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Short Communication

Pattern of Drug Resistance of Pathogenic Microbes in the Street Foods of Dhaka City, Bangladesh

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ABSTRACT: The ready-to-eat (RTE) foods sold by the street vendors cause numerous public health hazards. A pilot study was carried out in three areas of Dhaka city, namely Dhanmondi (posh residential area), Lalbag (Low income group residential area) and Motijheel (Commercial area) to assess the microbial load of seven randomly chosen commonly consumed street-vended foods (SVFs) – Bhelpuri, Cake, Cholaboot, Ghugni, Samucha, Singara and Sugarcane juice. The mean aerobic plate count (APC), total coliform (TC) and total *Escherichia coli* (EC) count ranged from 2.74-3.78, 1.77-2.58 and 1.57-2.64 $\log_{10}$ CFU/g respectively for all the foods tested. According to the guidelines for RTE foods, the APC for Bhelpuri, Cholaboot, Cake and Ghugni is in the satisfactory level whereas for Samucha and Singara it is in the acceptable level. However, the APC, TC and EC count for sugarcane juice are 3.55-4.16, 2.76-3.54 and 2.46-3.39 $\log_{10}$ CFU/ml respectively and all these values fall in the unsatisfactory level. Taking 10 bacterial isolates randomly from each food item of all three areas, a total of 210 colonies were isolated to check the antimicrobial resistant pattern. 79.52% of isolates did not show any antibiotic resistance while the remaining 20.47% of isolates showed resistance to single/multiple antibiotics. Electrophoresis of DNA extract from antibiotic-resistant-isolates showed the presence of plasmid DNA of various sizes. One of the isolate from Bhelpuri of Dhanmondi showed resistance to ampicllin (Amp), neomycin (Neo) and penicillin (Pen) and was identified biochemically as *Hafnia alvei.* Another isolate from Sugarcane juice of Motijheel showed resistance to Amp, gentamycin (Gen), kanamycin (Kan), Neo and Pen and was identified biochemically as *Klebsiella pneumoniae.*

KEYWORDS: Street food, Microbes, Antimicrobial resistant bacteria, Dhaka, Bangladesh

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INTRODUCTION

Street foods are commonly consumed by people in all the cities of Bangladesh. Foods and beverages that are prepared and sold by vendors in the street for immediate or later consumption without further processing are called street-vended foods (SVFs).[1] SVFs are sold under low hygienic condition in developing countries without any strict rules and regulations. The safety of food is affected by several factors including the starting raw materials, handling of food during processing and selling, and storage of the food. The majority of food vendors do not maintain the proper food handling practices. Their ignorance about the time of spoiling, proper storage conditions and protecting foods from open air exposure lead to easy cross-contamination of food items.[2] Here we carried out a study to assess the microbial load of some commonly consumed street-vended foods of Dhaka city – like bhelpuri, cake, cholaboot, ghugni, samucha, singara and sugarcane juice.

Microbial contaminations in food might cause nausea, vomiting, colic and diarrhea as well as other infectious diseases. Several studies have shown that drug-resistant bacteria exist in foods and emerge with multiple antibiotic resistances.[3] So, it is necessary to validate the condition of foods to check if they are microbiologically safe or not. In last few decades the SVFs have been analyzed for their microbiological safety in several countries like Brazil, India, Malaysia and South Africa. Bacterial contaminations have been observed in different foods and drinks in Bangladesh, like weaning foods and drinking water and carbonated soft drinks.[4,5] Several instances of microbial contamination in the street foods of Dhaka city have also been highlighted in different news papers of

Bangladesh. It is quite unfortunate, that still there is no microbial standard to maintain the food safety in Bangladesh. Therefore, here we focus to evaluate the microbiological risk of the commonly consumed SVFs in Dhaka city Bangladesh.

MATERIALS AND METHODS

10g each of seven ready-to-eat food samples (Supplementary Table 1) were collected from the streets of three different areas (Lalbag: low-income residential area, Dhanmondi: high-income residential area and Motijheel: commercial area) of Dhaka city. Foods were then crushed and diluted with sterile water and spread on multiple petri dishes, containing – agar (aerobic plate count, APC), violet red bile lactose agar (coliform), McConkey agar (*Escherichia coli*), Baird-Parker agar (*Staphylococcus aureus*) and selective agar (*Bacillus cereus*) (HiMedia, India). The same sterilized water was used as negative control. Cultures were incubated at 30° C for 48 hours for APC and at 37° C for 24-36 hours for the remaining. From these different selective media, pathogenic bacteria were counted as colony forming unit (CFU) and expressed as $\log_{10}$ CFU/g or ml of respective food.

