Working Paper

Microbial Contamination of Fixed and Mobile Street Food Vended around the Campus of East West University in Dhaka, Bangladesh

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ABSTRACT

This study was carried out to find out the presence of common enteric bacteria, such as, *Shigella*, *Salmonella*, pathogenic *E. coli* and *Vibrio* spp. in the street vended foods around the student campus of East West University (EWU) in Dhaka, Bangladesh. The study focused on the socio-economic, behavioral characteristics and practices with street foods among the students of EWU and the street food vendors around the campus. A total of 50 street food samples were randomly collected from fixed and mobile vendors around the EWU area and tested for the presence of microorganisms following conventional microbiological processes. In order to describe the characteristics and practices of students of EWU, 225 students were randomly selected and interviewed by using a structured questionnaire. Based on the availability, 150 vendors were also interviewed by using a structured questionnaire. Among 50 food samples, 46 (92%) had bacterial contamination of which, 6 samples (12%) were confirmed to contain different species of *E coli* and *Shigella*. Four types of food samples, namely shingara, cake, butter bun were found to be contaminated with Enteroaggregative *E coli* (EAEC) and lemon juice was contaminated with Enterotoxigenic *E coli* (ETEC). Two samples-khichuri and water collected from fixed vendor were contaminated with *Shigella flexneri* X-variant and *Shigella flexneri* 2a respectively. Among 55% students who consumed street vended foods, it has been observed that more female students (68%) consumed street foods than the male students (45%). Sixty five percent students washed hands before eating food and 56% of the students (n=207) who consumed street foods washed hands after using toilet. Fifty nine percent (n=22) of the students suffered from stomach ache and vomiting after consumption of the street vended food. About 61% vendors kept food open and 12% of them sold stale food. Nineteen percent of the vendors did not remove flies while roaming and 53% stored water in open pot. Among 150 vendors 23% were illiterate, 32% had educational status below the primary level and 45% of them had education above the primary level (5^{th} standard). Fifty-nine percent of the vendors washed their hands after coming from the toilet and 11% washed their hands with soap before preparing food. A significant relationship has been observed between the educational status of the vendors and their hand washing practice before preparing food ($p= 0.0008$). There was also a significant difference between the educational status and hand washing practice of the vendors after coming from toilet ($p=0.029$). The present study revealed that 92% of the street vended foods around an educational institution of Dhaka city had bacterial contamination. Six types of foods (12%) were found to be contaminated with Enteroaggregative *E coli* (EAEC) Enterotoxigenic *E coli* (ETEC), *Shigella flexneri* X-variant and *Shigella flexneri* 2a which could be a potential cause for food-borne diseases. Contaminated hands of food vendors can also be potential sources of pathogen transmission during the handling of the foods.

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1. Introduction

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors especially in the streets and other similar public places (**Artemis P. et al 2000, Redmond, E.C. et al 2003)**. Street food consumption is common in rapidly growing urbanized countries. The consumption of street foods is also common in countries where unemployment is high, salaries and work opportunities are low and social programs are limited **(Oladipo IC, 2010)**.

It is recognized that street foods play an important socio-economic role in terms of employment potential, particularly for women. The foods are served to consumers at prices affordable to the lower and middle income groups. Therefore, street foods are well appreciated by consumers, mainly people from low socioeconomic bracket and students because of their taste, reasonable price and easy availability **(Chauliac et al 1998)**. In contrast to these potential benefits, these foods are perceived to be a major public health risk due to lack of basic infrastructure and services and also difficulty in controlling the large numbers of street food vending operations. The food vendors' diversity, mobility and temporary nature; low socioeconomic and educational status, lack of knowledge of safe food handling also contribute to a public health risk **(WHO 1996**, **Artemis P. et al 2000).**

A number of studies found that the street foods are prepared and stored at places that are not always clean and well lit and also not far from sources of contamination. Most of the foods are not covered and are exposed to flies and dust which may also harbor food-borne pathogens **(WHO, 2001, 2003; Muinde and Kuria, 2005; Ghosh et al., 2007, Rane S, 2011)**. Wastewaters and garbage are discarded nearby, providing nutrients for insects and rodents, which may carry food borne pathogens **(Tambekar et al. 2009)**.

The quality of raw materials used for preparing street foods is also important as their contamination can persist through preparation and cooking **(Rane S., 2011).** Studies carried out in different regions of Asia, Africa and South America frequently pointed the unavailability of potable water for various activities at the vending site as a major concern. Due to the shortage of potable water, many vendors tend to re-use the water, especially for cleaning utensils and used dishes **(Rane S., 2011).** A number of studies showed heavy bacterial contamination of waters used by street vendors. A study carried out in Trinidad and Tobago revealed that 57.5% of water used by vendors was contaminated by coliforms **(Agard et al, 2002; Mankee et al, 2003)**. Similarly 29.6% water samples from storage tanks

used by some vendors at different localities in Pune, India were not conforming to the WHO standards of potability and had coliform counts of more than 16/100 ml, while fecal coliform counts were more than 16/100 ml in 15.5% of water samples, 4.5% of samples were positive for *E. coli* and 2.7% for Enteropathogenic *E. coli* **(Bhat and Waghray, 2000)**.

The serving utensils used at the vending site are often contaminated with *Staphylococcus sp*., which may have been transmitted from the vendors' hands when they touch the food preparation areas, dish washing cloth and the water during dish washing and hand washing which indicates cross contamination between dish water, food preparation surfaces, and the food itself **(Mensah P, et al 2002)**. All of these may contribute to microbial contamination of street foods. In addition, the vendors practice poor personal hygiene and may serve as a potential source of transmission of enteric fever. Unsanitary handling of street foods by some of the vendors has been commonly found to be the source of contamination. The hands of the food handlers are the most important vehicle for the transfer of organisms from feces, nose and skin to the food. A study in Columbia revealed that more than 30% of a group of food handlers were carriers of pathogenic organisms including *Salmonella typhi*, *Staphylococcus aureus*, and *Shigella spp.* **(Buchanan RL, 1998)**.

Food borne and waterborne diarrheal diseases are leading causes of illness and globally kill an estimated 2.1 million people annually, most of whom are children in developing countries **(WHO, 2001)**. A study carried out by Ghosh found the presence of *Staphylococcus aureus*, *Shigella dysenteriae* type 1 and *Shigella flexneri* 2a in coriander sauce, ready to eat salad and coconut slices collected from 75 fixed and mobile vendors of India. Another study revealed that 93% of the samples of panipuri were contaminated with pathogenic organisms like *E coli, S aureus, Klebsiella spp, pseudomonas spp and Yeast* **(Tambekar et al 2011)**. The causes of food borne illnesses are bacteria, viruses, parasites, and chemicals, and bacterial contamination is the most common cause of illness **(Su et al., 2005; Lynch et al., 2006)**.

Data collected from East West University medical centre in 2009 and 2012 revealed that a good number of students suffered from diseases like hyperacidity, diarrhea, dysentery, viral hepatitis and enteric fever which may be related to contaminated food. The occurrences of these diseases may be related to the consumption of street vended foods, although it is not known for sure that the occurrences of diseases are due to the consumption of street food. To

our knowledge no study has been carried out on the food samples sold by the fixed and mobile street vendors around the EWU community.

Lack of knowledge, social, behavioral and other factors of students as well as vendors might also be responsible for the increasing number of the diseases.

Considering the occurrence of the diseases and the consequences, the present study was aimed at finding out the presence of enteric organisms in foods sold by the street vendors around the EWU community.

