Microbial Quality Analysis of Panipuri Samples Collected from Different Parts of Bhaktapur

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ABSTRACT

Objectives: The study was aimed to identify potential bacterial pathogens from the panipuri samples collected from different vendors of Bhaktapur district and determine their antibiogram patterns.

Methods: Altogether 120 (40 puri, 40 stuffing and 40 pani) samples of panipuri were collected from different vendors of Bhaktapur district in a cold chain and were transported to the microbiology laboratory. Puri and stuffing were then pre-enriched, enriched and cultured while pani samples were processed using the MPN method. All the isolates were identified following standard microbiological procedure and subjected to antibiotic susceptibility testing following CLSI guidelines.

Results: The result revealed contamination of 77.5% stuffing, 67.5% puri and 52.5% pani samples with pathogenic bacteria. Among bacterial pathogens, the highest number was *Staphylococcus aureus* followed by *Escherichia coli* and *Salmonella* spp. 100% of *S. aureus* were found to be resistant to ampicillin and novobiocin. Similarly, 94.1% of *E. coli* were resistant to ampicillin followed by ciprofloxacin (64.7%). A very few isolates of *Salmonella* spp*, Shigella* spp and *Vibrio* spp were resistant to tetracycline. The highest number of multidrug-resistant bacteria were *S. aureus*, followed by *Klebsiella* spp and *E. coli.*

Conclusion: The study showed that the panipuri samples from street vendors were found to be highly contaminated with pathogenic bacteria which might affect consumers' health. Thus, to prevent any food-borne illness in the future, frequent evaluation and regulation of the quality of such foods should be carried out.

Keywords: Street foods, panipuri, bacterial pathogens, antimicrobial-resistant, multidrug-resistant

INTRODUCTION

According to (FAO 1997), street food is "ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in the street and other similar public places". People nowadays want to save time and money, whenever possible, thus the consumption of street food is growing enormously (Tuladhar and Singh 2012). Consequently, in developing countries, drinks, meals, and snacks sold by street food vendors are widely consumed (FAO 1988).

Street foods are becoming more popular these days in Nepal and the most popular street foods in Nepal are "Panipuri" or "Phulki" and "Chatpate". Although it is very popular, easily available and cheap, it is frequently associated with various food-borne diseases in India and elsewhere (FAO 1988, Estrada-Garcia et al. 2004, Chumber et al. 2007, Ghosh et al. 2007).

Panipuri and its constituents support growth of microorganisms since these are suitable media for the microorganisms (Dassana 2010; Pearce 2016; Ladage 2017). Panipuri is usually sold in open unhygienic surroundings often dwelt with houseflies and air-borne dust and vending places are usually congested. Water is a very critical raw material for street food but its supply is also limited. Many times, biological, physical and chemical contamination is also common in water that is used for processing street food, washing the containers and utensils and even water used for drinking (WHO 1996). Contact with sewage water contaminates street food with bacteria like *Salmonella* spp*, Shigella* spp, *Campylobacter* spp*.* and *E. coli* (Frelund et al. 1987; Blostein 1993; Beuchat 1996 and Gayler et al. 1955). Apart from this, it is often sold at roadsides without running water sources that require utensils and hands to be washed in a single bucket, unhygienic preparation and handling, negligence like they do not use gloves while serving the food also easily contaminate the food and open-drain area (Tambekar et al. 2009) can cause food-borne disease like diarrhoea, vomiting, etc. Hence, due to a lack of knowledge about health and hygiene between vendors, inappropriately prepared, stored and served food products have raised a question regarding their microbiological quality (Nyenje et al. 2012).

Food-borne illness associated with the consumption of street food has been reported in various places of India (Das et al. 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 et al. 2012). Studies conducted in America, Asian and African countries have revealed increased bacterial pathogens in the food (Das et al. 2010). Panipuri is displayed openly in the streets and its preparation is also questionable. Thus, from the consumer health point-of-view, the microbial quality of street vended food becomes very important as food acts as a major source for transmission of food intoxications (Barro et al. 2006). Street food vendors are mostly uninformed of good hygiene practices and causes of diarrheal diseases (Mensah et al. 2002), which can increase the risk of street food contamination (Bhaskar et al. 2004; Tambekar et al. 2009). Therefore, Street foods are the major cause of health problems due to the lack of basic infrastructure and services, and difficulty in controlling a large number of vendors because of their diversity, mobility and temporary nature (De sausa 2008). To analyze the microbial quality of the panipuri, this research has been conducted to isolate the potential bacterial pathogens in those samples and to determine their antibiogram patterns.

METHODS

Study site and duration: The laboratory investigation of this project was carried out in Sainik Awasiya Mahavidhyalaya, Sallaghari: Bhaktapur, Nepal. The study was conducted from October 2017 October to 2018 February.

