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Article

Identification and antibiogram study of bacteria isolated from different street food

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Abstract: Food borne diseases are an increasingly recognized problem involving a wide spectrum of illnesses caused by bacterial contamination of food.Microorganism poses potential human health problems and is mainly transmitted through consumption of contaminated foods .Bangladesh is one of the densely populated country where majority of the people consume cheap foods prepared in unlicensed food selling points. The objective of the present study was to investigate the microbiological quality of different street food (Amra, Fusca, Chanachur and Guava) sold by various street vendor at Dinajpur, 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. Among the samples all had bacterial contamination. The total viable count (TVC) in diferrent street food samples was ranged from 8.0×10^5 CFU/g to $6.7x10^7$ CFU/g. Among samples *Escherichia coli* 7(35%), *Staphylococcus* spp. 6(30%), *Klebsiella* spp. 5(25%), *Salmonella* spp. 1(5%) and *Shigella* spp. 1 (5%) were isolated. *Escherichia coli* were found highest 3(50%) Amra samples and 2(40%) Guava samples, then another organisms *Staphylococcus* spp. was found highest 2(50%) Chanachur and 2(40%) Fusca samples and then *Klebsiella* spp. was found highest 2(40%) Fusca samples. Antibiotic sensitivity test showed that *Shigella* spp., *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp. and *Escherichia coli* were sensitive to Ciprofloxacin and *Salmonella* spp. were resistance to Gentamycin. All isolates found resistant to Cefixime, Cefalexin, Erythromycin, Fusidic acid, Cefuroxime and Aztreonam. All of the sample harbor multidrug resistant food borne bacteria which might cause public health hazards if these antibiotic resistance transfer to human.

Keywords: street food; antibiotic; resistant; sensitivity; contamination

1. Introduction

Street vended foods mean ready to eat foods and beverages that are prepared and sold especially in streets or similar public places by the street vendors or merchants for consumption at the location or later without any further preparation. The street vended foods are usually under unhygienic conditions and displayed openly to a high degree of contamination. In most cases running water is not available at vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap. Thus, from the health point of view, selling foods in the street is very controversial (Bereda *et al.*, 2016). These street foods could be main vehicles for the transmission of severe food borne infections and fatal disease that could be life-threatening (Rane, 2011). In developing countries food sold by street vendors is the major source of food-borne illness. Although food items from these outlets are appreciated mostly for their unique flavor and for their convenience, their

