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Article

Isolation and identification of bacteria in different street vended foods collected from selected areas of Bangladesh

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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. 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. The objective of the study was to explore the microbiological quality of different street food; Chotpoti, Chanachur, Amra (*Spondias mombin*) and Jolpai (*Elaeocarpus serratus* (Veralu / Ceylon Olive) sold by various street vendor at Khulna city, Bangladesh. A total of 20 samples were collected randomly from street vendors and tested for the presence of bacteria following standard microbiological method used for isolation, enumaration and identification of bacreria. All the examined samples were contaminated by various types of bacteria. The total viable count (TVC) in different street food samples was ranged from 9.6 x 10 CFU/g to 5.9x10 CFU/g. Among samples *Escherichia coli* 8(40%), *Staphylococcus* spp. 5(25%), *Klebsiella* spp. 4(20%), *Salmonella* spp. 1(5%) and *Shigella* spp. 2 (10%) were isolated. *Escherichia coli* were found highest 3(50%) Jolpai samples, then another organisms Staphylococcus spp. was found highest 2(50%) in Chotpoti and then *Klebsiella* spp. was found 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; contamination; Khulna city; *E. coli; Staphylococcus* spp.*; Klebsiella* spp.*; Shigella* spp.*; Salmonella* spp.

1. Introduction

Street foods are defined as ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, especially in streets and other similar public places (Bereda *et al.,* 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). However, food borne illnesses of microbial origin are a major health problem associated with street foods (Islam *et al.,* 2015). 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).

The traditional processing methods that are used in preparation, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended foods.

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). Wastewater and garbage are therefore discarded nearby, providing nutrients for insects and other household rodents, which may carry food borne pathogens (Barro *et al.*, 2006).

Thus, potential health risks are associated with contamination of food by *Escherichia coli*, *Salmonella typhi*, *Pseudomonas* species, *Staphylococcus aureus, Proteus* species and other species during preparation, post-cooking and various handling stages (Tambekar *et al.*, 2008; Schmidt *et al.*, 2003). As a result, food borne illness associated with the consumption of street foods has been reported in several places such as Bangladesh, India (Khairuzzaman *et al.*, 2014), Mexico and Ethiopia. In Bangladesh, health risks associated with street foods are common. Salmonella, Shigella and other food-borne pathogens were identified in similar studies on street-vended foods in this country (Garode and Waghode, 2012).

On the other hand, the prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades (FAO and WHO, 2005; Sarker *et al.*, 2018). A study revealed that 15.18% of *S. aureus* stains isolated from street foods were resistant to methicillin. All isolated bacterial colonies were resistant to Penicillin G. The bacterial colonies resistant to methicillin were also resistant to kanamycin, gentamycin, tobramycin and erythromycin (Mamun *et al.*, 2013; Islam *et al.*, 2018). Moreover, the Salmonella species isolated from street foods vended in Khulna were sensitive to most of the drug tested. In a similar study, the isolated Shigella species showed multiple drug resistance patterns against ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, streptomycin and tetracycline. Since the popularity of street foods is increasing in the towns/cities in particular and in the country in general, this study was intended to assess the microbiological quality and antibiogram status of bacterial isolates from ready- to- eat foods sold by street venders to ensure health status of the consumers.

2. Materials and Methods

2.1. Sample collection

A total of 20 food samples were randomly chosen and collected from street vendors in the area around Khulna city. These samples were collected in different sealed poly bags to prevent their contact with any other source that can contaminate the samples. Food samples included Chotpoti, Chanachur, Amra and Jolpai. Approximately 200g of each food sample was collected using the vendors serving utensils, take parcel and placed into sterile plastic bags. All the collected samples 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.

2.2. Preparation of sample

Adequate amount of different street food (Chotpoti, Chanachur, Amra and Jolpai) 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. A quantity of 10 gm homogenate sample of each different street was taken aseptically with a sterile spoon and transferred carefully into a sterile pastle containing 90 ml of PBS. Thus 1:10 dilution of the samples was obtained.

2.3. Enumeration of total viable 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.

2.4. Isolation of associated bacteria

Bacteriological examination was carried out using standard method for aerobic bacteria (Brown, 2005). Each sample of Chotpoti, Chanachur, Amra and Jolpai samples were inoculated separately in nutrient broth (NB) to

promote growth of bacteria. 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 Nutient 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.

2.5. Identification of associated bacteria

The cultural examination of street food (Chotpoti, Chanachur, Amra and Jolpai) 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).

3. Results and Discussion

3.1. Results of microbial load by total viable count (TVC)

The (TVC) of different street food (Jolpai, Chotpoti, Chanachur and Amra) samples collected from different vendors are shown in Table 1. The highest numbers of bacterial colonies were observed in Jolpai sample ($6.7*10^7$ CFU/g) followed by Chotpoti sample ($6.3x10^7$ CFU/g), Chanachur sample ($6.1x10^7$ CFU/g) and Amra sample ($5.9x10^7$ CFU/g).

