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Original Research Article

Incidence of psychrotrophs bacteria with potential public health implications in ice cream sold in Zakho markets

Yousif Abdulla AlBany[1], Reem Qasim Mohammed[2], Nagham Mohammed Azzo[2], Mohammad Ismail Al-Berfkani[3]*

[1]Department of Nursing, Bardarash Technical Institute, Duhok Polytechnic University, Duhok, Iraq
[2]Department of Biology, University of Zakho, Duhok, Iraq
[3]Department of Medical Laboratory Technology, Zakho Technical Institute, Duhok Polytechnic University, Duhok, Iraq

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***Correspondence:**
Dr. Mohammad Ismail Al-Berfkani,
E-mail: berfkani85@gmail.com

ABSTRACT

Background: Foodborne illness are caused by the consumption of food that is contaminated with either bacteria or their toxins and the most common causes are psychotropic bacteria which cause major public health problem. The aim was to detect and enumerate pathogenic bacteria in locally made ice cream.
Methods: 250 locally made ice cream samples were randomly collected from automatic soft machines and dipping cabinets in the markets of Zakho city over a period of ten months starting in July 2016 to May 2017. All collected samples were transported to the microbiology laboratory. The most probable number methods used for detection and enumeration of pathogenic psychotropic bacteria.
Results: In general, both types of ice cream samples were showed heavy contamination with aerobic bacteria. The *Staphylococcus aureus* (MRSA) counts exceeded the standard limits in all samples; the count in dipping cabinets' samples was higher than automatic soft machines samples whereas Coliform count in automatic soft machines samples was higher than dipping cabinets' samples. High incidence of *Salmonella* and *Listeria monocytogenes* had been detected in all ice cream samples and the range in samples from automatic soft machines were higher than samples obtained from dipping cabinets.
Conclusions: This study indicates a high prevalence of pathogenic psychrotrophs in unpacked ice cream and poor hygiene during production, which make local ice cream unsuitable for consumption and it is indicating the need to monitor the hygienic quality of markets and the health authorities must be informed about such cases to avoid food poisoning.

Keywords: Coliform, *E. coli*, Ice cream, *Listeria monocytogenes*, Psychrotrophs, *Staphylococcus aureus* MRSA, *Salmonella typhi*

INTRODUCTION

Foodborne diseases are caused by the consumption of food that is contaminated with either bacteria or their toxins. The most common causes of foodborne illness are *Salmonella spp.*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*.[1-4] Psychrotrophic bacteria have an optimal temperature for growth above 15˚C and maximum temperature above 20˚C which make these bacteria widespread in natural environments and in foods.[5] The nutritional components of ice cream are enriched with minerals, proteins and fatty acids thus making it a perfect growth medium for a large spectrum of microorganisms, including a range of

Psychrotrophic bacterial species. Many studies have reported the prevalence of pathogenic Psychrotrophic in ice cream.[1,6-12] Despite the effective removal of *Staphylococcus aureus* via pasteurization in milk, its low infective dose and ability to survive unfavourable environments make this bacterium a serious risk to consumers.[10,13,14] Ice cream can be contaminated with *Staphylococcus aureus* by the introduction of flavourings and ingredients (nut and chocolate) during ice cream production or from the nasal cavities and hands of carriers during food production.[14] The pathogenicity and the ability of *Staphylococcus aureus* to cause diseases is attributed to the their heat stable enterotoxins.[15] *Staphylococcus aureus* produces fifteen enterotoxins. Although the role of newly identified enterotoxins in food poisoning is not fully clarified, 95% of staphylococcal food poisoning are caused by the five classical enterotoxins (SE, type A, B, C, D and E).[16]

