

Microbiological Hazard Analysis and Exposure Assessment of Street Vended Ready-to-Eat Foods in Dhaka City, Bangladesh

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Abstract: A total of 48 samples of street vended ready-to-eat (RTE) food products from Dhaka city were examined to find out the microbiological quality of these foods based on standard techniques (e.g., culturing on selective media) by determining total bacterial load, total coliforms, *E. coli* and total fungi showed that, almost all food samples remained hygienically poor since bacterial loads (aerobic plate counts, total coliforms counts, *E. coli* counts and total fungal counts) on the whole were abnormally high (APC 4.11×10^2 - 1.66×10^7 CFU g^{-1} ; TC 12-2075 MPN g^{-1} ; EC-1590 MPN g^{-1} ; TFC 81-6596 CFU g^{-1}). Among the 48 samples, 95.8 % contained mesophilic bacterial cells range of $>10 gm^{-1}$ while only somosa samples contained bacterial cells range $<10 gm^{-1}$. Only 4.2 % were found to be safe within APC range and 33 % were dangerously contaminated. All the 48 samples were found coliforms positive and only 29.16 % samples were in the acceptable range, whereas 83.33 % were found to be *E. coli* positive. A significant number of fungi were also found in sola, hog-plum, betel-leaf and jhal-muri. The results of our study indicated that most of the RTE food samples examined did not meet bacteriological and fungal quality standards. Hence, it is recommended that a more close supervision of such food type should be carried out by relevant authorities to avoid any future pathogen outbreaks.

Key words: Microbiological quality • Ready-to-eat (RTE) food • Bacterial load • Coliforms • Fungal Count

INTRODUCTION

Foods from street-vendors are ready-to-eat (RTE) foods, prepared and sold on streets and other public places [1]. The types of street-vended food vary significantly on countries and cultures [2]. Foods from the street provide a source of readily available, inexpensive meals with good nutritional values for the consumer. However, questions have been raised about the safety and microbiological quality of these food products. Food borne illnesses are a widespread public health problem globally. Developing countries bear the brunt of the problem due to the presence of a wide range of food-borne diseases. RTE foods pose a health threat to consumers, since any microorganisms present will be consumed as compared to food that is cooked before eating which should kill most of the microorganisms [3].

The basic human requirement for the intake of food places every human being at risk of contracting infection by food-borne pathogens. This fact is true not only in developing countries but in many developed countries. Food experts and public health agencies have put emphasis on control of food-borne pathogens in industries during processing [4]. Improper in home food handling, preparation and consumption practices by consumers [5-7], inadequate hygiene practices such as hand washing [8] and use of unhygienic utensils and materials [9-11], consumption of raw or unsafe food [12, 13], as well as cross-contamination via inanimate surfaces by raw food are some of the factors and practices that have been implicated in food-borne outbreaks in the home. The pathogens isolated most often in such outbreaks have been *Staphylococcus*, *Salmonella*, *Campylobacter*, *Escherichia coli*, *Clostridium perfringens* and *Vibrio cholera* [15-18].

In Bangladesh, the most popular and traditional street-vended foods includes; cup-cake, jhal-muri, bun, hog-plum, betel-leaf, sola, peaju, sweet, sheek-kabab, singara, somosa and vhel-puri. Dhaka city has a great number of street food vendors. These vendors congregate primarily in the central business areas and at major points of transit such as train and bus stations, as well as in front of school where several people enjoy these traditional foods. In Dhaka streets, food vending is ubiquitous; however there is a lacking of information regarding food borne diseases related to street-vended foods. The vendors in Bangladesh lack education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore dish and hand washing is done using the same bucket, sometimes even without soap. Garbage and waste water is typically discarded in the streets nearby and thus attracting and providing food for rodents and insects. Toilets are not available nearby in several cases thus forcing the vendors to eliminate their body wastes in nearby areas and return to their vending sites without washing their hands [19]. Environmental condition and practices like this often lead to contamination of cooked food. Vendors may purchase raw materials from doubtful sources which may either be contaminated with food borne pathogens or be unfit for consumption due to other reasons [1]. *Escherichia coli* infection leading to diarrhea is prevalent in Bangladesh. Reports have suggested that, Enteropathogenic *E. coli* (EPEC) is a predominant cause of diarrhea in Bangladesh and is spreads through contaminated water and food [20]. The major factor contributing to the spread and transmission of the disease include poor hygiene and lack of proper sanitation [21].

