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Prevalence and Antibiotic Resistance Patterns of *Salmonella enterica* Isolated from Diverse Food Commodities

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Abstract

Salmonella enterica remains a major foodborne pathogen globally, contributing to significant morbidity and economic losses. The increasing use of antimicrobials in food animals has accelerated the emergence of multidrug-resistant strains, posing critical challenges to food safety. This study investigated the prevalence and antimicrobial resistance profiles of S. enterica isolated from selected food commodities marketed in District Dera Ghazi Khan, Punjab, Pakistan. A total of 100 samples, including poultry, dairy, beef, fruits, vegetables, bakery products, and tap water, were collected under aseptic conditions and processed using standard microbiological procedures. Selective media, Gram staining, and biochemical assays were employed for bacterial confirmation, followed by antimicrobial susceptibility testing using the Kirby–Bauer disc diffusion method. Of the 100 samples examined, 30% were positive for S. enterica, with the highest contamination in poultry (60%), followed by dairy (33.3%) and beef (30%). Antibiotic susceptibility testing demonstrated high resistance to penicillin (90%), ampicillin (86.7%), oxacillin (83.3%), tetracycline (80%), and erythromycin (70%). Moderate resistance was recorded for streptomycin (50%) and neomycin (66.7%), while ciprofloxacin exhibited the lowest resistance (16.7%), indicating its retained therapeutic value. Resistance patterns varied significantly across food categories, with poultry and dairy products showing the highest multidrug resistance.

These findings highlight widespread contamination and substantial antimicrobial resistance among S. enterica isolates in the local food chain. Strengthened hygiene practices, regulated antibiotic use in livestock, and enhanced surveillance systems are urgently needed to mitigate public health risks.

Introduction

Foodborne diseases remain a persistent global public health concern, with *Salmonella enterica* being recognized as one of the leading bacterial agents responsible for human gastroenteritis, systemic infections, and foodborne outbreaks (Lamichhane and Mawad, 2024). The World Health Organization reports that over 1.4 billion cases of non-typhoidal salmonellosis occur annually, contributing to an estimated 33 million disability-adjusted life years (Majowicz et al., 2010). *Salmonella* comprises two species, *S. bongori* and *S. enterica*, the latter encompassing more than 2,600 serovars, with *S. Typhimurium* and *S. Enteritidis* being the most frequently implicated in human disease

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(Brown et al., 2021). These serovars have been widely associated with contaminated poultry, dairy products, raw meat, and fresh produce, reflecting their ability to persist in multiple environmental and food-processing (Ferrari et al., 2019).

Pakistan, like many low- and middle-income countries, faces significant challenges related to food hygiene, unregulated animal production practices, widespread antibiotic misuse, and inadequate monitoring systems (Mirha and Ali, 2024). These factors collectively create favorable conditions for the persistence and transmission of *Salmonella* throughout the food chain (Arshed and Umair, 2025). Numerous studies conducted across Pakistan have documented high levels of *Salmonella* contamination in retail poultry, meat, milk, and water sources (Siddique et al., 2021). The emergence of multidrug-resistant (MDR) strains further complicates disease management, as resistance to β-lactams, macrolides, tetracyclines, and aminoglycosides has become increasingly widespread.

Antimicrobial resistance (AMR) in *Salmonella* has significant public health implications (Starkova et al., 2025). Resistant strains compromise the efficacy of first-line antibiotics, prolong the duration of illness, increase hospitalization rates, and elevate treatment costs. Several studies across Asia, including Iran (Narimisa et al., 2024, Hosseininezhad et al., 2020), China (Yang et al., 2022), and Thailand (Trongjit et al., 2017), have reported alarming resistance patterns associated with contaminated raw meat, vegetables, eggs, and ready-to-eat foods. In Pakistan, resistance to commonly used antimicrobials such as ampicillin, tetracycline, and ciprofloxacin have been documented at varying levels, highlighting the influence of antibiotic misuse in livestock and human healthcare (Arshed and Umair, 2025).