Summer season in Duhok city this year was extremely hot, with an average high temperature of approximately 43ºC (109.4 °F) and frequent electrical outages, which led to temperature abuses during the storage, transportation and distribution of ice cream. Under these conditions, injured bacterial cells within ice cream have an opportunity for recovery, which was considered as the main cause of foodborne illness.[14,17] The main microorganism activated under these conditions is *Salmonella spp*., which causes Salmonellosis. Salmonellosis is the most common foodborne illness and is associated with the major outbreaks from ice cream consumption.[9,18-20] It is responsible for one million domestically acquired foodborne illnesses and more than 300 deaths each year in the United States.[3] *Salmonella* can survive for very long periods under less than optimal growth conditions and may originate from the inadequate pasteurization of milk, use of poultry eggs or cross-contamination of pasteurized ice cream in tankers.[18,21]

Listeriosis is food poisoning caused by eating foods contaminated with the *Listeria monocytogenes* bacterium and ranging from flu-like syndrome or gastroenteritis to deadline infection of nervous system causing meningitis and abortion in pregnant women.[22] *Listeria* is a facultative intracellular psychrotrophs bacterium that can survive and persist in a variety of frozen foods and food processing environments.[23] *Listeria* species are incredibly and hardy able to grow in refrigerator at 4°C to 37°C the body's internal temperature.[24] Low temperate around 4°C is not sufficient to prevent growth of *L. monocytogenes* as even small inoculums of bacteria held at these temperatures may outgrow competing organisms and capable of produce an infective dose sufficient to cause disease.[25] The presence of any *Listeria monocytogenes* in food may be an indicator of poor hygiene.[26] Many studies cited the *incidence of Listeria monocytogenes* in dairy milk and ice cream.[1,23,27]

Other foodborne pathogenic bacteria such as *Escherichia coli* are also present in the ice cream,[14,28,29] which is the main cause of diarrhoea, vomiting and food poisoning during summer. No microbial cells can resist long pasteurization conditions, but there is an opportunity for bacterial contamination due to the poor personal hygiene of people handling the ice cream and from contaminated water.[30]

Because ice cream is the most consumed product during summer, especially by children, it must be microbiologically safe.[6,31] Therefore, a microbial investigation of this product can provide valuable information on the quality of the raw milk, the ingredients and the sanitary conditions during the processing and packing stages. The aim of this study was to assess the prevalence of foodborne pathogenic bacteria in locally made ice cream sold at Zakho markets.

METHODS

This research was carried out over a period of ten months starting in July 2016 to May 2017. Two hundred and fifty samples of unpacked locally produced ice cream were randomly collected from five different markets of Zakho city. The locally produced ice cream was divided into two types; one collected from automatic soft machines and other collected from dipping cabinets. All samples were transported to the microbiology laboratory in Zakho and Bardarash technical institute in an icebox within one hour of collection and were kept frozen at -15°C until further microbiological analysis.

Microbiological analysis

To assess the prevalence of pathogenic bacteria in ice cream samples, the most probable number method was used for the detection and enumeration of pathogenic aerobic bacteria, *Staphylococcus aureus* and Coliform, *Salmonella* and *Listeria monocytogenes* from unpacked locally produced ice cream.

Detection and enumeration of aerobic bacteria

Serial dilutions up to 10^{-4} was made by adding 10 mL of ice cream sample to 90 mL of buffered peptone water (0.85% NaCl+0.1% peptone) then 1 mL of each dilution add to each three tubes containing 9 mL of nutrient broth (as enrichment broth) then incubate at 37°C for 24 hours. Following incubation period, a loopful was streaked on Nutrient Agar.[32] The plates were incubated at 35˚C for 48 hours and presence of typical colony on an agar plate was considered a positive result for the corresponding MPN tube. All typical colonies confirmed by standard biochemical reactions.[33] The number of tubes that contain the target bacteria had been recorded to obtain the most probable number.

Detection and enumeration of Staphylococcus aureus

Pre-enrichment step made by addition of 25 mL of each sample to 225 mL of Ringer solution and serial dilutions

up to 10^{-3} then 1 mL of each dilution add to each of the three tubes containing 9 mL of Brain Heart Infusion broth (as selective enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on Mannitol Salt Agar.[32] All plates were incubated at 35°C for 48 hours and yellow colony was selected and their identity was confirmed by standard biochemical reactions and their susceptibility to methicillin were tested.[33,34] the number of tubes that contain the target bacteria had been recorded to obtain the most probable number.

