www.bti.org.in
ISSN 0974-1453
Research article

BACTERIOLOGICAL ANALYSIS OF STREET FOOD AND OBSERVATION OF HYGIENE PRACTICES FOLLOWED BY STREET FOOD VENDOR AT DEHRADUN CITY

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ABSTRACT

Street food is a major and common part of the human lifestyle, which plays an important role in society. Due to poor hygiene practices during handling and cooking, these foods are highly contaminated with different pathogens that may cause food borne illness. The bacteriological analysis was performed for different street food and water provided by street food vendors and hygiene practices followed by them. The food and water samples were collected from different places of Dehradun city and were transported to the laboratory in sterile conditions for bacteriological analysis. *Escherichia coli, Staphylococcus aureus, Enterobacter, Salmonella typhi, Micrococcus, Shigella flexneri, Bacillus cereus,* and *Proteus vulgaris* were identified from different food samples. *Salmonella typhi,* and *Staphylococcus aureus* were the dominant isolated species. The microbial load was observed highest in batashe-ka-paani (5.8×10^8cfu/ml) followed by raw salads (4.2×10^8cfu/ml), fruit chaats (3.9×10^8 cfu/ml) and chutney (1.8×10^8 cfu/ml). Among the water sample of tap water (80%) which was used for drinking, washing, and cooking purposes showed highest microbial loads of 5.1×10^8 cfu/ml while lowest microbial loads was 4.5×10^8 was observed in filtered tap water. The present study highlighting the practices followed by street food vendors and the microbial load in different food items is sold by these vendors. It was concluded that due to mishandling and improper hygiene measures, street foods can get contaminated by pathogens that can cause serious food borne illness. Thus, best practices should be followed for a healthy life as street food plays major role in the society.

Keywords: Bacteriology, Street food, Food safety, *Escherichia coli*, Vendors.

INTRODUCTION

According to Food and Agriculture Organization (FAO), street food describes a wide range of 'Ready to Eat Food and Beverages' (FAO, 2018). These foods are prepared and sold by vendors which are easily available on street and public areas such as markets; outside school and 2.5 million people eat street food daily (Gadi *et al.,* 2013). Street food has become a major and common part of the human lifestyle across the globe, and its consumption is increasing day by day.

The reason of the increasing consumption scale of these foods is its important role in society as well as consumers busy schedule which is the best way to save time as well as its low price make it available for everyone to consume. But these foods due to mishandling have accordingly become an increasing threat to the public health in the developing countries around the world. Microorganisms are the primary cause of food spoilage and food borne illness (Hemalata and Virupakshaiah, 2015). According to the Centre for Science and Environment, India, the contamination is the greatest challenge for food safety in India. The major cause of contamination of these foods is poor hygiene measure followed by vendors. The risk of serious food poisoning outbreaks linked to street food remains a threat in many parts of the world. A lack of knowledge among street food vendors about the cause of food borne disease is the major risk factor (Rane, 2011; NASVI, 2018).

Major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, water used for washing the utensils, time and temperature, abuse of cooked food and personal hygiene of vendors (Lopez-Campos and Joaquin, 2102; Kumar and PJangir, 2017). The street food vendors convene in the crowded areas such as bus stands, outside schools/colleges, marketplaces where there are a high number of customers and thus high chances for sale of their food. But these crowded areas have a high risk of contamination and transmission of pathogens causing food borne infections.

According to World Health Organization (WHO) food handling plays an important role in ensuring food safety throughout the process of food production and storage. Mishandling of food and not following proper hygiene measures enables pathogenic bacteria to come into contact with food causing food borne illness (Nganga *et al*., 2017; Sabbithi *et al*., 2017; Verma and Gaur, 2017). Hygiene practices include the practices followed by the vendor which can prevent the contamination, cross contamination and transmission of pathogen which can cause serious food borne illness to the consumer. It includes practices like the cleanliness of utensils used for storing and cooking of food, covering of hands, face, head while cooking and serving the food, food utensils covered or not, type of utensils used for serving the food. Most vendors are uneducated, untrained in food hygiene.

Bacteria can cause severe food borne illness in humans with symptoms like vomiting, headache, fever, stomach cramps, and loss of appetite and can become more serious if left untreated. Various foods borne pathogenic microbe belongs to the genus *Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria, Salmonella etc.* Health agencies like the Environmental protection agency (EPA) and WHO "have avoided standards for plate counts, possibly for lack of pathogenicity and great variation in density encountered. A recommended microbial count limit (MCL) for human drinking water has not yet been proposed, but EPA does recognize the water quality deterioration implied by high plate counts. The upper limit for potable water is usually 500 colony forming unit or cfu/ml. According to DeZuane (1990), water with counts less than 100 cfu/ml should be considered "portable" and values 100-500/ml "questionable" (John De Zuane, 1997). In Dehradun city, it was observed that most of the public consumes street foods, but due to unhygienic practices

followed by street food vendors can cause food to get contaminated from different bacteria that can cause food borne illness to consumers.

