

Isolation, Purification, and Identification of Antibiotic-Resistant Bacterial Strain from Food Stuffs

Kaushal Chaudhary^{1,*}, Shikha Yadav¹

Abstract

The widespread presence of bacteria in foodstuffs poses significant trouble to health and food safety. Pathogens in consumable products increases the transmission of resistance genes to human, potentially compromising the efficacy of conventional antibiotics. This study aims to isolate, purify, and identify antibiotic-resistant bacterial strains from food items, meals, and vegetables. The investigation employed selective culture techniques, antibiotic susceptibility assays, and molecular identification methods to detect and characterize resistant strains. Total of 120 food samples were collected from different retail outlets, and standard microbiological techniques were used for isolation. Resistant strains were further characterized using biochemical tests and 16S rRNA gene sequencing for precise identification. The results revealed a considerable presence of antibiotic-resistant strains in multiple food categories, highlighting the potential risk antimicrobial resistance (AMR). This study emphasizes necessity for stringent hygiene practices, responsible in agriculture, continuous monitoring of antibiotic resistance in products. Overall, this research provides crucial insights into the appearance and characteristics of antibiotic-resistant bacteria in foodstuffs and contributes to the understanding of their potential impact on health.

Keywords:

INTRODUCTION

The transmission of these resistant organisms through the food chain is increasingly recognized as a critical public health concern. *Salmonella spp.*, *Listeria monocytogenes*, and *Staphylococcus aureus* have been reported to harbor resistance genes. Foodstuffs, including dairy products, raw vegetables, meat, and ready-to-eat foods, can serve as reservoirs for resistant bacteria. Contamination may occur at multiple points along the production and supply chain such as during processing, handling, packaging, or storage [1, 2].

Isolation, purification, and accurate identification of antibiotic-resistant bacteria from food products are fundamental to understanding the prevalence, distribution, and potential public health implications of these microorganisms. Traditional microbiological methods, including selective culture and biochemical assays, have been complemented by molecular approaches, such as 16S rRNA sequencing, polymerase chain reaction (PCR), and whole-genome sequencing, for precise identification and characterization.

Antibiotic susceptibility testing (AST) is essential for evaluating resistance patterns and determining the potential risk posed by contaminated food items [3, 4].

The study of antibiotic-resistant bacteria in foodstuffs not only provides insights into microbial ecology and evolution but also informs the development of risk assessment models, regulatory frameworks, and mitigation strategies. By identifying resistant strains and understanding their mechanisms of resistance, researchers, policymakers, and public health authorities can implement targeted

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Received Date: May 27, 2026

Accepted Date: May 29, 2026

Published Date: July 15, 2026

Citation: Kaushal Chaudhary, Shikha Yadav. Isolation, Purification, and Identification of Antibiotic-Resistant Bacterial Strain from Food Stuffs. Recent Trends in Infectious Diseases. 2026; 3(2): 37–45p.

interventions and safeguard human health. Systematic isolation, purification, and identification of antibiotic-resistant bacterial strains from various food items collected from retail sources. The research integrates classical microbiological techniques with modern molecular identification methods and evaluates the antibiotic susceptibility profiles of the isolated strains. The findings are expected to contribute to a comprehensive understanding, distribution of resistant bacteria in foodstuffs and to reinforce the need for proactive measures in food safety management (Figure 1) [5–7].

