

Microbiological Analysis of Street Foods Sold in Kathmandu, Nepal

Sumantee Baidya¹, Melina Nagarkoti¹, Sarita Kunwor¹, Rabin Paudyal^{2*}

¹Department of Microbiology, Kathmandu College of Science and Technology, Kamalpokhari, Kathmandu, Nepal.

² Department of Microbiology, Birendra Multiple Campus, Bharatpur, Chitwan, Nepal

*Corresponding author: Rabin Paudyal, Department of Microbiology, Birendra Multiple Campus, Bharatpur Chitwan, Nepal; E-mail: rpaudyal@kist.edu.np

ABSTRACT

Objectives: To investigate whether the street foods are potential source of pathogens or not.

Methods: A total of 36 samples of different street foods were aseptically collected from different areas of Kathmandu Valley and was transported to laboratory. The bacterial load was enumerated using pour plate technique on plate count agar. Fungal load was enumerated using pour plate technique on potato dextrose agar and coliform load was also calculated using pour plate technique on VRBA. While *Staphylococcus aureus* was selectively detected on Mannitol Salt Agar. *Escherichia coli* was detected by observation of metallic sheen on Eosin Methylene Blue Agar.

Results: The highest bacterial load was found on pani-puri with 70.4×10^3 cfu/ml and highest fungal load was found on samosa with 34.5×10^3 cfu/ml whereas the presence of coliforms was only detected in panipuri and chana chatpate. The food samples consisted of *E. coli* and *S. aureus*. *E. coli* was only detected from chana-chatpate. While the average Staphylococcal load was found to be highest in pani-puri (91.6×10^3 cfu/ml) and lowest in mo: mo (1.7×10^3 cfu/ml).

Conclusion: This study concludes that *E. coli* and coliforms are the most common contaminant in channa chatpate sold in streets. Panipuri and channa chatpate sold in streets have the highest microbial load and hence more chances of harboring potential pathogens. Street food sample in Kathmandu is mostly contaminated with the *S. aureus*.

Key words: Street food, Bacterial load, Fungal load, Coliform. Nepal

INTRODUCTION

Drinking Street food are ready-to-eat foods and beverages which are prepared in the streets and sometimes prepared from home as well and sold in many different public places in carts, trucks or in moving stalls by vendors for immediate consumption (FAO 2000). These street foods provide different variety of cuisines that provides a link between food, place and people. These foods are popular among the people all across the globe due to its taste, flavor, easy availability and low cost. For

many people with limited income, street foods are most accessible way of obtaining a nutritionally balanced, providing the consumer with appropriate combination of foods. Street foods are important and essential for maintaining the nutritional status of the population. Millions of people consume snacks, meals and drinks sold by street vendors in the developing countries (Fellows and Hilmi 2011). It was estimated that about 2.5 billion people consume street food each day (FAO 2007).

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Despite being popular, cheap, readily available, street foods are perceived to be a major health problem and major cause of death in developing countries (Garode and Waghode 2012). In fact, street foods have often been associated with traveler's diarrhea and other food borne diseases (Bhowmik 2010). In the past decade, food borne outbreaks associated with consumption of street food have increased. This is due to the contamination of the food by pathogenic microorganisms during preparation, post-cooking and various handling stages and using of contaminated machines, utensils and other serving accessories (Schmidt et al 2003; Tambekar et al 2008).

Food borne bacterial pathogens mainly include *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *E. coli*, *Listeria monocytogenes*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholera*, *Staphylococcus aureus*, *Proteus species* etc. Among these bacterial etiologic agents, *Salmonella* spp. stands out as a common bacterium, being responsible for foodborne diseases in many countries including Nepal and is the main cause of hospitalization and death (Breuil 2000). Even some fungi, viruses and parasites are responsible for causing food borne diseases. In addition to the prevalence of food borne pathogens, resistance of these foodborne microorganisms to multi-drug made the food safety situation more vulnerable in public health (Khairuzzaman et al 2014)

Street food safety is essential, and yet it has been rarely studied in Nepal. Hence this study was conducted with the purpose to assess the microbial load of commonly sold street food in the market of Kathmandu valley. This study will highlight microbiological contamination and risk factors associated with consumption of street foods. This study may also help in bringing awareness for consumers consuming street food.