Street food vending is a prevailing and distinctive part of a large informal sector in Bangladesh. It is commonly viewed in public spaces particularly in the cities and distinctive in the sense that it provides a basic need to the urban inhabitants. The items made available by the street vendors comprise a diverse range of selection, starting from small snacks such as biscuits, tea, nuts and phuchka/chotpoti to whole meals such as ruti-bhaji, rice and khichuri/tehari. Each street food enterprise is generally small in size, requires relatively simple skills, basic facilities, and small amount of capital. Therefore, they hold tremendous potential for generating income and employment for the rapidly rising urban population of Dhaka city, the capital of Bangladesh **(Muzaffar, et al 2009)**. In Bangladesh, a large number of workers have chosen their occupation as street food vendors and there are as many as 200, 000 street food vendors working in Dhaka city **(Faruque et al 2010)**. It has been observed that many street food vendors are available around different educational institutions targeting the students. According to Gina Kennedy of FAO's Nutrition and Consumer Protection Division (AGN), street foods make up a significant part of the dietary intake of children during the school day **(FAO, 2007)**. Majority of the students going to college or universities do not prepare food themselves or take it along with them to the university. As a result large number of students consumes street vended food. In developing countries, most of the street food vendors are illiterate or semi-illiterate. Thus, the general perception is that their understanding of food safety issues is inadequate **(Mensah P, et al 2002)**. It has been shown from other studies that most of the vendors lack any formal education or have few years of schooling. Therefore, they have inadequate knowledge on proper food handling and their role in the transmission of pathogens **(Mensah et al., 2002)**.

A cross-sectional study was carried out to determine the level of knowledge and awareness regarding food safety issues among the 250 school-based street food vendors in Dhaka city, where the authors revealed that food vendors had an inadequate level of knowledge and awareness regarding the food safety issues **(Mamun et al 2013)**. These school-based street food vendors were selected conveniently from surrounding areas/streets of the pre-selected primary and high schools in Dhaka city. This study was focused only on the knowledge and awareness of the street-food vendors in Dhaka city. There was also a need to evaluate the knowledge and awareness of the students regarding the street food safety. In addition behavioral characteristics of the vendors were not evaluated in this study. It is also important to evaluate the relationship between the knowledge of contamination, educational status and behavioral aspects of vendors which might have influence on the food safety issue. The relationships between characteristics/practices with street food consumptions of the students are also yet to be evaluated. Microbial evaluation of the street vended food samples should be carried out to find out the level of contamination in the street food sold by the vendors around the educational areas of Dhaka city.

To our knowledge, no study has been carried out on the pathogens present in street vended foods and to describe the socio-economic, behavioral characteristics and practices regarding street foods among the students of educational institutions in Dhaka city. A good number of street food vendors can be seen around the educational institutions of Dhaka city and also street foods always attract the students of young age due to their low cost and taste.

2. Objectives

The objectives of the present study were -

1. To find out the presence of enteric bacteria especially *Shigella*, *Salmonella*, pathogenic *E. coli* and *Vibrio* spp. in food samples from fixed and mobile vendors around the student campus of EWU.
2. To describe the characteristics and practices with regard to street food among the students of EWU.
3. To describe the characteristics and practices concerning street food among vendors around the EWU campus

3. Research Design and Methods

This study was conducted around the student campus of East West University. The food samples were collected from the old campus at Mohakhali and the new campus at Aftabnagar. A total of 50 food samples were collected in sealed poly bag from fixed and mobile vendors' bags to prevent their contact with any other source that can contaminate the samples. The collected food samples were: Singara, Samosa, Beguni, Alurchop, Pakora, Puri, Egg Fry, Vegetable Rolls, Noodles, Tehari, Khichuri, Parata, and Vaji, Butter Bun, Cake, Biscuits, Patties, Amra pickles, Chotpoti, Goava pickles, Chhola, Fish bhorta, Jhalmuri, Tamarind water of Chotpoti and Velpuri, Pickles, Drinking water, Lemon juice, Sugarcane juice, Velpuri and Fuchka.

A total of 225 students of EWU and 150 vendors were interviewed by using a structured questionnaire to know about their characteristics and practices on street vended foods.

3.1 Laboratory Testing

3.1.1 Isolation and Identification of pathogenic enteric organisms in food samples

After collection, the food samples were pre-enriched and/or enriched with specific media that have been supplemented with highly nutritious materials for the purpose of cultivating targeted organisms, i.e., *Salmonella*, *Shigella*, *Vibrio* and *E. coli.*

3.1.2 Bacteriological Subculture

Salmonella Species

Five grams (for solid and semi-solid) or, 5 ml (for liquid) of food samples were homogenized/mixed well with 45 ml of BPW (Buffered Peptone Water) broth and incubated at 37 ºC for 18-24 h. This pre-enrichment broths were then transferred to enrichment broths, 0.1 ml of BPW was mixed with 9.9 ml of RVS (Rappaport Vassiliadis) broth and incubated at 42 ºC for 18-24h and 1 ml of BPW was mixed with Selenite Cysteine broth and incubated at 37 ºC for 18-24h. Enrichment broths were then inoculated onto BGA (Brilliant Green Agar) and XLD (Xylose Lysine Deoxycholate) agar plates and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism **(Anonymous, 2001, 2002, 2004)**.

Shigella Species

Five grams (for solid and semi-solid) or, 5 ml (for liquid) of food samples were homogenized/mixed well with 45 ml of Trypticase Soy Broth (TSB) + 0.3% yeast extract

(YE) supplemented with 0.3 µg/ml Novobiocin and incubated at 37ºC for 18-24 h. Enrichment broths were then inoculated onto MacConkey and Salmonella-Shigella (SS) agar plates and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism **(Anonymous, 2001, 2002, 2004).**

Vibrio spp.

Five gram (for solid and semi-solid) or, 5 ml (for liquid) of food samples were homogenized/mixed well with 45 ml of APW (Alkaline Peptone Water) and incubated at 37 ºC for 18-24 h for enrichment. Enrichment broths were then inoculated in selective culture media TTGA (Taurocholate-Tellurite-Gelatin Agar) and incubated at 37 ºC for 18-24 h in aerobic condition for selective growth of the organism **(Anonymous, 2001, 2002, 2004).**

E. coli

Five grams (for solid and semi-solid) or, 5 ml (for liquid) of food samples were homogenized/mixed well with 45 ml of Trypticase Soy Broth (TSB) and 0.3% yeast extract (YE) and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism. Enrichment broths were then inoculated onto MacConkey agar plates and incubated in aerobic condition at 37 ºC for 18-24 h for selective growth of the organism **(Anonymous, 2001, 2002, 2004).**

3.1.3 Colony Morphology Observation

After overnight incubation of the specific media, organisms were selected based on the following criteria:

Organism	**Media**	**Appearance**
E. coli	MacConkey	Lactose fermenting pink colonies
Salmonella	BGA	Typical red colonies
	XLD	Red or clear colonies with black centers
Vibrio Cholerae	TTGA	Grey flattened black centered halo around colony
Shigella	SS	Smooth non-lactose fermenting transparent colony
	MacConkey	Smooth non-lactose fermenting transparent colony

3.1.4 Biochemical Tests

The biochemical reactions of the strains were determined by standard methods described in manual for laboratory investigation of acute enteric infections (WHO, 1987). The following biochemical tests were performed:

- Kliger's Iron Agar (KIA) Test
- Motility Indole Ornithine (MIO) Test
- Simmon's Citrate Agar (SCA) Test
- Urea Test
- Oxidase Test

Kliglar Iron Agar Test (KIA Test)

Freshly prepared Kliglar's Iron Agar was poured into the screw cap test tubes in such an amount that slant with a deep butt (1 inch) is produced.

