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Microbiological Risk of Street Foods in Gopalganj, Dhaka, Bangladesh: Impact on Younger Population

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Abstract

Food-borne illness is a significant public health concern in Bangladesh, especially among the younger generation. They often consume spicy street foods, such as Fusca, Hog plum bedfellow, and Chotpoti. These foods can be contaminated with harmful bacteria, which can cause various diseases, including diarrhea, cholera, and dysentery. This study investigated the microbiological quality of three commonly consumed street foods in Bangladesh.We employed microbiological, biochemical, and molecular testing to identify bacteria in samples collected from street food vendors in Gopalganj, Dhaka, Bangladesh. We also assessed antibiotic sensitivity. Additionally, we investigated the food production system and the practices involved in selling and serving these street foods.We found that all three of the food samples were contaminated with a variety of bacteria, including *Bacillus spp*., *Haemophilus spp.*, *Salmonella spp.*, *Klebsiella spp.*, *Staphylococcus spp*., and *Streptococcus spp*. Some of these bacteria, such as *Haemophilus spp.*, were resistant to the antibiotic ciprofloxacin.Our findings have revealed a high risk of foodborne illnesses associated with the consumption of these foods. We strongly urge vendors and consumers to take steps to enhance food safety, including proper food handling, cooking, and the use of clean water and utensils.

Keywords Microbial assay, Street food, Foodborne diseases, Multi-drug resistance, Health hazards.

Introduction

Street food refers to food and beverages prepared and sold by fast vendors or retailers, typically on-site or in public spaces, for immediate local consumption or later consumption without additional preparation[1]. It is often prepared in mobile food carts or food trucks and is intended for immediate consumption. Vendors or manufacturers often use mobile food carts or food trucks to prepare street food, which is intended for immediate customer consumption. Street food is immensely popular among consumers due to its delicious taste, affordability, nutritional value, and easy accessibility[2].

In Bangladesh, street food plays a crucial role in meeting the daily nutritional needs of individuals with busy schedules who are unable to cook meals at home [3]. However, it is worth noting that street food sold in fast-paced environments often lacks proper sanitation, leading to high levels of contamination. In many cases, retail stores selling street food do not have access to running water, and hand washing is sometimes done in a dish or bucket, often without soap [4]. Insufficient food handling practices among vendors, who lack knowledge and skills in food safety management and formal food safety training, can result in contamination [5]. Numerous studies have reported that street food,

especially in densely populated countries, can harbor a wide range of bacteria [4]. Therefore, from a health perspective, the marketing of street food remains controversial, and street food may play a significant role in the transmission of risky and potentially fatal foodborne infections [5].

Food sold by fast vendors in developing countries is a major contributor to foodborne diseases (FBD) [6]. These illnesses not only pose a significant threat to public health but also have economic consequences. Contamination can occur during storage, transportation, display, handling, preparation, or sale of food in unsanitary environments, lacking potable water, proper sanitation facilities, and adequate waste removal. Individuals who regularly consume street food are at a higher risk of food poisoning, including diarrhea, cholera, and typhoid [7]. According to several studies, street food contains dangerous bacteria such Escherichia coli, Salmonella spp., Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, and Vibrio cholera [8]. Staphylococcus aureus and Clostridium perfringens, which cause stomach pains and diarrhea, as well as Bacillus cereus, which causes vomiting and diarrhea, are commonly found in street food. Additionally, Salmonella is responsible for typhoid fever, food poisoning, and inflammation of the gastrointestinal tract. Antibiotic-resistant foodborne bacteria pose a global health concern, with over 19% of human clinical specimens testing positive. These bacteria form biofilms, impacting the economies of low- and middle-income countries by $110 billion annually. In the Indian subcontinent, particularly in Bangladesh, the younger generations have a fondness for street foods such as Fusca (maize stalk borer), hog plum bedfellow (spondias mombin), and Chotpoti (white peas), which they often enjoy during leisure time. While different studies have demonstrated contamination in their research locations, there was no assessment of the food safety situation in Gopalganj. There were only a few locally-based food safety assessments in Bangladesh. Moreover, there were no available data regarding all concerns regarding these meals in the Gopalganj region. Many people consume this popular dish every day, yet many are unaware of its safety. Therefore, we conducted this study to raise awareness of health concerns among the general population.

However, our research indicates that common street foods are not always safe due to inadequate hygiene practices during their preparation, cooking, and serving. These foods may harbor various pathogenic bacteria, posing potential life-threatening risks to humans. This study aims to identify the types of pathogenic bacteria present in fast food and determine the level of antibiotic resistance among these pathogens by testing them against different generations of antibiotics used for treating human diseases.

Materials and Methods

The entire study was conducted in two sequential steps. The initial step involved the isolation and identification of bacteria from food samples using a range of microbiological tests, biochemical assays, gram-staining, and molecular techniques. Subsequently, in the second step, the isolated bacteria underwent assessment for antibiotic sensitivity.

Sample Collection

Three food samples were collected from different fast-food vendors in Ghonapara, Nabinbag, and Launch Ghat, located in the Gopalganj district of Bangladesh. These food samples included Hog plum bedfellow (Spondias mombin), Fusca, and Chotpoti. We collected approximately 300g of each food sample using the provided serving utensils from the vendors. These samples were then packaged and placed in sterile plastic bags. The testing of these samples was promptly conducted within 24 hours of collection.

