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Isolation and Identification of *E Coli* and *V Cholerae* from Street Fruits and Juices from Different Areas in Dhaka City, Bangladesh

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ABSTRACT

Microbial contamination of ready-to-eat foods and beverages sold by street vendors and hawkers has become a global health problem. The study aims to evaluate microbial status in street vended food samples in Bangladesh and identify the presence of common food borne pathogens such as *E coli* and *Vibrio cholerae* by conventional cultural, microscopic and biochemical tests. A total of twenty samples (hog plum, guava; and sugarcane and lemon juices) were collected from mobile vendors from five different locations in Dhaka city. The total viable count (TVC) in all food samples was ranged from 8×10^3 to 2.1×10^8 cfu/ml. The total coliform count (TCC) varied between 1.5×10^4 to 2.2×10^8 cfu/ml. In addition, 8 *E coli* and 8 *V cholerae* strains were identified in 8 (40%) different samples out of 20 samples tested. They were found highest in sugarcane juice (50%) and lowest in lemon juice (12.5%) among different food samples. Our results demonstrated the non hygienic quality of most popular types of street vended foods suggesting the urgent need for government participation in developing suitable intervention measures to improve microbial quality.

KEYWORDS: *Total viable count (TVC), total coliform count (TCC), foodborne pathogens*

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INTRODUCTION

According to the WHO, each year 600 million people around the world, or 1 out of 10, become ill after consuming contaminated food. In Bangladesh, about 30 million people suffer from food borne illnesses each year of which diarrheal diseases kill approximately 2.2 million people including many children yearly [1]. Over the years, food borne diseases have been considered a major public health problem considering the socio-economic status in developing countries [2].

Street food vending has become an important public health issue and a great concern due to widespread food borne diseases. In developing countries food sold by street vendors is the major source of food borne illness [3]. People who patronize street food, have been reported to suffer from food borne diseases like diarrhea, cholera, typhoid fever and food poisoning [4–8]. Studies have been carried out in different countries throughout the world to investigate the microbiological quality of street-sold food. Many previous reports claim that consumption of non-homemade foods, especially street foods which can easily be contaminated by food borne pathogens, is mostly responsible for food borne diseases [9].

Major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors [10]. Also the foods are not effectively protected from flies and dust [11, 12]. Street-food vendors are often poor, uneducated and lack knowledge in safe food handling practices, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials, and use of portable water. Consequently, street foods are perceived to be a major public health risk [13].

E coli and *Vibrio cholerae* are pathogenic bacteria commonly found in various contaminated sources and pose a major health risk, causing a range of human enteric infections and pandemics, especially among infants in Africa [14]. Many previous reports identified high prevalence of *E. coli* into the different street foods in Bangladesh [15]. A report published by Victorian Government Department of Human services, Australia (2005) reported survival of *E.coli* 0157:H7 in apple juice for upto 24 days at 4°C. A cholera epidemic in Pune city, India, was related to street vended sugarcane juice containing ice that was contaminated with *Vibrio cholerae* [16]. Therefore, this study was designed to evaluate the detailed microbial status including enteric pathogens like *E coli* and *V cholerae* from different street foods of Dhaka city.

METHODOLOGY

A. Study area and sample collection

Five different areas of Dhaka city were selected as sampling sites for the investigation such as Uttara, Mohakhali, Mirpur, Airport and Farmgate. A total of twenty street fruit and juice samples (S1-S20) were collected from mobile vendors of these areas, at least 4 samples from each site. Two fruit samples (guava and hog plum) and juice samples (sugarcane and lemon) were collected in pre-sterilized stomacher bags and Duran bottles, kept in ice-boxes. Further, these samples were transported and analyzed in the laboratory, department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh.

B. Enumeration of total viable count (TVC) and total coliform count (TCC)

Serial dilutions of samples were made up to 10^{-7} with sterile normal saline. 1 mL of each homogenate from samples was added in 9 ml NaCl solution. For the enumeration of total viable bacteria and coliforms, 0.1 ml of each dilution was evenly spread on the nutrient agar (NA) medium for total viable count (TVC) and MacConkey agar for total coliform count (TCC). Then, the agar plates were incubated at 37^0C for 24 hours. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml).

C. Isolation of enteric pathogens like *E coli* and *Vibrio cholerae*

10 gm of homogenized fruit or 10ml of juice sample was pre-enriched in 90 ml buffered peptone water (incubated overnight at 37ºC), followed by enrichment in lactose broth for *E. coli* and alkaline peptone water for *Vibrio cholerae*. Then the culture media were incubated at 37^0 c for 24 hours. For presumptive isolation of *E. coli*, the enrichment culture was streaked onto Eosine Methylene Blue (EMB) agar media and incubated for 24 hours at 37ºC. For *Vibrio cholerae* isolation, the enrichment culture was streaked onto Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar media and incubated for 24 hours at 37ºC.

