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CONTENTS

Microbial contamination in panipuri ingredients and utensils

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ABSTRACT: The present study evaluates microbial contamination in panipuri, a popular street food in Uttarakhand, India. A total of 2, 250 samples from various sources, including water, masala, utensils and hands, were analyzed for contamination by *Escherichia coli* (*E. coli*), *Salmonella* spp. and *Staphylococcus* spp. Results revealed significant microbial presence, with 16.04% of samples testing positive for *E. coli*, 11.20% for *Salmonella* and 13.38% for *Staphylococcus*. The findings highlight the need for improved hygiene practices to mitigate health risks associated with street foods. Significant regional variations in contamination were observed, with Rudrapur showing the highest positivity rates. The study highlights the urgent need for improved hygiene practices, clean water supplies and antimicrobial resistance monitoring to ensure food safety and mitigate public health risks.

Key words: Antimicrobial resistance (AMR), foodborne pathogens, microbial contamination, panipuri contamination, public health risks

Panipuri, a beloved Indian snack, is increasingly associated with foodborne illnesses due to unhygienic preparation and serving practices. Contamination by pathogens such as *E. coli*, *Salmonella* and *Staphylococcus* poses significant public health risks. This study investigates the prevalence of microbial contamination in panipuri ingredients, utensils and handling practices in Uttarakhand.

MATERIALS AND METHODS

Study site and Sample Collection

Samples were collected from six locations in Uttarakhand: Pantnagar (n=375), Nagla (n=375), Haldi (n=375), Lalpur (n=375), Rudrapur (n=375) and Kiccha (n=375). A total of 2250 samples 450 samples were collected from each of the 5 sources i.e., main water source, masala water containers, serving spoons, bowls and food handlers' hand were collected. Sampling was conducted during morning and evening shifts.The samples were collected in sterile containers containing ice packs; and were transported to the laboratory within an hour and were processed immediately following standard procedure. The study was carried out from July 2023 to May 2024.

Microbiological Analysis

All the samples were analyzed for the presence of three common pathogens vis a vis. *Escherichia coli* (*E. coli*), *Salmonella* spp. and *Staphylococcus* spp.

Isolation and Identification

The pathogens were isolated by culturing the samples first on pre-enrichment media such as MacConkey broth for *E. coli*, Buffered Peptone Water for *Salmonella* and Nutrient Broth for *Staphylococcus*, followed by inoculating the presumptive isolates on selective media as EMB agar for *E. coli*, Brilliant Green Agar for *Salmonella* and Baird Parker Agar for *Staphylococcus* for appropriate growth. The suspected colonies were observed for morphological characteristics. They were subjected to biochemical confirmation using standard biochemical tests (IMVIC pattern)(Silva *et al.*, 1980).

Antibiotic susceptibility testing

The bacterial isolates were subjected to antibiotic susceptibility testing by using modified Kirby Bauer disc diffusion method (antibiotic discs, Hi Media) on MHA plates following CLSI guidelines (CLSI, 2024). The isolates resistant to 3 or more different classes of antibiotics were considered multidrug-

resistant strains (Magiorakos *et al.,* 2012).

RESULTS AND DISCUSSION

Of the total 2, 250 samples (Table 1) tested, *E. coli* had the highest contamination rate (16.04%), followed by *Staphylococcus* (11.30%) and *Salmonella* (11.20%). Main water sources showed the highest contamination across all three pathogens (62.44%; 281/450). Contamination was also high in masala water containers (44.22%; 199/450), hands (34.4%; 155/450) serving spoons (26%; 117/450) and bowls (26%;117/450). The *E. coli* was the highest contaminant among the four sources i.e., main water source (22.88%), water container (18.22%), serving spoon (17.30%) and bowls (14.20%) whereas hands exhibited significant contamination with *Salmonella* (14.8%). While *Salmonella* spp. was highest in hand samples (14.80%).

Evening samples had higher contamination rates compared to morning samples for all three pathogens. It was 68.60% for *E. coli*, 65.40% for *Salmonella* and 65.20% for *Staphylococcus*. However, in the morning 31.30%, 34.50% and 34.70% for *E. coli, Salmonella* and *Staphylococcus* respectively.

Across six locations (Table 2), Rudrapur had the highest contamination rates for all pathogens (70.40%) followed by Kiccha (47.46%), Lalpur (45.60%), Pantnagar (29.86%), Nagla (24.53%) and Haldi (13.86%).

On disk diffusion assay, the antimicrobial resistance patterns of isolated *E. coli*, *Salmonella* and *Staphylococcus* spp. revealed maximum resistance against penicillin (45.5% for *E. coli*, 42.7 %, against *Salmonella* spp. and 47.6% against *Staphylococcus* spp.) and least resistance was recorded against ceftriaxone (9.8 % for *E. coli,* 10.6% for *Salmonella*

Table 1: Source wise microbial contamination obtained from panipuri shop

Pathogens	Main water source(n=450)		Panipuri masala water container(n=450)		Serving spoon(n=450)		Bowl (Dona/ pattal)(n=450)		Hands (n=450)		Total
	No. of positive	Percent (%)	No. of positive	Percent (%)	No. of positive	Percent (%)	No. of positive	Percent (%)	No. of positive	Percent (%)	Percent (%)
E. coli	103	22.88	82	18.22	78	17.30	64	14.2	34	7.50	16.04
Salmonella spp.	92	20.44	64	14.22	13	2.80	16	3.5	67	14.8	11.2
Staphylococcus spp.	86	19.11	53	11.77	26	5.70	37	8.2	54	12.00	11.3
Total no. of isolates	281	62.44	199	44.22	117	26.00	117	26	155	34.40	33.91

