



Bacterial contamination and risk factors associated with street-vended Panipuri sold in Bharatpur, Nepal

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ABSTRACT

This study was designed to investigate the bacterial contamination of street-vended Panipuri and their possible risk factors. In a cross-sectional study, 300 samples (100 each of Pani, Puri and Masala) were aseptically collected from various locations of Bharatpur and processed at Microbiology Laboratory of Birendra Multiple Campus using standard microbiological techniques. Microbial analyses revealed that 164(54.6%) samples were contaminated with 13 different species of bacterial pathogens of which *Staphylococcus aureus* 50(16.6%) followed by *Escherichia coli* 30(10.0%) and *Citrobacter* spp. 28(9.3%) were the most prevalent ones. The highest proportion of both Pani (12.0%) and Puri (20.0%) were contaminated by *S. aureus* whereas the highest incidence of *E. coli* (22.0%) was seen in Masala. On antibiotic susceptibility testing, of the 212 isolates, 11(36.7%) *E. coli*, 8(50.0%) *Pseudomonas* spp., 6(33.3%) *Salmonella* Typhi and 4(14.8%) *S. aureus* were found to be multi-drug resistant (MDR). Significant association was noted between the rate of contamination of Panipuri with various factors pertaining to the vendors such as gender, literacy level and personal hygiene as well as with the location of sampling ($p < 0.01$). Vendors' awareness to improve their hygienic behavior during preparation, handling, serving and storing of street-foods is recommended.

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INTRODUCTION

Food and Agriculture Organization (FAO) defined street foods as "ready-to-eat foods and beverages which are prepared and/or sold by vendors especially in streets and other similar public places" (FAO, 1989). Streets vendors sell the varieties of food items such as drinks, meals and snacks which are consumed by millions of peoples in many countries (Fellows and Hilmi, 2011). Even though they are too famous, easily accessible and economical, they are regularly associated with numerous food-borne diseases. Food-borne diseases resulting from the consumption of unhygienic street-vended foods, drinks, meals and snacks have been reported by Estrada-Garcia

et al. (2004), Chumber et al. (2007) and Ghosh et al. (2007).

Panipuri, which is known as Golgappa and by some other names, comprises three different components: i) golgappa/patasha/puri/papri, ii) filling or masala, iii) spicy water or pani. Wheat flour is used to make Puri. In Masala, boiled or mashed potatoes mixed with spices are used. The Pani is sour water to which spices like salt, pepper, mango powder, jaljeera etc. are added. In every Puri, Masala are added after making a hole in it and then spicy water is filled in this Puri and served to the consumers in plate (Saxena and Agarwal, 2013).

Contamination of street-vended food like Panipuri is associated with poor sanitary practices including poor personal hygiene of the vendors, unhygienic management of foods, and use of dirty dishes and improperly

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washed raw vegetables (Das et al., 2012). Unhygienic Panipuri is harbored by potentially life-threatening bacteria like *Salmonella* Typhi and *Escherichia coli* (Garode and Waghode, 2012). The spicy water of Panipuri is found to be contaminated with different bacterial pathogens like *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and yeast. Food-borne and diarrheal diseases are caused by these bacterial pathogens (Tambekar et al., 2011). Bacteria isolated from Panipuri in some cities in India were found to be MDR (Mary and Usha, 2013; Gulati and Chakraborty, 2017). Street-vended foods like Panipuri are sold almost in all the cities in Nepal and are popular among people of all the ages, especially the adolescent girls. However, there are very few reports published regarding the bacteriological conditions of Panipuri sold in the streets throughout the country. In light of this background, this study was carried out to investigate the bacterial contamination of street-vended Panipuri sold in Bharatpur city and to determine the possible risk factors.

MATERIALS AND METHODS

Study design and area

A cross-sectional study was carried out in the major streets and markets in Bharatpur metropolis, Nepal from May 2017 to September 2017. The study covered six different areas in Bharatpur city (Narayani riverside, Sahidchowk, Belchowk, Lionschowk, Dipendrachowk and Bypass road) where the number of Panipuri vendors and their customers are comparatively higher.