With a sterile straight wire suspected colony was stabbed into the butt to inoculate and the slant was streaked and incubated at 37°C for up to 24 hours. After 24 hours of incubation, the tubes were observed for any change.

Figure 1: KIA test

MIO Test

For motility test, about 5 ml of MIO agar medium was poured into open mouth test tubes and kept straight. Indole paper was kept on the mouth and closed through cotton.

Suspected colonies were inoculated by stabbing the medium with the help of sterile straight wire. The tubes were incubated at 37°C for 24 hours.

Figure 2: MIO Test

Citrate Test

For citrate test about 5 ml of citrate agar medium was poured into screw caped test tubes in such a way that slant was produced with small butt. With a sterile straight wire suspected colony was streaked onto slant and incubated at 37°C for up to 24 hours.

Figure 3: Citrate Test

Urea Test

For Urea test about 3ml of Urea agar medium was poured into screw capped test tubes and kept straight. Suspected colonies were inoculated by stabbing the medium with the help of sterile straight wire. The tubes were incubated at 37°C for 24 hours.

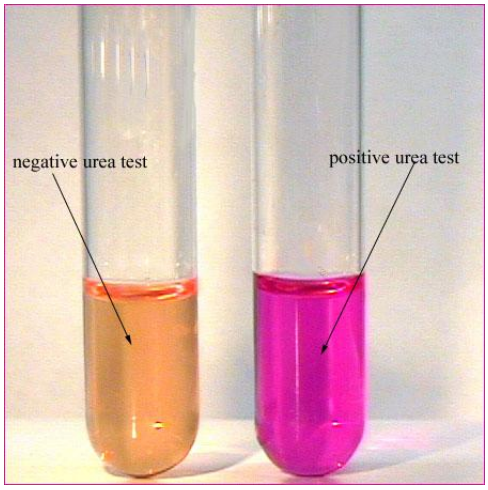


Figure 4: Urea Test

Oxidase Test

After preparation of oxidase reagent (phenylenediamine), a filter paper was placed in a petridish. 2 to 3 drops of reagent were added to the center of the paper. A pinch of suspected colony was dipped on the reagent.

Figure 5: Oxidase Test

Table 1 : Observation of Biochemical tests

Biochemical tests	**Observation after incubation**	
KIA	**Black color H_2S +** **No Black color H_2S -**	
SCA(Simmon's Citrate agar)	**Blue color citrate +**	**No change Citrate -**
Urea Test	**pink color Urea+**	**No color Urea -**
Oxidase Test	**Blue colony Oxidase +**	**No color change Oxidase -**

3.1.5 Confirmation Test

Serotyping for *Shigella, Salmonella* and *Vibrio spp*.

For final identification for *salmonella,* typical colonies were subjected to agglutination test using several polyvalent sera. Using a glass pencil, a glass slide was divided into several parts. Normal saline was placed in each of the parts. Then the bacteria to be tested were taken with loop and mixed with the saline. Then the serum was added to the bacteria. The slide was tilted back and forth to observe the agglutination. Only strong agglutination occurring within one minute was considered as a positive result.

3.1.6 PCR (Polymerase Chain Reaction) for *E coli*

The Polymerase Chain Reaction (PCR) allows the fast and direct detection of virulence genes in a sample even without prior cultivation of the microorganisms. This is particularly useful for the detection of low-number contamination in food and also for the analysis of clinical samples. PCR primers have been developed successfully for several of the categories of diarrheagenic *E. coli*. Advantages of PCR include great sensitivity in *in situ* detection of target templates.

Primers and PCR parameters

The primers for detection of the EPEC, EAEC, ETEC, EHEC and EIEC strains containing *eae, bfp, aat, aai, lt, ,st, stx1,stx2 ipaH* genes by PCR method are presented in Table 2.

Table 2: Primers for the detection of virulent genes of E. coli

Virulence genes	Primer sequence (5'-3')	Product size (bp)
Lt	LT: 'F'CACACGGAGCTCCTCAGTC LT: 'R'CCCCCAGCCTAGCTTAGTTT	508
St	ST: 'F' GCTAAACCAGTAGAGGTCTTCAAAA ST: 'R' CCCGGTACAGAGCAGGATTACAAC A	147
Bfp	bfp: 'F' GGAAGTCAAATTCATGGGGG bfp: 'R' GGAATCAGACGCAGACTGGT	300
Eae	eae: 'F' CCCGAATTCGGCACAAGCATAAGC eae: 'R' CCCGGATCCGTCTCGCCAGTATTCG	881
Aat	*aat*: 'F' CTGGCGAAAGACTGTATCAT	650

	aat: 'R' CAATGTATAGAAATCCGCTGTT	
Aaic	aaic: 'F' ATTGTCCTCACGCATTTCAC aaic: 'R' ACGACACCCCTGATAAACAA	215
stx1	stx1: 'F' CAGTTAATTTGGTGGCGAAG stx1: 'R' CTGCTAATAGTTCTGCGAATC	584
stx2	stx2: 'F' CCTCGGTATCCTATTCCCGG stx2: 'R' GGATGCATCTCTGGTCATTG	348
IpaH	ipaH: 'F' TGGAAAAACTCAGTGCCTCT ipaH: 'R' CCAGTCCGTAAATTCATTCT	423

Supplies and Equipment:

- **i)** Qiagen multiplex PCR KIT
- **ii)** Microcentrifuge tubes and racks
- **iii)** Nuclease free wáter (nfw)
- **iv)** Primers 20 μM F and R primers (lt, st, bfp, eae ,aat, aaic, ipaH, stx1, stx2)
- **v)** P200, P20, P10 pipette and tips
- **vi)** Vortex
- **vii)** Microcentrifuge & Thermocycler
- **viii)** PCR Plates, seals
- ix) Gel electrophoresis set up with 100 bp ladder and camera

Procedure

Template DNA isolation

Bacterial strains were grown overnight in trypticase soy agar (TSA) at 37°C. A loopfull colony of each isolate was suspended in 1ml distilled water and triton X into an eppendorf tube. After mixing the suspension by vortexing and boiling the suspension for 20 min; this was followed by ice depth and subsequently centrifuged at 10,000 rpm for 10 min to pellet the cell debris. The supernatant was used as template DNA for amplification reaction. The DNA was stored at -20°C.