Detection and enumeration of coliform

For the enumeration of Coliform, serial dilutions up to 10^{-3} were made by adding 10 mL of each sample to 90 mL of buffered peptone water as pre-enrichment step then 1 mL of each dilution add to each of the three tubes containing 9 mL of MacConkey broth (as selective enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on MacConkey Agar plates.[32] The plates were incubated at 35°C for 48 hours and pink colony was selected and their identity was confirmed by standard biochemical reactions.[33] The number of tubes that contain the target bacteria had been recorded to obtain the most probable number

Detection and enumeration of salmonella

For detection and enumeration of *Salmonella spp*. Most-probable-number (MPN) technique was used. Serial dilutions up to 10^{-2} were made by adding 25 gram of each ice cream sample to 225 ml sterile buffered peptone water (0.85% NaCl+0.1% peptone) as pre-enrichment broth, then 1 mL of each dilution was add to each of the three tubes containing 9 mL of Tetrathionate broth (as selective enrichment broth) and incubate at 37°C for 24 hours than a loopful was streaked on Salmonella Shigella Agar.[32] The plates were incubated at 35°C for 48 hours and black centre colony was selected and their identity was confirmed by standard biochemical reactions. 33 Subculture on Triple Sugar Iron (TSI) slants and incubated at 37°C for 24 hours. The number of tubes that contain the target bacteria had been recorded to obtain the most probable number.

Detection and enumeration of Listeria monocytogenes

The Most-probable-number (MPN) technique was used to detect and enumerate L. monocytogenes. The ice cream samples were left at room temperature for 30 min until fully melted. 25 grams of each sample was suspended in 225 ml sterile buffered Listeria enrichment broth and serial dilutions made up to 10^{-2} then 1 ml of each dilution add to each MPN tubes and incubated at 30˚C. Selective supplements (10 mg/liter acriflavin hydrochloride, 40 mg/liter nalidixic acid, and 50 mg/liter cycloheximide) were added after 4 hours and incubation continued for an additional 44 hours. Following incubation, a loopful of Enriched cultures was streaked on Gum base-nalidixic acid tryptone soya Agar which was incubated at 37˚C for up to 48 hours. The presence of typical L. monocytogenes colonies on an agar plate was considered a positive result for the corresponding MPN tube. A subset of typical colonies was picked and Confirmation of Listeria isolates were done by Latex Agglutination test (Oxoid, UK), and differentiation of *Listeria monocytogenes* from other Listeria spp. was done by Oxoid Biochemical Identification System (O. B. I. S.mono test kit, Oxoid).[35,36]

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software version 16. Chi-square tests used to compare the mean counts of the total aerobic plate, total Coliform plate, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella spp*.

RESULTS

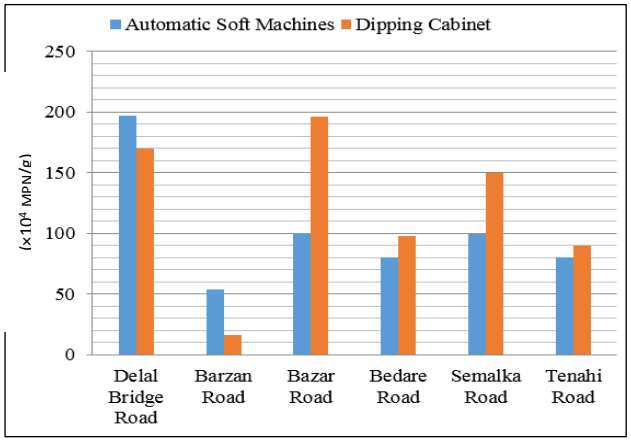
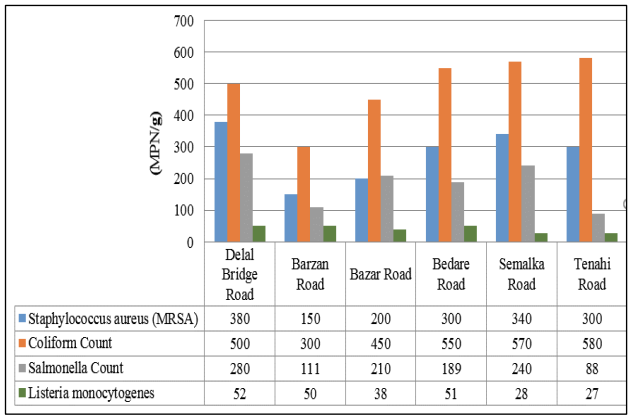


Figure 1: Total aerobic bacteria count (MPN/g).

