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Short Communication

EVALUATION OF MICROBIAL CONTAMINATION IN ICE ACQUIRED FROM DIFFERENT AREAS IN BHIWANDI CITY

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ABSTRACT

When food establishments and restaurants make ice for consumption, it is important to use water free from pathogens or any elements causing health hazard. Freezing can reduce number of culturable microbes but does not eliminate them completely. Coliforms are indicator of water quality. The Bureau of Indian Standards (BIS) establishes standards for safe drinking water and has set goal for zero coliforms. The objective of present study was to determine the microbial contamination of ice in food establishments and restaurant's in Bhiwandi, a town near Thane district in Maharashtra. Ten ice samples from local food establishments and restaurants (5 each) were analyzed using BIS standard method. Out of 10 samples one Ice sample contained *E.coli* (10%), 2 samples contained *Corynebacterium* (20%), 1 sample contained *Streptococcus Pyogenes* (10%), 2 contained *Streptococcus Agalactiae* (20%), 1 contained *Salmonella Paratyphi B* (10%), 2 contained *Enterococcus* (20%), 3 contained *Staphylococcus Epidermidis* (30%), 2 contained *Viridans Streptococci* (20%), 3contained *Peptostreptococcus* (30%), 1 contained *Streptococcus Pneumoniae* (10%), 1 contained *Pseudomonas Aeruginosa* (10%) and 1 sample also contained Fungal contaminant i.e. *Aspergillus Spp*. (10%). The current methods for inspecting ice at food establishments are insufficient for determining microbial contamination. Contaminated ice may contain pathogens and is a public health concern. Further research should be conducted to reduce the public health risk of ice and drinking water contamination in food establishments.

Keywords: Ice, Heterotrophes, Contamination, Coliforms, *E.coli*, Drinking water

1. INTRODUCTION

The ability of ice to be a vehicle for the transmission of pathogenic microorganisms has been recognized for some time. When food establishments make ice for consumption, it is important to use water free of pathogens and to employ hygienic practices. Ice may become contaminated through the use of contaminated water, by food service staff, by customers, and due to environmental factors within ice machines.

A study that placed contaminated ice in beverages found that, even with high alcohol content, not all pathogens were eliminated [1]. In the late 1990s, after an outbreak on three cruise ships affected more than 1,300 individuals, an epidemiological investigation was launched. Isolates of enterotoxigenic Escherichia coli (ETEC) were recovered from stool samples. The investigation identified contaminated water used to make ice as the vehicle for ETEC transmission [2].

There have been several well-known case reports that demonstrate contaminated ice machines spread disease. Ice from a poorly maintained machine at a Louisiana correctional facility is suspected for causing a Francis Ella novicida outbreak among inmates [3]. In this outbreak, three immunocompromised inmates were infected with the pathogen and one died after being admitted to the hospital. Several epidemiological investigations in hospitals have implicated ice machines as the most likely cause for the spread of Legionella [4-6]. Total *coliforms* are groups of closely related bacteria and include faecal coliform bacteria, which are normally found in the intestines of warm-blooded animals. The presence of faecal *coliforms* indicates that human or animal faecal materials, and pathogens associated with faecal material, are present. Due to the variety of bacteria, parasites, and viruses that can cause disease when ingested, *coliforms* are used as an indicator of sanitary quality. Testing for each pathogen individually is too expensive and impractical.

Another commonly used tool to measure water quality is the heterotrophic plate count (HPC). Heterotrophs are a broad group of microorganisms that include bacteria,

yeasts, and molds. The microorganisms cultured from HPC can vary greatly between locations, seasons, and consecutive samples at the same location [7]. Although concentrations of heterotrophic bacteria may not have a direct health effect, they can be used as an analytical tool to determine the amount of bacterial contamination in the water, and lower concentrations of bacteria may indicate a well maintained system [8]. The EPA limit for HPC is 500 colony forming units (CFU) per millilitre. High concentrations of heterotrophs can also interfere with the detection of pathogens [9]. The Bureau of Indian Standards (BIS) establishes standards for drinking water, and has set a goal for zero total *coliforms*. Another commonly used tool to measure water quality is the heterotrophic plate count (HPC). The BIS limit for HPC is 100 colony forming units (CFU) per millilitre .the standard limit of Conventional methods of culturing microorganisms to determine water contamination have limitations.