Antibiogram analysis was performed following Kirby-Bauer method (1966).[6] Plasmid DNA was isolated by conventional alkaline lysis method[7] and analyzed by electrophoresis on 0.8 % agarose gel (Sigma, USA). We also used Gelatin liquefaction test, Phenylalanine deaminase test, Lysine, Ornithine decarboxylase test and other biochemical tests.[8] Depending on the results of biochemical test, the isolates were identified with the help of text[9,10] and online program ABIS6 (Advanced Bacterial Identification Software; http://www.tgw1916.net/-bacteria_logare.html). Statistical data were analyzed using Microsoft Office 2007 Excel sheet.

Table 1. Aerobic plate count (APC), Total coliform (TC) and *E. coli* (EC) from the street foods.

Food Items	Area	$\log_{10}$ CFU/g or mL					
		APC		TC		EC	
		Mean	SD[a]	Mean	SD[a]	Mean	SD[a]
Bhelpuri	Dhanmondi	3.60	0.04	2.32	0.11	2.08	0.06
	Lalbag	3.78	0.03	2.42	0.29	2.02	0.16
	Motijheel	3.25	0.07	2.41	0.29	2.03	0.10
Cake	Dhanmondi	3.32	0.08	2.24	0.19	1.92	0.11
	Lalbag	3.58	0.07	1.88	0.19	1.57	0.20
	Motijheel	3.13	0.12	2.31	0.12	1.94	0.13
Cholaboot	Dhanmondi	3.55	0.06	2.34	0.13	2.20	0.14
	Lalbag	3.72	0.03	2.32	0.22	1.87	0.06
	Motijheel	3.39	0.07	2.89	0.13	2.64	0.13
Ghugni	Dhanmondi	3.69	0.03	2.58	0.19	2.18	0.09
	Lalbag	3.42	0.06	2.42	0.16	2.27	0.14
	Motijheel	3.21	0.11	2.54	0.20	2.13	0.15
Samucha	Dhanmondi	3.12	0.13	2.45	0.19	1.86	0.11
	Lalbag	3.19	0.10	2.24	0.20	1.94	0.13
	Motijheel	2.83	0.13	1.77	0.12	1.57	0.20
Singara	Dhanmondi	2.83	0.13	2.51	0.20	1.92	0.12
	Lalbag	3.30	0.05	2.22	0.17	1.90	0.09
	Motijheel	2.74	0.25	1.80	0.14	1.61	0.23
Sugarcane juice	Dhanmondi	3.55	0.05	3.38	0.07	3.02	0.08
	Lalbag	4.16	0.06	2.76	0.15	2.46	0.12
	Motijheel	3.93	0.03	3.54	0.06	3.39	0.07

[a] Standard deviation (SD) from four independent experiments.

RESULTS AND DISCUSSION

The mean of $\log_{10}$ CFU/g for solid food items and $\log_{10}$ CFU/mL for liquid item with their standard deviation (SD) for APC, TC and EC are presented in Table 1. The mean of $\log_{10}$ CFU/g of APC for the solid food items was in the range from 2.74 to 3.78 whereas that for sugar cane juice was from 3.55 to 4.16 in different areas. The mean of $\log_{10}$ CFU/g of TC and EC counts were in the range of 1.77 to 2.89 and 1.57 to 2.64 respectively for solid food items. Whereas the mean of $\log_{10}$ CFU/mL of TC and EC counts were in the range of 2.76 to 3.54 and 2.46 to 3.39 respectively for sugar cane juice and it is found that these values are higher than those of solid foods.

Ten random colonies were selected from each sample of all the areas (thus 10x21=210 colonies) to verify their antibiotic resistance pattern for ampicillin (Amp), chloramphenicol (Chlo), gentamicin (Gen), kanamycin (Kan) and penicillin (Pen) (Table 2). Most of the isolates (167) showed no antibiotic resistance, while single to double antibiotic resistance was shown by 29 and 10 isolates respectively. Most of the double resistant isolates showed resistance against Amp and Pen. Two isolates from bhelpuri of Dhanmondi and cholaboot of Lalbag were resistant against Amp, Neo and Pen. Surprisingly, at the same area of Motijheel five different antibiotic resistances were found in two isolates, one of which was against Amp, Chlo, Kan,

Neo and Pen (ghugni) and the other was against Amp, Gen, Kan, Neo and Pen (sugarcane juice).

