

Assessment of Bacterial (*E.coli* & *Salmonella*) Contamination in Street Food in Barishal City Corporation Area

FARJANA SIMI

Dr. Md. JAMAL HOSSAIN¹

*Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN)
Regional Station, Barishal*

Abstract

Street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. The present study was conducted to determine the total bacterial load with special emphasis on Escherichia coli and Salmonella spp in street fast foods sold in Barishal City Corporation area, Bangladesh. A total of 20 samples were collected randomly from street vendors included Chotpoti, Fucka, Biriani and Chanachur and tested for the presence of bacteria following standard microbiological method used for isolation, enumeration and identification of bacteria. All the examined samples were contaminated by various types of bacteria. The total viable count (TVC) in different street food samples was ranged from 9.8×10^5 CFU/g to 6.0×10^7 CFU/g. Among samples Escherichia coli 12 (60%) and Salmonella spp. 8 (40%) were isolated. Escherichia coli were found highest 3(60%) Chotpoti and Chanachur samples, then another organisms Salmonella spp. was found highest 3 (43%) in Biriani one in each sample. Therefore, application of sound risk analysis policies is being advocated to provide a scientific base to the host of risk management option which Bangladesh may need to explore to ensure public health and safety.

Keywords: Street food, *E. coli*; *Salmonella* spp.; Bacterial.

INTRODUCTION

Street fast food refers to the state of food and beverages that are prepared or sold by vendors in streets and other public places for direct human consumption immediately or later, with or without need for further processing and preparation (Tsang, 2002). Street vended foods are appreciated for their unique flavors as well as their convenience, they are also important in contributing to the nutritional status of the population especially low income level community (Habib, 2016). This industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries. It feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible (Rane, 2011). In developing country like Bangladesh, it is getting popular day by day due to cheap price and easily available and convenient in urban habitant. For this reason, without following the rule of food safety and hygiene, an increasing number of shops are getting established throughout the

¹ Corresponding author email: ssobarisal@gmail.com

Bangladesh (Ahmed *et al.*, 2008). In contrast to these potential benefits, it is also recognized that street food vendors are often poor, uneducated, and lack of knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials, and use of potable water. Consequently, street foods are perceived to be a major public health risk (Bhowmik, 2010 and Habib, 2016). On the other hand, street fast foods contribute a significant role in foodborne illness to consumers. However, food borne illnesses of microbial origin are a major health problem associated with street foods (WHO, 2002 and Isabella *et al.*, 2016). In addition, resistant to multi-drug against these organisms is a great concern of public health (Tabashsum *et al.*, 2013). It is stated that approximately 30 million people in Bangladesh getting infected from foodborne illness each year (FAO, 2012). The incidence of foodborne illness is on the rise worldwide and becomes an alarming issue involving broad range of disease caused by the pathogenic organisms (De Vogli *et al.*, 2014). Numerous reports have revealed the risks associated with consuming contaminated street-vended foods that have high levels of coliform bacteria and pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Vibrio cholera* (Cho *et al.*, 2011; Hanashiro *et al.*, 2005 and Mankee *et al.*, 2005). Food contamination with antibiotic resistant bacteria can also be a major threat to public health, since the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially comprising the treatment of severe bacterial infections (Hasan *et al.*, 2018; Sharma *et al.*, 2015). Due to bacterial contamination causes like- Diarrhoea, Nausea, Abdominal pain, Constipation, Fever, Vomiting, Central Nervous System.

Consumers who depend on such foods are more interested in its convenience and usually pay little attention to its safety, quality and hygiene (Muleta and Ashenafi, 2001). Ready-to-eat street foods are also subjected to cross-contamination from various sources such as utensils, knives, raw foodstuffs, flies that sporadically landing on the foods, vendors bare hand serving and occasional food handling by consumers (Muzaffar *et al.*, 2009; Karmaker *et al.*, 2018). In most cases, tap water is not available for washing hands and utensils at vending sites; hand and utensil washing are usually done in one or more buckets-sometimes without soap. Toilets, waste disposal and refrigeration facilities are rarely available (Hassan *et al.*, 2018). Waste water and garbage are therefore discarded nearby, providing nutrients for insects and other household rodents, which may carry food borne pathogens (Barro *et al.*, 2006).

