

Original Research Article

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Screening and Profiling of Antibiotic-Resistant Pathogenic Bacteria from Hot Processed Street-Vended Foods

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ABSTRACT

Street-vended foods are widely consumed for their affordability and convenience, yet they are often prepared under unhygienic conditions that increase the risk of contamination. Hot-processed items such as shawarma, pani puri, and sandwiches were collected from Chennai and analyzed using microbiological methods. Multiple bacterial isolates were recovered, with shawarma and pani puri showing the highest contamination levels. Screening with antibiotic-impregnated nutrient agar and disc diffusion testing revealed varied resistance profiles. Some antibiotics remained effective, while reduced sensitivity and complete resistance to others were observed. Nearly half of the isolates were identified as multidrug-resistant, highlighting their role in the spread of antimicrobial resistance. These findings emphasize that hot-processed street foods act as reservoirs of resistant microorganisms. Strengthening hygiene practices, enforcing food safety regulations, and routine monitoring are essential to protect consumer health.

Introduction

Street food has become a fast-growing sector in India, offering inexpensive and convenient meals for urban consumers. These foods are prepared and sold directly in public spaces, requiring no further processing before consumption. However, food safety standards are often overlooked by vendors, as well as by some hotels and restaurants.

While demand continues to rise, street foods are a major source of foodborne illness. Many handlers lack proper training in hygiene and safe practices, which increases

the risk of contamination. Contributing factors include the use of unclean utensils that promote cross-contact, unhygienic vending environments, reliance on untreated tap water, and careless waste disposal that attracts pests. Rodents, insects, and flies frequently contaminate exposed foods, and vendors handling items with bare hands further amplify the risk (Akilan *et al.*, 2020). Foodborne diseases arise not only from bacteria but also from fungi, viruses, and parasites. Their impact has grown more severe with the spread of multidrug-resistant microorganisms. Street-vended foods are frequently reported to carry pathogenic contamination, including common bacterial agents.

Unhygienic practices such as unsafe handling of raw

materials, contaminated surfaces, poor storage, and unsanitary preparation environments are major contributors. Studies have shown that many street foods harbor fungi and opportunistic bacteria, often with high microbial loads resistant to routine antibiotics.

Evidence from Ethiopia revealed varying levels of contamination in ready-to-eat foods, with most isolates resistant to commonly prescribed drugs. These findings highlight that street foods can act as vehicles for antimicrobial-resistant pathogens, posing a serious public health challenge (Acharya *et al.*, 2025).

Antimicrobial resistance (AMR) has become a pressing global health challenge, complicating treatment and intensifying disease burden. Understanding resistance trends in street foods is vital, yet in Bhimdatt Municipality, Nepal, recent data are scarce and antibiotic profiles of foodborne bacteria remain unexplored. This work sought to identify pathogens in local street foods and assess their resistance, aiming to fill critical knowledge gaps and guide public health policy (Upadhyay *et al.*, 2025).

Although antibiotics have revolutionized the management of bacterial infections, many foodborne organisms have developed survival mechanisms that render these drugs ineffective. AMR arises through genetic changes, making infections harder to treat and increasing risks of severe illness, disability, and death in humans and animals. Addressing this issue requires a coordinated “One Health” approach involving both scientific communities and the public (Singh *et al.*, 2023).

The lack of new therapeutic options against multidrug-resistant pathogens further heightens concern. Excessive antibiotic use drives higher healthcare costs, mortality, and environmental harm, accelerating the threat of a “post-antibiotic era”.

Vulnerable groups, particularly school-aged children and impoverished populations, carry a disproportionate burden of foodborne illnesses, while traveler’s diarrhoea remains a major obstacle for tourism in developing nations.

Therefore, isolating and characterizing resistant bacteria from street-vended foods is essential to evaluate safety, monitor AMR patterns, and provide evidence for stronger food safety regulations, vendor training, and resistance

control strategies.

Hot-processed foods, such as shawarma, fried snacks, pani puri fillings, sandwiches, noodles, and rice-based dishes, undergo cooking or heating at high temperatures, which initially reduces microbial loads in raw ingredients. However, contamination may reoccur during cutting, serving, or storage, especially when cooked foods are left at room temperature for extended periods. The popularity of these foods in crowded urban centers underscores the importance of maintaining strict hygiene during preparation and distribution.

