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Multi-antibiotic resistant bacteria in frozen food (ready to cook food) of animal origin sold in Dhaka, Bangladesh

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PEER REVIEW

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Comments

This work provides a better picture on the status of the RTE foods in Bangladesh. Moreover, it also provides information regarding the course of treatment and the type of food borne disease that can be suspected. This paper provides valuable insight for public health application.

Details on Page S271

ABSTRACT

Objective: To investigate the bacterial load and antibiotic resistance pattern of bacterial isolates obtained from (ready to cook) frozen food samples of animal origin in Dhaka, Bangladesh.

Methods: A total of 20 samples of frozen ready to cook food of animal origin were purchased from different separate grocery stores in Dhaka, Bangladesh. Bacteria were isolated and identified based on the basis of biochemical properties.

Results: A total of 57 isolates has been isolated from 20 samples, of them 35.08% were Gram positive and 64.92% were Gram negative organisms. Highest percentages of isolated organisms were *Staphylococcocus* spp. (24.56%), *Alcaligene* spp. (17.54%), *Klebshiella* spp. (12.28%) and the lowest percentages of organisms were *Enterococcus* spp., *Actinobacillus* spp. and *Proteus* spp. Antibiogram results clearly showed that levofloxacin and imipenem were the most effective drug against the isolates. The less effective antibiotics were chloramphenicol and nalidixic acid and resistance was highest against ciprofloxacin. The most contaminated food was chicken nuggets.

Conclusions: This type of frozen food contaminated with multi-antibiotic resistant microorganisms can be potential vehicles for transmitting food-borne diseases.

KEYWORDS

Animal origin, Antibiogram, Antimicrobial resistance, Frozen food, Food-borne disease, Multi-antibiotic

1. Introduction

The demand for ready-to-eat convenient food items is rising due to changing lifestyle. To cater the need, a range of frozen food products is being introduced in the market continually[1]. However, maintenance of quality of this product is of key importance to the continued development of this sector[2]. Quality and safety of frozen snack food are the aspects affecting the overall consumer acceptability in terms of flavor, texture, aroma, color and appearance besides microbiological safety and nutritional quality[3]. Microbial food safety is an increasing public health concern worldwide. It is estimated that food contamination with pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing and handling or preparation[4]. It was reported that numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens[5]. Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food borne pathogens[6].

In recent decades, antimicrobial resistance and reduced

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sensitivity in bacteria have become a major public health problem in many countries[7-9]. Limited information is available about the antimicrobial resistance bacteria in frozen food of Bangladesh. Drug resistance is spreading fast mainly due to overuse of antibiotics, incomplete and under use of medications and widespread practice of feeding livestock low levels of antibiotics to promote growth. Therefore, vigilance is needed in screening the drug resistance pattern of different antibiotics which should be a continuous process.

This study aims to evaluate bacterial load and antibiotic resistance pattern in bacterial isolates in frozen food samples of animal origin (ready to cook) in Dhaka, Bangladesh.

2. Materials and methods

2.1. Study area and sample collection

A total of 20 samples of frozen food (ready to cook) of different commercial brands were purchased from different supermarkets in Bangladesh at temperature 35 °C-37 °C. The food samples were taken in sterile plastic bags in ice-box. Samples were transported to the laboratory without delay and preserved in a freeze till analysis.

2.2. Preparation of samples

After collecting the samples, from each sample 10 g was aseptically weighed and stomached with 90 mL of sterile distilled water. Dilution with distilled water from 10^{-1} to 10^{-9}.

2.3. Enumeration of microorganisms

Each sample was tested for total bacterial count and total coliforms count. The total viable bacterial count was carried out by the pour plate technique according to ISO/DIS 4833-1:2009. Total coliform count was performed in most probable number technique according to ISO 4831:2006.

2.4. Isolation and identification of microorganisms

Stomached sample (25 mL) was added to 225 mL buffered peptone water and incubated at 37 °C overnight. For the isolation of microorganisms, the enriched sample was cultured on selective and non-selective media and incubated at 37 °C for 24 h. Morphologically typical colonies (at least 4 per plate) were taken into nutrient agar for further identification. The shape and type of Gram reaction are microscopically studied using 18 h culture from agar plate. The biochemical tests involved Kligler iron agar, Simmon's citrate slant, motility indole urease, lysine iron agar, urea broth, peptone water, methyl red, vogesproskauer, nutrient nitrate broth. Carbohydrate fermentation test was done for lactose, sucrose, glucose and starch, oxidase, and catalase tests. Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and differential media and; according to the procedures recommended in the Bergey's manual of determinative bacteriology[10].