Most of the food handlers and worker are uneducated and lack of the knowledge about handling practice, sanitation and hygiene so that food can easily be contaminated (Tabashsum *et al.*, 2013). There are several reason for contamination of street fast foods includes the utensils and equipment that enhance cross contamination, vending sites that are filthy in nature, tap water used in preparation of food, waste and garbage produce in restaurants discard nearby that attract the rodents and insect which may carry foodborne pathogens, flies that land on food sporadically and finally handling of food by vendors with bare hand (Nicolas *et al.*, 2007; Tambekar *et al.*, 2009). The present work was designed to isolate and characterize the microbes in the street foods and find out the association of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* with the status of vendors, vending site and their food handling practices. This research was assessment the bacterial contamination from street food in Barishal city corporation area.

MATERIALS AND METHODS

Sample Collection

The study was carried out on street drinking water, street foods sold in Barishal city corporation area during the period of January 2022 to September 2022. For this purpose, a total of 20 food samples comprising four items (Chotpoti, fukka, biriani and chanachur) and street drinking water, sugarcane juice were randomly collected from different street vended including launch ghat area, 30 godawon, sadar road, chawkbazar, Bangabandhu Uddan in Barishal city corporation area. These items and places were selected as per as consumer preference and their availability. Approximately 20g of each food sample and 10 ml street drinking water, sugarcane juices was collected using the vendors serving utensils, take parcel and placed into sterile plastic bags. After collection, samples were kept in labeled sterile polyethylene containers, were kept on an icebox during transportation to the laboratory and stored at 4°C until testing. They were analyzed within 24 hours of sampling.

Sample Processing

For analysis, adequate amount of different street food samples were uniformly homogenized in mortar and pastel using a sterile diluent as per recommendation of (Balamurugan et al., 2013). A homogenized suspension was made with the help of mortar and pastel. After that samples were homogenized properly with 90ml of phosphate buffered saline (PBS) solution. Serial dilutions of 10^{-1} to 10^{-6} were made depending upon contamination level using 10ml of homogenized sample with 90 ml of PBS.

Enumeration of Total Variable Count (TVC)

50µl of each fivefold dilution was transferred and spread onto Plate Count Agar using a micropipette for each dilution for the determination of total bacterial count. The diluted samples were spread as quickly as possible on the surface of the plate. The plates were kept in an incubator at 37°C for 24 hrs. After incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of colony forming units (CFU) per ml of food samples.

Isolation of Bacterial Species

After collection, the samples were grown in the freshly prepared Buffered Peptone Water. Subsequently then the samples were streaked into MacConkey and Eosin Methylene Blue (EMB) agar for isolation of *Escherichia coli* and also MacConkey agar and XLD agar media for *Salmonella* spp. Each group of these media were incubated at 37°C for overnight. The colonies on primary cultures were repeatedly subcultured by streak plate method (Cheesbrough, 1985) until the pure culture with homogenous colonies were obtained. Media such as Nutrient agar, MacConkey agar, Eosin Methylene Blue agar, *Salmonella Shigella* (SS) agar, and Manitol Salt Agar (MSA) were used for sub-cultures and incubated at 37°C for 24 hours for growth.

Identification of Bacterial Species

The cultural examination of street food samples for bacteriological study was done according to the standard method International Commission on Microbiological

Specifications for Foods (ICMSF, 1985). Identification of bacteria was performed on the basis of colony morphology Gram's staining reaction and biochemical test. Biochemical tests, such as sugar fermentation, coagulase, catalase, MR, VP, and indole tests, were performed as per the standard methods (Cheesbrough, 1985).

RESULTS AND DISCUSSION

During the investigation, a total of 20 street fast food samples were investigated to determine the microbial load. From this study, it was revealed that food samples examined from the various street vended restaurants were contaminated with indicator organisms including *E. coli*. and *Salmonella* spp.

Results of microbial load by total viable count (TVC)

The (TVC) of different street food (Chotpoti, fucka, biriani and chanachur) samples collected from different vendors are shown in Table 1. The highest numbers of bacterial colonies were observed in Chotpoti sample (6.5×10^7 CFU/g) followed by Fucka sample (6.1×10^7 CFU/g), Chanachur sample (6.0×10^7 CFU/g) and Biriani sample (5.8×10^7 CFU/g).