Although there is a growing demand for RTE food products, no recent information is available regarding the microbiological quality of these products in Dhaka city, Bangladesh. The present study was hence undertaken to determine the microbiological quality and safety of a variety of street- vended RTE food products collected from several typical vendors surrounding the busiest street of four different areas of Dhaka city.

MATERIALS AND METHODS

Samples Collection: A total of 48 samples were collected for bacteriological and fungal examination. The street vendors on the busiest street areas were chosen for sample collection. The samples were congregated during the months of June to August 2010, when the average

ambient temperature is $30-32\pm 0.5^{\circ}\text{C}$. The food samples were collected from vendors on Sunday afternoon between 1:30 and 2:30pm. The time interval between sampling from each vendor was approximately 15 minutes. Eight samples were collected on every sampling day. Food samples included cereal based (jhal-muri), cooked food (sheek-kabab, singara, peaju, somosa and vhel-puri), vegetable (betel-leaf) and fruits (hog-plum), baked goods (bun, sweet and cup-cake). Approximately 300 g of each food sample was collected using the vendors serving utensils, take parcel and placed into sterile plastic bags. Food temperatures were recorded at the time of sampling using a portable thermometer. All the collected samples were kept on an ice-box during transportation to the laboratory and stored at 4°C until testing. They were analyzed within 24 hours of sampling.

Processing of Samples: Before analysis, 10 grams each of sample were added to 90 ml lactose broth (Oxoid Ltd., Hampshire, England). The samples were emulsified separately using a Seward 400 Lab Stomacher (London, U.K.) at high speed for 120 seconds followed by serial dilutions as required.

Determination of Aerobic Plate Count (APC): The lactose broth emulsion was then used to determine the APC, by making serial dilutions using sterile saline and surface plating on nutrient agar plates and eosin methylene blue (EMB) agar (Oxoid Ltd., Hampshire, England) in duplicate. Inoculated plates were incubated aerobically at 37°C for 48 h after which the colonies were enumerated using a Quebec Darkfield Colony Counter (Cambridge Instruments Inc., Buffalo, USA). The mean count of colonies on duplicate plates was determined.

Detection of Total Coliforms (TC): Total coliforms and faecal counts of food sample were determined using the Most Probable Number (MPN) technique [22].

Presumptive Test: Fermentation tubes with appropriate quantity (10ml) of lauryl tryptose broth (Sisco Ltd., Mumbai, India) medium were distributed with different strengths. The tubes were inoculated with 10ml, 1 ml with 10 ml, 1ml and 0.1 ml amount of sample and incubated at 37°C for 24 ± 1 hr. Production of gas indicates the tubes to give positive presumptive test.

Confirmative Test: Tubes showing positive presumptive test were further tested for confirmation by inoculating in the Brilliant green lactose bile broth

(Oxoid Ltd., Hampshire, England) and streaking on the eosine methylene blue (EMB) agar (Oxoid Ltd., Hampshire, England).

Detection of *E. coli*: A number of tests were carried out to determine the presence of *E. coli* in the samples including indole test, Voges-Proskauer (VP) test and methyl red test.

Determination of Total Fungal Count (TFC): The spread-plate method was used to prepare plates for the determination of TFC. 0.2 ml of 10^{-1} dilution of the homogenate sample was spread onto each Petri dish containing Potato Dextrose Agar (PDA) (HiMedia, Mumbai, India) plate and incubated at room temperature for about four days, after which the plate was examined to detect fungal growth. Following equation was used to calculate APC and TFC:

$$\text{Total no of organism} = \frac{\text{no of colony/ml} \times \text{dilution factor} \times \text{TV}}{\text{amount of solute dissolve/ TV}}$$

RESULTS

The microbial analyses of various RTE foods in Dhaka city are presented in Table 1. In the present study, 12 types RTE food samples, including cup-cake, jhal-muri, bun, hog-plum, betel-leaf, sola, peaju, sheek-kabab, sweet, singara, somosa and vhel-puri were tested.

