



Assessment of the Microbiological Quality of Ready-To Fry Frozen Chicken-Based Snack Items Sold in Bangladesh

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Abstract

Microorganisms that are capable of spoiling the product during chill storage as well as several foodborne pathogens can be present in frozen snacks. The present study attempted to investigate the presence of microbial contaminants along with their antibiotic resistance pattern in frozen snacks. In this regard, a total of 15 ready-to-fry frozen chicken-based snack items including three each of chicken pops, chicken samosa, chicken lemongrass lollypop, chicken nuggets and chicken meatball were collected from super shops of Dhaka, Bangladesh. Microbiological analysis revealed that all the samples were highly contaminated with total viable bacteria and fungi in an average of 6 and 4 log cfu/g, respectively which exceeded the standard microbial limit. *Klebsiella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. were encountered in all samples. *E. coli*, *Vibrio* spp. and *Salmonella* spp. were also found in a majority of the samples. Besides, all the isolates were found to be multidrug-resistant. The isolates showed almost 100% resistance against Cefuroxime and Cefixime. A higher proportion of resistance was also reported against Vancomycin and Azithromycin. Whereas, Gentamycin, Ceftriaxone, Colistin and Levofloxacin sensitivity were found in all the isolates. Such chicken-based frozen snack items contaminated with multi-drug resistant microorganisms could be potential vehicles for transmitting food-borne diseases.

Keywords: Frozen foods; Ready-to-fry foods; Snacks; Microbiological quality; Drug resistance.

1. Introduction

Owing to changing habits and lifestyle, demand for ready-to-eat and -cook food items is growing. To satisfy the demand, a number of frozen food items are increasingly being introduced to the market [1, 2]. Reduced time for food preparation and consumption, decreased family size, increased disposable income levels, broader travel abroad and market adjustment to new cuisines are key factors for the ever accelerated use and increasing ranges of frozen foods [1]. Snack foods provide consumers with a convenient and healthy meal option [3]. The most popular among the frozen snack items eaten worldwide is ready-to-cook chicken meals. However, safety and quality of such widely eaten food commodity has been a significant point of concern around the world for the maintenance of mass public health and consumers' acceptance [4, 5].

Microorganisms have the ability to contaminate a wide variety of frozen snack items during harvesting, processing, post-processing, transportation, retailing, storage or handling [2, 4]. Moreover, chicken-based products are considered one of the most perishable foods because they provide enough nutrients to sustain microorganism development [4]. Spoilage and disease causing microorganisms inclusive of *Staphylococcus aureus*, *Salmonella* spp., *Clostridium botulinum*, *Clostridium perfringens*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Mucor* spp., *Campylobacter* spp., *Pseudomonas* spp., *Micrococcus* spp., *Moraxella* spp., lactic acid bacteria and various genera of *Enterobacteriaceae* family are the major contaminants of such food products [4, 5]. Food transmitted diseases or intoxications, such as enteric symptoms, stomach pain, fever, hemorrhagic colitis, bloodstream infection, meningitis, joint infection, renal disease, coma, infertility, etc. are caused by food-borne pathogenic microorganisms [4]. Spoilage-causing bacteria cause the frozen food product to deteriorate, resulting in unwanted odors, tastes, and textures [6].

Freezing keeps food fresh for longer by inhibiting the development and proliferation of microorganisms that cause food spoilage and foodborne disease, as well as inhibiting the food's own enzyme production, which would otherwise cause it to decay [7]. However, freezer burn, product dehydration, rancidity, drip degradation, and product bleaching are some of the risks of frozen storage [8], which may have an overall influence on the consistency of frozen foods [7]. Moreover, frozen foods must be thawed and reheated before being eaten [1]. Once thawed, the residing bacteria can reactivate, spreading to levels that can trigger foodborne illness under the right circumstances [7]. Frozen ready-to-cook snack food is usually served without or with minimal processing rather than reheating. Since freezing, these frozen snacks can be cooked in oil without thawing [1].

