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Bacteriological Identification of Salmonella In Street Vended Food Panipuri In Bhopal Awadhpuri Region

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Abstract:- Street-vendor food, such as Panipuri, is popular among people but is often prepared and sold under unhygienic conditions, making it susceptible to microbial contamination. In this study, ten samples from various zones of the Awadhpuri area in Bhopal were collected aseptically and analyzed within one hour of procurement. Standard procedures were followed for isolation, enumeration, and identification of bacteria. The bacteria were initially isolated on NAM plates, and DNA extraction was carried out using the phenol-chloroform method. Pathogenic bacteria such as Escherichia coli (40%), Salmonella typhi (30%), and Salmonella paratyphi A (20%) were detected in the samples.

Additionally, Escherichia coli (40%) and Salmonella typhi (20%) were isolated in the potato masala. The bacteriological analysis of Panipuri sold in Bhopal city highlights significant hazards to human health. The assay was validated using positive controls and compared to conventional culture-based methods to establish its accuracy and sensitivity. The PCR and qPCR methods targeted the conserved regions of Salmonella's ST gene to identify and quantify the pathogen in the samples. The qPCR emerged as a rapid and reliable method for Salmonella detection in food samples, providing crucial insights for food safety authorities to implement targeted interventions and minimize the risk of foodborne illnesses.

Keywords:- Salmonella, Panipuri, Street Food, Bacteria, Food Contamination

1. INTRODUCTION

In India, the streets offer a plethora of unique street food options, attracting millions of people from various classes and economic backgrounds. Street food holds a significant place in the daily diet of the urban population, contributing to approximately 35% of their food intake (Consumer International, 2011; Pmal). It is widely regarded as finger and fast food, offering a more affordable alternative to restaurant meals. Street food typically refers to ready-to-eat food or beverages sold in public places such as streets, markets, fairs, parks, or other outdoor venues. However, the consumption of street food poses potential risks of foodborne diseases due to the lack of proper hygiene practices during preparation and vending. These foods are often sold by hawkers or vendors from portable stalls, carts, or food trucks, increasing the risk of food contamination. According to a 2007 Food and Agriculture Organization study, an estimated 2.5 billion people consume street food daily. In urban areas of India, ready-to-eat street food has become a staple due to its convenience and

affordability. Among India's most popular street foods are Panipuri or Golgappas, which are often prepared and served under unhygienic conditions, posing a high risk of contamination, particularly through water, a common ingredient in Panipuri. This study aims to analyze potential risk factors associated with Salmonella in Panipuri water, as Salmonella is a common pathogen responsible for foodborne illnesses.

Salmonella belongs to the Enterobacteriaceae family and is a genus of rod-shaped, gram-negative bacteria. Salmonella enterica is the type species and comprises over 2,600 serotypes. Named after Daniel Elmer Salmon, an American veterinary surgeon, Salmonella species are non-spore-forming, motile bacteria with peritrichous flagella. They are chemotrophs, deriving energy from oxidation and reduction reactions using organic sources. Typhoidal Salmonella serotypes can cause foodborne illnesses, typhoid fever, and paratyphoid fever. Salmonella can thrive in aerobic and anaerobic environments, with optimal growth conditions at approximately 37°C and a pH of 4.0-9.0. The microbiological quality of street-vended foods is crucial as they can serve as significant sources of foodborne infections and intoxications. However, street food vendors often lack awareness of good hygiene practices, and hygiene facilities are limited at vending sites. Therefore, this study evaluates the bacteriological quality of Panipuri, aiming to identify and isolate risk factors associated with street food trade in Bhopal city and recommend safety interventions to ensure safe food practices intervention to ensure safe food practices.

2. MATERIALS AND METHODS

A total of 10 Panipuri samples were collected in autoclaved collection tubes from various vendors in and around Awadhpuri, Bhopal, to prepare pure cultures for further analysis. The hygiene and cleanliness status of the vending sites were assessed through structured interviews and observations. The pH of panipuri water and potato masala was determined using a pH paper strip directly at the vendor and confirmed using a pH meter (Systronics-361) in the laboratory.

4. SCREENING OF BACTERIA SPECIES IN NAM MEDIA

Nutrient Agar media(NAM): Nutrient Agar is a nutrient medium that can cultivate microorganisms and support the growth of various non-fastidious organisms. Nutrient agar is widely used because it can support the development of a wide range of bacteria and contains numerous nutrients required for bacterial growth.

4.1 Preparation of NAM

1. Suspend 28g of nutrient agar powder in 1 litre of distilled water.
2. Heat the mixture while stirring to dissolve all components fully.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once autoclaved, allow the nutrient agar to cool but not solidify.
5. Pour the nutrient agar into each Petri dish and leave the plates on a sterile surface until the agar has solidified.
6. Replace the lids of each Petri dish and store the plates in a refrigerator.