Reaction mixture preparation

Preparation of master mix and PCR plate

a) Qiagen multiplex MM, Q solution, and primers were thawed in a DNA free room.

b) Qiagen multiplex MM, Q solution, and primers were vortexed.

c) Master mix was prepared in a 1.5 ml microcentrifuge tube (eppendorf)

9 plex Master Mix for E. coli assay:

Qiagen Multiplex MM	12.5 µl
20 µM lt F	0.25 µl
20 µM lt R	0.25 µl
20 µM St F	0.25 µl
20 µM st R	0.25 µl
20 µM bfp F	0.25 µl
20 µM bfp R	0.25 µl
20 µM eae F	0.25 µl
20 µM eae R	0.25 µl
20 µM aaic F	0.25 µl
20 µM aaic R	0.25 µl
20 µM aat F	0.25 µl
20 µM aat R	0.25 µl
20 µM ipaH F	0.25 µl
20 µM ipaH R	0.25 µl
20 µM stx1 F	0.25 µl
20 µM stx1 R	0.25 µl
20 µM stx2 F	0.25 µl
20 µM stx2 R	0.25 µl
Q solution	2.5 µl
Nfw	2.5 µl

d) 20 µl of master mix was taken in each PCR tubes

e) 5 µl of extracted DNA (Template) was added

f) Tube wss sealed and PCR was run

PCR Cycling Condition:

1X	15 min.	950 C
40X	30 sec	940 C
	90 sec	580 C
	90 sec	720 C
1X	10 min.	720 C
1X	for cooling	40 C

Gel electrophoresis

Amplification products were subjected to horizontal gel electrophoresis in 1% agarose gel in TBE (Tris-borate EDTA) buffer at room temperature at 100 volt (50 mA) for 1h. Briefly, 10 μl of amplified DNA for each sample was mixed with 1 μl of tracking dye and loaded into an individual well of the gel (5 mm thick). DNA bands were detected by staining the gel with ethidium bromide (0.5 μg/ml) for 30 minutes at room temperature and photographs were taken according to the procedure. 1 kb and 100 bp DNA size standard (Invitrogen, USA) was used as marker to measure the molecular size of the amplified products.

Table 3 : Interpretations

Pathotypes of E.coli	Virulence genes
EAEC: Enteroaggregative *E. coli*	***aat*** **(**650 bp) and/or **aaic (**215 bp)
ETEC: Enterotoxigenic *E. coli*	***lt*** **(**508 bp) and/or ***st*** (147 bp)
EPEC: Enteropathogenic *E. coli*	***eae*** (881 bp) and/or ***bfpA*** (300 bp)
EIEC: Enteroinvasive E. coli	***ipaH*** (423 bp)
EHEC: Enteroheemorrhagic *E.coli*	***stx1*** (348 bp) and/or ***stx2*** (584 bp)

3.2 Socioeconomic and socio-cultural parameters

3.2.1 Study Population

A total 225 students of EWU and 150 vendors were interviewed by using a structured questionnaire to know their social, behavioral and other factors and also health safety awareness about the street vended foods.

3.2.2 Study site

The study was conducted at the old (Mohakhali) and new (Aftabnagar) campus of East West University.

3.2.3 Sampling procedure

The students of EWU were randomly selected and vendors were selected based on their availability.

3.2.4. Limitations

One of the limitations of this study is the collection of food samples only from EWU campus. Therefore, the collections of foods are not generalizable for the street food samples available in whole Dhaka city or in other university campuses. In this study we only included the students of EWU campus. This is a biased group of populations and they are neither the representative of the whole population in Dhaka city nor other university campuses. During the food sampling collection, we did not consider the time of keeping the foods (e.g. duration of foods on display) in the vending stall which may be a reason for contamination.

Moreover, this study design does not permit establishing relationship between the street food contamination, behavioral characteristics/practices of the street food vendors and also the students who consumed street food. Further study is needed to establish this kind of relationship. Future study will be designed to overcome all the limitations of this study including an observation checklist in the project.

3.2.5 Data Analysis

Collected data were analyzed by SPSS version 17.0. Univariate analysis was done for estimating proportion and bivariate analysis was done to compare between two groups. X^2 Test was carried out to see the significance level.

4. Results

Table 4: Number of food samples with growth of organisms

	No of samples with +ve growth		**No of samples with targeted organism (suspected)**	
Total no of samples	**Old campus (n=30)**	**New Campus (n=20)**	**Old campus (n=30)**	**New Campus (n=20)**
50	**29**	**17**	**19**	**13**

Table 5: Presence of suspected organisms in no of food samples

No. of samples	***Salmonella Spp***	***E coli***	***Shigella Spp***	***Vibrio cholerae***
Old Campus	**14**	**14**	**04**	**00**

(n=30)				
New campus (n=20)	**11**	**09**	**06**	**01**

Among the total number of food samples, 30 were collected from fixed and mobile food vendors around the old campus and 20 from vendors around the new campus of EWU. The characteristics of the street food vendors and students have been described later on. About 46 (92%) food samples were contaminated with pathogenic or non-pathogenic micro-organisms (Table 4). Of which, 32 samples (19 of old and 13 of new campus) were suspected to be contaminated with our targeted organisms (*Salmonella Spp*. *E coli, Shigella Spp, Vibrio cholerae*).

Table 5 shows the number of food samples contaminated with the targeted organisms. In total 25 samples (14 from old and 11 from new campus), 23 samples (14 from old and 9 from new), 10 samples (4 from old and 6 from new) and only 1 sample from new campus were suspected to be contaminated with *Salmonella spp., E coli, Shigella spp.* and *Vibrio cholerae* respectively.

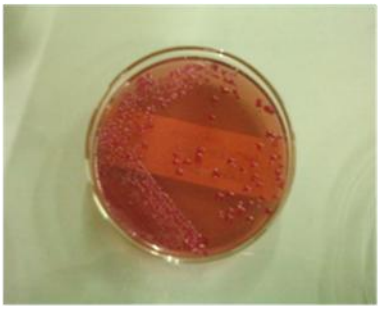
Table 6: Presence of organisms in different food samples collected from EWU Mohakhali Campus

Sample	*Salmonella spp* (XLD)	*E coli* (MacConkey)	*Vibrio spp*. (TTGA)	*Shigella* (SS)
Beguni	lot of colonies	-	-	-
Pakora	lot of colonies	-	-	-
Samosa	lot of colonies	-	-	lot of colonies
Alur Chop	lot of colonies	-	-	-
ButterBun	-	7	-	-
Cake	lot of colonies	8	-	-
Singara	-	8	-	-
Noodles	lot of colonies	lot of colonies	-	lot of colonies
Roll	lot of colonies	lot of colonies	-	-
Patis	lot of colonies	lot of colonies	-	-
Amra Vorta	lot of colonies	6	-	-
Fish Mix	-	lot of colonies	-	lot of colonies
Jhal Muri	lot of colonies	lot of colonies	-	-
Khichuri	-	lot of colonies	-	-
Tamarind water	-	lot of colonies	-	-
Lemon Juice	lot of colonies	lot of colonies	-	-
EWU Water	lot of colonies	-	-	-
Velpuri	lot of colonies	lot of colonies	-	lot of colonies
Sugarcaine Juice	lot of colonies	lot of colonies	-	-

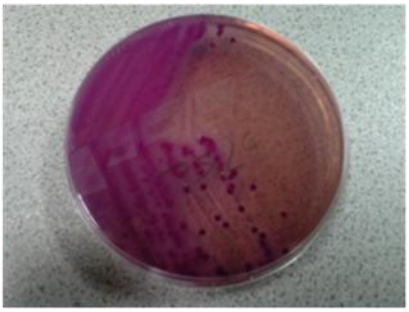
Table 7: Presence of organisms in different food samples collected from EWU Aftabnagar Campus

Sample	***Salmonella* spp (XLD)**	***E coli* (MacConkey)**	***V.* spp. (TTGA)**	***Shigella* (SS) or,(MAC)**
Beguni	lot of colonies	9	-	-
Samucha	lot of colonies	-	-	-
Butter Bun	lot of colonies	8	-	-
Cake	-	8	-	-
Singara	lot of colonies	7	-	-
Noodles	lot of colonies	-	-	-
Roll	-	10	-	-
Chotpoti	lot of colonies	-	-	lot of colonies
Khichuri	lot of colonies	6	-	lot of colonies
Lemon Juice	lot of colonies	7	-	9
Sugarcane Juice	lot of colonies	8	-	8
Vel. Tok	lot of colonies	-	-	8
Water from vendor	lot of colonies	8	8	lot of colonies

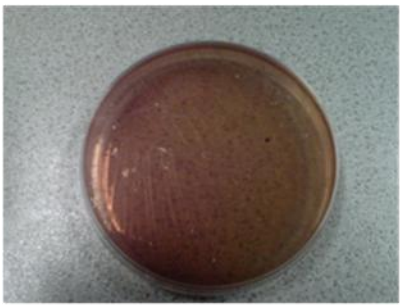
Table 6 and Table 7 show a lot of colonies of different microorganisms in different types of food samples collected from Mohakhali and Aftabnagar campus of EWU. We have found bacterial growth in 46 samples out of 50. Majority of the samples yielded multiple pathogens. Seven samples were contaminated with only one organism like *proteus, E. coli, klebsiella* and *Bacillus* species. We did not find any growth in four food samples.