Table 2: Location of sample collection from panipuri shop

S.No.	Location of sample collection	Total no. of samples	Number of positive samples			Total percent (%)
			E. coli	*Salmonella* spp.	*Staphylococcus* spp.	
1.	Pantnagar	375	53	28	31	29.86
2.	Nagla	375	48	23	21	24.53
3.	Haldi	375	21	17	14	13.86
4.	Lalpur	375	63	52	56	45.65
5.	Rudrapur	375	97	83	84	70.40
6.	Kiccha	375	79	49	50	47.46
	Total	2250	361	252	256	38.62

Table 3: Antimicrobial resistance patterns of isolates

Pathogen	Penicillin (%)	Amoxicillin (%)	Tetracycline (%)	Ciprofloxacin (%)	Streptomycin (%)	Gentamicin (%)	Ceftriaxone (%)
E. coli	45.5	39.8	37.4	20.7	18.6	12.4	9.8
Salmonella	42.7	38.5	34.2	22.5	21.3	15.2	10.6
Staphylococcus	47.6	40.2	39.1	18.3	16.9	11.9	8.7

spp and 8.7% for *Staphylococcus* spp.). Against *Staphylococcus* spp., amoxicillin showed (40.2%) and tetracycline (39.1%). Moderate resistance was seen for ciprofloxacin, amoxicillin, Tetracycline, Streptomycin and Gentamicin (Table 3).

The high prevalence of *E. coli* reflects fecal contamination, likely due to poor water quality and handling practices. The contamination by *Salmonella* indicates improper cooking and handling of ingredients. *Staphylococcus*, known for its presence on human skin, underscores the importance of personal hygiene among food handlers. Similar findings were reported in Indian street foods by Tandon *et al.* (2020).

Water sources being a major contaminant suggest inadequate filtration or storage practices. Masala water and utensils are secondary reservoirs due to improper handling. Hands were heavily contaminated with *Salmonella* spp., consistent with findings by Kumar and Singh (2018) , highlighting the lack of proper hygiene among vendors. Regular sanitization of utensils and awareness campaigns for food handlers can reduce contamination risks.

The higher contamination in evening samples might be due to prolonged storage of ingredients and utensils, allowing microbial growth. The morning samples, although less contaminated, still exhibited significant pathogen presence. These findings support the importance of proper storage and frequent cleaning, as emphasized in WHO guidelines (2021).

Therefore, results clearly showed multiple sources and factors contributing to the microbial contamination of panipuri. The primary source of contamination which may be untreated or poorly maintained water, aligning with Gupta and Sharma (2022), who emphasized the role of water quality in foodborne illnesses. Secondly, contaminated hands and utensils are significant contributors, reflecting inadequate training in hygiene. Similar results have been observed in other street food studies by Anuradha (2019). Thirdly, high evening contamination rates highlight the risks associated with prolonged storage of ingredients.

Since this study is first of its kind in the area hence, any comparison cannot be made. Moreover, selection of shops was based on the vendor's willingness. Even though, highest positive cases at Rudrapur indicate significance of hygiene training, clean water supply and regular monitoring to reduce contamination risks.

The antimicrobial resistance (AMR) patterns observed in this study reveal a concerning trend of resistance among isolates of *E. coli*, *Salmonella* and *Staphylococcus* spp. Resistance was highest against penicillin (47.6%) across all pathogens, consistent with its longstanding and widespread use, which often leads to diminished efficacy. Amoxicillin (40.2%) and tetracycline (39.1%) also showed substantial resistance, likely due to their frequent application in both human and veterinary medicine. Moderate resistance levels to ciprofloxacin (20.7% for *E. coli*) and streptomycin (18.6% for *E. coli*) highlight the ongoing pressure from commonly prescribed antibiotics. Resistance rates for gentamicin (12.4% for *E. coli*) and ceftriaxone (9.8% for *E. coli*) suggest that these antibiotics remain more effective, possibly due to their limited and targeted usage compared to older-generation antibiotics. The results align with global studies indicating that AMR is exacerbated by the misuse of antibiotics in both clinical and agricultural settings. For instance, Samtiya *et al.* (2022) noted that AMR in street food pathogens stems from unregulated antibiotic use, poor hygiene and cross-contamination. Similarly, Fusaro *et al.* (2024) emphasized that substandard sanitation practices in food preparation exacerbate resistance development among pathogens.
These findings emphasize the need for targeted interventions, such as clean water supply, regular sanitization of utensils and training for food handlers.

CONCLUSION

This study provides critical insights into the microbial contamination of panipuri. The results reveal that poor water quality, inadequate hygiene

practices and improper storage are the major contributors to foodborne risks. *E. coli* contamination indicates fecal pollution, *Salmonella* suggests handling-related issues and *Staphylococcus* highlights personal hygiene lapses. Immediate actions, including clean water supply, hygiene training and strict monitoring of street food practices, are essential to protect public health. The findings underscore the urgent need for AMR monitoring in foodborne pathogens, stricter antibiotic regulation and public awareness campaigns. Educating food handlers about the risks associated with improper antibiotic use and ensuring hygienic food preparation can help mitigate the spread of resistant strains.

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