MATERIALS AND METHODS

Materials

Nutrient Broth (NB), Nutrient Agar (NA), selective media viz. Brain Heart Infusion Agar (BHI), MacConkey agar. Biochemical kits Hi25^{TM} Enterobacteriaceae identification kit-KB003 and HiBacillus ^{TM} identification kit-KB013 were procured from Hi-Media, Mumbai and were used as per manufacturer's instructions.

Methods

The work was undertaken in the four stages, including the collection of food samples, isolation and differentiation of bacteria on culture media plate, morphological analysis followed by biochemical analysis; and membrane heterotrophic plate count method (HPC) for water sample (Figure 1).

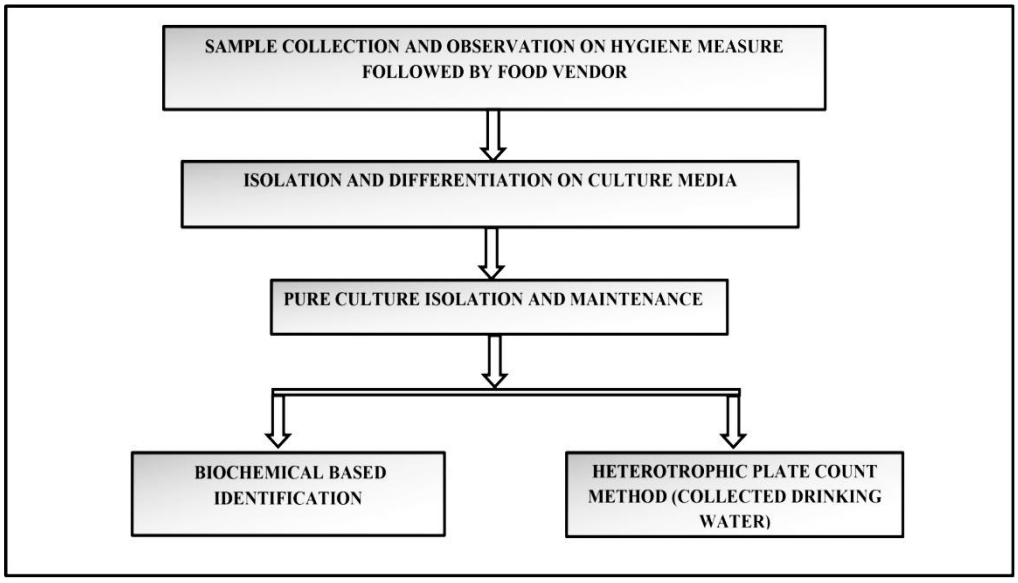

Figure 1. Methodology used in the present study

Sample collection

Five different locations of Dehradun city were chosen for the collection of different samples. The locations were ISBT bus stand, Dehradun; Near Clock Tower area, Dehradun; Subhash-Nagar market, Dehradun; Stalls outside Graphic Era (Deemed to be University), Dehradun and Buddha Temple Market, Clement Town, Dehradun. A total 25foods and water samples were collected viz. Batashe and batashe-ka-paani (C01), raw salad including cucumber (C02), momos chutney (C03), fruit chaats (C04) and drinking water samples (C05). The samples were collected in sterile zip-lock bags and sterile bottles and were transported to the laboratory within 1 hour and were processed.

Observation of hygiene practices followed by the vendors

Personal hygiene of the food vendors were obtained that includes the observation of cleanliness of food vendor (clothes condition, nails, hair, as well as the health status of vendor, fever, cough or cold etc.). The environment of food stall includes the cleanliness of food stalls, nearby surroundings, the preventive measure followed by the food vendor to make the surroundings of the stalls neat and clean.

Isolation and differentiation of bacteria on culture media

The collected samples were serially tenfold diluted in sterile water and 0.1 ml of dilution was spread by spread plate technique. Culture media used were Nutrient agar, Brain Heart Infusion agar, MacConkey agar. These plates were incubated at 37°C for 24 hours. Microbial load in food samples was determined using formula: [cfu/ml= (no. of colonies × dilution factor/volume of inoculum)]. Colony characteristics were observed like shape, size, margin and elevation. The selected colonies were picked up and inoculated in culture broth and incubated at 37°C for 24 hours. Overnight grown bacterial culture was viewed under microscope after negative staining as well as Gram's staining and separately streaked on suitable culture media.

Biochemical based identification

The isolated bacterial colonies were confirmed by biochemical kits Hi25^{TM} Enterobacteriaceae identification kit-KB003 and Hi-*Bacillus*^{TM} identification kit-KB013. The result was interpreted as per interpretation chart and identification index following kit protocol.