Studies have identified several bacterial species in hot-processed street foods, including *Staphylococcus aureus*, *Klebsiella spp.*, *Proteus spp.*, *Enterococcus spp.*, *Pseudomonas spp.*, *Shigella spp.*, and *Enterobacter spp.* Their presence highlights the potential health risks associated with inadequate sanitation and poor food-handling practices.

Ultimately, while hot processing reduces initial microbial contamination, improper handling and storage can quickly negate these benefits. Ensuring safe water, clean utensils, hygienic preparation, and proper storage conditions is essential to safeguard public health. Strengthening vendor training, enforcing food safety regulations, and routine monitoring are critical steps toward minimizing contamination and ensuring the safety of street-vended foods.

Hence, this research was carried out to detect, characterize and analyze the occurrence of antibiotic-resistant pathogens in hot-processed street-vended foods, with emphasis on their incidence and implications for consumer safety.

Materials and Methods

Sample Collection

Street-vended food samples were aseptically collected from five different locations across Chennai. Sterile gloves and containers were employed during collection to minimize external contamination. A total of 10 samples of each food type were obtained, clearly labelled, and promptly transported to the laboratory for microbiological testing. The study focused on commonly consumed items, including Shawarma (Figure 1), Pani puri (Figure 2), and Sandwiches (Figure 3).

Processing of Food Samples

Approximately 25 g of each solid food sample was aseptically transferred into 100 mL of sterile distilled water. For liquid samples, an equivalent measured volume was used.

The mixture was thoroughly homogenized to obtain a uniform suspension, which served as the stock preparation for subsequent microbiological analyses.

Serial Dilution

To obtain countable colonies, 1 g of solid food or 1 mL of liquid sample was transferred into 9 mL of sterile saline to prepare the first dilution. Successive ten-fold dilutions were made, with thorough mixing at each step.

A sterile saline tube served as the negative control. The dilution series was then used for bacterial isolation and enumeration by the pour-plate method.

Screening & Isolation

A variety of culture media were prepared to facilitate bacterial growth, differentiation, identification, and antibiotic resistance assessment. Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Salmonella–Shigella Agar, Tributyrin Agar, and Mueller–Hinton Agar were applied for cultivation and sensitivity testing.

For biochemical characterization, media such as Tryptone Broth, MR-VP Broth, Simmons Citrate Agar, Christensen's Urease Agar, Triple Sugar Iron Agar, Nitrate Broth, and Carbohydrate fermentation broths were utilized to analyze metabolic functions and confirm bacterial identity.

A preliminary screening was performed using nutrient agar medium supplemented with a low concentration 10–20 µg/mL of Aminoglycosides.

Maintenance of Pure Culture

Pure cultures were obtained through the pour-plate method and subsequently maintained by streaking on nutrient agar plates. Isolated colonies were further purified using the quadrant streak technique. Stable cultures were preserved on nutrient agar slants for further study.

Identification of Organisms

- Gram staining was performed using the bacterial isolates obtained from the nutrient agar plates to determine their Gram reaction and cellular morphology.
- Motility testing was carried out by the hanging drop method to determine the motility of the bacterial isolates.
- Biochemical identification was performed using a series of appropriate biochemical tests to characterize and identify the bacterial isolates.
- Additional verification was achieved through the application of selective culture media in combination with specialized confirmatory assays, ensuring precise differentiation and validation of the isolates.

Incidence of Organisms

The incidence of microbial isolates was determined by recording their frequency across the collected food samples. Identification was carried out using Gram staining, motility testing, and biochemical characterization. The distribution analysis revealed that Type 1, Type 2, Type 3, Type 4, Type 5, Type 6 and Type 7 isolates occurred at varying proportions in shawarma, pani puri, and sandwiches. These variations reflected differences in preparation, handling, and hygiene practices, thereby providing insight into the overall microbial burden present in hot-processed street-vended foods (Table 1).

The bacterial isolates with higher prevalence were subjected to antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method, employing discs of Colistin, Aminoglycosides, Fluoroquinolones, and Chloramphenicol to determine their resistance profiles.

Results and Discussion

Sample Collection

Street-vended foods were obtained from different roadside outlets to capture variety. Popular ready-to-eat items such as Pani puri, Sandwiches, and Shawarma were included.

All samples were aseptically placed in sterile containers to avoid contamination and promptly transported to the laboratory for microbiological analysis.

Screening & Isolation

Pure cultures were successfully established using the pour-plate method, followed by streaking on nutrient agar plates for maintenance (Figure 4). Distinct colonies were purified through the quadrant streak technique to ensure isolation. Stable isolates were preserved on nutrient agar slants for subsequent analyses. Among the tested samples, Shawarma and Pani puri exhibited the highest microbial loads, indicating greater contamination risk compared to other foods (Table 2).