4.1.1. Protocol for enumeration of bacteria

1. Autoclave 9 test tubes at 121°C for 15 minutes.
2. Add 9 ml autoclaved distilled water to each tube.
3. Mix 1 ml of Panipuri water into the first test tube.

4. Transfer 1 ml of the mixed solution into the second tube containing 9 ml of distilled water and mix well, resulting in a dilution factor 10^-1.
5. Repeat the process until the seventh tube by transferring 1 ml of mixture from the previous tube.
6. Plate 100µl from the 10^-7 tube onto the Nutrient Agar Medium (NAM).
7. Repeat the process for all water samples.
8. Incubate the NAM plates in an incubator at 37°C overnight for desired bacterial growth and PDA plates at 25°C for 2-3 days for fungal growth. The total viable count (TVC) is then counted and reported.
9. Store the isolated colonies in pure culture for further use.
10. The dilution factor of each tube is calculated as follows:
11. Dilution Factor = Volume of Sample / (Volume of Sample + Volume of Diluent)

4.2 DNA EXTRACTION FROM BACTERIAL CELLS

The selected colonies, comprising ten randomly chosen samples, were cultured in an LB broth liquid medium to facilitate DNA extraction. Isolating DNA from bacteria is a relatively straightforward process, requiring optimal cultivation conditions in a suitable medium and temperature, typically during the late log to early stationary phase, to ensure maximum yield. Genomic DNA isolation involves the separation of total DNA from RNA, proteins, lipids, and other cellular components.

4.2.1 REQUIREMENT

1. Sol. B (pH 8.0)
2. 20% SDS
3. Proteinase K
4. Phenol-Chloroform-Isoamyl alcohol mixture (25:24:1)
5. 5 M Sodium Acetate (pH 5.2)
6. Chilled Isopropanol (IPA)
7. 70% ethanol
8. Nuclease-free water
9. Eppendorf tubes (2ml)
10. Micropipette

4.2.2 PREPARATION OF REAGENTS

1. Sol. B (pH 8.0): Dissolve 40 ml of 1M Tris HCl (pH 8), 15 ml of 1M NaCl, and 10 ml of 0.5 M EDTA in 95 ml distilled water. Autoclave the content and cool it down. Add 5 ml of 20% SDS solution and mix well.
2. 20% SDS: Dissolve 20 g of SDS in 100 ml autoclaved distilled water.
3. Proteinase K: Dissolve 10 mg of Proteinase K in 1 ml of autoclaved distilled water.
4. Phenol: Mix 25 ml of saturated phenol with 24 ml of Chloroform, then add 1 ml of isoamyl alcohol.
5. 5 M Sodium Acetate: Dissolve 41g of Sodium acetate in 100 ml distilled water and adjust pH to 5.2 with dilute acetic acid.
6. Isopropanol: Keep the IPA at -20°C before use.
7. 70% Ethanol: Dissolve 70 ml absolute ethanol in 30 ml distilled water.

4.2.3 PROCEDURE

1. Centrifuge the grown bacterial broth for 20 mins at 5000 RPM to extract the bacterial pellet.
2. Discard the supernatant and add 1 ml of solution B, 50 µl of 20% SDS, and 5 µl of Proteinase K.

3. Mix the above mixture well and incubate at 56°C for 60 mins.
4. Transfer the supernatant to a fresh Eppendorf tube and add 250 µl of sodium acetate and 500 µl of PCI mixture to the contents. Mix well by inverting and incubating.
5. Centrifuge the mixture at 10,000 rpm for 15 minutes.
6. Three layers will form. Carefully transfer the upper layer containing DNA to a fresh tube.
7. Add 500 µl of Chilled IPA to precipitate the DNA. Incubate the mixture at -20°C overnight.
8. Centrifuge the mixture at 10,000 RPM for 10 min. Discard the supernatant.
9. Wash the pellet with 500 µl of 70% ethanol by centrifuging at 10,000 RPM for 5 mins.
10. Discard the supernatant and air-dry the pellet.
11. Dissolve the DNA pellet in 15 µl of Nuclease-free water.
12. Visualize the dissolved DNA under UV in agarose gel electrophoresis.

Fig-1:Showing PCR machine used in the experiment

5. PCR AMPLIFICATION OF SALMONELLA BACTERIA USING A SPECIFIC PRIMER

In the present study, Polymerase Chain Reaction (PCR), as shown in Figure 1, was employed to examine specific ST primers targeting Salmonella. This technique amplifies DNA sequences, enabling the detection and identification of the target organism. By utilizing conventional PCR methodology, the study aimed to validate the presence of Salmonella using specific genetic markers.

