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## **Original Research Article**

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# Assessment of Bacteriological Quality of Street Vended Fast Foods and their Antimicrobial Resistance

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# ABSTRACT

#### Keywords

Street vended fast food, Total bacterial load, Antimicrobial susceptibility test

**Article Info** 

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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). In developing country like Bangladesh, it is getting popular day by day due to cheap price and easily available and convenient in urban habitant.

The present study was conducted to determine the total bacterial load with special emphasis on Staphylococcus spp., Salmonella spp. and Escherichia coli in street vended fast foods sold in Bangladesh Agricultural University campus, Mymensingh and to evaluate the antibiotic resistance of isolates. A total of 72 samples from six street vended fast food items included Shingara, Samosa, Piazu, Puri, Potato chop and Beguni were collected. Bacterial enumeration and antimicrobial susceptibility test were made using the following standard microbiological methods. The mean total bacterial count, Staphylococcus spp., Salmonella spp. and E. coli count in all the fast food samples ranged from  $1.0 \times 10^7$  to  $2.4 \times 10^7$ ,  $6.3 \times 10^3$  to  $4.2 \times 10^4$ ,  $2.4 \times 10^3$  to  $3.2 \times 10^4$  and  $2.7 \times 10^4$  to 4.0x  $10^4$  cfu/g respectively. Among the 60 isolates, *Staphylococcus* spp. was most detected isolate (40%) followed by E. coli (33.3%) and Salmonella spp. (26.7%). All the isolates were tested to antibiotic susceptibility against ten commonly used antibiotics and the results showed most of the bacterial isolates were resistant to ampicillin, amoxicillin, nalidixic acid, tetracycline and erythromycin whereas many of the isolates of these organisms were sensitive to ciprofloxacin, gentamycin, azithromycin and chloramphenical. Thus the study revealed that street vended fast food in this area is contaminated with foodborne bacteria that can pose a serious health problem.

For this reason, without following the rule of food safety and hygiene, an increasing number of shops are getting established throughout the Bangladesh (Ahmed *et al.*, 2008).

Fast foods are easy to prepare and serve. Samosa, shingara, vegetable roll, chicken roll, sandwich, pakura, puri, chop, cake, patties and burgar are most popular fast food in Bangladesh (Rahman *et al.*, 2014). Fast foods are served as snacks in meeting, seminar, any festival and most of the students take those during the tiffin period as alternative regular food items. Most of the ingredients used in fast food are nutritious but they harbor various pathogenic organism including *Escherichia coli*, *Enterobacter* spp. *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogens* (Ahmed *et al.*, 2014).

Street fast foods contribute a significant role in foodborne illness to consumers. 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).

Foodborne illness is occurred after consumption of contaminated foods containing microorganisms and their toxin (Clarence et al., 2009). It is now becoming a great concern involving a wide range of illness caused by bacterial, viral, parasitic and chemical contamination of food. 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). Most of the bacteria present in foods are resistant to antibiotic and it is very difficult to control them and

sometimes it reaches at such as level that can cause death of an individual stated by Ahmed *et al.*, (2008).

Studies have been carried out in different countries throughout the world to investigate the microbiological quality of street fast food. Eromo et al., (2016) isolated three bacteria from six street food items in Hawassa, Ethiopia where 29.6% of E. coli followed by 12.7% of Salmonella spp. and 9.9% of S. aureus. Adimasu et al., (2016) identified E. coli, S. aureus and Salmonella spp. in street vended foods in Gondar, Ethiopia. Manguiat and Fang (2013) were found higher number of coliforms, E. coli and S.aureus in pork and chicken based food samples. Kibret and Tadesse (2013) also identified contamination in lupin by Salmonella spp., Shigella spp. and E. coli. Sharma and Mazumdar (2014) carried out the experiment on street food samples in Silchar city, India and the isolates recovered was 37.5% E. coli, 5.35% Salmonella, 19.94% Shigella and remaining percentage was other microbial species. In Orissa, India E coli, Klebsiella spp. Vibrio spp. Shigelladysenteriae and Salmonella paratyphi were detected in Panipuri samples by Das et al., (2012).

Popularity of street fast foods is increasing day by day among the all ages. About 10 thousand students are studying in Bangladesh Agricultural University. The university is considered as one of the densely populated areas in Mymensingh and is surrounded by a number of food vendors and restaurants. So, there is lots of chance of getting infection through consumption of fast food from these street vended restaurants. Considering above mentioned facts, this study was carried out to assess the total bacterial load and also isolation, identification and antimicrobial susceptibility of common bacterial pathogen with special emphasis on Staphylococcus spp., Salmonella spp. and Escherichia coli in street vended fast foods.

