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MICROBIOLOGICAL AND ANTIBIOGRAM PROFILING OF FROZEN FOODS AND ICE CREAM PRODUCTS FROM SUPER-SHOPS OF DHAKA, BANGLADESH

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The current study was conducted to assess the microbiological loads and antibiotic resistance patterns among 20 frozen food and ice cream samples that were gathered from multiple super-shops in Dhaka, Bangladesh. A total of 20 samples (11 frozen food samples and 9 ice cream samples) were collected from different supershops in Dhaka, Bangladesh during the period from July 2024 to September 2024. Microbiological analyses were performed to determine the total viable bacterial count (TVBC), total coliform count (TCC), and total staphylococcal count (TSC). The highest total viable bacterial count (2.12×10^9 cfu/g), highest coliform count (3.7×10^7 cfu/g), and highest staphylococcal count (2.04×10^8 cfu/g) were found in the frozen fish sample. Bacteria were isolated and identified based on their morphological and biochemical characteristics. While *E. coli* and *Klebsiella* spp. were observed in seven samples, *Staphylococcus aureus* was identified in twelve. Additionally, different antimicrobial agents were used to determine the isolates' susceptibility towards them. Ciprofloxacin, Imipenem, and Gentamicin have been demonstrated to be the most effective drugs against each isolate. All of the *Klebsiella* isolates exhibited sensitivity to Chloramphenicol, whereas most of the *E. coli* isolates were sensitive to Erythromycin, Chloramphenicol, and Nalidixic acid. However, erythromycin and amoxicillin were found to be less effective against *Staphylococcus aureus* isolates. The presence of several different microorganisms suggests that the majority of frozen food samples did not meet public health standards. These frozen foods, contaminated with multi-antibiotic-resistant microorganisms, have the potential to transmit food-borne diseases.

Keywords: Frozen food, Food-borne disease, Coliform, Microbiological quality

INTRODUCTION

Microbiological standards have often been linked to consumer safety concerns (1). Freezing and refrigeration techniques preserve foods by limiting the growth of microorganisms that cause foodborne illness (2). These methods considerably slow down and also inhibit the growth and spread of microorganisms (3). Since frozen meat and fish are stored and processed by direct contact, there is an increased possibility of contamination with potential food-borne pathogens, which has led to their involvement in outbreaks of foodborne illness (4). These microorganisms cause sour flavors, foul aromas, and other negative effects on frozen food products. They transform the textures or flavors of food that make them undesirable for eating (5). Because of its high nutritional content, almost neutral pH (pH 6–7), and extended shelf life, ice cream can be regarded as a suitable medium for microbial growth. Microbiological contamination can arise from several ice cream manufacturing processes, either coming from the addition of contaminated ingredients or, careless handling (6). However, the majority of these risks can be minimized by time-dependent heating methods like pasteurization, freezing, and hardening procedures, which can lower the vegetative forms of bacteria (7).

Several studies have found a direct association between contaminated food and a variety of gastrointestinal disorders, including typhoid fever and tuberculosis. Food-borne poisoning can also occur as a result of eating frozen foods contaminated with higher toxin-producing bacterial and fungal counts, which cannot be eliminated even by high-temperature boiling (8). Frozen foods of animal origin are a major reservoir of *Escherichia coli*, and it is not unknown that human infection is commonly linked to contamination of food or water with animal waste (9). As an opportunistic pathogen, *Staphylococcus aureus* (*S. aureus*) is the most subjected to grow on foods like meat products, curd, and yogurt that have a higher percentage of water-phase salt and less water activity (10). The presence of *S. aureus* is associated with cross-contamination or raw material contamination that results from improper handling during processing and storage (11). Because of their large numbers, coliforms, particularly *E. coli*, are bacteria that are harmful to almost every food product. Furthermore, pathogens in ice cream samples are mostly

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transmitted by devices and instruments, water, workers, the environment, packing materials, and contamination during ice cream transportation and distribution (12).

Numerous studies have identified the presence of harmful microbes in ice cream, including *Salmonella*, *Staphylococcus aureus*, and *E. coli*. The studies showed that as ice cream is consumed by individuals of all age groups, a more consistent microbiological analysis of ice creams is required (13). Since the determinants of antibiotic resistance can be passed on to other pathogenic bacteria, affecting the treatment of severe infections, food contamination by antibiotic-resistant bacteria may pose a serious risk to public health. Over the past few decades, food-borne bacteria have become more common in their antibiotic resistance (14).

