

**A Comparative Study on Microbial Contamination in Selected Street Foods
(Pani Puri and Samosa) From Different Regions of Hyderabad and Its Health
Impact: An Indian Knowledge System Perspective on Food Hygiene Practices and
Safety**

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ABSTRACT

Street foods like Pani Puri and Samosa are widely consumed across India for their affordability and taste. However, their preparation and storage often occur under unhygienic conditions, making them potential carriers of harmful microorganisms. Such contamination can act as a vector for foodborne diseases, posing serious public health risks. The present study aims to compare the levels and types of microbial contamination in Pani Puri and Samosa collected from different areas of Hyderabad, and to assess their associated health impacts with reference to Indian Knowledge System (IKS)- based Food hygiene and food safety practices. Food Samples of Pani Puri water and Samosa were collected aseptically from selected locations: Nacharam, Boduppall, and Mallapur. The samples were then subjected to serial dilution and cultured on selective and differential media such as Nutrient Agar, Eosin Methylene Blue (EMB) Agar, Czapek-Dox Agar, and Potato Dextrose Agar to isolate and identify bacterial and fungal species. Gram staining was used to determine bacterial morphology, while fungal species were identified microscopically. Bacterial isolates were further confirmed through IMVIC tests. The analysis revealed two major bacterial species: *Staphylococcus* (Gram-positive cocci) and *Escherichia coli* (Gram-negative bacilli). Fungal species such as *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., and *Lactobacillus* spp. were also identified, particularly in Samosa samples. Alarmingly, a parasitic nematode (*Ascaris*) was detected in one Samosa sample, indicating fecal contamination and severe hygiene lapses. This study highlights significant microbial contamination in both Pani Puri and Samosa, underlining the urgent need for better hygiene practices in street food preparation. Public awareness campaigns and vendor training in safe food handling, proper storage, and the use of potable water are essential to minimize risks. The study also highlights the relevance of traditional Indian Practices such as use of copper vessels, earthen pots, natural antimicrobial spices, fresh food

preparation and hygienic food handling, which are emphasized in Indian Knowledge System. Copper vessels, in particular, demonstrate oligodynamic action, effectively Killing pathogenic microorganisms in drinking water. Strengthening these practices can enhance food safety and contribute to national well-being and disease prevention.

Traditional Indian methods offer eco-friendly, cost-effective, and sustainable solutions for preventing microbial contamination. Integrating these traditional knowledge-based practices with modern scientific food safety can help in improving the microbiological quality of street foods and can safeguard public health.

Keywords: Street food, microbial contamination, foodborne pathogens, public health, IMVIC test, Traditional methods, oligodynamic action.

BACKGROUND:

Microbial food-borne illnesses are a global health concern, especially affecting vulnerable groups like children, the elderly, pregnant women and the immunocompromised. Street foods like Pani Puri and Samosa are ready-to-eat items sold by vendors in open environments, prepared unhygienically, increasing the risk of microbial contamination. In developing countries, many street food vendors lack proper food safety knowledge due to limited education. In Hyderabad, street foods such as Pani Puri and Samosa are widely consumed due to their affordability, taste and easy availability. However, food safety remains a major concern as these street foods often are prepared and sold under **unhygienic conditions**, like **exposure to dust, use of contaminated water, improper storage and frequent manual handling**. These practices increase the risk of microbial contamination, making street foods a significant source of **food-borne diseases**.

Microorganisms such as **Escherichia coli, Salmonella, Staphylococcus aureus and various Fungi** have been frequently reported in street-vended foods and consumption of these contaminated street foods can lead to health problems ranging from mild gastro-intestinal discomfort to severe infections among various age groups. Hence, monitoring the microbiological quality of street foods is essential in safeguarding public health.

