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Original Research Article

Prevalence and characterization of selected foodborne pathogens in frequently consumed street foods of Hyderabad, India: An exploratory study

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ABSTRACT

Background: Emerging foodborne pathogens in recent times are been a major public health concern for consumers. Food establishments such as street foods, which cater to larger sections of the population in developing countries, have become potential vehicles for emerging foodborne pathogens. The present study aimed to identify high-risk/emerging pathogens in street-vended foods from the south Indian city –Hyderabad.

Materials & Methods: A cross-sectional study was carried out on 150 Chinese fast food samples, and 150 Bhelpuri- (mixed of puffed rice vegetables and sauces) samples, collected by stratified random sampling method. Foodborne pathogens/hygiene indicators were analyzed using USFDA-BAM (United States Food and Drug Administration-Bacteriological Analytical Manual) methods.

Results: Enteropathogens such as *Shigella* spp. and *Salmonella* spp. were identified in 45.3% (68/150) and 20% (30/150) respectively in Bhel puri samples. Among Chinese fast foods, *Bacillus cereus* was detected in 90-92% of samples. About 40% samples were observed positive for *S. aureus* in Chinese fried rice. Among the isolates of *Shigella* (n=20), 50% were resistant to Co-Trimoxazole and Amoxicillin. *Salmonella bongori* and *Shigella sonnei* were identified in the food samples.

Conclusion: Periodic evaluation and monitoring of street foods are very essential to estimate the risk and toxicity of foodborne pathogens.

1. Introduction

Street foods consist of ready-to-eat items and drinks prepared and sold by vendors in urban and public settings.[1] They are a vital part of modern eating habits and a significant contributor to the informal urban sector in developing countries. Approximately 2.5 billion people eat street food every day globally[2] and India is no exception with an estimated 20 lakh vendors serving street foods.[3]

However, there are many concerns about its safety. People consuming street-vended foods are known to experience foodborne diseases like diarrhea, typhoid fever, and food poisoning[4] and the majority of them go unreported. Global estimates of foodborne diseases revealed that The African and South-East Asia regions are the most affected by foodborne diseases.[5] A recent report indicated that foodborne disease cases in India are projected to increase from 100 million to between 150 and 177 million by 2030. This implies that, on average, one in nine people is expected to fall ill from foodborne illnesses by 2030.[6]

Diarrheal disease agents were the leading cause of foodborne disease burden, and non-typhoidal *Salmonella enterica* is an important burden globally. Other main organisms include enteropathogenic *Escherichia coli*,

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enterotoxigenic *Escherichia coli* and *Vibrio cholerae,* and *Campylobacter* spp. These disease-causing agents have been reported in street foods.[7–9]

Street foods in recent times are becoming a new food vehicle for emerging foodborne diseases. Emerging, re-emerging, or drug-resistant pathogens have recently appeared in the population. While these pathogens have been widespread for many years, they have only recently been identified due to advancements in knowledge and methods for detecting and analyzing the disease agents.[10] Emerging foodborne pathogens can include both Gram-negative and Gram-positive bacteria.[11] Reports have shown emerging strains of bacteria in street foods as well.[12–14]

Microbial contamination in street-vended foods has become a pressing concern. Thorough studies of the microorganisms found in these foods are crucial for assessing the toxicity of foodborne pathogens. At present, there is limited data on the microbial contamination of street foods in developing countries like India. This study aimed to identify high-risk and emerging foodborne pathogens in commonly consumed street foods in Hyderabad, a city in South India.

2. Materials and Methods

2.1. Study area

Hyderabad, the capital of the state of Telangana, has a population of approximately 6.9 million, with a metropolitan population of around 7.75 million. This makes it the fourth most populous city and the sixth most populous urban agglomeration in India. The city features a significant informal economy, with 40,000 to 50,000 street vendors and a growing number. In this study, 300 street food samples were collected from five zones of Hyderabad—east, west, north, central, and south—encompassing 18 circles and 150 wards under the Greater Hyderabad Municipal Corporation (GHMC) for microbiological analysis.

2.2. Study design, sampling technique, Inclusion criteria

It was a cross-sectional study. A stratified random sampling technique was applied and samples were stratified into zones and randomly collected from each zone. Street food vendors who are typically on streets without basic infrastructure (Cement roof, Furniture, Drinking water supply, etc.)

