MICROBIAL CONTAMINATION OF STREET VENDED FOODS FROM A UNIVERSITY CAMPUS IN BANGLADESH

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Abstract. The microbiological quality of street vended food samples from Dhaka, Bangladesh was evaluated. The objective of the study was to identify the presence of common pathogens (*Escherichia coli*, *Shigella* spp, *Salmonella* and *Vibrio* spp) and to describe the molecular characterization of *E coli*, a commonly found pathogen in various street foods. Fifty food samples were collected from fixed and mobile vendors from two sampling locations (Mohakhali and Aftabnagar) in Dhaka city, Bangladesh. The tested samples included deep fried and fried snacks; quick lunch items; pickles; fruit chutney; baked items; spicy, sour and hot snacks etc. Juices, tamarind water and plain drinking water were also tested. Sterile polythene bags were used for collecting 200 g of each category of samples. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests followed by serology were done for the confirmation of *Shigella* and *Salmonella*. Serological reaction was carried out for confirmation of *Vibrio* spp. DNA was isolated for the molecular characterization to detect the pathogenic *E. coli* by polymerase chain reaction (PCR). Out of 50 food samples, six (12%) were confirmed to contain different species of *E. coli* and *Shigella*. Molecular characterization of *E. coli* revealed that three samples were contaminated with enteroaggregative *E. coli* (EAEC) and one was contaminated with enterotoxigenic *E. coli* (ETEC). *Shigella flexneri* X variant was detected in one food item and *Shigella flexneri* 2a was found in drinking water. All these enteric pathogens could be the potential cause for food-borne illnesses.

Keywords: street food, *Shigella*, *Salmonella*, *Escherichia coli, Vibrio* spp, Bangladesh

INTRODUCTION

In developing countries food sold by street vendors is the major source of food-borne illness (Kibret and Tadesse, 2013). Although food items from these outlets are appreciated mostly for their unique flavor and for their convenience (Nyenje *et al*, 2012), their microbiological safety is not always certain. The major sources contributing to microbial contamination of such food are infrastructure, preparation and storage, cooking, cleaning and serving utensils, quality of water and per-

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sonal hygiene of food handlers (Mensah *et al*, 2002; Muinde and Kuria, 2005; Ghosh *et al*, 2007; Rane, 2011). Other sources of contamination include place and surface of food preparation, flies and dust on uncovered food items, lack of facilities for drainage of waste water and garbage disposal, dish washing cloths, contaminated raw materials and water, unavailability and shortage of potable water, time–inadequate reheating of cooked food, and improper and unsanitary food handling by vendors.

Studies have been carried out in different countries throughout the world to investigate the microbiological quality of street-sold food. Microbiological quality of chicken and pork-based street-food samples were found to be unsatisfactory due to high levels of coliforms, *Escherichia coli* and *Staphylococcus aureus* (Manguiat and Fang, 2013). *E. coli*, *Salmonella* and *Shigella* spp have been identified in white lupin (Kibret and Tadesse, 2013). It has been documented recently that street-sold water is a significant risk factor for cholera infection in cholera epidemic regions of Sierra Leone (Nguyen *et al*, 2014). A study in Silchar city, Assam, India has reported detection of isolates of *E. coli* (37.5%), *Salmonella* (5.36%), *Shigella* (19.64%) and other microbial species from 37 street-sold food samples (Sharma and Mazumdar, 2014). In Orissa, India *E coli*, *Klebsiella* spp, *Salmonella paratyphi*, *Shigella dysenteriae*, and *Vibrio* spp were detected in 12 Panipuri samples (Das *et al*, 2012).

Food from street vendors are perceived to be a major public health risk due to the lack of basic infrastructure and services and also the difficulty in bringing the large numbers of street food vendors under effective control measures (Rane, 2011). In view of the health risk posed from street-sold food in densely populated cities of a developing country such as Bangladesh, a cross sectional study has been conducted revealing that food vendors have inadequate knowledge and awareness regarding food safety issues (Mamun *et al*, 2013). To the best of our knowledge, no study has been carried out on the pathogens present in street-sold food in Dhaka city, Bangladesh. Although it is a preliminary study, it is designed to identify the presence of common pathogens (*viz*, *E coli*, *Shigella*, *Salmonella* and *Vibrio* spp) and to describe the molecular characteristics of *E coli*.

MATERIALS AND METHODS

Sample collection

Food samples (200 g of each category), namely, different types of fried and deep fried snacks, quick lunch items, pickled native fruits, baked items, spicy and sour items, different types of liquids including plain drinking water, and other snack items, were collected from mobile and fixed vendors in two areas of Dhaka, Mohakhali and Aftabnagar. The food samples were collected in sterile plastic bags, which were sealed and transported to the laboratory and processed within 2-3 hours of collection.

