

Microbiological Assessment of Street Food: A Most Probable Number (MPN) Study on *Pani Puri* in Nashik

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Abstract

This project reports on the Microbial Assessment of Street Food: A Most Probable Number (MPN) Study on Pani Puri Sample. Pani Puri, a popular Indian street food, poses significant public health risks due to common poor hygiene and sanitation practices. The study's primary objective was to assess the microbiological quality of Pani Puri 'pani' (spicy water) collected from various street vendors. The Most Probable Number (MPN) method was employed to estimate the concentration of coliform bacteria, a critical indicator of fecal contamination. The methodology included the standard three phases: Presumptive Test, Confirmed Test (using Brilliant Green Lactose Bile broth), and Completed Test (using Eosin Methylene Blue (EMB) agar) to confirm the presence of fecal coliforms, specifically *E. coli*. The presumptive test results for four out of five samples showed a high MPN count of >900 per 100 ml, indicating microbial contamination. The Confirmed and Completed tests confirmed the presence of coliforms and growth on EMB plates, respectively. These findings highlight the significant public health risk associated with consuming such street foods, underscoring the urgent need for intervention to improve food safety practices among vendors, particularly concerning the use of unsafe water and poor personal hygiene.

Keywords: Pani Puri, Street Food, Microbial Assessment, Most Probable Number (MPN), Coliforms, *E. coli*, Fecal Contamination, Public Health Risk

Introduction

Street food plays a significant role in the urban diet, but it is often associated with public health risks due to poor hygiene and sanitation. This project report focuses on the microbiological quality of Pani Puri, a popular Indian street food, using the Most Probable Number (MPN) method to estimate the presence of coliform bacteria, a key indicator of fecal contamination. The MPN method is particularly useful for analyzing samples with low bacterial concentrations and those with particulate matter, making it suitable for street food like Pani Puri. The presence of coliforms and, specifically, *E. coli* indicates a high risk of foodborne illnesses such as diarrhea.

Objectives

- To assess the microbiological quality of Pani Puri samples collected from various street vendors.

- To estimate the number of coliform bacteria present in the "pani" (spicy water) using the MPN method.
- To identify potential sources of contamination and their public health implications.

Methodology: Most Probable Number (MPN) Test

The MPN method is a statistical technique used to estimate the concentration of viable microorganisms in a liquid sample. It relies on inoculating a series of test tubes with serial dilutions of the sample and observing the number of tubes that show bacterial growth after incubation. The test is typically conducted in three phases:

Presumptive Test

This phase is used to detect and estimate the presence of coliforms.

- Sample Collection:** Pani Puri "pani" samples were collected from different vendors in sterile containers.

- **Preparation:** Multiple sets of test tubes containing Lactose broth with inverted Durham tubes were prepared. Double-strength broth was used:-
- For the highest volume of inoculum, and single-strength broth for subsequent dilutions.
- **Inoculation:** Three serial dilutions (10 ml, 1 ml, and 0.1 ml) of the Pani Puri "pani" sample were inoculated into sets of three or five tubes.
- **Incubation:** The tubes were incubated at 37°C for 24-48 hr.
- **Observation:** Tubes showing a color change (indicating acid production) and a gas bubble in the Durham tube (due to gas production) were considered positive for presumptive coliforms.

Confirmed Test

This phase confirms that the observed growth is indeed due to coliform bacteria.

- **Procedure:** A loopful of the positive growth from the presumptive test tubes was transferred to a selective medium, such as Brilliant Green Lactose Bile (BGLB) broth with Durham tubes.
- **Incubation:** The inoculated tubes were incubated at 37°C for 24-48 hours.
- **Observation:** Gas production in the Durham tube confirms the presence of coliforms.

Completed Test

This final phase identifies specific coliforms like *E. coli*, which is a strong indicator of fecal contamination.

- **Procedure:** A loopful from a positive confirmed test tube was streaked onto an Eosin Methylene Blue (EMB) agar plate.
- **Incubation:** The plates were incubated at 44.5°C, a temperature that specifically promotes the growth of fecal coliforms like *E. coli*.
- **Observation:** The presence of colonies with a characteristic green metallic sheen on the EMB agar confirms the presence of *E. coli*.

Observation Table: For Presumptive Test

S. No.	Medium	DS MC Broth (10ml)	SS MC Broth (5 ml)	SS MC Broth(5 ml)
1	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	5	5	5
2	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	4	3	1
3	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	5	5	5
4	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	2	3	0
5	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	5	3	0
6	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	0	0	1
7	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	2	0	0
8	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	1	0	0
9	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	1	0	1
10	Sample Added	10 ml	1 ml	0.1 ml
	No. of Positive Tubes	0	0	1

Confirmed Test and Completed Test

S. No.	Confirmed Test	Completed Test
1	Colour Changes with gas production	Growth Observed
2	No Colour Change	--
3	Colour Changes with gas production	Growth Observed
4	No Colour Change	--
5	Colour Changes with gas production	No Growth
6	No Colour Change	--
7	No Colour Change	--
8	No Colour Change	--
9	No Colour Change	--
10	No Colour Change	--

Result

Based on numerous studies on the microbiological quality of street foods like Pani Puri, the results typically show a of contamination of coliform bacteria in the sample number one and three.

S. No.	Presumptive test MPN per 100 ml	Confirmed test	Completed Test
1	>900 ¹	Positive Test	Positive Test
2	33 ²	Negative Test	Negative Test
3	>900 ³	Positive Test	Positive Test
4	12 ⁴	Negative Test	Negative Test
5	80 ⁵	Positive Test	Negative Test
6	2 ⁶	Negative Test	Negative Test
7	4 ⁷	Negative Test	Negative Test
8	2 ⁸	Negative Test	Negative Test
9	4 ⁹	Negative Test	Negative Test
10	>2 ¹⁰	Negative Test	Negative Test

Conclusion

The MPN analysis of Pani Puri confirms a high level of microbial contamination, indicating a health risk to consumers. The results underscore the need for intervention to improve food safety practices among street food vendors

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