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Microbiological Quality Assessment of Ready-To-Eat Fried Chicken and Chicken Soup Samples Sold in Dhaka Metropolis, Bangladesh

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Abstract

Contamination of ready-to-eat foods with pathogenic microorganisms depicting them unacceptable for human consumption and has become a global health problem. Current study attempted to investigate the occurrence of bacterial contamination in ready-to-eat fried chicken and chicken soup samples. In this regard, a total of fifteen fried chicken samples and twelve chicken soup samples were collected from Chinese restaurants, fast food shops and street food shops along with homemade samples. Microbiological analysis of fried chicken revealed that homemade samples and samples from Chinese restaurants and fast food shops were safe for consumption as microbial load was found to be within the acceptable limit. While, the load of total viable bacteria and fungi exceeded the standard microbial limit in the samples collected from University canteen and street food shops. E. coli and Klebsiella spp. along with Staphylococcus spp. and Pseudomonas spp. were also encountered in those samples. In case of chicken soup, homemade samples were found to be good for consumption as only total viable bacteria was found which was in acceptable range. On the other hand, chicken soup samples from street food shops were found to be unacceptable for consumption. Along with total viable bacteria and fungi, specific bacteria including Klebsiella spp., E. coli, Pseudomonas aeruginosa, Pseudomonas putida and Staphylococcus spp. above the acceptable limit were encountered in that samples. In addition, all the isolates from both the types of samples were found to be multidrug resistant. In case of fried chicken samples, the isolates showed resistance against Cefuroxime and Novobicin. Whereas, Azithromycin, Cefiprime, Ceftriaxone, Ciprofloxacin, Meropenem and Amikacin sensitivity were found in all the isolates. For chicken soup samples, all the pathogenic bacterial isolates were found to be resistant against Cefuroxime, Vancomycin, Amoxycilin and Novobicin. On the other hand, the isolates were found to be sensitive against Azithromycin, Kanamycin, Ceftriaxone, Ciprofloxacin, Meropenem and Amikacin. Presence of microbial contaminants in some tested samples and the occurrence of drug resistance in the bacterial isolates portray the public health risk in the consumption of such food.

Keywords: Ready-to-eat foods; Fast food; Microbiological quality; Drug resistance; Chicken soup; Fried chicken.

1. Introduction

Safety of commonly consumed food items has attracted a major concern globally for the maintenance of mass public health [1]. Foods are essential factors for every living being including microorganisms, as foods are full of nutrients such as protein, fat, carbohydrates, minerals and vitamins which likely make foods easier to be contaminated by microorganisms. Foods are usually subjected to contamination by various bacterial species and fungi during harvesting; processing; and using of associated machines, utensils, water, etc. [2-5]. Foods contaminated with pathogenic microorganisms are responsible for food borne infections or intoxications including the enteric complications, abdominal pain, fever, hemorrhagic colitis, bloodstream infection, meningitis, joint infection, kidney failure, paralysis, miscarriage, etc. [6-14].

Ready-to-eat or fast food is a kind of food that can be prepared and served very quickly [15, 16]. Such foods are ready for immediate consumption at the point of sale that can be raw or cooked, hot or chilled and can be consumed without further heat treatment [17, 18] . Ready-to-eat foods are commercially provided snacks inclusive of pastries, burger, sandwitch, salad or coleslaw, fried meat, fried chicken, milk and milk products etc. Fast foods are getting increasing popularity in the last two decades, particularly in metropolitan areas. However, read-to-eat foods are consumed without additional treatment. Improper handling of such kind of foods may result in foodborne disease outbreaks upon its consumption. Previous studies revealed the presence of various foodborne pathogens in the fast food products and found ready-to-eat foods to be a source of bacterial foodborne outbreaks [19, 20]. The numbers of

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microorganisms can be affected by the methods of storage, processing, handling and distribution of fast food items [21, 22].

Chicken and ready-to-eat chicken meals are the main type of meat consumed worldwide [23]. Poultry meat and meat products are considered as one of the most perishable foods since it contains sufficient nutrient required to support the growth of microorganisms [24, 25]. Poultry meat comprises of water, fat, phosphorus, protein, iron and vitamins. Also, most poultry meat have high water content with water activity of 0.99 which promote microbial growth [26]. Microorganisms such as *Staphylococcus aureus*, *Salmonella* spp., *Mucor* spp., *Campylobacter* spp., *Pseudomonas* spp., *Micrococcus* spp., *Moraxella* spp., lactic acid bacteria and various genera of *Enterobacteriaceae* family are the major contaminants of poultry products [27-29]. Contamination of poultry meat with foodborne pathogens pose a public health concern, as it can lead to illness if there are malpractices in handling, cooking or post-cooking storage of the product. It is evident from several studies that foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry are particularly a defined source of transmission of these food borne pathogens [30, 31]. Moreover, the occurrence of antimicrobial resistance among food-borne pathogens has increased during recent decades [32, 33]. There is increasing proof that animals constitute a reservoir of antimicrobial resistance as antimicrobials are using to raise food producing animals.