Data collection

Altogether, 100 Panipuri vendors from six different locations in Bharatpur who gave their approval were recruited into the study and the vendors were assured of total confidentiality. Vending sites' hygiene and salubrious status were studied by brief interview using semi-structured questionnaire and through observations as well.

Sample collection and processing

A total of 300 samples (100 Masala, 100 Pani and 100 Puri) were collected from the street vendors. Pani, Puri and Masala samples were collected separately in sterile zip-locked plastic bags and transported aseptically to Microbiology Laboratory of Birendra Multiple Campus for further processing within 1 h. Each Puri and Masala items was homogenized prior to inoculation onto culture media. For this, 1 g sample was mixed with 9 mL sterile water,

crushed and ground in sterile mortar and pestle. Whereas, a loopful of Pani sample was directly inoculated onto culture media without homogenization. Various culture media like nutrient agar, MacConkey agar, thiosulphate citrate bile-salt sucrose agar, xylose lysine deoxycholate agar, mannitol salt agar and eosin methylene blue agar were used for inoculation of samples and the plates were incubated at 37°C for 24 h (Forbes et al., 2007).

Identification of bacterial isolates

All the isolates were first differentiated by colony morphology and Gram's staining reaction. They were further identified by an array of biochemical tests. For Gram-positive isolates, various biochemical tests such as oxidase, catalase, coagulase, DNase, oxidative/fermentative, methyl-red and Voges-Proskauer were performed. For Gram-negative isolates, indole, methyl-red, Voges-Proskauer, citrate, triple sugar iron agar, catalase, oxidase, urease and oxidative/fermentative tests were performed (Forbes et al., 2007).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute using Mueller-Hinton agar (CLSI, 2015). Antibiotics such as tetracycline (30 µg), nitrofurantoin (300 µg), cotrimoxazole (30 µg) and, cefotaxime (30 µg) were tested against Gram-negative isolates whereas azithromycin (15 µg), cefoxitin (30 µg) and vancomycin (10 µg) were tested against *S. aureus*. In addition, gentamicin (10 µg), ciprofloxacin (5 µg) and ampicillin (10 µg) were tested against both Gram-positive as well as Gram-negative bacteria. Resistance to three or more than three classes of antimicrobials tested was considered as MDR (Magiorakos et al., 2012). For identification and standardization of the Kirby-Bauer test, standard culture of *E. coli* ATCC 25922 was used as a reference strain.

Data analysis

All the collected data were tabulated and analyzed by using SPSS version 20. P-value < 0.01 was considered to have significant association.

Ethical considerations

Permission to conduct the work was obtained from the Department of Microbiology, Birendra Multiple Campus.