Sample Preparation

Approximately 15 grams of each street food sample (Hog plum bedfellow, Fusca, and Chotpoti) were homogenized uniformly in a mortar and pestle using sterile phosphate-buffered saline (PBS) as a diluent. This homogenization process resulted in a uniform suspension. To create a standard solution, 1.0 gram of each homogenized sample was mixed with PBS. This solution was then serially diluted

(1:10) to a dilution of 10^-3 in test tubes, with 100 µl of the stock solution added to 900 µl of normal saline. Subsequently, 100 µl of the homogenized samples from each dilution were spread onto nutrient agar medium using a plastic spreader and incubated at 37°C for 24 hours. The diluted samples were applied to the plate surface as quickly as possible. After 24 hours of incubation, the plates were examined for bacterial growth.

To calculate the total number of bacteria present, 0.1 ml of each tenfold dilution was transferred using a micropipette and spread onto a nutrient agar plate. A sterile glass spreader was used to evenly distribute the diluted samples across the plate's surface. This process was repeated for each dilution. The plates were then incubated at 37°C for 24 hours, and the quantity of colony-forming units (CFU) per gram of the sample under examination served as an indicator of the total bacterial count.

Bacteria Identification

For the bacteriological study of street food samples (Hog plum bedfellow, Fusca, and Chotpoti), we followed the standard procedure recommended by the International Commission on Microbiological Specifications for Foods. Pure colonies were subjected to gram staining and microscopic examination using a UNITRON 14711-PS microscope from the USA to assess their morphological and staining characteristics. Identification of these bacteria was further confirmed using various biochemical tests, including indole, MIU, TSI, methyl red, Voges-Proskauer, citrate utilization, and urea utilization.

Molecular Identification

E. coli was identified using readily available primers, polymerase chain reaction (PCR), and a boiling bacterial DNA extraction method. PCR-amplified fragments were visualized through agarose gel electrophoresis, and the resulting UV light bands were examined.

The standard number of PCR cycles typically ranges from 20 to 40, with each cycle often involving two or three distinct temperature increments. Before initiating the cycling process, a single high-temperature step (above 90°C) is commonly performed, followed by a subsequent hold for the extension of the final product or for short-term storage. The specific temperatures and their durations during each cycle are influenced by various factors, including the DNA synthesis enzyme, the quantity of bivalent ions and dNTPs involved in the procedure, as well as the melting temperature (Tm) of the primers.

Bacterial DNA Isolation

To isolate bacterial DNA, 1000 µl of cultured broth from each Escherichia coli (E. coli) culture was transferred to an Eppendorf tube. The DNA extraction process was carried out using the boiling method after centrifuging the cell suspensions at 4,500 rpm for 5 minutes at 40°C. The resultant material was placed in a tube containing 100 µl of nuclease-free water and was then subjected to boiling at 100°C for five minutes. Following this, the mixture was centrifuged at 3000g for 10 minutes. Subsequently, the DNA-containing upper aqueous phase was transferred to a separate 2 ml Eppendorf tube, and 0.7 µl of cold 100% ethanol was added. Centrifugation was employed to separate the aqueous phase for 20 minutes at 10,000 rpm. Genomic DNA was precipitated through ethanol after transferring the supernatant. The pellet was rinsed in cold 70% ethanol, and the ethanol was subsequently removed via an additional centrifugation process at 14,000 rpm for 15 minutes. The pellets were dried until all traces of alcohol had dissipated. Re-suspension of the pellet was carried out in 50 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA was stored briefly at 4°C and for an extended period at -20°C.

Polymerase Chain Reaction

A typical PCR reaction entails 20 to 40 cycles, which involve periodic temperature variations, with two or three unique temperature increments frequently incorporated into each cycle. Prior to initiating the cycling process, a single high-temperature step (above 90°C) is usually performed, followed by a subsequent hold for final product extension or short-term storage. The specific temperatures and their durations during each cycle are determined by factors such as the DNA synthesis enzyme, the

concentration of bivalent ions and dNTPs in the reaction, as well as the melting temperature (Tm) of the primers. The temperatures used and the duration of their application during each cycle are all influenced by the DNA synthesis enzyme, the amount of bivalent ions and dNTPs involved in the procedure, as well as the melting temperature (Tm) of the primers. The 16S rRNA of E. coli was amplified using a genus-specific PCR approach employing previously reported primers with both forward and reverse primers [9].

Antibiotic Susceptibility Test

For the assessment of drug sensitivity and resistance patterns in the isolated bacterial strains, commercially available antibiotic discs (6 mm in diameter) containing Ciprofloxacin (5 µg/disc), Cefixime (5 µg/disc), Azithromycin (30 µg/disc), and Gentamicin (10 µg/disc) were employed. These antibiotics were selected based on their efficacy in treating bacterial infections in patients from Gopalganj, Bangladesh. Antibiotic resistance was evaluated using the Kirby-Bauer disc diffusion technique, following the recommendations of the Clinical and Laboratory Standards Institute [10]. The zones of inhibition surrounding each antimicrobial disc were measured in millimeters, recorded, and categorized as resistant (R), intermediate (I), or sensitive (S) following an overnight incubation at 37°C.