Identification of microbial load in water by heterotrophic plate count method (HPC)

Water is the basic need in all the operations done in the food stalls like drinking, cooking, etc. WHO considers that "drinking-water" should be suitable for human consumption and for all the domestic purposes including personal hygiene**.** Contaminated water can create a public health risk when it is used for drinking, washing vegetables and fruits and used for washing equipment, utensils and hands (WHO). HPC method was followed for the analysis of microbial load present in the drinking water sample. 1 ml water sample was serially diluted and 1 ml of 10^{-5} diluted sample was membrane filtered and placed in sterile nutrient agar plates. It was incubated for 12-24hrs at 37°C and colonies were counted as cfu/ml.

Results and discussion

The present study describes the observation of street food vendor personal hygiene and hygiene measures followed by him/her to prevent contamination, recontamination and cross contamination of the food. The study also describes microbial load and types of bacteria present in the food that can cause food borne illness.

The study showed that out of 25 vendors from where the samples were collected, 22 vendors (80%) were in healthy conditions, wearing clean clothes, and having proper garbage disposal facility. Whereas, 3 vendors (20%) were having infections like the common cold, and were not wearing clean clothes and having poor personal hygiene. Only 15 food vendors (60%) followed hygiene practices like covering of the head, hands, and cooked food. They were using clean utensils for cooking and storing the food. Whereas, 10 food vendors (40%) were not following any hygiene practices, among these 10 food vendors, 5 food vendors were using clean utensils for storing and serving the food items. 20 street food vendors (80%) used tap water for cooking the food and drinking, whereas 5 street food vendors (20%) used filtered water for cooking and drinking purposes.

The bacteriological analysis of food samples has been depicted in table 1 and table 2. The microbial load was observed higher in batashe-ka-paani (5.8×10^{8}cfu/ml) followed by raw salads (4.2×10^{8}cfu/ml), fruit chaats (3.9×10^{8}cfu/ml), chutney (1.8×10^{8}cfu/ml). Eight different bacterial isolates were identified from different food

samples i.e. *Escherichia coli, Staphylococcus aureus, Enterobacter, Salmonella typhi, Micrococcus, Shigella flexneri, Bacillus cereus, Proteus vulgaris*. Among the isolated bacterial species, *Salmonella typhi, Staphylococcus aureus* were the dominant species. All the bacteria identified can cause food borne illness if ingested in high number (Hemelata *et al*., 2015).

Table 1. Macroscopic and microscopic identification of bacterial isolates

S.No.	Sample	Colonies	Colonies isolated/ Growth characteristic	Negative staining	Gram staining	Bacteria identified (Biochemical testing)
1	C01	1A	White, convex, circular and smooth margin growth on NAM	Rod	Gram negative	*Escherichia coli*
		2A	Golden yellow color, convex entire margin colony on NAM	Cocci	Gram positive	S*taphylococcus aureus*
		3A	Transparent colony with white center on MacConky agar media color changes to pink	Rod	Gram negative	*Enterobacter aerogenes*
		4A	Transparent colorless colony on BHI	Rod	Gram negative	*Salmonella typhi*
		5A	Dark yellowish growth on BHI	Cocci	Gram positive	*Micrococcus*
2	C02	1B	Transparent colony with white center on MacConky agar media color changes to pink	Rod	Gram negative	E*nterobacter aerogenes*
		2B	Transparent colorless colony on BHI	Rod	Gram negative	*Salmonella typhi*
		3B	White, shine and entire margin growth	Rod	Gram positive	*Bacillus cereus*
		4B	Round, smooth growth	Rod	Gram negative	*Proteus vulgaris*
3	C03	1C	White, convex, circular and smooth margin growth on NAM and pink color colonies on MacConkey agar	Rod	Gram negative	*Escherichia coli*
		2C	Transparent colorless colony on BHI	Rod	Gram negative	*Salmonella typhi*

		3C	Dark yellowish growth on BHI	Cocci	Gram positive	*Micrococcus*
		4C	White, shine and entire margin growth	Rod	Gram positive	*Bacillus cereus*
4	C04	1D	Golden yellow color, convex entire margin colony growth on NAM	Cocci	Gram positive	*Staphylococcus aureus*
		2D	Opaque round translucent growth	Rod	Gram negative	*Shigella flexneri*
		3D	Transparent colony growth	Rod	Gram negative	*Enterobacter aerogenes*

Table 2. Organism identified through biochemical test (Hi-25TM Enterobacteriaceae identification kit-KB003), where + shows positive test and - shows negative test.