Identification of Bacterial Isolates

- Gram staining revealed the cellular morphology and Gram reaction of the isolates, distinguishing them as either Gram-positive or Gram-negative. Motility testing further differentiated the organisms by identifying whether they exhibited active movement or remained non-motile.
- Biochemical characterization revealed distinct reactions that supported species identification. Catalase, oxidase, urease, TSI, nitrate reduction, and carbohydrate fermentation tests each produced characteristic outcomes. These responses enabled differentiation and classification of the bacterial isolates.
- Colonies developed were differentiated based on their morphology and named as type 1, type 2, type 3, etc... (Table 3)

Based on biochemical profiles, the isolates were identified as:

- *Staphylococcus aureus*
- *Salmonella* spp.
- *Proteus* spp.
- *Klebsiella pneumoniae*
- Type 1 was identified as *Staphylococcus aureus*. It produced small, opaque, smooth, circular, convex colonies with a characteristic golden-yellow colour on nutrient agar.
- Type 2 was identified as *Salmonella* spp. It produced medium-sized, smooth, circular, opaque, greyish-white colonies with a slightly raised elevation.
- Type 3 was identified as *Klebsiella pneumoniae*. It produced large, circular, smooth, convex, shiny and highly mucoid creamish-white colonies.
- Type 4 was identified as *Proteus* spp. It produced large, spreading, greyish-white colonies with characteristic swarming growth and concentric rings.

- For further confirmation, selective media were employed along with specialized diagnostic tests to validate the identity of the bacterial isolates.

Selective Media

Growth on selective media confirmed the identity of the isolates. *Staphylococcus aureus* produced yellow colonies on Mannitol Salt Agar due to mannitol fermentation (Figure 5). *Salmonella* formed colourless colonies on Salmonella–Shigella Agar (Figure 6), consistent with non-lactose fermentation. *Klebsiella pneumoniae* developed pink colonies on MacConkey Agar, indicating lactose fermentation. *Proteus* showed clear zones on Tributyrin Agar, demonstrating lipolytic activity.

Antibiotic Sensitivity (Kirby–Bauer Method)

All isolates were tested for antibiotic susceptibility on Mueller–Hinton agar using the Kirby–Bauer disc diffusion method. Antibiotic discs were applied to the inoculated plates, and the inhibition zones were measured to assess resistance patterns (Table 4).

Determination of Drug-Resistant Isolates

Most of the multidrug-resistant isolates were identified as *Salmonella* spp. and *Staphylococcus aureus*, underscoring their major contribution to antibiotic resistance in street-vended food contaminants (Table 6).

Street-vended foods play a vital role in the daily diets of urban populations in developing countries, largely due to their affordability and convenience. Yet, the lack of strict hygiene practices during preparation and sale makes them highly susceptible to microbial contamination. In this study, all food samples exhibited considerable bacterial loads, with shawarma and pani puri showing the highest counts. These elevated levels can be attributed to repeated manual handling, exposure to contaminated water, and storage under unhygienic conditions.

The wide range of bacterial isolates recovered highlights multiple contamination pathways, including raw materials, poor sanitation, and direct environmental exposure. Biochemical and morphological analyses confirmed *Staphylococcus aureus*, *Salmonella* spp., *Proteus* spp., and *Klebsiella pneumoniae* as the dominant organisms.

Table.1 Incidence of Organisms

Food Samples	Bacterial Isolates	Percentage (%)
Pani puri, Sandwich & Shawarma	Type 1	60%
Pani puri & Shawarma	Type 2	40%
Pani puri & Sandwich	Type 3	40%
Shawarma & Sandwich	Type 4	40%
Pani puri	Type 5	14%
Pani puri	Type 6	20%

Table.2 Mean Bacterial Load of Street Vended Food Samples

Food Sample	Mean Colony Count (CFU/ml or g)
Shawarma	7.1×10^5
Pani Puri	6.8×10^5
Sandwich	5.2×10^5

Table.3 Antibiotic Susceptibility Test by Kirby-Bauer Disc Diffusion Technique

Bacterial Isolates	Colistin	Aminoglycoside	Flouroquinolones	Chloramphenicol
<i>Staphylococcus aureus</i>	Resistant	Sensitive	Resistant	Sensitive
<i>Salmonella spp.</i>	Resistant	Sensitive	Sensitive	Sensitive
<i>Klebsiella pneumoniae</i>	Resistant	Sensitive	Resistant	Resistant
<i>Proteus spp.</i>	Resistant	Sensitive	Sensitive	Sensitive