Results

Isolation of Bacteria from Samples

After 24 hours of incubation, numerous bacterial colonies were observed on the nutrient agar plates (supplement materials). In the Hog plum bedfellow samples, only five identical colonies and a few confluent bacterial colonies were found. In contrast, Fusca exhibited a substantial number of confluent colonies, along with approximately 90 identical bacterial colonies. Chotpoti, on the other hand, showed a significant number of bacterial colonies, ranging from 3 to 300 colonies per gram.

Total Viable Count We have presented the total viable cell counts (supplement materials), where Fusca contains a higher number of microbes, nearly 37.3% and 45.8% more than Chotpoti and Hog plum bedfellow. On the other hand, Hog plum bedfellow held the second position among these food items, approximately 8.5% higher than Hog plum bedfellow (**Figure 1**).

Identification of associated bacteria characteristics by morphological study and gram staining

Based on morphological examination under a microscope, Gram staining, and other biochemical assays, six different bacterial species were identified. These include Bacillus spp., Haemophillus spp., Salmonella spp., Klebsiella spp., Staphylococcus spp., and Streptococcus spp. In Chotpoti (**Figure 2**), two types of bacteria were found: (a) Bacillus spp. and (b) Salmonella spp. Hog plum bedfellow (**Figure 3**) yielded only one type of bacteria, Staphylococcus spp. Fusca (**Figure 4**) revealed four types of bacteria: (a) Klebsiella spp., (b) Haemophillus spp., (c) Streptococcus spp., and (d) Staphylococcus spp.

Characteristics by biochemical tests of isolated samples

The gram-staining test was performed for the microscopic characterization of bacteria and the results has been presented (**Table 1**). The identified isolates were further confirmed by seven biochemical tests, such as indole, MIU, TSI, methyl red, Voges-Proskauer, citrate utilization, and urea utilization (**Table 2**).

Results of Molecular Identification

PCR amplification using E. coli specific primers targeting the 16S rRNA gene produced positive bands at the desired locations, confirming the presence of suspected isolates. For the detection of pathogenic E. coli isolates, suspected isolates from biochemical and staining results were subjected to 16S rRNA targeted PCR amplification. A 584bp DNA fragment was amplified from the 16S rRNA gene using the forward primer GGGAGTAAAGTTAATCCTTTGCTC and the reverse primer TTCCCGAAGGCACATTCT. All the isolates tested positive in PCR (supplement material). The

results showed that amplification fragment sizes 584bp. Suspected 25 Bacillus isolates were used to detect E. coli and 14 isolates found to be positive.

Results of Antibiotic Susceptibility Test

Antibiotic sensitivity assays were conducted on a total of six isolates (Bacillus spp., Haemophillus spp., Salmonella spp., Klebsiella spp., Staphylococcus spp., and Streptococcus spp.). The tests revealed that all the isolated bacterial species were sensitive to ciprofloxacin. However, all the isolates exhibited resistance to cefixime, except for Haemophillus spp. Bacillus spp., Haemophillus spp., Salmonella spp., and Streptococcus spp. displayed resistance to the antibiotic azithromycin, while Staphylococcus spp. showed sensitivity to azithromycin, and Klebsiella spp. exhibited intermediate sensitivity. Gentamycin sensitivity varied among all species of isolated bacteria, with Staphylococcus spp. showing only intermediate sensitivity.

Discussion

Over the past decade, there has been growing recognition of the pivotal role played by street food in various facets of public health and hygiene [11]. One area that has garnered significant attention and understanding is how street foods contribute to the transmission and acquisition of foodborne diseases. There has been a global increase in the incidence of foodborne illnesses. Compelling evidence, primarily drawn from global data on illnesses caused by Salmonella and Campylobacter species, underscores this point. These species are responsible for a wide spectrum of diseases, including gastrointestinal disorders and fever (Martins). Foodborne illnesses such as hemorrhagic colitis, listeriosis, campylobacteriosis, shigellosis, and toxoplasmosis can all be traced back to pathogens commonly found in street food[12]. Numerous research studies on street cuisine have been conducted in specific regions of Bangladesh [13], revealing contamination of street foods like Chotpoti, Chanachur, Amra (Spondias mombin), and Jolpai (Elaeocarpus serratus) with bacteria such as E. coli, S. aureus, K. pneumoniae, S. typhimurium, S. salmonella, and S. shigella[12]. Among these bacteria, Staphylococcus spp. and Escherichia coli have demonstrated prevalence [14].