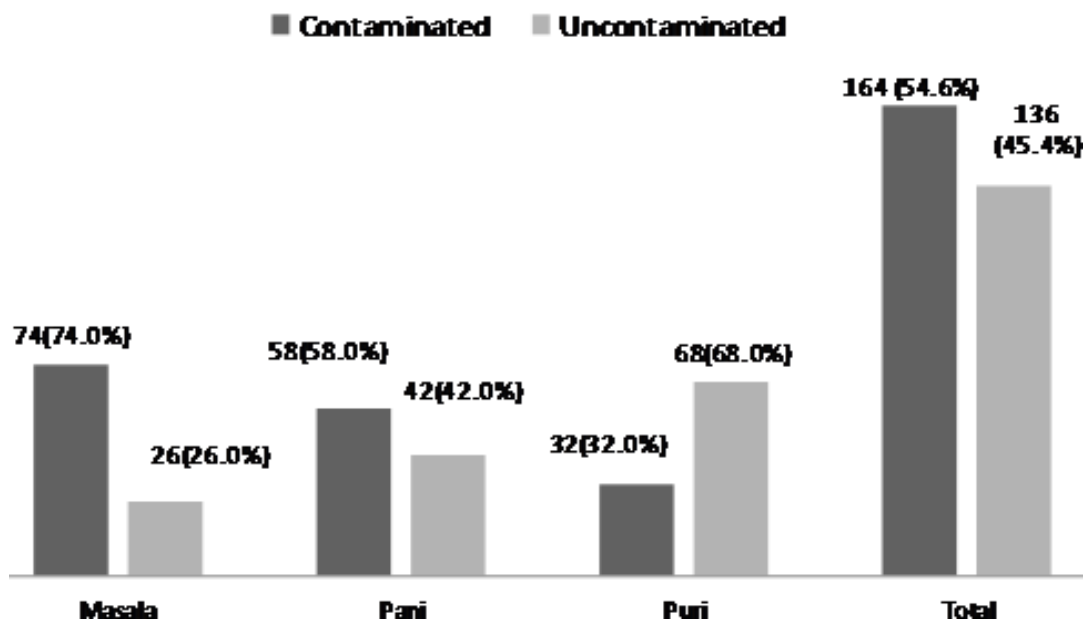


Figure 1. Frequency of contamination of Masala, Pani and Puri.

Table 1. Distribution of contaminated Masala, Pani and Puri samples.

S/N	Bacterial isolates	Masala (N=100)	Pani (N=100)	Puri (N=100)	Total (N=300)
1	<i>E. coli</i>	22	6	2	30(10.0%)
2	<i>Klebsiella</i> spp.	8	-	-	8(2.6%)
3	<i>Enterobacter</i> spp.	4	6	-	10(3.3%)
4	<i>Citrobacter</i> spp.	18	6	4	28(9.3%)
5	<i>Acinetobacter</i> spp.	-	6	-	6(2.0%)
6	<i>Pseudomonas</i> spp.	8	8	-	16(5.3%)
7	<i>P. mirabilis</i>	-	6	-	6(2.0%)
8	<i>P. vulgaris</i>	2	6	-	8(2.6%)
9	<i>S. Typhi</i>	8	10	-	18(6.0%)
10	<i>S. Paratyphi</i>	6	4	-	10(3.3%)
11	<i>Shigella</i> spp.	6	2	-	8(2.6%)
12	<i>S. aureus</i>	18	12	20	50(16.6%)
13	<i>B. cereus</i>	4	-	10	14(4.6%)

Verbal informed consent was also sought from each study participant after explaining the objective of the study. All the information about the study participants were kept strictly confidential.

RESULTS

Out of 300 samples analyzed, 164(54.6%) samples were contaminated by pathogenic bacteria that comprised of 74 Masala, 58 Pani and 32 Puri items (Figure 1).

Altogether, 13 different bacterial species were identified in the study. Higher incidence of *E. coli* was observed in Masala items (22.0%) whereas *S. aureus* was more prevalent in both Pani (12.0%) and Puri (20.0%). The most contaminating bacteria was *S. aureus* (16.6%) followed by *E. coli* (10.0%) and *Citrobacter* spp. (9.3%) whereas *Acinetobacter* spp. and *Proteus mirabilis* were the least prevalent bacterial pathogens both contaminating only 2.0% of the total samples (Table 1).

Masala (67.6%) and Pani (79.4%) sold by the female vendors were more contaminated by bacteria compared

Table 2. Association of various variables with the rate of bacterial contamination of Masala, Pani and Puri items.

