



ANTIBIOGRAM OF BACTERIAL PATHOGENS ISOLATED FROM ONE OF THE MOST POPULAR STREET FOOD (PANIPURI) OF DEHRADUN

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ABSTRACT

India is a country where each city boasts of its own unique street food and a large percentage of population relishes these delicacies. The most popular street foods in India are Panipuri or Gol gappas and Papdi chaat among others. The present study attempts to assess the bacteriology of khatta pani and masala sold with Gol Gappas in Dehradun district of Uttarakhand, India, in an attempt to throw light on the inherent risk associated with such foods. Panipuri samples were collected from different locations of Dehradun and analysed for the presence and count of pathogenic bacteria and their susceptibility to various antibiotics. Total Viable Count, Total Coliform Count and Total Staphylococcal Count were found to be in the range of 65×10^4 to 74×10^5 , 36×10^2 to 64×10^5 and 62×10^4 to 45×10^6 respectively. Six isolates were tentatively identified as *Aeromonas sp.*, *Klebsiella sp.*, *Bacillus sp.*, *Escherichia sp.*, *Micrococcus sp.* and *Pseudomonas sp.* Antibiotic sensitivity test was performed against 8 antibiotics, in which *Klebsiella sp.* was inhibited by all the antibiotics used. *Aeromonas sp.* and *Micrococcus sp.* showed resistance to Penicillin G and Vanomycin and were inhibited by the rest, while *Bacillus sp.*, *Escherichia sp.* and *Pseudomonas sp.* showed resistance to Vanomycin. Based on this test, MRI% was calculated and was found to be in the range of 12.5 to 25% for all the isolates. Our study shows high level of contamination in one of the most popular Indian street food, *Panipuri*. Therefore, regular monitoring is required for safety against these food-borne pathogens.

KEYWORDS: Antibiotic sensitivity test, Hygienic practices, Microbial quality, Panipuri, Street-food vendors



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INTRODUCTION

Street foods are common and important features of urban centres in many developing countries. Vast changes in the social and cultural milieu and long hours spent away from home, make eating out a necessary part of people's daily life.¹ People who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene. All kinds of food are sold by food vendors, presenting the options for variety and choice for customers. The most popular street foods in India are Panipuri or Gol gappas and Papdi chaat among others. Gol Gappa is a traditional chaat product and is preferred widely in several regions of South Asia. It consists of a round, hollow puri made of semolina or refined wheat flour, fried crisp and filled with a mixture of flavoured water (*pani*), tamarind chutney, chilli, chaat masala, potato, onion and chickpeas. While street vended foods are appreciated for their unique flavours as well as their convenience, they are also important in contributing to the nutritional status of the population to the majority of people, especially the low-income group in the developing countries. In contrast to these potential benefits, it is also recognized that vendors are often poorly educated, unlicensed, untrained in food hygiene, and work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease.² The unhygienic conditions in which these foods are prepared, stored and served provide a suitable nutritional and physical environment for the growth and multiplication of microorganisms. The largely unregulated nature of street food vending, and poor hygienic practices as well as lack of running water, toilet, proper storage and waste disposal facilities at preparation and services points has resulted in poor unsanitary conditions, exposure to potential contaminants and an increased risk to public health.³ Food borne illness of microbial origin is a major international health problem associated to food safety and an important cause of death in developing countries.⁴ Food-borne bacterial pathogens commonly detected in street-vended foods are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella sp.*, *Proteus sp.*, *Klebsiella sp.*, *Enterobacter sp.* and *Shigella sp.*⁵ The resistance of food-borne microorganisms to drugs has further made the food safety situation more vulnerable in public health.⁶ India is a country where each city boasts of its own unique street food and a large percentage of population relishes these delicacies. However, despite their widespread prevalence and use, only a very few organized attempts have been made to study them. Therefore, the present study attempts to assess the bacteriology of khatta pani and masala sold with Gol Gappas in Dehradun district of Uttarakhand, India, in an attempt to throw light on the inherent risk associated with such foods.

MATERIALS AND METHODS

Sample collection

Panipuri samples (both potato masala and khatta pani) were collected from street food vendors from local area at Dehradun, Uttarakhand. Both parts of the samples were collected separately in sterilized zip lock plastic bags and immediately kept in ice bucket. After reaching

the laboratory they were transferred to freezer and stored till further analysis was done.