In our research, we have successfully isolated and characterized foodborne pathogens from three popular street food items in Bangladesh: Hog plum, Fusca, and Chotpoti. We identified six bacterial species (Bacillus spp., Haemophilus spp., Salmonella spp., Klebsiella spp., Staphylococcus spp., Streptococcus spp.). Chotpoti was found to contain Bacillus spp. and Salmonella spp., Hog plum harbored Staphylococcus spp., and Fusca was contaminated with Haemophilus spp., Klebsiella spp., Staphylococcus spp., and Streptococcus spp. We observed that there was no predominant presence of either gram-positive or gram-negative bacteria, as three of each were identified among the bacterial species. These bacteria are responsible for a range of diseases. Staphylococcus spp. produces toxins that can induce vomiting shortly after ingestion. Staphylococcus bacteria, responsible for staph infections, can be present on the skin or in the nasal passages of healthy individuals. Serious staph infections occur when these bacteria invade vital organs like the heart, lungs, or bloodstream, and they are becoming increasingly common even among seemingly healthy individuals, with some infections carrying a risk of fatality [15]. Streptococci, which are gram-positive aerobic organisms, can cause various diseases, including pharyngitis, pneumonia, wound and skin infections, septicemia, and endocarditis. The severity of symptoms depends on the affected organ, with potential complications such as rheumatic fever and glomerulonephritis (Van Emmenis). Klebsiella, a gram-negative bacterium, has been associated with a range of healthcare-associated disorders, including pneumonia, bloodstream infections, surgical site infections, and meningitis [16]. Our primary focus is on salmonellosis, an infection caused by most Salmonella strains, including those responsible for typhoid and paratyphoid fever [15]. While H. influenzae most commonly causes pneumonia, it can also lead to serious conditions like meningitis and bloodstream infections. Haemophilus influenzae can cause a wide array of illnesses, ranging from common ear infections to life-threatening infections that invade the bloodstream. Although anthrax is the most well-known Bacillus disease, other Bacillus species

have been increasingly linked to various infections in recent years, including abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmic infections, osteomyelitis, peritonitis, respiratory and urinary tract infections. These include abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmic infections, osteomyelitis, peritonitis, respiratory and urinary tract infections.

Furthermore, we conducted antibiotic susceptibility tests on these bacterial species using antibiotics such as ciprofloxacin (CIP), cefixime (CFM), azithromycin (AZN), and gentamycin (GEN). All isolated species were found to be sensitive to ciprofloxacin. However, all bacteria exhibited resistance to cefixime, except for Haemophilus species. Azithromycin resistance was observed in Bacillus spp., Haemophilus spp., Salmonella spp., and Streptococcus spp., while Klebsiella spp. displayed intermediate resistance, and Staphylococcus spp. showed susceptibility. Gentamycin sensitivity was evident in all species, except for Staphylococcus spp., which demonstrated only intermediate sensitivity.

Most hospital-acquired and community-acquired infections are caused by Gram-positive and Gram-negative pathogens, including Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Enterococcus faecalis, and Pseudomonas aeruginosa [9, 17]. Moreover, bacterial proliferation refers to the growth of harmful bacteria strains on or within the body, which can lead to severe infectious diseases [18]. Antibiotics are crucial for treating bacterial infections, but resistance has developed due to long-term, inappropriate use and abuse, worsening the situation [18, 19]. Annually, 700,000 drug-resistant pathogen-related deaths occur, with the number potentially increasing to 10 million by mid-century if current trends persist [20]. By 2050, drug-resistant infections could cause 10 million global fatalities, up from the current 0.7 million annually [21]. Drug-resistant pathogens pose a significant public health threat, necessitating the development of new antibacterials with superior activity against both drug-sensitive and drug-resistant pathogens [21].

Conclusions

Our research underscores the need for caution when consuming popular street foods. While these street food items are delicious and widely available in our country, immediate steps should be taken to improve the hygienic practices of street food vendors in their food preparation and serving processes. Otherwise, the consequences could be dire for both current and future generations. Additionally, more research in our country is warranted to address these critical health concerns. The lack of species-specific primers limited our ability to identify specific species; however, future research could employ such primers for individual species identification and conduct further antibiogram studies.

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Conflict of Interests Statement

The authors declare no conflicts of interest.

Preprint Statement

A preprint has previously been published of this work [22].

Data Availability Statement

All data generated or analysed during this study are included in this article.

Authors' Contribution Statement

MIH and MSA designed the experiment and LBM, MIH, MASMT, MOF, MS and MSA performed, analyzed and interpreted the data, and prepared the manuscript. SRA, KRI, MS and MSA reviewed the manuscript.