2.3. Sample collection and processing

A total of 300 street food samples were collected, including 150 samples of Chinese fast foods (comprising 50 chicken noodles, 50 chicken fried rice, and 50 chicken - 65) and 150 samples of Bhelpuri (a mix of puffed rice, vegetables, and sauces). The samples were transported in sterile polythene bags, kept at 4-10°C, and analyzed within 2-4 hours of arrival at the laboratory. Each sample, weighing 25 grams, was added to 225 ml of sterile buffered peptone water. The resulting dilutions were then inoculated onto appropriate media for further analysis.

2.4. Pathogen and indicator organism analysis

The identification and enumeration of foodborne pathogens and hygiene indicators—*E. coli*, Fecal coliforms, *S. aureus*, *Salmonella* spp., *Shigella* spp., and *Bacillus cereus*—were conducted following the standard procedures outlined in the Bacteriological Analytical Manual (USFDA-BAM).

E. coli: Identified by spreading 0.1 mL of a diluted sample onto sterile MacConkey Agar (Hi Media Laboratories Pvt. Ltd.) and incubating at 35°C for 24 hours. Pink colonies were considered presumptive *E. coli*. Further confirmation was done by streaking colonies onto EMB agar for metallic sheen and performing biochemical carbohydrate fermentation tests.

S. aureus: Detected by spreading 0.1 mL of a diluted sample onto sterile Baird Parker Agar (Hi Media Laboratories Pvt. Ltd.) and incubating at 35°C for 24 hours. Golden yellow colonies indicated presumptive *S. aureus*. The presence of coagulase was tested to confirm coagulase-positive strains.

B. cereus: Isolated on Bacillus Cereus Agar (Hi Media Laboratories Pvt. Ltd.) supplemented with egg yolk and Polymyxin B. Diluted samples (0.1 mL) were spread on plates and incubated at 30°C for 48 hours. Blue colonies were presumptive *B. cereus* and were confirmed through Gram staining and nitrate reduction tests.

Salmonella spp.: Detected by spreading a 1:10 dilution of the food samples onto Salmonella-Shigella Agar (Hi Media Laboratories Pvt. Ltd.) and incubating at 37°C for 24 hours. Jet black colonies with translucent edges were indicative of *Salmonella* spp., confirmed by a Latex agglutination test.

Shigella spp.: Identified by spreading a 1:10 dilution of food samples onto Salmonella-Shigella Agar (Hi Media Laboratories Pvt. Ltd.) and incubating at 37°C for 24 hours. Transparent colonies were considered presumptive *Shigella* and confirmed by biochemical tests.

2.5. Enterotoxin detection of S. aureus

The *S. aureus* cultures were tested for toxin production This was done by an ELISA kit provided by R-Biopharma AG, Darmstadt, Germany.

Pure cultures have been prepared and stored on slant agar tubes and glycerol stocks. Selected isolates have been subjected to further characterization.

2.6. DNA isolation & 16s rRNA sequencing

DNA isolation was done using a column-based DNA extraction kit supplied by Bioserve Biotechnologies Pvt.

Limited, Hyderabad. Quantification of DNA was done using the NanoDrop spectrophotometer. Sequencing was performed with 5 different primers designed in the conserved regions on 16s rRNA i.e., 16SEQ2R, 16SEQ3F, INS16SREV, 16SEQRF, 16SEQ4R.

2.7. Antibiotic sensitivity of isolated foodborne pathogens

Antimicrobial sensitivity was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Hi Media Pvt. Ltd.). The study employed eight of the most commonly prescribed antibiotics by physicians for this evaluation.

Ethical approval: This study was granted ethical clearance by the Institutional Ethical Committee at the National Institute of Nutrition, Hyderabad *(Registration No: ECR/35/Inst/AP/2013)/* Participation was voluntary, with all street vendors being fully informed about the study's objectives and procedures. To ensure confidentiality, participants were identified by code numbers instead of their names.

3. Results

3.1. Prevalence of foodborne pathogens and hygiene indicator organisms

Bhel puri samples showed a high prevalence of indicator organisms, with *E. coli* detected in 60.6% of the samples and fecal coliforms in 67.3%. *Bacillus cereus* which is another most common pathogen in cereals and cereal products was detected in 67.3% of the bhelpuri samples. Enteropathogens such as *Shigella* and *Salmonella* were identified in 45.3 and 20% of samples respectively.