Water and fresh produce also serve as important vehicles for *Salmonella* transmission. Contaminated irrigation water, polluted soil, and improper handling practices can facilitate cross-contamination, leading to outbreaks linked to fruits and vegetables. The increasing reliance on untreated surface and groundwater sources in agricultural production further amplifies these risks (Alegbeleye et al., 2018).

Despite growing concerns, limited data exist on the prevalence and AMR profiles of *S. enterica* in several understudied regions of Pakistan, including District DG Khan. The present study aims to provide a comprehensive assessment of *S. enterica* in diverse food commodities sold in Dera Ghazi Khan, Punjab, Pakistan. By examining the prevalence across multiple food categories and evaluating antimicrobial resistance profiles, the study offers critical insights for public health authorities, veterinarians, and policymakers.

Materials and Methods

Sample Collection

An experimental cross-sectional study was conducted to investigate the prevalence and antibiotic resistance patterns of *Salmonella enterica* in selected food commodities sold in District Dera Ghazi Khan, Pakistan. A total of 100 samples representing seven food categories, dairy products, poultry, beef, fruits, vegetables, bakery goods, and tap water were collected from commercial markets under aseptic conditions. Samples were placed in sterile plastic bags, labelled appropriately, and transported immediately for analysis to the Microbiology Laboratory, Faculty of Veterinary and Animal Sciences, MNS University of Agriculture, Multan, Punjab, Pakistan.

Enrichment and Culture

Samples were enriched in nutrient broth prepared by dissolving 37.5 g of medium in 1 L of distilled water, followed by autoclaving at 121°C and 15 psi for 15 minutes. After sterility confirmation through incubation at 37°C for 24 hours, enriched samples were streaked onto nutrient agar and incubated for an additional 24 hours.

Selective plating was performed on Eosin Methylene Blue (EMB) agar and Salmonella-Shigella (SS) agar. Both media were prepared according to standard protocols, autoclaved, poured into sterile petri dishes, and incubated overnight at 37°C for sterility evaluation. Suspected colonies non-lactose fermenting, colorless on SS agar with black centers indicating H_2S production were selected for further analysis.

Biochemical Characterization

Gram staining was performed to confirm Gram-negative rod-shaped bacteria. Colonies showing pink-stained bacilli were subjected to biochemical tests, including Triple Sugar Iron (TSI) reactions, citrate utilization, urease activity, and indole production. Typical *Salmonella* isolates exhibited alkaline slants with acid butts and H_2S production on TSI, positive citrate utilization, and negative urease and indole tests.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was assessed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Commercial antibiotic discs representing multiple drug classes were used. After incubation at 37°C for 18-24 hours, inhibition zones were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Pierce et al., 2023).

Statistical Analysis

Data were compiled in SPSS (Version 20). Descriptive statistics were used to calculate prevalence across food categories. Chi-square analysis assessed differences in contamination rates, while logistic regression evaluated

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associations between food source and antibiotic resistance profiles. Statistical significance was accepted at $P < 0.05$.

Results

Confirmation of *Salmonella Enterica*

The suspected isolates displayed characteristic morphology on Salmonella-Shigella (SS) agar, presenting as pale, colorless colonies with distinct black centers indicative of H_2S production. On MacConkey agar, isolates appeared as non-lactose-fermenting colonies. Gram staining revealed Gram-negative, pink-stained bacilli. Biochemical tests including TSI, citrate, urease, and indole assays confirmed the identity of *S. enterica*.

Prevalence in Food Commodities

Among the 100 food samples tested, 30 (30%) were positive for *S. enterica*. Poultry exhibited the highest contamination rate (60%), followed by dairy products (33.3%) and beef (30%). Lower contamination was observed in vegetables (26.7%), bakery products (20%), fruits (20%), and tap water (6.7%), (Table 1, Fig. 1).

Antibiotic Resistance Profiles

The resistance was highly prevalent across multiple antibiotic classes Table 2. The highest resistance was recorded for penicillin (90%), ampicillin (86.7%), oxacillin (83.3%), tetracycline (80%), and erythromycin (70%). Moderate resistance was observed for neomycin (66.7%), streptomycin (50%), and kanamycin (30%). Ciprofloxacin showed the lowest resistance (16.7%).

