

Microbiological Quality Analysis of Some Frozen Food Items Collected from Various Locations of Dhaka City, Bangladesh

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Frozen foods of different categories make a huge contribution to meet the sufficient demands for nutrition in our daily lives. For this reason, this study was conducted to emphasize the existence of indicator and pathogenic bacteria through the isolation, identification, and characterization of such microorganisms obtained from frozen food samples collected from different local markets of Dhaka City, Bangladesh. A total of 12 frozen food samples were analyzed for the detection of total viable bacterial counts (TVBC), total fungal count (TFC), total staphylococcal count (TSC), and total coliform count (TCC). Biochemical testing was conducted for the presumptive identification of the bacterial isolates. All food samples harbored TVBC ranging from 10^5 to 10^7 CFU/g, and TFC were within the range of 10^2 to 10^3 CFU/g. Among the others, high loads of *Staphylococcus*, *Escherichia coli*, and *Klebsiella* were consistently observed. Additionally, most of the isolates exhibited resistance to commonly used antibiotics, raising concerns about antimicrobial resistance in these foodborne pathogens. Such findings will provide valuable insights into the microbial risks associated with ready-to-cook frozen food consumption, highlighting the need for enhanced safety practices and stricter regulatory policies in their processing and handling.

Keywords: Ready to cook frozen food, TVBC, TFC, TCC, TSC, Food safety

INTRODUCTION

Food is one of the five essential human needs, making its safety and quality a priority in terms of microbial load and nutrient balance (1). With changing lifestyles and dietary habits, the demand for ready-to-cook (RTC) food is increasing (2). In response to this growing demand, a wide range of frozen food products is being rapidly introduced to the market (3). Other factors have contributed to the growing demand for ready-to-fry frozen food items, including a combination of socioeconomic and lifestyle factors, such as limited time for meal preparation, higher disposable incomes, globalization and exposure to international cuisines, extended working hours, increased female workforce participation, and shifting dietary and culinary practices (4, 5).

Ready-to-cook foods are rich in high-quality protein, essential minerals, and vitamins. These food items are shelf-stable, flavorful, affordable, and convenient, offering immediate accessibility to customers without the need for lengthy preparation or processing (6). Ready-to-cook frozen food items can be processed from animal, plant, vegetable, and wheat-based products (5). To provide enough nutrients for the growth of microorganisms, chicken-based products are considered one of the most spoilable foods (4). There are various types of ready-to-cook frozen food items available in our country, and they are the most convenient way to enjoy fresh-like food all year round, regardless of the season. Despite their availability, maintaining the quality of frozen food items remains quite challenging. Food

quality includes all influences that enhance the value of a discoloration, origin, color, flavor, texture, and processing methods of the foods (4). Due to the lack of awareness about food safety, appropriate storage, and hygiene practices, frozen food items can be contaminated and spoiled. If food is contaminated with harmful microbes and chemicals, or toxins, then food can be a disease-causing agent. WHO estimated that 600 million people, nearly one in ten, fall ill after eating food contaminated with bacteria each year, and 420,000 people die from these illnesses, among them 40% or 125,000 of whom are children (7, 8). In Bangladesh, diarrhea is responsible for one-third of childhood deaths; the actual number of casualties might be underestimated in the absence of a national health database (9-11).

Numerous reports revealed that frozen food items are contaminated with various types of microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus*, *Klebsiella*, *Salmonella*, *Flavobacterium*, *Clostridium botulinum*, *Clostridium perfringens*, *Micrococcus*, *Moraxella*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Mucor*, and others (12). Presence of pathogenic microorganisms in food can cause a large spectrum of these infections, such as enteric complications, abdominal pain, fever, hemorrhage, bloodstream infection, meningitis, joint infection, kidney failure, miscarriage, coma, infertility, renal disease, stomach pain, colitis, and paralysis (9, 13-15). Moreover, the presence of antibiotic-resistant bacteria in food can significantly impact the treatment of serious infections, posing a considerable public health risk. In recent decades, antibiotic resistance among food-borne bacteria

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has become gradually dominant (16, 17). Sultana *et al.* (11) investigated the bacterial load and antibiotic resistance profiles of isolates from 20 ready-to-cook (RTC) frozen animal-based food samples sold in Dhaka. Proliferation of microorganisms can occur in various steps, such as during food production or harvesting, processing, post-processing, storage, distribution, retail marketing, and food handling. To confirm food safety and ensure consumer protection, food manufacturers should implement GMP or HACCP to eliminate risks associated with microbial contamination, pesticide residues, food additives, and adulterants. Meanwhile, food quality encompasses all factors that enhance a product's value, including the absence of spoilage and contamination (5). Despite previous research, there is limited knowledge regarding the microbial quality of widely consumed RTC frozen foods in Bangladesh. Studies have reported the presence of *Salmonella*, *Vibrio*, and *Shigella* in frozen foods, but these reports are often inadequate in terms of the variety and number of food samples tested, as well as the microbiological parameters analyzed. Thus, our study aims to assess the presence of bacterial population in frozen food items along with their drug resistance pattern collected from various location of Dhaka city, Bangladesh.