Among Chinese fast foods, *Bacillus cereus* was detected in 90-92% of samples. About 28-40% of samples were observed positive for *S. aureus* with the highest in Chinese fried rice. Indicator organisms were detected in 16-24% of Chinese foods followed by *Shigella* spp. *Salmonella* spp. was detected in only 3 samples, as detailed in Table 1.

3.2. Zone (East, West, North, South, and Central) wise comparison of contamination in street food samples of Hyderabad

Bhelpuri- Among all the zones, north and south zone samples have shown high percentages of bacterial contamination. The lowest prevalence (not exceeding 30%) was observed in the samples from the central zone. *Bacillus cereus* (85%) and Fecal coliforms (92%) were more prevalent (Figure 1). Chinese fast foods- *Bacillus cereus* was more prevalent (80-99%) in all the samples of Chinese fast foods and followed the same trend in all the five zones of Hyderabad (Figure 2).

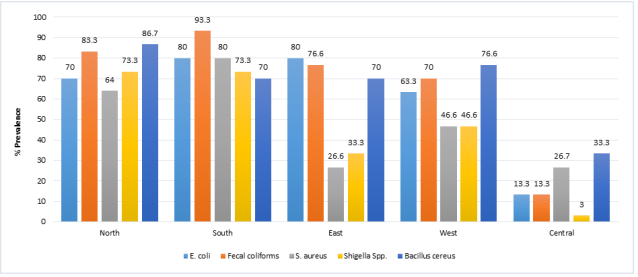

Figure 1: Prevalence of foodborne pathogens/ indicator organisms in Bhel puri samples in different zones of Hyderabad

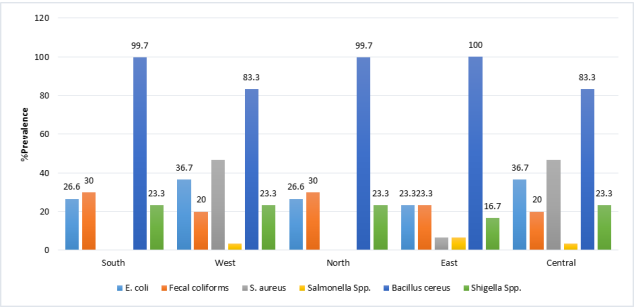

Figure 2: Prevalence of foodborne pathogens/ indicator organisms in chinese fast foods in different zones of Hyderabad

3.3. Microbial population in high-risk street foods

Bhelpuri samples were contaminated with fecal coliforms (2.59 log) and *Bacillus cereus* (2.47 log) followed by *E. coli* (2.27 log) and *Salmonella* spp. (2.16 log). Chinese fast foods were contaminated with *Bacillus cereus* (3.65-4.01 log). The minimum and maximum load of bacteria were also presented in Table 1. The minimum log value was two and the maximum value was five log units.

3.4. Antibiotic sensitivity of isolates from street foods

About 81 isolates have been used to check the antibiotic sensitivity with nine commonly prescribed antibiotics. All the isolates (n=6) of *Salmonella* spp. were sensitive to the antibiotics except for one isolate which was resistant to Co-Trimoxazole. Among the isolates of *Shigella* (n=20), 50% were resistant to Co-Trimoxazole, and the majority of 11 isolates to Amoxicillin. All the *E. coli* isolates have been sensitive to antibiotics, except for Amoxicillin, to which there was a 71% resistance rate. Compared to the other isolates *S. aureus* was more resistant to the antibiotics with the highest of 85% resistance to Furazonidole, Gentamycin, and Ampicillin. Among the four types of isolates, *Salmonella* spp. and *E. coli* showed greater sensitivity to antibiotics, whereas *Shigella* and *S. aureus* exhibited higher resistance, as shown in Table 2.