S/N	Attributes (vendors)		Number of contaminated items				Total contaminated items (N=164)		
			Masala	P-value	Pani	P-value		Puri	P-value
1	Gender	Male	24(32.4%)	<0.01	12(20.6%)	<0.01	8(25.0%)	0.018	44(26.8%)
		Female	50(67.6%)		46(79.4%)		24(75.0%)		120(73.2%)
2	Age	15-30	38(51.3%)	0.1	24(41.4%)	0.2	20(66.7%)	0.043	82(50.0%)
		31-45	28(37.8%)		30(51.8%)		12(33.3%)		70(42.6%)
		46-60	8(10.9%)		4(6.8%)		-		12(7.4%)
3	Literacy rate	Literate	30(40.5%)	<0.01	16(27.5%)	<0.01	10(31.2%)	0.052	56(34.1%)
		Illiterate	44(59.5%)		42(72.5%)		22(68.8%)		108(65.9%)
4	Use of gloves	Use of plastic gloves on hands	15(20.3%)	<0.01	18(31.0%)	<0.01	12(37.5%)	0.077	45(27.4%)
		Bare hands	59(79.7%)		40(69.0%)		20(62.5%)		119(72.6%)
5	Food storage	Covered	22(29.7%)	<0.01	12(20.7%)	<0.01	8(25.0%)	0.034	42(25.7%)
		Uncovered	52(70.3%)		46(79.3%)		24(75.0%)		122(74.3%)
6	Area of sample collection	Narayani riverside	24(32.5%)	<0.01	26(44.8%)	<0.01	14(43.7%)	0.018	64(39.0%)
		Lionschowk	8(10.8%)		10(17.2%)		2(6.3%)		20(12.2%)
		Sahidchowk	10(13.5%)		10(17.2%)		8(25.0%)		28(17.0%)
		Belchowk	12(16.2%)		6(10.4%)		4(12.5%)		22(13.4%)
		Dipendrachowk	12(16.2%)		0(0.0%)		0(0.0%)		12(7.4%)
	Bypass road	8(10.8%)	6(10.4%)	4(12.5%)	18(11.0%)				
7	Nails cutting	Nail cutters	26(35.1%)	<0.01	16(27.6%)	<0.01	10(31.2%)	0.029	52(31.7%)
		Non-nail cutters	48(64.9%)		42(72.4%)		22(68.8%)		112(68.3%)

to those sold by the males ($p < 0.01$). Similarly, Panipuri sold by illiterate vendors showed higher incidence of bacterial contamination in Pani (72.5%) and Masala (64.9%) items ($p < 0.01$). Higher incidence of contamination was revealed in Masala (64.9%) and Pani (72.4%) items sold by the vendors with long nails ($p < 0.01$). Masala (70.3%) and Pani (79.3%) items which were left and stored uncovered harbored higher proportions of bacteria ($p < 0.01$). Masala (32.5%) and Pani (44.8%) items sold at Narayani riverside were more contaminated by bacteria than other areas ($p < 0.01$) (Table 2).

Antibiotic sensitivity test was performed for all the isolates except for *Bacillus cereus*. Gentamicin was the most effective antibiotic whereas ciprofloxacin was the least effective antibiotic for Gram-negative isolates. Majority of the *E. coli* isolates (93.3%) were sensitive to gentamicin whereas a larger proportion of them (60.0%) were resistant to ciprofloxacin. Nitrofurantoin was the least effective antibiotic for both *Pseudomonas* spp. (62.5%) and *S. Typhi* (77.7%). For *S. aureus*, vancomycin (100.0%) was the most effective antibiotic. The number of methicillin resistant *S. aureus* (MRSA) detected was 16(32.0%). Altogether, 11(36.7%) *E. coli*, 8(50.0%) *Pseudomonas* spp., 6(33.3%) *S. Typhi* and 4(14.8%) *S. aureus* were found to be MDR (Table 3). No any MDR isolate was detected from Puri samples (Table 4).

DISCUSSION

In the present study, the most frequent contaminating bacteria in Panipuri was *S. aureus* (16.6%). About 20.0% of Puri was contaminated by *S. aureus*. In a similar work, Subhashini et al. (2014) in Tamil Nadu, India, showed that Pani was highly contaminated by *S. aureus* and *Bacillus* spp. Majority of the vendors were found to serve with ungloved hands in our study. *S. aureus* in the food samples might be from direct human interaction, such as skin and diseased cuts, or indirectly through spices or tools (Akharaiyi and Adeyanju, 2016). The prevalence of *E. coli* and *S. Typhi* in Panipuri was 10.0 and 6.0%, respectively. A similar study carried out by Garode and Waghode (2012) in Buldana, India, documented comparatively higher prevalence of *E. coli* (80.0%) and *S. Typhi* (40.0%) in Panipuri; whereas a study performed by Saxena and Agarwal (2013) in Rajasthan, India, reported that *Salmonella* spp. and *Bacillus* spp. were absent from all Pani, Puri and Masala items.

