

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.033>

Isolation and Detection of Antibiotic Resistant Bacteria from Cold Processed Street Vended Foods

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Keywords

Cold-processed foods, street-vended products, ice cream and fruit juices, microbial contamination, antimicrobial resistance, hygienic practices, sanitation

Article Info

Received:

22 August 2026

Accepted:

28 September 2026

Available Online:

10 October 2026

ABSTRACT

This study investigates the microbiological quality and antibiotic resistance profiles of cold-processed street-vended foods, specifically ice cream and fruit juices sold across Chennai. Samples were analyzed for bacterial contamination and antimicrobial susceptibility using standard microbiological and biochemical techniques. Six bacterial species were isolated—*Staphylococcus aureus*, *Salmonella* spp., *Klebsiella pneumoniae*, *Proteus* spp., with *Staphylococcus aureus* showing the highest prevalence. Antibiotic sensitivity testing revealed complete resistance to colistin, moderate resistance to fluoroquinolones (50%), and low resistance to chloramphenicol (25%), while aminoglycosides remained fully effective. The findings highlight significant microbial contamination and the emergence of antibiotic-resistant strains in ready-to-eat chilled foods, underscoring the need for stringent hygiene, proper storage, and continuous surveillance to safeguard public health.

Introduction

The street-vending sector is an important part of the urban food system in developing countries, including India, where affordable and readily available foods are widely consumed. The demand for convenient foods has increased the availability of cold and ready-to-eat products, such as fresh fruit juices, ice cream, and other chilled foods.

As these products are usually consumed without further heat treatment, their microbiological quality depends greatly on preparation, handling, storage, transportation, and vending conditions.

Cold foods can be contaminated through poor-quality water, contaminated ingredients, unclean utensils, food-contact surfaces, unhygienic vending environments, improper waste disposal, insects, rodents, airborne particles, and frequent manual handling. Inadequate refrigeration and improper storage conditions may further support the survival and multiplication of microorganisms.

Foodborne bacterial contamination remains a major food safety concern, as several bacteria can survive in food and cause illness after consumption. Nutrient-rich ingredients, dairy products, fresh fruits, and contaminated water may provide favourable conditions

for bacterial survival. Common food-associated bacteria include *Salmonella spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella spp.*, *Klebsiella spp.*, and *Pseudomonas spp.* Their consumption may cause diarrhea, vomiting, abdominal pain, and fever, with severe effects possible in vulnerable groups such as children, elderly individuals, pregnant women, and immunocompromised persons.

The increasing prevalence of antimicrobial resistance (AMR) has created an additional concern in food safety. AMR enables microorganisms to survive antimicrobial treatment, thereby reducing the effectiveness of available therapies. Resistant bacteria may enter food through contaminated water, raw materials, food handlers, processing surfaces, environmental sources, and inadequate sanitation. Thus, street-vended foods may act as an interface for the transmission of antimicrobial-resistant microorganisms between the environment, food, vendors, and consumers.

The emergence of antibiotic resistance bacteria, particularly those resistant to multiple antimicrobial classes, can complicate the treatment of infections. Their detection in food-associated environments is therefore important from a food safety and One Health perspective, which recognizes the interconnection between human health, animal health, food systems, and the environment. Against this background, The Hidden Threat in Chilled Foods: Isolation of Antibiotic Resistance Profiling of Bacterial Contaminants focuses on assessing the microbiological quality of commonly consumed chilled products, particularly fresh fruit juices and ice cream. Isolation and identification of bacterial contaminants, followed by antibiotic resistance profiling, can provide valuable information about their potential microbiological risks.

Materials and Methods

Cold-processed food samples were collected from multiple vending points located in different areas of Chennai to ensure geographical variation in the study. Sampling was carried out aseptically using sterile gloves and pre-sterilized containers to prevent contamination during handling. A total of 10 samples of each ice cream and juice were obtained from selected vendors. Each specimen was appropriately labelled with relevant sampling details and transported to the microbiology laboratory under suitable conditions for subsequent microbiological analysis.

Pre-Analytical Sample Preparation

Approximately 25 g of ice cream and 25 mL of juice samples were aseptically transferred into 100 mL of sterile distilled water and homogenized thoroughly. The homogenates obtained were considered as the initial stock suspensions for microbiological analysis. Serial ten-fold dilutions were prepared by transferring 1 mL of the stock suspension into 9 mL of sterile distilled water successively. Appropriate dilution levels were selected for inoculation onto suitable culture media for enumeration and further identification of bacterial isolates.