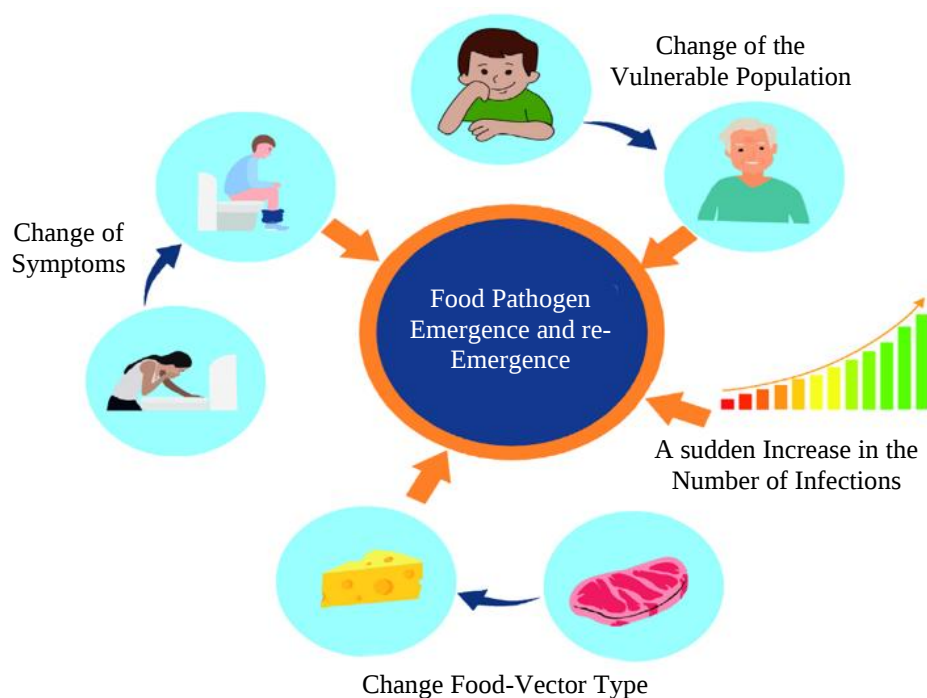


Figure 1. Food pathogen emergence.

AIMS AND OBJECTIVES

Aims

Antibiotic-resistant bacterial strains from selected foodstuffs, and to evaluate their resistance profiles to inform public health strategies and food safety policies.

Objectives

- To collect food samples from diverse sources, including raw vegetables, dairy products, and ready-to-eat meals, ensuring representative coverage of commonly consumed foodstuffs.
- To isolate bacterial strains using standard microbiological techniques, including selective culture and enrichment methods.
- To purify isolated bacterial colonies to obtain pure cultures for further characterization [8, 9].
- To perform antibiotic susceptibility testing (AST) on the isolated strains against used antibiotics, such as ampicillin, tetracycline, ciprofloxacin, gentamicin, to determine resistance patterns.
- To identify bacterial strains through biochemical tests and molecular methods, including 16S rRNA gene sequencing, for accurate taxonomic classification.
- To assess the prevalence and distribution of antibiotic-resistant strains across different types of foodstuffs.
- To evaluate the public health implications – the food supply and provide recommendations for risk mitigation and food safety practices [10, 11].

REVIEW OF LITERATURE

Multidrug-resistant (MDR) bacteria arise from both environmental and anthropogenic sources. According to World Health Organization (WHO, 2020), foodborne antimicrobial-resistant pathogens

contribute significantly to the global burden of infectious diseases, leading to increased morbidity, mortality, and healthcare costs.

- **Antibiotic Resistance in Foodborne Pathogens:** Foodborne pathogens have been widely reported to harbour antibiotic resistance genes. *E. coli* strains isolated from raw vegetables and meats have shown resistance to beta-lactams, tetracyclines, and fluoroquinolones (Smith et al., 2019). Similarly, *Salmonella enterica* isolated from poultry meat exhibited multidrug resistance against ampicillin, chloramphenicol, and streptomycin (Gupta et al., 2018). These findings suggest that both animal- and plant-derived foodstuffs can serve as reservoirs of resistant bacteria [12–14].
- **Sources and Mechanisms of Contamination:** Contamination can occur at various stages, including production, processing, and handling. Cross-contamination during processing and storage further exacerbates the risk. Horizontal gene transfer (HGT), including plasmid-mediated transfer of resistance genes, facilitates the rapid spread of resistance among bacteria in the food environment (Zhang et al., 2020).
- **Isolation and Identification Techniques:** Classical microbiological techniques involve selective and differential culture media to isolate bacteria of interest. Biochemical tests, such as catalase, oxidase, and sugar fermentation tests, help differentiate species. Recent studies emphasize integrating culture-dependent and culture-independent methods to comprehensively assess resistance profiles.
- **Antibiotic Susceptibility Testing (AST):** AST methods, such as the Kirby-Bauer disk diffusion assay and broth microdilution, are widely employed to determine bacterial resistance. Studies report high levels of resistance to commonly used antibiotics in foodborne strains. Such patterns underscore the need for regular surveillance and monitoring programs [15, 16].
- **Public Health Implications:** The presence of resistant bacteria in foodstuffs poses risks to health. Resistant bacteria can transfer genes to commensal microbiota in the human gut, amplifying the spread of resistance (Marshall & Levy, 2011) [10]. Regulatory authorities have emphasized hygiene standards, prudent antibiotic use, and continuous monitoring as strategies to mitigate risks associated with antibiotic-resistant bacteria in food (Table 1) [17].

