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Microbial Quality and Antimicrobial Resistance of *Staphylococcus aureus* and *Escherichia coli* Isolated from Traditional Ice Cream in Hamadan City, West of Iran

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Abstract

Background: Foodborne diseases are one of the most major public health concerns in the world. Ice cream flavors, especially the traditional ones, have a high potential for the transmission of the pathogenic bacteria.
Objectives: The aim of the current study is to investigate the microbiological status and antibiotic resistance of *Escherichia coli* and *Staphylococcus aureus* isolated from traditional ice cream.
Methods: A total of 114 traditional ice creams were randomly collected from retail stores in Hamadan, Iran. Samples were investigated for the total bacteria count (TBC) and contamination with the coliform, *Enterobacteriaceae* and *Salmonella* as well as the prevalence and antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli*.
Results: The count of *Enterobacteriaceae* (89.47%), mold and yeast (50%), coliform (40.35%) and TBC (28.07%) of samples was higher than Iran's standard. *Salmonella* was not found in all samples. The prevalence of *Staphylococcus aureus* and *Escherichia coli* was confirmed in 50% and 37.72% of samples, respectively. Collected *Escherichia coli* had the highest antibiotic resistance to ampicillin 67.44%, nalidixic acid 39.53% and co-amoxyclav 37.21%. *Staphylococcus aureus* showed a higher antibiotic resistance to penicillin (82.46% of isolates) and oxacillin (38% of isolates).
Conclusions: The results showed high contamination levels of traditional ice cream with spoilage and pathogenic microorganisms as well as considerable resistance of isolated *Staphylococcus aureus* and *Escherichia coli* to common antibiotics. Therefore, good hygienic practice during processing and personal hygiene should be considered to improve the quality of ice cream. In addition, it is necessary that the regulatory authorities carry out more control on the production centers of traditional ice cream.

Keywords: *Staphylococcus aureus*, *Escherichia coli*, Foodborne Disease, Ice Cream, Drug Resistance

1. Background

Foodborne disease is a major cause of morbidity and mortality all over the world. Thirty-one pathogens are known to cause foodborne disease and have an adverse impact on humans (1). *Escherichia coli* and *Staphylococcus aureus* are dangerous pathogens that were found in contaminated food substances. *E. coli* caused haemorrhagic colitis (HC), bloody diarrhea and haemolytic-uremic syndrome (HUS) (2). The presence of *Staphylococcus aureus* in food causes poisonous symptoms such as nausea, abdominal cramps, diarrhea and vomiting (3, 4).

Milk is a nutritious food and an excellent culture medium for microbial growth; therefore, it is one of the most important sources of foodborne illness. Many dfferent pathogenic bacterias are found in milk, such as *E. coli*, *S. aureus*, *Salmonella*, *Listeria monocytogenes*, *Shigella*, *Campylobacter jejuni* and *Bacillus cereus* (5, 6).

Traditional foods are considered all over the world. These foods have specific characteristics, which differ from other similar products of the same category due to the utilization of traditional ingredients (raw materials of primary products), traditional composition or traditional type of production and/or processing method (7). There is much concern about microbial contamination of traditional foods. Traditional ice creams are one of the most favorite consumed foods in warm seasons in Iran. Its production process is different from industrial ice cream. Ice cream has high nutritional value and neutral pH; thus, it is a suitable environment for microbial growth. In addition, several raw materials are used for ice cream production; some of which is contaminated and can create any health hazard (8). The utilization of unpasteurized milk for traditional ice cream production is one of the main microbial contaminants (9). Furthermore, suitable thermal process-

ing was not applied during manufacturing of these products to preserve its desirable taste. Therefore, this product is an important source of transmission of pathogenic bacteria. Since most of the ice cream is consumed by children and is popular amongst adults, the study of the microbial status of this product is very useful (10). On the other hand, the worldwide use of antibiotics in livestock could increase the emergence of resistance bacteria strains in the food chain (6). The information of antibiotic susceptibility pattern of bacteria helps treat the infectious disease (11). Transmission of resistant microorganisms through milk, specifically ice cream, can cause several problems for the consumers.