Preliminary Detection & Isolation

To support bacterial isolation, identification, and resistance profiling, a diverse set of culture media was employed. Growth and sensitivity testing were carried out on Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Salmonella–Shigella Agar, Tributyrin Agar, and Mueller–Hinton Agar. For metabolic and biochemical analysis, specialized 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 used to characterize enzymatic activity and confirm species identity.

Initial screening was performed on nutrient agar supplemented with aminoglycosides at concentrations of 10–20 µg/mL to detect resistant strains.

Pure Culture Stabilization

Isolates were first obtained through the pour-plate technique and refined by repeated streaking on nutrient agar plates. Colonies were further purified using quadrant streaking to ensure single-strain cultures. For long-term preservation and continued investigation, stable cultures were maintained on nutrient agar slants.

Microbial Isolate Profiling

- **Microscopic Examination:** Gram staining was carried out on isolates obtained from nutrient agar plates to determine their Gram response and cellular morphology.
- **Motility Assessment:** The hanging drop technique was employed to evaluate the motility of the bacterial cultures.

- **Biochemical Profiling:** A range of biochemical assays was performed to establish the metabolic traits and aid in the identification of the isolates.
- **Indicator media:** Target organisms were recovered using selective culture media tailored for specific species—for example, Mannitol Salt Agar for *Staphylococcus aureus*, Salmonella–Shigella Agar for *Salmonella* spp., MacConkey Agar for *Klebsiella pneumoniae*, and Tributyrin Agar for *Proteus* spp.
- **Confirmatory Testing:** Specialized assays were conducted to validate the identity of the isolates. The coagulase test confirmed *Staphylococcus aureus* by its plasma-clotting ability, while the urease test was applied to *Salmonella* spp. to detect urea hydrolysis activity.

Antibiotic Susceptibility Testing

The Kirby–Bauer disc diffusion assay was employed to determine the antimicrobial susceptibility profiles of the bacterial isolates. A standardized inoculum was prepared by adjusting the turbidity of the bacterial suspension to the 0.5 McFarland standard, thereby ensuring uniform cell density across all experimental replicates.

The inoculum was aseptically spread over the surface of Mueller–Hinton agar plates using sterile cotton swabs to achieve a confluent lawn of growth.

Sterile paper discs impregnated with defined concentrations of selected antibiotics—namely colistin, aminoglycosides, fluoroquinolones, and chloramphenicol—were carefully positioned on the agar surface with sterile forceps to avoid cross-contamination. During incubation at 37 °C for 18–24 hours under aerobic conditions, the antibiotics diffused radially into the medium, establishing concentration gradients.

Zones of growth inhibition surrounding the discs were subsequently measured in millimetres using a calibrated ruler or digital caliper.

The diameters of inhibition zones were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, enabling classification of each isolate as susceptible, intermediate, or resistant. This approach provided a reproducible and quantitative assessment of the resistance phenotypes, facilitating comparative evaluation of multidrug resistance patterns among the tested isolates.

Results and Discussion

Sample Collection

Cold-processed street-vended foods such as ice cream and fruit juices were collected from various roadside outlets to capture diversity. All samples were aseptically transferred into sterile containers to prevent contamination and promptly delivered to the laboratory for microbiological evaluation.

Profiling of Bacterial Isolates

Microscopic examination through Gram staining revealed the structural features and differential staining properties of the isolates, categorizing them as Gram-positive or Gram-negative. Assessment of motility further distinguished the cultures by determining whether they exhibited active locomotion or remained stationary.

Biochemical profiling demonstrated unique metabolic reactions that aided in species determination. Enzymatic and substrate utilization assays—including catalase, oxidase, urease, Triple Sugar Iron (TSI), nitrate reduction, and carbohydrate fermentation—produced characteristic responses.

These diagnostic outcomes provided the basis for accurate differentiation and systematic classification of the bacterial isolates.

Colony Features of Isolates

- **Isolate I** (*Klebsiella pneumoniae*) formed large, round, smooth colonies that were shiny, mucoid, and cream-white in colour.
- **Isolate II** (*Staphylococcus aureus*) produced small, compact colonies that were opaque, smooth, circular, slightly raised, and golden-yellow on nutrient agar.
- **Isolate III** (*Salmonella* spp.) showed medium-sized, smooth, circular colonies that were opaque, greyish-white, with a slight elevation.
- **Isolate IV** (*Proteus* spp.) developed spreading, greyish-white colonies with swarming growth and concentric rings.