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

Type of food	Dilution	Number of colony	Total viable count (TVC)
Chotpoti	10^{-3}	96	$9.6x10^5$ CFU/g
	10^{-4}	71	$7.1x10^6$ CFU/g
	10^{-5}	64	$6.4x10^7$ CFU/g
	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
Chanachur	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	84	$8.4x10^5$ CFU/g
	10^{-4}	74	$7.4x10^6$ CFU/g
	10^{-5}	60	$6.0x10^7$ CFU/g
Amra (*Spondias mombin*)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	80	$8.0x10^5$ CFU/g
	10^{-4}	67	$6.7x10^6$ CFU/g
	10^{-5}	59	$5.9x10^7$ CFU/g
Jolpai (Ceylon Olive)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	91	$9.1x10^5$ CFU/g
	10^{-4}	76	$7.6x10^6$ CFU/g
	10^{-5}	68	$6.8x10^7$ CFU/g

3.2. Results of bacteriological investigation

A total of 20 different street food (Jolpai, Chotpoti, Chanachur and Amra) samples were collected from different places in Khulna for this study. Among 20 different street food (Jolpai, Chotpoti, Chanachur and Amra) samples, *Shigella* spp., *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp. and *Escherichia coli* were found.

3.3. Results of isolation of bacteria from street vended food

Five genera of bacteria such as *Shigella* spp., *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* and *Escherichia coli* were isolated from different street food (Jolpai, Chotpoti, Chanachur and Amra) samples. During the study period a total 20 samples were collected from different street food. In case of Jolpai 3(50%) positive for *Escherichia coli*, 1(16.66%) positive for *Staphylococcus* spp., 1(16.66%) positive for *Klebsiella* spp. and 1(16.66%) were positive for *Shigella* spp. In case of Chanachur 2(50%) positive for *Staphylococcus* spp., 1(25%) positive for *Escherichia coli* and 1(25%) were positive for *Klebsiella* spp. In case of Chotpoti 2(40%) positive for *Staphylococcus* spp. and *Klebsiella* spp. and 1(20%) were positive for *Escherichia coli*. In case of Amra 2(40%) positive for *Escherichia coli*, 1(20%) positive for *Staphylococcus* spp. and 1(20%) were positive

for *Klebsiella* spp. respectively. In 20 street food samples *Escherichia coli* were found highest 3(50%) Jolpai samples and 2(40%) Amra samples, then another organisms *Staphylococcus* spp. was found highest 2(50%) Chanachur and 2(40%) Chotpoti samples and then *Klebsiella* spp. was found highest 2 (40%) Chotpoti samples which were shown in Table 2.

Table 2. Results of isolation of bacteria from street vended food.

Bacterial isolate	Types of sample					Percentage (%)
	Jolpai	Chanachur	Chotpoti	Amra	Total	
E. coli	3(50%)	2(50%)	1(20%)	2(40%)	8	40%
Staphylococcus spp.	1(16.66%)	1(25%)	2(40%)	1(20%)	5	25%
Klebsiella spp.	1(16.66%)	1(25%)	1(20%)	1(20%)	4	20%
Shigella spp.	1(16.66%)	0 (0%)	1 (20%)	0 (0%)	2	10%
Salmonella spp.	0 (0%)	0 (0%)	0 (0%)	1(20%)	1	5%
Total bacteria identified	**6**	**4**	**5**	**5**	**20**	**100%**

3.4. Results of isolation of 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 *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp. and *E. coli* exhibited on the media are presented in Table 3.

Table 3. Cultural characteristics of the bacterial isolates of different street food.

Suspected case of bacteria	Name of media	Cultural characteristics
Klebsiella spp.	MacConkey agar	Large, mucoid, bright pink lactose fermented colony
Staphylococcus spp.	MS agar	Medium yellowish colony
Shigella spp.	SS Agar	Small non-lactose fermented colony
Salmonella spp.	SS Agar	Small non-lactose fermented with black center colony
Escherichia coli	EMB agar,	Metallic sheen (greenish black) - colony

Notes: MS Mannitol salt, EMB = Eosin methylene blue, SS=*Salmonella Shigella*

3.5. 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 Jolpai, Chotpoti, Chanachur and Amra samples by Gram's staining are presented in Table 4.

Table 4. Morphological and staining properties of the bacterial isolates by Gram's staining.

Staining characteristics			Remarks
Shape	**Arrangement**	**Gram's staining character**	
Rod in shape	Single, pairs or cluster	(-) ve	*Klebsiella* spp.
Cocci in shape	Arranged in grapes like cluster	(+) ve	*Staphylococcus* spp.
Rod in shape	Single or pair	(-) ve	*Shigella* spp.
Rod in shape	Single or pair	(-) ve	*Salmonella* spp.
Short plump rods	Single, paired or in short chain	(-) ve	*E. coli*

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

3.6. Results of biochemical tests of different isolates

Isolated *E. coli, Salmonella* spp., *Staphylococcus* spp. were positive and *Klebsiella* spp. *was negative for* methyl red test. All isolates were positive for catalase test with gas bubble formation. All isolates were negative for oxidase test with no colour change except *Staphylococcus* spp. *E. coli, Staphylococcus* spp. were negative and *Salmonella* spp. *and Klebsiella* spp. were positive for voges-proskauer test.

4. Conclusions

In general, this study demonstrated that street vended foods which are sold on the streets of studied areas were considerably contaminated. The foodborne bacteria and antibiotic resistance isolates 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 venders 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.

Conflict of interest

None to declare.

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