The basic objective of the study was to determine microbial contamination of ice as it could lead to improved sanitation practices which could improve public health.

2. MATERIALS AND METHODS

2.1. Description and collection of sample

Total 10 samples were collected from 5 food establishments and 5 restaurants of Bhiwandi city (Table 1). A single ice sample was taken from each location. Sterile Zip lock bags were used for sample collection. The collected samples were then shifted in a cold box to the laboratory of Department of Biotechnology, K. M. E. Society's G.M Momin Women's College, Bhiwandi.

2.2. Microbiological analysis

2.2.1. Bacterial Screening and Analysis

For aerobic enumeration of colonies, 0.1 ml sample suspension was cultured on Nutrient agar (HIMEDIA-M001) and incubated at 37°C for 24 h. The following day, total number of colonies were counted and read morphology and Gram Stained for their identification. Separated colonies were sub culture on freshly prepared Nutrient agar (HIMEDIA-M001), MacConkey (HIMEDIA-M081B), Blood Agar, Mannitol Salt Agar (HIMEDIA-M118), Endo Agar (HIMEDIA-M029),Urea Broth Base (HIMEDIA-M111), EMB Broth (Eosin Methylene Blue Broth)(HIMEDIA-M317), Slanetz & Bartely Medium (HIMEDIA-M549), Differential Reinforced Clostridial Agar (HIMEDIA-M1603) and Brilliant Green Agar Medium plates. Further identification of the Gram negative colonies was performed by biochemical tests such as production of urease, utilization of sugars, carbon and production of indole, H_2S gas, oxidase and the gram positive bacteria colonies were identified by coagulase and catalase tests. The Antibiotic Sensitivity (AST) was carried out on Mueller Hinton Agar (M173).

2.2.2. Fungal Screening And Analysis

0.1 ml sample suspension was cultured on Sabouraud Dextrose Agar (HIMEDIA-GM063) and incubated at 37°C for 24 h. The following day, total number of colonies were counted and read morphology and Lactophenol Stained for their identification. Separated colonies were sub culture on Sabouraud Dextrose Agar (HIMEDIA-GM063) and Potato Dextrose Agar (HIMEDIA-MH096).

2.3. Colony counts

Microbial counts were done with digital Gallenkamp colony counter. Total count was expressed as colony forming units per millilitre (CFU/ml).

2.4. Characterization and identification of isolates

Bacteria isolates was identified by the methods described in Bergey's manual of determinative bacteriology (7th Ed.)And Barnett & Hunter - Illustrated Genera of Imperfect Fungi (4th ed.) were used to identify fungi respectively.

3. RESULTS

The total viable counts in samples of the ten locations of Bhiwandi City are presented in Table 1. The samples of many of the areas show heavy contamination of bacteria ranging from > $1.2x10^3$ CFU/g to $5.7x10^4$ CFU/g (Table2).

Table 3 shows the general characteristics and identities of the bacteria in the investigated ice samples. *Streptococcus sp*. is the commonest species and was isolated from 03 samples of different areas i.e. in sample of Khadak Road, Old Fish Market and Ekta Chawk (Khadipar). *Staphylococcus sp*. was isolated from 03 samples i.e. samples of Old Fish Market, Food Inn Restaurant, Pioneer Juice Center.

Overall isolation of *Peptostreptococcus* was from 03 samples *i.e*. in sample of Khadak road, Pioneer Juice Center and Ekta Chawk (Khadipar). *Corynebacterium* was isolated from 2 samples, i.e. in sample of Khadak road, and Pioneer Juice Center. *Escherichia coli* was isolated from 01

sample i.e. Old Fish Market. *Salmonella* sp. was isolated from 01 sample i.e. Old Fish Market. *Enterococcus* sp was isolated from 02 samples i.e. Pioneer Juice Center and Ekta Chawk (Khadipar). *Pseudomonas* sp was isolated from 01 sample *i.e.*Ekta Chawk (Khadipar). *Aspergillus* sp. was isolated from just 01 sample *i.e.*Domino's Pizza.