Table.4 Biochemical test

Biochemical & Isolated Organisms	TYPE 1	TYPE 2	TYPE 3	TYPE 4
Indole	+	-	-	-
Methyl Red	+	+	-	-
Voges-Proskauer	+	+	+	-
Citrate	+	+	+	+
Urease	+	-	+	+
TSI	(A/A)	(K/A)	A/A	K/A
Gas production	-	+	+	+
H ₂ S production	-	+	-	+
Oxidase	-	-	-	-
Catalase	+	+	+	+
Nitrate reduction	+	+	+	+
Organisms	<i>Staphylococcus aureus</i>	<i>Salmonella spp.</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus spp.</i>

Table.5 Incidence of Organisms

Food Samples	Location	Bacterial Isolates	Percentage (%)
Pani puri, Sandwich & Shawarma	Kodungaiyur	<i>Staphylococcus aureus</i>	60%
Pani puri & Shawarma	MKB Nagar	<i>Salmonella spp.</i>	40%
Pani puri & Sandwich	Erukkancherry	<i>Klebsiella pneumoniae</i>	40%
Shawarma & Sandwich	Moolakadai	<i>Proteus spp.</i>	40%
Pani puri	Kodungaiyur	<i>Pseudomonas spp.</i>	14%
Pani puri	MKB Nagar	<i>Bacillus subtilis</i>	20%

Table.6 Detemination of Drug-Resistant Isolates

Food Sample	MDR Isolates (%)
Pani Puri	75
Shawarma	72
Sandwich	55



Figure 1: Sharwarma



Figure 2: Pani Puri



Figure 3: Sandwich



Figure 4: Pure Culture



Figure 5: MSA Agar with Yellow colonies



Figure 6: SS Agar with Black centered colonies

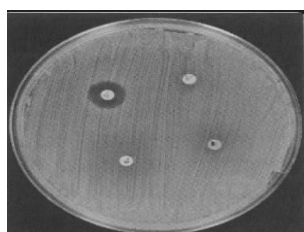


Figure 7: *Klebsiella pneumoniae*



Figure 8: *Staphylococcus aureus*

The frequent detection of *S. aureus* points to contamination from food handlers, while *Salmonella* suggests fecal origin or cross-contact with raw foods. The presence of *Proteus* and *Klebsiella* further emphasizes environmental contamination, with *K. pneumoniae* raising concern as an opportunistic pathogen capable of causing serious infections. Antibiotic resistance testing revealed diverse susceptibility patterns. Aminoglycosides and chloramphenicol retained effectiveness against most isolates, whereas fluoroquinolones showed reduced activity and colistin resistance was widespread. The identification of multidrug-resistant (MDR) strains, particularly among *Salmonella* and *S. aureus*, underscores the growing public health challenge posed by antimicrobial resistance in street foods. Food-specific analysis indicated that pani puri and shawarma were most frequently contaminated with MDR organisms, while sandwiches displayed moderate levels. These variations highlight how preparation techniques, storage conditions, and vendor hygiene practices directly influence contamination risks.

Taken together, the findings demonstrate that street-vended foods act as reservoirs for pathogenic and drug-resistant bacteria. Elevated microbial loads, the presence of foodborne pathogens, and MDR isolates collectively reflect inadequate food safety measures. To mitigate these risks, stricter enforcement of hygiene regulations, routine monitoring, vendor training, and consumer awareness initiatives are essential. Strengthening food safety standards is critical to protecting public health and preventing outbreaks linked to contaminated ready-to-eat foods.

In conclusion, the investigation successfully recovered four bacterial species—*Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella* spp., and *Proteus* spp.—from hot-processed street-vended food samples, with their prevalence carefully documented. Initial screening was carried out using nutrient agar media impregnated with antibiotics, which aided in the selective isolation of resistant strains. Notably, nearly half of the recovered organisms (around 50%) were identified as multidrug-resistant (MDR), highlighting their significant role in the dissemination of antimicrobial resistance.

Author Contributions

S. Divya Bharathi: Initiated the research concept, formulated the study design, and prepared the manuscript. K. Hemalatha: Contributed to the research

work and helped revise the manuscript. C. Ganga: Participated in the experimental work and supported the preparation revision of the manuscript. J. Sangeethalakshmi: Established and optimized the experimental procedures and critically reviewed the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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