The results of this study illustrate the prevalence of psychrotrophs pathogenic bacteria in local made ice cream sold in markets in Zakho city. According to food safety and standard regulations, all samples showed a heavy contamination of aerobic bacteria, exceeding the standard limits $25*10^4$ MPN/g. The bacterial load in samples obtain from the Automatic Soft Machines is shown in Figure 1. The counts of total pathogenic aerobic bacteria ranged between lowest values $54*10^4$ MPN/g to highest value $197*10^4$ MPN/g obtain from Delal Bridge Road, followed by samples from Bazar Road. however, the bacterial counts for samples obtained from the dipping cabinets is ranged from $16.3*10^4$ MPN/g to $196*10^4$ MPN/g, and the highest count observed from samples taken from Bazaar Road followed by samples from Delal Bridge Road (Figure 1).

All local ice cream samples exceeded 100 MPN/g of *Staphylococcus aureus* (MRSA), which is considered heavily contaminated, with the counts for samples from Automatic Soft Machines ranged between 150 MPN /g

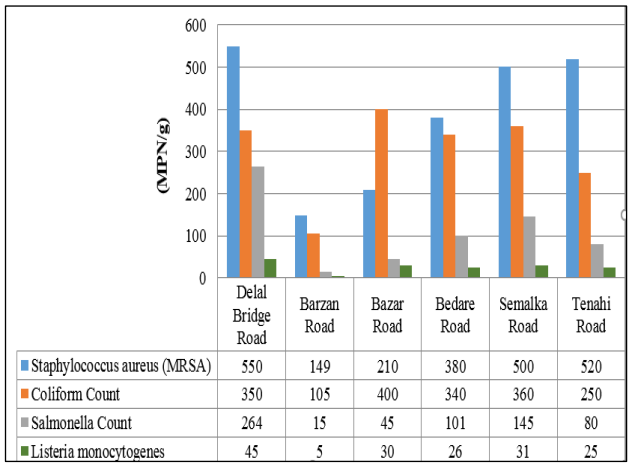
and 380 MPN /g, the highest load of value 380 MPN/g observed in the samples from Delal Bridge Road (Figure 2).



	Delal Bridge Road	Barzan Road	Bazar Road	Bedare Road	Semalka Road	Tenahi Road
Staphylococcus aureus (MRSA)	380	150	200	300	340	300
Coliform Count	500	300	450	550	570	580
Salmonella Count	280	111	210	189	240	88
Listeria monocytogenes	52	50	38	51	28	27

Figure 2: Bacteriological quality of ice cream samples collected from automatic soft machines.

The *Staphylococcus aureus* (MRSA) counts for samples from dipping cabinets ranging between 149 MPN/g and 550 MPN/g; the highest load was 550 MPN/g, in the samples obtained from Delal Bridge Road as shown in Figure 3.



	Delal Bridge Road	Barzan Road	Bazar Road	Bedare Road	Semalka Road	Tenahi Road
Staphylococcus aureus (MRSA)	550	149	210	380	500	520
Coliform Count	350	105	400	340	360	250
Salmonella Count	264	15	45	101	145	80
Listeria monocytogenes	45	5	30	26	31	25

Figure 3: Bacteriological quality of ice cream samples collected from Dipping Cabinets.

According to the industry standards, the presence of Coliform (*Escherichia coli*) in ice cream should not exceed 100 MPN/g. the range of Coliform (*E. coli*) in the samples obtained from Automatic Soft Machines was 300 MPN/g to 580 MPN/g, which exceeded the standard limits (Figure 1). The samples from dipping cabinets for Coliforms (*E. coli*) ranged between 105 MPN/g and 400 MPN/g, with the highest Coliform load observed in samples from Barzan Road, followed by samples from Delal Bridge Road (Figure 2).