## **Materials and Methods**

## Sample collection

The study was carried out on street vended fast foods sold in Bangladesh Agricultural University, Mymensingh, Bangladesh during the period of January 2017 to December 2017. For this purpose, a total of 72 fast food samples comprising six items (Shingara, Samosa, Piazu, Puri, Potato chop and Beguni) were randomly collected from different street vended restaurants including Jabbarer More (5 Kamal-Ranjit restaurants). Market (3 restaurants), Kewatkhali (2 restaurants) and Shesh More (2 restaurants). These fast foods items and places were selected as per as consumer preference and their availability. After collection, samples were kept in labeled sterile polyethylene containers and transported to the Bacteriology laboratory, Department of Microbiology and Hygiene, Bangladesh Agricultural University in cold box (contain ice blocks) and processed immediately for bacteriological analysis.

# Sample processing

For analysis, 10g of samples were weighed using electric balance and blended in mortar and pestle. 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 1ml of homogenized sample with 9 ml of PBS.

# Microbial analysis

All the media were prepared according to Manufacture's instruction, and sterilized by autoclaving for 15 mins at 121°C. Salmonellashigella agar (SS) which did not require autoclaving, were sterilized by boiling for 15 mins according to the instruction of manufacturer's. Nutrient agar was used to count the total bacterial colonies. A 0.1 ml aliquot from each dilution was inoculated onto the center of petridishes of nutrient agar and spread by a glass stick smoothly. Then it was kept in incubator at 37 °C for 24 to 48hrs in an inverted position. After incubation, colonies on nutrient agar were counted and recorded in cfu/g of fast food samples. Similar method was applied to enumerate the *Staphylococcus* spp., *Salmonella* spp. and *E. coli* using Mannitol-salt agar, Salmonella-shigella agar and Eosin methylene blue agar respectively.

# Isolation and identification of bacteria

For obtaining pure culture, bacterial colonies of different agar were streaked in their respective media and incubated overnight at 37°C. Bacteria was identified on the basis of colony characteristics, morphological characteristics by Gram's staining, sugar fermentation test and a series of biochemical including Methyl red tests (MR). VogesProskauer (VP), Catalase and coagulase test (Cheesbrough, 2006).

# Molecular detection by PCR

Genus specific PCR was performed to identify microorganisms previously using the published primers. The lists of primers and their oligonucleotide sequences are shown in Table 1. Genomic DNA was extracted from each isolate by heat lysis method as described previously by Dashti et al., (2009). A 20µl reaction mixture was used comprising 4ul PCR master mix (MP Bio Medical), 1µl forward primer, 1ul reverse primer, 9ul nuclease free water and 5µl DNA template. Thermal condition used for amplification was 5 mins at 94°C for initial denaturation followed by 35 cycles comprising of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 mins in case of Staphylococcus spp. For Salmonella spp.

amplification condition was 5 mins at 94°C for initial denaturation followed by 30 cycles of reaction comprising denaturation at 94°C for 30s, annealing at 66°C for 30s, extension at 72°C for 45s and final extension at 72°C for 10 mins.

Thermal condition used for amplification of *E. coli* was 5 mins at 95°C for initial denaturation followed by same reaction cycle used in *Salmonella* spp. consisting at denaturation at 94°C for 40s, annealing at 56°C for 30s, extension at 72°C for 30s and final extension at 72°C for 7 mins. After that 5  $\mu$ l of PCR products was loaded in 1.5% agarose gel well and electrophoresed in 1X TAE buffer for 25 mins at 100V. Ethidium bromide was used to stain the DNA and then visualized under ultraviolet trans-illuminator (Biometra, Germany).

#### Antimicrobial susceptibility test

Kirby-Bauer disc diffusion method described by Bauer et al., (1966) was used to perform antimicrobial susceptibility test on Mueller Hinton agar using the following antibiotic discs includes ampicillin (25µg), gentamycin (10µg), ciprofloxacin (5µg), azithromycin (30µg), chloramphenicol (30µg), tetracycline neomycin (30µg), erythromycin  $(30 \mu g),$ (5µg), nalidixic acid (30µg) and amoxicillin (30µg). Antimicrobial agents tested in this study were selected on the criteria of commercially availability and widely used as treatment of bacterial diseases. The zone sizes of bacteria were compared with standard of Clinical and Laboratory Standards Institute (CLSI, 2016).