Biochemical Test	***E.coli***	***S.typhi***	***Enterobacter aerogenes***	***Shigella dysenteriae***	***Proteus vulgaris***
ONPG	+	-	-	-	-
Lysine	+	+	+	-	-
Ornithine	+	-	+	-	-
Urease	-	-	-	-	+
TDA	-	-	-	-	+
Nitrate	+	+	+	+	+
H2S	-	+	-	-	+
Citrate utilization	-	-	+	-	+
Voges Proskauer's	-	-	+	+	-
Methyl red	+	+	-	+	+
Indole	+	-	-	-	+
Malonate	-	-	+	-	-
Esculin hydrolysis	+	-	+	-	+
Arabinose	+	-	+	+	-
Xylose	+	+	+	-	+
Adonitol	-	-	+	-	+
Rhamnose	+	-	+	-	-
Cellobiose	-	-	+	-	-
Melibiose	+	+	+	+	-
Saccharose	+	-	+	-	+
Raffinose	+	-	+	+	-
Rhalose	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+	-	+	-	-

Table 3. Organism identified trough biochemical test (HiBacillus™ identification kit-KB013), where + shows positive test and – shows negative test.

Test no.	Biochemical Test	*Bacillus cereus*	Test no.	Biochemical Test	*Bacillus cereus*
1	Malonate	-	7	Arginine	+
2	Voges Proskauer's	+	8	Sucrose	+
3	Citrate	+	9	Mannitol	-
4	ONPG	-	10	Glucose	+
5	Nitrate reduction	+	11	Arabinose	-
6	Catalase	+	12	Terhalose	+

Bacterial contamination may be present due to the improper handling and unhygienic practices followed by the vendor. Presence of bacterial contamination in sample 'Batashe-ka-paani' was due to the water used while its preparation and dipping of hands in the paani while filling the Batasha for serving since *Staphylococcus aureus* are the common microbial flora of human skin. The presence of *Salmonella typhi* and other bacteria in 'Batashe-ka-paani' and other samples was due to improper handling practices, material used or environment of the stall.

Out of 25, 20 water samples 80% of tap water were used for drinking, washing and cooking purposes and had shown highest microbial load of 5.1×10^8cfu/ml having 4.5×10^8cfu/ml microbial loads as compared to filtered water and collected 5 samples (20%) which shows low microbial load. The present study revealed that the batashe-ka-pani is more contaminated than other samples. The contamination in batashe-ka-pani is higher because of the conditions under which it is prepared and vended. Vendors usually prepare and serve the food through bare and unwashed hands, which is one of the most potable sources of contamination. Cross contamination of street foods also increases by unsanitary processing and preservation. The use of raw vegetables also contributes to the bacterial load (Terry and Overcast, 1976).

Presence of high number of bacterial loads was reported in 80% of the samples, indicating a high risk of contamination from pathogenic microorganisms. Our findings are in perfect correlation with the previous reports of high incidence of bacterial loads in street vended Fruit chaats in Patiala city, India (Kumar *et al*., 2006). In a few similar studies, Das-Mohapatra *et al*, reported the presence of *Escherichia coli*, *Shigella sp.*, *Staphylococcus sp.* and *Bacillus sp*. in four popular street-foods (including batashe-ka-pani) of Bhubaneswar city (Das-Mohapatra *et al*., 2002) , whereas, Das *et al* showed that street foods such as Panipuri, Bhelpuri and Chaat in Bangalore city, were contaminated with high loads of pathogens viz. *Streptococcus faecalis, Escherichia coli, Staphylococcus aureus, Bacillus sp., Klebsiella sp*. and *Pseudomonas sp* (Das *et al*., 2010).

Seth *et al.*, in their study showed the presence of high bacterial colony count and presence of *Staphylococcus* and *Escherichia coli* in street food samples in Vadodra city (Seth *et al*., 2005). Further, the occurrence of *Bacillus* species in batashe-ka-pani (khattapani) implicated the ubiquitous nature

of bacterial spores especially in dusty road side locations. Both *Bacillus* and *Staphylococcus sp.* normally exhibit tolerance to a wide range of temperature and pH, which justifies their presence in batashe-ka-pani even at highly acidic conditions (Hanashiro *et al*., 2005; De-Barros *et al*., 2009; Desai and Varadaraj, 2010).

CONCLUSION

Food-borne illness has made a worldwide impact. Due to their easy availability as "Ready to eat" and low on price these foods are becoming part of the human life style day by day. But mishandling of these foods and beverages has become a great concern for the public health. It is an accepted fact that street foods are heavily contaminated with microorganisms due to the wrong practices followed by the vendor.

From the present study, it was concluded that mishandling and improper hygiene measure can contaminate the food by harmful bacteria which can cause serious food borne illness. Preventive measures should be followed by the vendors as these street foods play an important role in society by which the health of the consumer can be maintained and the chances of food borne illness can be prevented or reduced in the country.

ACKNOWLEDGMENTS

Authors are thankful to Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India for providing all necessary laboratory facilities and support.

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