Indian Knowledge Systems (IKS) offer valuable insights into traditional food safety, health, and hygienic practices that were traditionally used to prevent contamination of food. According

to Ancient Indian texts and traditional practices, emphasis was laid on **Ahara Vidhi (dietary rules)**, maintaining cleanliness during food preparation, consumption of freshly prepared food and use of **natural antimicrobial agents** such as turmeric, ginger, garlic, clove, pepper lime, ash and neem for cleaning hands and utensils, as well as serving food on fresh banana leaves, which possess natural antibacterial properties. Additionally **traditional use of copper and silver utensils** which are known for its **oligodynamic action**, has been scientifically proven to reduce microbial load in food and water. These methods, rooted in cultural and Ayurvedic principles, played a significant role in reducing microbial contamination even before the advent of modern microbiology.

In context of increasing urbanization and dependence on street foods because of everybody's busy life, integrating these **IKS-based preventive practices** with modern microbiology assessment becomes highly relevant. This study, therefore, aims to evaluate the degree of microbial contamination in selected street foods of Hyderabad while highlighting the potential role of traditional Indian food safety practices in reducing health risks and improving food hygiene.

MATERIALS AND METHODS:

Materials required: Inoculation loop, spreader, Glassware like Sterile conical flasks, Petri plates and test tubes.

Laboratory Equipment: Autoclave, Incubator, Microscope (compound), Hot plate, Balance, Bunsen burner, Glass slides

Requirements for Gram staining: Bacterial culture, Crystal violet, Gram's iodine solution, 95% ethyl alcohol, Safranin, Distilled water, Dropper, Inoculation loop.

METHODOLOGY:

Collection of the sample: A total of six street food samples were collected aseptically from different vendors from three locations:

- Nacharam (2 samples of Panipuri and samosa)
- Boduppall (2 samples of Panipuri and samosa)
- Mallapur (2 samples of Panipuri and samosa)

The Samples were collected aseptically using sterile hand gloves and transferred into sterile test tubes containing 10 mL of saline water (0.85% NaCl). Then test tubes were vigorously shaken for 2 minutes to facilitate the dislodgement of bacteria from the surface of the food samples into the saline solution. The saline suspension was then used for further microbiological analysis. First each sample of Pani puri and Samosa was homogenized in sterile distilled water to create a uniform suspension.

METHODOLOGY FOR MICROBIAL ANALYSIS:

Isolation of Bacterial colonies: We prepared Serial dilutions (e.g., 10^{-1} , 10^{-2} , 10^{-3}) to isolate individual colonies. Culture medias of Nutrient agar and EMB (Eosin Methylene Blue) agar was prepared to isolate the bacterial colonies. EMB Agar was used to selectively isolate Gram-negative bacteria, while Nutrient Agar supported the growth of a wide range of bacteria. The streaking method was used to isolate colonies on Nutrient Agar and EMB Agar. Spread plating was performed to ensure the even distribution of microorganisms. These petri plates were then incubated at 37°C for 24-48 hours in an incubator for bacterial growth. After the growth was observed, the colony morphology of the bacteria was identified. Further identification was done by gram staining to know the gram nature of the bacteria and the confirmation of bacteria was done by IMViC Test.

Isolation of Fungal species from Pani puri and Samosa samples:

The same six samples of pani puri and samosa were used for fungal isolation. Samples were homogenized in sterile distilled water first and then serial dilutions (e.g., 10^{-1} , 10^{-2} , 10^{-3}) were prepared. Potato Dextrose Agar (PDA) and Czapekdox Agar were used for fungal isolation. PDA is selective for fungi, while Czapekdox Agar inhibits bacterial growth, allowing for better fungal isolation. Streaking was performed in the slant test tubes containing solidified agar media to isolate fungal colonies. The test tubes were then incubated at $25-28^{\circ}\text{C}$ (room temperature) for 5 to 7 days to promote fungal growth. Fungal colonies were identified based on morphological characteristics, such as colony colour, texture, and spore formation.

Gram Staining Technique to study the morphological characteristics of the microorganisms: Gram staining is a staining technique used for the identification and classification of the bacteria into two major groups i.e. Gram-positive (purple) and Gram-negative (pink) bacteria.