Plasmid DNA isolation and electrophoresis showed that colonies of double to multiple antibiotic resistances carry from single to multiple plasmid DNAs of different sizes irrespective of their antibiotic resistance pattern (Figure 1). The isolate resistant against five different antibiotics (Amp, Gen, Kan, Neo and Pen; Figure 1, lane 1) showed different plasmid sizes compared to the other isolate showing five antibiotics resistance (Amp, Chlo, Kan, Neo and Pen; Figure 1: lane 6). Only in the case of three antibiotic resistances (Amp, Neo and Pen), the two different isolates showed similar plasmid profile in size (Figure 1: lane 2 and 3). The samples that were resistant to double antibiotics (Amp and Pen) carried an almost similar plasmid DNA profile (Figure 1: lane 4, 5 and 7) except for an extra plasmid of ~1.7kb at lane 4. The isolates that showed multiple and maximum antibiotic resistances were numbered Isolate-01 (Amp, Gen, Kan, Neo and Pen; lane 1 of Figure 1) and Isolate-02 (Amp, Neo and Pen; lane 2 of Figure 1), and were subjected to biochemical analysis (Supplementary Table 2). The results of biochemical test were analyzed (online program ABIS6, normal mode) inferring that the Isolate-01 is *Hafnia alvei* (~ 96 %; acc: 60 %) and Isolate-02 is *Klebsiella pneumoniae subsp. Rhinoscleromatis* (~ 95 %; acc: 60 %) respectively. *Hafnia alvei* is not highly pathogenic but may cause disease in immunocompromised patients, while *Klebsiella pneumoniae subsp. rhinoscleromatis* causes destructive changes to human lungs.

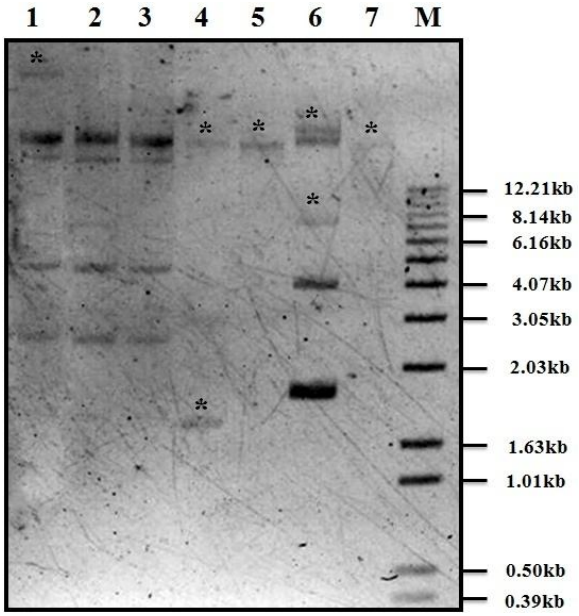

Figure 1. Plasmid profile of some representing antimicrobial resistant isolates. Mini scaled plasmids from 3.0 ml antimicrobial resistant bacteria cultures (10^8 CFU/mL) were isolated and separated on 0.8 % agarose gel in 1x TAE. The name of antibiotics against which microbial isolates were resistant and the food source from where they were isolated are described as follows, lane: 1 (Amp, Gen, Kan, Neo and Pen resistant from sugarcane juice, Motijheel); 2 (Amp, Neo and Pen resistant from bhelpuri, Dhanmondi); 3 (Amp, Neo and Pen resistant from cholaboot, Lalbag); 4 (Amp and Pen resistant from ghugni, Motijheel); 5 (Amp and Pen resistant from ghugni, Dhanmondi); 6 (Amp, Cam, Kan, Neo and Pen from ghugni, Motijheel) and 7 (Amp and Pen resistant from sugarcane juice, Dhanmondi). *Note the weak signal of plasmid DNA. M indicates 1 kb DNA ladder where only major DNA fragments are indicated.

Table 2. Bacterial isolates those were resistant from none to multiple antibiotics.

Food Items	Area	Name/Number of Antibiotics										Total Isolates
		None	Amp	Chlo	Gen	Kan	Pen	2	3	4	5	
Bhelpuri	Dhanmondi	5	1	0	0	0	2	1	1	0	0	10
	Lalbag	6	0	0	0	0	3	1	0	0	0	10
	Motijheel	7	0	1	0	1	1	0	0	0	0	10
Cake	Dhanmondi	7	2	0	0	1	0	0	0	0	0	10
	Lalbag	8	0	0	0	0	2	0	0	0	0	10
	Motijheel	7	1	0	2	0	0	0	0	0	0	10
Cholaboot	Dhanmondi	10	0	0	0	0	0	0	0	0	0	10
	Lalbag	7	1	1	0	0	0	0	1	0	0	10
	Motijheel	10	0	0	0	0	0	0	0	0	0	10
Ghugni	Dhanmondi	7	0	0	0	0	1	2	0	0	0	10
	Lalbag	9	0	0	0	0	0	1	0	0	0	10
	Motijheel	8	0	0	0	0	0	1	0	0	1	10
Samucha	Dhanmondi	10	0	0	0	0	0	0	0	0	0	10
	Lalbag	7	0	0	0	0	3	0	0	0	0	10
	Motijheel	8	1	1	0	0	0	0	0	0	0	10
Singara	Dhanmondi	9	0	0	0	0	0	1	0	0	0	10
	Lalbag	10	0	0	0	0	0	0	0	0	0	10
	Motijheel	9	1	0	0	0	0	0	0	0	0	10
Sugarcane juice	Dhanmondi	9	0	0	0	0	0	1	0	0	0	10
	Lalbag	7	1	0	0	0	0	2	0	0	0	10
	Motijheel	7	0	0	0	0	2	0	0	0	1	10
Resistant Isolates		**167**	**8**	**3**	**2**	**2**	**14**	**10**	**2**	**0**	**2**	**210**

