Multi-Disciplinary Publishing Institute Pakistan **Vol.** 4, **N0.** 1

Submitted: 13 JUN 2025 **Accepted: 18 JUL 2025** **Published: 19 JUL 2025**

Genomic Analysis of Cephalosporin Resistance in Salmonella: A One Health Perspective on the Transmission of Resistance Genes between Animals, Humans, and the Environment in Punjab, Pakistan

Original Article

Mahnoor[1], Bhajan Kumar[2], Ayesha Tariq[3], Umair Abbas[4], Ieman Tariq[3], Muhammad Waseem Akram[4], Muhammad Ashraf[5]

[1]Department of Zoology, Government College University, Lahore 54000, Pakistan.

[2]Department of Biotechnology Quaid-i-Azam University Islamabad 45320 Pakistan.

[3]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan.

[4]Industrial Biotechnology Division National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad 38000 Pakistan.

[5]Associate Professor, Institute of Microbiology, University of Faisalabad, Pakistan.

Corresponding Author email:
bhajankumar@bs.qau.edu.pk

Citation

Mahnoor, Kumar, B., Tariq, A., Abbas, U., Tariq, I., Akram, M.W., & Ashraf, M. (2025). Genomic analysis of Cephalosporin resistance in Salmonella: A one health perspective on the transmission of resistance genes between animals, humans, and the environment in Punjab, Pakistan. *Open Access Public Health and Health Administration Review*, 4(1), 72-85.

WEBSITE: www.mdpip.com
ISSN: Print: 2959-619X
ISSN: Online: 2959-6203
PUBLISHER: MDPIP

Abstract

Antimicrobial resistance (AMR) in *Salmonella*, particularly to third-generation cephalosporins, is a rising public health threat, especially in developing regions with dense human-animal-environment interactions. This study evaluates cephalosporin resistance in *Salmonella* through a One Health lens in Punjab, Pakistan, using whole-genome sequencing (WGS) to uncover resistance mechanisms and transmission dynamics. Phenotypic resistance was determined through antimicrobial susceptibility testing, while genotypic resistance was characterized using WGS. Phylogenetic analysis and multilocus sequence typing (MLST) were used to map potential transmission routes. *Salmonella* was isolated from 33.4% of samples, with the highest prevalence in poultry (42%). Cephalosporin resistance was phenotypically observed in 52.7% of isolates, with 72.7% being ESBL-producers. WGS revealed the presence of CTX-M-15 (58%), TEM-1 (29.5%), and CMY-2 (20.5%) among resistant strains. Significant SNP-based phylogenetic relatedness was observed between human, animal, and environmental isolates, indicating active cross-sector transmission. Notably, poultry and wastewater sources were major transmission hubs. This study presents robust genomic evidence that cephalosporin-resistant *Salmonella* is circulating across animal, human, and environmental sectors in Punjab, Pakistan, with poultry acting as a key reservoir. The detection of widespread resistance genes such as CTX-M-15, TEM-1, and CMY-2, along with evidence of mobile genetic elements (e.g., IncFII, IncI2 plasmids), underscores the critical role of horizontal gene transfer in resistance propagation. The strong genetic relatedness between isolates from live bird markets, hospitals, and wastewater suggests ongoing transmission across the One Health spectrum.

Keywords: Antimicrobial Resistance (AMR), Cephalosporin, Livestock, Poultry, *Salmonella*.

Introduction

The emergence and rapid global spread of Antimicrobial Resistance (AMR) presents a momentous and pressing concern, threatening public health, economic welfare, and food security worldwide (Aqeel *et al*., 2024). This crisis is exacerbated by the capability of microorganisms, including bacteria, to endure the impact of antimicrobial medications, thereby diminishing their efficacy. The issue is universal, affecting all countries regardless of wealth or status, as resistant organisms know no geographical borders and can spread through the movement of people, animals, food, or water (Mahmood, Rasoo, Khurshid, & Aslam, 2025). Reports indicate that AMR is projected to cause millions of deaths annually, for example, an estimated 4.95 million deaths globally in 2019, with projections suggesting up to 10 million deaths per year by 2050 if current trends continue. A significant driver of this alarming rise is the irrational and excessive use of antibiotics in both human and veterinary medicine. This widespread misuse creates selective pressure, allowing resistant bacterial strains to accumulate and thrive within livestock production and pet healthcare, making traditional antibiotic treatments increasingly ineffective (Habib, 2023). The problem is further complicated by the zoonotic transmission of resistant pathogens between animals and humans, leading to a complex global AMR crisis. Resistant infections in livestock operations can result in lowered productivity, increased veterinary expenses, and higher mortality rates, with the challenge of antibiotic residue elimination through animal and poultry waste posing significant environmental concerns for soil and water ecology (Malik *et al*., 2024). The absence of new antimicrobials with increased efficacy further compounds this serious issue, highlighting the urgent need for effective control measures.