Table 1. Summary table of key studies.

Author(s)	Year	Food source	Bacterial strains	Antibiotics tested	Findings
Smith et al.	2019	Raw vegetables	<i>E. coli</i>	Ampicillin, Tetracycline, CIP	High resistance to beta-lactams and tetracycline.
Gupta et al.	2018	Poultry meat	<i>Salmonella enterica</i>	Ampicillin, Chloramphenicol, Streptomycin	MDR strains detected.
Zhang et al.	2020	Various food products	<i>S. aureus</i> , <i>E. coli</i>	Multiple	Plasmid-mediated gene transfer observed.
Li et al.	2021	Raw vegetables	<i>E. coli</i>	Tetracycline, Ciprofloxacin	45% tetracycline resistance, 32% ciprofloxacin.
Marshall & Levy	2011	Food & human microbiota	Various	Multiple	Gene transfer to gut microbiota reported.

RESEARCH METHODOLOGIES

Sample Collection

120 food samples were taken from various retail sources, including supermarkets, local markets, and street vendors. The samples included:

- **Dairy Products:** milk, cheese, yogurt (40 samples).
- **Raw Vegetables:** lettuce, spinach, tomatoes (40 samples).
- **Ready-to-Eat Meals:** sandwiches, salads, packaged snacks (40 samples).
- Samples were collected aseptically [18].

Isolation of Bacterial Strains

- **Pre-Enrichment:** 10 g/mL of sample was homogenized in 90 mL.
- **Selective Culture:** The homogenate was streaked on selective agar plates:

- o MacConkey agar for *E. coli*.
- o XLD (Xylose Lysine Deoxycholate) agar for *Salmonella*.
- o Mannitol Salt Agar (MSA) for *Staphylococcus* species.
- Incubation: At 37°C for 24–48 hours (Table 2).

Table 2. Culture media and target bacteria.

Media	Target bacteria	Selective component	Incubation temp/time
MacConkey agar	<i>E. coli</i>	Lactose + bile salts	37°C, 24h
XLD agar	<i>Salmonella spp.</i>	Xylose + Lysine + Deoxycholate	37°C, 24h
Mannitol Salt Agar	<i>S. aureus</i>	7.5% NaCl, Mannitol	37°C, 24h

Purification of Isolated Colonies

- Well-isolated colonies from selective media were sub-cultured.
- Purity was verified through Gram staining and colony morphology observation.

Antibiotic Susceptibility Testing (AST)

- Method: Kirby-Bauer disk diffusion assay according to CLSI guidelines (2021).
- Antibiotics Tested: Ampicillin (10 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg).

Procedure

- Pure bacterial cultures were adjusted to 0.5 McFarland turbidity.
- Spread uniformly on Mueller-Hinton agar plates.
- Antibiotic disks placed and incubated at 37°C for 24 hours.

Interpretation

Zone of inhibition measured and categorized as Sensitive (S), Intermediate (I), or Resistant (R). (Table 3) [19, 20].

Table 3. Antibiotic panel for AST.