The occurrence of *E. coli* in foods is mainly due to a lack of hygiene during the manufacturing process, poor storage conditions, and post-process contamination (15). Therefore, in order to ensure the safety and hygienic conditions of the food products, the minimally acceptable levels of coliforms and *E. coli* (10 cfu/g) have been established in the national standards of the majority of countries (16). For products to be free of these pathogens or to have their presence reduced to the lowest possible level prior to freezing, conventional food processing, storage, and handling procedures must be followed (17). An effective system tool for ensuring food safety is Hazard Analysis and Critical Control Point (HACCP). Despite the implementation of Good manufacturing practice (GMP) and HACCP guidelines, coliforms can nevertheless occasionally be observed in products after cooking, even though they can be completely removed during the pasteurization step. Since contamination in the processing environment can originate from a variety of sources, identifying these sources is essential to establishing efficient control mechanisms and reinforcing the GMP and HACCP procedures (18).

Although many studies have been conducted on the microbiological analysis of frozen fish or any one category of food, there has been no adequate study to identify viable coliform and staphylococci in all types of frozen foods and ice cream, as well as their antibiogram (13, 19). This study aimed to determine the bacteriological profile of frozen foods and ice creams from various brands that are available in supershops to assess the products' microbiological quality. To evaluate the degrees of contamination and sanitation, the total viable bacteria, total coliform, and total staphylococci were identified and enumerated. The prevalence of antimicrobial resistance, which could potentially affect people's health, was also investigated.

MATERIALS AND METHODS

Sample collection: A total of twenty samples were collected for the analysis of total viable bacteria, total coliform, and staphylococci from Dhaka city, Savar, Gazipur, and Narayanganj. Among them, eleven samples were frozen food items (Samples 1 to 11) and the other nine samples were ice cream (Samples 12 to 20). Among them, samples 12, to 14 were cup ice cream, samples 15, 16, and 17 were hard chocobars and samples 18, 19, and 20 were soft cone ice cream. The sampling period was from July 2024 to September 2024. Frozen food samples were collected in a sterile container using a sample collector ice box at 4°C (20) and ice cream samples were collected in a cool box stored at -20°C and transported to the laboratory without delay.

Samples processing: From each sample, 10g was aseptically weighted and mixed with 90 ml of sterile distilled water. Serial dilutions of samples were made up to 10^{-7} (curd, yogurt, and ice cream) and 10^{-9} (meat and fish) (21).

Microbiological analysis: The total viable bacterial count was carried out by the spread plate technique (22). The sample (0.1 ml) of five dilutions from 10^{-3} to 10^{-7} was taken onto each sterile petri dish evenly spread on the solid nutrient medium and incubated at 37 °C for 24 hours. The plates were screened for the presence of discrete colonies after the incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/g). Then the results per dilution were recorded. Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media (23). Total coliform count (TCC) and total staphylococcal count (TSC) were done in the same way using MacConkey agar medium and Mannitol Salt Agar medium (4), respectively. All the viable counts were the average of at least three independent experiments. Bacterial isolates were then identified according to Bergey's manual of determinative bacteriology, and the manual for the identification of medical bacteria (24).

Enumeration of Microorganism: The bacterial count was performed by the standard method (25). The microbiological condition of safety and hygiene were then assayed using the methods recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) (26).

Biochemical tests: From all the isolates, one representative isolate was selected from each of the microorganisms (*E. coli*, *Klebsiella* spp., and *S. aureus*).Identification of the isolates was done by major biochemical tests, for example- Triple Sugar Iron (TSI), Motility Indole Urease (MIU), Methyl-Red (MR), Voges-Proskauer (VP), and Citrate Utilization were performed following the standard methods (27).