microbiological safety is not always certain (Islam *et al*., 2015). Food borne bacterial pathogens commonly detected in street vended foods are Bacillus cereus causes vomiting and diarrhea, *Clostridium perfringens* causes abdominal cramps and diarrhea, *Staphylococcus* spp. causes vomiting, diarrhea, loss of appetite, severe abdominal cramps and mild fever and *Salmonella* species causes typhoid, food poisoning and irritation and inflammation in the gastrointestinal tract (Hasan *et al*., 2018; Sharma *et al*., 2015). Knowing the microbiological quality of street vended foods is important factor to appreciate the safety problems related to street foods so that concerned bodies may take appropriate steps to improve safety and sanitation with respect to this economic sector (Muleta and Ashenafi, 2001). Vendors are often poorly educated, unlicensed, untrained in food hygiene, and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease (Barro *et al*., 2007). The street foods provide a source of affordable nutrients to the majority of the people specially the low earning group in the developing countries (Muzaffar *et al*., 2009). Ready-to-Eat (RTE) foods could be raw overcooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002). Increased consumption of RTE foods result in food-borne illness (Sivapalasingam *et al*., 2004). Street foods are frequently associated with diarrhoeal diseases due to their improper handling and serving practices (Barro *et al*., 2006). Microbial contamination of RTE sold by street vendors and hawkers has become a major health problem for the consumers (Tambekar *et al.*, 2008). In developing countries, drinks, meals and snacks sold by street food vendors are widely consumed by millions of people and a considerable percent of consumers have been suffering from disease like dysentery, diarrhea, enteric fever etc. (Ali *et al*., 2011; Das *et al*., 2011; Rath and Patra, 2012). In addition, multi-drug resistance of food borne microorganisms made the food safety situation more vulnerable in public health (Khairuzzaman *et al*., 2014). Approximately, 30 million people in Bangladesh are suffering from food borne illnesses each year (FAO, 2012). Food borne illnesses caused by microorganisms are a major national and international health problem and an important cause of death in developing countries (Garode and Waghode, 2012). Street foods in some African countries have been tested for various microorganisms of public health concern, including faecal coliforms, *Escherichia coli*, *Staphylococcus* spp., *Salmonella* species and *Bacillus cereus*. *Escherichia coli* and *Staphylococcus* spp. were recovered in a significant proportion of the food, water, hands and surface swabs tested in Harare (FAO and WHO, 2005). Foods from street-vendors are usually ready-to-eat (RTE) foods, prepared and sold on streets and other public places (Dawson and Canet, 1991). The types of street-vended food vary significantly on countries and cultures (Moy *et al*., 1997). According to a study from the Food and Agriculture Organization (FAO), 2.5 billion people eat street food every day. It is also recognized that street food vendors are often poor, uneducated, and lack 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 (Hassan *et al.*, 2018). Consequently, street foods are perceived to be a major public health risk (Bhowmik, 2010). As food is biological in nature, it is capable of supporting the growth of microorganisms and food borne diseases result from the ingestion of contaminated foods and food products (Sheth *et al*., 2005). In Bangladesh, street foods are mostly prepared and processed manually and sold to the public at various lot terminals, by the roadside or by itinerant vendors (Mamun *et al*., 2013). The vendors in Bangladesh lack of education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore dish and hand washing is done using the same bucket, sometimes even without soap. Garbage and waste water are typically discarded in the streets nearby and thus attracting and providing food for rodents and insects (Bryan *et al*., 1988). Foodborne bacterial agents are the leading cause of severe and fatal foodborne illnesses. Of the many thousands' different bacterial species more than 90% of food-poisoning illnesses are caused by species of *Staphylococcus*, *Salmonella*, *Clostridium*, *Campylobacter*, *Listeria*, *Vibrio*, *Bacillus*, and entero pathogenic *Escherichia coli* (Schmidt *et al*., 2003). In addition, resistance of foodborne microorganisms in multi-drug made the food safety situation more vulnerable in public health (Ali *et al*., 2011). Street food feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible (Latham, 1997; Tambekar *et al*., 2011). Street food is intimately connected with take-out, junk food, snacks, and fast food (Lues *et al*., 2006). Street food is food obtained from a street side vendor, often from a makeshift or portable stall (FAO, 2007). The objective of this research work was to isolate and identify the bacteria that present in different street vended foods and study of their antimicrobial susceptibility patterns.

2. Materials and Methods

The entire study is divided into three steps. The first step includes the total viable counts of the collected samples. The second steps includes isolation and identification of the bacteria from the sample by cultural, morphological and biochemical test. Third step includes evaluation of antibiotics sensitivity against the isolated bacteria.

2.1. Collection of samples

The present research work was conducted during the period from July to December 2017, in the Bacteriology Laboratory of the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh science & Technology University (HSTU), Dinajpur. A total number of twenty Food samples samples were included in this study. Sample was collected from different street vended foods in Lilly More, Basharhat, Doshmile and College More of sadarupazilla at Dinajpur district. Food samples included Amra, Fusca, Chanachur, and Guava (pearah). Approximately 300g 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 ice-box 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 (Amra, Fusca, chanachur, and Guava) 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 37^0C 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 Amra, Fusca, chanachur, and Guava 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 (Amra, Fusca, Chanachur, and Guava) 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).

2.6. Antibiogram study

To determine the drug sensitivity and resistance patterns of isolated organisms used different types of commercially available antibiotic discs, (Mast diagnostics Mersey side, UK.). The antibiotic resistance was determined by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (Difco), according to the guidelines of clinical and Laboratory Standards Institute (CLSI, 2007). After overnight incubation at 37 °C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant, intermediate and sensitive in accordance with company recommendations (Cappuccino and Carpenter, 2005).

3. Results and Discussion

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

The (TVC) of different street food (Amra, Fusca, Chanachur and Guava) samples collected from different vendors are shown in Table 1. The highest numbers of bacterial colonies were observed inAmra sample

(6.7×10^7 CFU/g) followed by Fusca sample ($6.3x10^7$ CFU/g), Chanachur sample ($6.0x10^7$ CFU/g) and Guava sample ($5.8x10^7$ CFU/g).