Isolation of bacteria

The samples were serially diluted from 10^{-1} to 10^{-5} and plated out on three media: Nutrient agar (NA), MacConkey's agar (MAC) and Eosin methylene blue (EMB) agar. The plates were incubated at 37°C for 24-48 hours. The colonies so obtained on these media were marked and numbered. They were then streaked on their respective medium from which they were isolated until pure cultures were obtained. Bacterial pure cultures were maintained on slants, stored at 4°C and sub cultured every month.

Characterization of the bacterial isolates⁷

Isolated pure strains were identified on the basis of morphological and physiological characteristics and by biochemical tests. Simple and Gram staining was carried out. Catalase test, citrate utilization test, indole production test, methyl red-voges proskeur test, urease test, gelatinase test, nitrate reduction test, hydrogen production test, and oxidase tests were carried out for the identification.

Microbial analysis of the samples

Total viable count (TVC)

Pour plate technique was used to enumerate the total bacterial population count of the samples. The samples were serially diluted and 1 mL each of the aliquots of appropriate dilutions was pipetted out into sterile petridishes. 15-20 mL of sterile nutrient agar medium was poured into each petriplate. Duplicate plates were also maintained. The plates were then allowed to solidify and incubated at 37°C for 48 hours. The number of bacterial colonies were counted after 48 hours and the result was expressed as colony forming units (cfu) per ml.

Total coliform count (TCC)

Presence of coliform was studied both in masala and khatta pani of the samples collected. Samples were serially diluted and 1 mL each of the aliquots of appropriate dilutions was pipetted out into sterile petridishes. 15-20 mL of sterile EMB agar was poured into each petriplate. Duplicate plates were maintained. The plates were then allowed to solidify and incubated at 37°C for 48 hours. The number of bacterial colonies were counted after 48 hours and the result was expressed as colony forming units (cfu) per ml.¹

Total Staphylococcal count (TSC)¹

The samples were serially diluted and 1 mL each of the aliquots of appropriate dilutions was pipetted out into sterile petridishes. 15-20 mL of sterile mannitol salt agar medium was poured into each petriplate. Duplicate plates were maintained. The plates were then allowed to solidify and incubated at 37°C for 48 hours. The number of bacterial colonies were counted after 48 hours and the result was expressed as colony forming units (cfu) per ml. CFU was calculated from the spread plates of NA (Total Viable Count), EMB (Total Coliform Count) and MSA (Total Staphylococcal Count) using the following formula:

$$CFU = \text{No. of colony} \times \frac{\text{Dilution factor}}{\text{Quantity of sample}}$$

Antibiogram pattern

The antibiotics used in the study were Trimethoprim (5mcg), Penicillin G (10units), Ciprofloxacin (10mcg), Streptomycin (10mcg), Vanomycin (30 mcg), Cefotaxime (10mcg), Erythromycin (15mcg) and Gentamicin

(50mcg). Mueller Hinton Agar medium was used for susceptibility testing using Kirby-Bauer disc diffusion method.⁸ Multiple-antibiotic Resistance Index (MRI%) was determined, using the following formula:

$$\%MRI = \frac{\text{No. of antibiotics which pathogens showed resistance}}{\text{No. of antibiotics used}} \times 100$$

RESULTS AND DISCUSSION

Plating and enumeration

TVC, TSC and TCC results were calculated as an average of the duplicate plates respectively and reported in Table 1.