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Furthermore, food contamination with antibiotic-resistant bacteria poses a significant risk to public health because antibiotic susceptibility determinants may be passed on to other pathogenic bacteria, compromising the treatment of serious infections [7]. Antimicrobial resistance has become more widespread among food-borne pathogens in recent decades [4, 7, 9]. Over use of antibiotics and widespread application of antimicrobials in feed of animal and livestock to promote their growth may contribute to the spread of antibiotic resistance [2, 4]. It is crucial to identify food-borne pathogenic bacteria including the drug-resistant ones in food rapidly and reliably, both for quality assurance and to monitor pathogens in the food chain. Considering the facts, the present study was aimed to screen the presence of microorganisms in ready-to-fry frozen chicken-based snack samples along with determining the drug susceptibility pattern of the isolates.

2. Materials and Methods

2.1. Study Period and Sampling

Fifteen ready-to fry frozen chicken-based snack items including 3 samples each of chicken pops, chicken samosa, chicken lemongrass lollypop, chicken nuggets and chicken meatball were collected from different super shops of Dhaka city, Bangladesh during January and February 2020. The samples were kept in ice-box after collection, transported immediately to the laboratory and then stored at refrigerator before processing. Following complete thawing and homogenization, the frozen chicken-based snack samples were serially diluted up to 10^{-4} for the microbiological assay according to the standard protocol [4, 10-14].

2.2. Isolation and Identification of Microorganisms from the Ready-to-Fry Frozen Chicken-Based Snack Items

Owing to enumerate total viable bacteria (TVB) and fungi, 0.1 ml of each sample from the dilutions 10^{-3} and 10^{-4} was introduced onto the Nutrient Agar (NA) and Sabouraud's Dextrose Agar (SDA) plates by means of spread plate technique. From the raw sample and dilution 10^{-2} , 0.1 ml of each sample was spread onto the Membrane Fecal Coliform (MFC) agar, MacConkey agar, Starch agar, Mannitol Salt Agar (MSA) and *Pseudomonas* Agar (PA) for the isolation and enumeration of total fecal coliform (TFC), coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), *Bacillus* spp., *Staphylococcus* spp. and *Pseudomonas* spp., respectively. SDA and MFC agar plates were incubated at 25 °C for 48 hours and at 44.5 °C for 24 hours, correspondingly. All the other plates were incubated at 37 °C for 24 hours [4, 10-17].

For the isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp., 10 ml of sample was first transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW), respectively for the enrichment. Following incubation at 37 °C for 6 hours, the samples were diluted up to 10^{-4} and then 0.1 ml of samples from each of the 10^{-2} and 10^{-4} dilutions were spread onto *Salmonella-Shigella* (SS) agar and Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar [4, 10-17]. Plates were incubated at 37 °C for 24 hours for the detection of typical colonies. Finally, all the isolates were biochemically identified following standard procedures as described earlier [4, 13, 14].

2.3. Antibiotic Susceptibility Pattern of the Isolates

The standard agar-disc-diffusion method (Kirby Bauer technique) was used to examine the antibiotic susceptibility of the isolates (either sensitive or resistance) on Mueller-Hinton agar (Difco, Detroit, MI) [4, 10, 11, 13, 18-20]. The commercial antibiotic discs employed over the bacterial lawns were Cefuroxime (CXM, 30 µg), Vancomycin (VA, 30 µg), Gentamycin (GEN, 10 µg), Amikacin (AK, 10 µg), Cotrimoxazole (SXT, 25), Ceftriaxone (CRO, 30), Azithromycin (AZM, 15), Colistin (CL, 10 µg), Cefixime (CFM, 5 µg), Levofloxacin (LE, 5 µg). After incubation at 37 °C for 24 hours at inverted position, the plates were examined and the zone of inhibition was measured in mm.

2.4. Statistical Analysis

The data generated in this study were statistically validated using SPSS statistics version 20.0 (IBM, Georgia, USA) and Microsoft Office Excel Professional Plus 2016 (Microsoft Corporation, Redmond, Washington, USA) program packages. The mean values and standard deviations (SD) were determined.