Salmonella should be absent in 25 grams of a sample, as recommended by the food safety and standard regulations.[37] In this study, all samples showed *Salmonella* typhi contamination in as low as 1 gram of the sample. The range of *Salmonella typhi* in samples from Automatic Soft Machines ranged from 88 MPN/g to 280 MPN/g, with the highest load observed for samples taken from Delal Bridge Road (280 MPN/g) (Figure 1). The count of *Salmonella typhi* from samples obtained from dipping cabinets also showed heavy contamination, ranging between 15 MPN/g and 264 MPN/g, with the highest count of 264 MPN/g from samples taken from Delal Bridge Road (Figure 2).

The count of *Listeria monocytogenes* in ice cream should be absence in 25 grams of sample and in present study, all samples showed *Listeria* contamination in as low as 1 gram of the sample. The range of *L. monocytogenes* in samples from Automatic Soft Machines ranged from 27 MPN/g to 52 MPN/g, with the highest load observed for samples taken from Delal Bridge Road (52 MPN/g) as shown in Figure 1. Heavy contamination with *L. monocytogenes* was shown also in samples obtained from dipping cabinets with ranging between 5 MPN/g and 45 MPN/g, with the highest count of 45 MPN/g from samples taken from Delal Bridge Road (Figure 2).

DISCUSSION

Daily product such as ice cream is a quite complex frozen food containing milk, sugar, fats and emulsifiers and on condition that no bacteria contaminate any of the milk and ingredients after or during processing then ice cream while frozen is one of the safer commodities.[38] Depending on storage condition and available water, bacterial grow could be rapid in melted ice cream. If melted ice cream were contaminated and allowed to remain at elevated temperatures, freezing temperatures later would not make the product safe.[39]

This research shows the current status of bacterial quality of ice cream being sold in Zakho city, all 250 ice cream samples showed heavy contaminated with pathogenic aerobic bacteria. Similar results had been reported by many researches in different countries.[40,41] the level of the contamination was higher in samples obtain from dipping cabinets than in those from Automatic Soft Machines, which suggests poor sanitation, the use of contaminated equipment (e.g., spoons) and poor personal hygiene during production.[40]

AlBerfkani et al, reported presence of *S. aureus* in all packed ice cream samples sold in Zakho.[14] We found the microorganism at a higher rate than the above mentioned study. The levels of contamination by *S. aureus* (MRSA) in the dipping cabinet samples was higher than those in the Automatic Soft Machines samples, which indicate the poor personal hygiene and hard contaminated of the manufacturer. The main reservoir of *S. aureus* (MRSA) in human is the nasal cavity and skin from these sources; staphylococci find their way into air and dust, into clothing and in other place from which foods are contaminated. Since staphylococcal food poisoning is

intoxication and depends on the ability of food concerned to support the growth of the staphylococci, which produce the toxin. It is therefore important to consider that the processing and handling of the food products should be so designed to minimize contamination and to make unfavourable medium for the growth of these organisms. When susceptible foods are produced with low numbers of staphylococci, they will remain free of enterotoxin if kept either below 4℃ until consumed. The factors that contribute mostly to staphylococcal food-borne outbreaks may be due to inadequate refrigeration, preparing food far in advance of planned service, infected persons practicing poor personal hygiene, inadequate heat processing and holding food in warming devices at bacterial growth temperature.[42,43]

The present study revealed that ice cream samples were heavily contaminated with Coliforms. Although using Automatic Soft Machines for the production of ice cream minimizes direct hand manipulation and cross-contamination, the contamination levels were higher than in the samples from dipping cabinets due to unpasteurized milk, contaminated water and contaminated machine tanks.[44] The levels of Coliform contamination also exceeded the standard limits in samples obtained from dipping cabinets due to a lack of personal hygiene of the manufacturer and improper storage (e.g., frequent electrical cut off in the city). Similar, finding was also reported high incidence of *E.coli* form count in ice creams marketed in Iraq, Turkey, India and other countries.[40,45-47]