D. Biochemical tests

For the biochemical characterization of the isolates, different tests including Triple Sugar Iron (TSI), Indole, Voges Proskeur (VP), Methyl red (MR), Motility, H_2S production, Oxidase and Citrate tests were done. **D. Biochemical tests**

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RESULTS

A. Enumeration of total viable count (TVC) and total coliform count (TCC)

The total viable and coliform count of different street food samples are given in Table 1. The highest number of bacterial colony was observed in guava sample (2.1×10^8cfu/ml) collected from Mohakhali, the lowest total bacterial count was found in hog plum (8×10^3cfu/ml) from Mirpur. The highest total coliform count was observed in sugarcane juice (2.2×10^8 cfu/ml) collected from Farmgate, the lowest count was 1.5×10^4 cfu/ml in lemon juice which was collected from Mohakhali.

B. Isolation of E coli and V cholerae

All the 20 samples were found to be heavily contaminated with enteric pathogens like *E coli* and *V cholerae*. Based on the rapid biochemical tests (Table 6), the identities of *E coli* and *V cholerae* on different selective media of both fruit and juice samples were confirmed.

Total 8 *E. coli* strains (EC1- EC8) were isolated from 8 (40%) samples out of 20 analyzed in this study (Table 2). *E coli* contamination (40%) was observed among sugarcane juice, lemon juice and guava samples collected from Mohakhali (12.5%), Uttara (25%), Mirpur (12.5%), Airport (25%) and Farmgate (25%). Out of 8 positive samples, *E coli* strains were isolated from 4 (50%) sugarcane samples, 3 (37.5%) guava samples and 1(12.5%) lemon juice sample (Table 3).

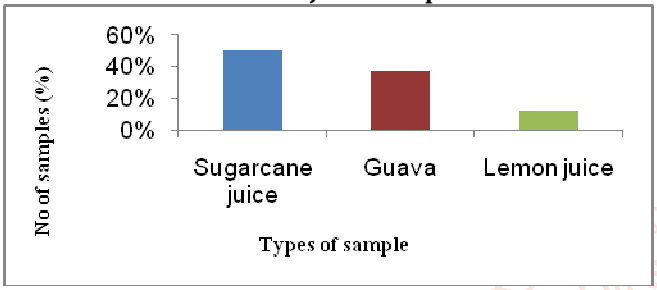
TABLE 1: Total viable and coliform count from street fruit and juice samples (n=20)

Sample ID	Sampling Area	Type of Sample	TVC (cfu/ml)	TCC (cfu/ml)
S1	Mohakhali	Hog plum	2×10^6	8×10^5
S2		Guava	2.1×10^8	1.8×10^8
S3		Sugarcane Juice	2.4×10^6	4×10^7
S4		Lemon Juice	2.5×10^6	1.5×10^4
S5	Uttara	Hog plum	8×10^7	4×10^7
S6		Guava	8×10^5	2.5×10^6
S7		Sugarcane Juice	5.5×10^6	4×10^5
S8		Lemon Juice	1.5×10^8	0
S9	Mirpur	Hog plum	8×10^3	0
S10		Guava	8.5×10^6	0
S11		Sugarcane Juice	1.2×10^8	5×10^7
S12		Lemon Juice	6×10^7	1.2×10^6
S13	Airport	Hog plum	7×10^5	0
S14		Guava	1.8×10^8	1.2×10^8
S15		Sugarcane Juice	4×10^7	1.2×10^6
S16		Lemon Juice	3×10^5	3×10^5
S17	Farmgate	Hog plum	5×10^7	2.1×10^4
S18		Guava	1.2×10^8	5×10^7
S19		Sugarcane Juice	8×10^7	2.2×10^8
S20		Lemon Juice	1.8×10^6	1.8×10^4

TABLE 2: E. coli strains isolated from different areas in Dhaka city

Stain ID	Types of sample	Collection area
EC1	Guava	Mohakhali
EC2	Guava	Uttara
EC3	Sugarcane juice	Uttara
EC4	Sugarcane juice	Mirpur
EC5	Guava	Airport
EC6	Sugarcane Juice	Airport
EC7	Sugarcane juice	Farmgate
EC8	Lemon Juice	Farmgate

TABLE 3: E. coli strains isolated from different street fruit and juice samples



Similarly, *V cholerae* was most frequently detected in different food samples and 8 (40%) samples out of 20 were found to be *V cholerae* contaminated (Table 4). Total 8 *V. cholerae* strains (VC1- VC8) were found among sugarcane juice, lemon juice, guava and hog plum samples which were collected from Mohakhali (25%), Uttara (12.5%), Mirpur (25%), Airport (12.5%) and Farmgate (25%). The frequency of *V cholerae* was found to be highest in sugarcane juice from 4 (50%) samples, followed by guava 2 (25%), lemon juice and hog plum 1 (12.5%) samples respectively (Table 5).