It was observed that 10.0% of Pani and 8.0% of Masala samples were contaminated by *S. typhi* whereas they were completely absent from Puri. A large number of Puri showed the presence of *B. cereus*. The occurrence of *B. cereus* in Panipuri sample may be of great concern due to the ubiquitous nature of their spores particularly in and

Table 3. Antibiotic resistant and MDR patterns of the isolates.

Isolates	GEN	CIP	NIT	COT	TE	AZ	CX	VA	CTX	AMP	MDR
	R	R	R	R	R	R	R	R	R	R	
<i>E. coli</i>	2	18	5	16	7	NT	NT	NT	5	6	11(36.7%)
<i>Klebsiella</i> spp.	-	2	2	1	-	NT	NT	NT	2	3	
<i>Enterobacter</i> spp.	-	4	-	4	1	NT	NT	NT	-	1	
<i>Citrobacter</i> spp.	-	4	4	2	-	NT	NT	NT	-	2	8(50.0%)
<i>Acinetobacter</i> spp.	-	2	-	2	-	NT	NT	NT	4	2	
<i>Pseudomonas</i> spp.	4	9	10	8	3	NT	NT	NT	8	9	
<i>P. mirabilis</i>	-	2	-	2	1	NT	NT	NT	-	-	6(33.3%)
<i>P. vulgaris</i>	-	2	2	-	-	NT	NT	NT	-	-	
<i>S. Typhi</i>	-	4	14	12	2	NT	NT	NT	2	4	
<i>S. Paratyphi</i>	-	-	9	4	-	NT	NT	NT	1	1	4(14.8%)
<i>Shigella</i> spp.	-	5	-	2	-	NT	NT	NT	-	-	
<i>S. aureus</i>	2	4	NT	NT	NT	5	16*	-	NT	4	
<i>B. cereus</i>	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	29(14.6%)
Total											

Note: NT, Not tested; R, resistant; GEN, gentamicin; CIP, ciprofloxacin; NIT, nitrofurantoin; COT, co-trimoxazole; TE, tetracycline; AZ, azithromycin; CX, ceftioxin; VA, vancomycin; CTX, cefotaxime; AMP, Ampicillin.

Table 4. Distribution of MDR isolates among Masala, Pani and Puri items.

MDR isolates	Distribution of MDR isolates			Total isolates
	Masala	Pani	Puri	
<i>E. coli</i>	8	3	0	11
<i>Pseudomonas</i> spp.	4	4	0	8
<i>S. Typhi</i>	2	4	0	6
<i>S. aureus</i>	2	2	0	4
Total	16	13	0	29

polluted sites. Das et al. (2012) reported that *Enterobacter* spp. (28.8%) was the most predominant in Panipuri followed by *E. coli* (13.6%) and *Klebsiella* spp. (10.6%). They also found that *S. Paratyphi* (1.5%), *Micrococcus* (3.0%) and *Bacillus* spp. (3.0%) were the least prevalent bacteria. In this study, Panipuri items were contaminated by *Enterobacter* spp. (3.3%), *Klebsiella* spp. (2.6%) and *Salmonella* Paratyphi (3.3%). *Proteus vulgaris* (2.6%) and *P. mirabilis* (2.0%) were isolated from Panipuri items. In a previous study conducted by Kumar and Jangir (2017) in Rajasthan, India, similar proportion of *Proteus* spp. (2.0%) were noted in the street-vended foods like Samosa and Masala dosa. Hassan et al. (2016) documented a lower prevalence rate of *Proteus* spp. (0.9%) in Chotpoti, a kind of street-food in Dhaka, Bangladesh. In our work, 2.6% of Panipuri items were contaminated by *Shigella* spp. In