Supplementarymaterials

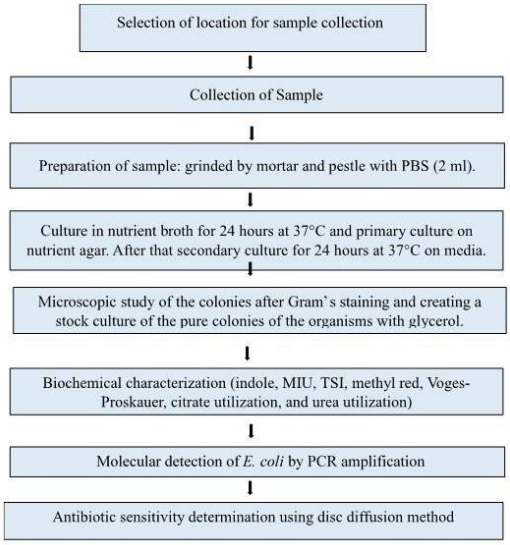
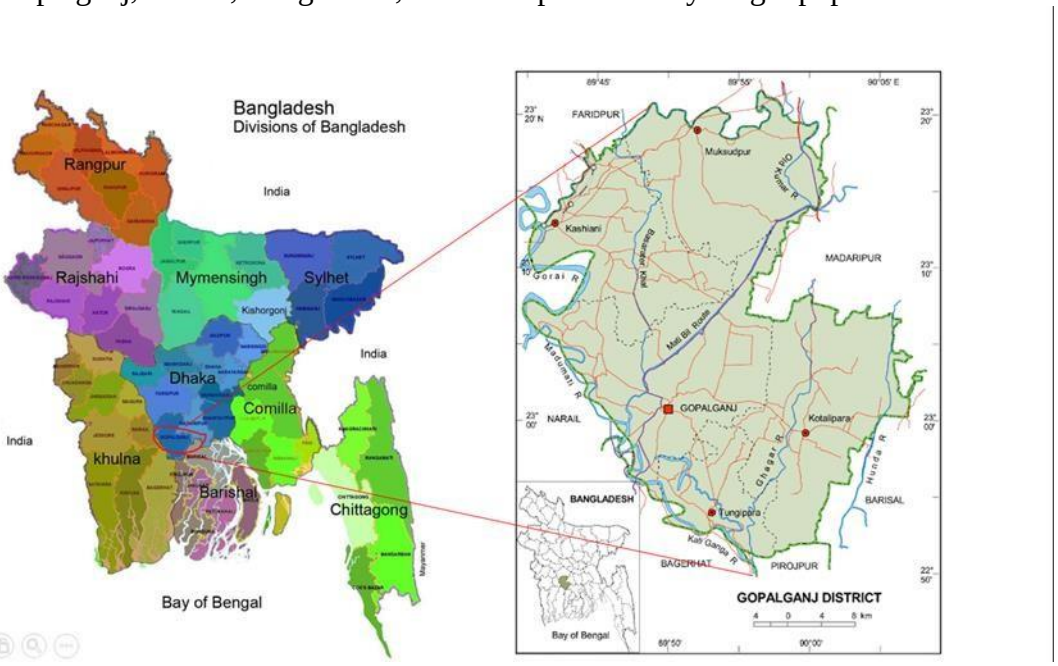


Figure S1. A general experimental design investigated the microbiological risk of street foods in Gopalganj, Dhaka, Bangladesh, and its impact on the younger population.



FigureS2. Map depicting the location and boundary of the study area, Gopalganj, within Bangladesh.

FigureS3. Real-time image of a Chotpoti stall in Gopalganj, Dhaka, Bangladesh, captured on January 23, 2023.

FigureS4. Real-timeimageof Fusca.

FigureS5. Real-timeimageofHogplum bedfellow (*Spondias mombin*).

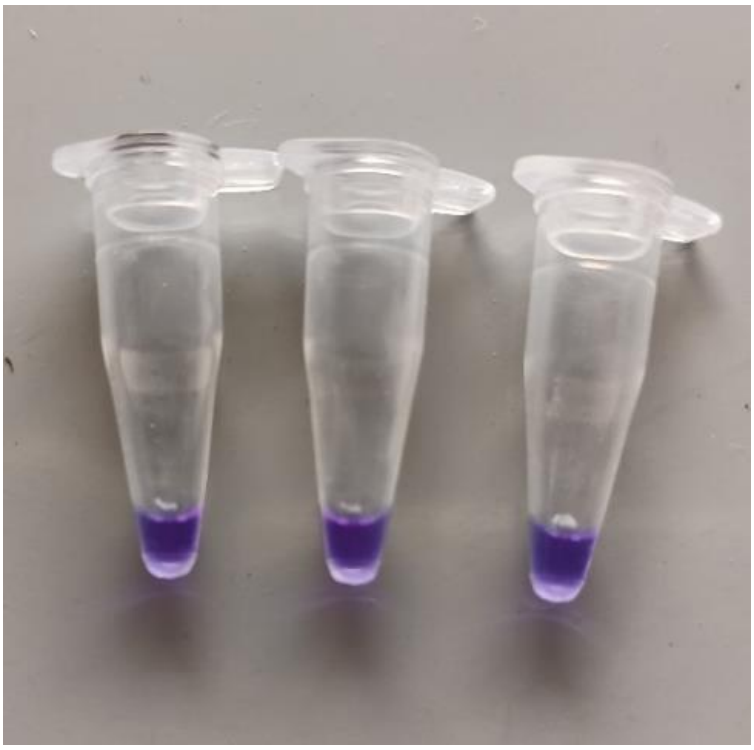


Figure S6. PCR products contained within PCR tubes are utilized to identify *E. coli*. These tubes encapsulate the amplified genetic material indicative of the presence of *Escherichia coli*, providing a visual representation of the molecular diagnostic process.