Sample collection, transport and processing: A total of 120 panipuri samples were collected from different parts of Bhaktapur district, Nepal. The samples were collected separately as pani, puri and stuffing (every 40 samples) in sterile containers containing ice packs; and were transported to the laboratory within an hour and were processed immediately following standard procedure.

Laboratory procedure

Pre-enrichment and enrichment: For the bacterial analysis, the solid samples (puri and stuffing) were pre-enriched on buffered peptone water and further enrichment was done on selenite F broth and alkaline peptone water.

Isolation and identification of bacterial pathogens: For the isolation of *S. aureus* and non-fastidious Gram-negative pathogens, the pre-enriched samples were cultured on Mannitol Salt Agar (MSA) and Mac-Conkey agar (MA). The isolated colonies from MSA and MA were identified based on standard microbiological procedures including colony morphology and biochemical tests. Whereas the enriched samples on selenite broth were cultured on XLD for isolation and identification of *Salmonella* spp and *Shigella* spp. Likewise, the enriched samples in alkaline peptone water for cultured on TCBS agar plates. The bigger golden-yellow colonies were further processed by biochemical tests and serotyping for identification of *V. cholerae* (Cheesbrough 2006).

The pani samples were subjected to the MPN method for isolation and identification of coliforms (Cappuccino and Sherman 2009). The colonies obtained from the culture on EMB agar were identified by using standard microbiology methods including colony morphology, Gram staining and biochemical tests.

Antibiotic susceptibility testing and multidrug-resistant: The identified bacterial isolates were subjected

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to antibiotic susceptibility testing by modified Kirby Bauer disc diffusion method on MHA plates following CLSI guidelines (CLSI 2017). Similarly, the isolates that were resistant to 3 or more different classes of antibiotics were considered multidrug-resistant strains (Magiorakos et al. 2011).

RESULTS

Growth on panipuri samples: Out of 40 stuffing, 40 puri samples, 77.5% of stuffing and 67.5% of puri samples showed bacterial growth on different culture media. The pH range of pani was 2.0 to 3.9. Out of 40 samples processed by the MPN method, 21 samples from pani were contaminated with faecal coliforms*, E. coli*. Because of the acidic nature of pani samples, only *E. coli* was studied in pani samples (Figure 1).

Frequency of bacterial pathogens: A total of seven different types of bacterial pathogens have been identified from 120 panipuri samples. The highest number of bacterial pathogens were *S. aureus* (37/117) followed by *E. coli* (34/117) and *Salmonella* spp (19/117). Only 2 *V.*

cholerae isolates were confirmed from stuffing samples (Table 1).

Antibiotic susceptibility pattern: All *S. aureus* were resistant to ampicillin followed by chloramphenicol (67.6%) and cotrimoxazole (67.6%). Among Gram-negative pathogens, the highest number of isolates were resistant to ampicillin. 100% of *Klebsiella* spp and *V. cholerae* were non-susceptible to ampicillin. Likewise, 94.1% of *E. coli* isolates were resistant to ampicillin. Among *Salmonella* isolates, 42.9% of *S.* Typhi and 66.7% of *S.* Paratyphi were resistant to ampicillin respectively. All *Salmonella* isolates were sensitive to methicillin and novobiocin. Erythromycin, gentamicin and tetracycline were found to be effective drugs against Gram-negative bacterial pathogens isolated in this study (Table 2)

Multidrug-resistant pathogens: The highest number of multidrug-resistant bacteria were *S. aureus* which was 67.6% followed by *Klebsiell*a spp (57.1) and *E. coli* (52.9%). Out of 2 *V. cholerae* isolates, 1 isolate was found to be MDR strain (Table 3).
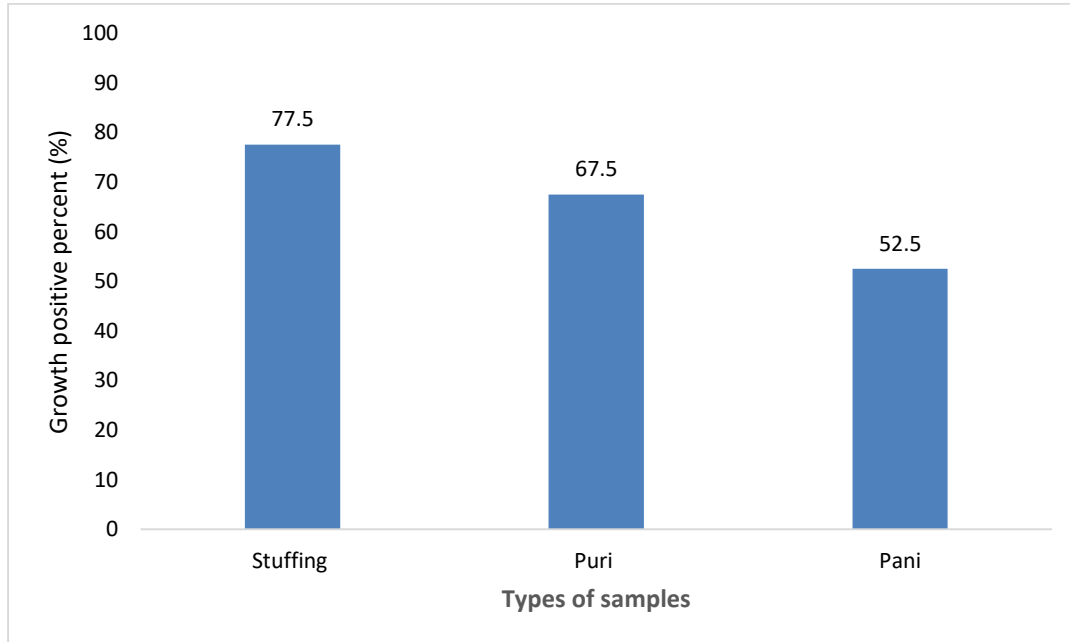