Table 1: Prevalence of foodborne pathogens and hygiene indicator organisms in street foods with the percentage in parenthesis in Hyderabad

Type of food	*E. coli*	Fecal coliforms	*S. aureus*	*Shigella* spp.	*Salmonella* spp.	*Bacillus cereus*
***Bhelpuri* (n=150)**						
Prevalence n (%)	91(60.6%)	101(67.3%)	40(26.6%)	68(45.3%)	30(20%)	101(67.3)
Log mean±SD	2.27±1.90	2.59±1.90	0.94±1.61	1.74±1.93	2.16±1.25	2.47±1.77
(Max-Min)	(2.30-4.54)	(2.60-4.77)	(2.47-4.54)	(2.69-4.54)	(2.02-3.95)	(2.77-4.54)
Chinese fast foods (n=150)						
Chicken fried rice (50)						
Prevalence n (%)	8(16%)	8(16%)	20(40%)	5(10%)	2(4%)	46(92%)
Log mean±SD	0.78±1.62	0.75±1.60	1.58±1.85	0.37±1.17	0.11±0.61	4.01±0.72
(Max-Min)	(2.30-5.17)	(2.00-5.17)	(2.77-4.65)	(2.30-4.54)	(1.71-3.95)	(2.77-5.23)
Chicken noodles (50)						
Prevalence n (%)	12(24%)	12(24%)	14(28%)	8(16%)	1(2%)	45(90%)
Log mean±SD	0.84±1.58	0.94±1.67	0.96±1.60	0.57±1.28	0.04±0.34	3.65±1.28
(Max-Min)	(2.30-5.11)	(2.60-5.17)	(2.30-4.69)	(2.30-4.54)	(0.01-2.47)	(2.30-5.20)
Chicken 65 (50)						
Prevalence n (%)	9(18%)	11(22%)	19(38%)	5(10%)	ND	46(92%)
Log mean±SD	0.65±1.48	0.87±1.71	1.34±1.79	0.38±1.19	ND	3.75±1.22
(Max-Min)	(2.00-5.21)	(2.30-5.20)	(2.30-4.54)	(2.47-4.87)		(2.60-5.17)

Table 2: Antibiotic sensitivity of foodborne pathogens isolated from street foods with the percentage in parenthesis

Antibiotics	*Salmonella* spp.(n=6)	*Shigella* spp.(n=20)	*E. coli* (n=17)	*S. aureus* (n=38)
Cefatoxime (CTX)	6(100%)	14(70%)	17(100)	36(94.8%)
Furazonidole (FR)	6(100%)	13(65%)	17(100)	6(15%)
Norfloxacin (NX)	6(100%)	14(70%)	17(100)	37(97.45)
Cotrimoxazole (COT)	5(83.4%)	10(50%)	16(94%)	19 (50%)
Gentamycin (GEN)	6(100%)	14(70%)	17(100)	6(15%)
Ampicillin (AMP)	6(100%)	11(55%)	17(100%)	6(15%)
Amikacin (Ak)	6(100%)	14(70%)	17(100%)	37(97.4%)
Amoxicillin (AMC)	6(100%)	9(45%)	5(29%)	37(97.4%)

Table 3: Toxic profile of *S. aureus* cultures isolated (n=38) from street vended foods

Test conducted	Cultures detected positive	Cultures detected negative
Gram staining	38(100)	0(0)
Coagulase test	24(63.15)	14(36.85)
Toxin detection (ELISA)	1(2.63)	37(97.37)

3.5. Toxic profile of S. aureus

Out of 38 isolates of *S. aureus,* about 63.18% were coagulase-positive and 36.85% were coagulase-negative (Table 3). Only 1 isolate was detected positive for enterotoxin production and 37 isolates were negative for toxin production.

3.6. Molecular characterization of isolates

Of the 300 samples, 11% tested positive for *Salmonella* spp. and 28.6% for *Shigella* spp. selected isolates of these pure cultures were processed for molecular characterization. Results have been identified as *Salmonella bongori* and *Shigella sonnei*. The dendrograms of these are shown in Figures. 3 and 4.