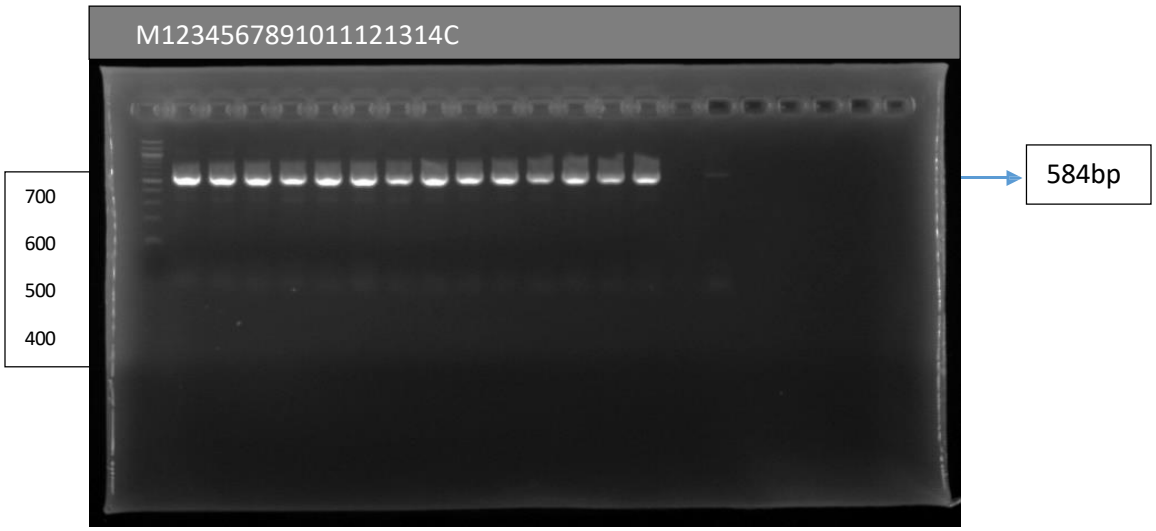
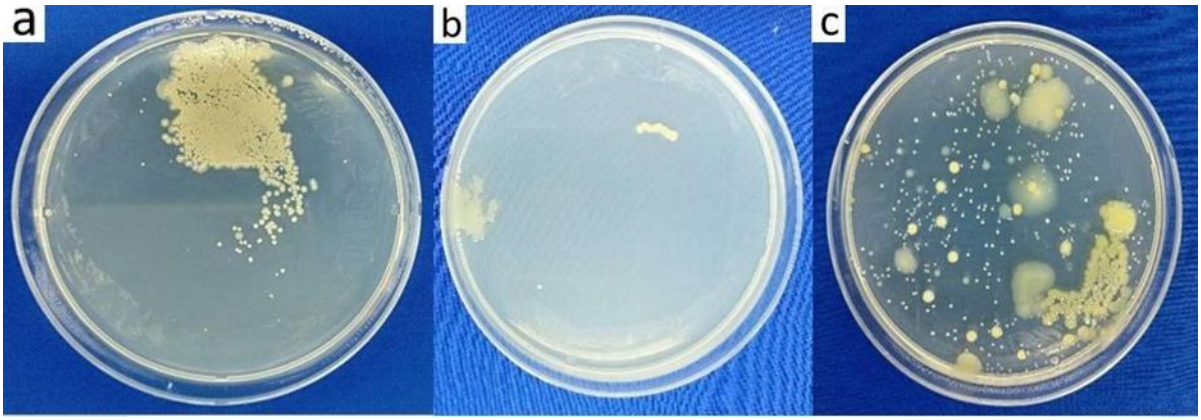


Figure S7. Amplification of a 584bp DNA fragment from the 16S rRNA gene of E. coli. The gel electrophoresis results are shown with Lane M containing a 100bp DNA ladder for size reference. Lanes 1 to 14 display the test samples, while Lane C is the Negative Control. This visualization aids in assessing the presence and size of the target DNA fragment in the tested samples.



FigureS8. The process of isolating bacterial colonies from three distinct street food samples: a) Chotpoti, b) Hog plum, and c) Fusca.

Sample	**100 µl inoculum**
Chotpoti (cfu/g)	50
Hogplum bedfellow (*Spondias mombin*) (cfu/g)	90
Fusca(cfu/g)	463

TableS1. Totalviablecountofbacteriafromcollectedthreesamples.

Primer	Sequences(5´-3´)	Size(bp)	References
*E.coli*16E1-E2	F: GGGAGTAAAGTTAATCCTTTGCTC R: TTCCCGAAGGCACATTCT	584bp	[23]

TableS2. PCR primers and their corresponding sequences are specifically designed to amplify *E. coli* DNA.

Bacteria Name	Nameof theantibiotics	Zoneof inhibition(mm)	Interpretation
*Salmonella*spp.	Ciprofloxacin(CIP)	25	S
	Cefixime(CFM)	0	R
	Azithromycin(AZN)	11	R
	Gentamycin (GEN)	23	S
Staphylococcus spp.	Ciprofloxacin(CIP)	22	S
	Cefixime(CFM)	0	R
	Azithromycin(AZN)	22	S
	Gentamycin(GEN)	14	I
*Bacillus*spp.	Ciprofloxacin(CIP)	27	S
	Cefixime(CFM)	0	R
	Azithromycin(AZN)	0	R

	Gentamycin (GEN)	20	S
*Klebsiella*spp.	Ciprofloxacin(CIP)	28	S
	Cefixime(CFM)	0	R
	Azithromycin(AZN)	15	I
	Gentamycin (GEN)	21	S
*Haemophilus*spp.	Ciprofloxacin(CIP)	26	S
	Cefixime(CFM)	19	S
	Azithromycin(AZN)	0	R
	Gentamycin (GEN)	19	S
*Streptococcus*spp.	Ciprofloxacin(CIP)	26	S
	Cefixime(CFM)	0	R
	Azithromycin(AZN)	10	R
	Gentamycin (GEN)	18	S

TableS3. Antibioticsensitivitytestagainst isolated bacteria.