Table 1. Microbial load by total viable count (TVC).

Type of food	Dilution	Number of colony	Total viable count (TVC)
	10 ⁻¹	Over 300	TNTC
	10 ⁻²	Over 300	TNTC
Chotpoti	10 ⁻³	94	9.4x10 ⁵ CFU/g
	10 ⁻⁴	70	7.0 x10 ⁶ CFU/g
	10 ⁻⁵	65	6.5x10 ⁷ CFU/g
	10 ⁻¹	Over 300	TNTC
	10 ⁻²	Over 300	TNTC
Fucka	10 ⁻³	98	9.8 x10 ⁵ CFU/g
	10 ⁻⁴	70	7.0 x10 ⁶ CFU/g
	10 ⁻⁵	61	6.1 x10 ⁷ CFU/g
	10 ⁻¹	Over 300	TNTC
	10 ⁻²	Over 300	TNTC
Biriani	10 ⁻³	96	9.6x10 ⁵ CFU/g
	10 ⁻⁴	79	7.9 x10 ⁶ CFU/g
	10 ⁻⁵	58	5.8 x10 ⁷ CFU/g
	10 ⁻¹	Over 300	TNTC
	10 ⁻²	Over 300	TNTC
Chanachur	10 ⁻³	83	8.3 x10 ⁵ CFU/g
	10 ⁻⁴	74	7.4 x10 ⁶ CFU/g
	10 ⁻⁵	60	6.0 x10 ⁷ CFU/g

RESULTS OF BACTERIOLOGICAL INVESTIGATION

A total of 20 different street food (Chotpoti, fucka, biriani and chanachur) samples were collected from different places in Barishal city corporation area for this study. Among 20 different street food (Chotpoti, fucka, biriani and chanachur) samples, *Salmonella* spp. and *Escherichia coli* were found.

Total *E. Coli* Count

From results, *E. coli* count was estimated 2.1×10^3 to 3.8×10^3 which crossed the acceptable range where the range is 2 log CFU/g (ICMSF, 2002). This indicates the high amount of *E. coli* were present on investigated fast food samples which can induce

infection. Similar results were also observed by other researchers include Nguendo (2018) who reported 4.10×10^3 cfu/g of bacteria in fast samples in three location of Yaounde, Cameroon and Tabashsum et al., (2013) who seen 10^2 to 10^7 cfu/g of *E. coli* in different street foods of Dhaka city. Similarly, Amissah and Owusu (2012) carried out a study in Koforidua Polytechnic campus, Ghana and the results showed 1.0×10^3 cfu/g of *E. coli* in fast foods which was comparable to our study. Such variation in study could be linked with the use of unhygienic water for cleaning utensils and cooking of foods, secondary contamination via contact with contaminated equipment's.

Total salmoella spp. Count

In this study, 50% (10/20) samples were found to be contaminated with *Salmonella spp.* and all of them crossed the acceptable limit as according to ICMSF (2002) no Salmonella will be detected in 25g of samples. Total Salmonella count ranged from 2.5×10^3 to 3.1×10^3 cfu/g. The count of Salmonella was the highest in biryani and lowest in chanachur samples whereas no Salmonella was detected in chotpoti and fucka samples. The present finding agrees with several previous reports such as Akusu et al., (2016) in Rivers State, Nigeria who obtained 10^3 cfu/g of Salmonella in egg burger, fried rice, meat pie and vegetable salad samples and Adimasu et al., (2016) was also found 1.6×10^3 cfu/g in street vended fast food samples in Gonder, Ethiopia. However, No Salmonella was detected in street food samples in different locations of Chennai city reported by Arunaand Rajan (2017). The presence of Salmonella spp. in this study may due to in clothing, handling and serving practices of vendors.

Table 2 Mean total bacteria, Staphylococcus spp., Salmonella spp. and E. coli counts in street vended fast foods.

