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BACTERIAL LOAD AND MULTI-DRUG RESISTANCE PATTERNS OF SOME READY-TO-EAT STREET FOODS OF DHAKA CITY

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Abstract

Bacterial load and drug resistance pattern associated with some ready-to-eat (RTE) street foods such as Chatpoti, Fuchka, Singara, Panipuri, Ghugni-muri, Chola and water of Dhaka South City Corporation were investigated. Most of the samples were found to be contaminated and the bacterial load ranged from 2.4×10^4 - 9.2×10^6, 1.2×10^3 - 7.3×10^5 and 1.1×10^3 - 1.6×10^6 cfu/g of aerobic heterotrophic, coliform bacteria and *Staphylococcus*, respectively. The highest coliform load (7.3×10^5 cfu/ml) was found in the water of Gulistan. The highest aerobic heterotrophic bacteria (9.2×10^6 cfu/g) and *Staphylococcus* (1.6×10^6 cfu/g) were observed in the Chatpoti of Nilkhet. Among the isolated 100 different bacterial colonies, 20 Gram-positive and 8 Gram-negative isolates were studied in details. Based on the morphological and biochemical analysis, the Gram-positive isolates were identified as *Staphylococcus* (9), *Bacillus* (4), *Kurthia* (3), *Planococcus* (1), *Micrococcus* (1), *Listeria* (1) and *Renibacterium* (1). Gram-negative isolates were identified as *Klebsiella pneumoniae* (3), *Yersinia pestis* (1), *Y. pseudotuberculosis* (1), *Escherichia coli* (1), *Enterobacter aerogenes* (1) and *Plesiomonas shigelloides* (1). The multi-drug resistance (MDR) pattern was found to be diverse. Among the MDR bacteria, *Enterobacter aerogenes* was found to be resistant against six common antibiotics. *Plesiomonas shigelloides* and *Yersinia pestis* were found to be resistant against five antibiotics. The multiple antibiotic resistant (MAR) indices of Gram-negative isolates ranged in between 22.22 and 66.67%. Conventionally identified five bacterial isolates with significant MAR indices were further identified with 16S rDNA sequencing and found to be as *Enterobacter cloaceae* Ecl1, *Plesiomonas shigelloides* CIFRI, *Aeromonas* sp. TIL_WAK_4, *Aeromonas* sp. 280 and *Klebsiella pneumoniae* KPS77. Conventional identification was found to be accurate for three isolates but the two *Yersinia* sp. were identified to be as *Aeromonas* sp. in 16S rDNA sequencing.

Introduction

In Bangladesh, Chatpati, Fuchka, Velpuri, Panipuri, Alupuri, Samosa, Singara, Beguni, Chop etc. are very common and popular ready-to-eat (RTE) food items[(1)]. Selling street foods is an important occupation in many cities of developing countries and

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consumption of street foods is also common here as unemployment is high, salaries are low, work opportunities are limited and rapid urbanization is taking place[2]. Several observational studies have shown that street foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings that make them prone to contamination and frequently cause diarrhoeal diseases[3]. Majority of poor people in developing countries obtain food from informal markets but these are often neglected by food safety authorities and little is known about their impacts on public health[4]. Street food vendors are mostly uninformed of good hygiene practices (GHP) and contribute in the spread of diarrhoeal diseases[5].

The number of food poisoning notifications rose steadily worldwide since the inception of *E. coli* O157:H7 outbreak in the 1980s to date[6]. Approximately, 30 million people in Bangladesh are suffering from food borne illnesses each year[7]. In addition, multi-drug resistance (MDR) of food borne microorganisms has made the food safety situation more vulnerable in public health[6]. Food borne illnesses caused by MDR microorganisms are a major national and international health problem and an important cause of death in developing countries[8]. Considering all these facts and situation, the present study was undertaken to assess the bacterial load and their multi-drug resistance pattern of RTE street foods and water of public rushed areas of Dhaka South City Corporation.

