

Detection of drug resistant bacteria in different ice creams available in local market of Dhaka city

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Sound microbiological quality of ice cream should be ensured for being a widely popular dairy food in the world. Present study was conducted to determine the microbiological quality of different ice cream samples available in Dhaka, Bangladesh. Total 3 ice cream samples were collected and processed to detect the microbiological quality as well as drug resistant trait of the isolates through several conventional Kirby Bauer method. All the samples were found to be contaminated with the total viable bacteria and fungi within the range of 1.2×10^4 cfu/ml to 4.3×10^6 cfu/ml. The presence of *E. coli*, *Staphylococcus aureus* was also observed up to 10^4 cfu/ml. However, the fecal contamination was totally absent in all the samples. Antibiotic profile of two isolates was measured against 8 commonly used antibiotics and both *E. coli* and *Staphylococcus aureus* were found to be resistant against more than one antibiotics. Appropriate hygienic and storage condition should be maintained concerning the health safety of consumers.

Key words: Ice Cream; Contamination; Drug Resistance

Being a tropical country, Bangladesh usually has long summer periods and the ice cream consumption rate increases in a large amount on that time. Ice cream is a very popular ready to eat frozen dairy food. It is consumed by people of all ages and so it should be microbiologically safe because the huge popularity of this food item among the children (1). On the basis of several previous studies, the microbiological quality of ice cream samples in Bangladesh is not quite satisfactory from the public health perspective (2). The poor quality of ice cream samples in Bangladesh have been observed at some previous studies conducted on available ice cream samples in the market at different cities (3).

Milk, sweetening, stabilizing agents with colorings and flavorings are the main ingredients of ice cream (4) and presence of these rich ingredients makes ice cream a nutritious food item for human as well as a perfect medium for growth of many pathogenic microorganisms (5). Along with the high milk content, the nearly neutral pH value also makes ice cream an ideal medium for bacterial growth (6). These pathogens sometimes can survive pasteurization and cause outbreaks of food borne diseases like cholera, typhoid, diarrhea, listeriosis etc. (7).

Along with the faulty manufacturing process, the raw materials used for the production of sometimes serve as

a main source of bacterial contamination to the ice creams (8). Besides these, leakage in the processing tanks, post sterilization contamination, improper storage conditions, faulty packaging process and transportation ways contributes the presence of pathogens into these products (9). Presence of pathogenic bacteria like: *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp, *Shigella* spp and coliform bacteria enhance the potential threat to ice cream quality for human (1, 10).

On the other hand, the constant failures of antibiotics on pathogens due to the presence of resistance genes in the developing countries compelled the researchers to establish the common bacterial species that occur in the dairy products (especially ice cream) and their antibiotic resistance patterns (11). This study was designed to determine the microbiological properties and antibiotic susceptibility pattern of the resistant bacteria present in the Ice Cream samples.

MATERIALS AND METHODS

Sampling plan. This study was carried out during the summer period at Dhaka, Bangladesh. In this time, people consume ice cream in a large amount. Three Ice Cream samples of different brands were collected from the local market of Shantinagar randomly. The samples were aseptically collected from -16°C refrigerator and the valid expiry dates and intact seals were ensured. The collected ice cream samples were then transported to the laboratory as soon as possible for further experiments by following the method suggested by American Public Health Association (12).

Samples preparation and processing. After transportation of the ice cream samples to the laboratory, we kept these in the water bath at 45°C for the thawing process. The top parts of the packets were opened carefully and samples were collected by using a sterile micro pipette aseptically when the samples became liquid completely (13, 14). Afterwards, each of the collected samples were

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transferred to 9ml normal saline in sterile test tubes (15). The samples were then diluted up to 10^5 dilutions for the enumeration of total viable bacterial count.

Microbiological Analyses. For the enumeration of total viable bacterial count (TVBC), an aliquot of 0.1 ml from 10^{-3} dilutions of each sample were introduced onto the nutrient agar (NA) plates by the spread plate technique (16, 17). After that, the inoculated nutrient agar plates were incubated at 37°C for 24 hours. For the enumeration of fecal coliform count, an aliquot of 0.1ml from 10^2 dilutions of each sample were introduced onto the m-FC agar plates by the spread plate technique and incubated at 44.5°C for 24 hours.

An aliquot of 0.1ml from 10^{-2} dilutions of each sample were introduced onto MacConkey agar and Manitol Salt agar plates by the spread plate technique for the enumeration of total coliform and *Staphylococcus aureus*. The inoculated agar plates were then kept at 37°C in an incubator for 24 hours. And, for the detection of any fungal count, an aliquot of 0.1 ml from 10^{-3} dilutions of each samples were introduced onto the Saboured Dextrose Agar plate by following the spread plate method and incubated at 25°C for 48 hours.