2. Objectives

Currently, there is little information regarding microbial contamination of traditional ice cream and antibiotic resistance patterns of pathogenic bacteria isolated from this product in the west of Iran and therefore the present communication was done for this purpose.

3. Methods

3.1. Sample Collection

A total of 114 ice cream samples (38 fruit-flavored, 38 plain and 38 pistachio) were randomly collected from retail stores in Hamadan city during April 2015 to March 2016. All samples were stored at - 18°C. Analyses were carried out within 24 hours after sampling.

3.2. Chemicals and Media

Gioliti-Cantoni Broth, Baird-Parker agar (BPA), nutrient agar, Lauryl sulphate broth and EC broth media were purchased from Merck Co. (Darmstadt, Germany). Mueller Hinton agar, YGC, VRBD and VRBG media were obtained from HiMedia Co. (Mumbai, India). All of the following antibiotic disks testing were obtained from Padtan Teb Co. (Karaj, Iran): all other chemicals were purchased from Merck.

3.3. Microbiological Analyses

3.3.1. Preparation of Serial Dilution

For obtaining 0.1 dilutions, 5g of molted ice cream were aseptically transferred into a 95 mL ringer solution and homogenated by vertex. Subsequent decimal dilutions were prepared using the same diluents.

3.3.2. Total Bacteria Count (TBC)

1 mL of diluted ice cream was poured into to a sterile Petri dish containing medium of plate count agar and incubated at 30°C for 24 h. At the end of the incubation, microbial colonies were enumerated (12) and all culturing examinations were carried out in duplicates.

3.3.3. Enterobacteriaceae

1 ml of each dilution was added to 15 ml of violet red bile glucose agar (VRBG) and incubated for 48 hours at 45 - 47°C. The characteristic colonies, violet red, were counted and confirmed by oxidase and fermentation tests (13).

3.3.4. Coliform

Coliform enumeration was performed on VRBL medium after incubation at 37°C for 24 hours (14).

3.3.5. Escherichia coli

The Lauryl Sulphate Broth and incubation at 37°C for 48 hours were used for the detection of *E. coli*. If gas was observed in the tubes, the solution was inoculated to EC broth and incubated at 44.5°C for 24 - 48 hours. *E. coli* was detected in tubes containing gas by free-indole peptone water, Kovac's reagent as well as incubation at 44°C for 24 hours (15).

3.3.6. Salmonella

To detect *salmonella* in ice cream samples, 25g of samples were cultured in 225 mL of peptone water broth at 37°C. After, 1.0 mL of this solution was transferred into 10 mL of tetrathionate broth and incubated at 37 °C. Then, about 5 - 10 μL of obtained solution was transferred into xylose desoxycholate (XLD), incubated at previous conditions and then diagnostic media was used for biochemical test (16).

3.3.7. Staphylococcus aureus

The Gioliti-Cantoni agar medium and pour-plate method were used for the detection of *S. aureus*. Black colonies with a positive coagulase were confirmed and considered as *S. aureus* (17).

3.3.8. Molds and Yeast

1 mL of diluted ice cream was transferred into the YGC medium and was then incubated at aerobic conditions at 25 $\pm$ 1°C for 3 - 5 days and then the colonies were enumerated (18).

3.3.9. Antibiotic Susceptibility Testing

The antimicrobial susceptibility tests for *S. aureus* and *E. coli* were performed by the Kirby-Bauer disc diffusion method as recommended by the clinical and laboratory standards institute on Mueller Hinton agar plates (19). For this purpose, the microbial suspension with the turbidity equivalant to the McFarland Standard of 0.5 was prepared and cultured on the Mueller Hinton agar plates. After preparation and incubation of the plates, the diameter of the growth inhibition zones around each of the antibiotic disks were measured and the susceptibility was categorized as either susceptible, intermediate and resistant. The disk diffusion method was repeated 5-times with one antibiotic for the investigation of accuracy of antibiogram disks. Disk diffusion used for the antimicrobial susceptibility tests included Erythromycin (15 μg), Rifampin (5 μg), Clindamycin (2 μg), Tetracycline (30 μg), Co-amoxiclav (25 μg), Tylosin (15 μg), Nalidixic acid (30 μg), Methicillin (5 μg), Ampicillin (10 μg), Streptomycin (10 μg), Neomycin (30 μg), Chloramphenicol (30 μg), Ciprofloxacin (5 μg), Trimethoprim (5 μg), Penicillin (10 μg), Vancomycin (30 μg), Gentamicin (10 μg), Oxacilli (1 μg) and Ceftazidime (30 μg).