Materials and Methods

Samples of RTE foods as Chatpoti, Fuchka, Boiled Chola, Ghugni-muri, Panipuri and Singara were collected from nine different populated areas of Dhaka South City Corporation *viz.* Shahabag, Nilkhet, Gausia market, Polashi, New Market, Chankharpul, Gulistan, Bokshi Bazar and Press Club. Samples were collected in sterile polythene bags and water samples were collected in sterile plastic bottles and brought to the laboratory immediately for bacteriological analysis.

Ten g of RTE food samples were taken in a sterile conical flask containing 100 ml of sterile water. In case of water, 1 ml of sample water was added in sterile conical flask containing 99 ml sterile water. Bacterial load of the samples was then measured by ten-folds serial dilution technique[9]. Nutrient agar (NA) medium was used for aerobic heterotrophic bacteria while MacConkey agar (Scharlau Chemie, Japan) and Mannitol Salt Agar (MSA) (Oxoid) were used for coliform bacteria and *Staphylococcus* sp., respectively. The plates were incubated at 37°C in an incubator (Memmert GmbH + Co Kg 8540 Sehwabach) for 24 hrs. After incubation, the well discrete colonies were counted by a colony counter (DC-8 OSK 100086, Kayagaki, Japan).

Important physiological and biochemical tests were carried out and conventional identifications of the isolates were made following standard laboratory manuals[10-13]. Antibiotic susceptibility test was performed by the Kirby-Bauer disc diffusion method[14]

on Mueller-Hinton agar. Zone diameter around the discs was measured and the isolates were classified as susceptible (S), intermediately resistant (I) and resistant (R). Nine antibiotics *viz*., cefuroxime (CXM 30µg), neomycin (NEO 30µg), gentamycin (GEN 10µg), kanamycin (KAN 30µg), doxycycline (DOX 30µg), ciprofloxacin (CIP 5µg), rifampicin (RIF 5µg), erythromycin (ERY 15µg) and chloramphemicol (CHL 30µg) were tested. Multiple antibiotic resistance (MAR) index % of the isolates was determined using the following formula[15]:

$$\text{MAR index \%} = \frac{\text{No. of antibiotics to which pathogen showed resistance}}{\text{No. of antibiotics used}} \times 100$$

Molecular identification of MDR bacterial strains was conducted based on 16S rDNA sequence analysis. The following primer pairs - 5'-16S rRNA: CCAGACTCCTACGGG AGGCAGC, 3'-16S rRNA: CTTGTGCGGGCCCCCGTCAATTC were used to amplify a part (~600 bp) of the rRNA gene. Supernatant of heat lysed cell suspension was used as the source of template DNA for PCR amplification following protocol as described in our previous work[16]. The amplified products were separated electrophoretically on 1% agarose gel. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Microdoc DI-HD, MUV21-254/365, Cleaver Scientific). The sequence generated from automated sequencing of PCR products were analyzed through NCBI-BLAST database (http://blast.ncbi.nlm.nih.gov/) and rRNA BLAST (http://bioinformatics.psb.ugent.be/cgi-bin/rRNA/blastform.cgi) programs to find out possible similar organisms in the data bases. The data were analyzed to determine the descriptive statistics *viz.* statistical mean and standard deviation (Sd) with SPSS v.16.0 for windows (SPSS, SAS Institute Inc. Cary, USA).

Results and Discussion

The aerobic heterotrophic bacterial load of the samples ranged between 2.4×10^4 and 9.2×10^6 cfu/g (Table 1). Maximum heterotrophic bacterial count (9.2×10^6 cfu/g) was observed in the Chatpoti of Nilkhet. The coliform and staphylococcal bacterial load of the samples ranged from 1.2×10^3 to 7.3×10^5 and 1.1×10^3 to 1.6×10^6 cfu/g, respectively. The highest coliform load (7.3×10^5 cfu/ml) was found in the water sample of Gulistan and highest *Staphylococcus* (1.6×10^6 cfu/g) was observed in the Chatpoti of Nilkhet. Similar findings were also observed by other workers[17-20]. In most cases, potable water supply was not available to the vendors and thus hand and dish washing are usually done in one and same bucket. Considering coliform contamination, the water of the RTE food shops was not safe for drinking. Vendors usually prepare and serve the food in bare and unwashed hands which could be the most probable sources of contamination[6]. The use of raw vegetables *viz.* cucumber, tomato, carrot, onion, green chili, tamarind and

coriander leaf samples also contribute to the bacterial load[17]. The contamination may originate also from the utensils or through transportation.

