germany) was used to evaluate the growth rate of *Listeria* bacteria. At this time, 1 ml of sterile distilled water was added to a vial containing PALCAM culture supplement, and then the contents of the vial were added to PALCAM culture medium, which was 60 °C in Erlenmeyer, after uniformity. After that, under sterile conditions, this culture medium was poured into sterile plates, and after closing the medium, the plates were kept at refrigerator temperature until used. Then 1 cc of *Listeria* selective enrichment broth was added to PALCAM *Listeria* selective agar and incubated for 24 hours at 37 °C.^{6,7}

To separate the coliform, 1 cc of the mixture of cheese and diluent solution was introduced into the MacConkey agar culture medium (Merck, Germany) and spread well on the surface of the culture medium with the help of a curved glass rod. They were then incubated at 37° C for 48 hours.⁸ Gram staining was used to distinguish *Listeria*-positive bacteria from gram-negative coliforms.⁹

Data Analysis: Frequency distribution table with number and percentage and one-way analysis of variance (ANOVA) with SPSS software (version 21, IBM Corporation, Armonk, NY, USA) were used for statistical analysis ($P < 0.05$).

Results

***Listeria* Contamination:** Out of 20 cases of Khiki cheese, 13 (65%), out of 18 cases of *Lactic* cheese, 12 (67%), and out of 22 cases of Tabrizi cheese, 12 (55%) cases had positive contamination with *Listeria* (Table 1, Figure 1).

***Coliforms* Contamination:** Out of 20 cases of Khiki cheese, 7 (35%) cases were positive in terms of contamination with coliforms.

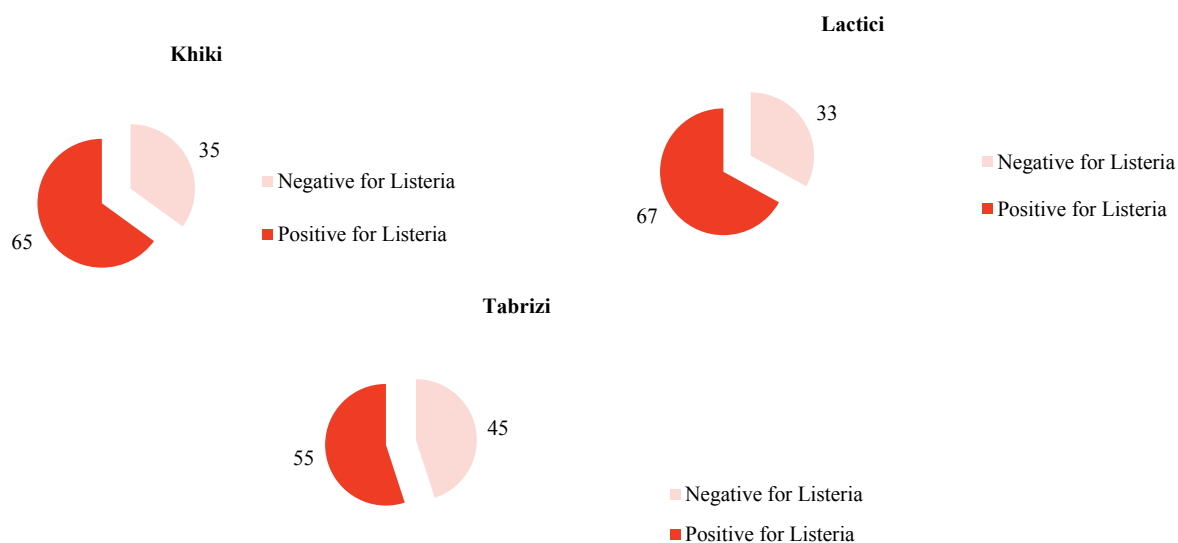


Figure 1. Contamination of traditional cheese samples in Babol City, Iran, with *Listeria* species

Table 1. Contamination of traditional cheese samples in Babol City, Iran, with *Listeria* species

Cheese types	Total (number)	Positive for <i>Listeria</i> [n (%)]	Negative for <i>Listeria</i> [n (%)]
Khiki	20	13 (65)	7 (35)
Lactic	18	12 (67)	6 (33)
Tabrizi	22	12 (55)	10 (45)

Out of 18 cases of Lactic cheese, 5 (28%) and out of 22 cases of Tabrizi cheese, 13 (59%)

cases were contaminated with coliforms (Table 2, Figure 2).

Table 2. Contamination of traditional cheese samples in Babol City, Iran, with coliforms

Cheese types	Total (number)	Positive for coliforms [n (%)]	Negative for coliforms [n (%)]
Khiki	20	7 (35)	13 (65)
Lactic	18	5 (28)	13 (72)
Tabrizi	22	13 (59)	9 (41)