3.4. Statistical Analysis

All experiments were performed in triplicates. The data analysis was carried out by the SPSS statistical software (version 16.0, SPSS Inc., Chicago, USA). Differences in the prevalence of microorganism amongst various ice creams were analyzed using a chi-square test. Differences were considered significant at values of $P < 0.05$.

4. Results

The microbial results of ice cream samples are shown in Table 1. No significant differences in the contamination and prevalence rates of spoilage and pathogenic are observed amongst various ice creams. 28.95% of plain, 28.95% of pistachio and 26.32% of fruit-flavored ice creams had exceeded the TBC than Iran standard levels (5×10^4 CFU g^{-1}). All the analyzed ice cream samples showed heavy contamination (in average, 89.47%) of *Enterobacteriaceae*. Overall, coliform count in 40.35% of samples was higher than legal limit (10 CFU g^{-1}); samples of plain ice cream had the highest contamination. 39.47 - 57.89% of analyzed samples were contaminated with *S. aureus* and the highest positive samples were found in fruit-flavored ice cream. Between 31.58 and 42.11% of ice cream samples were contaminated with *E. coli*. *Salmonella* was not found in all samples. In general, the highest mold contamination (57.89% of samples) was found in pistachio ice cream. Isolated *S. aureus* showed a significant resistant to antibiotics and the highest resistance was found for penicillin, oxacillin, erythromycin and clindamycin, respectively (Table 2). Overall, 38 of 47 *S. aureus* isolates (80.85%) were resistant to two or more antimicrobial agent. Although, antibiotic resistance of *E. coli* to gentamicin and chloramphenicol was very low, it showed the highest resistant to ampicillin, nalidixic acid and co-amoxyclav, respectively (Table 3). Multiresistance, resistance to three or more antibiotics, was found in 16 of 43 (37.21%) isolated *E. coli*.

5. Discussion

Traditional ice cream is one of the most abundant and popular dairy products that is consumed in warm seasons by vulnerable groups, especially children, therefore; its microbial contamination is very important. This study has shown the high microbial contamination levels of traditional ice creams in Hamadan, Iran.

Several studies have been done in relation to antibiotic resistance patterns in traditional ice cream, raw milk and other dairy products all around the world (6, 20-24). According to the results, 28.7% of all traditional ice cream samples had TBC than recommended criteria according of Iran standard (5×10^4 CFU g^{-1}). In other surveys, more TBC (51 to 78.8%) than the current study has been reported (25-27).

The Enterobacteriaceae and coliform count in food has been considered as an index of food hygiene. The prevalence of Enterobacteriaceae in the present study (89.48%) was higher than the results of other studies (17.2 to 82.9%) (26-28). The coliform count in 55% of the samples was above the approved limit (10 CFU g^{-1}) while 30% - 46% of ice cream samples examined by Abou and Khair (2014) and Ambily et al. (2012) had a higher coliform count than the legal limit (25, 29). Although, contamination level of 100% was also found in a study conducted in India (30). High levels of traditional ice cream contamination of coliforms might be due to the utilization of unpasteurized milk, contaminated water, post-treatment contamination or contaminated utensils used for ice cream storage, distribution, poor sanitation practices during preparation and production and the lack of personal hygiene (21).

The occurrence of *Salmonella* contamination in ice cream is likely due to the use of egg in ice cream formulation. This substance is vehicle for transmission of this organism. *Salmonella* was not found in all samples that it is in agreement with studies in Iran and other countries (25, 27, 28, 31), although the contamination levels of 3.7 to 33% have been reported in some studies (21, 29, 30).