MATERIALS AND METHODS

Study area, sampling, and sample processing: Twelve different RTC frozen food items (Aloo Puri, French Fries, Chicken Nuggets, Chicken Samosa, Chicken Sausage, Chicken Meat Ball, Chicken Sami Kabab, Chicken Shingara, Chicken Momo, Chicken Roll, Chicken Popcorn, Pizza) were randomly collected following standard protocol (18, 19). All the samples were quickly transported to the laboratory. Before the microbiological assay, 10g of each sample was homogenized with 90 ml of distilled water in a 1:9 ratio and serially diluted up to 10^{-5} .

Microbiological analysis of each sample: A volume of 0.1 ml from each sample suspension was spread onto nutrient agar (NA) and incubated at 37°C for 24 hours to enumerate the total viable bacterial count (TVBC). Following inoculation, Sabouraud dextrose agar (SDA) (Oxoid Ltd., Basingstoke, Hampshire, England) was incubated at 25°C for 48 to 72 hours for the isolation of fungi.

Isolation indicator and pathogenic microorganisms: For the isolation of coliform bacteria, 0.1 ml of each sample suspension was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar and incubated at 37°C for 24 hours (20). 0.1 ml of each sample suspension was spread on Mannitol salt agar (MSA; Oxoid Ltd., Basingstoke, Hampshire, England) for the isolation and enumeration of *Staphylococcus aureus*, and the plates were incubated at 37°C for 24 hours (21).

Enrichment and inoculation of pathogens: The *in vitro* cultivation of the species of *Salmonella*, *Shigella* and *Vibrio* often appears difficult or with false-

negative due to their viable but non-culturable (VBNC) attributes (22, 23). Therefore, enrichment was used prior to isolating these bacteria (19, 24).

Enrichment for *Salmonella* and *Shigella* was performed using selenite cysteine broth (SCB). From the mixture of the homogenized sample, 1 ml suspension was transferred to SCB, followed by incubation at 37°C for 4 hours, and serial dilutions were made up to 10^{-4} , and from this dilution, 0.1 ml was spread onto *Salmonella Shigella* (SS) agar (Hi media, India) followed by the incubation at 37°C for 24 hours. For the enrichment of *Vibrio*, 1 ml of the homogenized sample suspension was transferred to alkaline peptone water (APW) and incubated at 37°C for 4 hours and serial dilutions were made up to 10^{-4} and from 10^{-4} dilution 0.1 ml was spread onto TCBS (Oxoid Ltd., Basingstoke, Hampshire, England) agar followed by the incubation at 37°C for 24 hours (24).

Presumptive identification of the bacterial isolates: Finally, all isolates were presumptively detected by a number of confirmatory biochemical tests, like the triple sugar iron (TSI) test, motility, indole urease (MIU) test, methyl-red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, and oxidase tests.

Determination of antimicrobial susceptibility of the isolates: Bacteria isolated from frozen food samples were tested for antibiotic susceptibility on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) using the modified Kirby-Bauer disk diffusion method (19, 20). The antibiotics tested included Ampicillin (10 µg), Ciprofloxacin (5 µg), Streptomycin (10 µg), Cephalosporin (30 µg), Imipenem (30 µg), Penicillin (10 µg), Gentamicin (10 µg), Azithromycin (15 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Chloramphenicol (10 µg), Trimethoprim/sulfamethoxazole (25 µg). Following 24 h of incubation, inhibition zone diameters were recorded, and results were interpreted as susceptible or resistant (Figure 1) (19).

RESULTS

Microbial analysis of a frozen food sample: In this study, all samples were found to be severely contaminated with both bacteria and fungi. Chicken Shami Kabab (1.3×10^8 CFU/g), along with Chicken Singara (3.1×10^7 CFU/g) and French Fries (3.0×10^7 CFU/g), showed the highest total viable bacterial counts (TVBC). Such high bacterial counts could indicate poor handling or inadequate temperature control during storage. On the other hand, Chicken Popcorn (1.8×10^5 CFU/g) and Chicken Nuggets (2.8×10^5 CFU/g) had relatively lower bacterial loads, suggesting better microbiological quality (Table 1). Fungal contamination was detected in several samples, with the highest TFC found in Chicken Meat Ball (9.6×10^3 CFU/g) and French Fries (2.2×10^3 CFU/g). Nevertheless, some frozen foods like Aloo Puri, Chicken Sausage, Chicken Sami Kabab, and Pizza had no detectable fungal count. In the case of specific bacteria, the presence of *E. coli* and *Staphylococcus spp.* was most predominant in all the samples. *E. coli* was found in all samples except Chicken Sami Kabab, indicating widespread fecal contamination. The highest count was observed in Pizza (5.0×10^4 CFU/g), which raises safety concerns. *Vibrio* and

Table 1: Microbiological analysis of different types of microorganisms in RTE frozen food sample (CFU/g).