Procedure: We made a thin smear on the glass slides of the culture isolated and let the smear air dry. Then we heat fixed the smear. Then we covered the smear with crystal violet for 30 seconds followed by washing the slide with distilled water for a few seconds using a wash bottle. Then we covered the smear with Iodine solution for 60 seconds. Then we washed off the iodine solution with 95% ethyl alcohol. at last, adding ethyl alcohol drop by drop until no more colour flows from the smear. The gram-positive bacteria are not affected while all the gram-negative bacteria are completely decolorized. Then we wash the slides with distilled water and drain. Then adding safranin to smear for 30 seconds and washing it with distilled water and letting the stained slides air dry. Now we, examine the slides microscopically using oil immersion and note down the observations. The bacteria that appear purple in colour are referred to as gram-positive bacteria whereas those that appear pink in colour are referred to as gram-negative bacteria.

Fungal Staining Technique to study and identify fungal infections of the microorganisms:

Procedure: On a clean slide we placed a drop of 70% ethanol. Then using a sterile inoculation loop, we added the fungal specimen to the drop of ethanol followed by teasing apart the fungal sample gently with a needle to ensure it mixes well. Then we added one or two drops of prepared LPCB solution before the ethanol dries and then carefully placing a cover slip over the specimen to avoid air bubbles. Fungal staining observations and results for pani puri and samosa indicated significant contamination levels.

IMViC Test: The IMViC test is a series of four biochemical tests which is generally used to identify and differentiate members of the Enterobacteriaceae family, especially between *Escherichia coli* and *Enterobacter* species. The name IMViC stands for **Indole**, **Methyl Red**, **Voges-Proskauer**, and **Citrate** tests. To perform this test, a pure culture of the bacterium is inoculated into four different media: tryptone broth for the Indole test, MR-VP broth for the Methyl Red and Voges-Proskauer tests, and Simmons citrate agar for the Citrate test. After incubation, specific reagents are added—Kovac's reagent for Indole, methyl red indicator for Methyl Red, and Barritt's reagents (VP-A and VP-B) for Voges-Proskauer—to observe color changes that indicate positive or negative results. The Citrate test is read by observing a color change in the medium from green to blue, which indicates citrate utilization. The pattern of results from all four tests helps in identifying the bacterial species.

Total Viable Count(TVC): Total Viable Count (TVC) is a measurement used to estimate the total number of living microorganisms like Bacteria, Yeast or Mold spores in a sample.

TVC of microorganisms was determined using **standard plate count method**. Plates showing 30-300 colonies were selected for counting and the results were expressed as colony forming unit (CFU) per unit volume (ml) or mass(g). High TVC levels can indicate poor product quality or health risks.

$CFU/ml \text{ or } CFU/g = (\text{No. of colonies} \times \text{Total dilution factor}) / \text{Volume of culture plated in ml}$

CFU is a measure of the number of viable living cells in a sample that can multiply and form a visible colony when cultured & Dilution factor is the factor by which the original sample was diluted before plating.

RESULTS:

IDENTIFICATION OF MICROORGANISMS IN DIFFERENT CULTURE MEDIA

Table no. 1: Nutrient agar Panipuri sample

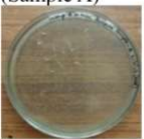
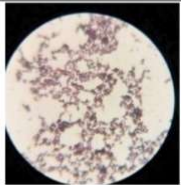
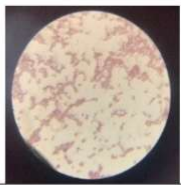
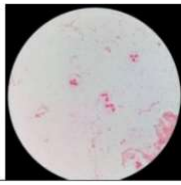
S.no	Medium	Dilution	Number of Colonies	Colony Morphology	Gram's Nature	Microscopic Observation
1	Nutrient agar (Sample A) 	10^{-4}	220	Pinpointed cream colored colonies	Gram positive	
2	Nutrient agar (Sample B) 	10^{-4}	175	Pinpointed cream-coloured colonies	Gram negative	
3	Nutrient agar (Sample C) 	10^{-4}	150	Pinpointed cream-coloured colonies	Gram negative	