A significant bacterial count (CFU g^{-1}) was detected in jhal-muri ($1.66 \times 10^7 \text{ CFU g}^{-1}$), betel-leaf ($1.49 \times 10^7 \text{ CFU g}^{-1}$), hog-plum ($1.87 \times 10^6 \text{ CFU g}^{-1}$), sweet ($3.39 \times 10^5 \text{ CFU g}^{-1}$) and bun ($3.11 \times 10^5 \text{ CFU g}^{-1}$). Sola ($6.24 \times 10^4 \text{ CFU g}^{-1}$), cup-cake ($6.19 \times 10^4 \text{ CFU g}^{-1}$), peaju ($4.96 \times 10^4 \text{ CFU g}^{-1}$), sheek-kabab ($2.63 \times 10^4 \text{ CFU g}^{-1}$) and vhel-puri ($1.96 \times 10^4 \text{ CFU g}^{-1}$) found to be contained moderate bacterial count whereas, singara ($8.93 \times 10^3 \text{ CFU g}^{-1}$) and somosa ($4.11 \times 10^2 \text{ CFU g}^{-1}$) found to be contained least bacterial count (Table 1). The total coliforms count was done using the MPN method. 100% (48 samples) showed positive results for coliforms. Although betel-leaf and jhal-muri showed excessive incidence of total coliforms, i.e., 2075 MPNg^{-1} and 1750 MPNg^{-1} , respectively, the counts were least for somosa (12 MPNg^{-1}), singara (16 MPNg^{-1}) and cup-cake (16 MPNg^{-1}) (Table 1). All samples except singara and somosa, showed positive results for *E. coli* count. Jhal-muri (1590 MPNg^{-1}) and betel-leaf (1265 MPNg^{-1}) had the higher level of contamination with *E. coli* among the 12 types of samples tested in this study (Table 1). 100% of the tested raw items showed significant amount fungal growth (CFU g^{-1}). Sola showed the highest incidence of total fungal count 6596 CFU g^{-1} followed by hog-plum 6197 CFU g^{-1} , betel-leaf 3856 CFU g^{-1} and jhal-muri 2312 CFU g^{-1} , whereas the rest 8 samples showed less fungal contamination when compared (Table 1).

The microbial evaluation and distribution of RTE food products are presented in Table 2.

Table 1: Microbial analysis of various RTE foods in Dhaka city.

Name of Sample	Total Bacterial Count (CFUg^{-1})	Total Coliforms (MPNg^{-1})	<i>E. coli</i> (MPNg^{-1})	Total Fungi (CFUg^{-1})
Cup-cake	6.19×10^4	16	2	544
Jhal-muri	1.66×10^7	1750	1590	2312
Bun	3.11×10^5	100	21	729
Hog-plum	1.87×10^6	66	20	6197
Betel-leaf	1.49×10^7	2075	1265	3856
Sola	6.24×10^4	1105	188	6596
Peaju	4.96×10^4	1050	4	451
Sheek-kabab	2.63×10^4	23	18	89
Sweet	3.39×10^5	22	11	81
Singara	8.93×10^3	16	0	198
Somosa	4.11×10^2	12	0	205
Vhel-puri	1.96×10^4	1750	124	839

Table 2: Microbial evaluation and distribution of RTE food products ^a

CFUg^{-1}	Cup cake (4) ^b	Jhal muri(4)	Bun (4)	Hog plum (4)	Betel leaf (4)	Sola (4)	Peaju (4)	Sheek kabab (4)	Sweet (4)	Singara (4)	Somosa (4)	Vhel puri (4)	Total Samples	Incidence (%) ^c
10 to $<10^2$	0	0	0	0	0	0	0	0	0	0	2	0	2	4.2
10^2 to $<10^4$	2	0	0	0	0	0	2	1	0	3	2	3	13	27.1
10^4 to $<10^5$	1	0	3	0	2	3	1	3	2	1	0	1	17	35.4
$\geq 10^5$	1	4	1	4	2	1	1	0	2	0	0	0	16	33.3
Incidence (%) ^d	25	100	25	100	50	25	25	0	50	0	0	0	33.3	-

^a 48 samples were tested; ^b number of samples of each food items; ^c incidence (%) indicates the percentage of positive samples for a given microorganisms; ^d incidence (%) indicates the percentage of highly contaminated food samples.