#### Statistical analysis

All data was incorporated in SPSS software (SPSS-20.0) and analyzed. Descriptive analysis was performed to calculate mean and frequency.

#### **Results and Discussion**

During the investigation, a total of 72 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 *Staphylococcus* spp., *Salmonella* spp. and *E. coli*. Bacteria enumerated from different types of food samples are shown in Table 2.

#### Total bacterial count (TBC)

In present study, all the six food items were examined for determination of total bacterial load and all of them were found to be contaminated. Total bacterial count in all the food items was recorded ranged from 1.0 x  $10^7$  to 2.4 x  $10^7$  cfu/g whereas highest one was estimated from piazu samples and lowest one from puri samples. TBC of samples from all the tested street vended restaurants were not within acceptable limit recognized by the International Commission for Microbiological Specification for Foods  $(10^6 \text{ to } < 10^7 \text{cfu/g})$ (ICMSF, 2002). The count of TBC was relatively higher than the findings of Nguendo (2018) who found TBC ranged from 1.71 x  $10^{5}$  cfu/g to 3.71 x  $10^{5}$  cfu/g for different street foods and beverages in three location of Yaounde, Cameroon. Khandoker et al., (2014) was also reported TVC ranging from 10<sup>4</sup>to  $10^{5}$  cfu/g for spicy puffed rice sold by street vendors at Bangladesh Agricultural University campus, Bangladesh. Sharma and Mazumder (2014) examined the TBC in street fast food samples ranged from  $4.5 \times 10^5$  to  $1.12 \times$ 10<sup>6</sup> cfu/g in Silchar city, Assam, India. On the other hand, mean TBC obtained in present study was quite comparable wiith the findings of Madueke et al., (2014) who observed average mean bacterial count ranging from 5.0 x  $10^4$ to 2.08 x  $10^7$ cfu/g forakara and fish, respectively in Lokoja-Abuja Express Way,

Lokoja. The variations of TBC with the other research might be due to types of sampled food items, number of food samples, food content, way of preparation, handling and serving of foods by vendors. Environment and personal hygiene along with prolong exposure of foods to the different environmental conditions might be other cause of differences.

## **Total Staphylococcal count**

Among the all tested food samples, 95% (68/72) of them was found to be contaminated with Staphylococcus spp. The Staphylococcal countin food items ranged from  $6.3 \times 10^4$  to 4.2x  $10^{\circ}$  cfu/g. Among the all samples highest Staphylococcus spp. was found in puri and lowest in piazu samples. This finding was some extent comparable with the findings of Adimasu *et al.*, (2016) who obtained  $1.5 \times 10^4$ cfu/g of S. aureus from street vended foods in Goinder, Ethiopia. Similarly, Wimalasekara and Gunasena (2016) also found  $10^4$  cfu/g in meat based fast food samples in Colombo, Srilanka which was quite similar to present research. But present findings disagreed with the research of Khater-Dalia et al., (2013) and Cho *et al.*, (2011) that documented  $10^2$  cfu/g and  $10^{3}$  cfu/g of *Staphylococcus* spp. in fast food samples of Tanta city, Egypt and Korea respectively which was comparatively lower than the present findings. Hoque *et al.*, (2012) noted 10<sup>5</sup>cfu/g of Staphylococcal count in sandwich collected from fast food outlets of Dhaka and Mymensingh city, Bangladesh that was slightly higher than the current study. Among the *Staphylococcus* spp. contaminated food samples, 78% (53/68) samples exceed acceptable range  $(10^4 \text{cfu/g})$  of *Staphylococcus* spp. which indicates that tested fast food samples are hazardous to health. This variation might happen because it is stated that the presence of Staphylococcus was an indication of contamination from the skin and mouth or nose of food handlers. Tambekar et al., (2009) stated that lack of adequate

knowledge on hygiene practices and food safety and contaminated hands of vendors may enhance the contamination.

## Total Salmonella spp. count

In this study, 27% (20/72) 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 2.4 x  $10^3$  to 3.2 x  $10^4$  cfu/g. The count of Salmonella was the highest in potato chop and lowest in samosa samples whereas no Salmonella was detected in piazu 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 x  $10^{3}$  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.