This study will further help to determine policy regarding selling and usage of street foods in terms of presence of potential pathogens.

MATERIALS AND METHODS

The organism that was sub-cultured from EMB showed

gram negative short rod shaped morphology while an organism that was sub-cultured from Mannitol Salt Agar showed gram positive cocci in cluster morphology.

E. coli was identified using Catalase test, Oxidase test, Indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, TSIA test, Citrate utilization test and Urease test. *Staphylococcus aureus* was identified using Catalase test, Oxidase test, Oxidative-Fermentative (O/F) test The study was conducted on street food samples collected from different locations of Kathmandu, Bhaktapur and Lalitpur. The study was conducted between September 2019 to November 2019. A total of 36 street food samples were collected for analyzing the microbial quality of street food

The study was conducted in Microbiology Laboratory of Kathmandu College of Science and Technology (KCST), Kamalpokhari, Kathmandu, Nepal About 50 gram of food samples was collected separately in sterile bottles for liquid and aseptically in sterile plastic bags for solid food samples and was kept in sterile ice box and transported with proper labeling. Food samples that were collected and transported on the same day to the laboratory were included in the study. While those samples that were not collected properly in sterile plastic and leaking samples were excluded from the study. After collection, the samples were transported to the Microbiology Laboratory of Kathmandu College of Science and Technology holding in an ice box at about 4°C. They were examined as soon as possible on arrival or within 6 hours of collection. If immediate analysis was not possible then the samples were preserved at 4°C until analysis. One gram of each collected street food samples was weighed and then they were homogenized in sterile normal saline of 10 ml using mortar and pestle for about five minutes. All the aseptic conditions were maintained throughout the process. After homogenization, the street food homogenates were serially diluted in a sterile normal saline upto a dilution of 10⁻⁶. One ml diluted sample (10³) were placed in the center of a sterile petri dish and using pour plate technique about 20 ml of molten Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) used for enumeration of bacterial and fungal load was poured into the petri dish and mixed well and was allowed to solidify before

Incubation. The PCA plate was incubated at 37°C for 24 hours while PDA plate was incubated at 28°C for 3-5 days. Similarly, again using pour plate technique, Violet Red Bile Agar (VRBA) was poured in the sterile petri dish containing inoculum and after solidification the plate was incubated at 37°C for 24 hours for enumeration of coliform load. One ml of sample was spread in Eosine Methylene Blue (EMB) Agar Mannitol Salt Agar (MSA) for isolation of *E.coli* and *Staphylococcus aureus* respectively. Both plates were incubated at 37°C for 24hrs.

E.coli was presumptively identified as an organism giving greenish metallic sheen in EMB agar whereas *S. aureus* was presumptively identified as an organism giving golden yellow colored colonies and yellow coloration of the media on MSA. The organisms were then sub-cultured in Nutrient Agar to obtain pure culture Gram staining was performed from Nutrient Agar. and Coagulase test

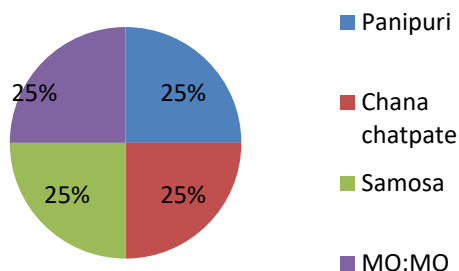
The bacterial and fungal loads were calculated as colony forming unit per milliliter (cfu/ml) using the formula as given and expressed in cfu/ml. The simple percentage was used to determine the occurrence frequency of bacteria and fungi isolated from different street food samples collected from different places around Kathmandu, Lalitpur and Bhaktapur. The results obtained were analyzed by calculating various arithmetic mean values.