Table 1. pH and bacterial load of the samples.

Sample		Bacterial load (cfu/g of RTE foods and cfu/ml of water)		
Sites	Type	Aerobic heterotrophic bacteria on NA	Coliforms on MacConkey agar	*Staphylococcus* on MSA
Shahabag	Chatpoti	8.2×10^6	4.4×10^5	2.4×10^4
	Fuchka	8.7×10^6	2.4×10^5	2.2×10^4
Nilkhet	Chatpoti	9.2×10^6	5.0×10^4	1.6×10^6
	Fuchka	8.8×10^6	7.4×10^4	2.0×10^5
Gausia Market	Chatpoti	4.1×10^6	1.5×10^4	2.4×10^4
	Fuchka	2.9×10^6	2.0×10^3	3.1×10^4
Polashi	Chola	1.5×10^6	2.6×10^5	2.4×10^5
New Market	Ghugni-muri	8.3×10^6	5.2×10^4	5.9×10^4
Chankharpul	Water	5.0×10^4	1.2×10^3	Nill
Gulistan	Water	2.5×10^4	7.3×10^5	Nill
Bokshibazar	Panipuri	8.5×10^4	2.9×10^4	4.9×10^4
Press club	Singara	2.4×10^4	Nill	1.1×10^3
Azimpur	Chola	7.7×10^6	3.0×10^5	2.2×10^5

NA = Nutrient agar, MSA = Mannitol salt agar.

During this study, a total of 100 colonies were primarily isolated and finally 28 were selected randomly of which 20 were Gram-positive bacteria and 8 were Gram-negative. These isolates were purified for detailed study for their important biochemical characters for provisional identification (Table 2). Among the Gram-positive isolates, 9 were rod shaped, spore former and remaining 11 isolates were coccus. Fig. 1 clearly indicated that among the Gram-positive bacteria, *Staphylococcus* was the dominant genus (31%) having 3 distinct species *viz., S. auricularis* (3), *S. caseolyticus* (1) and *S. epidermidis* (5). Wertheim *et al.*[21] mentioned that the different species of *Staphylococcus* are one of the most predominantly virulent human pathogens causing a wide range of diseases. Other Gram-positive isolates were identified as different species of *Bacillus* (14%), *Kurthia* (10%), *Listeria* (4%), *Micrococcus* (4%), *Planococcus* (4%) and *Renibacterium* (4%). All the 8 Gram-negative isolates were short rods and non-spore former and identified as *Enterobacter aerogenes* (4%), *Escherichia coli* (4%), *Klebsiella pneumoniae* (10%), *Plesiomonas shigelloides* (4%) and *Yersinia* spp. (7%). In our previous investigation more or less similar results were observed in some fresh vegetables, fruits and some RTE foods[19-20, 22]. Street foods

are also not protected from the various pathogens and contaminant carriers like flies and other insects, multifunctional hands and own health status of vendors. Potential health risks are reported to be associated with contamination of food by *E. coli*, *Salmonella typhi* and *Staphylococcus aureus* during preparation, post cooking and other handling stages[8].

Table 2. Major biochemical characteristics and provisional identification of bacteria associated with the RTE foods and water.