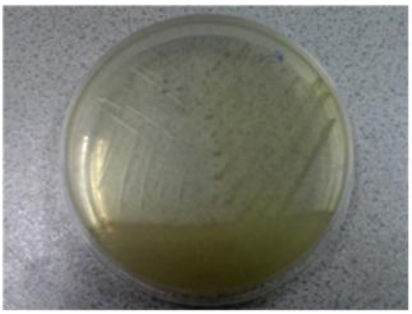
Red colony of suspected *Salmonella* from Butterbun



Pink colony of suspected *E. coli* from khichuri



Transparent colony of suspected *Shigella* from fixed vendor water



Grey, flattened, black centered colony of suspected *Vibrio* spp. from fixed vendor water

Figure 6: Suspected organisms in different media

Table 8: Biochemical test of samples collected from Mohakhali and Aftabnagar Campus for suspected *Salmonella* spp

Sample	Location	KIA	Motility (M)	Indole (I)	Ornithine (O)	Urea (U)	Citrate	Oxidase	Gas	H_2S
Alur Chop	M	K/A	+	–	–	–	–	–	–	–
Cake	M	K/A	+	–	–	–	–	–	–	–
Jhalmuri	M	K/A	+	–	–	–	–	–	–	–
Ewu water	M	K/A	+	–	–	–	–	–	–	–
Khichuri	A	K/A	+	–	–	–	–	–	–	+
Water(fixed vendors)	A	K/A	+	–	–	–	–	–	–	+
Roll	M	K/A	+	–	–	–	–	–	–	+
Amra vorta	M	K/A	+	–	–	–	–	–	–	+
Sugar cane Juice	A	K/A	+	–	–	–	–	–	–	–

Table 9: Biochemical test of samples collected from Aftabnagar Campus for suspected *Shigella sp.*

Sample	Location	KIA	Motility (M)	Indole (I)	Ornithine (O)	Urea (U)	Citrate	Oxidase	Gas	H_2S
Khichuri	A	K/A	–	–	–	–	–	–	–	–
Water from vendors	A	K/A	–	–	–	–	–	–	–	–

Preliminary confirmation of *salmonella and shigella spp.* was done by performing different biochemical tests. Based on the standard guideline, organisms were confirmed. Of 25 samples that were suspected to contain S*almonella spp*, 9 samples gave positive results (table 8). Out of 10 samples, 2 were confirmed by biochemical tests for the presence of *shigella spp* (Table 9). Biochemical test was not done for all samples because there are some specific criteria for specific media (Table 10) on the basis of which biochemical test is done. During the colony morphology observation of bacterial culture on media, it was decided which will be taken for further confirmation test. Biochemical tests were not performed for *E coli* and *Vibrio spp*. since they were directly confirmed by Polymerase Chain Reaction (PCR) of specific genes and serotyping respectively.

Table 10: Criteria of Organisms on Specific Media for Biochemical Test

Organism	Media	Colony Morphology
Shigella spp.	**SS (Salmonella-Shigella Agar)**	**Small, round, transparent colony**
Shigella spp.	**MacConkey**	**Small, round, transparent colony**
Salmonella spp.	**XLD (Xylose lysine deoxycholate)**	**Red with black centered colony**
Salmonella spp.	**BGA (Brilliant Green Agar)**	**Typical red colony**
Vibrio spp.	**TTGA (Taurocholate-Tellurite-Gelatin Agar)**	**Grey, flattened ,black centered , with halo around colony**
E.coli. spp.	**MacConkey**	**Flat, dot centered , pink colony**

Table 11: Serology of samples collected from Aftabnagar Campus for *Shigella*

Sample	Polyvalent Serotype	Group Antigen	Type-Antigen	Subtype
Khichuri	Poly B	7(8)	-	*S. flexneri* X-variant
Water collected from fixed vendor	Poly B	(3)4	II	*S. flexneri* 2a

Serotyping of 9 *Salmonella spp* and 1 *vibrio spp* were negative after serological tests. After biochemical tests, 2 samples were positive for *Shigella spp.* Serotyping of these 2 samples confirmed the presence of *Shigella flexneri.* After serotype level identification, it was revealed that Khichuri was contaminated with *Shigella flexneri* X-variant and Water collected from fixed vendor was contaminated with *Shigella flexneri* 2a (Table 11).

Table 12: PCR Test for *E coli* suspected samples from Mohakhali and Aftabnagar Campus

Sample	Location	Result
Singara	Mohakhali	EAEC (*aat*)
Cake	Mohakhali	EAEC (*aat*)
Butter bun	Mohakhali	EAEC (*aat*)
Lemon juice	Aftabnagar	ETEC (*lt*)

Of the total food samples, 23 were suspected to be contaminated with *E coli.* All the samples were subjected to Polymerase Chain Reaction (PCR) for detecting the presence of specific gene present specifically in different types of *E coli.* For example, to confirm the presence of Enteroaggregative *E coli* (EAEC), *aat* gene needs to be detected. The PCR amplicons were then subjected to gel electrophoresis for the visualization, 4 samples (3 collected from old campus and 1 collected from new campus of EWU) were confirmed to contain *E coli*. Three samples collected from the old campus showed the presence of *aat* gene and confirmed the presence of EAEC and 1 sample collected from the new campus showed the presence of *lt* gene and thereby confirmed the presence of Enterotoxigenic *E coli* (ETEC) (Table 12).