Table 1
Bacterial enumeration

Sample		TVC (cfu/ml)	TSC (cfu/ml)	TCC (cfu/ml)
Sample 1 (Suddhowala Chowk)	Pani	74 x 10 ⁵	62 x 10 ⁴	55 x 10 ⁵
	Masala	56 x 10 ⁵	45 x 10 ⁵	64 x 10 ⁵
Sample 2 (Premnagar Mandi)	Pani	65 x 10 ⁴	82 x 10 ⁴	64 x 10 ⁴
	Masala	72 x 10 ⁵	75 x 10 ⁵	36 x 10 ²
Sample 3 (Paltan Market)	Pani	42 x 10 ⁵	34 x 10 ⁵	48 x 10 ⁵
	Masala	53 x 10 ⁵	36 x 10 ⁵	32 x 10 ⁵
Sample 4 (Ghantaghar)	Pani	96 x 10 ⁴	23 x 10 ⁵	34 x 10 ⁴
	Masala	63 x 10 ⁵	22 x 10 ⁵	22 x 10 ³
Sample 5 (Rajpur Road)	Pani	52 x 10 ⁵	65 x 10 ⁵	58 x 10 ⁵
	Masala	13 x 10 ⁵	34 x 10 ⁵	42 x 10 ⁵
Sample 6 (Near Parade Ground)	Pani	76 x 10 ⁴	42 x 10 ⁵	52 x 10 ³
	Masala	32 x 10 ⁵	12 x 10 ⁵	85 x 10 ³
Sample 7 (Near Saharanpur Chowk)	Pani	10 x 10 ⁵	40 x 10 ⁵	45 x 10 ⁴
	Masala	23 x 10 ⁵	72 x 10 ⁵	82 x 10 ⁴
Sample 8 (Hathibarkala)	Pani	82 x 10 ⁴	42 x 10 ⁵	75 x 10 ⁵
	Masala	31 x 10 ⁵	80 x 10 ⁵	34 x 10 ³
Sample 9 (Chakrata Road)	Pani	40 x 10 ⁵	10 x 10 ⁵	36 x 10 ⁴
	Masala	57 x 10 ⁵	76 x 10 ⁵	23 x 10 ⁵
Sample 10 (Panditwari)	Pani	20 x 10 ⁵	54 x 10 ⁵	22 x 10 ⁵
	Masala	47 x 10 ⁵	62 x 10 ⁵	15 x 10 ⁵

The highest TVC, TSC and TCC were found in Sample from Suddhowala Chowk. Overall TVC was found in the range of 65 x 10⁴ to 74 x 10⁵ cfu/ml, TSC in the range of 62 x 10⁴ to 45 x 10⁵ cfu/ml and TCC in the range of 36 x 10² to 64 x 10⁵ cfu/ml.

Isolation of bacteria

Isolation was done by streaking selected colonies on 3 different media plates: Nutrient Agar (NA), Mac Conkey Agar (MAC) and Eosin Methylene Blue Agar (EMB), for obtaining pure cultures of the isolates. Results obtained is represented in Table 2 and Figure 1.

Table 2
Isolation of bacteria

Isolate No.	Sample	Media	Source
DD01	Pani	EMB	Suddhowala Chowk
DD02	Masala	EMB	Suddhowala Chowk, Chakrata Road, Panditwari, Parade Ground, Saharanpur Chowk
DD03	Masala	NA	Premnagar Mandi
DD04	Pani	EMB	Premnagar Mandi, Rajpur Road, Hathibarkala
DD05	Masala	NA	Paltan-market, Ghantaghar
DD06	Pani	MAC	Paltan-market, Ghantaghar

Isolation of bacteria on different media

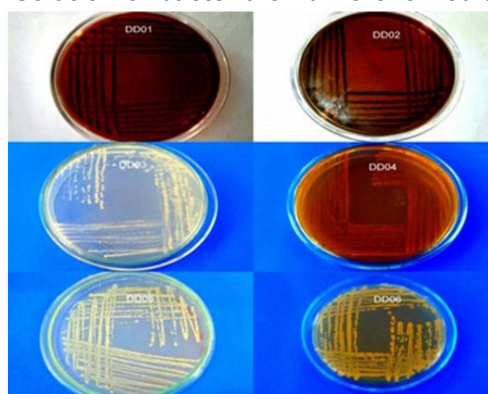


Figure 1
Master plates of bacterial isolates

Characterization of bacterial isolates

The isolates were characterized for their colony morphology on nutrient agar plates and nutrient agar slants. Biochemical characterization was done for their tentative identification. Results obtained are depicted in Table 3.

Table 3
Biochemical characterization of isolates

Isolates	Nitrate Reduction	Gelatinase	Urease	MR	VP	Indole	Citrate Utilisation	Catalase	Oxidase	H ₂ S	Organisms Identified
DD01	+	-	+	-	-	+	+	+	-	+	<i>Aeromonas sp.</i>
DD02	+	-	+	-	-	+	+	-	-	-	<i>Klebsiella sp.</i>
DD03	-	-	+	+	-	+	+	-	-	-	<i>Bacillus sp.</i>
DD04	+	-	+	-	-	+	-	+	-	-	<i>Escherichia sp.</i>
DD05	-	-	-	-	-	+	+	-	+	-	<i>Micrococcus sp.</i>
DD06	-	-	-	+	-	-	-	-	+	-	<i>Pseudomonas sp.</i>