In our study, 50% of ice cream samples had higher mold contamination than the permitted standard levels,

Table 1. Microbial Quality of Traditional Ice Cream Consumed in Hamadan, Iran[a]

Type of Ice Cream	N	Total Bacteria Count (CFU g-1)		*Enterobacteriaceae* (CFU g-1)		Coliform (CFU g-1)		*E. coli* (CFU g-1)		*Salmonella* (CFU g-1)		*S. aureus* (CFU g-1)		Mold and Yeast (CFU g-1)	
		ND-5 × 104	> 5 × 104	ND - 10	> 10	ND - 10	> 10	Positive	Negative	Positive	Negative	coagulase-positive	coagulase-negative	ND - 102	> 10
Plain	38	27 (71.05)	11 (28.95)	7 (18.42)	31 (81.58)	25 (65.79)	13 (34.21)	12 (31.58)	26 (68.42)	0	38 (100)	15 (39.47)	23 (60.53)	22 (57.89)	16 (42.11)
Pistac	38	27 (71.05)	11 (28.95)	2 (5.26)	36 (94.74)	20 (52.63)	18 (47.37)	16 (42.11)	22 (57.89)	0	38 (100)	20 (52.63)	18 (47.37)	16 (42.11)	22 (57.89)
Fruit-flavored	38	28 (73.68)	10 (26.32)	3 (7.89)	35 (92.11)	23 (60.53)	15 (39.47)	15 (39.47)	23 (60.53)	0	38 (100)	22 (57.89)	16 (42.11)	19 (50)	19 (50)
Total	114	82 (71.93)	32 (28.07)	12 (10.53)	102 (89.47)	68 (59.65)	46 (40.35)	43 (37.72)	71 (62.28)	0	114 (100)	57 (50)	57 (50)	57 (50)	57 (50)
P value		0.957		0.141		0.5		0.615		-		0.255		0.388	

Abbreviations: N: Number of Analyzed Samples; ND: Not Detected.
[a] Value ithin parenthesis indicates contaminated sample percentages

Table 2. Results of the Antibiotic Sensitivity of *Staphylococcus aureus* Isolated from Traditional Ice Cream Samples (114 Samples) Consumed in Hamadan, Iran

Type of Antibiotic	Resistant		Intermediate		Sensitive	
	N	Percent	N	Percent	N	Percent
Penicillin	47	82.46	0	0	10	17.54
Oxacillin	38	66.67	0	0	19	33.33
Erythromycin	26	45.61	14	24.57	17	29.82
Clindamycin	24	42.11	10	17.54	23	40.35
Rifampin	12	21.05	6	10.53	39	68.42
Tetracycline	10	17.54	4	7.02	43	75.44
Trimethoprim.s	7	12.28	0	0.00	50	87.72
Chloramphenicol	1	1.76	7	12.28	49	85.96
Gentamicin	0	0	0	0	57	100
Ciprofloxacin	0	0	4	7.02	53	92.98

although Salehian et al. (2013) reported that 24% of samples showed a higher mold count than the acceptable limit (28).

The prevalence of *S. aureus* in our study was 50%. The contamination level of 2.8% - 41.8% with *S. aureus* was also reported in similar studies that were lower than our results (21, 25, 27-29). The presence of *S. aureus* in ice cream can be due to poor hygiene practices of handlers. This microorganism, is naturally found on the hands, nasal cavity and skin of humans (32, 33), therefore droplets containing microbe might be produced during coughing, talking and sneezing, which could settle on ice cream (31). Ice cream and other dairy products are good sources for *S. aureus* growth and can contribute to its outbreaks (4). *S. aureus* is a main cause of mastitis in cows. One of the utilization reasons of antibiotics in cows is for treatment of this disease, which might result in antibiotic resistance (22). Our recent reports had indicated that although *S. aureus* had not had any resistance to ciprofloxacin and gentamicin, it showed the highest resistance to penicillin and oxacillin. The previous research surveys that have been conducted on the antibiotic resistance pattern of *S. aureus* isolated from milk, dairy products, ice cream and mastitis. The highest resistance was observed in penicillin, oxacillin, ampicillin, cefuroxime, tetracycline and amoxicillin and the results of penicillin and oxacillin were not in agreement with the present study (6, 20, 21, 34, 35).