Isolation and molecular characterization of *E. coli*

Five grams (solid and semi-solid items) or 5 ml (liquids) of food samples were homogenized/mixed with 45 ml of trypticase soy broth (TSB) and 0.3% yeast extract (YE) and incubated under aerobic condition at 37°C for 18-24 hours. The enriched broths were then inoculated onto MacConkey agar plates and incubated as described above. DNA was isolated for detection of pathogenic *E. coli* spp by PCR according to the standard procedures (Lüscher and Altwegg, 1994; Nguyen *et al*,

Table 1
Primers used in multiplex PCR for detection of diarrheagenic *E. coli* (DEC).

DEC	Target gene	Primer	Primer sequences (5'—>3')	Amplified product size (bp)
ETEC	*elt*B	ETEC 508F	5'-CACACGGAGCTCCTCAGTC-3'	508
		ETEC 508R	5'-CCCCCAGCCTAGCTTAGTTT-3'	
	*est*A	ETEC 147F	5'-GCTAAACCAGTAGGTCTTCAAAA-3'	147
		ETEC 147R	5'-CCCGGTACAGCAGGATTACAACA-3'	
EPEC	*Eae*	EPEC 881F	5'-CCCGAATTCGGCACAAGCATAAGC-3'	881
		EPEC 881R	5'-CCCGGATCCGTCTCGCCAGTATTCG-3'	
	*bfp*A	EPEC 300F	5'-GGAAGTCAAATTCATGGGGGTAT-3'	300
		EPEC 300R	5'-GGAATCAGACGCAGACTGGTAGT-3'	
EAEC	*aat*A	EAEC 650F	5'-CTGGCGAAAGACTGTATCAT-3'	630
		EAEC650R	5'-CAATGTATAGAAATCCGCTGTT-3'	
	*aai*C	EAEC 215F	5'-ATTGTCCTCAGGCATTTCAC-3'	215
		EAEC215R	5'-ACGACACCCCTGATAAACAA-3'	
EHEC	*Stx*1	EHEC 348F	5'-CAGTTAATGTGGTGGCGAAGG-3'	348
		EHEC348R	5'-CACCAGACAATGTAACCGCTG-3'	
	*Stx*2	EHEC 584F	5'-ATCCTATTCCCGGGAGTTTACG-3'	584
		EHEC584R	5'-GCGTCATCGTATACACAGGAG -3'	
EIEC	*ipa*H	EIEC 423F	5'-TGGAAAAACTCAGTGCCTCT-3'	423
		EIEC 423R	5'-CCAGTCCGTAAATTCATTCT-3'	

2005) using primers sequences listed in Table 1.

Isolation of *Shigella, Salmonella* and *Vibrio* spp

For isolation of *Shigella* spp, 5 g or 5 ml of food samples were homogenized/mixed with 45 ml of TSB containing 0.3% YE and 0.3 μg/ml novobiocin and incubated as described above. The enriched broths were inoculated onto MacConkey and Salmonella-Shigella (SS) agar plates and incubated as described. For *Salmonella* spp isolation, food samples were homogenized/mixed with 45 ml of buffered peptone water (BPW) broth and incubated as described. Enriched broths were inoculated onto brilliant green agar (BGA) and xylose-lysine-deoxycholate (XLD) agar plates and incubated as described for selective growth of the organism. For detection of *Vibrio* spp, homogenized/mixed samples were mixed with 45 ml of alkaline peptone water (APW) and incubated. Enriched broths were then inoculated onto taurocholate-tellurite-gelatin agar (TTGA) and incubated as described.

Typical colonies identified on the selective agar plates were confirmed using standard biochemical tests (WHO, 1987). All biochemically-positive isolates were serologically confirmed using commercially available antisera kits specific for all groups and type-factor antigens (Denka Seiken, Tokyo, Japan). Isolates were sub-

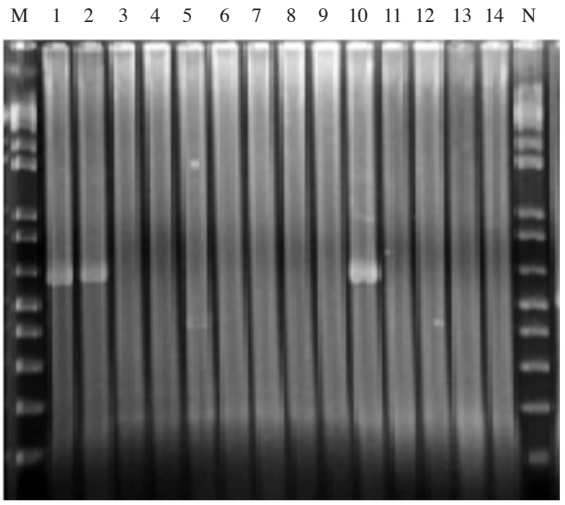

Fig 1 - PCR amplification of pathogenic *E. coli aat* in food samples. DNA extracted from *E. coli* isolates were subjected to PCR using gene-specific primers and amplicon was analyzed by gel-electrophoresis and ethidium bromide staining. The presence of a 650 bp amplicon of *aat* indicates the presence of enteroaggregative *E. coli* (EAEC) in singara (lane 1), cake (lane 2) and buttered bun (lane 10).