Isolate	Biochemical characteristics						Identified bacteria
	VP	MR	Starch	Tyrosine	Urease	Lecithinase	
S/C/N/1	-	+	+	+	ND	+	*Bacillus brevis*
S/C/N/2	+	-	+	-	ND	-	*B. circulans*
P/S/N/4	+	+	+	-	ND	+	*B. firmus*
P/S/N/5	+	-	+	-	ND	+	*B. lentus*
P/S/N/1	+	-	+	-	ND	+	*Kurthia gibosonii*
N/C/N/2	+	-	+	-	ND	+	*K. zopfii*
P/S/N/2	+	+	+	-	ND	+	
N/F/N/1	+	-	-	+	ND	-	*Listeria welshimeri*
B/P/N/1	+	-	+	-	ND	+	*Micrococcus luteus*
G/C/N/2	+	+	-	+	ND	-	*Planococcus citreus*
C/W/N/1	+	+	-	+	ND	+	*Renibacterium salmoninarum*
S/C/Ms/1	+	+	-	-	ND	+	*Staphylococcus auricularis*
N/F/Ms/2	-	+	-	-	ND	-	
N/C/Ms/3	+	-	+	-	ND	-	
G/F/Ms/3	-	-	-	+	ND	+	*Staphylococcus caseolyticus*
B/P/Ms/1	-	+	+	-	ND	+	*Staphylococcus epidermidis*
B/P/Ms/3	-	-	+	-	ND	-	
S/F/Ms/1	+	-	-	-	ND	+	
B/P/Ms/2	-	+	-	-	ND	-	
N/M/Ms/1	+	+	-	-	ND	+	
N/M/Mc/1	+	-	-	ND	-	-	*Enterobacter aerogenes*
P/C/Mc/1	+	+	+	ND	-	-	*Escherichia coli*
G/C/Mc/3	+	+	-	ND	-	-	*Klebsiella pneumoniae*
B/P/Mc/2	+	-	-	ND	+	-	
B/P/Mc/3	+	-	-	ND	+	-	
S/F/Mc/1	-	+	+	ND	-	+	*Plesiomonas shigelloides*
B/P/Mc/1	-	-	-	ND	-	-	*Yersinia pestis*
S/C/Mc/2	+	-	+	ND	+	-	*Y. pseudotuberculosis*

+ = Positive result, - = Negative result, ND = Not done.

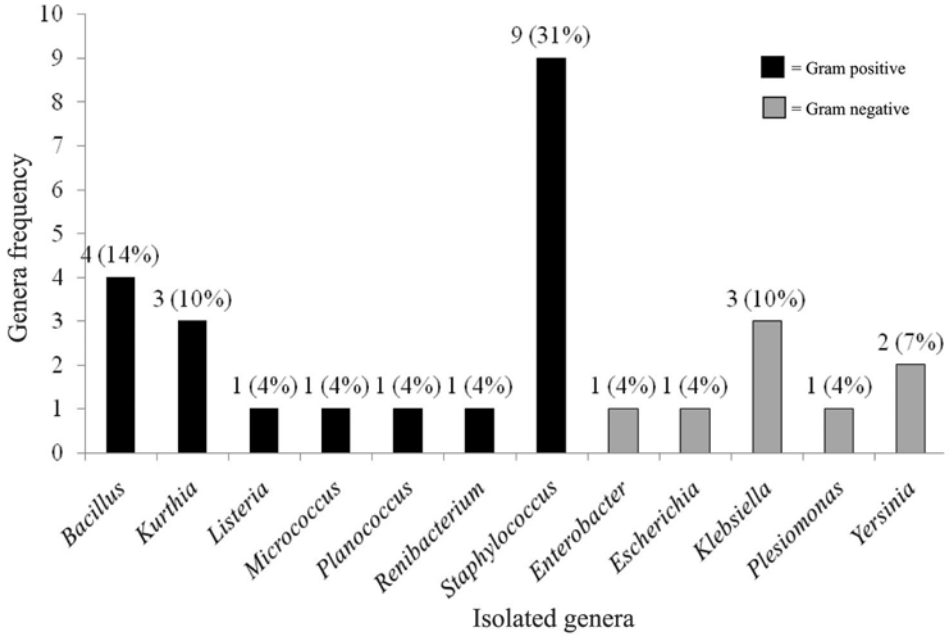

Fig. 1. Frequency of the identified bacterial isolates associated with RTE food and water.