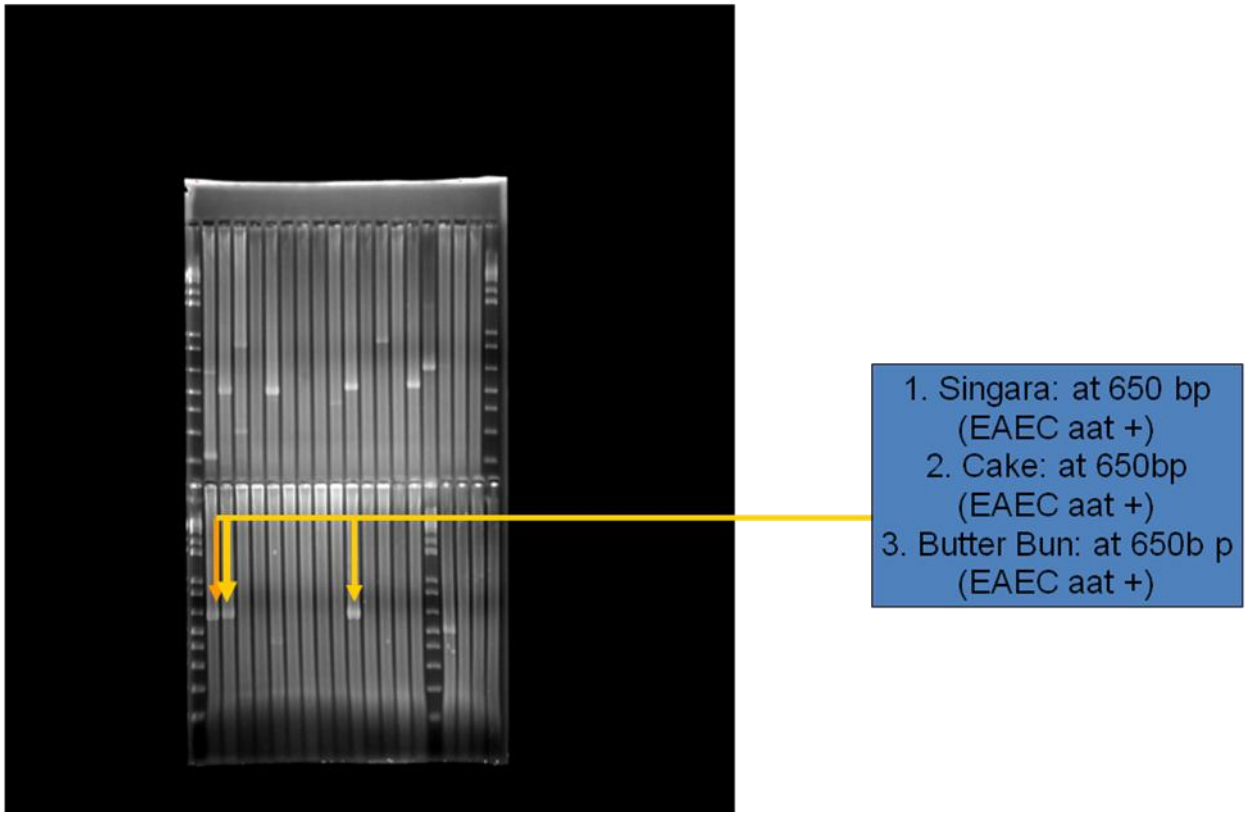


Figure 7: Agarose gel electrophoresis showing the PCR amplification products of *aat* gene of *E. coli*

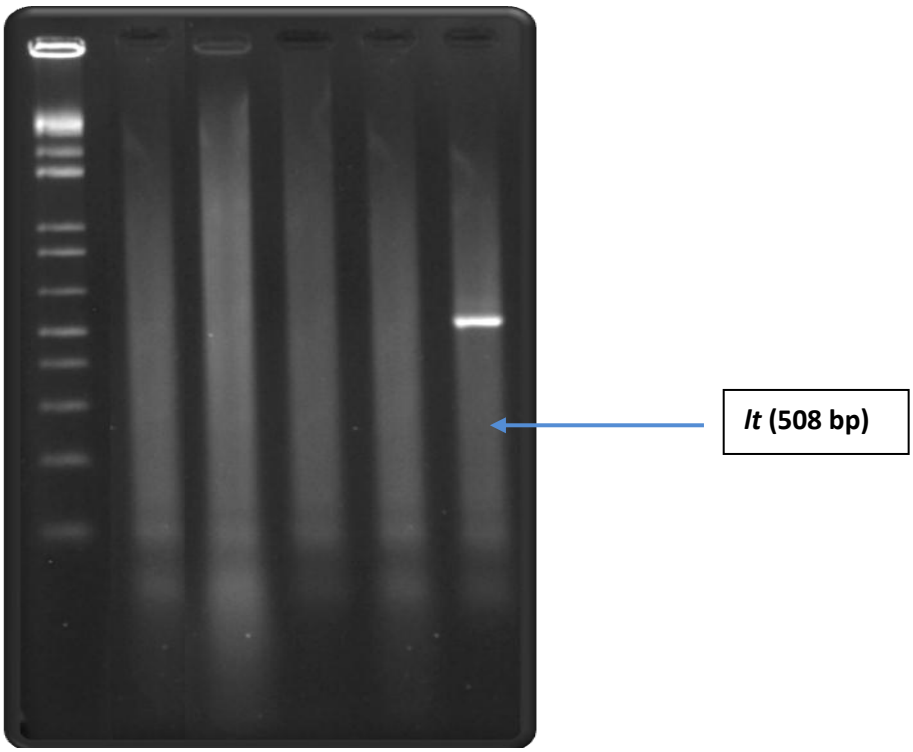


Figure 8: Agarose gel electrophoresis showing the PCR amplification products of *lt* gene of *E. coli* in lemon juice

Table 13: Summary of the findings

No. of samples tested	No. of samples positive for organisms			
	Shigella	*Salmonella*	*Vibrio spp*	*E Coli*
50	02	0	0	04
Total	**06 (12%)**			

As a whole, out of 50 food samples, 6 (12%) were found to contain the targeted organisms. Four (4) samples were contaminated with *E coli* and 2 were contaminated with *Shigella spp.* After doing all the experiments we have confirmed that 6 samples (out of 50) were contaminated with pathogenic organisms although we have found organisms in most of the samples.

Table 14: Characteristics of the students

Variables		**(%)**
Age	16-19 Yrs	20.9
	20-22 Yrs	49.3
	23-25 Yrs	29.8
Sex	Male	58.2
	Female	41.8
Living Area in the City	Near University	38
	At a moderate distant	38
	Far from university area	24
Monthly Family Income (Taka) (n=213)*	<40000	31.1
	40000-59000	24.4
	60000-79000	16.9
	>80000	22.2
Monthly pocket expenditure (n=196)*	Tk Less than 1000	30.6
	Tk 1000 – less than 3000	40.8
	Tk 3000 – 5000	17.3
	Tk More than 5000	11.2
Students consumed street food (n=223)*		55
Students wash hands with soap after coming from toilet (n=224)*		92
Students wash hands with soap before eating food (n=222)*		65.8
Knowledge of contamination of street food (n=158)*		93.7

Students cannot afford to take food from good restaurant	17.0

*The data does not add to 225 cases as some students failed to respond to these questions

Table 14 shows the characteristics of the students of EWU. Majority of the students (49%) were in the age range of 20-22 years. About 58% of the students were male and 42% were female. Among the students, 76% lived near the university or at a moderate distance of 2 km. Thirty one percent students had a monthly family income of less than Tk. 40, 000 and 41% students got monthly pocket expenditure between Tk 1000 and less than 3000. About 55% of the students consumed street food. Ninety two percent of the students washed hands with soap after coming from toilet and 66% washed hands with soap before eating food. 94% students had knowledge about contamination of street food and 17% of the students cannot afford to take food from good restaurant.

Table 15: Relationship between characteristics/practices and street food consumptions of students

Variables		**Consumed street food**	**Did not consume street food**	***P***
Age	16-19 yrs (n=46)	47.8	52.2	
	20-22 yrs (n=110)	54.5	45.5	0.460
	23- 25 yrs (n=67)	59.7	40.3	
Sex	Male (n=129)	45	55	0.001
	Female (n=94)	68	32	
Reported Diarrhoea in the past 3 months (n=36*)		52.8	47.2	0.855
Reported stomachache/ vomiting (n=22*)		59	41	0.822
Have knowledge about street food contamination (n= 147*)		78.2	21.8	0.056
Wash hands after toilet (n= 207*)		56.5	43.5	0.067

*Only those respondents were included who gave a positive answer to the questions

Table 15 shows the relationship between characteristics/practices and street food consumptions of students. It has been observed that female students (68%) consume street foods more than the male students (45%). Among 36 reported cases of diarrhea and 22 reported cases of stomachache and vomiting, street food consumption was 53% and 59% respectively. Majority of the students (78%) consume street food in spite of having knowledge about street food consumption. About 57% of the students, who consumed street foods, washed their hands with soap after toilet.