RESULTS

Source-wise distribution of coliforms

A total of A total of 36 samples were collected from various places, out of which 9 sample each of panipuri, chanachatpate, samosa, MO: MO were collected respectively as shown in the chart below

As shown in Table 1, the highest distribution of bacterial



load was found in panipuri (70.4x10³cfu/ml) and the lowest bacterial load was observed in MO: MO samples (2.5x10³cfu/ml).

Table 1: Distribution of average bacterial and fungal load in various street foods samples

Type of sample	Total number of sample	Average bacterial load (cfu/ml)	Average fungal load (cfu/ml)
Panipuri	9	70.4x10 ³	5.3x10 ³
Chanachatpate	9	19.6x10 ³	2x10 ³
Samosa	9	45.3x10 ³	34.5x10 ³
MO:MO	9	2.5 x10 ³	11.2x10 ³

As shown in Table 2, the maximum bacterial load was collected from Bhaktapur (45.25x10³cfu/ml). And maximum fungal load was collected from Lalitpur (31.5x10³cfu/ml).

Table 2: Area-wise distribution of average bacterial and fungal load (cfu/ml) in various street food samples.

Area	Total number of samples	Average bacterial load in all samples	Average fungal load in all samples
Kathmandu	12	44.75x10 ³	8.33x10 ³
Lalitpur	12	16.33x10 ³	28.08x10 ³
Bhaktapur	12	41.08x10 ³	3.41x10 ³

Table 3: Distribution of coliforms in various street foods

Types of samples	Total number of samples	Number of coliform positive samples	Mean	Number of coliform negative samples
Panipuri	9	2(22.22)	2.5	7
ChanaChatpate	9	3(33.33%)		6
Samosa	9	0		9
MO:MO	9	0		9

As shown in Table 3, the highest coliform was found in chanachatpate (33.33%) followed by panipuri (22.22%).

Among the samples collected, *E. coli* was only found in Chanachatpate while none other samples showed the growth of *E. coli* (as shown in Table 4).

Table 4: Detection of *E. coli* in various street food samples

Types of sample	Total	<i>E.coli</i> positive	<i>E.coli</i> negative
Panipuri	9	0	9
Chanachatpate	9	3 (33.3%)	6
Samosa	9	0	9
Mo:Mo	9	0	9

As shown in Table 5, the highest *S. aureus* load was seen in panipuri (91.6×10^3 cfu/ml) followed by Samosa (37.3×10^3 cfu/ml). The lowest *S. aureus* load was seen in Mo:mo (1.7×10^3 cfu/ml).

Table 5: Distribution of average load (cfu/ml) of *S. aureus* in street food samples

Type of sample	Total number of samples	Average load of <i>S. aureus</i>
Panipuri	9	91.6×10^3
Chanachatpate	9	3.6×10^3
Samosa	9	37.3×10^3
MO:MO	9	1.7×10^3

From the table below, the maximum Staphylococcal load was found in Kathmandu (70.25×10^3 cfu/ml), followed by Lalitpur and then by Bhaktapur being the least contaminant area for *S. aureus*.

Table 6: Area-wise distribution of average load (cfu/ml) of *S. aureus* in street food samples

Area	Total number of samples	Average load of <i>S. aureus</i>
Kathmandu	12	70.25×10^3
Lalitpur	12	25.75×10^3
Bhaktapur	12	5.33×10^3