Most of the Gram-positive isolates were susceptible and some were intermediate resistance to the tested antibiotics (data not shown). The Gram-negative isolates were found to be significant against antibiotic resistance pattern (Table 3). The MAR index of all the Gram-negative bacteria ranged in between 22.22 and 66.67%. Most of the isolates were found to be resistance against two common antibiotics, doxycyclin and ciprofloxacin. *Enterobacter aerogenes* was found to be resistant against six antibiotics *viz*. rifampicin, doxycycline, erythromycin, ciprofloxacin, gentamycin and kanamycin having the highest MAR index (66.67%). In an earlier work, Khan and Saha[20] found the MAR index in between 14.28 and 71.43% of the bacteria isolated from Chatpoti and *Enterobacter* sp. showed the maximum MAR index. In present study, both *Plesiomonas shigelloides* and *Yersinia pestis* showed resistance against five antibiotics and intermediate against two antibiotics. Similarly, *Y. pseudotuberculosis* was resistant against four antibiotics and intermediate against one antibiotic with MAR index of 44.44%. In another study Tabassum *et al.*[22] found *Pseudomonas syringae* to be resistant against five common antibiotics. Kim *et al.*[23] isolated a total of 132 *Klebsiella pneumoniae* isolates and all were found to be resistant against ampicillin, tetracycline, streptomycin, gentamycin and kanamycin. In the present study *Klebsiella pneumoniae* was found to be resistant against ciprofloxacin and doxycycline.

Table 3. Antibiotic sensitivity pattern of the Gram-negative isolates.

Bacteria	Zone diameter (Mean ± Sd) mm									MAR index (%)
	CXM (30 µg)	CHL (30 µg)	NEO (30 µg)	RIF (5 µg)	DOX (30 µg)	ERY (15 µg)	CIP (5 µg)	GEN (10 µg)	KAN (30 µg)	
Enterobacter aerogenes	52.00 ± 0.00 S	25.00 ± 0.00 S	19.66 ± 0.58 S	16.33 ± 0.58 R	7.33 ± 0.58 R	13.00 ± 1.00 R	0 R	12.33 ± 0.58 R	13.0 ± 1.00 R	66.67
Escherichia coli	26.33 ± 0.58 S	30.66 ± 0.57 S	25.33 ± 2.08 S	19.66 ± 0.58 I	9.33 ± 1.52 R	17.00 ± 1.00 I	0 R	15.00 ± 0.00 S	17.33 ± 0.57 S	22.22
Klebsiella pneumoniae	44.66 ± 2.00 S	25.00 ± 0.00 S	24.66 ± 0.58 S	18.33 ± 2.51 I	8.00 ± 1.00 R	15.00 ± 0.00 I	9.33 ± 0.57 R	13.00 ± 1.00 I	15.0 ± 1.00 I	22.22
K. pneumoniae	0 R	30.66 ± 1.15 S	19.33 ± 1.15 S	19.33 ± 1.15 I	9.66 ± 0.58 R	16.00 ± 1.00 I	9.66 ± 0.57 R	14.66 ± 1.52 I	15.33 ± 2.30 I	33.33
K. pneumoniae	28.00 ± 2.00 S	31.00 ± 1.70 S	28.00 ± 0.00 S	18.33 ± 1.52 I	10.33 ± 1.52 R	16.66 ± 1.10 I	9.33 ± 0.57 R	16.66 ± 2.88 S	17.33 ± 1.52 I	22.22
Plesiomonas shigelloides	30.00 ± 0.00 S	12.33 ± 0.57 R	28.66 ± 1.15 S	10.00 ± 1.00 R	0 R	16.66 ± 1.50 I	0 R	14.33 ± 0.58 I	11.66 ± 2.88 R	55.56
Yersinia pestis	21.00 ± 0.00 I	26.33 ± 0.57 S	21.00 ± 1.00 S	14.66 ± 1.15 R	8.33 ± 0.58 R	13.66 ± 1.10 R	0 R	10.00 ± 7.80 R	13.0 ± 1.00 I	55.56
Y. pseudo-tuberculosis	27.33 ± 0.58 S	10.33 ± 2.80 R	24.33 ± 0.58 S	9.00 ± 1.00 R	0 R	18.66 ± 0.57 I	7.33 ± 0.57 R	16.33 ± 0.58 S	20.33 ± 0.58 S	44.44

Zone diameters were studied in triplicates (n = 3) and presented as mean ± standard deviation (SD). CXM = Cefuroxime, CHL= Chloramphenicol, NEO = Neomycin, RIF = Rifampicin, DOX = Doxycycline, ERY = Erythromycin, CIP = Ciprofloxacin, GEN= Gentamycin and KAN = Kanamycin. S = Susceptible, I = Intermediate resistance and R = Resistant. MAR index % = Multiple Antibiotic Resistance Index percentage.