Table 1: Total Viable count (CFU/g) of various bacteria isolated from ice samples

Sample no.	Area	Total viable Bacterial count cfu/g
Sample 1	Khadak Road	$4.2X10^3$
Sample 2	Old Fish Market	$5.5X10^4$
Sample 3	Food In Restaurant	$1.2X10^3$
Sample 4	Hotel Regent Garden	00
Sample 5	Pioneer Juice Center	$4.9X10^3$
Sample 6	Café Nine	00
Sample 7	EktaChawk(Khadipar)	$5.7X10^4$
Sample 8	The Flora Family Restaurant	00
Sample 9	Domino's Pizza	$4.9X10^3$
Sample 10	Sarika Hotel	00

Table 2. Characteristics of bacteria isolated from ice samples on bacteriological medium (Nutrient Agar)

Sample Number	Colony characteristics	Grams Nature
1.1,2.5	Cream, moist, circular, smooth, convex, opaque colonies	Gram +ve Cocci in chains
1.2	Small, smooth and shiny low convex, pale yellow colonies	Gram +ve cocci, nonspore forming
1.5,5.3	Small, smooth, yellowish/creamy, granular, translucent, convex colonies	Gram +ve Rods, non-spore forming
1.7,5.4,7.1	Light yellow colonies vary from raised, dull and smooth to convex and dull.	Gram +ve cocci, nonspore forming
2.2	Large, thick, greyish white, moist, smooth, opaque or translucent colonies	Gram –ve Rods
2.3	Smooth, pale, opaque, raised colonies	Gram –ve Rods
2.6,3.5,5.1	Pale, raised, cohesive, smooth, opaque colonies	Gram +ve Cocci in grape like clusters
2.8	Smooth, pale yellow, raised, moist colonies	Gram +ve cocci
5.2,7.2	Small, circular, smooth and shiny cream colonies	Gram +ve Cocci in pairs, chain
7.3	Irregular, smooth, cream, translucent or mucoid colonies	Gram +ve Diplococci, lancet shaped
7.4,7.5	Pale yellow, circular, smooth, flat, mucoid colonies	Gram +ve Cocci in chains
7.6	Pale, rough, flat, dry colonies	Gram –ve Rods

Key: + positive, - negative, V-Variable

Cat: Catalase; Coa: Coagulase: Hem: Haemolysis; Oxi: Oxidase; Ind: Indole; MR: Methyl Red; VP:Voges Proskauer's Test; Cit: Citrate Utilization; L:Lactose; S:Sucrose; G:Glucose; MSB: Mannitol Salt Broth; Nit: Nitrite Reduction; Opt: Optochin Sensitivity Test; Bac; Bacitracin Sensitivity Test; Nov: Novobiocin Sensitivity Tes

Table 4 shows incidence of different bacteria isolated during the investigation. The infestation ratios were: *Escherichia coli* 1(10) 10%**,** *Corynebacterium 2*(10) 20%, *S. Pyogenes* 1(10)10%, *Streptococcus Agalactiae 2*(10)20%, *Salmonella sp.1*(10) 10%, *Enterococcus sp. 2*(10)20%, *Staphylococcus Epidermidis* 3(10) 30%, *Viridans Streptococci* 2(10)20%, *Peptostreptococcus 3*(10) 30%, *Streptococcus pneumoniae* 1 (10)10%), *Pseudomonas aeruginosa*.

Table 3: Bio-chemical Characteristics of bacteria isolated from ice samples

Sample No.	Biochemical Characteristics															
1.1,2.5	Cat	Coa	Hem	Oxi	Ind	MR	VP	Cit	L	S	G	MSB	Nit	Opt	Bac	Nov
1.2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1.5,5.3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1.7,5.4,7.1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2.2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2.3	+	-	-	**V**	+	+	-	-	+	**v**	+	+	-	-	-	-
2.6,3.5,5.1	+	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-
2.8	+	-	-	+	+	+	-	-	-	-	+	**v**	-	-	-	-
5.2,7.2	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
7.3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7.4,7.5	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7.6	+	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-

Key: + positive, - negative, V-Variable

Cat: Catalase; Coa: Coagulase: Hem: Haemolysis; Oxi: Oxidase; Ind: Indole; MR: Methyl Red; VP:VogesProskauer's Test; Cit: Citrate Utilization; L:Lactose; S:Sucrose; G:Glucose; MSB: Mannitol Salt Broth; Nit: Nitrite Reduction; Opt: Optochin Sensitivity Test; Bac; Bacitracin Sensitivity Test; Nov: Novobiocin Sensitivity Test

Table 4: Identity and percentage infestation of heterotrophs isolated from Ice samples