Amp = Ampicillin; Chlo = Chloramphenicol; Gen = Gentamycin; Kan = Kanamycin; Pen = Penicillin.
2: Amp and Pen; 3: Amp, Neo (neomycin) and Pen; 4: Amp, Chlo, Neo and Pen; 5: Amp, Chlo, Kan, Neo and Pen

Based on the microbial load, street foods can be categorized into food category 1 (APC satisfactory $<10^3$/gm), food category 2 (APC satisfactory $<10^4$/gm) and food category 3 (APC satisfactory $<10^5$/gm).[11] Food items tested in this study belong to three food categories. Bhelpuri, cholaboot, ghugni, samucha and cake belong to food category 2; singara belongs to food category 1; sugarcane juice belongs to food category 3. The mean $\log_{10}$ CFU/g aerobic plate count (APC) for the studied solid foods was from 2.74 to 3.78. All the SVFs contain an acceptable microbial count with respect to the food categories supporting the data of Gilbert and his co-workers in 2000.

A study in the central Accra city of Ghana showed that Kebab, an oil-fried meat food contained 3.36-6.25 $\log_{10}$ CFU/g of bacteria in total plate count (TPC) (Ghana standard for pre-cooked meat is 4.0 $\log_{10}$ CFU/g).[12] The mean total APC and TC for the fruit chats in Patiala city of India were 8.46 and 6.67 $\log_{10}$ CFU/g respectively.[13] In Pune of India, 88% of the food samples showed the presence of bacterial pathogens.[14] In Atbara city of Sudan, cooked meal and juice were shown to be contaminated with total viable counts of 4.6 $\log_{10}$ CFU/g and 4.1 $\log_{10}$ CFU/g respectively.[15] Other study from Brazil showed that maximum level of $\log_{10}$ CFU/mL for total coliform in sugarcane juice was 3.38[16] but in our case that value ranged from 2.76 to 3.54 depending on the source area. The vendor's food stored at ambient temperature during summer stimulates the growth of mesophilic organisms including many pathogens.[17] The hands and finger tips are the most important vehicles even after normal washing for the transfer of organisms from faces, nose, skin or other body parts of food vendor.[18] Bare and unwashed hands were used to sell the foods even in developed city like New York City.[19]

The widespread use of antibiotics in animals which are used as human food, has resulted in the emergence of novel antibiotic resistant microorganisms that can be transmitted to human through the food chain and involve human health threat.[20-22] We extended our study to reveal the antibiotic resistance patterns of the isolated colonies and data showed that 79.52 % of the colonies had no resistance to the antibiotics tested here. The other 20.48 % colony showed resistance to antibiotics – from single to as much as five different antibiotics.

Biochemical tests were carried out for two isolates that showed resistance to multiple antibiotics. Analysis of these results suggested that Isolate-01, which was isolated from bhelpuri of Dhanmondi and was found to be resistant against Amp, Neo and Pen is *Hafnia alvei*. Isolate-02, which was isolated from sugarcane juice of Motijheel and was found to be resistant against Amp, Gen, Kan, Neo and Pen is *Klebsiella pneumonia*. In a previous study it was shown that *Streptococcus pneumoniae* was resistant against penicillin, macrolides, cephalosporins and tetracyclines.[23] When the isolates were resistant against Amp and Pen, the signal resulting from plasmid DNA was weak (marked by *, Figure 1: Lane 4, 5 and 7). Only a single isolate with five antibiotic resistances showed discrete plasmid profile (Figure 1: Lane 6). A large plasmid in an *E. coli* isolate with eight antimicrobial resistances was reported in 2005.[24] A further study in a large scale for a variety of street vended foods is necessary to establish a standard for Bangladesh and to regulate the quality of the foods. Moreover, it will be more interesting to further characterize the isolated microbes with molecular biology techniques.

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