Table 2. Antibiotic Resistance of *Salmonella Enterica* Isolates **(n=30)**

Antibiotic	Resistant Isolates	Resistance (%)
Tetracycline	24	80.0%
Penicillin	27	90.0%
Ciprofloxacin	5	16.7%
Augmentin	10	33.3%
Neomycin	20	66.7%
Erythromycin	21	70.0%
Kanamycin	9	30.0%
Streptomycin	15	50.0%
Oxacillin	25	83.3%
Ampicillin	26	86.7%

Antimicrobial Susceptibility

The susceptibility profiles represented resistance patterns. Ciprofloxacin showed the highest susceptibility rate, followed by kanamycin and augmentin. β-lactam antibiotics demonstrated markedly reduced efficacy, with widespread resistance Table 3.

Correlation Between Food Category and Resistance

Poultry isolates exhibited the highest MDR patterns, particularly toward penicillin, ampicillin, tetracycline, and oxacillin. Dairy products also produce isolates resistant to penicillin, erythromycin, and neomycin. Vegetables and fruits showed lower resistance levels; however, penicillin resistance persisted, suggesting environmental contamination. Tap water isolates exhibited resistance to ampicillin and oxacillin but remained susceptible to ciprofloxacin and neomycin Table 4.

Table 4. Correlation between food source and antibiotic resistance

Food Commodity	Most resistant antibiotics (≥70%)	Least resistant antibiotics (≤30%)
Poultry	Penicillin, Ampicillin, Oxacillin, Tetracycline	Ciprofloxacin
Dairy	Penicillin, Erythromycin, Neomycin	Kanamycin
Beef	Ampicillin, Oxacillin	Ciprofloxacin, Streptomycin
Vegetables	Penicillin, Erythromycin	Ciprofloxacin, Kanamycin
Fruits	Penicillin	Ciprofloxacin
Tap Water	Ampicillin, Oxacillin	Ciprofloxacin, Neomycin
Bakery	Oxacillin, Penicillin	Ciprofloxacin, Kanamycin

Discussion

The understanding of the distribution and resistance behavior of *Salmonella enterica* within locally consumed foods is essential for identifying critical control points in the food supply chain (Qamar et al., 2020). This study documents substantial contamination of food commodities with *Salmonella enterica* in District DG Khan, highlighting both food safety shortcomings and widespread antimicrobial resistance. The overall prevalence of 30% is consistent with reports from various regions of Pakistan, although higher than some international estimates. Poultry demonstrated the highest contamination burden (60%), aligning with numerous studies indicating poultry as a major reservoir of *Salmonella* due to intensive production systems, poor hygiene practices, and cross-contamination during slaughter and processing (Samad et al., 2018). Live bird markets, common in Pakistan, further facilitate bacterial spread through improper handling and inadequate sanitation.

The high contamination rate in dairy products (33.3%) is concerning. Raw milk consumption is widespread in the region, and lack of pasteurization, poor hygiene during

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milking, and contaminated water sources contribute significantly to bacterial persistence. Comparative studies in China report lower contamination rates (He et al., 2023), likely attributable to more stringent regulations and improved pasteurization systems. Contamination in beef (30%) also exceeded several previous findings from urban centers, suggesting localized handling issues and potential lapses in abattoir sanitation.

The contamination of fruits (20%), vegetables (26.7%), and bakery products (20%) indicate that fresh produce and processed foods are also vulnerable to cross-contamination. Unhygienic food handling, contaminated irrigation water, soil fertilized with untreated manure, and unclean food preparation environments may facilitate pathogen transfer. Tap water contamination (6.7%), although comparatively low, indicates underlying water safety issues that can introduce bacterial contaminants into the food chain.

Antimicrobial resistance patterns observed in this study revealed a high proportion of MDR strains. Resistance was most prevalent against β-lactams (penicillin 90%; ampicillin 86.7%; oxacillin 83.3%), consistent with national trends in Pakistan and neighboring countries (Iqbal et al., 2022, Fatima et al., 2023). Resistance to tetracycline (80%) and erythromycin (70%) further supports claims of widespread misuse in poultry and dairy farms. Aminoglycoside resistance, including neomycin (66.7%) and streptomycin (50%), indicates selection pressure within animal husbandry systems.