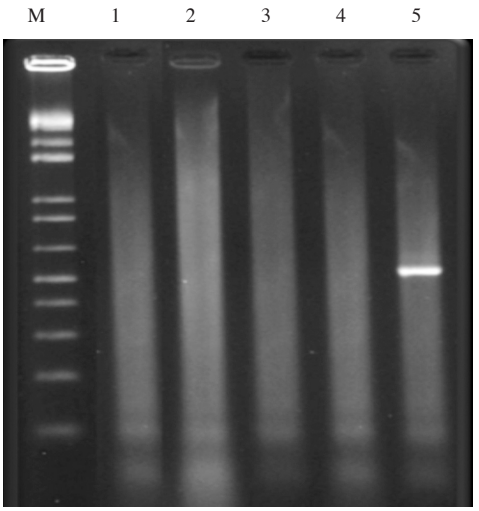

Fig 2 - PCR amplification of pathogenic *E. coli lt* in food samples. DNA extracted from *E. coli* isolates were subjected to multiplex PCR using gene-specific primers and amplicon was analyzed by gel-electrophoresis and ethidium bromide staining. The presence of a 508 bp amplicon of *lt* indicates the presence of enterotoxigenic *E. coli* in lemon juice (lane 5).

cultured onto MacConkey agar (Difco; Becton Dickinson, Sparks, MD) plates and after about 18 hours of incubation serological tests were performed using a glass slide agglutination method for identification of *Shigella* spp (El-Gendy *et al*, 1999; Talukder *et al*, 2007) and the Manual of Clinical Microbiology for identification of *Vibrio* and *Salmonella* spp (Murray, 2007).

RESULTS

The majority of 50 food samples tested were found to contain the presence of different species of pathogenic microorganisms indicating poor bacteriological quality of the food samples. Using culture and biochemical assay techniques, bacteria identified as being present in the food samples included *Salmonella* spp (50% of the samples), *E. coli* (46%), *Shigella* spp (20%), and *Vibrio* spp (2%). However, the single putative *Vibrio* isolate and all of the putative *Salmonella* isolates were negative when tested serologically. Only two samples showed contamination with *S. flexneri* X-variant and *S. flexneri* 2a. Among the 23 *E. coli* isolates, PCR-based assay revealed the presence of EAEC gene in singara, cake and buttered bun (13%) (Fig 1) and ETEC gene in lemon juice (4%) (Fig 2).

DISCUSSION

The present study conducted in Dhaka city, Bangladesh showed the presence of *E coli* and *Shigella* spp, but not *Salmonella* or *Vibrio* spp, in 12% of food

samples from street vendors. Pathogenic *E. coli* was detected only in 4/23 (17%) isolates. In developing countries, fruit juices, drinks, meals and snacks sold by street food vendors are widely consumed by millions of people. In Trinidad and Tobago 35% of food were contaminated by *E. coli*, and 57.5% of water used by street vendors were contaminated by coliforms (Rane, 2011). Several studies have reported contamination of street-sold food, such as chicken- and pork-based items, sauces, salads, coconut slices, panipuri, fruit juices, chat, and water by different types of bacteria (Kaul and Agarwal, 1988; Mosupye and Holy, 1999; Ghosh *et al*, 2007; Tambekar *et al*, 2009, 2011; Manguiat and Fang, 2013; Nguyen *et al*, 2014).

The findings of this pilot study demonstrate that the food sold by street vendors in Dhaka city were contaminated with pathogenic bacterial organisms, which are likely to pose a potential hazard to consumers, an issue that needs to be addressed. Provision of health education to the street food vendors on personal hygiene, safe food handling practice and proper disposal of waste would improve food quality and thereby reduce the risk of contamination of street-sold food. Infrastructure development for access to potable water, public toilet, washing and waste disposal facilities also would reduce the health hazards to consumers.

One limitation of the present study has been the sampling of food products from only two locations, which may not be fully representative of the situation of street-sold food in Dhaka city. Moreover, this study design does not permit the isolation and confirmation of the presence of other microorganisms present in the food samples. Therefore, future studies will be needed to determine the presence of various microorganisms responsible for food-borne illnesses and their confirmation in the laboratory.

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