Food Items	Number of Samples	Mean Bacterial colony count (cfu/g)		
		Total Bacterial Count (cfu/g)	<i>E. coli</i> count (cfu/g)	<i>Salmonella spp.</i> count (cfu/g)
Chotpoti	5	1.2×10^7	2.2×10^4	-
Fucka	5	1.1×10^7	2.1×10^4	-
Biriani	5	2.0×10^7	3.8×10^3	3.1×10^3
Chanachur	5	1.7×10^7	3.5×10^3	2.5×10^3

Note: According to the International Commission for Microbiological Specification for Foods (ICMSF, 2002), Acceptable range of TBC, Staphylococcus and *E. coli* are 10^6 cfu/g (6 log cfu/g); 10^4 cfu/g (4 log cfu/g); and 10^2 cfu/g (2 log cfu/g) respectively and detection of *Salmonella spp.* in 25g sample is unacceptable.

Results of isolation of bacteria from street food

Two genera of bacteria such as *Escherichia coli* and *Salmonella spp.* were isolated from different street food (Chotpoti, fucka, biriani and chanachur) samples. During the study period a total 20 samples were collected from different street food. In case of Chotpoti 3(60%) positive for *Escherichia coli*, 2(40%) positive for *Salmonella spp.* respectively. In 20 street food samples *Escherichia coli* were found highest 4(57%) Biriani samples and 3(60%) Chotpoti and Chanachur samples, then another organisms *Salmonella spp.* was found highest 3(43%) Biriani and 2(40%) Chotpoti and Chanachur samples which were shown in Table 2. In case of Chotpoti 3(60%) positive for *E. coli* and 2 (40%) were positive for *Salmonella spp.*. In case of Fucka 2(66.67%) positive for *Escherichia coli*, 1(33.33%) positive for *Salmonella spp.* respectively. In 20 street food samples *Escherichia coli* were found highest 4(57%) Biriani samples and 3(60%) Chotpoti and

Chanachur samples, then another organisms *Salmonella spp.* was found highest 3(43%) Biriani and 2(40%) Chotpoti and Chanachur samples which were shown in Table 3.

Table 3. Results of isolation of bacteria from street vended food.

Bacterial isolate	Types of sample					Percentage(%)
	Chotpoti	Fucka	Biriani	Chanachur	Total	
<i>E. coli</i>	3 (60%)	2 (66.67%)	4 (57%)	3 (60%)	12	60 %
<i>Salmonella spp.</i>	2 (40%)	1(33.33%)	3 (43%)	2 (40%)	8	40 %
Total bacteria identified	5	3	7	5	20	100

Result of isolation bacteria

Cultural characteristics of each type of bacteria isolated from different street food were studied for the determination of size, shape and colony characteristics in various bacteriological media. The staining property of primary culture of each of the different street food samples indicated the presence of more than one type of bacteria in the same smear. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media for study. The cultural characteristics of *Salmonella spp.* and *E. coli* exhibited on the media are presented in Table 4.

Table 4: Cultural characteristics of the bacterial isolates of different street food.

Suspected case of bacteria	Name of media	Cultural characteristics
<i>Escherichia coli</i>	EMB agar	Metallic sheen (greenish black) - colony
<i>Salmonella spp.</i>	SS agar	Small non-lactose fermented with black center colony

Notes: EMB = Eosin methylene blue, SS=Salmonella Shigella

Results of staining characteristics of the isolated bacteria

The staining characteristics of the isolated organisms were determined according to Gram's staining technique. Morphological and staining characteristics of bacteria recorded from Chotpoti, Fucka, Biriani and Chanachur samples by Gram's staining are presented in Table 5.

Table 5. Morphological and staining properties of the bacterial isolates by Gram's staining

Staining characteristics			Remarks
Shape	Arrangement	Gram's staining characters	
Short plump rods	Single, paired or in short chain	(-) ve	<i>E. coli</i>
Rod in shape	Single or pair	(-) ve	<i>Salmonella spp.</i>

Notes: (+)Ve = Positive; (-) Ve= Negative

CONCLUSION

This study results showed that, the four types of assessed street food items in Barishal City Corporation area were contaminated with at least two pathogenic bacteria. The foodborne bacteria detected in this study are also evident that street foods might pose a major problem for public health. Lack of training (orientation) on the proper handling and processing of food, poor personal hygiene of vendors and unhygienic surroundings could be possible factors for observed problems in that locality. Therefore, education for vendors on food safety and hygienic practices is essential to reduce contamination rate. In addition, regular inspection on food vending practices and safety of street foods is required to improve the health standards of consumers.

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