3. Results and Discussion

The main goal of the food processing industry is to provide consumers with healthy, wholesome, and acceptable food. Therefore, microbial control is crucial. Many bacteria that cause food poisoning and spoilage will bind to food and survive washing and disinfection. This may have a significant impact on the quality and safety of the packaged food, as well as posing a danger to consumers [2, 5, 21]. Due to the wide acceptability and convenience, ready-to-eat frozen food products are becoming increasingly popular around the world as well as in Bangladesh [22]. There are a variety of spoilage-causing bacteria that can thrive at low temperatures. The odor, flavor, and texture of frozen snacks can be changed by a vast number of bacteria and their products [6]. Therefore, the present study was carried out to investigate the microbiological quality of uncooked frozen chicken-based snack items for the estimation of overall scenario.

In present study, ready-to-fry frozen chicken-based snacks samples contained total viable bacteria and fungi in an average of 6 log cfu/g and 4 log cfu/g, respectively (Table 1) which were well exceeded the acceptable limit according to FSANZ ((Food Standards. Australia New Zealand) [23]. Specific bacterial isolates were also recovered to a large extent and in majority of the cases the count was above the standard microbial limit. All the samples were

found to contain *Staphylococcus* spp. *Pseudomonas* spp. and *Klebsiella* spp. in an average of 3 log cfu/g. *E. coli* and *Salmonella* spp. were encountered in almost all samples except of chicken samosa and chicken nugget, respectively (Table 1). *Vibrio* spp. was harbored in samples of three frozen chicken-based snack items. However, all the samples were devoid of the presence of fecal coliform and *Shigella* spp.

Table-1. Microbial load in the ready-to-fry frozen chicken-based snack samples

Frozen chicken-based snack samples (n=15)	Microbial load (log cfu/g)							
	TVB	Fungi	<i>Staphylococcus</i> spp.	<i>Klebsiella</i> spp.	<i>E. coli</i>	<i>Pseudomonas</i> spp.	* <i>Vibrio</i> spp	* <i>Salmonella</i> spp
Chicken Pops (n=3)	6.1±0.28	4.7±0.12	3.4±0.29	3.1±0.12	3.5±0.43	4.1±0.26	3.4±0.13	3.1±0.46
Chicken Samosa (n=3)	7.1±0.11	4.3±0.27	3.3±0.22	3.5±0.11	0±0	3.7±0.28	3.1±0.12	3.7±0.21
Chicken Lemongrass Lolloypop (n=3)	6.2±0.12	4.1±0.33	3.2±0.09	3.1±0.18	3.1±0.22	4.2±0.37	4.1±0.08	3.1±0.34
Chicken Nugget (n=3)	6.1±0.26	4.5±0.12	3.3±0.15	3.3±0.19	3.3±0.14	3.1±0.27	0±0	0±0
Chicken Meatball (n=3)	6.2±0.32	4.2±0.18	3.1±0.32	4.1±0.23	4.2±0.11	3.2±0.29	0±0	3.2±0.32

TVB- Total viable bacteria

Mean±SD count (log₁₀ cfu/mL) of microorganisms from all the samples have been shown here.

*Bacterial load after enrichment (Prior to enrichment, the count of bacteria was nil).

Fecal coliform and *Shigella* spp. were absent in all samples.

Microbial acceptable limit for the frozen foods according to FSA NZ Food Standards. Australia New Zealand FSA NZ [23] is the following:

Standard plate count: <5 log cfu/g

Enterobacteriaceae: 2 log cfu/g to 4 log cfu/g

E. coli and coliforms: 3 to <2 log cfu/g

Other pathogens: 2 log cfu/g to <3 log cfu/g

Similar to the current study, Chakraborty, *et al.* [6] found total viable bacteria in an average of 10⁶ cfu/g along with *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella aerogenes* and *Proteus mirabilis* in frozen snacks in their study in Bangladesh. Another study carried out by Banik, *et al.* [22] reported more than 6 log cfu/g total viable bacteria in addition to coliforms in ready-to-eat chicken-based and other snack samples. Sultana, *et al.* [2] in their study on ready-to-cook frozen foods of animal origin found total viable bacteria in a range of 10⁴-10⁶ cfu/g. They also revealed the presence of a range of Gram positive and Gram negative bacterial isolates among which *Staphylococcus* spp., *Alcaligenes* spp. and *Klebsiella* spp. were predominant. Enayat, *et al.* [5] documented the presence of several enteric bacteria with a higher prevalence of *E. coli* in chicken-based and other frozen food samples. Several other pieces of research also reported similar findings [24, 25]. Contamination of soil, air, flies, water, manufacturing content, staff, harvesting, and transportation equipment may have contributed to the bacterial population in these frozen snacks [6].