Antibiotic susceptibility testing. The disc diffusion method was applied to determine the antibiotic susceptibility of the isolated *E.coli* and *Staphylococcus aureus* colonies (18). Muller Hinton (MH) Agar plates were used for this experiment and the agar plates were seeded by earlier prepared cell suspension with a turbidity of 0.5 McFarland standards. The suspensions were streaked onto the MH agar plates by sterile cotton swabs carefully. After that, the antibiotic discs were placed on the agar plates and kept at 30°C for 24 hours in the incubator. Total eight types of antibiotics were used for the experiment. They are: Oxacillin (OX1), Kanamycin (K30), Ampicillin (AM10), Cefixime (CFM5), Erythromycin (E15), Azithromycin (AZM30), Tetracycline (TE 30) and Chloramphenicol (C30). After the incubation period, the zones of inhibition were measured and recorded (11).

RESULTS AND DISCUSSIONS

In the present study we detected total viable bacterial count, total coliform count, *Staphylococcus aureus* count, total fungal count and total fecal coliform

count in three ice cream samples. Total viable bacteria ranged from 2.26×10^6 cfu/ml to 4.3×10^6 cfu/ml. Total coliform count was ranged from 1.1×10^4 cfu/ml to 8×10^3 cfu/ml. Rahman et al. (2) reported the differential distribution of microbial contaminations on various ice cream samples. On that study, the highest total viable bacterial count was 9.5×10^4 cfu/ml and the maximum total fecal coliform count was 4 cfu/ml whereas in this study there was no fecal coliform present in the ice cream samples. Total Fungal Growth ranges from 1.2×10^4 cfu/ml to 1.7×10^4 cfu/ml. The presence of *Staphylococcus aureus* was from 1.2×10^4 cfu/ml to 7×10^3 cfu/ml and the reason could be, their widespread presence in the environment. The human nasal cavity and skin is their largest reservoir and they disseminates in the contacted dust, air and food products (10). (Table 1).

Antibiotics are natural or semi synthetic of synthetic drugs which has the ability of killing bacteria and fungus. For the treatment of food poisoning and infection, keeping track of medically important antibiotics by the antibiotic susceptibility test of food products is necessary (11). Table 2 shows the zone diameter of inhibition of the selected antibiotics against 2 isolated bacteria from 3 ice cream samples. The degree of susceptibility of these test organisms to each antibiotic was determined and

TABLE 1. Microbial Prevalence of microorganisms in ice cream samples (cfu/ml)

Ice Cream Sample	Total Viable Count	Total Staphylococcus Count	Total Coliform Count	Total Fecal Coliform Count	Total Fungi Count
Brand 1	3.65×10^6	1.2×10^4	4×10^3	Nil	1.2×10^4
Brand 2	4.3×10^6	7×10^3	8×10^3	Nil	1.7×10^4
Brand 3	2.26×10^6	1.3×10^4	1.1×10^4	Nil	1.2×10^4

TABLE 2. indicates the Sensitivity of the *E. Coli* and *Staphylococcus aureus* isolates against 8 commonly used antibiotics

Antibiotics	<i>E. coli</i>			<i>Staphylococcus aureus</i>		
	R	I	S	R	I	S
OX1	100%	0%	0%	0%	0%	100%
K30	0%	0%	100%	0%	0%	100%
AM10	100%	0%	0%	0%	0%	100%
CFM5	0%	0%	100%	0%	0%	100%
E15	100%	0%	0%	100%	0%	0%
AZM30	0%	0%	100%	100%	0%	0%
TE30	100%	0%	0%	0%	0%	100%
C30	0%	0%	100%	0%	0%	100%

OX1= Oxacillin, K30= Kanamycin, AM10= Ampicillin, CFM5= Cefixime, E15=Erythromycin, AZM30= Azithromycin, TE30= Tetracycline and C30= Chloramphenicol.

R= Resistant, I= Intermediate, S= Sensitive.

interpreted as sensitive (S), resistant (R) or intermediate (I) by calculating zones of inhibition around the antibiotic discs (19). Study of antibiogram showed that isolated *E. coli* was resistant to 4 antibiotics- OX1, AM10, E15 and TE30, while, *Staphylococcus aureus* was resistant against 2 antibiotics (E15 and AZM 30) and there was no intermediate response against the antibiotics by any of these isolates. Both *E. coli* and *Staphylococcus aureus* were resistant against Erythromycin only which is similar to a recent study conducted by Nwinyi et al, (11).

CONCLUSION

Along with the contamination of different pathogenic bacteria in available ice cream samples, the resistance ability of these pathogens is increasing in an alarming rate day by day. More focus should be tuned for controlling multidrug resistance of pathogens isolated from food samples. Proper manufacturing protocols should be ensured during the processing, transportation and handling of food products to decline the contamination which can act as perilous for the human being after consumption

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