Table. No. 2: Nutrient agar - Samosa sample

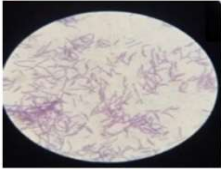
S.No	Medium	Dilution	Number of Colonies	Colony Morphology	Gram's Nature	Microscopic Observation
1	Nutrient agar (Sample A) 	10^{-4}	15	Pinpointed cream-coloured colonies	Gram negative	
2	Nutrient agar (Sample B) 	-	0	-	-	No observation
3	Nutrient agar (Sample C) 	-	0	-	-	No observation

Table no.3 EMB agar- panipuri sample

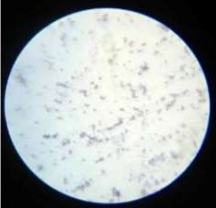
S.No	Medium	Dilution	Number of colonies	Colony morphology	Gram's nature	Microscopic observation
1	Eosin Methylene Blue 	10^{-2}	100	Pink lavender colour colonies with dark centre	positive	
2	Eosin Methylene Blue 	10^{-2}	220	Pink lavender colour colonies with dark centre	negative	
3	Eosin Methylene Blue 	10^{-2}	150	Colourless or translucent colonies	negative	

Table No. 4 EMB agar - samosa sample

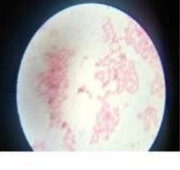
S.No	Medium	Dilution	Number of Colonies	Colony Morphology	Gram's nature	Microscopic observation
1	Eosin Methylene Blue 	10^{-2}	18	Pinpointed cream-coloured colonies	positive	
2	Eosin Methylene Blue 	10^{-2}	60	Bacterial lawn	negative	
3	Eosin Methylene Blue 	10^{-4}	180	Pin pointed colonies	negative	

Table No. 5: PDA - panipuri sample

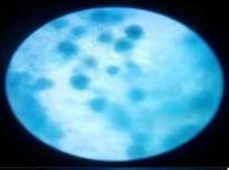
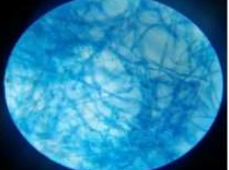
S.No	Medium	Dilution	Colony Morphology	Microscopic Observation
1	Potato Dextrose agar	10^{-2}	Cottony dry colonies	
2	Potato Dextrose agar	10^{-2}	Grey coloured, Powdery colony of spore	
3	Potato Dextrose agar	10^{-2}	Circular smooth colonies	

Table no. 6: PDA samosa sample

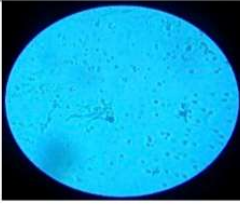
S.No	Medium	Dilution	Colony Morphology	Microscopic Observation
1	Potato Dextrose agar (Sample A)	10^{-2}	Powdered black coloured colony	
2	Potato Dextrose agar (Sample B)	10^{-2}	Green coloured powdered colonies	
3	Potato Dextrose agar (Sample C)	10^{-2}	Smooth cream coloured colony	

Table no.7 Czapekdox Panipuri sample

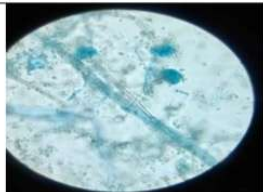
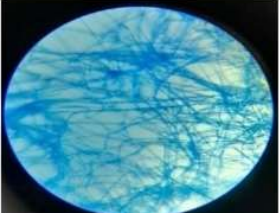
S.No	Medium	Dilution	Colony Morphology	Microscopic Observation
1	Czapekdox Agar	10^{-4}	Isolated black coloured colonies	
2	Czapekdox Agar	10^{-4}	Powdery black colour colonies	
3	Czapekdox Agar	10^{-4}	Pointed green coloured colonies	