Sample Name	TVBC	TFC	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Salmonella</i> spp.	<i>Vibrio</i> spp.	<i>Staphylococcus</i> spp.
Aloo Puri	2.1×10^7	0	4.2×10^2	6.8×10^2	0	8.0×10^3	6.3×10^4
French Fries	3.0×10^7	2.2×10^3	1.0×10^2	5.0×10^3	0	0	7.3×10^4
Chicken Nuggets	2.8×10^5	2.0×10^3	1.6×10^2	7.6×10^3	0	0	2.8×10^3
Chicken Samosa	3.3×10^6	1.5×10^2	2.0×10^2	8.0×10^3	0	0	1.2×10^4
Chicken Sausage	2.8×10^6	0	1.5×10^2	3.5×10^3	4.0×10^3	2.0×10^2	1.9×10^4
Chicken Meat Ball	1.1×10^6	9.6×10^3	2.0×10^3	0	4.0×10^3	0	8.0×10^4
Chicken Sami Kabab	1.3×10^8	0	0	0	5.0×10^4	0	1.6×10^4
Chicken Shingara	3.1×10^7	2.2×10^3	1.0×10^2	4.0×10^3	3.5×10^2	0	7.3×10^4
Chicken Momo	2.8×10^5	2.0×10^3	1.6×10^2	5.6×10^3	2.5×10^2	0	2.8×10^4
Chicken Roll	3.5×10^6	1.5×10^2	2.0×10^2	6.0×10^3	0	0	1.2×10^4
Chicken Popcorn	1.8×10^5	2.0×10^3	2.6×10^2	5.3×10^3	2.5×10^2	0	2.8×10^4
Pizza	2.7×10^7	0	5.0×10^4	0	5.0×10^2	4.5×10^5	5.0×10^5

Salmonella were also present in some samples. Their presences were assumed by biochemical test (Table 1). Prior enrichment, *Vibrio* in food and fish samples was found to be nil in many studies (25, 16, 17). In the present study, *Salmonella* was detected in five samples, and *Vibrio* was only discovered in Aloo Puri, Chicken Sausage, and Pizza, with the Pizza sample showing a significantly high load (4.5×10^5 CFU/g), posing serious health risks. The presence of *Salmonella* is particularly notable in chicken-based foods, as some bacteria are part of the normal intestinal flora of chicken or other animals (26-28). According to the International Commission on Microbiological Specifications for Foods (ICMSF), the permissible limit for *Salmonella* in frozen food samples is 1 per 25 g of product, a standard that our samples failed to meet (8). The use of various spices, edible coatings, and preservatives is a well-established method for eliminating such pathogens, which could be possible reasons for the low counts observed (29, 30).

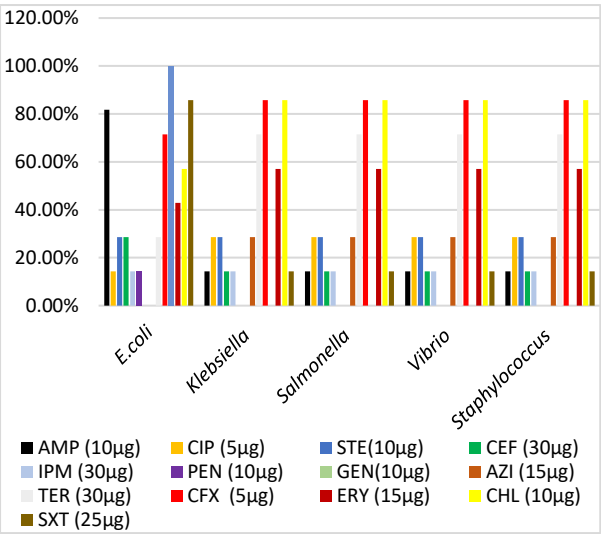


Figure 1: Antibiotic Resistance Pattern.

Antibiotic susceptibility results: Antibiotic susceptibility testing was conducted using the disc diffusion method. As shown in Figure 1, most isolates exhibited resistance to a wide range of antibiotics. Notably, streptomycin (STE), chloramphenicol (CHL), and ciprofloxacin (CIP) showed consistently high resistance rates (typically exceeding 80%) across the majority of bacterial species, indicating limited therapeutic efficiency. In contrast, imipenem (IPM)

demonstrated the lowest resistance levels among all tested antibiotics, suggesting it remains a highly effective treatment option. Additionally, gentamicin (GEN) and trimethoprim-sulfamethoxazole (SXT) displayed relatively high efficacy in comparison (Table 2). These findings underscore the importance of appropriate antibiotic selection, routine susceptibility testing, and robust antimicrobial stewardship to address the growing challenge of antibiotic resistance.