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

Place of vendor (Type of food)	Dilution	Number of colony	Total viable count (TVC)
Basherhat (Chanachur)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	83	$8.3x10^5$ CFU/g
	10^{-4}	75	$7.5x10^6$ CFU/g
	10^{-5}	60	$6.0x10^7$ CFU/g
Doshmile (Guava)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	80	$8.0x10^5$ CFU/g
	10^{-4}	66	$6.6x10^6$ CFU/g
	10^{-5}	58	$5.8x10^7$ CFU/g
College more (Fusca)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	97	$9.7x10^5$ CFU/g
	10^{-4}	70	$7.0x10^6$ CFU/g
	10^{-5}	63	$6.3x10^7$ CFU/g
Lilymore (Amra)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	92	$9.2x10^5$ CFU/g
	10^{-4}	76	$7.6x10^6$ CFU/g
	10^{-5}	67	$6.7x10^7$ CFU/g

3.2. Results of bacteriological investigation

A total of 20 different street food (Amra, Fusca, Chanachur and Guava) samples were collected from different places in Dinajpur for this study. Among 20 different street food (Amra, Fusca, Chanachur, and Guava) 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 (Amra, Fusca, Chanachur and Guava) samples. During the study period a total 20 samples were collected from different street food. In case of Amra 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 Fusca 2(40%) positive for *Staphylococcus* spp. and *Klebsiella* spp. and 1(20%) were positive for *Escherichia coli*. In case of Guava 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%) Amra samples and 2(40%) Guava samples, then another organisms *Staphylococcus* spp. was found highest 2(50%) Chanachur and 2(40%) Fusca samples and then *Klebsiella* spp. was found highest 2 (40%) Fusca samples which were shown in Table 2.

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

Bacterial isolate	Types of sample					Percentage (%)
	Amra	Chanachur	Fusca	Guava	Total	
E. coli	3(50%)	1(25%)	1(20%)	2(40%)	7	35%
Staphylococcus spp.	1(16.66%)	2(50%)	2(40%)	1(20%)	6	30%
Klebsiella spp.	1(16.66%)	1(25%)	2(40%)	1(20%)	5	25%
Shigella spp.	1(16.66%)	0 (0%)	0 (0%)	0 (0%)	1	5%
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 by cultural test

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 individual cultural characteristics of bacterial isolates are presented in Table 5. 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.

Sl. No	Suspected case of bacteria	Name of media	Cultural characteristics
01	*Klebsiella* spp.	MacConkey agar	Large, mucoid, bright pink lactose fermented colony
02	*Staphylococcus* spp.	MS agar	Medium yellowish colony
03	*Shigella* spp.	SS Agar	Small non-lactose fermented colony
04	*Salmonella* spp.	SS Agar	Small non-lactose fermented with black center colony
05	*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 Amra, Fusca, Chanachur and Guava samples by Gram's staining are presented in Table 4.

Table 4. Morphological and staining properties of the bacterial isolates from Amra, Fusca, Chanachur and Guava 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.

3.7. Results of antibiotic sensitivity tests

A total of five isolates such as *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp. and *E. coli* were subjected to antibiotic sensitivity assay. The results of antibiotic sensitivity assay are presented in Tables 6, 7, 8, 9 and 10. Antibiotic sensitivity test showed that *Shigella* spp., *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp. and *Escherichia coli* were sensitive to Ciprofloxacin. *Staphylococcus* spp., *Klebsiella* spp., *Escherichia coli* were sensitive to Gentamycin and *Salmonella* spp. *were* resistance to Gentamycin. *Klebsiella* spp. and *Staphylococcus* spp. were sensitive to Neomycin and *Shigella* spp. were resistance to Neomycin. *Klebsiella* spp. and *Shigella* spp. were intermediate sensitive to Kenamycin. All isolates found resistant to Cefixime, Cefalexin, Erythromycin, Fusidic acid, Cefuroxime and Aztreonam.

Table 5. A total of 5 bacterial species were identified from 20 isolates.