Molecular identification of five MDR bacterial isolates was conducted based on 16S rDNA sequence analysis and in the gel, approximate size of the amplified DNA band was 600 bp (Fig. 2). The automated sequencing of PCR products was analyzed and identified as *Enterobacter cloaceae* Ecl1 (N/M/Mc/1), *Plesiomonas shigelloides* CIFRI (S/F/Mc/1), *Aeromonas* sp. TIL_WAK_4 (B/P/Mc/1), *Aeromonas* sp. 280 (S/C/Mc/2) and *Klebsiella pneumoniae* KPS77 (G/C/Mc/3) (Table 4). Though molecular identification of three among the five matched with their conventional identification but both the provisionally identified *Yersinia* were identified to be as *Aeromonas* sp. Abdelraouf and Ferwana[24] mentioned difficulties in differentiating *Yersinia enterocolitica* and *Aeromonas hydrophila* through conventional techniques obtained from food and environmental samples.

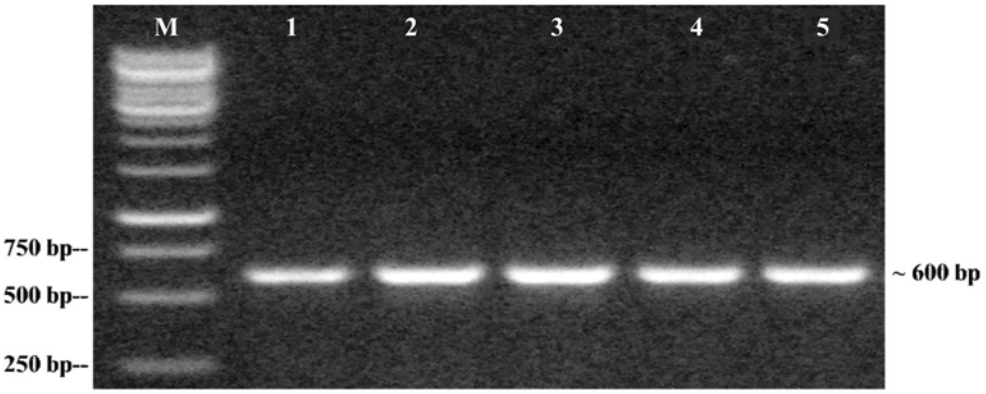

Fig. 2. PCR amplification of part of the 16S rRNA gene. Lane M is the 1.0 kb ladder and lanes 1-5 are representing 5 different bacterial isolates.

Table 4. Conventional and molecular identification of selected bacterial isolates.

Isolate	Conventional identification	Molecular identification				
		Scientific name	Strain	Identity match (%)	Max. coverage score	E-value
N/M/Mc/1	*Enterobacter aerogenes*	*E. cloaceae*	Ecl1	89	309	1e-42
S/F/Mc/1	*Plesiomonas shigelloides*	*P. shigelloides*	CIFRI	99	989	0.0
B/P/Mc/1	*Yersinia pestis*	*Aeromonas* sp.	TIL_WAK_4	98	872	0.0
S/C/Mc/2	*Y. pseudo-tuberculosis*	*Aeromonas* sp.	280	93	845	7e-158
G/C/Mc/3	*Klebsiella pneumoniae*	*K. pneumoniae*	KPS77	92	754	0.0

The bacterial load, the presence of some particular bacteria *viz. E. coli, Klebsiella, Enterobacter, Staphylococcus* and *Aeromonas* associated with the RTE street foods and water and their MDR pattern as well as the high MAR index indicate significant health hazard for the RTE food consumers and needs immediate public attention.

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