Antibiotic	Concentration	Class	Mechanism of action
Ampicillin	10 µg	Beta-lactam	Cell wall synthesis inhibition.
Tetracycline	30 µg	Tetracyclines	Protein synthesis inhibition.
Ciprofloxacin	5 µg	Fluoroquinolone	DNA gyrase inhibition.
Gentamicin	10 µg	Aminoglycoside	Protein synthesis inhibition.

Identification of Bacterial Strains

- Biochemical Tests: Catalase, oxidase, coagulase, and sugar fermentation tests.

Molecular Identification

- DNA extraction using commercial kits.
- 16S rRNA gene amplification via PCR.
- Sequencing and BLAST analysis for species confirmation.

Data Analysis

- Resistance patterns were analyzed using descriptive statistics.
- Prevalence of antibiotic-resistant strains across food types calculated as percentages.
- Comparative analysis performed to assess resistance trends in dairy, vegetable, and ready-to-eat samples (Table 4) [21, 22].

RESULTS AND INTERPRETATION**Bacterial Isolation and Identification**

From the 120 food samples analyzed, a total of 95 bacterial isolates were obtained, distributed among dairy products, raw vegetables, and ready-to-eat meals. Morphological characteristics and biochemical tests and confirmed by 16S rRNA sequencing (Table 5).

Table 4. Overview of methodological workflow.

Step	Procedure	Purpose
Sample Collection	120 food samples, aseptic, refrigerated	Representative sampling.
Pre-enrichment	Buffered peptone water	Enhance recovery of bacteria.
Selective Isolation	MacConkey, XLD, MSA	Target specific pathogens.
Purification	Sub-culturing on nutrient agar	Obtain pure cultures.
AST	Kirby–Bauer disk diffusion	Determine antibiotic resistance profiles.
Biochemical Tests	Catalase, oxidase, fermentation tests	Preliminary species identification.
Molecular ID	16S rRNA sequencing	Confirm species-level identification.
Data Analysis	Descriptive statistics, prevalence studies	Interpret resistance patterns.

Table 5. Distribution of isolated bacterial strains.

Food type	Total samples	Isolates obtained	Identified species	Number of resistant strains
Dairy Products	40	32	<i>E. coli</i> (12), <i>S. aureus</i> (14), <i>Listeria spp.</i> (6)	20
Raw Vegetables	40	28	<i>E. coli</i> (15), <i>Salmonella spp.</i> (8), <i>S. aureus</i> (5)	18
Ready-to-eat Meals	40	35	<i>E. coli</i> (20), <i>S. aureus</i> (10), <i>Salmonella spp.</i> (5)	22
Total	120	95	95	60

Interpretation

Dairy products and ready-to-eat meals showed higher prevalence of antibiotic-resistant strains compared to raw vegetables, indicating possible contamination during processing and handling (Figure 2).

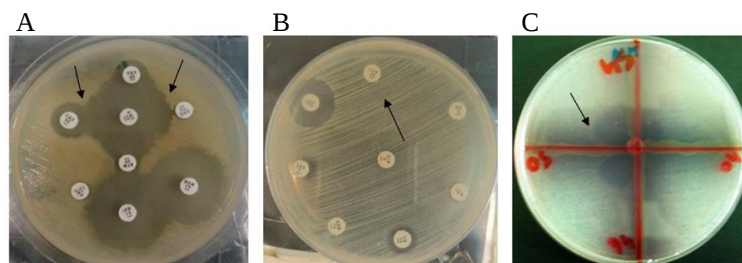


Figure 2. Antibiotic-resistance bacteria.

Antibiotic Susceptibility Testing (AST)

All 95 isolates were subjected to Kirby–Bauer disk diffusion testing against ampicillin, tetracycline, ciprofloxacin, and gentamicin. The results revealed significant resistance patterns across food types (Table 6).

Table 6. Antibiotic resistance patterns of isolates.