Salmonella spp. are resistant to freezing temperatures and consumption of ice cream contaminated with enterobacteria such as *Salmonella* has been the cause of several outbreaks. The nationwide outbreak of Salmonellosis was most likely the result of contamination of pasteurized ice cream premix during transport in tanker trailers that had previously carried non-pasteurized liquid eggs containing *S. enteritidis*.[9] In our research the *Salmonella typhi* count was exceeded the standard limits in every sample tested, which suggests that all of the products are unsuitable for consumption. These results can be supported by the work of various other researchers.[14,40,46] These bacteria can survive for a long time in ice cream but cannot survive in pasteurized milk. Their presence indicates post-process contamination and tanks that are contaminated with raw ice cream. The consumption of such products may result in case of food poisoning.[47,48]

Listeria monocytogenes is Psychrotrophic pathogenic bacterium and can cause listeriosis, which is characterized by fever, muscle ache and gastrointestinal symptoms, such as nausea or diarrhea. The infection can affect the nervous system causing headache, confusion, loss of balance or even convulsion. Healthy people are occasionally infected with *L. monocytogenes*, but rarely present serious symptoms, which make presence of *L. monocytogenes* in ice cream is a public health concern. The count of *L. monocytogenes* was exceeded the standard limits in every sample tested, which indicate that all ice cream samples are unsuitable for human consumption. Although *L. monocytogenes* cannot survive in pasteurized milk, *L. monocytogenes* have the ability to survive for a long time in frozen product such as ice cream and their presence indicates poor hygiene during manufacture. Presence of such pathogenic bacteria in ice cream presume that there is a potential health risk to the consumer and indicate the need for the implementation of monitoring of these microorganisms as much by producers as by health inspectors.

In the present study *Listeria monocytogenes* isolated from all locally produced ice cream samples and the load of bacteria in samples obtain from Automatic Soft Machines were higher than Dipping Cabinet samples either due to poor hygienic measurements and poor surveillance on product or contamination during production, processing, transportation and storage. Similar results were reported by other research[1,23,27].

CONCLUSION

The current investigation illustrates heavy contamination with Psychrotrophic pathogenic bacteria in ice cream due to low standards of hygiene in the service of locally made ice cream sold in Zakho markets. The high load of total microorganisms exceeds the standards of food safety and regulations; therefore, it is necessary to develop hygienic statutes for locally made ice cream. The pathogenic aerobic bacteria count was high in locally made ice cream, which indicates contamination during storage. The heavy contamination of locally made ice cream with Coliform and Salmonella illustrates poor sanitation during the preparation and storage of the products. The high load of *Staphylococcus aureus* (MRSA) in almost all of the samples represents the poor personal hygiene and the use of contaminated flavors and integrates, and presence of *L. monocytogenes* indicates poor hygiene during manufacture. It is clear from the results that ice cream from both dipping cabinets and Automatic Soft Machines is unsuitable for consumption in the Zakho markets, Duhok province, Kurdistan region, Iraq.

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REFERENCES

1. Chen Y, Burall LS, Macarisin D, Pouillot R, Strain E, De Jesus AJ. Prevalence and level of listeria monocytogenes in ice cream linked to a listeriosis outbreak in the United States. J Food Prot. 2016;79(11):1828-32.
2. Altekruse SF, Cohen ML, Swerdlow DL. Emerging foodborne diseases. Emerg Infect Dis. 1997;3(3):285-93.

3. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States-Unspecified agents. Emerg Infect Dis. 2011;17(1):16-22.
4. Vasavada PC. Pathogenic bacteria in milk- a review. J Dairy Sci. 1988;71(10):2809-16.
5. Moyer CL, Morita RY. Psychrophiles and Psychrotrophs. Encycl Life Scienes. 2007:1-6.
6. Warke R, Kamat A, Kamat M, Thomas P. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. Food Control. 2000;11(2):77-83.
7. de Oliveira GB, Favarin L, Luchese RH, McIntosh D. Psychrotrophic bacteria in milk: How much do we really know? Brazilian J Microbiol. 2015;46(2):313-21.
8. Pooran A, Seepersadsingh N, Georges K, Adesiyun AA. Evaluation of the bacteriological quality of ice cream sold in Trinidad. J Food Agric Environ. 2012;10:39-45.
9. Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, et al. A national outbreak of Salmonella enteritidis infections from ice cream. Investigation Team J Med. 1996;334(20):0-5.
10. Gucukoglu A, Cadirci O, Terzi G, Kevenk TO, Alişarli M. Determination of enterotoxigenic and methicillin resistant Staphylococcus aureus in ice cream. J Food Sci. 2013;78(5):M738-41.
11. Chug K. Salmonella outbreak from ice cream. Indian Pediatr. 1997;33:976-7.
12. Todd EC. Epidemiology of foodborne diseases: a worldwide review. World Heal Stat quarterlyRapport Trimest Stat Sanit Mond. 1997;50(1-2):30-50.
13. Gogov I, Slavchev G, Peeva T. Cold resistance of S. aureus and staphylococcal enterotoxins A and C2 in ice cream. Vet Nauk. 1984;21:46-50.
14. Al-Berfkani MI, Al-Bany YA, Mohammed RQ. Recovery of Freeze Injured Pathogenic Bacteria From Packed Ice Cream. J Food Heal Sci. 2016;2(3):123-129.
15. Sandel MK, McKillip JL. Virulence and recovery of Staphylococcus aureus relevant to the food industry using improvements on traditional approaches. Food Control. 2004;15(1):5-10.
16. Aitichou M, Henkens R, Sultana AM, Ulrich RG, Sofi Ibrahim M. Detection of Staphylococcus aureus enterotoxin A and B genes with PCR-EIA and a hand-held electrochemical sensor. Mol Cell Probes. 2004;18(6):373-7.
17. Abou-El Khair E, Salama AA, Radwan H, Khalafallah A, Arafa H. Bacteriological Quality of Packaged Ice Cream in Gaza City, Palestine. J Food Nutr Sci. 2014;2(3):68.
18. Dodhia H, Kearney J, Warburton F. A birthday party, home-made ice cream, and an outbreak of Salmonella enteritidis phage type 6 infection. Commun Dis Public Health. 1998;1(1):31-4.
19. Vought KJ, Tatini SR. Salmonella enteritidis contamination of ice cream associated with a 1994 multistate outbreak. J Food Prot. 1998;61(1):5-10.
20. Indar-Harrinauth L, Daniels N, Prabhakar P, Brown C, Baccus-Taylor G, Comissiong E, et al. Emergence of Salmonella enteritidis phage type 4 in the Caribbean: case-control study in Trinidad and Tobago, West Indies. Clin Infect Dis. 2001;32(6):890-6.
21. Zubair AI, Al-berfkani MI, Issa AR. Prevalence of Salmonella species from poultry eggs of local stores in Duhok. Int J Res Med Sci. 2017;5(6):2468-71.
22. Liu D. Handbook of Listeriamonocytogenes. 1st ed. USA: CRC Press; 2008.
23. Abrahao WM, Abrahao PR da S, Monteiro CLB, Pontarolo R. Occurrence of Listeria monocytogenes in cheese and ice cream produced in the State of Paraná, Brazil. Rev Bras Ciências Farm. 2008;44(2):289-96.
24. Cartwright, E. J., K. A. Jackson, S. D. Johnson, L. M. Graves, B. J. Silk, and B. E. Mahon. Listeriosis outbreaks and associated food vehicles, United States, 1998-2008. Emerg Infect Dis. 2013;19:1-9.
25. Jones D. Foodborne listeriosis. Lancet. 1990;336(8724):1171-4.
26. Kathariou S. Listeria monocytogenes virulence and pathogenicity, a food safety perspective. J Food Prot. 2002;65:1811-29.
27. Al-shamary AHA. Prevalence of Listeria in ice creams in Baghdad province Ali. Iraqi J Vet Med. 2010;34(2):39-44.
28. Foschino R. Freezing injury of Escherichia coli during the production of ice cream. Ann Microbiol. 2002;52:39-46.
29. Pooran A, Seepersadsingh N, Georges K, Adesiyun A. Evaluation of the bacteriological quality of ice cream sold in Trinidad. J Food Agric Environ. 2012;10(2):39-45.
30. Wilson IG, Heaney JC, Weatherup ST. The effect of ice-cream-scoop water on the hygiene of ice cream. Epidemiol Infect. 1997;119(1):35-40.
31. Çağlayanlar GE, Kunduhoğlu B, Çoksöyler N. Comparison of the Microbiological Quality of Packed and Unpacked Ice Creams Sold in Bursa, Turkey. J Arts Sci. 2009;(12):93-102.
32. Barrow GI, Feltham R. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Great Britain: Cambridge University Press; 1993.
33. Roberts D, Greenwood M. Practical Food Microbiology. 3rd ed. london: Blackwell; 2003.
34. Nccls. Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals: Approved Guideline M49-A. Vol 26. Wayne, Pennsylvania, USA: CLSI. Clinical and Laboratory Standards Institute.; 2006. Available at http://demo.nextlab.ir/getattachment/17f670fd-88b7-4f99-a720-2ab857361d54/M42-P.pdf.
35. Pagotto, F, Daley E, Farber J, Warburton D. 2001. Isolation of Listeria monocytogenes from all food