Figure 1: Bacterial growth percentage on different samples

Table 1: Number of bacterial pathogens isolated from different samples

Bacterial pathogens	Stuffing samples (n=40)		Puri samples (n=40)		Pani samples (n=40)		Total no.
	No.	Percent %	No.	Percent %	No.	Percent %	
Staphylococcus aureus	25	62.5	12	30.0	ND	ND	37
Klebsiella spp	11	27.5	3	7.5	ND	ND	14
Salmonella Typhi	7	17.5	0	0	ND	ND	7
Salmonella Paratyphi	9	22.5	3	7.5	ND	ND	12
Shigella spp	8	20.0	3	7.5	ND	ND	11
Vibrio cholerae	2	5.0	0	0	ND	ND	2
E. coli	8	20.0	5	12.5	21	52.5	34
Total no. of isolates	**70**		**26**		**21**		**177**

n= no of samples used, ND: not done

Table 2: Antibiotic susceptibility testing of bacterial pathogens isolated from the samples

Antibiotics used	Number of resistant bacteria (%)						
	S. aureus	*Klebseilla* spp	*S.* Typhi	*S.* Paratyphi	*Shigella* spp	*V. cholerae*	*E. coli*
	n=37	n=14	n=7	n=12	n=11	n=2	n=34
Ampicillin	37 (100)	14 (100)	3 (42.9)	8 (66.7)	9 (81.8)	2 (100)	32 (94.1)
Chloramphenicol	25 (67.6)	7 (50)	1 (14.3)	1 (8.3)	2 (18.2)	0 (0)	8 (23.5)
Ciprofloxacin	23 (62.2)	8 (57.1)	4 (57.1)	11 (91.7)	3 (27.3)	0 (0)	22 (64.7)
Cotrimoxazole	25 (67.6)	12 (85.7)	1 (14.3)	2 (16.7)	1 (9.1)	2 (100)	18 (52.9)
Erythromycin	7 (18.9)	6 (42.9)	0 (0)	1 (8.3)	0 (0)	1 (50)	13 (38.2)
Gentamycin	17 (45.9)	7 (50)	0 (0)	1 (8.3)	0 (0)	0 (0)	18 (52.9)
Methicillin	11 (29.7)	3 (21.4)	0 (0)	0 (0)	1 (9.1)	0 (0)	7 (20.6)
Novobiocin	4 (10.8)	5 (35.7)	0 (0)	0 (0)	0 (0)	0 (0)	8 (23.5)
Tetracycline	16 (43.2)	3 (21.4)	1 (14.3)	2 (16.7)	2 (18.2)	0 (0)	16 (47.1)

Table 3: Multidrug-resistant strains among the bacterial pathogens

Organisms	Total no. of isolates	No. of MDR (%)
Gram-negative organisms		
Escherichia coli	34	18 (52.9)
Klebsiella spp	14	8 (57.1)
Salmonella Typhi	7	1 (14.3)
Salmonella Paratyphi	12	2 (16.7)
Shigella spp	11	2 (18.2)
Vibrio cholerae	2	1 (50)
Gram-positive organisms		
Staphylococcus aureus	37	25 (67.6)

DISCUSSION

Readily prepared food in the street for consumption varies from place to place according to their cultural practices and social traditions. Among them, panipuri is one of the popular street foods sold in South- Asian countries (Pearce 2016). It originated in India and is very popular in Nepal as well. The present research work was undertaken to find out the presence of pathogenic bacteria, especially *S. aureus*, *E. coli, Klebsiella* spp, *Salmonella* spp, *Shigella* spp, and *Vibrio* spp from panipuri collected from different places of Bhaktapur district of Nepal. Our studies show higher bacterial growth in stuffing and puri than in pani; this result is in agreement with the study done by Tambeker et al (2008). It is explained by the low acidity (6.1) and more nutrient content of stuffing (Campbell 2017). In most cases running tap water is not available at vending sites; hand and dishwashing are usually done in the same buckets and without soap water. The serving plates or the container are not properly washed. Unhygienic food handling practices increase contamination.