Antibiotic Susceptibility Testing: The Kirby-Bauer method was used to examine bacterial susceptibility to antimicrobial agents (28). The Muller-Hinton agar plates (pH 7.2–7.4) were used for all tests. A sterile cotton swab stick was used to consistently and gently inoculate the surface. The swab stick was dipped into a bacterial suspension that met the 0.5 McFarland requirements for visually comparable turbidity before being inoculated. The excess suspension was then disposed of by removing the swab stick and squeezing it against the test tube wall. For 16-18 hours, inoculated plates were incubated at 37°C and the diameters of the zones of complete inhibition were measured in millimeter scale. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate, and resistant categories.

RESULTS

Microbiological Analysis

The highest total viable bacterial count was 2.12×10^9 cfu/g, found in sample no-5, (fish-2) and the lowest total bacterial count was 1×10^4 cfu/g, which had been collected from yogurt. (Table 2). According to the FDA guidelines of the frozen food samples exceeded the highest acceptable microbiological limit. In contrast, all of the ice cream samples exceeded the highest acceptable microbiological limit. The ice-cream sample-13 (cup ice cream) had the maximum total viable bacterial count (4.5×10^6 cfu/g) and the minimum total bacterial count was 1.3×10^4 cfu/g found in sample no-16, collected from hard chocobar (Table 3).

Total Coliform Count (TCC)

Coliform was isolated from 5 frozen food samples out of 11 samples. The highest coliform bacterial count (3.7×10^9 cfu/g) was found in sample no. 5, collected from fish-2 and the lowest total coliform count was 5.2×10^4 cfu/g which was collected from curd (Table 2).

Among the ice-cream samples, coliform was isolated from 2 samples, the highest (5.2×10^5 cfu/g) was found in sample no.12 (cup ice cream) and the lowest total coliform count was 3.8×10^3 cfu/g, found in sample no. 14 (hard chocobar) (Table 3).

Total Staphylococcal Count (TSC)

In this experiment, Staphylococci were found in 9 frozen food samples out of 11 samples. (Table 2). The highest staphylococcal count was also found in sample no. 5, collected from fish (2.04×10^8cfu/g) and the lowest staphylococcal count was 1.30×10^3 cfu/g, found in sample no. 4 (Shoal fish). Among the ice-cream samples, staphylococci were isolated from 3 samples, the highest (5×10^5 cfu/g) was found in sample no.13 and the lowest total coliform count was 1.1×10^2 cfu/g, found in sample no. 14 (Table 3).

Biochemical tests

The representative isolate from *E. coli*, *Klebsiella* spp., and *Staphylococcus aureus* exhibited similar biochemical characteristics to the ATCC isolates (Table 4).

Antibiogram of the Isolates. From the frozen food and icecream samples, seven isolates of *E. coli*, seven isolates of *Klebsiella* spp., and twelve isolates of *Staphylococcus* spp. were obtained. All of the isolates exhibited sensitivity to Gentamicin, Ciprofloxacin, and Imipenem (Figure 1a, 1b, 1c). Each of the *Klebsiella* spp. showed sensitivity to Chloramphenicol (Figure 1b). Most of the *Staphylococcus aureus* were found to be resistant to Erythromycin (83.3%) and Amoxicillin (75%) (Figure 1c). On the other hand, about 85.7% of the *E. coli* isolates were sensitive to Erythromycin and Chloramphenicol and 71.5% were sensitive to Nalidixic Acid (Figure 1a). Ceftazidime was found to be effective for most of the isolates (>70%).

DISCUSSION

A total of 11 frozen food and 9 ice cream samples were tested for total viable bacteria, coliform, and staphylococcal counts. Tables 2 and 3 indicated the total number of bacteria, staphylococci, and coliform found in the samples. According to Table 1, the frozen food samples' microbiological quality was assessed. With the exception of food that is not heated before refrigeration, the quality is poor if there are more than 10^5 cfu/g bacteria found in the sample. On the other hand, the fish samples were not heated prior to refrigeration. The presence of coliform bacteria, such as *E. coli*, is a common indicator of fecal contamination (29).