and environmental samples. HPB Method MFHPB-30. 2001.
36. Liu D.Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J Med Microbiol. 2006;55:645-59.
37. Kaferstein F, Abdussalam M. Food safety in the 21st century. Bull World Heal Organ. 1999;77(4):347-51.
38. Microbiological Risk Assessment of Ice-Cream. Risk Assessment Studies Report No. 7, Hong Kong; 2001. Available at http://www.cfs.gov.hk/english/programme/programme_rafs/files/ice_e.pdf.
39. Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology. 7th ed. India: Springer; 2005;790.
40. Yaman H, Elmali M, Ulukanli Z, Tuzcu M, Genctav K. Microbial quality of ice-cream sold openly by retail outlets in Turkey. Rev Med Vet (Toulouse). 2006;157(10):457-62.
41. Ambily R, Beena AK. Bacteriological quality of icecream marketed in Thrissur town, Kerala, India. Vet World. 2012;5(12):738-41.
42. Hossain KM, Kabir SL, Rahman MM, Rahman MB, Choudhury KA. Organoleptic and Microbial Quality of Ice Cream Sold at Retail Stores in Mymensingh, Bangladesh. J Microbiol Res. 2012;2(4):89-94.
43. Dagnew M, Tiruneh M, Moges F, Tekeste Z. Survey of nasal carriage of Staphylococcus aureus and intestinal parasites among food handlers working at Gondar University, Northwest Ethiopia. BMC Public Health. 2012;12(1):837.
44. Jadhav AS, Raut PD. Original Research Article Evaluation of microbiological quality of ice creams marketed in Kolhapur city, Maharashtra, India. Int J Curr Microbiol Appl Sci. 2014;3(9):78-84.
45. Anuranjini C, Geethu S, Dhanashree B. Bacteriological analysis of ice creams from Mangalore, south India. Indian J Med Res. 2008;127(1):91-2.
46. Abou E, Khair E, Salama AA-R, Mezyed HIA, Mohsen SM, Arafa H. Microbiological quality of artisanal-ice cream produced in Gaza city- Palestine. Int J Nutr Food Sci. 2014;3(3):222-9.
47. Ahmed K, Hussain A, Imran, Qazalbash MA, Hussain W. Microbiological quality of ice cream sold in Gilgit town. Pakistan J Nutr. 2009;8(9):1397-400.
48. Baylis CL, MacPhee S, Betts RP. Comparison of methods for the recovery and detection of low levels of injured Salmonella in ice cream and milk powder. Lett Appl Microbiol. 2000;30(4):320-4.

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