Table no.8. Czapekdox Agar samosa sample

S.No	Medium	Dilution	Colony Morphology	Microscopic Observation
1	Czapekdox Agar	10 ⁻⁴	Orange coloured smooth colonies	
2	Czapekdox Agar	10 ⁻⁴	White coloured creamy colonies	
3	Czapekdox Agar	10 ⁻⁴	Orange coloured powdery colonies	

GRAM'S NATURE, MICROSCOPIC OBSERVATIONS, AND IMVIC TEST RESULTS OF PANI PURI AND SAMOSA SAMPLES COLLECTED

S.No.	SAMPLE	GRAM'S NATURE & MICROSCOPIC OBSERVATION	INDOLE TEST	METHYL TEST	VOGES PROSKAUER TEST	CITRATE TEST	ORGANISM
1	Panipuri (Nacharam)	Gram +VE Cocci	-	-	-	+	staphylococcus
2	Panipuri B (Mallapur)	Gram -VE Bacilli	+	+	-	-	E. coli
3	Panipuri C (Boduppall)	Gram -VE Bacilli	+	+	-	-	E. coli
4	Samosa A (Nacharam)	Gram +VE Cocci	-	-	-	+	staphylococcus
5	Samosa B (Boduppall)	Gram -VE Bacilli	+	+	-	-	E. coli
6	Samosa C (Mallapur)	Gram -VE Bacilli	+	+	-	-	E. coli

3.3 To characterize the bacteria based on their biochemical test [IMViC]



Panipuri sample A – IMViC test



Samosa sample A – IMViC test



Panipuri sample B -IMViC test



Samosa sample B – IMViC test



Panipuri sample C- IMViC test

Fig.3.3.1. IMViC Test Results For Panipuri Samples



Samosa sample C- IMViC test

Fig.3.3.2 IMViC Test Results for Samosa

3.4 To compare the degree of microbial contamination based on the growth of microorganisms and by TVC.

TABLE No. 10 TOTAL VIABLE COUNT OF BACTERIA IN PANIPURI AND SAMOSA SAMPLES COLLECTED FROM 3 DIFFERENT AREAS

Food Sample Type & area	Observation Date	Number of colonies	Dilution	Total Viable Count (TVC) (CFU/g)
Panipuri 1 (Nacharam)	17/2/2025	100	10^{-2}	10×10^4 CFU/g
Panipuri 2 (Mallapur)	17/2/2025	220	10^{-2}	22×10^4 CFU/g
Panipuri 3 (Boduppall)	17/2/2025	150	10^{-2}	15×10^4 CFU/g
Samosa 1 (Nacharam)	17/2/2025	18	10^{-4}	18×10^3 CFU/g
Samosa 2 (Mallapur)	17/2/2025	60	10^{-4}	6×10^4 CFU/g
Samosa 3 (Boduppall)	17/2/2025	180	10^{-2}	18×10^{10} CFU/g
Panipuri 1 (Nacharam)	21/2/2025	220	10^{-4}	22×10 CFU/g
Panipuri 2 (Mallapur)	21/2/2025	175	10^{-4}	17.5×10 CFU/g
Panipuri 3 (Boduppall)	21/2/2025	150	10^{-4}	15×10 CFU/g
Samosa 1 (Nacharam)	21/2/2025	15	10^{-4}	15×10 CFU/g
Samosa 2 (Mallapur)	21/2/2025	0	10^{-4}	0 CFU/g
Samosa 3 (Boduppall)	21/2/2025	0	10^{-4}	0 CFU/g

SUMMARY: The Total Viable Count (TVC) test measures the number of microorganisms in a sample to assess contamination levels and quality. Colony counts directly reflect contamination. High TVC levels can indicate poor product quality or health risks.

Key findings includes:

- Panipuri Sample 2, collected from Mallapur (on 17.2.25) showed the highest contamination.
- Samosa Sample 3, collected from Boduppall (17.2.25) had the highest contamination among samosa samples.
- Panipuri Sample 2 from Mallapur (21.2.21) had the highest contamination compared to samples from Nacharam and Boduppall.
- Samosa Sample 1 from Nacharam (21.2.25) had 15 colonies, indicating contamination, while other samples from Mallapur and Nacharam showed no colony growth and were uncontaminated.