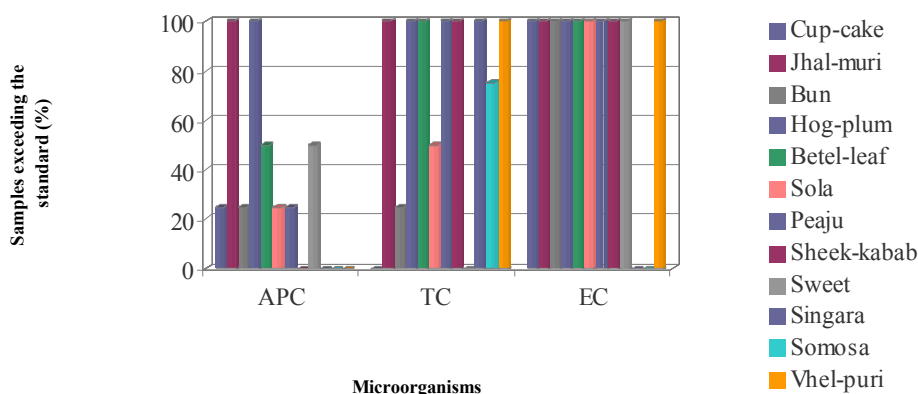


Fig. 1: Percentage of aerobic plate count (APC), total coliforms (TC) and *E. coli* (EC) not meeting the microbiological standards imposed by the Bangladesh Government in RTE food products.

Table 3: Microbiological quality of food from street-vends on the basis of total coliforms

Name of Sample	N ^a	N ^b	N ^c	Incidence ^d (%)
Cup-cake	4	4	0	0.0
Jhal-muri	4	0	4	100.0
Bun	4	3	1	25.0
Hog-plum	4	0	4	100.0
Betel-leaf	4	0	4	100.0
Sola	4	2	2	50.0
Peaju	4	0	4	100.0
Sheek-kabab	4	0	4	100.0
Sweet	4	4	0	0.0
Singara	4	0	4	100.0
Somosa	4	1	3	75.0
Vhel-puri	4	0	4	100.0
Total	48	14	34	70.84

^a number of samples of each food items; ^b number of acceptable food samples; ^c number of unacceptable food samples; ^d incidence (%) indicates the percentage of unacceptable food samples.

Table 4: Microbiological quality of food from street-vends on the basis of total *E. coli*.

Name of Sample	N ^a	N ^b	N ^c	Incidence ^d (%)
Cup-cake	4	0	4	100.0
Jhal-muri	4	0	4	100.0
Bun	4	0	4	100.0
Hog-plum	4	0	4	100.0
Betel-leaf	4	0	4	100.0
Sola	4	0	4	100.0
Peaju	4	0	4	100.0
Sheek-kabab	4	0	4	100.0
Sweet	4	0	4	100.0
Singara	4	4	0	0.0
Somosa	4	4	0	0.0
Vhel-puri	4	0	4	100.0
Total	48	8	40	83.33

^a number of samples of each food items; ^b number of acceptable food samples; ^c number of unacceptable food samples; ^d incidence (%) indicates the percentage of samples of unacceptable range.

Regarding the distribution of microbial populations, 35.4 % (17 of 48) of the samples were found to have an aerobic plate count of 10^4 to $<10^5$ CFU g^{-1} followed by 33.3% have $=10^5$ CFU g^{-1} , 27.1 % have 10^2 to $<10^4$ CFU g^{-1} and 4.2 % have 10 to 10^2 CFU g^{-1} (Table 2). In comparison to the various samples, 100 % of jhal-muri and hog-plum samples were found to be highly contaminated. Betel-leaf and sweets contained 50 % of highly contaminated samples whereas any sample of sheek-kabab, singara, somosa and vhel-puri were highly contaminated (Fig. 1).

Jhal-muri, hog-plum, betel-leaf, peaju, sheek-kabab, singara and vhel-puri were found to be 100 % contaminated with coliforms with an unacceptable range, as compared to somosa (75 %), sola (50 %) and bun (25 %) (Fig. 1). But cup-cake and sweet were free from contamination with coliforms (0 %). So among the 48 RTE food samples, 29.16 % of them did not contain coliforms (Table 3).

All the food samples but singara and somosa were found to be 100% contaminated with an unacceptable range with *E. coli* (Fig. 1). Among the 48 RTE food samples, 16.67% of the samples were free of *E. coli* (Table 4).