Samples	Name of the isolate	Percentage infestation
1.1,2.5	Streptococcus agalactiae	20%
1.2	Streptococcus pyogenes	10%
1.5,5.3	Corynebacterium	20%
1.7,5.e4,7.1	Peptostreptococcus	30%
2.2	Escherichia coli	10%
2.3	Salmonella sp.	10%
2.6,3.5,5.1	Staphylococcus epidermidis	30%
2.8	Unknown	10%
5.2,7.2	Enterococcussp	20%
7.3	Streptococcus pneumoniae	10%
7.4,7.5	Viridans streptococci	20%
7.6	Pseudomonassp	10%
9.1	Aspergillus sp	10%

4. DISCUSSION

The objective of this study was to determine the microbial contamination of ice at food establishments in Bhiwandi, Maharashtra. The contamination of ice at food establishments was compared to the EPA and WHO standards for water quality. Heterotrophs and indicator organisms, such as *coliforms*, were used to determine the sanitary quality of ice and water. The results obtained in this study represent the current status of microbiological quality of ice being sold in Bhiwandi city. The higher rate of ice contamination found in this study compared with previous research may be due to a smaller sample size.*Coliform* and *E.coli*counts from Bhiwandi food establishments were relatively low than the Heterotrophs count. None (00%) of the 10 ice samples contained *coliforms*. Analysis of the samples determined that of the samples determined that 1(10%) of these samples also contained *E.coli*. But when the samples were analyzed for heterotrophs, it was found that the samples contained 20% of *S. agalactiae*,10% *S. pyogenus*,20% *Corynebacterium*, 30% *Peptostreptococcus,* 10% *S. paratyphi B*, 30% *S.epidermi dis*, 20% *Enterococcus*, 20% *Viridans Streptococci*, 10% *S. Pneumoniae,* 10% *P. aeruginosa and also 10% of Aspergillus* fungi. Such high numbers of positive heterotrophs samples indicated ice machines maybe contaminated and can become a public health issue

There was a significant difference in the number of *coliforms* in ice compared to number of heterotrophs in ice samples, indicating that water used to make ice is not only the source of contamination, this may be attributed to a local contamination near where the water is dispensed. The large number of contaminants found in ice indicates that ice machines may not be cleaned as often as recommended and nozzles where water is dispensed may be contaminated or the accessories, such as ice scoops and buckets, may have contamination.

Food establishments in Bhiwandi, Maharashtra, may need more regular inspection and cleaning of ice machines.

The contamination found in this study was higher than previous studies (in Mumbai). This may have been due to the limitations of a small sample size. These results may not be representative of Bhiwandi food establishments or restaurants. Further research should be conducted to determine the source of contamination and how to reduce the microbial risk of ice and drinking water contamination in food establishments. Similar study has been carried out with some variations which support the investigation in present study. There are many limitations associated with diagnosing waterborne disease, such as individuals not seeking medical attention, as the disease is usually self-limiting. This makes it even more challenging to determine if disease is linked to ice.

In one of the study by Jadhav *et al*. [10] all samples showed positive growth of bacteria ranging from 1.2 $x10^3$ to 8.0 x 10^7 cfu/g. The study revealed that all the samples showed positive growth of coliform bacteria while 40% ice cream samples were contaminated by the *E. coli*, 33% samples showed positive growth of *Salmonella*, 40% showed growth of *Staphylococcus aureus*and 53% *Shigella species*.A study was conducted regarding bacteriological quality of local made open scoop ice creams sold by street hawkers indifferent areas of Jalandhar city, Punjab which showed heavy contamination of bacteria ranging from $0.1\times10^9$to 10.2×10^9cfu/g [11].

5. CONCLUSION

The objective of this study was to determine the microbial contamination of ice at food establishments & restaurants in Bhiwandi, Maharashtra. There are various ways that microorganisms can be introduced into ice. Therefore, ice was collected from each food Establishment in the study. Culture analysis showed that 10%of the ice samples contained *E.coli*. HPC testing indicated that 6 of the 10 (60%) samples exceeded EPA limits for heterotrophs. The current methods for inspecting ice at food establishments are insufficient for determining microbial contamination.

This was a pilot study using a convenience sample. Ice contamination levels found in this study does not necessarily represent all the food establishments in Bhiwandi, Maharashtra.

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