Although huge knowledge is available on the food borne pathogens around the world and extensive researches are continually being conducted globally, a few work on the microbiological contamination of the fast foods especially on ready-to-eat chicken meals has been carried out in Bangladesh, not even with a minimum public health priority [3, 34, 35]. Therefore, the present study was aimed to investigate the occurrence of pathogenic bacteria and fungi in ready-to-eat fried chicken and chicken soup samples collected from different retailer shops of Dhaka city. Drug susceptibility pattern of the isolates was also determined.

2. Materials and Methods

2.1. Study Period and Sampling

A total of fifteen fried samples including 3 homemade and 3 samples each from University canteen, Chinese restaurants, fast food shops & street food shops, and twelve ready-to-eat chicken soup samples including 3 homemade and 3 samples each from Chinese restaurants, fast food shops & street food shops were collected from different locations of Dhaka city, Bangladesh during October and December 2018 following standard protocol [36]. The samples were homogenized and subjected to serial dilution up to 10^{-4} according to the standard methods as described by Cappuccino and Sherman [37].

2.2. Isolation and Identification of Microorganisms

2.2.1. Estimation of Total Viable Bacteria, Fungi, Escherichia Coli, Klebsiella Spp., Pseudomonas Spp., Staphylococcus Spp. And Bacillus Spp

For the enumeration of 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. Plates were incubated at 37° C for 24 hours for total viable bacteria and 25° C for 48 hours for fungi [11-13, 36, 37]. From the dilutions 10^{-1} and 10^{-2}, 0.1 ml of each sample was spread onto the membrane fecal coliform (MFC) agar and MacConkey agar for the enumeration of total fecal coliform (TFC), and coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hours at 44.5°C and 37°C for fecal coliform and coliforms, correspondingly. Likewise, *Bacillus* spp., *Staphylococcus* spp. and *Pseudomonas* spp. were isolated onto Starch agar, Mannitol Salt Agar (MSA) and *Pseudomonas* agar, respectively by adding 0.1 ml of each sample from dilutions 10^{-1} and 10^{-2}, and all the plates were then incubated at 37°C for 24 hours [11-13, 37].

2.2.2. Isolation of Salmonella spp., Shigella spp. and Vibrio spp

Ten (10) ml of each sample was transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella*, and *vibrio* spp., respectively and incubated at 37°C for 6 hours. After incubation, 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 for the isolation of *Salmonella* spp. *and Shigella* spp., *and Vibrio* spp., consecutively. Plates were incubated at 37°C for 48 hours for the detection of typical colonies. Finally, all the isolates were biochemically examined following standard procedures as described earlier [11-13, 36, 37].

2.3. Antibiotic Susceptibility Test 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) [38-40]. The antibiotic discs used in this experiment were Amoxicillin (AMX, 30 µg), Amikacin (AK, 10), Meropenem (MEM, 10 µg), Azithromycin (AZM, 30 µg), Ciprofloxacin (CIP, 5 µg), Cefixime (CFM, 5 µg), Cefuroxime (CXM, 30 µg), Ceptriaxone (CTR, 30 µg), Trimethoprime-Sulfamethoxazole (COT, 25 µg), Novobicin (NV, 30 µg), Aztreonam (ATM, 30 µg), Cefiprime (CEF, 30 µg), Rifampicin (RIF, 5 µg) and Amoxy Cillin/Clavulanic Acid (20/10 µg). The plates were then inverted and incubated at 37 °C for 24 hours. After incubation, the plates were examined and the zone of inhibition was measured in mm.

3. Results and Discussion

Recently, consumption of ready-to-eat foods has been increased extensively and fast food sectors are becoming an important industry [41]. Due to improper handling and management such kind of foods can easily become contaminated with pathogenic microorganisms which often lead to food borne illnesses and even death. Therefore the present study was carried out to evaluate the microbiological quality of ready-to eat fried chicken and chicken soup samples.

3.1. Occurrence of Pathogenic Microorganisms in the Tested Fried Chicken and Chicken Soup Samples

In case of fried chicken, homemade fried chicken samples and samples from Chinese restaurant and fast food shop contained total viable bacteria and fungi in an average of 10^4 cfu/g and 10^3 cfu/g, respectively (Table 1). Among the pathogenic bacteria, *Staphylococcus* spp. and *Pseudomonas* spp. were present in Chinese restaurant and fast food samples in an average of 10^2 cfu/g, while homemade samples only contained *Pseudomonas* spp. These samples were safe to consume as the microbial load was in the acceptable limit (standard plate count: $<10^5$ cfu/g) according to FSANZ (Food Standards. Australia New Zealand) [42]. On the other hand, samples from University canteen and street food shop rendered to be acceptable for consumption as they had total viable bacterial count of 3.3×10^5 cfu/g and 3.3×10^5 cfu/g, respectively (Table 1). Fungi were present in both the types of the samples in an average of 10^4 cfu/g. In addition, the samples contained *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *E. coli*, and the count was above the standard microbial limit ($<10^2$ cfu/g for coliforms and $<10^3$ cfu/g for other pathogens) provided by FSANZ (Food Standards. Australia New Zealand) [42]. *Bacillus* spp., fecal coliforms, *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. were absent in all the samples (Table 1).