DISCUSSION:

Street food, though affordable and popular, poses significant health risks due to microbial contamination from poor hygiene and handling. The present study evaluated the degree of microbial contamination in commonly consumed street foods like Pani puri and Samosa, collected from different regions of Hyderabad (Nacharam, Boduppall & Mallapur) and assessed their potential health impact with reference to IKS-based food safety practices. The findings reveal that both the food items collected from three different regions of Hyderabad, showed varied level of microbial contamination.

- **Bacterial Contamination:** All samples showed bacterial growth on EMB agar, including *Staphylococcus aureus*, indicating potential for foodborne illness.
- **Fungal Contamination:** Fungi like *Aspergillus niger* and *Aspergillus flavus* were found on Czapekdox and PDA media, suggesting spoilage and risk of mycotoxin exposure.
- **Parasitic Contamination:** *Ascaris lumbricoides* eggs were detected, pointing to fecal contamination likely from unsafe water and unhygienic conditions.
- **Comparison between Pani puri and Samosa:** Among the two food items analyzed, Pani puri exhibited a significantly higher microbial load compared to Samosa. This may be because of the use of untreated or contaminated water, repeated manual handling during servings and prolonged exposure of pani to the open environment. In contrast, Samosa, being a deep fried food, showed relatively lower microbial counts, possibly due to high temperature during frying, which reduces microbial survival.

The presence of bacterial, fungal, and parasitic contaminants indicates inadequate personal hygiene, unsafe water usage and improper food handling practices among street food vendors. Many of these risks could be minimized by adopting basic hygienic measures traditionally followed in Indian households, such as thorough handwashing before food preparation or handling during serving, use of clean water, proper cleaning of utensils which are used for storage and consumption of freshly prepared food.

The findings of this study support the importance of traditional Indian Food safety practices described in IKS. Practices such as use of Copper vessels for water storage, fresh preparation of food every time, avoidance of prolonged storage, washing utensils thoroughly used for storage and incorporation of natural anti-microbial spices like turmeric, ginger, garlic, clove and pepper to inhibit the growth of harmful bacteria and fungi, can significantly reduce microbial contamination. We must also educate vendors about these simple traditional practices along with basic hygiene training, promote proper food handling, washing, and cooking practices, conduct regular food safety inspections, raise public awareness.

CONCLUSION:

The present study concludes that street vended foods such as Samosa and Pani-puri sold in different regions of Hyderabad showed significant microbial contamination, including both Gram-positive and Gram-negative bacteria and toxin-producing fungi indicating potential public health risks. The observed microbial contamination raises questions on the inadequate hygienic practices considered during the food preparations, unsanitary handling of the ingredients and potential contamination from environmental source. These findings indicate a risk of foodborne illnesses and highlight poor hygiene practices, such as the use of untreated water and unsanitary food handling. Consuming these street foods on regular basis can increase the risk of food-borne diseases like diarrhoea, gastroenteritis and food poisoning among vulnerable populations.

To mitigate these risks, there is an urgent need for:

- Food safety training for street vendors
- Strict hygiene enforcement and regular inspections
- Public awareness about safe food practices

Importantly, the study demonstrates the relevance of IKS-based food safety practices in preventing microbial contamination in these street foods. Traditional methods such as fresh preparation of food, use of copper vessels, incorporation of anti-microbial spices and sticking to basic hygiene principles described in Ahara vidhi can effectively complement modern food safety measures. Integrating these traditional practices with scientific approaches can contribute to safer street food consumption and improved public health outcomes.

Today, modern processing industries continue to draw inspiration from these age-old techniques to develop sustainable and chemical-free preservation methods. As the demand for organic and naturally preserved food grows, these time-tested methods are finding renewed relevance in both rural and urban households. The findings highlight the importance of reviving traditional Indian hygienic and food preparation practices alongside modern food safety measures to promote better health outcomes.

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