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Investigation of the Rate of the Bacterial Contamination of the Ice Factories in Bandar Abbas, Iran

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A R T I C L E I N F O

A B S T R A C T

Background: Pollution of drinking water and ice is one of the most serious ways of water borne diseases spread. The purpose of this study was to investigate the bacterial contamination of the ice produced by ice factories in Bandar Abbas.
Methods: In this descriptive, cross-sectional study samples were collected from seven ice factories in Bandar Abbas. Sampling was done by standard method. Amount of the Contamination of ice and water to coliforms was investigated by the Multiple Tube method to determine the MPN, isolate bacteria and identify the microorganisms using conventional bacteriological techniques and counting the total count of bacteria by the Plate Count method on a nutrient agar medium. The data was analyzed by the SPSS software.
Results: In this study, a total of 84 samples were investigated. Gram-positive bacteria (65.5%) and gram-negative bacteria (34.5%) were separated from each other. The MPN rate in samples and total count of bacteria were 0->1100 and $2×10^1×10^4$ CFU/mL, respectively.
Conclusion: The results suggest that necessary precautions be taken by environmental health specialists and other public health authorities in production, transportation and distribution of the ice blocks to reduce the rate of bacterial contamination.

1. Introduction

Ice is used for cooling beverages, as well as storing and packaging food, especially fresh seafood. For a long time, transmission of the pathogens through the use of ice has been taken into consideration. Pollution of ice usually occurs through the contaminated water, inappropriate methods of distribution, transportation, and improper maintenance.

Various studies have shown the microbial contamination of drinking water and ice products, while the ice contamination by the contaminants like bacteria, family Entrobacteriacae, gram-positive pathogenic bacteria, Vibrio cholera etc. have been reported as well. These contaminants cause disease outbreak transmitted through contaminated water and ice.

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Epidemics caused by Salmonella and Shigella due to the use of polluted water in ice products have been also reported repeatedly [1].

There are Reports about separating the bacteria of water-borne pathogens such as the species of *Mycobacterium fortuitum*, *Mycobacterium gordona* and *Legionella pneumophilla* [2]. Cases of ice contamination with *Stenotrophomona smalthophilia* were reported that can cause septicemia in patients with leukemia [3].

In addition, other water-borne pathogens such as *Legionella pneumophilla* and various species of *Mycobacterium* such as *Mycobacterium fortuitum* and *Mycobacterium gordona* are separated from the ice [2]. The city of Bandar Abbas is potentially predisposed to the appearance of these diseases, due to its hot and humid climate and people's urgent need for ice consumption (it should be noticed that the ice must be provided from the drinking water). The aim of this study was to determine the ice contaminating organisms, which play an important role in the transmission of these diseases and have been less considered.

2. Materials and Methods

During the cross-sectional study of the ice factories in Bandar Abbas through census method samples were taken from 7 factories. In total, 84 samples were collected, because the pollution may vary with the seasons. Hence, three samples were taken in each season (including the water in the ice molds before freezing, consumable water and water reservoirs in the factories), and each factory was sampled four times. Samples of 200 mL volume held in special containers in 0.2 mL Sodium thiosulfate1% and sterile conditions were collected and transferred to the microbiology laboratory under standard conditions.

Turbidity of the samples was measured by a turbid meter (Hach 2100-p), pH by pH meter (Cp-501 Elmetron) and residual chlorine was measured by the DPD kit. Coliform bacteria were counted based on the Multiple Tube Fermentation method to determine the MPN. Total count of bacteria was performed by Plate Count Method, samples were inoculated with1 mL of the medium. In addition to the direct inoculation, the samples of 1 / 10 were diluted and the volumes of 1 mL were inoculated in the nutrients agar and incubated at 37°C [4]. To isolate and identify the bacteria, 2 mL of samples was inoculated in Brain heart infusion broth and peptone water. The samples were kept in a 37ºC incubator for 12-24 hours and were cultured on the blood agar and Eosin- Methylene Blue agar to separate the gram-positive and gram-negative bacteria and from peptone water on the Thiosulfate citrate bile salt sucrose Agar to separate Vibrio Cholera. After observing colony morphology and Gram stain, the initial screening tests including catalase, oxidase and coagulase were performed. Other differential biochemical tests were used according to the type of the bacteria. SPSS software was used for data analysis.

3. Results and Discussion

In this study, 16 bacterial genera were isolated from samples. Altogether, 84 samples, including drinking water, ice molds and reservoirs, were collected from seven ice factories in the city of Bandar Abbas, among which there were a wide range of bacteria including gram-positive bacteria (65.5%). Isolated in 12 cases (14.4%), coagulase-negative Staphylucoccus indicates that the environmental pollution and contamination is a result of maintenance and distribution. These bacteria are mostly skin normal flora [5].