#### Total E. coli count

From results, *E. coli* count was estimated from 2.7 x  $10^4$  to 4.0 x  $10^4$ cfu/g 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 x  $10^3$ 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  $1.0 \ge 10^3$  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.

#### Table.1 Oligonucleotide sequence of primers for PCR

Primer	Sequence	Size (bp)	References	
<i>Staphylococcus</i> spp.16S (F)	5'-GGAGGAAGGTGGGG ATGACG-3'	241	Stuhlmeier and Stuhlmeier, 2003	
Staphylococcus spp. 16S (R)	5'-ATGGTGTGACGGGC GGTGTG-3'	4		
Salmonella common (F)	5'- ACTGGCGTTATCCCTTTCTCTGGTG-3'		Cohen <i>et al.</i> , 1993	
Salmonella common (R)	5'- ATGTTGTCCTGCCCCTGGTAAGAGA- 3'			
Escherichia coli (F)	5'-AATTGAAGAGTTTGATCATG-3'	704	Guan et al., 2002	
Escherichia coli (R)	5'-CTCTACGCATTTCACCGCTAC-3'			

Note: F=Forward, R= Reverse and bp= Base pair

# **Table.2** Mean total bacteria, *Staphylococcus* spp., *Salmonella* spp. and *E. coli* counts in streetvended fast foods

Food	Number	Mean bacterial colony count (cfu/g)						
items	of	Total	Staphylococcus	Salmonella	E. coli			
	samples	bacterial	spp. count	spp. count	(cfu/g)			
		count	(cfu/g)	(cfu/g)				
		(cfu/g)						
Shingara	12	$1.6 \ge 10^7$	$1.0 \ge 10^5$	$5.0 \times 10^3$	$3.2 \times 10^4$			
Samosa	12	$1.7 \ge 10^7$	$1.5 \ge 10^5$	$2.4 \times 10^3$	$4.0 \times 10^4$			
Piazu	12	$2.4 \times 10^7$	$6.3 \times 10^4$	-	$2.7 \times 10^4$			
Puri	12	$1.0 \ge 10^7$	$4.2 \times 10^5$	$2.0 \ge 10^4$	$3.7 \times 10^4$			
Potato	12	$2.0 \times 10^7$	$1.1 \ge 10^5$	$3.2 \times 10^4$	$3.8 \times 10^4$			
chop								
Beguni	12	$1.7 \ge 10^7$	$8.4 \times 10^4$	$2.1 \times 10^4$	$2.9 \times 10^4$			

\* 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.

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Identified organism	Food items						Total	
	Shingara	Samosa	Piazu	Puri	Potato chop	Beguni	bacterial isolates (%)	
Staphylococcus spp.	5 (20.8)	4 (16.7)	2 (8.3)	6 (25)	4 (16.6)	3 (12.5)	24 (40)	
Salmonella spp.	3 (18.7)	1 (6.2)	0 (0)	4 (25)	4 (25)	4 (25)	16 (26.7)	
E. coli	4 (20)	4 (20)	3 (15)	3 (15)	4 (20)	2 (10)	20 (33.3)	
Total	12 (20)	9 (15)	5 (8.3)	13 (21.7)	12 (20)	9 (15)	60 (100%)	

#### Table.3 Bacterial isolates of street vended fast foods

\* Percentages were calculated by dividing the number of isolates in a particular food items by total number of isolates

#### Table.4 Antimicrobial resistance pattern of bacterial isolates from ready to eat fast food samples

	Antibiotics tested									
Organisms	No (%) of resistance									
	AMP	GEN	CIP	AZM	С	TE	Ν	Ε	NA	AMX
Staphylococcus	24	6	0	4	8	20	14	20	24	24
spp. (n=24)	(100)	(25)	(0)	(16.7)	(33.3)	(83.3)	(58.3)	(83.3)	(100)	(100)
Salmonella spp.	16	4	0	2	4	14	8	12	16	16
( <b>n=16</b> )	(100)	(25)	(0)	(12.5)	(25)	(87.5)	(50)	(75)	(100)	(100)
E. coli	20	б	0	4	6	16	6	16	18	20
( <b>n=20</b> )	(100)	(30)	(0)	(20)	(30)	(80)	(30)	(80)	(90)	(100)

Legends: AMP=Ampicillin; GEN=Gentamycin; CIP=Ciprofloxacin; AZM=Azithromycin; C=Chloramphenicol; TE=Tetracycline; N=Neomycin; E=Erythromycin; NA=Nalidixic acid and AMX=Amoxicillin