The present investigation was aimed at determining the count of potentially hazardous bacteria in one of the most popular street foods of India. Our study revealed high loads of bacteria such as *Aeromonas sp.*, *Klebsiella sp.*, *Bacillus sp.*, *Escherichia sp.*, *Micrococcus sp.*, and *Pseudomonas sp.* in the panipuri samples. In a similar study microbiological quality of 8 different samples of street foods like *panipuri* and *bhelpuri*, collected from 4 different locations of Bangalore, India, was investigated. The analysis revealed high loads of bacterial pathogens such as *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp.*, *Klebsiella sp.* and *Pseudomonas sp.* Total viable counts of bacteria in all the samples was found to vary between $0.4-3.0 \times 10^4$ cfu g⁻¹, faecal coliforms between $0.03-0.14 \times 10^4$ cfu g⁻¹ and faecal streptococci between $0.2-11 \times 10^4$ cfu g⁻¹.⁹ Microbiological quality of forty samples of *panipuri* sold in eleven different locations of Amravati, India, was investigated. Analysis of the food samples revealed that 93% of *panipuri* water samples had high loads of bacterial pathogens such as *Escherichia coli* (41%), *Staphylococcus aureus* (31%), *Klebsiella sp.* (20%), *Pseudomonas sp.* (5%) and yeast (3%).¹⁰ Microorganisms present in panipuri water samples

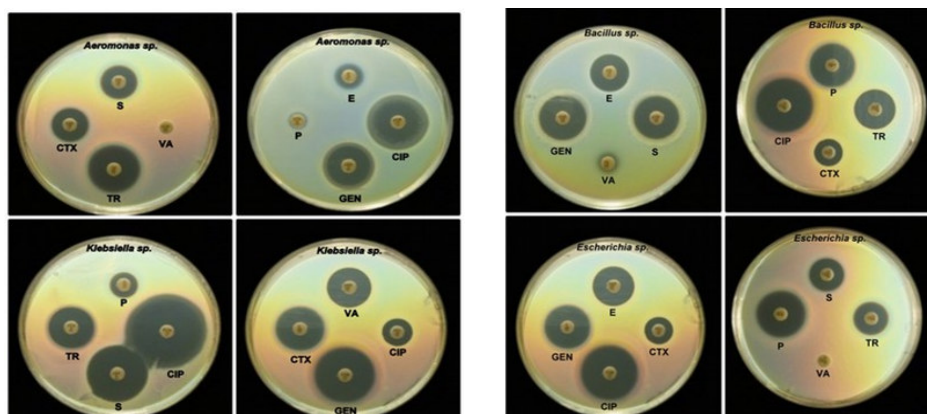
collected from the twin cities (Hyderabad and Secunderabad) were enumerated and identified. *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Salmonella* and *Shigella* were the organisms observed in the samples collected, and from CFU/mL calculations the colonies were found ranging from 40–4320.¹¹ Five different food samples (*chicken fry*, *vada*, *samosa*, *panipuri* and *biryani*) were collected from street vendors in Mayiladuthurai area, Tamilnadu, India. High aerobic bacterial load was observed in the samples. Pathogenic bacteria like *Escherichia coli*, *Enterobacter sp.*, *Klebsiella pneumoniae*, *Pseudomonas sp.*, *Salmonella typhi*, *Shigella sp.*, *Staphylococcus sp.* *Streptococcus pyogenes* and fungi like *Aspergillus niger*, indicative of fecal and water borne contamination of food samples were observed.¹²

Antibiotic sensitivity test

Antibiotic sensitivity testing was done using 8 different antibiotic disks for each species. The obtained result is presented in Table 4 and Figure 2. This test reveals resistance of a pathogen against an antibiotic, which can be very helpful in treatment purposes of food borne diseases.