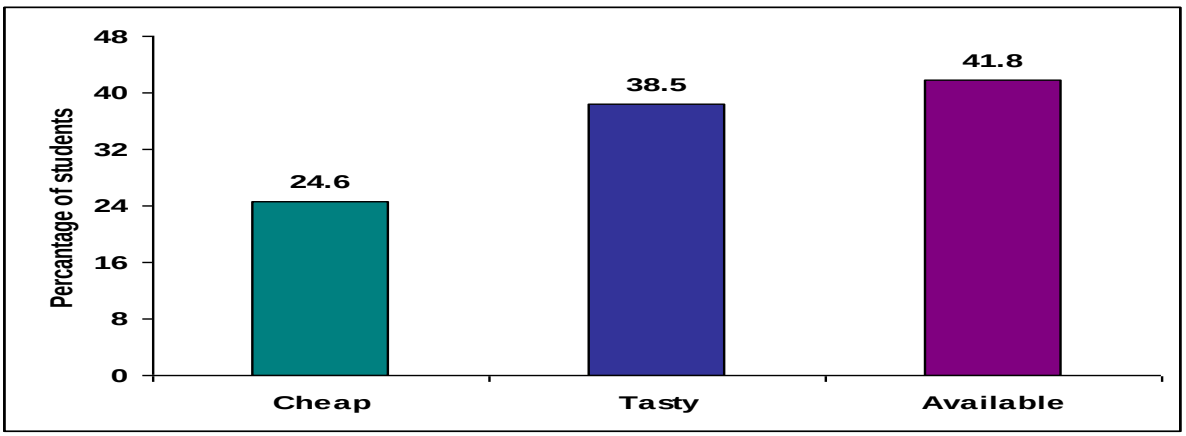


Figure 9: Reasons for taking street vended food (n=122)

Among the students who took street vended food (n=122), 25% consumed because these foods were cheap, 39% because they were tasty and 42% because they were readily available (Figure 9).

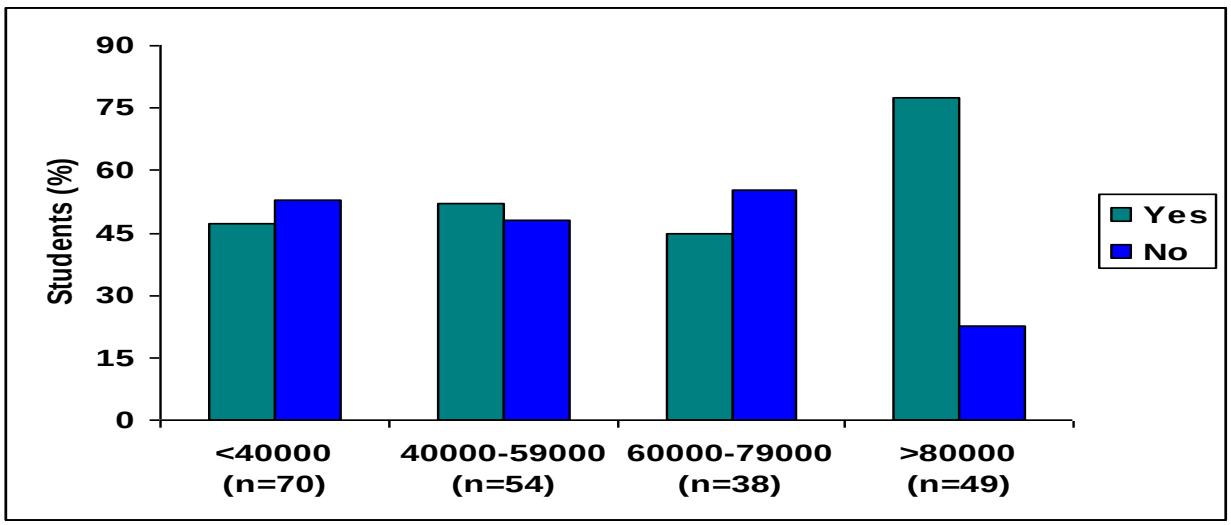


Figure 10: Monthly family income in relation to consumption of street vended food

A significant relationship ($p<0.003$) was detected between the monthly family income of the students and their street food consumption rate (Figure 10).

Table 16: Socio-economic characteristics of the vendors

Characteristics (n=150)		**%**
Age in Yrs	16-29 yrs	48
	30-45 Yrs	38
	46-60 Yrs	14
Educational Status	Illiterate	23.3
	Below Class 5	32.0
	Class 5 – HSC	44.7
Monthly family income (Taka)	Less than 5000	12.7
	5000-7999	24.7
	8000-12000	54.7
	More than 12000	8.0
Living condition	Slum	58.7
	Other than slum	41.3

Table 16 shows the socio-economic characteristics of the vendors. About 150 fixed and mobile food vendors who were available around the old and new campus of EWU were interviewed to know about their characteristics and practices with regard to street foods. About 48% of the vendors were in the age range of 16-29 years. Only 14% of the vendors were in the age range of 46-60 years. About 23% vendors were illiterate and 32% had an educational status below class 5. Monthly family income of 55% vendors was Tk 8000-12000. About 59% vendors lived in slums.

Table 17: Behavioral characteristics of the vendors

Behavioral characteristics (n=150)	**Yes**	**No**
Keep food open	60.7	39.3
Sell stale food	12.0	88.0
Prepare food at home	15.3	84.7
Remove flies when roaming	80.7	19.3
Store Water in an open pot	53.3	46.7
Suffering from infectious disease recently	79.3	20.7
Wash hands with soap after toilet	59.3	40.7
Wash hands with soap before preparing food	10.7	89.3

Have knowledge of contamination	46	54

From Table 17, we get information regarding the behavioral characteristics of the vendors. About 61% vendors kept food open and 12% of them sold stale food. Nineteen percent of the vendors did not remove flies while roaming and 53% stored water in open pot. Of the vendors, 79% suffered from infectious diseases recently and 46% had knowledge of food contamination. About 59% of the vendors washed hands with soap after toilet and only 11% washed hands with soap before preparing food.

Table 18: Relationship between the knowledge of contamination, educational status and hand washing practice of vendors before preparing food

Variables		Wash hand with soap before preparing food	P value
Educational Status	Illiterate (n=35)	2.9	<0.008
	Below Class 5 (n=48)	4.2	
	Class 5 – HSC (n=67)	19.4	
Have Knowledge about street food contamination	Yes (n=69)	17.4	0.017
	No (n=81)	4.9	

Table 18 shows the relationship between the knowledge of contamination, education status of the vendors, and their behavioral aspects. A significant relationship ($p<0.008$) was found between the educational status of the vendors and their hand washing practice before preparing food. It was observed that with the increase in educational status, more vendors washed their hands with soap before preparing food. A significant relationship ($p<0.017$) was also obtained between the knowledge of the vendors about street food contamination and hand washing practice before preparing food.