4. Discussion

Street foods are highly susceptible to contamination by various foodborne pathogens and indicator organisms. A study conducted in Egypt on street vended meat sandwiches by Lidya et al.[14] had *Listeria monocytognes* and *E. coli* O157 H: 7. Another study by Maustafa et al.[15] had *L. monocytogenes* in 78/576 samples analyzed. A popular street snack Panipuri samples had another emerging foodborne pathogen i.e. *Yersinia enterocolitica*.[13] *Campylobacter jejuni* was detected in street-vended grilled chicken samples in Mexico.[12,16]

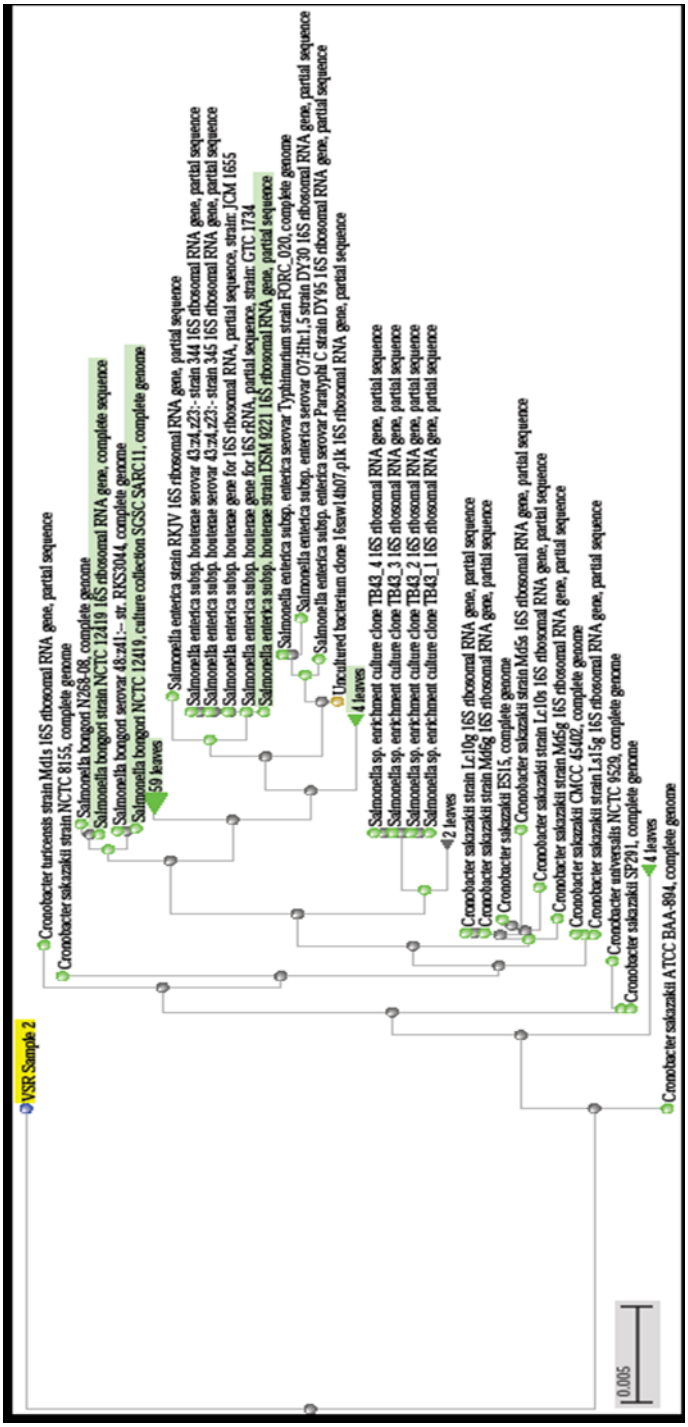

Figure 3: Dendrogram of *Salmonella bongori*

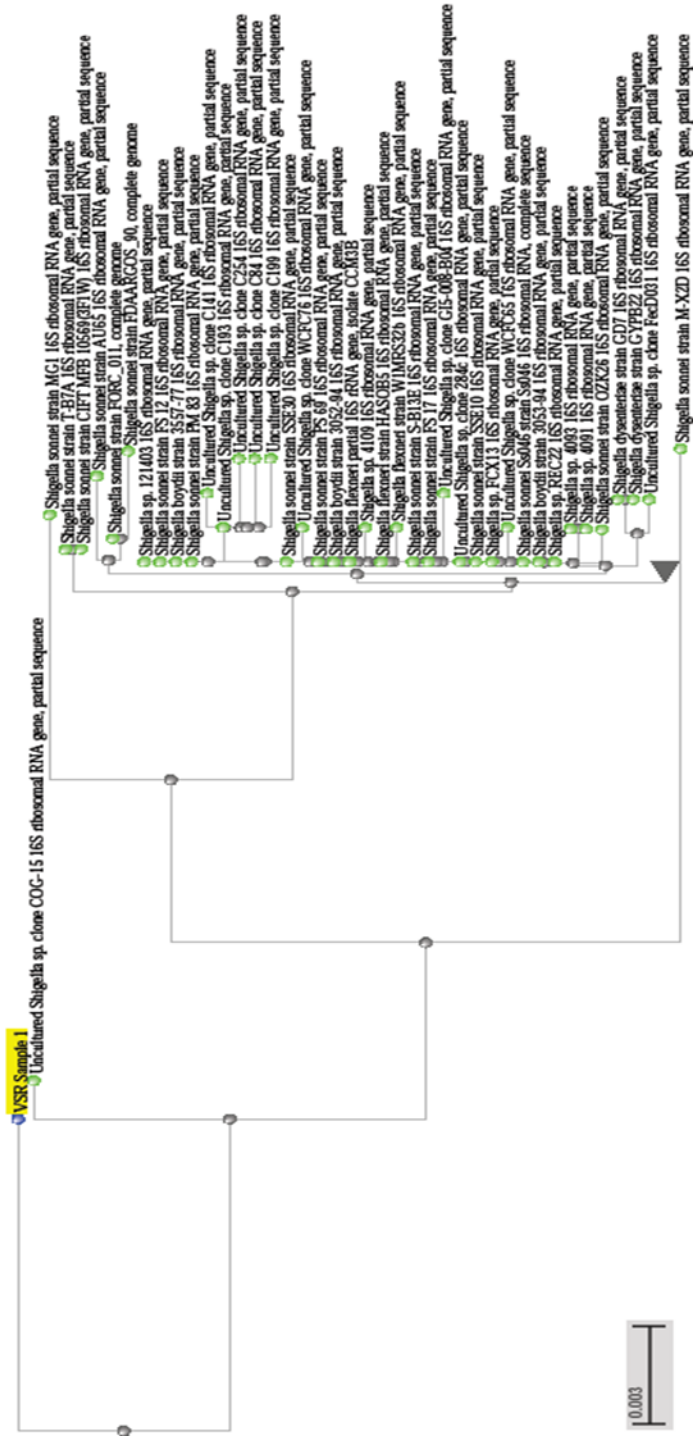

Figure 4: Dendrogram of *Shigella sonnei*

In this study, approximately 67% of the Bhel puri samples were contaminated with *Bacillus cereus*, likely due to the widespread presence of bacterial spores, especially in dusty roadside environments. The mesophilic spore-formers are introduced into the food through contaminated puffed rice. Previous research by Frazier and Westhoff[17] indicated a count of 0.01-0.9 x 10^4 CFU/g of mesophilic bacilli in street-vended foods, attributed to the wheat and rice flour used in preparation. The presence of *Bacillus cereus* is concerning due to its production of heat-sensitive (diarrheal) and heat-stable (emetic) toxins associated with food poisoning.[18] Hanashiro's findings also revealed that 35% of street food samples were deemed unsuitable for consumption due to high *B. cereus* loads.[19]

Staphylococcus aureus and *Bacillus cereus* are known for their tolerance to a wide range of temperatures and pH levels, which helps them persist even in highly acidic environments. In this study, *S. aureus* was detected in 26% of samples, while *Shigella* spp. was found in 45% and *Salmonella* spp. in 15%. The presence of *Shigella* spp. and *Salmonella* spp. may be attributed to inadequate cleaning of vegetables and raw produce used in food preparation.

Chinese fast foods in this study showed a high prevalence of *Bacillus cereus,* followed by *S. aureus*, aligning with findings from Sudershan et al.[20] The presence of *S. aureus* could be due to unclean hands or vendors coughing during food preparation. *Bacillus cereus* contamination is primarily due to spores in raw vegetables, which survive cooking.[18] The contamination of street foods with *Salmonella* spp. is often due to improper washing of salads,[21] as observed in Hyderabad, where 41% of vendors used unclean and unpeeled vegetables.[22] This practice, coupled with poor personal hygiene, contributes to cross-contamination and reflects poor hygiene standards.[23]