Salmonella is recognized as a major zoonotic foodborne pathogen, holding significant economic status in both animals and humans (Qamar, Ismail, & Akhtar, 2020). Its natural habitat is the gastrointestinal tract (GIT) of domestic and wild animals, and it can readily infect various foodstuffs of both animal and plant origin, even in animals showing no signs of illness. Contaminated poultry products, particularly meat and eggs, have been identified as primary sources of infection for humans, responsible for a substantial percentage of foodborne diseases. *Salmonella* causes a diverse range of illnesses, from enteritis and septicemia to abortion and, in rare cases, meningitis. Globally, *Salmonella* infections account for approximately 95 million cases and an estimated 150,000 deaths annually, with low- and middle-income countries experiencing higher infection rates due to inadequate sanitation and food safety standards. Among the over 2,600 identified serovars, *Salmonella enteritidis* and *Salmonella typhimurium* are the most prevalent serovars associated with human infections (Fatima *et al*., 2023). Direct contact with infected animals, consumption or handling of contaminated animal products like raw meat or eggs, and interaction with contaminated equipment or vectors are the main routes of transmission to humans (Yasmeen, Aslam, Fang, Baloch, & Liu, 2023).

Cephalosporins are critically important antimicrobials due to their broad-spectrum activity against various Gram-positive and Gram-negative bacteria. Historically, third-generation cephalosporins, such as Ceftriaxone, became the recommended first-line treatment for multidrug-resistant (MDR) *Salmonella typhi* infections, especially after increased resistance to conventional antibiotics like ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (Hameed *et al*., 2024). However, the emergence of extensively drug-resistant (XDR) *Salmonella typhi* strains, which are a type of MDR H58 strain, has alarmingly acquired resistance to ceftriaxone and other third-generation cephalosporins, posing a significant threat to global public health by severely limiting treatment options. This resistance is often mediated by enzymes like extended-spectrum beta-lactamases (ESBLs), which hydrolyze these critical antibiotics, with genes such as *blaTEM*, *blaSHV*, and *blaCTXM* being responsible (Rahim *et al*., 2024). Specifically, the *blaCTXM* gene governs resistance to third-generation cephalosporins, a class of antibiotics prioritized by the World Health Organization (WHO) as critically important for human use. The increasing prevalence of ESBL-producing *Salmonella*, *Escherichia coli*, and *Shigella* poses substantial public health risks. While carbapenems remain one of the few effective treatment options, high resistance to some (e.g., Imipenem) has also been observed in *Salmonella* strains, further narrowing the available therapeutic arsenal (Sattar *et al*., 2024).

The One Health framework is fundamentally essential for understanding and combating AMR, particularly in regions like Punjab, Pakistan. This integrated approach acknowledges the profound interconnectedness between human, animal, and environmental health, which is crucial for controlling zoonotic diseases like salmonellosis. Punjab is a prominent region for livestock and poultry production, making it a critical interface for AMR transmission (Arshed et al.). Pakistan's substantial poultry industry, with billions of broilers and millions of layers and breeding stock annually, involves extensive antibiotic use, making the poultry sector a significant reservoir for the emergence and dispersal of antimicrobial-resistant bacteria. In this densely populated region, the high level of contact between humans and animals, coupled with often unregulated antibiotic use, inadequate sewage facilities, and poor hygiene practices, creates an ideal environment for the proliferation and transmission of resistant pathogens (Hashmi, 2019). Studies have highlighted the high prevalence of *Salmonella* spp. in raw milk and environmental samples from dairy farms in Southern Punjab, directly contaminating farm environments and produce, which then contribute to more frequent infections in both farm animals and humans. The environmental dissemination of resistance, notably through poultry litter containing unmetabolized antibiotics and resistant bacteria, and the bidirectional flow of AMR genes, further underscores the need for a comprehensive One Health approach to mitigation and surveillance (Naheed, Sultan, & Barvi, 2025). This integrated approach acknowledges the profound interconnectedness between human, animal, and environmental health (Figure 1), which is crucial for controlling zoonotic diseases like salmonellosis (Ismail, Qamar, & Ismail, 2020).

Figure 1

The One Health Concept. This diagram illustrates the interconnectedness of human, animal, and environmental health. The overlapping circles demonstrate that the health of each domain is intrinsically linked, forming the foundation of the One Health approach to tackling complex public health issues like antimicrobial resistance.