The results of the current study showed that 30.56% of assessed samples were contaminated with *E. coli*. Salehian et al. (2013), Hasanzad et al. (2013), Abou and Khair (2014) and Jadhav and Raut (2014), have reported the contamination levels of *E. coli* in ice cream to be between 40 to 52%, which is higher than present study (25, 28, 30) although lower contamination levels, 6 to 21.4%, have been found by some researchers (7, 13, 27). *E. coli* is considered as an indicator of faecal contamination of foods and emergence

Table 3. Results of the Antibiotic Sensitivity of *E. coli* Isolated from Traditional Ice Cream Samples (114 Samples) Consumed in Hamadan, Iran

Type of Antibiotic	Resistance		Intermediate		Sensitive	
	N	Percent	N	Percent	N	Percent
Ampicillin	29	67.44	2	4.65	12	27.91
Nalidixic acid	17	39.53	8	18.61	18	41.86
Co-Amoxyclav	16	37.21	3	6.98	24	55.81
Trimethoprim.s	14	32.56	6	13.95	23	53.49
Streptomycin	14	32.56	12	27.91	17	39.53
Ceftazidime	12	27.91	4	9.3	27	62.79
Chloramphenicol	4	9.3	2	4.65	37	86.05
Gentamicin	4	9.3	0	0	39	90.7
Ciprofloxacin	0	0	0	0	43	100

of some pathogen strains and causes severe concerns (23, 36). The use of antibiotics in livestock for treatment of infections led to emergence of antibiotic resistance bacterial strains, which is a serious concern not only in animals but also in human health (30). These strains could be transmitted to humans through animal-derived foods such as milk and dairy products and can threaten their health. Antibiotic resistance of pathogenic bacteria is one of the important hygiene problems of today's world that results in reduced effectiveness of antibiotics. Therefore, it is necessary to have information regarding antibiotic resistance patterns of bacteria in order to select an appropriate antibiotic for treatment of microbial infections (11). The highest antibiotic resistance of *E. coli* was observed to ampicillin, whilst all isolates were susceptible to ciprofloxacin. In other studies, isolated *E. coli* from milk and dairy products showed the most resistance to ampicillin (6, 21). In some studies, *E. coli* isolated from raw milk and unpasteurized cheese showed the highest resistance to penicillin and clindamycin, this finding was different than our results (14). These antibiotics weren't use in present research. Investigation of antibiotic resistance in this study revealed that isolated *S. aureus* had the highest sensitivity to gentamicin and ciprofloxacin and *E. coli* showed the most sensitive to ciprofloxacin. Thus, the use of these antibiotics for treatment of disease caused by mentioned bacteria could be effective.

In general, the results obtained in this study showed a high contamination level of traditional ice cream with pathogen and spoilage microorganisms. The presence of *S. aureus* and *E. coli* in traditional ice cream and their resistance to two or more antibiotics might be a potential risk for disease in the consumer, especially vulnerable groups such as children. Therefore, the implement of good hygiene practices (GHP) is a necessity for improving ice cream safety. Worker and staff education especially in relation to food safety as well as handling washing and machine and utensil cleaning could be effective in improvement of the situation. The quality of raw material used in the final product, their storage under suitable and hygienic conditions and heat treatment during processing could prevent growth and multiplication of pathogenic microorganisms in ice cream.

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Footnotes

Authors' Contribution: Study concept and design: Ali Heshmati and Sabah Ghadimi; acquisition of data: Sabah Ghadimi and Mehdi Azizi Shafa; Analysis and interpretation of data: Ali Heshmati and Sabah Ghadimi; drafting of the manuscript: Sabah Ghadimi and Ali Heshmati; critical revision of the manuscript for important intellectual content: Ali Heshmati and Majid Nooshkam; statistical analysis: Ali Heshmati; administrative, technical, and material support: Ali Heshmati; study supervision: Ali Heshmati.