Another aspect of the current study was to determine the antibiotic susceptibility of the isolates. Multidrug resistance was observed as all the pathogenic isolates were found to be resistant to two or more antibiotics (Table 2). All the isolates showed resistance against Cefuroxime. Cefixime resistance was found in almost all the isolates. While, Gentamycin, Ceftriaxone, Colistin and Levofloxacin were able to completely inhibit the growth of all the bacterial isolates (Table 2). A major proportion of bacterial isolates showed resistance against Vancomycin and Azithromycin. Whereas, the isolated showed sensitivity against Amikacin and Cotximoxazole in greater proportion than resistance (Table 2).

Table-2. Antibiotics susceptibility pattern of the isolates from the frozen snacks samples

Antibiotic disc	Bacterial Isolate (% resistant/sensitive)					
	<i>Staphylococcus</i> spp. (n=5)	<i>Vibrio</i> spp. (n=3)	<i>Salmonella</i> spp. n=4)	<i>E. coli</i> (n=4)	<i>Klebsiella</i> spp. (n=5)	<i>Pseudomonas</i> spp. (n=5)
CXM 30	100/0	100/0	100/0	100/0	100/0	100/0
VA 30	80/20	33.3/66.7	75/25	100/0	80/20	20/80
GEN 10	0/100	0/100	0/100	0/100	0/100	0/100
AK 10	20/80	33.3/66.7	25/75	25/75	20/80	80/20
SXT 25	20/80	0/100	25/75	100/0	80/20	20/80
CRO 30	0/100	0/100	0/100	0/100	0/100	0/100
AZM 15	80/20	33.3/66.7	50/50	75/25	40/60	80/20
CL 10	0/100	0/100	0/100	0/100	0/100	0/100
CFM 5	100/0	100/0	100/0	50/50	100/0	100/0
LE 5	0/100	0/100	0/100	0/100	0/100	0/100

CXM - Cefuroxime (30 µg), VA - Vancomycin (30 µg), Gen - Gentamycin (10 µg), AK - Amikacin (10 µg), SXT - Cotximoxazole (25 µg), CRO - Ceftriaxone (30 µg), AZM - Azithromycin (15 µg), CL - Colistin (10 µg), CFM - Cefixime (5 µg), LE -Levofloxacin (5 µg)

In agreement with our study, Sultana, *et al.* [2] found a higher proportion of resistance in bacterial isolates against cefixime, nalidixic acid, chloramphenicol and azithromycin. According to their study findings, most of the

isolates were sensitive to levofloxacin and imipenem. Banik, *et al.* [22] reported ampicillin-, sulfamethoxazole- and tetracycline-resistant *E. coli* isolates in frozen snacks. Enayat, *et al.* [5] also claimed a higher proportion of resistance against tetracycline and ampicillin. Tansuphasiri, *et al.* [26] reported multidrug-resistant isolates in frozen foods as well. According to the findings of the present study, the presence of drug-resistant bacteria in ready-to-cook frozen snack samples poses a risk to human health and the environment and seeks a need for regular monitoring.

4. Conclusions

All the ready-to-fry frozen chicken-based snack samples which were analyzed in the present study appear to be heavily contaminated with drug-resistant bacteria. If left unaddressed or neglected, this may result in a public health threat. To ensure high quality and safe foods, there is an absolute need for practicing good hygienic procedures, proper handling, packaging, and retail of frozen foods items in a sterile environment and at proper refrigeration temperature. A regulatory framework should be established to respond to emerging food safety and environmental issues, ensuring traceability through food laws, and performing regular inspection and surveillance operations.

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