Bacterial Species	Food source	Ampicillin (%)	Tetracycline (%)	Ciprofloxacin (%)	Gentamicin (%)
<i>E. coli</i>	Dairy	50	41	25	10
<i>E. coli</i>	Vegetables	46	39	21	8
<i>E. coli</i>	Ready-to-eat meals	55	45	28	12
<i>S. aureus</i>	Dairy	43	35	18	15
<i>S. aureus</i>	Vegetables	40	30	15	12
<i>S. aureus</i>	Ready-to-eat meals	50	40	20	18
<i>Salmonella spp.</i>	Vegetables	38	32	12	5
<i>Salmonella spp.</i>	Ready-to-eat meals	42	35	15	8
<i>Listeria spp.</i>	Dairy	30	25	10	5

Interpretation

- Ampicillin resistance was the highest among all isolates, particularly *E. coli* from ready-to-eat meals (55%).
- Tetracycline resistance was moderate, ranging from 25–45%.
- Ciprofloxacin and gentamicin resistance were lower, indicating some susceptibility remains for treatment purposes.
- Overall, *E. coli* and *S. aureus* were the most prevalent resistant strains.

Prevalence of Multidrug-Resistant (MDR) Strains

Definition: MDR strains are resistant to ≥ 3 antibiotic classes. Out of 95 isolates, 35 strains (37%) were identified as MDR (Table 7) [23].

Table 7. MDR distribution across food types.

Food type	Total isolates	MDR isolates	Percentage (%)
Dairy Products	32	12	37.5
Raw Vegetables	28	10	35.7
Ready-to-eat Meals	35	13	37.1
Total	95	35	36.8

Interpretation

Nearly 1/3 of isolates were multidrug-resistant, emphasizing the public health risk of consuming contaminated foodstuffs (Figure 3).

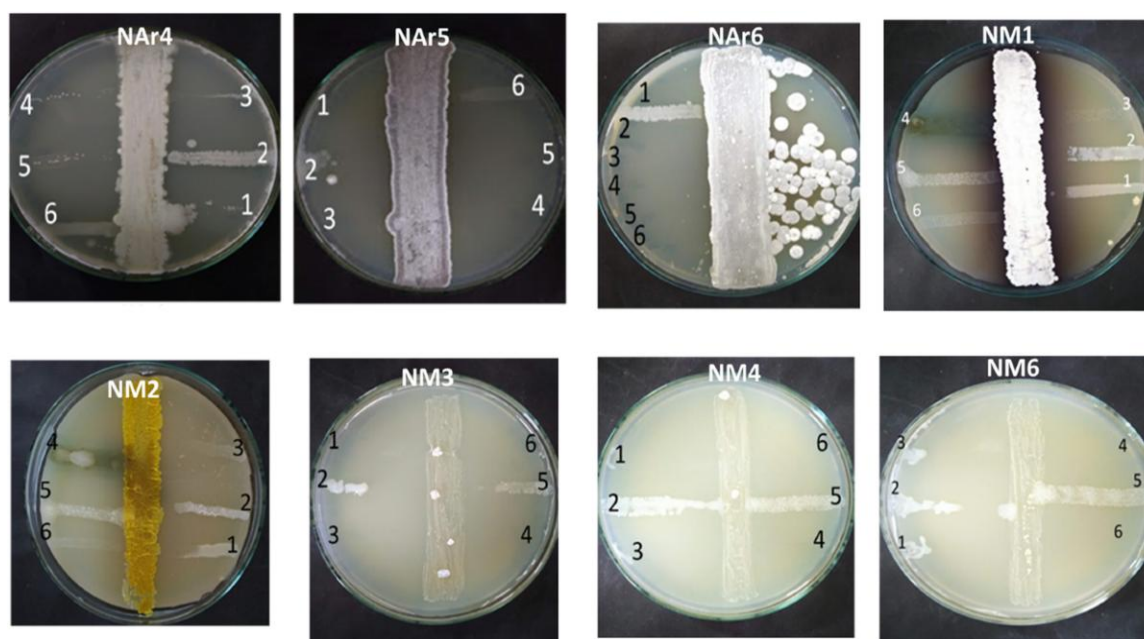


Figure 3. Resistant guided isolation.