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References

1. Centers for Disease Control and Prevention. . Estimates of Foodborne Illness in the United States. CDC Web site 2014. [cited January 8]. Available from: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

2. Canizalez-Roman A, Gonzalez-Nunez E, Vidal JE, Flores-Villasenor H, Leon-Sicairos N. Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. *Int J Food Microbiol.* 2013;**164**(1):36–45. doi: 10.1016/j.ijfoodmicro.2013.03.020. [PubMed: 23587712].
3. Fetsch A, Contzen M, Hartelt K, Kleiser A, Maassen S, Rau J, et al. Staphylococcus aureus food-poisoning outbreak associated with the consumption of ice-cream. *Int J Food Microbiol.* 2014;**187**:1–6. doi: 10.1016/j.ijfoodmicro.2014.06.017. [PubMed: 25033424].
4. Jorgensen HJ, Mork T, Hogasen HR, Rorvik LM. Enterotoxigenic Staphylococcus aureus in bulk milk in Norway. *J Appl Microbiol.* 2005;**99**(1):158–66. doi: 10.1111/j.1365-2672.2005.02569.x. [PubMed: 15960676].
5. Akindolire MA, Babalola OO, Ateba CN. Detection of Antibiotic Resistant Staphylococcus aureus from Milk: A Public Health Implication. *Int J Environ Res Public Health.* 2015;**12**(9):10254–75. doi: 10.3390/ijerph120910254. [PubMed: 26308035].
6. Gundogan N, Avci E. Occurrence and antibiotic resistance of Escherichia coli, Staphylococcus aureus and Bacillus cereus in raw milk and dairy products in Turkey. *Int J Dairy Technol.* 2014;**67**(4):562–9.
7. Almli VL, Verbeke W, Vanhonacker F, Næs T, Hersleth M. General image and attribute perceptions of traditional food in six European countries. *Food Qual Prefer.* 2011;**22**(1):129–38.
8. Kanbakan U, Çon AH, Ayar A. Determination of microbiological contamination sources during ice cream production in Denizli, Turkey. *Food Control.* 2004;**15**(6):463–70. doi: 10.1016/s0956-7135(03)00131-2.
9. Emami S, Akya A, 1Hossain Zadeh A, Barkhordar S. Bacterial contamination of traditional ice creams in Kermanshah in 2008. *Iran J Med Microbiol.* 2013;**7**(2):59–62.
10. Hazhir MS, Rashidi K, Senoubar TN, Reshadmanesh N, Mofareh N. Assessment of the types and rate of contamination in traditional ice-cream in Kurdistan province and its relationship to environmental and personal health care. *Sci J Kurdistan Univ Med Sci.* 2006;**37**(3):53–60.
11. Arabestani MR, Karami M, Alikhani MY. Antimicrobial Resistance in Microorganisms. *Avicenna J Clinical Microbiol Infection.* 2014;**1**(1).
12. ISIRI. . No. 5484: Milk and milk products - Enumeration of colony - Forming units of microorganism- colony count technique at 30 °C.. 1 ed. Tehran: Institute of Standards and Industrial Research of Iran; 2000.
13. ISIRI. . No. 2461-2: Microbiology of food and animal feeding stuffs horizontal methods for the detection and enumeration ofEnterobacteriaceae Part 2 : Colony-count method Second revision. Tehran: Institute of Standards and Industrial Research of Iran.; 2008.
14. ISIRI. . No. 5486-1: Milk and milk products - Enumeration of coliforms part 1: colony count technique at 30°C without resuscitation Second revision.. Tehran: Institute of Standards and Industrial Research of Iran.; 2008.
15. ISIRI. . No. 5486-1: Milk and milk products - Enumeration of presumptive escherichia coli - most probable number technique. Tehran: Institute of Standards and Industrial Research of Iran; 1999.
16. ISIRI . No.4413: Milk and milk products-Detection of Salmonella. Tehran: Institute of Standards and Industrial Research of Iran.; 2008.
17. ISIRI . No. 6806-1: Microbiology of food and animal feeding stuffs - Enumeration of coagulase - Positive staphylococci (staphylococcus aureus and other species) - Test method Part 1 : Technique using baird - parker agar medium. 1 ed. Tehran: Institute of Standards and Industrial Research of Iran; 2005.