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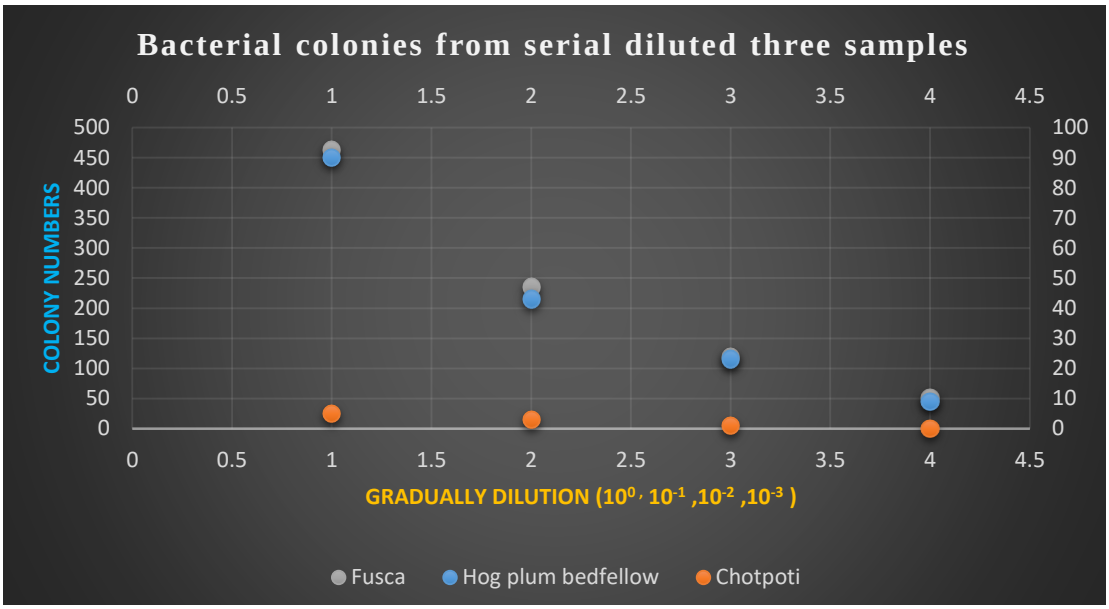

Figure 1. Scatter plot of bacterial colonies from serial diluted three samples (100, 10-1, 10-2 & 10-3).

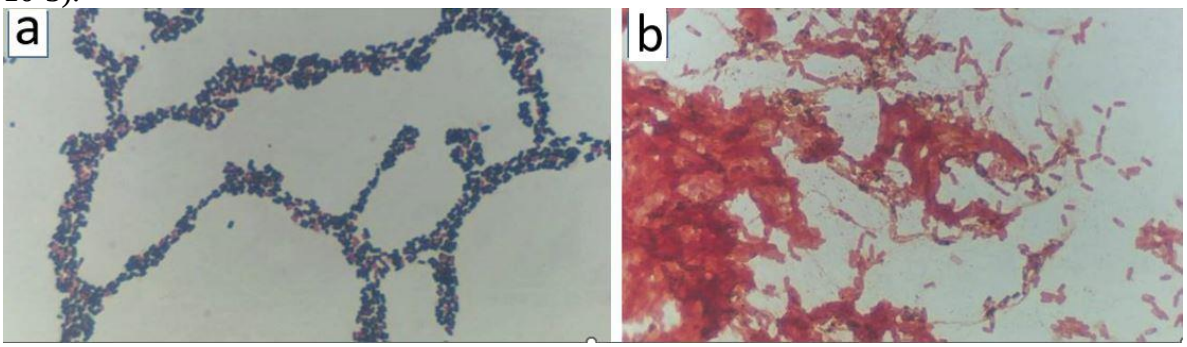

Figure 2. Light microscopy photograph at 100X magnification of isolated bacteria from Chotpoti (a) Bacillus spp., (b) Salmonella spp.

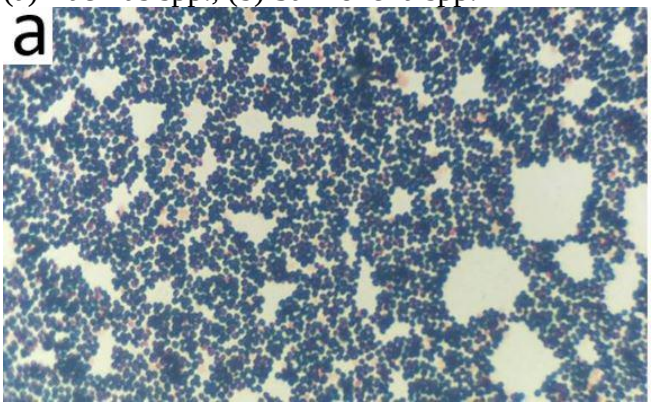

Figure 3. Light microscopy photograph at 100X magnification of isolated bacteria from Hog plum (a) Staphylococcus spp.