2.5. Antibiotic susceptibility testing

Antibiotic susceptibility was tested by the standard agar disc diffusion technique on Mueller-Hinton agar using commercial discs (Oxoid, UK)[11]. The following antibiotics with the disc strength in parentheses were used: ciprofloxacin (cip, 5 μg), nalidixic acid (nal, 30 μg), cefuroxime (cem, 30 μg), azithromycin (azt, 15 μg), imipenem (imp, 10 μg), cefixime (cep, 30 μg), ceftriaxone (cef, 30 μg), levofloxacin (lev, 5 μg), chloramphenicol (chl, 30 μg). A control strain of *Escherichia coli* ATCC 25922 was included in each plate. Antimicrobial breakpoints and interpretation were taken from the Clinical and Laboratory Standards Institute standards[12].

3. Results

3.1. Total bacterial count and total coliform count of the frozen food samples

Total bacterial count of the samples ranged from 10^4-10^8 CFU/g and total coliform count ranged from 3-39 most probable numbers (MPN)/g. Most of the samples are acceptable in terms of total coliform count. Total bacterial count and total coliform count of the samples were given in Table 1.

Table 1

Total bacterial count and total coliform count of the samples.

Sample No.	Type of sample	Total bacterial count (CFU/g)	Total coliform (MPN/g)
S-1	Chicken nuggets	1.55×10^4	<3
S-2	Sausage	1.50×10^7	27
S-3	Meatball	5.50×10^8	35
S-4	Fish finger	1.20×10^7	19
S-5	Crispy chicken drumstick	1.32×10^7	6
S-6	Burger pattie	6.50×10^6	28
S-7	Beef samocha	7.00×10^6	12
S-8	Kids nuggets	4.50×10^6	9
S-9	Kievs	5.80×10^6	11
S-10	Chicken cutlet	6.50×10^6	12
S-11	Mini chicken spring roll	1.00×10^9	<3
S-12	Burgur pattie	9.00×10^6	39
S-13	Chicken drumstick	3.50×10^6	23
S-14	Beef samocha	4.00×10^8	14
S-15	Chicken drumstick	1.05×10^7	19
S-16	Chicken nuggets	5.00×10^8	6
S-17	Sausage	3.20×10^8	23
S-18	Chicken kebab	9.50×10^6	11
S-19	Chicken cutlet	9.50×10^6	<3
S-20	Meatball	8.50×10^6	<3

3.2. Identification of microorganisms from frozen food samples

A total of 57 bacteria were isolated from the 20 frozen food samples and identified to genus level on the basis of cultural and biochemical properties according to Bergey's manual of determinative bacteriology. Of the isolates, 64.92% were

Gram negative and 35.08% were Gram positive. In total, 13 genus were identified as shown in Table 2.

Table 2

Microorganisms identified.

Names of organisms	Percentage (%)
Staphylococcus spp.	24.56
Enterobacter spp.	3.50
Alcaligens spp.	17.54
Shigella spp.	8.77
Klebsiella spp.	12.28
Haemophilus spp.	7.01
Enterococcus spp.	1.75
Micrococcus spp.	5.26
Pseudomonas spp.	8.77
Actinobacillus spp.	1.75
Salmonella spp.	3.51
Proteus spp.	1.75
Corynebacterium spp.	3.51

3.3. Antibiotic susceptibility of microorganisms isolated from frozen food

Antibiotic susceptibility of the bacteria isolated from frozen food samples were determined against 9 antibiotics (Table 3) and comparative higher resistance was found against ciprofloxacin (54.40%), cefuroxime (49.10%), nalidixic acid (70.20%), azithromycin (57.90%), chloramphenicol (66.70%) and cefixime (72.00%). Distribution of microorganisms according to the number of antibiotic they were resistant showed that most of the isolates were resistant to at least 3 antibiotics (Table 4).

Table 3

Antibiotic resistance of the isolated bacteria.

Name of antibiotic	Percentage (%)
Ciprofloxacin	54.40
Ceftriaxone	14.00
Cefuroxime	49.10
Nalidixic acid	70.20
Levofloxacin	0.00
Azithromycin	57.90
Chloramphenicol	66.70
Imipenem	1.70
Cefixime	72.00

Table 4

Distribution of antibiotic resistance.

No. of antibiotic	Percentage (%)
6 Types of antibiotics	26.36
5 Types of antibiotics	7.02
4 Types of antibiotics	31.58
3 Types of antibiotics	17.55
2 Types of antibiotics	1.76
1 Types of antibiotics	10.53
0 Types of antibiotics	5.26

4. Discussion

A major goal for the food processing industry is to provide safe, wholesome and acceptable food to the consumer. Control of microorganisms is essential to meeting this goal. Many food pathogenic and spoilage bacteria are able to attach to food and remain viable even after cleaning and disinfection. This can seriously affect the quality and safety of the food processed and poses a potential risk to the consumer[2].