Of 120 panipuri samples collected from 40 vendors during the study period at Bhaktapur district were categorized into stuffing, puri and pani samples. More than 50% of each sample was found to be contaminated with bacterial pathogens including *S. aureus, Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *V. cholerae* and *E. coli.* Out of 80 samples of stuffing and puri, 62.5% of both stuffing and 30% of puri were found contaminated with *S. aureus* which was the highest rate among bacterial pathogens. This indicated cross-contamination of the food with the skin flora of vendors due to their poor hygiene and improper handling of food. During sample collection, we observed more than 90% of vendors (37/40) did not wear globes during panipuri preparation. The panipuri might have been also contaminated by consumers and the environment since that stuff was kept open in those vendors. The presence of *Klebsiella* spp in our study was 27.5% and 7.5% on stuffing and puri respectively, which might be due to carrier vendors as found in the study conducted by Gieser et al. (2011) or improperly washing of raw ingredients used on potato stuffing with contaminated water which is supported by the result of the study conducted by Podschun et al. (2001) in which *Klebsiella* spp were isolated from the surface water sample. A total of 16 *Salmonella* spp on stuffing and 3 isolates on puri samples were isolated. Out of which, 7 were *S.* Typhi and 12 were *S.* paratyphi. The presence of *Salmonella* spp, *Shigella* spp and *V. cholerae* were directly associated with the use of faecal contaminated water during panipuri preparation. This statement was supported by our study on pani samples by MPN method. The MPN analysis of pani samples indicated 52.5% contamination with *E. coli.* In the earlier study on analysis of street foods of Kathmandu carried out by Tuladhar and Singh (2012) showed that all the food samples analyzed were contaminated with bacteria including *S. aureus* followed by *Bacillus alvei, Escherichia coli, Enterobacter aerogenes, Bacillus subtilis, Serratia* spp, *S. saprophyticus*. However, the result of this study is different from ours as the bacteria like *Salmonella* spp; *Shigella* spp, *Vibrio* spp and *Klebsiella* spp were not isolated in this study. This variation may be due to the environmental condition around the food stalls or due to the hygienic condition of the food vendors. Even the type of ingredients used on the stuffing i.e stuffing may cause variations in the result as suggested by the study conducted (Nyenje et al. 2012).

On antibiotic susceptibility testing, 100% isolates of *S. aureus* from both stuffing and puri were found to be resistant to ampicillin; this result was in agreement with the study done by Bouza et al (2002). The study conducted in India by Das et al. (2010) showed 92.3% and 72.4% antibiotic resistance on *Salmonella* spp and *Shigella* spp respectively. Our result showed that 100% of both *Salmonella* Typhi and *Shigella* spp isolated were sensitive to gentamicin which contradicts with the findings of Kumar et al. (2017) as their study showed that 20.88% and 2.08% *Salmonella* spp and *Shigella* spp respectively were resistant to gentamicin. Similarly, none of *V. cholerae* isolates were found to be resistant to tetracycline; however, the result shown by Raissy et al. (2012) contradicted our result in which 18.1% of *Vibrio* isolates were found to be resistant to tetracycline. It might be due to non-cholera *Vibrio* isolates in their study (Intyre and Feely 1965).

Out of 40 samples of pani, MPN test revealed that 52.5% of the samples were contaminated with *E. coli*. The absence of other bacteria in pani is due to the low pH of the pani, as most bacteria favor growing in neutral conditions (Thompson 2017). *E. coli* isolated from pani was found to be highly resistant to ampicillin, ciprofloxacin and cotrimoxazole; however, the study done by Nazir et al. (2007) showed that *E. coli* isolates were sensitive to chloramphenicol and ciprofloxacin. The presence of *E. coli* in pani indicates the use of fecal contaminated and untreated water. In addition, the pH of the water samples was found to be in the range of 2.0-3.9 which correlates with the pH range of tamarind water pH (range, 1.8 to 3.7; mean, 2.8) (Nassereddin and Yamani 2005).

All different classes of antibitics as recommended by CLSI guidelines couldn't be used in this study.

CONCLUSION

This study indicated panipuri of different parts of Bhaktapur district were highly contaminated with pathogenic bacteria which can contribute to potential health risks for consumers. Both vendors and consumers should be aware of the possible infections and good food hygiene practices for prevention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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