Ciprofloxacin displayed the lowest resistance level (16.7%), preserving its value as a viable therapeutic option. However, emerging fluoroquinolone resistance remains a public health concern, as highlighted by recent outbreaks associated with resistant *Salmonella* strains in Asia and Africa (Chen et al., 2019).

The strong association between food source and resistance patterns underscores the role of agricultural practices in shaping AMR emergence. Poultry and dairy products exhibited the highest MDR levels, reflecting intensive antibiotic use in these sectors (Begum et al., 2018). Conversely, lower resistance in fruits, vegetables, and tap water suggests environmental contamination rather than direct antibiotic exposure.

Comparisons with similar studies globally reveal that the prevalence and resistance levels observed here are higher than many reported values. For example, a study in Thailand reported 68-84% MDR prevalence in poultry and pork samples (Begum et al., 2018), while 26.7% MDR occurrence in raw vegetables in China (Yang et al., 2020), lower than the levels seen in DG Khan.

The persistence of MDR *S. enterica* in food commodities poses significant health risks, including prolonged illness, treatment failure, and increased hospitalization. These findings highlight the urgent need for integrated measures, including improved hygiene practices, stricter regulation of antibiotics in animal production, routine surveillance, and consumer education. Strengthening food safety monitoring systems at local and national levels is critical to reducing the burden of foodborne pathogens and combating AMR.

Conclusion

This study confirms widespread contamination of the local food supply with *S. enterica* and demonstrates high levels of antibiotic resistance across multiple drug classes. These findings underscore the need for improved hygiene practices, stricter regulation of antimicrobial use in livestock, and strengthened food safety monitoring. Future work should focus on molecular characterization of circulating *Salmonella* strains, identification of transmission pathways within the food chain, and development of targeted interventions to reduce antimicrobial resistance and enhance public health protection.

Author Contributions

AA designed the study. MTS, NA, IH and TI collected the samples. BK conducted lab experiments. MN, and MZ collected the data. RK, and SM performed statistical analysis. BK drafted the manuscript, and AA revised and finalized the manuscript.

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N/A

Conflict of interest

The authors declare no conflicts of interest.

Ethical statement

All animal handling procedures were approved by the Ethics Committee of the Department of Pathobiology & Biomedical Sciences, Faculty of Veterinary & Animal Sciences, MNS-University of Agriculture, Multan.

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Table 1. Prevalence of *S. enterica* in Food Samples

Food Category	Food product	Samples tested	Positive samples	Prevalence (%)
Bakery	Bread, pastries	15	3	20.0%
Dairy	Milk, yogurt, cheese	15	5	33.3%
Poultry	Raw chicken, meat	20	12	60.0%
Beef	Minced, cuts	10	3	30.0%
Fruits	Apples, bananas	10	2	20.0%
Vegetables	Tomatoes, spinach	15	4	26.7%
Tap water	Tap source	15	1	6.7%
Total	—	100	30	30.0%

Table 3. Antimicrobial Susceptibility of Salmonella Isolates (n = 30)

Antibiotic	Sensitive (n)	Intermediate (n)	Resistant (n)	Resistance (%)
Tetracycline	8	5	17	56.7%
Penicillin	6	4	20	66.7%
Ciprofloxacin	18	6	6	20.0%
Augmentin	12	5	13	43.3%
Neomycin	10	4	16	53.3%
Erythromycin	7	3	20	66.7%
Kanamycin	14	5	11	36.7%
Streptomycin	9	4	17	56.7%
Oxacillin	5	3	22	73.3%
Ampicillin	6	4	20	66.7%