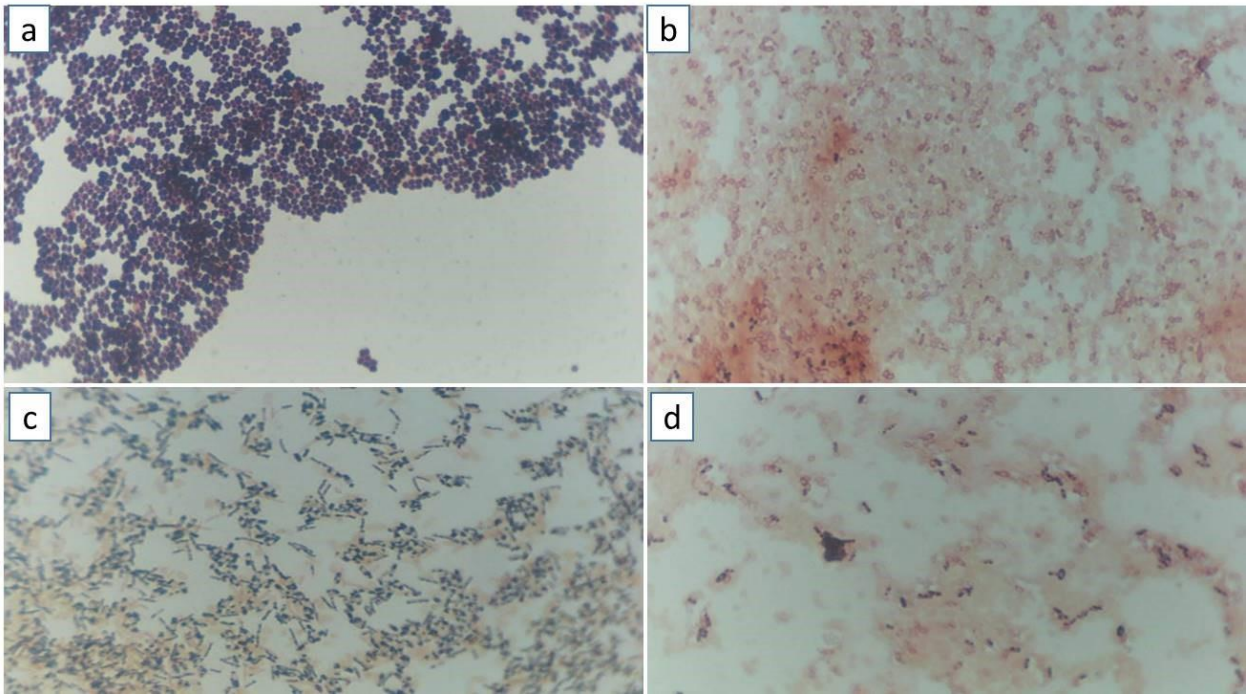

Figure 4. Light microscopy photograph at 100X magnification of isolated bacteria from Fusca; (a) Klebsiella spp., (b) Haemophillus spp., (c) Streptococcus spp. and (d) Staphylococcus spp.

Sample	Microscopic characterization	Gram's reaction	Identified isolates
Fusca	Non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium	Negative	*Klebsiella* spp.
	Nonmotile, Rod shaped	Negative	*Haemophilus* spp.
	Coccoid, nonmotile and non-spore forming.	Positive	*Streptococcus* spp.
	Nonmotile, non-spore forming, spherical shape	Positive	*Staphylococcus* spp.
Hog Plum	Nonmotile, non-spore forming, spherical shape	Positive	*Staphylococcus* spp.
Chotpoti	Rod-shaped, endospore-forming aerobic or facultatively anaerobic	Positive	*Bacillus* spp.
	Straight rod shaped, motile	Negative	*Salmonella* spp.

Table 1. Microscopic characterization of isolated bacteria after gram staining.

Item	Test	Result
*Bacillus*spp.	Indole	Negative (-)
	MIU	Negative (-)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (-)

	Citrate utilization	Positive (+)
	Urea utilization	Positive (+)
Haemophillus spp.	Indole	Positive (+)
	MIU	Negative (-)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (-)
	Citrate utilization	Negative (-)
	Urea utilization	Positive (+)
Salmonella spp.	Indole	Positive (+)
	MIU	Negative (-)
	TSI	Positive (+)
	Methyl red	Negative (-)
	Voges-Proskauer	Negative (-)
	Citrate utilization	Positive (+)
	Urea utilization	Positive (+)
*Klebsiella*spp.	Indole	Positive (+)
	MIU	Negative (-)
	TSI	Positive (+)
	Methyl red	Negative (-)
	Voges-Proskauer	Negative (-)
	Citrate utilization	Positive (+)
	Urea utilization	Positive (+)
*Staphylococcus*spp.	Indole	Negative (-)
	MIU	Positive (+)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (-)
	Citrate utilization	Negative (-)
	Urea utilization	Positive (+)
*Streptococcus*spp.	Indole	Negative (-)
	MIU	Negative (-)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (-)
	Citrate utilization	Negative (-)
	Urea utilization	Negative (-)

Table 2. Biochemical properties of isolated bacterial species.