Table 19: Relationship between the knowledge of contamination, educational status and hand washing practice of vendors after coming from toilet

Variables		**Wash hand with soap after toilet**	**P value**
Educational Status	Illiterate (n=35)	40	0.029
	Below Class 5 (n=48)	64.6	
	Class 5 – HSC (n=67)	65.7	
Have Knowledge about street food contamination	Yes (n=69)	68.1	0.04
	No (n=81)	51.9	

Table 19 also shows the relationship between the knowledge of contamination, education status of the vendors, and their behavioral aspects. A significant relationship ($p<0.029$) was found between the educational status of the vendors and their hand washing practice after coming from toilet. It was observed that with the increase in educational status, more vendors (65.7%) washed their hands with soap after coming from toilet. A significant relationship ($p<0.04$) was also obtained between the knowledge of the vendors about street food contamination and hand washing practice after coming from toilet.

Table 20: Relationship between hand washing practice of vendors and suffering from diarrhea/dysentery

Variables	**Hand washing practice after toilet**			
		With soap	**Water only**	**P**
Suffered from diarrhea/dysentery recently	Yes (N=54)	40.7	59.3	0.001
	No (N=96)	69.8	30.2	

A significant relationship ($p<0.001$) was observed between the number of vendors who suffered from diarrhea or dysentery recently and hand washing practices of the vendors after coming from toilet. It was observed that 59% of the vendors who suffered from diarrhea/dysentery recently washed their hands only with water (Table 20).

5. Discussion

Street food vending is a prevailing and distinctive part of a large informal sector in Bangladesh. It has a major economic impact in many countries and is a major source of employment **(Mahon BE, 1999)**. The present study provides information about the presence of enteric bacteria especially *Shigella, Salmonella, pathogenic E. coli* and *Vibrio spp*. in food samples from fixed and mobile vendors around the student campus of EWU. This study also provides information and reveals many issues about the characteristics and practices with regard to street food among the students and vendors in the same area.

We collected 50 food samples, of which 46 (92%) had bacterial contamination. Six (12%) of them were confirmed to contain the targeted organisms (*Salmonella spp, E coli, Shigella spp and Vibrio choleare*). We found that 4 food samples, namely shingara, cake, butter bun, were found to be contaminated with Enteroaggregative *E coli* and lemon juice was contaminated with Enterotoxigenic *E coli*. Two samples, khichuri and water collected from fixed vendor, were contaminated with *Shigella flexneri*. Khichuri contained *Shigella flexneri* X-variant and water collected from fixed vendor contained *Shigella flexneri* 2a. This study also revealed that 55% of the students consumed street vended foods. The reason for consuming the street vended foods by the students is their availability on the premises. The other reasons for

having the street foods were taste (39%) and low price (25%). Regarding the behavioral practices of the students, about 92% washed their hands with soap after coming from toilet and 65% wash hands before eating food. Ninety-two percent students had knowledge about food contamination. Reported cases of diarrhea and stomach ache/ vomiting among the students in the past 3 months were 36 and 22 respectively.

In our study, majority (48%) of the vendors were in the age range of 16-29 years. A similar trend in age was observed among the street food vendors in Accra, Ghana, where 66% of the vendors were below 30 yrs of age **(Odonkor et al, 2011)**. A significant relationship ($p<0.001$) was observed between the hand washing practice of vendors after coming from toilet and their suffering from diarrhea and dysentery.

In developing countries, fruit juices, drinks, meals and snacks sold by street-food vendors are widely consumed by millions of people. It was reported from the study conducted in Trinidad and Tobago that 35% of foods were contaminated by E. coli while 57.5% of water used by vendors was contaminated by coliforms **(Rane S, 2011)**. Study conducted on quality and safety of street vended fruit juices in Amravati city, India revealed presence of enteric bacterial pathogens in a total of 52 fruit juice samples. The dominant enteric bacterial pathogens were *Escherichia coli* (40%), followed by *Pseudomonas aeruginosa (25%), Salmonella spp (16%), Proteus spp (9%), Staphylococcus aureus (6%), Klebsiella spp (3%) and Enterobacter spp* (1%) **(Tambekar et al, 2009)**. Another study by Reddy and her coworkers (2009) also showed the presence of four different types of fecal coliforms namely, *Klebsiella pneumonia, Citrobacter freundii, Enterobacter aerogens and Escherichia coli* in all fruit juice samples collected from four different public areas of Bellary city, India. **Kaul and Agarwal (1988)** reported high microbial count in fruit chat sold by a street vendor in Chandigarh, India and a number of pathogens, such as *E. coli, Salmonella typhimurium, Salmonella gallinarum, Shigella dysentriae, Pseudomonas fluorescens and Klebsiella pneumoniae* were also found to be present in these samples. Similar result was observed in our study where 46 (92%) out of 50 samples were contaminated with different microorganisms. However, our objective was to find out the presence of enteric bacteria especially *Shigella, Salmonella, pathogenic E. coli and Vibrio spp*. in food samples. Therefore, we confirmed the presence of *Shigella* and *E. coli* in 6 food samples (12%) collected from the student campus of EWU.

This study revealed that 54% of the vendors stored water in open pot; 81% of them removed flies from the food and 12% sold stale food. Water is a critical raw material in many street

vending operations and contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient and used in the processing of food or used for washing equipment, utensils and hands. It is a well known vehicle for enteropathogens such as *E. coli, Salmonella spp.* and *Campylobacter spp.* amongst others **(Rane S, 2011)**. A study carried out by **Muinde OK and E Kuria (2005)** showed that houseflies were present in almost all the samples which were the vehicles for the contamination of food samples. In the same study the authors found that, most of the vendors kept cooked foods open and uncovered which conforms to our study where 61% of the vendors kept food open. A recent study conducted in Dhaka city revealed that about 58% of the street food vendors did not cover their food while selling, and 88% vendors used stored water for cleansing of utensils **(Faruque et al., 2010)**. Keeping food open may result in contamination due to dust and microbes. **Kinton and Ceserani (1992)** recommended that foodstuffs of all kinds should be kept covered as much as possible to prevent contamination from dust and flies.

Enteropathogens can survive on the hands for three hours or longer if not washed by disinfectant soap. Strains of *E coli* were detected in hands of high and low-income mothers in different countries **(Mathur and Reddy, 1983; Black et al, 1989)**. Diarrheal pathogens on the hands of mothers have been shown to be transmitted to infants. Therefore, bacteria present on the hands of vendors may contaminate foods and subsequently may be transmitted to the consumers. It was observed in our study that among the vendors who suffered from diarrhea and dysentery (n=54), 59% of them washed their hands with water without any disinfectant. Since we did not have the clinical data from this study regarding the pathogens present in vendors' hands, we cannot confirm that the foods were contaminated by the transmission of pathogens from the vendors.

Studies carried out in different countries showed that the foods are contaminated with different types of microorganisms like *Bacillus spp, Staphylococcus spp, Clostridium, Campylobacter, Klebsiella pneumonia, Citrobacter freundii, Enterobacter aerogens* etc. However, we confirmed the presence of only four enteric pathogens namely, *Salmonella spp, E coli, Shigella spp and Vibrio cholera* in our food samples. Although we have found other pathogens present in the food samples, they could not be confirmed in our study because of the limited resources. Future studies can be designed to evaluate the presence of all probable microorganisms in the food samples.

6. Conclusion

These findings demonstrate that street vended foods sold on the student campus of EWU contain some pathogenic organisms which are likely to be a potential hazard to the health of the EWU community. This study also reveals lack of knowledge regarding the food contamination by the vendors. Lack of vendors' hygiene practice can also make the consumption of street food a high risk to health.

Therefore, health education of the vendors on personal hygiene, safer food handling practice and the proper disposal of waste would improve food quality and thereby reduce the risk of contamination of street foods. Infrastructure developments for access to potable water, public toilet, washing and waste disposal facilities would reduce the health hazards.

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