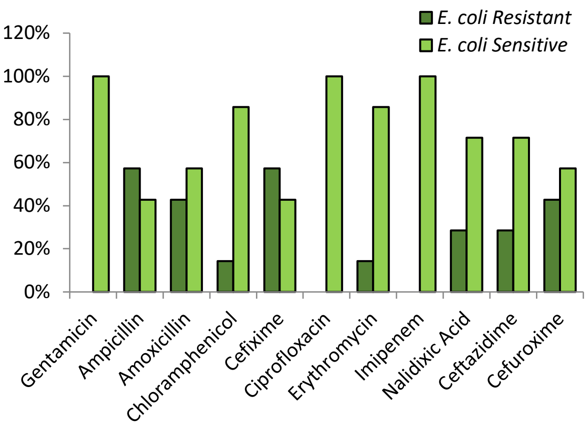

Figure 1a.: Antibiotic Susceptibility of the *E. coli* isolates.

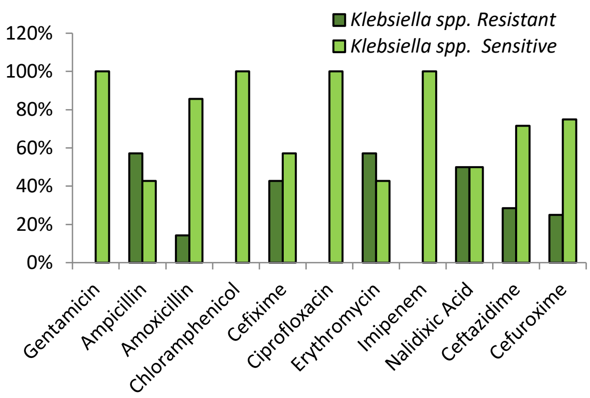

Figure 1b: Antibiotic Susceptibility of the *Klebsiella* spp. isolates.

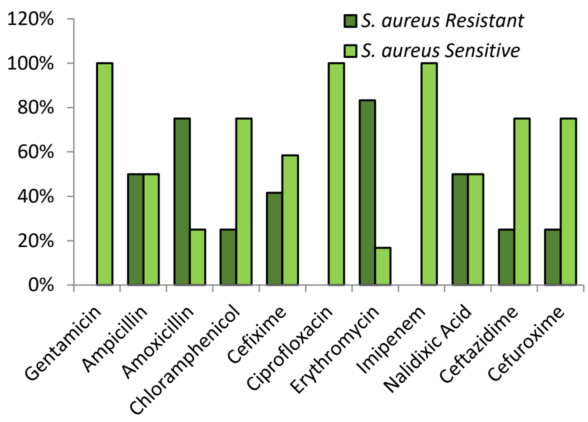

Figure 1c: Antibiotic Susceptibility of the *S. aureus* isolates.

Table 1: FDA guidelines for the acceptable microbiological limit for frozen foods (25).

Sample	**Total bacteria (cfu/g)**	**Coliforms (cfu/g)**	***S. aureus*** **(cfu/g)**
Meat products	10^5	10	10^2
Fish products	5×10^5	10	10^3
Milk products	5×10^4	10	$10\text{-}10^2$
Icecream	10^3	10	10

Table 2: Enumeration of microorganisms found in Frozen Food samples.

Sample	**Sample Type**	**Total Viable Bacterial Count (TVBC) (cfu/g)**	**Total Coliform Count (TCC) (cfu/g)**	**Total Staphylococcal Count (TSC) (cfu/g)**
Sample-1	Meat-1	8.7×10^6	1.7×10^6	2.8×10^6
Sample-2	Meat-2	1.76×10^6	0	7.5×10^3
Sample-3	Fish-1	7.4×10^6	2×10^5	4.2×10^6
Sample-4	Fish (Shoal)	1.30×10^5	0	1.30×10^3
Sample-5	Fish-2	2.12×10^9	3.7×10^7	2.04×10^8
Sample-6	Shrimp-1	1.4×10^7	0	2.1×10^6
Sample-7	Shrimp-2	1.94×10^6	8.1×10^4	2.6×10^3
Sample-8	Curd	1.78×10^6	5.2×10^4	1.2×10^4
Sample-9	Yogurt-1	1×10^4	0	0
Sample-10	Yogurt-2 (Aarong)	1.3×10^5	0	0
Sample-11	French Fries	1.32×10^7	0	1.2×10^6

Table 3: Enumeration of microorganisms found in Icecream samples.