#### **Identification of bacteria**

In this study microbiological methods were used to identify bacteria from the street fast foods. The results of cultural characteristics. Gram's staining, biochemical tests and genus specific PCR used identify to the microorganisms were similar to the findings of previously published paper. Konuku et al., (2012) stated that Staphylococcus spp. on mannitol salt agar showed colonies that fermented mannitol and appeared golden yellow. Salmonella spp. produced small round black center colonies on Salmonella-Shigella (SS) agar which was in agreement with the study of Muktaruzzaman et al., (2010). E. coli on Eosin Methylene Blue (EMB) agar plate appeared as dark with characteristics metallic sheen as indicated by El-Aziz et al., (2014).

Among the identified bacteria Staphylococcus spp. and E. coli were most frequently isolated whereas Salmonella spp. was prevalent in only five food items except piazu. A total of 60 isolates of three organisms were identified. the isolates, 40% Among (24)were Staphylococcus spp. followed by 33.3% (20) E. coli and 26.7% (16) Salmonella spp. (Table 3). The highest number of bacteria was isolated from Puri (13/60) and lowest number in piazu sample (5/60). This variation might be associated with the ingredient that was used for preparation of particular food. The percentages of different types of bacteria recovered from the food samples were incomparable with the findings of Bereda et al., (2016) who reported 84.6% of S. aureus, 51.5% of E. coli and 19.7% of Salmonella spp. in street vended foods of Jigjaj city of Ethiopia that was comparatively higher than our study. The same types of bacteria were isolated in several previous studies includes in street vended foods of Rivers state Nigeria by Akusu *et al.*, (2016), in selected street foods of Gondar town, Ethiopia by Adimasu *et al.*, (2016) and in street food of Silchar city, Assam, India by Sharma and Mazumdar (2014).

## Antimicrobial susceptibility test

From the antimicrobial susceptibility test results, it was revealed that 70-100% isolates of three organisms showed resistant to ampicillin, amoxicillin, nalidixic acid. tetracycline and erythromycin which would make the treatment of infections difficult (Table 4). However, ciprofloxacin was 100% sensitive to all isolates. The isolates of Staphylococcus showed sensitive against gentamycin (75%), azithromycin (83.33%) and chloramphenicol (66.67%) respectively. About 75% of isolates Salmonella spp. showed sensitive to gentamycin followed by azithromycin 87.5% to and 75% chloramphenicol respectively and similar results were also observed in E. coli.

That means this drug still can be used as a choice of treatment of foodborne illness in this area. This study completely agreed with the previous study conducted by Sultana et al., (2015) who observed highest resistance ampicillin, cephalexin rate for and vencomycin against S. aureus. This result was also some extent comparable with the findings of Eromo et al., (2016) who reported that 89% isolates of Salmonella spp. and 14.3% of S. aureus were resistant to chloramphenicol and vancomycin whereas respectively ciprofloxacin was sensitive against all isolates in street foods of Hawassa, Ethiopia. Similar reports were also described by following researchers Khandokar et al., (2014), Sharma and

Mazumdar (2014) and Kalantari *et al.*, (2012). Multi drug resistant bacteria pose a big challenge to human survival and continued existence in relation to bacterial infection and diseases. The results from this study show that street vended fast food samples are contaminated with various bacteria most of which are resistant to commonly used antibiotics and therefore represent risks and public health hazards.

The results of the current study clearly demonstrated that street vended fast foods sold in Bangladesh Agricultural University campus premises are contaminated with Staphylococcus spp., Salmonella spp. and E. *coli*. The existence of these bacteria in fast foods could cause potential public health problems for consumers. Poor personal hygiene, improper handling practices, prolong storage of foods in ambient temperature and lack of knowledge of restaurants operators towards food safety and foodborne infections were the associated factors to contamination of fast foods. Furthermore, the study revealed that majority of restaurants vendors did not have any food safety training. Moreover, multiple drug resistant bacteria from fast foods might act as a vector in the spread of antibiotic resistant strains in the community and environment. So, necessary information, education facilities and food safety training program should be ensured for all the restaurants vendors that can help to reduce the chance of infection and at the meantime provide safe and good quality foods to consumers.

Therefore, consumers should be aware of harmful effect of consumption street foods. Finally, regular inspection of fast foods vending site should be ensured by public health authority of respected country to improve the quality of street vended fast foods as well as to avoid foodborne disease outbreaks in community.

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