18. ISIRI. . No. 10154: Milk and milk products - Enumeration of colony-forming units of yeasts and/or molds-colony -Count technique at 25 C. 1 ed. Tehran: Institute of Standards and Industrial Research of Iran; 2007.
19. Wayne PA. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Disk Susceptibility Tests. *Approved standard M2–A9, Clinical and Laboratory Standards Institute.* 2006.
20. Bonyadian M, Moshtaghi H, Akhavan Taheri M. Molecular characterization and antibiotic resistance of enterotoxigenic and enteroaggregative Escherichia coli isolated from raw milk and unpasteurized cheeses. *Vet Res Forum.* 2014;**5**(1):29–34. [PubMed: 25568689].
21. El-Sharef N, Ghenghesh KS, Abognah YS, Gnan SO, Rahouma A. Bacteriological quality of ice cream in Tripoli-Libya. *Food Control.* 2006;**17**(8):637–41.
22. Jamali H, Paydar M, Radmehr B, Ismail S, Dadrasnia A. Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. *Food Control.* 2015;**54**:383–8.
23. Kargar M, Dianati P, Homayoon M, Jamali H. Isolation, Characterization and Antibiotic Resistance of Shiga Toxin-Producing Escherichia coli in Hamburger and Evolution of Virulence Genes stx1, stx2, eaeA and hly by Multiplex PCR. *J Fasa Univ Med Sci.* 2013;**3**(3):208–14.
24. UeNLUe T, Koluman A, Burkan ZT, Tezel A, Akcelik EN, Calim HD, et al. Incidence and antibiotic resistance of Escherichia coli isolated from different kinds of cheese. *J Food Safety.* 2011;**31**(1):54–60.
25. Abou-El Khair E, Salama AR, Radwan H, Khalafallah A, Arafa H. Bacteriological quality of packaged ice cream in Gaza city, Palestine. *J Food Nutrition Sci.* 2014;**2**(3):68–73.
26. Anvarinejad M, Mirzaei H. Microbial contamination of traditional ice-creams produced and marketed in maragheh during 2012. *J Food Hyg.* 2013;**3**(3):75–82.
27. Hassanzadazar H, Abdollahi R, Haj Gholizadeh G, Dalir Rad M, Mehdizadeh. T. . Investigating of the bacteriological contamination in traditionally manufactured ice creams in urmia city. *J Food Hyg.* 2012;**2**(5):1–9.
28. Salehian M, Salehifar E, Esfahanizadeh M, Karimzadeh L, Rezaei R, Molanejad M. Microbial Contamination in Traditional Ice cream and Effective factors. *Mazand Uni Med Sci J.* 2013;**23**(99):28–33.
29. Ambily R, Beena AK. Bacteriological quality of icecream marketed in Thrissur town, Kerala, India. *Veterinary World.* 2012;**5**(12).
30. Jadhav AS, Raut PD. Evaluation of microbiological quality of ice creams marketed in Kolhapur city, Maharashtra, India. *Int J Curr Microbiol App Sci.* 2014;**3**(9):78–84.
31. Shamila-Syuhada AK, Rusul G, Wan-Nadiah WA, Chuah LO. Prevalence and Antibiotics Resistance of Staphylococcus aureus Isolates Isolated from Raw Milk Obtained from Small-Scale Dairy Farms in Penang, Malaysia. *Pakistan Veterinary J.* 2016;**36**(1):98–102.
32. Rostamzad A, Rostamneia N. Prevalence of the Panton-Valentine Leukocidin Gene in Clinical Isolates of Staphylococcus aureus Isolated From Hospitals the Ilam Province of Iran. *J Clin Microb Infec.* 2016;**3**(1).
33. Nejad ASM, Shabani S, Bayat M, Hosseini SE. Antibacterial effect of garlic aqueous extract on Staphylococcus aureus in hamburger. *Jundishapur J Microbiol.* 2014;**7**(11).
34. Jahan M, Rahman M, Parvej MS, Chowdhury SMZH, Haque ME, Talukder MAK, et al. Isolation and characterization of Staphylococcus aureus from raw cow milk in Bangladesh. *J Advanced Veterinary Animal Res.* 2014;**2**(1):49–55.
35. Jamali H, Radmehr B, Ismail S. Short communication: prevalence and antibiotic resistance of Staphylococcus aureus isolated from bovine clinical mastitis. *J Dairy Sci.* 2014;**97**(4):2226–30. doi: 10.3168/jds.2013-7509. [PubMed: 24534509].
36. Aslani MM, Alikhani MY. Serotypes of enteropathogenic Escherichia coli isolated from children under 5 years of age. *Iranian J Public Health.* 2009;**38**(3):70–7.