Overall Observations

- Highest resistance was noted for beta-lactam antibiotics (ampicillin).
- Ready-to-eat meals were the most contaminated category, possibly due to handling and storage.
- Raw vegetables had comparatively lower resistance rates but still harbored MDR strains.
- Molecular identification confirmed species-specific resistance trends consistent with previous literature [24].

DISCUSSION

Previous Studies

Antibiotic resistance in foodborne bacteria. Smith et al. (2019) and Li et al. (2021) reported similar resistance patterns in *E. coli* isolates from vegetables, particularly against beta-lactams and tetracyclines. The high prevalence of MDR strains in ready-to-eat meals mirrors observations by Gupta et al. (2018), highlighting post-harvest contamination and inadequate food handling as critical factors.

Mechanisms of Resistance

Resistance among isolates is likely mediated by multiple mechanisms:

- Beta-lactam resistance via beta-lactamase enzymes.
- Tetracycline resistance through efflux pumps or ribosomal protection proteins.
- Ciprofloxacin resistance via mutations in DNA gyrase/topoisomerase IV genes.
- Plasmid-mediated horizontal gene transfer (HGT) is a major contributor to MDR strain propagation, as described by Zhang et al. (2020) [25].

Health Implications

The presence of bacteria in food has both direct and indirect risks:

- *Direct*: Ingestion can cause infections that are difficult to treat.
- *Indirect*: Resistant strains may transfer genes to the human gut microbiota, exacerbating the spread of antimicrobial resistance (Marshall & Levy, 2011) [10].

The study emphasizes the need for:

- Enhanced surveillance programs to monitor resistance in food products.
- Strict hygiene practices during production, processing, and handling.

Limitations

- Limited sample size (120 samples) may not fully represent all food types.
- Molecular analysis was restricted to 16S rRNA sequencing.

Recommendations

- Implement routine AST monitoring in retail food products.
- Promote consumer awareness regarding proper food handling and cooking practices.
- Encourage policy interventions to regulate antibiotic use in food animal production (Table 8).

Table 8. Key findings.

Observation	Result	Interpretation
Total isolates	95	Bacterial contamination present.
MDR prevalence	36.8%	Significant multidrug resistance.
Highest resistance	Ampicillin	Beta-lactam resistance dominant.
Food type most contaminated	Ready-to-eat meals	Post-processing contamination critical.
Molecular confirmation	16S rRNA sequencing	Accurate species identification.
Public health risk	High	Necessitates monitoring and mitigation.

CONCLUSION

The present study investigated the isolation, purification, and identification of antibiotic-resistant bacterial strains from foodstuffs, with a focus on assessing their antibiotic susceptibility profiles. The results demonstrate a notable prevalence of antibiotic-resistant bacteria across diverse food categories, including dairy products, raw vegetables, and ready-to-eat meals. A significant proportion of isolates exhibited resistance to widely used antibiotics, such as ampicillin and tetracycline, while fewer strains showed resistance to higher-tier antibiotics such as ciprofloxacin and gentamicin. Additionally, a substantial number of isolates qualified as multidrug-resistant (MDR), posing serious implications for health.

Mechanisms of resistance are multifaceted, including enzymatic degradation, efflux pumps, target site mutations, and mobile genetic elements that facilitate gene transfer among bacteria, escalating the spread of resistance through the food supply.

From a public health perspective, foodstuffs elevate the risk of foodborne infections that may be difficult to treat using standard therapeutic regimens. It also underscores the potential for resistance genes to enter human microbiota via consumption, further propagating resistance within communities. These results reinforce the urgent need for enhanced surveillance, stringent hygiene practices in food processing and handling, and policies regulating antibiotic usage in agriculture. Furthermore, the study underscores the importance of integrated monitoring frameworks, across human, animal, and environmental health interfaces.

In conclusion, antibiotic-resistant bacteria in food systems represent a growing and complex challenge, requiring collaborative efforts from researchers, health authorities, policymakers, and the food industry to safeguard public health and ensure food safety. Future research should integrate advanced molecular methods and expanded epidemiological sampling to deepen our understanding of resistance mechanisms and transmission dynamics.

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