Table-1. Microbial load in the tested fried chicken samples

Fried chicken samples (n=15)	**Microbial load ((cfu/g)**					
	TVB	**Fungi**	***Staphylococcus* spp.**	***Klebsiella* spp.**	***E. coli***	***Pseudomonas* spp.**
Homemade (n=3)	1.3×10^4	2.1×10^3	0	0	0	6.0×10^2
Chinese restaurant (n=3)	2.5×10^4	2.8×10^4	1.2×10^2	0	0	7.1×10^2
Fast food shop (n=3)	3.5×10^4	1.8×10^3	1.8×10^2	0	0	2.5×10^2
University canteen (n=3)	3.3×10^5	1.2×10^4	1.8×10^3	2.0×10^2	1.0×10^2	6.5×10^3
Street food shop (n=3)	2.8×10^6	2.3×10^4	1.2×10^4	1.6×10^2	2.5×10^2	5.4×10^4

TVB- Total viable bacteria
Average count (cfu/g) from all samples have been shown here.
Bacillus spp., Fecal coliform, *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. were absent in all samples.
Microbial acceptable limit for the cooked ready-to-eat foods according to FSANZ (Food Standards. Australia New Zealand) [42] is following:
Standard plate count: $<10^5$ cfu/g
Enterobacteriaceae: 10^2 to $<10^4$ cfu/g
E. coli and coliforms: 3 to $<10^2$ cfu/g
Other pathogens: 10^2 to $<10^3$ cfu/g

The findings in this study for the fried chicken samples was in agreement with Clarence, *et al.* [43], that six different bacterial pathogens including *Staphylococcus* spp., *E.coli*, *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp. and *Enterococcus* spp. Other researchers also found similar kind of results in their study on fried chicken samples [44-46]. The presence of microorganisms in fried chicken portrays a state of poor hygiene and sanitary practices in the retailer during the processing and sales of these food products.

On the other hand, microbial contaminations were evident in the ready-to-eat chicken soup samples. However, the level of contamination only exceeded the acceptable microbial limit in the case of soup samples collected from street food shops (Table 2). In those samples, total viable bacteria and fungi were found to be 3.2×10^5 cfu/g and 2.3×10^4 cfu/g, respectively. *Staphylococcus* spp., *Pseudomonas aeruginosa* and *Pseudomonas putida* encountered in an average of 10^4 cfu/ml. The samples also contained *E. coli* and *Klebsiella* spp. above the standard acceptable limit (Table 2). On the other hand, homemade chicken soup samples only carried total viable bacteria of 1.3×10^3 cfu/g. Total viable bacteria and fungi were present in an average of 10^3 cfu/g in the samples collected from Chinese restaurants and fast food shops. Among the specific bacteria, *Pseudomonas aeruginosa* and *Pseudomonas putida* were present in these two types of samples, but within the acceptable limit (Table 2). *Bacillus* spp., fecal coliforms, *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. were absent in all the samples. Previous studies carried out elsewhere also found higher rate of microorganisms in chicken soup samples collected from street food [44-46]. The extended bacterial count in chicken soup samples from street food shops would indicate that they were contaminated after cooking, during handling procedures, demonstrating an overall lack of hygiene.

Table-2. Microbial load in the tested cooked ready-to-eat chicken soup samples

Ready-to-eat chicken soup samples (n=12)			Microbial load ((cfu/g)				
	TVB	Fungi	*Staphylococcus* spp.	*Klebsiella* spp.	*E. coli*	*Pseudomonas aeruginosa*	*Pseudomonas putida*
Homemade (n=3)	1.3×10^3	0	0	0	0	0	0
Chinese restaurant (n=3)	2.4×10^3	1.3×10^3	0	0	0	1.2×10^2	1.8×10^2
Fast food shop (n=3)	5.0×10^4	1.8×10^3	0	0	0	3.2×10^2	2.5×10^2
Street food shop (n=3)	3.2×10^5	2.3×10^4	6.2×10^4	8.3×10^3	1.8×10^3	2.8×10^4	2.1×10^4