Table 1. PCR was performed using primers on Salmonella Bacteria Species

Primer	Sequence (5' to 3')
ST- Fw	AGCCAACCATTGCTAAATTGGCGCA
ST- Rv	GGTAGAAATTCCCAGCGGGTACTG

Table 2. represents the PCR Reaction mixture

S. No	Content	Volume(µl)
1	Buffer	2
2	MgCl2	0.5
3	dNTPs	0.5
4	Primer (forward)	0.5
5	Primer (reverse)	0.5
6	Taq polymerase	0.2
7	DNA Sample	1
8	D/W	14.8
9	Total volume	20

Rekha Bisht's research focuses on rapidly identifying Gyrase-resistant bacteria in food samples using real-time PCR techniques, and the sequence of bacteria is shown in Table 1. By employing real-time PCR, the study aims to swiftly and accurately detect bacterial strains resistant to Gyrase inhibitors, a class of antibiotics commonly used in food production. This method enables timely intervention to prevent the spread of antibiotic-resistant bacteria, safeguarding food safety and public health. Bisht's work underscores the importance

of molecular techniques in monitoring and managing bacterial resistance in foodborne pathogens.

Table 3. PCR Steps and Corresponding Time Duration

S.No	Treatments	Temperature	Duration
1	Pre- denaturation	95	5 min
2	Denuturation	95	30 Sec
3	Annealing	60	30 Sec
4	Primer extension	72	40 Sec
5	Final extension	72	7 min

Table 4: qPCR was performed using primers described

1	RTPCR Master mix (2x)	5 µl
2	Primer forward	1 µl
3	Primer reverse	1 µl
4	DNA	1 µl
5	Nucleare-free distilled water	2 µl
	Total Volume	10 µl

Figure 2. Real-Time PCR Machine Used in the Present Study

5.1 qPCR AMPLIFICATION FOR RAPID DETECTION OF SALMONELLA

Real-time PCR, also known as quantitative polymerase chain reaction (qPCR), is a molecular biology laboratory technique based on the polymerase chain reaction. It allows for the monitoring of DNA amplification in real-time during the PCR process rather than at its conclusion, as seen in conventional PCR. Real-time PCR can provide quantitative and semi-quantitative analysis.

Table 5. Description of PCR Performed Using Primers.

Primer	Sequence (5' to 3')
ST-Fw	AGCCAACCATTGCTAAATTGGCGCA
ST- Rv	GGTAGAAATTCCCAGCGGGTACTG

6. RESULT AND DISCUSSION

In the present study, a total of 10 Panipuri samples were collected from various public places in Awadhpuri, Bhopal. It was observed that vendors either fully or partially prepare their food items at home and store them at ambient temperature, encouraging the growth of mesophilic organisms, including foodborne pathogenic bacteria. Notably, none of the vendors used gloves or head coverings during food preparation and selling. The elevated acidity of the Panipuri water could be attributed to the addition of tamarind juice and other ingredients. Salmonellosis, historically associated with sources of animal origin such as water, meat, eggs, and poultry, has garnered significant regulatory attention and mitigation efforts. According to data from the European Food Safety Authority (EFSA) and the Food and Agriculture Organization (FAO)/World Health Organization (WHO), Salmonella spp. Remains a significant global risk for foodborne illness. In 2008, Salmonellosis was the second most commonly reported zoonotic disease in humans after campylobacteriosis, with 131,468 confirmed cases in the European Union (EU). Furthermore, this study investigated Salmonella species in water samples using rapid qPCR identification, which PCR subsequently validated. Various colonies were observed on nutrient agar media when water samples were inoculated onto culture plates.

Figure 3. Bacterial colonies on nutrient agar media plate.

The inappropriate and excessive use of antibiotics in humans and animals has contributed to the emergence of resistant strains of this pathogen. Consequently, treatment options for Salmonella infections may become limited, leading to prolonged illness and increased healthcare costs.

6. CONCLUSION

Identifying Salmonella in the examined samples highlights the imperative for sustained vigilance in the surveillance and managing of foodborne pathogens. Through adopting preventive strategies and disseminating awareness initiatives, strides can be made in fortifying public health defences and mitigating the prevalence of typhoid fever and other foodborne diseases in low and middle-income nations. Addressing the contamination of street-vendor food, particularly popular delicacies like Panipuri, demands comprehensive efforts encompassing stringent hygiene practices, robust regulatory frameworks, and public education campaigns. Furthermore, the study underscores the significance of integrating advanced molecular techniques, such as qPCR, into routine food safety protocols for expedited and accurate pathogen detection. By enhancing the capacity for early detection and intervention, healthcare systems can effectively curb the transmission of Salmonella and avert potential outbreaks. Moreover, the findings accentuate the critical role of interdisciplinary collaboration between health authorities, food regulatory agencies, and local communities to institute robust surveillance mechanisms and enforce adherence to food safety standards. Ultimately, concerted action on multiple fronts is essential to safeguarding consumer well-being and fostering a culture of food safety resilience in vulnerable populations.

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