Staphylococcus aureus (6%), formed 5 cases of isolated bacteria. Since this bacterium is a human flora, the sources of pollution, are those individual vectors that carry the bacteria in their body. Those bacterial strains that have enterotoxin gene can cause food poisoning by producing enterotoxins [6].

Most bacteria isolated from samples were Diphtheroids, Bacillus and Coagulase negative staphylococci. Gram-negative bacteria (34.5%) were separated from the samples. *Pseudomonas aeruginosa* 9(10.7%), *Acinetobacter genus*4(4.8%) included species of *A.baumanni* 2(2.4%), *A.calcoaceticus* 1(1.2%), *A.lowffii* 1(1.2%), *Enterobacter* genus 9(10.7%) included species of *E.sakazaki* 4(4.8%), *E.amnigenus* 1(1.2%) and *E.agglomerans* 4(4.8%), The

Klebsiella genus 3(3.6%), include species of *K.pneumonia 2(2.4%), K.terrigena 1(1.2%)* and the *Providentia* genus included species of

P.rustigionii 1(1.2%) and *P.heumbachae 1(1.2%)* (Table1).

Table 1: Frequency of bacterial contamination of the ice factories of Bandar Abbas.

Microorganism	Frequency of microorganisms isolated			Total frequency (%)
	Ice molds N=29	Reservoirs N=26*	Water N=29	
Diphtheroides	12	12	12	36(42.9%)
Bacillus spp	7	9	4	20(23.8%)
Flavobacter odoratum	4	0	1	5(6%)
Acinetobacter spp	1	2	1	4(4.8%)
Pseudomonas aeruginosa	6	2	1	9(10.7%)
Pseudomonas spp	0	0	1	1(1.2%)
Serratia marcescens	1	0	0	1(1.2%)
Micrococcus spp	2	3	0	5(6%)
Coagulase negative Staphylococcus	4	3	5	12(14.4%)
Citrobacter freundii	1	0	0	1(1.2%)
Entrobacter spp	5	2	2	9(10.7%)
Morganella morgani	1	0	0	1(1.2%)
Staphylococcus aureus	3	2	0	5(6%)
Tatomella ptyseos	1	2	0	3(3.6%)
Providencia spp	1	1	0	2(2.4%)
Escherichia coli	2	0	0	2(2.4%)
Klebsiella spp	3	0	0	3(3.6%)

* A factory has not reservoirs

Two cases of *E.coli* (2.4%) were taken apart from ice molds. These bacteria were introduced as the indicators of fecal contamination of water and food and some of the serotypes with different mechanisms were involved in causing diarrhea.

Separating them indicates that water and ice are contaminated by human or animals' fecal material through the use of contaminated water and also inappropriate distribution and maintenance [7].

Pseudomonas aeruginosa was separated from 9 samples (10.7%), although this bacterium has an environmental origin, it is able to create a wide range of diseases in humans. The bacteria cause fatal necrotizing colitis in neutropenic patients, and also enteritis similar to typhoid [8].

Other opportunistic bacteria that are normally found in the nature such as non-fermentative gram-negative bacilli, including *flavobacter,*

Acinetobacter, Pseudomonas species*,* gram-positive bacilli such Diphtheroids, Bacillus and gram-positive cocci such as *Micrococcus*, make 71 cases in total (84.7%). These microorganisms are the indicators of environmental pollution [9].

Acceptable levels of counting bacteria found in ice at temperatures under 37°C is 500CFU / mL [10]. Among 29 cases of ice molds as the samples of this study, 15 cases (51.7%) were non-consumable, since the number of bacteria was over the acceptable levels. *E.coli* (2.4%), was separated from 2 samples (MPN: 53, 240), *Enterobacter* (10.7%) from 9 samples (MPN: 9.1, 43, 75, 240, 460, >1100), *Klebsiella* (3.6%) from 3 samples (MPN: 9.1, 75, 75), and *Citrobactr freundii* (1.2%) from 1 sample (MPN: 460). The results of the total count of bacteria isolated from samples in different seasons   are shown in Table 2.

Table 2: The frequencies of bacteria isolated from ice factories of Bandar Abbas in different seasons.