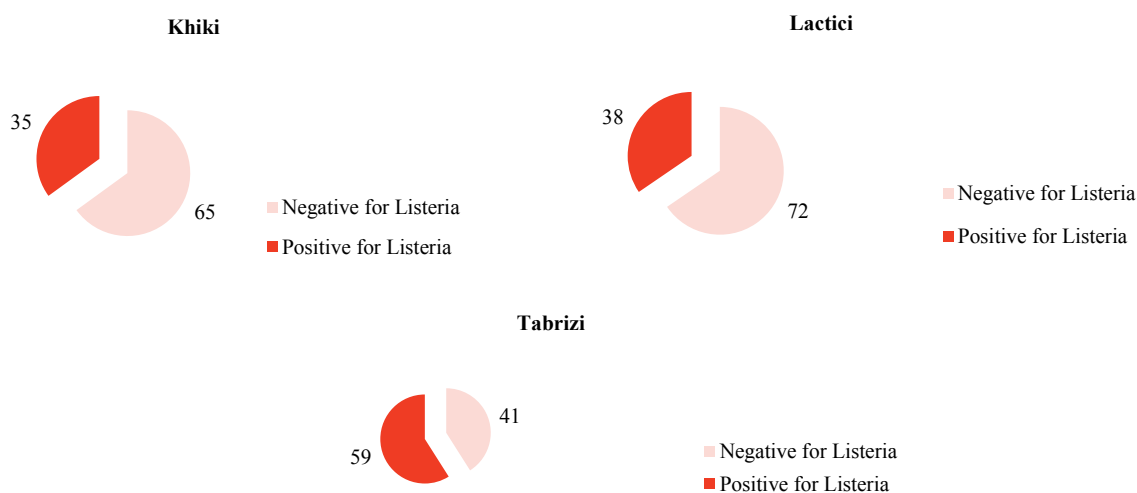


Figure 2. Contamination of traditional cheese samples in Babol City, Iran, with coliforms Table 3. Contamination of traditional cheese samples in districts (North, East, and West) of Babol City, Iran

District	Total (number)	Positive for contamination [n (%)]	Negative for contamination [n (%)]
North	20	15 (75)	5 (25)
East	20	17 (85)	3 (15)
West	20	20 (100)	0 (0)

Districts (North, East, and West) Contamination: In the North, East, and West regions, 15 (75%), 17 (85%), and 20 (100%) samples were contaminated with bacteria, respectively (Table 3, Figure 3).

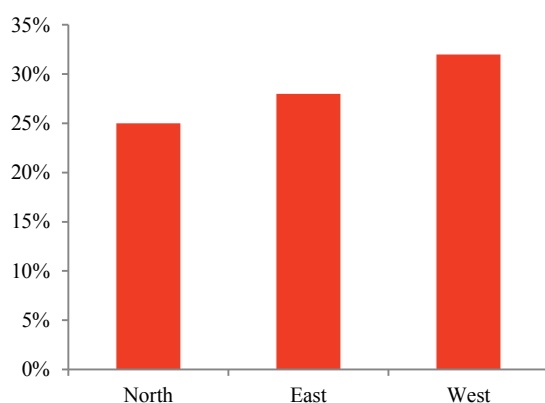


Figure 3. Contamination of traditional cheese samples in the North, East, and West districts of Babol City, Iran

Statistical Results: There was no significant relationship between microbial contamination and the type of bacteria isolated in the North and East regions with the type of cheese ($P > 0.05$). A significant relationship between microbial contamination and the West region was observed ($P = 0.04$).

Discussion

This study aimed to investigate traditional cheese contamination with coliforms and *Listeria*. In the present study, out of 60 traditional cheese samples, 37 (61.66%) samples were contaminated with *Listeria*, and 25 (41.66%) were contaminated with coliforms. In a study by Rezaei et al. in Iran, all 80 (100%) traditional cheese samples surveyed were contaminated with coliforms. This rate was higher than our study (41.66%); these results indicate the unfavorable condition of

traditional cheeses in terms of microbial contamination. Differences in the results obtained from reports can depend on the type of cheeses, unsanitary preparation, and improper storage of the product in different places; moreover, it can be related to the location of the animal, the milk storage containers, and the health of the animal.¹⁰ Al-Ghamdi et al. in Saudi Arabia showed that *Staphylococcus aureus*, yeasts, and molds were isolated from cheese samples at a low rate. The number of coliform bacteria isolated from cheese was significantly higher than other microbes.¹¹ The high concentration of cheese salt and the low pH are two other factors that can be used to justify the differences in the results of the reports.^{10,11} In the present study, 35%, 28%, and 59% of Khiki, Lactic, and Tabrizi cheese samples were contaminated with coliform, respectively.

Contrary to the findings of Al-Ghamdi et al.,¹¹ in the present study, samples contaminated with coliforms were less than *Listeria*. Additionally, in the study conducted by Rabinejad et al. in Iran, *Listeria monocytogenes* contamination was not observed in any of the traditional and industrial cheese samples.¹² These results were the opposite of the results of our study; 65% of Khiki cheese samples, 67% of Lactic cheese samples, and 55% of Tabrizi cheese samples in our study were contaminated with *Listeria*. Inadequate washing, inadequate sterilization of milk production equipment, infiltration of air into packages, improper milk storage temperature, inadequate environmental hygiene, lack of clean water used for washing dishes, and lack of cleanliness of livestock fodder cause contamination of dairy products.¹⁰⁻¹² It is important to educate farmers to learn how to use appropriate and hygienic

technology for the production of milk and cheese and their proper maintenance. It is also suggested that to remove these contaminants from these products, cheese made from raw milk should be stored in brine for two to three months before consumption. Pasteurizing milk helps prevent brucellosis.

One of the limitations of this study is the lack of a large number of samples. Besides, sampling should be done during several months and seasons, which we were not able to do due to lack of time and financial budget.

Conclusion

Contamination of cheese samples taken in this study was shown. This information also indicates the presence of coliform and *Listeria* in traditional cheese.

Conflict of Interests

Authors have no conflict of interests.

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