In terms of antibiotic sensitivity, *Salmonella* spp. in this study was sensitive to all tested antibiotics, contrasting with studies by Madhuchanda et al.[24] and Poonam et al.,[25] which reported resistance to Ampicillin, Norfloxacin, Polymyxin, Chloramphenicol, and Amoxicillin. *S. aureus* showed resistance to Furazolidone, Cotrimoxazole, Gentamicin, and Ampicillin, while Poonam et al.[25] noted resistance to Ampicillin and Cefuroxime. *E. coli* exhibited resistance to Amoxicillin in this study, consistent with Poonam et al.[25] but differing from Madhuchanda et al.,[24] who reported resistance to Ampicillin and Chloramphenicol. Most of the isolates from food samples were found to be sensitive to antibiotics, in contrast to isolates from human samples, which exhibited high resistance as reported by Sudershan et al.[26] The level of resistance in foodborne isolates was notably lower compared to those from human sources. This difference could be attributed to the extensive use of antibiotics in animal farming, agriculture, and human healthcare, which contributes to the emergence of resistant bacteria in these systems. While humans are less directly exposed to antibiotics than animals, the transmission of antimicrobial-resistant microorganisms at the human-animal interface remains complex.[27] Despite limited exposure, bacteria in food samples tend to remain sensitive to antibiotics but may acquire resistance after entering the human system.

The presence of *S. aureus* alone does not indicate food poisoning; enterotoxin production must be tested to assess toxicity. According to FSSAI standards, foods should not contain coagulase-positive *S. aureus*. In this study, 63% of isolates were coagulase-positive, similar to Salamandane et al.,[28] but only one isolate was positive for enterotoxin production. There are also reports of coagulase-negative[29] *S. aureus* producing toxins, indicating a need for further research in this area.

In this study, *Shigella sonnei* was identified as the predominant species. This pathogen had also been reported in earlier research by Mansah et al.,[30] who detected *S. sonnei* in street-vended macaroni, rice, and tomato stew samples. Other species, such as *Shigella flexneri*, were found in street food samples from studies conducted by Muleta et al.[31] and in ethnic street foods from the Himalayas.[32] Although *S. sonnei* has been infrequently found in street food samples, its presence seems to be on the rise.

Historically more common in developed countries, *S. sonnei* is now emerging in developing regions, including those previously dominated by other *Shigella* species. The exact reasons behind this shift and the global spread of *S. sonnei* remain unclear but may be linked to significant environmental pressures. The strain, which originated in Europe, has recently gained a global foothold. Additionally, localized antimicrobial use appears to have exerted strong selective pressure, contributing to the rapid evolution of the *S. sonnei* population. Consequently, this species is poised to become one of the more dominant and concerning gastrointestinal pathogens soon.[33]

Another significant organism detected in this study was *Salmonella bongori*. The *Salmonella* genus comprises two species: *S. enterica* and *S. bongori*, classified based on genetic similarities and biochemical properties.[34,35] While most serovars belong to *S. enterica* subspecies—such as *enterica* (about 60%), *salamae* (20%), *diarizonae* (13%), *arizonae* (3.8%), *houtenae* (2.8%), and *indica* (0.45%)—only 20 serovars (0.8%) are attributed to *S. bongori*.

Typically, *S. bongori* strains are primarily isolated from reptiles and other cold-blooded animals, and human cases involving this species are rare.[36] However, in recent years, several strains with the antigenic formula 48, identical to the original isolate, have been identified in hospital laboratories across three major cities in Sicily, Italy. Despite this, limited data exists to trace the specific sources of this serovar. *S. bongori* is predominantly found in cold-blooded animals,

but it can also infect humans. In evolutionary terms, some characteristics of *S. bongori* suggest that it occupies a middle ground between *E. coli* and *S. enterica*.[37]

5. Conclusion

This study highlighted critical insights into the prevalence and load of high-risk foodborne pathogens. It underscores the urgent need for training street food vendors in proper food safety and hygiene practices to reduce the risk of contamination. Research like this contributes to a better understanding of the hazards associated with consuming street foods and provides valuable information to help mitigate those risks.

6. Ethical Approval

This study was approved by Institute ethical Committee with ref. no. ECR/35/Inst/AP/2013.

7. Source of Funding

This study was funded by Indian Council of Medical Research (13FD01).

8. Conflict of Interest

The authors declare no conflict of interest to the present study.

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