Table 4
Disk diffusion test

Antibiotics (conc.)	DD01 <i>Aeromonas sp.</i>	DD02 <i>Klebsiella sp.</i>	DD03 <i>Bacillus sp.</i>	DD04 <i>Escherichia sp.</i>	DD05 <i>Micrococcus sp.</i>	DD06 <i>Pseudomonas sp.</i>
Trimethoprim (5mcg)	Inhibited (31mm)	Inhibited (30mm)	Inhibited (32mm)	Inhibited (32mm)	Inhibited (29mm)	Inhibited (32mm)
Streptomycin (10mcg)	Inhibited (30mm)	Inhibited (32mm)	Inhibited (33mm)	Inhibited (31mm)	Inhibited (30mm)	Inhibited (32mm)
Pencillin-G (10units)	Resistant	Inhibited (10mm)	Inhibited (40mm)	Inhibited (42mm)	Resistant	Inhibited (41mm)
Ciprofloxacin (10mcg)	Inhibited (43mm)	Inhibited (46mm)	Inhibited (45mm)	Inhibited (43mm)	Inhibited (44mm)	Inhibited (45mm)
Vancomycin (30mcg)	Resistant	Inhibited (25mm)	Resistant	Resistant	Resistant	Resistant
Cefotaxime (10mcg)	Inhibited (29mm)	Inhibited (31mm)	Inhibited (30mm)	Inhibited (30mm)	Inhibited (32mm)	Inhibited (30mm)
Gentamicin (50mcg)	Inhibited (35mm)	Inhibited (37mm)	Inhibited (38mm)	Inhibited (37mm)	Inhibited (39mm)	Inhibited (39mm)
Erythromycin (15mcg)	Inhibited (15mm)	Inhibited (12mm)	Inhibited (30mm)	Inhibited (32mm)	Inhibited (20mm)	Inhibited (30mm)

Effect of various antibiotics on the isolates

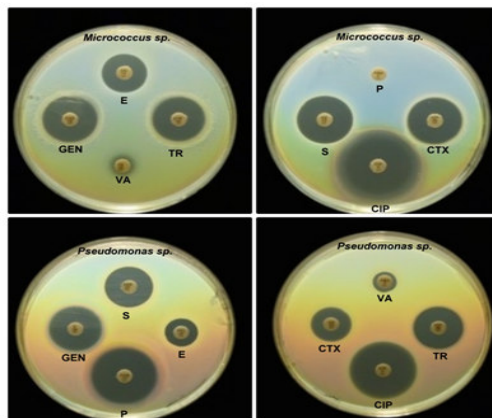


Figure 2

Disk diffusion test**Antibiotic sensitivity testing of the isolates against various antibiotics**

In a similar work, 37 street vended food samples were examined for bacterial contamination and the colony forming unit counts was found to range from 4.5×10^5 to 1.12×10^6 . Different isolates as *Escherichia coli* (37.5%), *Pseudomonas aeruginosa* (3.57%), *Staphylococcus aureus* (14.20%), *Salmonella sp.* (5.36%), *Klebsiella sp.* (10.71%), *Shigella sp.* (19.64%) and *Enterobacter sp.* (8.93%) were identified; all the isolates were susceptible

to ciprofloxacin while their susceptibility to the other drugs varied.¹³

Multiple-antibiotic resistance index

The MRI% of the isolates were calculated and the results obtained are as shown in Table 5. The MRI% is helpful in analyzing health risk, and is used to check the antibiotic resistance of a microbe.

Table 5

%Multiple-antibiotic resistance index of the isolates.

Isolates	%MRI
<i>Aeromonas sp.</i>	25.0%
<i>Klebsiella sp.</i>	00.0%
<i>Bacillus sp.</i>	12.5%
<i>Escherichia sp.</i>	12.5%
<i>Micrococcus sp.</i>	25.0%
<i>Pseudomonas sp.</i>	12.5%

In our study, the MRI% ranged from 12.5 to 25%. In a similar study, panipuri samples fractionated into two parts viz. *khatta pani* and smashed potato *masala* were collected from different locations in Baripada city, Orissa. Aerobic bacterial load in solid samples was observed to be more than in the liquid samples. Coliform-positive samples were found to be 80.33%. Pathogenic bacteria like *Escherichia coli*, *Klebsiella sp.*, *Enterobacter sp.*, *Bacillus sp.*, *Enterococcus sp.*, *Micrococcus tetragens*, *Salmonella paratyphi*, *Shigella dysenteriae* and *Vibrio sp.* were detected. Antibio gram studies of the isolates showed multiple antibiotic resistance index (MRI%) ranging from 15 to 92%.¹⁴

CONCLUSION

The present study show high level of contamination in one of the most popular Indian street food, *Panipuri*. The study throws a light on the potential risks of food poisoning, and under the hot and humid Indian weather even outbreaks of epidemics of gastro-intestinal diseases caused by these bacteria. To overcome this

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alarming situation, vendors should be taught to practice proper hygiene and sanitation. Regular monitoring at the level of municipal food inspectors should be done. Strict measures in terms of fine or cancellation of vending licences should be taken up to ensure compliance of hygiene and sanitation practices by vendors. Further, more of such experiments and investigations should be performed at different locations, towns or cities, time to time, to keep a record on the safety levels of street foods.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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