Sample	CFU/mL / Season	0-10	11-50	51-100	101-999	≥1000	Total
Ice mold	Spring	4(40%)	1(10%)	3(30%)	1(10%)	1(10%)	10(100%)
	Summer	5(83.3%)	1(16.7%)	0(0%)	0(0%)	0(0%)	6(100%)
	Fall	6(85.7%)	0(0%)	0(0%)	0(0%)	1(14.3%)	7(100%)
	Winter	5(83.3%)	0(0%)	0(0%)	0(0%)	1(16.7%)	6(100%)
	Total	20(68.9%)	2(6.9%)	3(10.3%)	1(3.4%)	3(10.3%)	29(100%)
Reservoirs	Spring	7(77.8%)	0(0%)	0(0%)	1(11.1%)	1(11.1%)	9(100%)
	Summer	6(100%)	0(0%)	0(0%)	0(0%)	0(0%)	6(100%)
	Fall	6(100%)	0(0%)	0(0%)	0(0%)	0(0%)	6(100%)
	Winter	5(100%)	0(0%)	0(0%)	0(0%)	0(0%)	5(100%)
	Total	24(92.3%)	0(0%)	0(0%)	1(3.8%)	1(3.8%)	26(100%)
water	Spring	10(100%)	0(0%)	0(0%)	0(0%)	0(0%)	10(100%)
	Summer	4(80.0%)	0(0%)	1(20.0%)	0(0%)	0(0%)	5(100%)
	Fall	6(85.7%)	0(0%)	0(0%)	11(14.3%)	0(0%)	7(100%)
	Winter	7(100%)	0(0%)	0(0%)	0(0%)	0(0%)	7(100%)
	Total	27(93.1%)	0(0%)	1(3.4%)	1(3.4%)	0(0%)	29(100%)

In Salek-Moghaddam and colleagues' study, the rate of non-consumable ice blocks was 68% and in the study performed by Wilson and colleagues, it was 25% [10-11]. The coliform existing in 100 ml of drinking water should not be detectable [11]. Two cases of the Coliforms, including *Enterobacter* found in the water used by the factories were less than 10 Coilforms per ml.

Environmental bacteria were isolated to the amount of 5 to 60 CFU / mL. Environmental bacteria were isolated to the amount of 5 to 60 CFU / mL. The Coliforms were isolated from the water used by those factories which were using the water derived from the wells and non-purified water. The factories are located in urban areas where the groundwater may get contaminated by the urban wastewater.

During the study which was implemented on ice blocks by Alireza Salek Moghaddam etal., in 1999 in Tehran, it has been found that most of the isolated bacteria belonged to the genera Diphtheroids (46%), *Pseudomonas aeruginosa* (42%), coagulase-negative *Staphylococcus* (40%), *Bacillus* (20%), *Escherichia coli* (10%) and Coliforms (6%). The bacteria were isolated from the whole samples of ice blocks. As a result, none of the samples were free of bacteria [10]. In the review on the ice blocks in Tehran, since the samples were collected from the city, the contamination with Environmental bacteria and *E.coli* was observed at higher levels. In the present study, since the sampling is done in local ice factories, primarily, less pollution will still be observed and the results will confirm this. In another study conducted in Mashhad by Abdolkrim Hamedi etal., among 200 cases of ice

and iced water, 24 cases were infected by a kind of Vibrio, of which 1% was *Vibrio cholera*. Also, Proteus (0.5%), Salmonella Para typhi (5%), *Escherichia coli* (13%) and *Aerobacter* (7%) were isolated [12]. In this study, *Vibrio cholera* was not isolated. *Salmonella* and *Shigella* were not isolated from the under-the-inspection samples which is consistent with the low rates of *E. coli*.

Measuring the turbidity is the key to control the water quality. Considering that the allowable turbidity of drinking water is 1 unit of NTU (Nephlometric Turbidity Unit), [13].

Minimum turbidity of samples was 0.39 NTU and Maximum turbidity of samples was 4.94 NTU, which are related to the consumable water and ice molds, respectively (Table 3).

Table 3: Frequencies of turbidity in the ice factoriesof Bandar Abbas.

Sample / Turbidity (NTU)	Ice molds N (%)	Reservoirs N (%)	Water N (%)	Total N (%)
0-0.5	1(1.2%)	2(2.4%)	8(9.6%)	11(13.2%)
0.51-1	12(14.4%)	16(19.2%)	16(19.2%)	44(52.8%)
1.1-2	14(16.8%)	6(7.2%)	4(4.8%)	24(28.8%)
2.1-3	1(1.2%)	1(1.2%)	1(1.2%)	3(3.6%)
3.1-4	0(0 %)	0(0 %)	0(0 %)	0(0 %)
4.1-5	1(1.2%)	1(1.2%)	0(0 %)	2(2.4%)
Total	29(34.5%)	26(31%)	29(34.5%)	84(100%)

As the results show 29 samples (34.5%) had the turbidity higher than the standard criteria and the highest frequency was observed in the ice molds (16.8%). The consumable water had one case of high turbidity that was related to the factory for which the water was supplied from wells. Chlorine was used to disinfect the water.