To ensure precision, selective culture media and confirmatory diagnostic assays were employed, validating the identity and reliability of the bacterial classifications.

Selective Media

Growth on selective culture media provided clear confirmation of the isolates. *Klebsiella pneumoniae* produced pink colonies on MacConkey Agar, reflecting its ability to ferment lactose. *Staphylococcus aureus* developed yellow colonies on Mannitol Salt Agar, a result of mannitol fermentation and acid production. *Salmonella spp.* formed pale, colourless colonies on Salmonella–Shigella Agar, consistent with non-lactose fermentation. *Proteus spp.* displayed distinct clear zones on Tributyrin Agar, indicating lipolytic activity associated with enzyme production.

Antibiotic Sensitivity (Kirby–Bauer Method)

Bacterial isolates were evaluated for antimicrobial susceptibility on Mueller–Hinton agar using the Kirby–Bauer disc diffusion assay. Antibiotic discs were positioned on the inoculated plates, and the resulting inhibition zones were measured to characterize resistance trends (Table 4).

The present study evaluated the microbiological quality and antibiotic resistance profiles of cold-processed street-vended foods, with particular emphasis on ice cream and fruit juices collected from different vending locations in Chennai. These products are generally consumed without further heat treatment, making hygienic preparation, handling, storage, and vending conditions important determinants of their microbiological safety. The occurrence of several bacterial contaminants in the analyzed samples indicates that cold-processed street foods may serve as potential vehicles for foodborne microorganisms and antimicrobial-resistant bacteria.

The microbial load observed in the present investigation was comparatively high in both categories of samples. Ice cream showed a mean colony count of 7.1×10^6 CFU/g, whereas juices showed a mean count of 6.8×10^5 CFU/mL. The higher microbial burden observed in ice cream may be associated with contamination of dairy ingredients, utensils, handling surfaces, repeated manual contact, inadequate sanitation, or inappropriate storage conditions. In fruit juices, contamination may similarly originate from raw fruits, water used for preparation or dilution, utensils, containers, and direct handling during vending. Since these products are commonly consumed directly, the presence of substantial bacterial populations highlights the importance of maintaining hygienic conditions throughout preparation and distribution. The

study methodology specifically included sampling from multiple vending points in Chennai to capture geographical variation.

Six bacterial groups were recovered from the analyzed food samples, demonstrating considerable bacterial diversity. *Staphylococcus aureus* (55%) was the most frequently represented isolate, followed by *Salmonella spp.* (50%), *Klebsiella spp.* (40%), and *Proteus spp.* (30%). *Escherichia coli* and *Micrococcus spp.* were detected at lower frequencies of 10% each. The predominance of *S. aureus* is of particular food-safety interest because its presence in ready-to-eat foods can be associated with contamination through human handling and poor personal hygiene. The detection of *Salmonella spp.* is also important because *Salmonella* is a recognized foodborne pathogen and its occurrence in foods intended for direct consumption represents a potential microbiological hazard.

The detection of *Klebsiella*, *Proteus*, and *E. coli* further indicates possible contamination from environmental, water-associated, raw-material, or handling-related sources. The combination of these organisms suggests that contamination of chilled street-vended foods may occur at multiple stages rather than through a single source. The study's introduction similarly identifies contaminated water, ingredients, utensils, food-contact surfaces, vending environments, insects, and manual handling as potential routes of contamination.

The bacterial isolates were characterized using microscopic, biochemical, selective-media, and confirmatory approaches. The organisms demonstrated characteristic colony morphology on nutrient agar, while selective media provided additional differentiation. *Klebsiella pneumoniae* produced pink colonies on MacConkey agar, *S. aureus* produced yellow colonies on Mannitol Salt Agar, *Salmonella spp.* produced pale colonies on Salmonella–Shigella Agar, and *Proteus spp.* showed clear zones on Tributyrin Agar. These observations, together with the biochemical profiling, supported the classification of the recovered isolates.

All four principal isolates showed resistance to colistin, while additional resistance was observed against chloramphenicol and/or other antimicrobial groups depending on the isolate. *Klebsiella pneumoniae* and *Staphylococcus aureus* showed the pattern of sensitivity to aminoglycosides and fluoroquinolones but resistance to colistin and chloramphenicol.