DISCUSSION

In this present study undertaken to observe the microbiological quality of different street food, the levels of total plate count (TPC) in all street food samples ranged

with a mean value from $(2.5 - 70.4) \times 10^3$ cfu/ml whereas according to the report provided by (Sharma and Mazumdar 2014), the TPC ranged from 4.5×10^5 to 1.12×10^6 colony-forming unit per gram (cfu/ml) which is beyond the acceptable limits set for microbiological quality of ready-to-eat foods. Similarly, the study conducted by (Das et al 2010) the average total viable counts was found to be varied between $(0.4 \text{ to } 3) \times 10^4$ cfu/ml and from the (Tuladhar and Singh 2015) study, the TPC counts of different street foods samples was found to be ranging from 8.83×10^5 to 260×10^5 cfu/gm which is more than our findings. While (Yadav et al 2019) found an average bacterial load ranged from $(90 - 182) \times 10^5$ cfu/ml in pani-puri samples from Janakpurdham street which is also higher as compared to our study where load is 70.4×10^3 cfu/ml. The higher degree of bacterial contamination is in pani-puri samples. It may be due to different unhygienic practice and high chance of contamination through its liquid and solid components.

From our data the average bacterial load represented in mo:mo samples which was found to be 2.5×10^3 cfu/ml is the lowest among all the street food samples. While pani-puri was the most contaminated food with mean bacterial count of 70.4×10^3 cfu/ml followed by samosa (45.3×10^3 cfu/ml) and the least contaminated was mo: (2.5×10^3 cfu/ml). It may be due to the high temperature it required to be properly cooked. From the study conducted by (Upadhyaya et al 2017) showed that the bacterial load was highest in pani-puri followed by Mo:Mo (3.7×10^2 cfu/ml). Samosa was found to be least contaminated.

In our study, the fungal population was found to be the highest in Samosa samples with a mean of 34.5×10^3 cfu/ml followed by mo:mo (11.2×10^3 cfu/gm), pani-puri (5.3×10^3 cfu/ml) and then chana-chatpate (2×10^3 cfu/ml). There are very limited studies conducted on the fungal isolation from street food samples. Most of the fungal organism isolated in this study cause deterioration of food and also some of them are able to produce toxic compounds that are responsible for causing diseases in human and animals. Deterioration in food causes alteration in nutritional value as well as change in taste, smell, color and appearance which cause very serious health problem.

From the area-wise data shown on table, the maximum bacterial load present in street food sample

(44.75×10^3 cfu/ml) was obtained from area Kathmandu. Similarly the highest fungal contamination of street food samples was obtained from area Lalitpur (28.08×10^3 cfu/ml). The lowest bacterial load and fungal load was 16.33×10 cfu/ml which was obtained from area Lalitpur and 3.41×10^3 cfu/ml from area Bhaktapur respectively. Contamination of bacterial and fungal load depends upon the hygienic practice during transportation, processing, handling and storage period and also may be due to the increasing pollution in Kathmandu valley.

Coliforms are indication of unsanitary conditions, unhygienic practices during and after production and poor source of water and food used (Beuchat 1996). They are considered as primary indicator of contamination of the water and food. In addition, the presence of coliform bacteria in food indicates whether the food is directly or indirectly contaminated by fecal matters. During this investigation for detecting the of presence and absence of coliform in food samples, we found out that panipuri and chana-chatpate consisted of coliform while in other sample there were no contamination of coliforms and were tested negative for coliform load. Among 9 sample each of panipuri and chanachatpate collected, 2(22.2%) samples tested positive for coliform in panipuri and 3(33.3%) samples tested positive for coliform in chanachatpate respectively.

In the 60 street food samples tested by (Bhaskar et al 2004), the Coliform count of more than 10^5 cfu/g was detected in 21 samples. According to the data provided by (Madueke et al 2014), the mean coliform count ranged between 5.0×10^5 cfu/g (yam) and 1.06×10^7 cfu/g (suya). The mean count of coliform in 160 ready-to-eat street foods samples like 40 each of firfir which is a mixture of majority of cabbage, watt, macaroni and injera; bread, injera (an Ethiopian traditional food) and sambussa samples study reported by (Reda et al 2017) was between 1.7 to 4.0 cfu/gm.