TVB- Total viable bacteria
Average count (cfu/g) from all samples have been shown here.
Bacillus spp., Fecal coliform, Vibrio spp., *Salmonella* spp. and *Shigella* spp. were absent in all samples.
Microbial acceptable limit for the cooked ready-to-eat foods according to FSANZ (Food Standards. Australia New Zealand) [42] is following:
Standard plate count: $<10^5$ cfu/g
Enterobacteriaceae: 10^2 to $<10^4$ cfu/g
E. coli and coliforms: 3 to $<10^2$ cfu/g
Other pathogens: 10^2 to $<10^3$ cfu/g

3.2. Antibiotic Resistance of the Isolates

Previously, drug resistant isolates were evident by many researchers in fast food and other food items [3, 34, 35]. In current study, multidrug resistance was observed in all the pathogenic isolates (Table 3). In case of isolates from ready-to-eat fried chicken, all the pathogenic isolates were found to be resistant against Cefuroxime and Novobicin (Table 3). While, Azithromycin, Cefiprime, Ceftriaxone, Ciprofloxacin, Meropenem and Amikacin were able to inhibit the growth of all the bacterial isolates. Aztreanam and Rifampicin resistance were only found in *Klebsiella* spp. which along with *E. coli* showed resistance against Cefixime and Amoxycillin. Trimethoprime/sulfamethoxazole resistance was only found in *Pseudomonas* spp. While *Klebsiella* spp. and *Staphylococcus* spp. were found to be resistant against Amoxycillin/Clavulanic Acid.

Table-3. Antibiotics susceptibility pattern of the isolates from the fried chicken and chicken soup samples

Isolates	Antibiotics													
	AZM	CXM	ATM	CEF	CTR	CFM	CIP	COT	MEM	AMC	NV	AK	AMX	RIF
Fried Chicken samples														
E. coli	S	R	S	S	S	R	S	S	S	S	R	S	R	S
Klebsiella spp.	S	R	R	S	S	R	S	S	S	R	R	S	R	R
Pseudomonas spp.	S	R	S	S	S	S	S	R	S	S	R	S	S	S
Staphylococcus spp.	S	R	S	S	S	S	S	S	S	R	R	S	S	S
Chicken soup samples														
E. coli	S	R	R	S	S	R	S	S	S	R	R	S	R	S
Klebsiella spp.	S	R	R	S	S	R	S	R	S	R	R	S	R	R
Pseudomonas aeruginosa	S	R	S	S	S	R	S	S	S	R	R	S	R	S
Pseudomonas putida	S	R	R	S	S	S	S	S	S	R	R	S	R	S
Staphylococcus spp.	S	R	S	S	S	S	S	S	S	R	R	S	R	S

R: Resistant; S: Sensitive

AZM = Azithromycin (30 µg); CXM = Cefuroxime (30 µg); ATM = Aztreonam (30 µg); CEF = Cefiprime (30 µg); CTR = Ceftriaxone (30 µg); CFM = Cefixime (5 µg); CIP = Ciprofloxacin (5 µg); COT = Trimethoprime/sulfamethoxazole (25 µg); MEM = Meropenem (10 µg); AMC = Amoxycillin/Clavulanic Acid (20/10 µg); NV = Novobicin(10 µg); AK = Amikacin (10 µg); AMX = Amoxicillin (30 µg); RIF = Rifampicin (5 µg).

In case of isolates from the ready-to-eat chicken soup samples, all the pathogenic isolates were found to be resistant against at least four drugs including Cefuroxime, Vancomycin, Amoxycilin and Novobicin (Table 3). Whereas, Azithromycin, Kanamycin, Ceftriaxone, Ciprofloxacin, Meropenem and Amikacin sensitivity were found in all the bacterial isolates. Trimethoprime-Sulfamethoxazole and Rifampicin resistance were only observed in *Klebsiella* spp. *E. coli* and *Klebsiella* spp. were also found to be resistant against Tetracycline and Cefixime. These trends of antibiotic susceptibility were in line with earlier observations carried out by some researchers [47, 48]. Antibiotic resistant bacteria could be transmitted to human if ready-to-eat chicken meals are improperly cooked or handled. Therefore, health professionals should generate plan for the reduction in the spread of antibiotic resistant foodborne pathogens through the food chain, with the aim to control their outbreaks in the community.

4. Conclusions

The findings of the present study revealed that the fried chicken samples from University canteen and street food shops and ready-to eat chicken soup sold in street food shops were constitutes a likely potential hazard to human health, though other samples were good or acceptable for consumption. Presence of drug resistant bacteria is alarming and may accelerate the health risk of the consumers. As such ready-to-eat foods are consumed by majority of population especially in the urban settings; it is obvious to maintain a good microbiological quality and safety of the foods. Public awareness programs are encouraged to be implemented for the food processors, food vendors and

personnel involved in food preparation. Training programs may also lead to an improvement in hygienic practices and implementing a hazard analysis system in street vended and restaurant premises.

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