DISCUSSION

The present investigation into the quality of frozen and ready-to-cook (RTC) foods revealed those available in super shops across Dhaka city. Among the analyzed items, the most contaminated was found to all animal-originated RTC frozen foods which is similar as Shamimuzzaman *et al.* 2022 (5). Across nearly all sampled frozen and RTC foods, various pathogenic microorganisms were detected in varying amounts, including *Escherichia coli*, *Klebsiella*, fecal coliforms, fungi, *Staphylococcus aureus*, *Salmonella*, and *Vibrio*. These pathogens are known to cause several foodborne illnesses such as food poisoning, diarrhea, dysentery, cholera, typhoid, and pneumonia (25, 31). Contamination was more frequent in chicken-based products. This indicates that frozen and RTC foods from markets are generally contaminated by unhygienic handling of raw chicken or meat and are potentially unsafe for direct consumption. Since many of these pathogenic microorganisms were found in both sources, it suggests that neither type of product is completely free from risk. Therefore, frozen and RTC foods, especially those that contain a high amount of protein or chicken, without further cooking, should be properly handled and, where applicable, reheated or washed according to recommended safety guidelines before consumption (32). Furthermore, the bacteria isolated in this study may have originated from the natural microbial flora of the ingredients or from contamination during various stages of food processing and handling. Possible sources include air, water, processing equipment, packaging materials, handlers' hands, hair, clothing, and storage conditions (32). Since RTC frozen items are typically heated or cooked before eating, most pathogenic microorganisms are expected to be eliminated during cooking, meaning the reported microbial contamination may not pose an immediate health risk to consumers. Nevertheless, the threat of microbial toxin production and the potential decline in the nutritional value of these foods due to prolonged microbial growth should not be ignored. It is

Table 2: Biochemical reaction chart of different isolates to identify different bacteria.

Assumed Organism	TSI				Indole test	MR test	VP test	Citrate test	Motility	Oxidase test
	Slant	Butt	Gas	H ₂ S						
<i>E. coli</i>	Y	Y	+	-	+	+	-	-	+	-
<i>Klebsiella</i> spp.	Y	Y	+	-	-	-	+	+	-	-
<i>Vibrio</i> spp.	Y	Y	-	-	+	+	-	+	+	+
<i>Staphylococcus</i>	Y	R	+	+	-	+	-	+	+	-

also important to acknowledge that the limited number and diversity of samples analyzed in this study restrict the authors from making broad conclusions regarding the overall microbial quality and safety of RTC frozen foods available in Dhaka. The authors expect that these findings will prompt regulatory bodies like BSTI or BFSA and manufacturers to strengthen hygiene standards throughout the production chain, encompassing raw material handling, processing, packaging, and maintenance of appropriate freezing conditions during storage or distribution.

On the other hand, most of the pathogens isolated from frozen food samples in the present study were resistant to commonly used antibiotics (Figure 1). The emergence of drug resistance may be attributed to the remarkable genetic adaptability of microbes, inappropriate use of antibiotics, and various epidemiological factors (16, 17). To effectively manage outbreaks associated with frozen food, such resistance must be addressed. Moreover, given the rising concern over antibiotic resistance and associated side effects, the use of herbal alternatives to control frozen food-borne pathogens may represent a safer, more cost-effective, and sustainable approach than conventional antibiotics (26, 27). Further experiments and investigations are necessary to reveal the mechanisms underlying these interactions, in order to gain a clearer understanding of the natural spoilage processes in ready-made frozen snacks. Such insights could contribute to the development of effective strategies to minimize product losses and reduce associated health risks in the future.

CONCLUSIONS

This study revealed a huge microbial load in RTC foods, frozen snacks sold in Dhaka, calling attention to the urgent need for improved food safety practices. Similarly, it is crucial that all stakeholders including food industry workers, regulatory bodies, storekeepers, transporters, retailers, and consumers, develop more awareness to prevent contamination by harmful bacteria. Key measures include ensuring proper personal hygiene among workers, implementing effective cleaning protocols for production areas, regularly fumigating production and storage facilities, and thoroughly cleaning and sterilizing manufacturing equipment. Furthermore, controlling insect and rodent access, maintaining appropriate storage and transportation temperatures, and adhering to the recommended retail storage temperature of -18°C are essential steps. These practices can significantly reduce bacterial load, prevent product spoilage, and minimize health risks to consumers.

CONFLICTS OF INTEREST

The author has declared that no competing interests exist.

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