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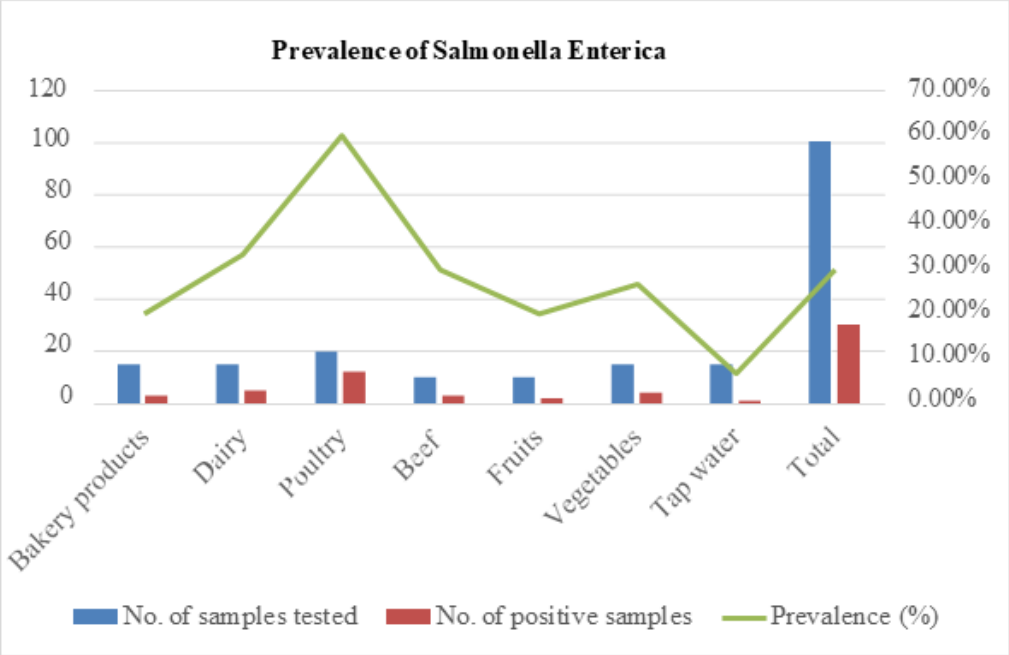


Fig. 1. Prevalence of *Salmonella enterica* across food categories

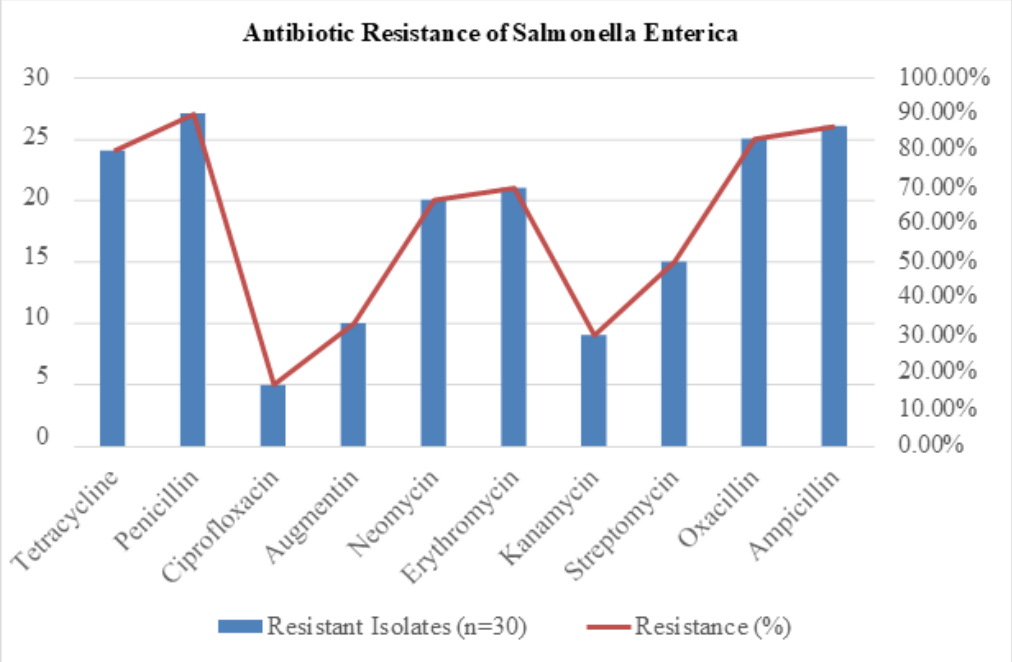


Fig. 2. Antibiotic resistance profile of *Salmonella enterica* isolates across different antibiotics

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