SL No.	Ca	Ox	Ind	Cit	MR	VP	MIU	Spore	TSI	Identification
1	+	-	-	+	-	+	-	-	Yellow butt, Yellow slant, Gas= (+ve) H_2S=(-ve)	*Klebsiella* spp.
2	+	+	-	-	+	+	-	-	Slant and Butt both acidicH_2S=(-ve) Gas=(-ve)	*Staphylococcus* spp.
3	+	-	-	+	+	-	+	-	Slant alkaline Butt acidic H_2S=(+v) Gas=(-ve)	*Salmonella* spp.
4	+	-	-	+	+	-	-	-	Slant alkaline Butt acidic H_2S=(-v) Gas=(-ve)	*Shigella* spp.
5	+	-	+	-	+	-	+	-	Yellow butt, Yello slant, Gas= (+ve) H_2S=(-ve)	*Escherichia coli*

Legends: SL No. = Serial Number, Cat: Catalase test, Ox: Oxidase test, md: Indole test, Cit: Citrate Utilization test, MR: Methyl red; VP: Voges-Proskauer, TSI: Triple Sugar Iron, MIU: Motility, Indole and Urease test, (+) = Positive; (-) = Negative, H2S= hydrogen sulphide.

Table 6. Antimicrobial profile of *Klebsiells* spp.

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Klebsiells spp.	Ciprofloxacin (CIP)	30	S
	Gentamycin (GEN)	23	S
	Erytromycin (E)	0	R
	Kanamycin (K)	25	S
	Neomycin (N)	22	S

Note: R=Resistant, S=Sensitive, I=Intermediate

Table 7. Antimicrobial profile of *Staphylococcus* spp.

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Staphylococcus spp.	Ciprofloxacin (CIP)	30	S
	Gentamycin (GEN)	25	S
	Erytromycin (E)	10	R
	Fusidic Acid (FD)	0	R
	Neomycin (N)	20	S

Note: R=Resistant, S=Sensitive, I=Intermediate

Table 8. Antimicrobial profile of *Shigella* spp.

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Shigella spp.	Ciprofloxacin (CIP)	35	S
	Gentamycin (GEN)	20	S
	Erytromycin (E)	09	R
	Kanamycin (K)	17	I
	Neomycin (N)	20	R

Note: R=Resistant, S=Sensitive, I=Intermediate

Table 9. Antimicrobial profile of *Salmonella* spp.

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
	Gentamycin (GEN)	5	R
	Ciprofloxacin (CIP)	22	S
	Cefixirne (CfM)	16	I
Salmonella spp.	Cefalexin (CN)	0	R
	Penicillin-g (P)	0	R
	Aztreonam (ATM)	0	R
	Cefuroxime (CXM)	0	R

Note: R=Resistant, S=Sensitive, I=Intermediate

Table 10. Antimicrobial profile of against *E. coli.*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
	Gentamycin (GEN)	25	S
	Ciprofloxacin (CIP)	21	S
	Cefixirne (CfM)	0	R
E. coli	Cefalexin (CN)	0	R
	Penicillin-G (P)	0	R
	Aztreonam (ATM)	0	R
	Cefuroxime (CXM)	0	R

Note: R=Resistant, S=Sensitive, I=intermediate

4. Conclusions

The present study was conducted for the isolation, identification and antibiotic sensitivity of the bacteria isolated from different street food. Presence of coliforms in the sample might be due to poor quality of water, unhygienic vendor places and poor personal hygiene of vendors. Most Street vendors were illiterate and they did not have a clear hygienic knowledge about the preparation, storage and serving of the food. All isolates found resistant to Cefixime, Cefalexin, Erythromycin, Fusaric acid, Cefuroxime and Aztreonam. The results of this study suggested that although RTE foods are cheap and economical but they are not healthy due to lack of hygienic measures, dirty utensils, and vendor‘s hygiene. These factors contributing many species of bacteria but major pathogen is *E. coli*, *Salmonella* spp. *Shigella* spp., *Klebsiella* spp. and *Staphylococcus* spp. Basic and main source of bacterial infection is poor hygienic measures and this problem may be solved by improving supervision in food handling procedure, extended consumer education on transmission of enteric food borne diseases and food safety risks. So that street vended RTE foods should be manufactured under Good Hygienic Practices and conservation practices should be developed in order to minimize the microbial contamination of food.

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Conflict of interest

None to declare.

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