Similarly, from the data provided by (Shaltot et al 2015) the mean values in the examined samples of street vended burger, kofta, sausage and hawawshi were 6.53×10^2 , 9.25×10^2 , 4.38×10^3 and 1.12×10^4 cfu/g for coliform count respectively. This results obtained were relatively agree to some extent with those obtained by (Khater et al 2013) who found that the mean value of total coliform count of RTE street vended grilled kofta was 2.92 log cfu/g. Also higher results were recorded by (Shaltout et al 2013) which was

2.6×10^5 cfu/g in the examined street vended kofta.

E. coli are the most common of the coliforms and it is primary indicator organism of fecal contamination in food and water and possible presence of other enteric pathogens. From the present study, the level of contamination of *E. coli* was recorded only in chana-chatpate (33.33%) which was found similar to that of study conducted by (Tuladhar & Singh 2015). Whereas in other samples, *E. coli* was not recorded this indicates that the foods are free from fecal contamination. In another similar study performed by (Yadav et al 2019) and by (Tambekar et al 2011). 38.09% and 41% of *E. coli* was present in pani-puri samples respectively. The presence of *E. coli* on different street food samples may be the indication of lack of hygiene and poor water quality during preparation and processing of food (Poojara and Krishna 2012). From the study conducted by (Khadka et al 2018) the prevalence rate of *E. coli* in Panipuri was found to be 10.0 while a similar study carried out by (Garode and Waghode 2012) in Buldana, India, documented comparatively higher prevalence of *E. coli* (80.0%) in Panipuri.

From our study, the highest load of *Staphylococcus aureus* was isolated from pani-puri which was 91.6×10^3 cfu/ml followed by samosa (37.3×10^3 cfu/ml) and least was observed in mo:mo 1.7×10^3 cfu/ml. In the study conducted by (Yadav et al 2019) and (Sharma and Mazumdar 2014), *Staphylococcus aureus* in the food samples was found to be 45.23% and 14.20% respectively. While in another study conducted by (Khadka et al 2018). *S. aureus* in Panipuri was found to be 16.6%. And in a similar work conducted by (Subhashini et al 2014) showed that Pani was highly contaminated by *Staphylococcus aureus*.

Staphylococcus aureus are the common cause of food poisoning in human due to its production of enterotoxin (Argudín et al 2010) and it also cause a diverse array of diseases, ranging from relatively harmless localized skin infections too life threatening systemic conditions (Bukowski et al 2010). The presence of *Staphylococcus aureus* in food product is mainly associated with poor personal hygiene, improper handling of cooked or processed foods, use of low quality food products, followed by improper storage of food under improper conditions. Although cooking at proper temperature for appropriate time may destroy the organism but the toxin it

produced is heat stable. The area wise distribution table showed that the food samples collected from area Kathmandu consisted of highest number of *S. aureus* with the load of 70.25×10^3 cfu/ml. The contamination of street food particularly with *S. aureus* may indicate contamination from skin, mouth and nose of sellers and customers since it is the normal flora of the human body.

The study demonstrated that popularly sold street foods collected from different areas of Kathmandu valley were found to be contaminated by microorganisms together with food-borne pathogens like *E. coli*, *S. aureus*, etc which is very risky to the health of human population.. The detection of fungal organism in food samples help in determining the shelf life duration of a food. Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage. The main source of Staphylococcal contamination may be due to the poor hygiene of vendor and customers and the use of polluted water during processing in the street foods

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CONCLUSION

This study concludes that *E. coli* and coliforms are the most common contaminant in channa chatpate sold in streets. Panipuri and channa chatpate sold in streets. This study concludes that *E. coli* and coliforms are the most common contaminant in channa chatpate sold in streets. Panipuri and channa chatpate sold in streets have the highest microbial load and hence more chances of harboring potential pathogens. A street food sample in Kathmandu is mostly contaminated with the *Staphylococcus aureus*.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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