Table.1 Microbial Load in Cold-Processed Foods

Analyzed Portion	Mean Colony Count (CFU/ml or g)
Ice cream	7.1×10^6
Juices	6.8×10^5

Table.2 Biochemical Interpretation

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

Table.3 Antibiotic Susceptibility Interpretation

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

Table.4 Resistance Patterns of Antibiotics

Antibiotic	Resistance (%)	Susceptibility (%)	Interpretation
Aminoglycoside	0%	100%	Fully susceptible - highly effective
Colistin	100%	0%	Completely resistant - ineffective for treatment
Chloramphenicol	25%	75%	Low resistant - still useful in some cases.
Fluoroquinolones	50%	50%	Moderate resistant-use with caution.

Table.5 Representation of Organisms

Food Samples	Location	Bacterial Isolates	Percentage (%)
Icecream & Juice	Thirumangalam	<i>Staphylococcus aureus</i>	55%
Icecream & Juice	Mogappair	<i>Salmonella spp.</i>	50%
Icecream & Juice	Anna nagar	<i>Klebsiella spp.</i>	40%
Juice	Vadapalani	<i>Proteus spp.</i>	30%
Juice	Koyambedu	<i>Escherichia coli</i>	10%
Icecream	Thirumangalam	<i>Micrococcus spp.</i>	10%



Figure 1: Juice



Figure 2: Ice Cream



Figure 3: Tributylin Agar with Lipolytic hydrolysis



Figure 4: SS agar with Black centered colonies



Figure 5: Antibiotic Plate 1

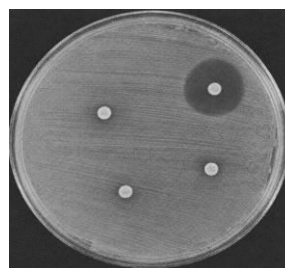


Figure 6: Antibiotic Plate 2

Salmonella spp. showed sensitivity to aminoglycosides and chloramphenicol but resistance to colistin and fluoroquinolones, whereas *Proteus* spp. showed sensitivity to aminoglycosides, chloramphenicol, and fluoroquinolones with resistance to colistin.

The occurrence of antibiotic-resistant isolates in foods is important from a food-safety and One Health perspective because food can provide an interface through which resistant microorganisms may circulate between environmental sources, food handlers, food products, and consumers. The identification of resistance phenotypes in bacteria recovered from ready-to-eat chilled foods therefore extends the significance of the study beyond conventional microbial quality assessment. However, the present findings represent the isolates and samples investigated in this study and should not be interpreted as prevalence estimates for all street-vended foods in Chennai.

The isolation of multiple bacterial species together with the observed antimicrobial resistance patterns emphasizes the need for improved hygienic practices among street-food vendors. Particular attention should be given to the quality of water and raw materials, cleaning and sanitation of utensils and food-contact surfaces, prevention of cross-contamination, personal hygiene, appropriate refrigeration, and proper storage conditions. Regular microbiological surveillance of ready-to-eat chilled foods could also help identify contamination trends and provide useful information for food-safety interventions.

In conclusion, the present study demonstrated that cold-processed street-vended foods, particularly ice cream and fruit juices, can harbour diverse bacterial contaminants of food-safety significance. The relatively high microbial loads observed in ice cream and juice, together with the recovery of *Staphylococcus aureus*, *Salmonella* spp., *Klebsiella pneumoniae*, *Proteus* spp indicate the presence of considerable microbiological contamination in the investigated samples. The antimicrobial susceptibility assessment further revealed varied resistance patterns among the recovered isolates, with resistance to colistin observed across the principal isolates tested. These findings highlight the importance of maintaining proper hygiene, safe water and raw-material quality, effective sanitation, appropriate storage, and prevention of cross-contamination during the preparation and vending of chilled foods. Continuous microbiological surveillance and antimicrobial resistance

monitoring of street-vended ready-to-eat foods are therefore important for strengthening food safety and reducing the potential public health risks associated with contaminated and antibiotic-resistant bacteria.

Author Contributions

V. Gayathri: Initiated the research concept, designed the study framework, and drafted the manuscript. T. Indira: Assisted with the research execution and contributed to revising and refining the manuscript. J. Sangeethalakshmi: Developed and optimized the experimental procedures, and provided critical feedback to strengthen 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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How to cite this article:

Gayathri V., Indira T. and Sangeethalakshmi J. 2026. Isolation and Detection of Antibiotic Resistant Bacteria from Cold Processed Street Vended Foods. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 310-317.
doi: <https://doi.org/10.20546/ijcmas.2026.sp13.033>