Sample	**Sample Type**	**Total Viable Bacterial Count (TVBC) (cfu/g)**	**Total Coliform Count (TCC)(cfu/g)**	***S. aureus*** **(cfu/g)**
Sample-12	Ice Cream-1	1.76×10^6	5.2×10^5	3.2×10^5
Sample-13	Ice Cream-2	4.5×10^6	0	5×10^5
Sample-14	Ice Cream-3	1×10^5	3.8×10^3	1.1×10^2
Sample-15	Hard Chocobar (Brand 1)	3.2×10^4	0	0
Sample-16	Hard Chocobar (Brand 2) Lot: 157	1.3×10^4	0	0
Sample-17	Hard Chocobar (Brand 2) Lot: 162	1.5×10^4	0	0
Sample-18	Soft (Regular Cup) (Brand A) Lot: 145	2.3×10^4	0	0
Sample-19	Soft (Regular Cup) (Brand A) Lot: 167	2.5×10^4	0	0
Sample-20	Soft (Regular Cup) (Brand B)	3.5×10^4	0	0

Table 4: Biochemical identification of the bacterial isolates from different frozen food samples.

Assumed Organism	**TSI**				**Indole test**	**MR test**	**VP test**	**Citrate Test**	**Motility**
	Slant	**Butt**	**Gas**	**H_2S**					
Escherichia coli	Y	Y	+	-	+	+	-	+	+
Klebsiella spp.	Y	Y	+	-	-	-	+	+	-
Staphylococcus aureus	Y	Y	-	-	-	+	-	-	-

Note: TSI, Triple Sugar Iron; Y, Yellow (Acid); R, Red (Alkaline); MR, Methyl red; VP, Voges-Proskauer.

Coagulase-positive staphylococci may cause human disease through the production of toxins (30). Additionally, antibiotic resistance has increased worldwide leading to failures in the treatment of human infectious diseases. Resistance against antibiotics by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals (31). In order to analyze the percentages of food-isolated bacteria that were resistant to different antibiotics, 11 commonly used antimicrobial drugs were used. The Kirby-Bauer disk diffusion test was used in this experiment to determine whether the isolated organisms were susceptible or resistant to a selection of antimicrobial agents. The susceptibility of isolated *E. coli*, *Klebsiella* spp., and *Staphylococcus* spp. to eleven different antimicrobial agents was measured *in vitro* by the Kirby-Bauer method. It allowed rapid determination of the efficacy of the drug by measuring the zone of inhibition that results from the diffusion of the antimicrobial agent into the medium surrounding the disc (32).

Foodborne infections are the most critical global challenge and produce significant morbidity and mortality every year (33). The majority of foodborne illnesses are caused by pathogenic bacteria (34). The purpose of this study was to evaluate the microbiological quality of various frozen food items and ice creams that were gathered from different supermarkets in Dhaka, Bangladesh. Despite worldwide initiatives and the use of modern technologies for ice cream production, the spread of infectious diseases brought on by these microorganisms remains an enormous concern (35). Accordingly, the current study attempted the identify and measurement of distinct pathogenic organisms from several kinds of refrigerated food items as well as their drug-resistance features. The findings of this study indicate that frozen foods might cause significant health risks to consumers. Many factors, such as transportation and storage, affect how safe frozen food products are. Therefore, we need to improve the conditions for food distribution and storage. The implementation of a food safety management system based on Hazard Analysis Critical Control Point (HACCP) standards should improve the quality of frozen foods and ice creams. In food industries, priority should be given to the quality of the raw materials before processing, manufacturing, and storing them in the right conditions. This is also crucial for preventing diseases and toxins carried out by pathogenic microbes (36). To prevent the rapid development of antibiotic resistance, many measures regarding the use of antibiotics in the food chain as well as antimicrobial agent-growth enhancers are required.

CONCLUSION

This study focuses on the microbiological analysis of frozen food and ice cream collected from a few super-shops in Dhaka, Bangladesh, where high levels of bacteria, coliforms, and staphylococci were found, indicating poor quality and significant health risks. Contamination of frozen foods is caused by a number of factors, including transportation, handling, and storage. To reduce the number of microorganisms and to prevent the spread of multidrug-resistant organisms especially pathogens that can contaminate these foods, additional monitoring is needed.

CONFLICTS OF INTERESTS

The authors have declared that no competing interests exist.

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LIMITATIONS

Due to some limitations of resources, we could not triplicate the results. Hence, there were no error bars in the graphs.

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