The optimal pH for the drinking water is 7 to 8.5. The highest pH range of the samples was

The optimal pH for the drinking water is 7 to 8.5. The highest pH range of the samples was between 7.6-7.8. Only one case of samples had pH=7.

However, the parameters do not have any significant effects on chlorination and Coliforms.

Residual chlorine was used as the safety factor to control the secondary pollution of water. The results of this study indicate that in 28 cases (33.6%) such certainty could not be found. Most

cases were related to the ice molds, 20 cases (24%) and in 4 cases (4.8%) of the water this

certainty was not observed either. The results of residual chlorine are shown in Table 4.

Table 4: Frequencies of residual chlorine in samples of ice factories of Bandar Abbas.

Sample Residual Chlorine(mg/L)	Ice molds N(%)	Reservoirs N (%)	Water N (%)	Total N (%)
0	20(24%)	4(4.8%)	4(4.8%)	28(33.6%)
o.1-0.5	8(9.6%)	12(14.4%)	7(8.4%)	27(32.4%)
o.6-1.0	1(1.2%)	10(11.8%)	18(21.4%)	29(34.5%)
Total	29(34.5%)	26(31%)	29(34.5%)	84(100%)

4. Conclusion

In the present study, coliform bacteria, that cause food poisoning and are environmental bacteria of ice and water sources of the ice factories and whose importance is often ignored, can be considered as an important factor for the transmission of infectious diseases.

It is recommended that the ice producing centers pay enough attention to health issues and microbiological standards, including timely and sufficient chlorination. Moreover, we recommend the environmental health officers pay great attention to the fact that they supervise production, distribution and maintenance routes properly.

Acknowledgments

References

1. Anonymous: Shiglla Sonnei Outbreak Associated with Contaminated Drink Water Island Park. *J Am Med Assoc*.1996; 275- 1071.

2. Stout JE. Yu VL, Muraca P. Isolation of Legionella Pneumophila from the Cold Water of Hospital Ice Machines. *Infect Control Hosp Epidemiol*. 1985; 6: 141-146.

3. Spencer RC. The Emergence of Epidemic, Multiple-Antibiotic Resistant Stenotrophomonas Maltophilia and Burkholderia Cepacia. *J Hosp Infect*.1995; 30: 453-464.

4. Gholomi M, Hamedi M. Microbiology of Water and Wastewater. *Hayan*. 1999; 188-189. [In Persian].

5. Elmer WK. A Color Atlas and Text Book of Diagnostic Microbiology. *15 ed, Philadelphia: Lippincott*; 1997; 122-130.

6. Forbes Betty A, Sahm Daniel F, Weiss Feld A. Bailey and Sott's Diagnostic Microbiology. *Tenth ed, Boston: Mosby;* 1998; 606-618.

7. Holmes B. The Enterobacteriaceae in: Parker Tom M. Collier Leslie H. To ply and Wilson's Principle of Bacteriology, Virology, Immunity. Tented, Edward Arnold. *London*. 2000; 920-938.

8. Porco FV, Visconte EB, Pseudomonas Aeruginosa as a Cause of Infectious Diarrhea

Successfully Treated with Oral Ciprofloxacin. *Ann Pharmacother*. 1995; 29: 1122-1123.

9. Committee on Microbiological Safety of Blood and Tissues for Transplantation. Guidance on the Microbiological Safety of Human Tissuses and Organs Used in Transplantation. *London: NHS Executive;* 1996; 1-16.

10. Salekmoghadam AR, Frooheshtehrani H. A Survey on Microbial Contamination on fifth Ice Samples from Different Areas of Tehran in 1999. *Iran UMS J*. 2002; 29: 239-244. [In Persian].

11. Wilson Q, Hogg G.M, Bar JG. Microbiological Quality of Ice in Hospital and Community. *J Hosp Infect*. 1997; 36: 171-180.

12. Hamedi A, Khosravi Khorashad A, Omidi A. Cantamination of Ice and Cold Water with Vibrio Cholera in Different Part of Mashhad. *Hakim J.* 2002; 3: 235-239. [In Persian].

13. Alipoor V, Dindarloo K, Zare S. Microbial Quality of Drinking Water of Bandar Abbas' Buses. *Hormozgan Med J.* 2005; 4: 215-219. [In Persian].