TABLE 4: V. cholerae strains isolated from different areas in Dhaka city

Strain ID	Types of sample	Collection area
VC1	Guava	Mohakhali
VC2	Sugar cane juice	Mohakhali
VC3	Guava	Uttara
VC4	Sugar cane juice	Mirpur
VC5	Lemon juice	Mirpur
VC6	Sugar cane Juice	Airport
VC7	Hog plum	Farmgate
VC8	Sugar cane juice	Farmgate

TABLE 5: V. cholerae strains isolated from different street fruit and juice samples

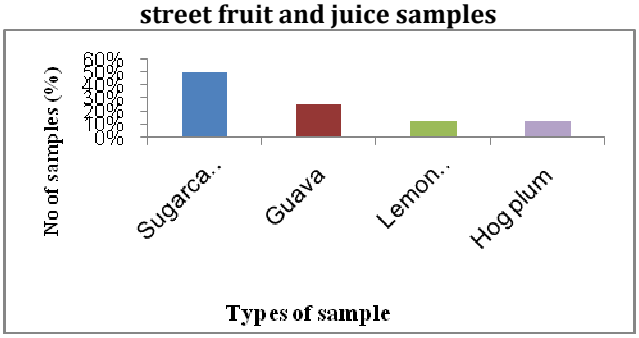


TABLE 6: Biochemical identification of isolated strains of E. coli and V cholerae results

Biochemical Tests		Isolated strains	
		E coli	V cholerae
TSI	Slant	Y	Y
	Butt	Y	Y
	Gas	+	_
H_2S reaction		-	-
Indole test		+	+
Citrate test		-	-
MR test		+	+
VP test		-	-
Motility test		+	+
Oxidase test		-	+

Legend: "+"=positive, "-"=negative, Y=yellow

DISCUSSION

Street foods are prepared, processed and handled in a more or less quick and casual way by non-professional personnel in developing countries, like in Bangladesh; tend to be vulnerable to the possible microbial attacks propagated from the surrounding environment. Therefore, the current study attempted to check the presence of pathogenic microorganisms in terms of total viable and coliform count as well as in the finding of two enteric pathogens such as *Vibrio cholerae* and *E coli* in various street foods which are being sold and prepared by various vendors in Dhaka city, Bangladesh.

This result highlights the fact that the prevalence of bacterial colony was highest in guava sample (2.1×10^8cfu/ml) which is higher than 2.1×10^4 CFU/ml in guava sample observed by Baishakhi *et al.* in Bangladesh [17]. Another major finding of the study was that the total coliform count was highest in sugarcane juice (2.2×10^8 cfu/ml) collected from Farm gate whereas Rashed *et al.* stated that the highest coliform count for vendor fruit juice was 1.58 $x10^6$ cfu/ml collected from Farmgate [18].

There are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe [19-21]. In the present study, 40% (8/20) food samples were found as *E. coli* and *V cholerae* positive. This finding *is consistent* with a previous *study conducted by Singh et al. in 2016 in India where* eight food samples out of 26 were found positive for *E. coli* showing 30.7% overall incidence [22]. Another study was conducted in Korea where *E. coli* was most frequently detected in convenient foods and 50% samples were found to be *E. coli* contaminated [23]. As the presence of coliforms and *E. coli* in food may indicate fecal contamination, presence of *E. coli* in 40% street food samples might be representing fecal contamination in the present study.

Vibrio cholerae has long been known to be responsible for the life threatening secretory diarrhoea termed as Asiatic cholera or epidemic cholera [24, 25]. Although cholera is primarily known as a waterborne disease in the endemic regions including Bangladesh, contamination of foods can also be an imperative mode for cholera transmission [26]. Upon analysis, the prevalence of *V. cholerae* in food samples was 40% in our study which is in agreement with another study demonstrated by Ubong *et al.* where the prevalence of *V cholerae* in fruit juices and flavored drinks for hawker stalls was 36.7% [27].

The frequency of *E. coli* and V *cholerae* was found to be highest in sugarcane juice (50%) in our study. This may result from the presence of microorganisms on the sugarcane that are introduced into the juices during processing [28, 29]. The most frequently isolated enteric bacteria such as *E. coli* (n=9; 36%) from sugarcane juices was also found by MWAMBETE in Tanzania [30]. In this study, the distribution percentages of *E. coli* and *Vibrio cholerae* in guava were 37.5% and 25%, which are higher than the findings of Sarker *et al.* [31], i.e. the frequency distribution of *E. coli* was 22.64% and *Vibrio spp*. was 16.03%.

CONCLUSION

Current study exhibited the microbiological status of available local fruits and juices to ensure food safety for a precise control over public health risk. We observed high prevalence of enteric pathogens among the street foods in different areas of Dhaka city. Lack of awareness and lack of adherence to the food laws and regulation together within the frequent implementation of existing regulations are contributing significantly to dissatisfactory food safety circumstances of Bangladesh. We call upon the regulatory authorities to re-enforce measures on microbiological food safety in processing of ready-to-eat foodstuffs.

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