The present study demonstrated that three major bacterial sp., thus *Staphylococcus* sp., *Alcaligenes* sp. and *Klebshiella* sp. and rest out of thirteen species were present in frozen food (ready to cook) samples of animal origin obtained from supermarkets in the Dhaka, Bangladesh. The most prevalent bacteria were *Staphylococcus* sp. A recent study reported that the prevalence of antimicrobial resistance in commensally microflora is very useful in monitoring and understanding the process of antimicrobial-mediated selection in a population[4]. A total of 57 isolates of 13 different genus had been isolated from 20 frozen samples by aerobic culture method. Among them 24.56% belong to *Staphylococcus* spp., 17.54% were *Alcaligens* spp., 12.28% *Klebsiella* spp., 8.77% *Shigella* spp., 8.77% *Pseudomonas* spp, 7.01% *Haemophilus* spp., 5.26% *Micrococcus* spp., 3.51% *Salmonella* spp., 3.51% *Corynebacterium* spp., 3.50% *Enterobacter* spp. and 1.75% *Enterococcus* spp., 1.75% *Actinobacillus* spp. and 1.75% were *Proteus* spp. Most of the isolates were Gram negative bacillus (64.92%) and few were Gram positive cocci (35.08%).

Most of the isolates were resistant against cefixime, nalidixic acid, chloramphenicol and azithromycin. But maximum isolates were sensitive against levofloxacin and Imipenem. Most of the bacterial isolates were moderately sensitive to ciprofloxacin, ceftrixone and cefuroxime sodium. Resistant rate of ciprofloxacin were 0%, levofloxacin 0%, ceftrixone was 14.00%, cefuroxime sodium 49.10%, nitrofurantoin 54.4%, nalidixic acid 70.2%, azithromycin 57.9%, chloramphenicol 66.7%, imipenem was 1.70%, cefixime was 72.00%. Most resistant antibiotic was nalidixic acid, cefixime, chloramphenicol, azithromycin and cefuroxime sodium. The numbers of antibiotics resistant rate were also found. Organism resistant against six types of antibiotic 26.36%, five types of antibiotic 7.02%, four types of antibiotic 31.58%, three types of antibiotic 17.55%, two types of antibiotic 1.76%, one types of antibiotic 10.53% and the 100% sensitive against antibiotic organism were 5.26%. The four types of antibiotic resistant rate were higher than other types. Levofloxacin and Imipenem were found to be the most effective antibiotic. On the other hand cefixime, nalidixic acid, and chloramphenicol were the less effective antibiotics.

The quality of frozen food samples of animal origin which were ready to cook has deteriorated widely in Bangladesh and if resistant pathogens are enriched in these food resources will be a threat in future. It is a useful tool for the simultaneous monitoring of several resistant pathogens in the consuming food product[13]. The spontaneous transfer of these genes to sensitive organism will ameliorate the existing ratio of drug resistance. It is a clear indication that frozen food samples of animal origin which were ready to cook are responsible for the development of bacterial resistance with the risk of human health and environment and has to be regularly monitored[14].

In conclusion, all the frozen food samples of animal origin which were ready to cook samples analyzed appear to be contaminated with bacteria. This culminates a potential public health danger if allowed unaddressed or unabated. The need for good hygienic practices, proper handling, storage and retail of frozen foods in clean environment and at refrigeration temperature cannot be over emphasized to ensure good quality and safe foods. A regulatory system should be developed to response emerging concerns for food safety and ecology, for ensuring traceability through food regulations and for monitoring and surveillance activities.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

With the increasing pace in human life, ready to eat food has come to play an important role in providing quick nutrition along with the tastes. The growing demand of ready to eat food has placed a burdening global demand in the microbial safety of such foods.

Research frontiers

The present work paints a picture on the microbial load, its type and its susceptibility to various antibiotics available in Bangladesh, against the common pathogens that are found in the ready to eat foods.

Related reports

Not many papers has been published on microbial contamination and antibiotic resistance in frozen foods of Bangladesh.

Innovations and breakthroughs

The main innovation in the paper is that it takes into consideration the resistance of the microorganisms to antibiotics. This provides the information on the type of microorganisms to be suspected and the selection of antibiotics for the treatment in case of food-borne disease.

Applications

This paper provides information that can be used in Public Health of the country. It will be a valuable paper for physician referencing.

Peer review

This work provides a better picture on the status of the ready to eat foods in Bangladesh. Moreover, it also provides information regarding the course of treatment and the type of food borne disease that can be suspected. This paper provides valuable insight for public health application.

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