DISCUSSION

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers [23]. The total plate count analysis is a useful tool in monitoring food process and the results may reflect the hygienic level of food handling and retail storage. The large number of aerobic plate count, indicator organisms (coliforms and *E. coli*) and fungi

detected in the street-vented RTE food samples surveyed in this investigation revealed that contamination of these foods presented a potential health hazard to consumers. Although RTE foods provide a source of readily available and nutritious meals for the consumers, the safety and microbiological quality of these foods should be the first priority, since they do not receive any heat treatment just before consumption. Several investigations regarding the microbiological quality of various RTE or ready-to-use food products, such as vegetable salads [24, 25] cold and hot meals served by airlines [26,27], cooked rice [28], street-vended foods [29, 30, 31], hot-held foods [32], catering dishes [33], sliced meat and meat products [34] and shrimp [35].

The present survey revealed that the incidence of coliforms and *E. coli* in 48 street vended RTE foods were 70.84 % and 83.33 %, respectively. There was a significant fungal incidence too. It is contended that, contamination is mainly due to poor quality of water used for dilution, prevailing unhygienic conditions related to washing of utensils, maintenance of the premises and storing conditions, location by the side of a busy road with heavy vehicular traffic and dust or by the side of the waste disposal system and overcrowding. Use of contaminated water associated with cross-contamination in home-made food [31, 36]. Pathogens can be transferred to food from utensils that are not properly cleaned with contaminated water. Therefore, the water might have contaminated the utensils during cleaning and then cross-contaminated the food in the same manner as the transfer of pathogens by chopping boards was observed. This study also observed that hand washing was mostly done without using any sanitizers. This could have promoted transfer of the pathogens from the hand to the food. Mechanical removal of pathogens with soap and water alone is not the most effective method for achieving hygiene, but that this should be supplemented with the use of an effective disinfectant⁸.

According to the microbiological standard of foods in Bangladesh, aerobic plate counts ranges from 10 to 10² CFU g⁻¹ can be said to be safe, 10² to < 10⁴ CFU g⁻¹ acceptable, 10⁴ to <10⁵ CFU g⁻¹- not acceptable and =10⁵ CFU g⁻¹ can be said to be extremely hazardous for public health. In addition, food borne pathogens such as *S. aureus*, *Salmonella* spp. and *L. monocytogenes* should not be detected in RTE foods [39] although these were not tested in this experiment. So, in this investigation, on the basis of APC, 27.1% showed an acceptable range, 4.2 % safe, 35.4 % non-acceptable and 33.3% were hazardous (Table 2). All the tested samples were positive for total coliforms and *E. coli*, that indicates the extremely poor

microbial quality of street vented RTE foods that are not desired and it is obviously an alert for public health that, 70.84 % and 83.33 % of the samples are completely unsuitable for consumption respectively. It is a great matter of consciousness that, jhal-muri, hog-plum, betel-leaf, peaju, sheek-kabab and vhel-puri were 100 % contaminated with both coliforms and *E. coli* in a hazardous range whereas only singara and somosa were in acceptable range for *E. coli* but not safe (Fig. 1). Fungal contamination in ready-to-eat rice with *Saccharomyces cerevisiae* and *Aspergillus niger* [40]. Although this study did not isolate fungal strain, but it has found that, sola (6596 CFU g⁻¹), hog-plum (6197 CFU g⁻¹), betel-leaf (3856 CFU g⁻¹) and jhal-muri (2312 CFU g⁻¹) were hazarously contaminated with fungi. Therefore, intake of such poor quality and unhygienic foods can lead to calamitous diseases.

CONCLUSION AND RECOMMENDATION

The results of the present study reveled that majority of the street foods in Dhaka city are extremely contaminated with microorganisms at an unacceptable limit especially the raw items (jhal muri, sola, betel leaf and hog-plum). Total coliform were found to be present all sample, indicating an alarming situation of health hazard. *E. coli* were detected in 83% of the food samples. A vast number of people in Dhaka city are taking contaminated street food without any awareness about the food borne diseases. The disease causing agents spread by these type of food products not only incapacitate large groups of people but also some times result in serious disability and death.

The results of this study suggested that street vended RTE foods should be manufactured under Good Hygienic Practices and conservation practices should be developed in order to minimize the microbial contamination of food so largely consumed. Moreover, it is recommended to develop an appropriate hazard analysis critical control point (HACCP) system and a more close supervision of RTE food should be carried out by relevant authorities to enhance food safety.

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