Baripada city of Orissa, India, Das et al. (2012) found that 4.5% of Panipuri samples were contaminated by *Shigella* spp. Fingers, flies or non-living objects such as utensils and cutting surfaces can act as the medium to blowout *Shigella* spp. (Levine and Levine, 1991). In our study, 2.0% of Panipuri harbored *Acinetobacter* spp. In Bangladesh, Hassan et al. (2016) reported that 66.6% of Chotpoti were contaminated by *Acinetobacter* spp. Contaminated hands and environmental surfaces indicating poor hand-hygiene of the vendors are the striking reasons for the transmission of *Acinetobacter* spp. (CDC, 2014). *Citrobacter* spp. is ubiquitous as it is a component of normal intestinal flora (Holmes and Aucken, 1998; Ryan, 2004). In our study, only 8.0% of Pani samples were found to be contaminated by *Citrobacter* spp. Vyas (2012) detected *Citrobacter diversus* from all Pani samples in India. There exists a

marked variation in the proportions of different bacterial isolates in Panipuri in different countries. This might be due to the differences in the hygienic conditions of the vendors and vending-sites.

Higher number of bacteria was isolated from Masala and Pani sold by female vendors ($p < 0.01$). This might be because female vendors had to look after their babies even during vending and thus got exposed to dirty environments. Poor hygienic practices leading to food contamination during handling, storage and food preparation are associated with the lower education level of street-vendors (Kitagwa et al., 2006). We noted a significant association between the literacy rate of the vendors and contamination of Pani ($p < 0.01$). This signifies that literate vendors are more conscious about their personal hygiene. Less contamination of Panipuri was noted from the vendors who used plastic gloves instead of bare-hands ($p < 0.01$). Use of plastic gloves can prevent the direct contact of Panipuri with dirty hands and hence may reduce the chance of contamination. In a research work presented in a meeting of the Infectious Disease Society of America in San Francisco, researchers showed that artificial and natural nails longer than 3 mm beyond the tip of the finger, or the length of a pencil tip, transport more harmful bacteria and yeast under them as compared to the short nails (Kauffman, 2016). The present study also showed vendors having long nails sold more contaminated Panipuri than the vendors who cut their nails ($p < 0.01$). Prevalence of microbes was seen higher in uncovered foods ($p < 0.01$). This might be because the vendors sold Panipuri at crowded environment where uncovered foods might get exposed in dust for a long time. A large number of Masala items (74.0%) were contaminated than Pani (58.0%) and Puri (32.0%). Masala items might be more suitable for the growth of bacteria than Pani which is very spicy sour water and Puri which are first fried and later made completely dry. Addition of tamarind and lemon juices to Pani de-creases the pH, which is not suitable for the growth of microbes (Rath and Patra, 2012). A significantly higher rate of contamination was found in those Panipuri vended at Narayani riverside ($p < 0.01$). This might be due to the fact that vendors of this area might have used river water for cleansing purposes.

Gentamicin was the most effective antibiotic for gram-negative bacteria whereas vancomycin for gram-positive bacteria. *E. coli*-11(36.7%), *S. Typhi*-6(33.3%), *Pseudomonas* spp.-8(50.0%) and *S. aureus*-4(14.8%) were found to be MDR. In a similar study, out of 100 samples of Panipuri collected in Bangalore, 74 showed the presence of *E. coli* and most of them were found to be MDR (Mary and Usha, 2013). Our study revealed 3 MDR *E. coli* from Pani samples. Similarly, 4 MDR *E. coli* were detected from Pani samples in Indore, India (Vyas, 2012). Occurrence of MDR isolates from Panipuri in Bharatpur may indicate potential health problems to the consumers

of this region.

Conclusion

The results of the study indicates that the street-vended Panipuri sold in Bharatpur are contaminated with pathogenic bacteria and few of them are MDR. This cautions for the prudent use of antibiotics by the health professionals. Poor personal hygiene of the vendors and improper handling and storage practices were found to be the key factors that contributed to the contamination of Panipuri. Hence, concerned authorities should impart health education to Panipuri vendors to improve their hygienic conditions during the preparation, handling, storing and serving.

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