To effectively track and understand the complex spread of resistance genes, genomic epidemiology is an indispensable tool. The power of Whole Genome Sequencing (WGS) lies in its ability to provide high-resolution insights into the emergence and transmission dynamics of AMR pathogens. WGS facilitates the identification of specific resistance genes, such as ESBLs like *blaCTX-M*, which is a predominant ESBL genotype found in *Escherichia coli* and *Klebsiella pneumoniae* in Pakistan and globally, as well as genes like *blaTEM-1*, *catA1*, and *gyrA* in *Salmonella(Subhani, Batool, Naveed, Shabbir, & Noor, 2023)*. Moreover, WGS allows for the precise tracking of mobile genetic elements, such as plasmids (e.g., IncHI1, IncY, IncFII, IncFIA) and integrons, which play a principal role in the horizontal transfer of antibiotic resistance genes between bacterial strains and even different bacterial species. Through comparative genomics and phylogenetic analysis, WGS helps to infer transmission pathways between human, animal, and environmental reservoirs, enabling a more comprehensive understanding of the origin and spread of resistant strains. This detailed genetic information is critical for designing targeted and effective interventions to control the growing threat of cephalosporin-resistant *Salmonella* within the One Health context in Punjab, Pakistan (Subhani *et al*., 2023).

The Study Rationale and Objectives are vital for confronting the escalating threat of antimicrobial resistance (AMR) in *Salmonella*, especially within Punjab, Pakistan, a region characterized by dense human populations, extensive livestock farming, and shared environmental resources. This research is fundamentally rooted in the One Health framework, which acknowledges the intrinsic link between human, animal, and environmental health, highlighting that interventions in one sector invariably impact the others. The study aims to utilize advanced genomic tools to comprehensively understand cephalosporin resistance in *Salmonella* and its transmission dynamics across these interconnected sectors (Siddique *et al*., 2021).

Firstly, to determine the prevalence of cephalosporin-resistant *Salmonella* in animal, human, and environmental sources in Punjab. *Salmonella* is a major zoonotic foodborne pathogen of global economic and public health significance, causing substantial economic losses and serious health concerns in Pakistan, particularly in Punjab and Sindh provinces (Sharma, 2022). Existing data already indicate an increased prevalence of *Salmonella* spp. in raw milk and environmental samples from dairy farms in South Punjab, with a noted overall prevalence of 25.89% in Multan district. Similarly, raw meat samples from Lahore, Pakistan, exhibited a high *Salmonella* recovery rate of 51.3%, with poultry gizzard (60%) and liver (55.5%) being highly contaminated. Raw vegetables in Karachi have also been identified as potential reservoirs for *Salmonella typhi* and multidrug-resistant (MDR)/extensively drug-resistant (XDR) strains. The specific focus on cephalosporin resistance is critical because third-generation cephalosporins, like Ceftriaxone, were once the recommended treatment for MDR *Salmonella typhi*, but the emergence of XDR *Salmonella typhi* strains, which are resistant to ceftriaxone, now poses a significant global public health threat by severely limiting treatment options. Phenotypic testing of *Salmonella* isolates from raw meat in Lahore has revealed alarmingly high resistance to Cefepime (98.24%) and Ceftriaxone (50%) (Nazir *et al.*, 2025).

Secondly, to identify the specific antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) responsible for this resistance using Whole Genome Sequencing (WGS). WGS is an indispensable tool for genomic epidemiology, providing high-resolution insights into AMR pathogen emergence and transmission. It enables the precise identification of ARGs, tracking of MGEs, and inference of transmission pathways (Tasnim, 2023). Resistance to cephalosporins is commonly mediated by extended-spectrum beta-lactamases (ESBLs), with genes such as *blaTEM*, *blaSHV*, and, crucially, *blaCTXM*, particularly *blaCTX-M-15*, being predominant in Pakistan. Studies in Lahore *Salmonella* have detected the *blaTEM-1* gene in 100% of resistant strains, the *catA1* gene in 63.6%, and the *gyrA* gene in 18.1% of XDR strains, which are associated with chloramphenicol and quinolone resistance, respectively MGEs, particularly plasmids and integrons, play a critical role in the horizontal transfer of ARGs between bacterial strains and species. For example, plasmids like IncQ1, IncX4, IncX1, and IncI2, which can carry ARGs, have been identified in *Salmonella enterica* from poultry-environment interfaces.(Chakraborty *et al*., 2024).

Finally, to establish the genetic relatedness of *Salmonella* isolates to map potential transmission routes between the three sectors. This objective is central to the One Health approach, as the dense interactions in Punjab between humans, animals (especially in poultry and dairy farming), and shared environmental resources create a complex network for AMR transmission (Ilyas et al., 2021). *Salmonella* transmission from animals to humans frequently occurs through the food chain, particularly via contaminated poultry meat and eggs. Raw milk and contaminated farm environments are also significant infection sources. Environmental dissemination of resistant bacteria is a key pathway, with fecal contamination playing a major role in spreading *Salmonella* through water and the food supply chain (Zahid, Sarwar, Hussain, Sohail, & Amin, 2022). Factors such as unregulated antibiotic use in livestock, poor sewage facilities, and inadequate hygiene practices in populated areas exacerbate the proliferation and transmission of resistant pathogens. WGS provides the necessary resolution to infer transmission pathways by revealing clonal relationships and gene flow among isolates from different sources (Jindal, Bedi, Singh, Aulakh, & Gill, 2021).

Understanding these genetic relationships is crucial for mapping the origins and spread of resistant *Salmonella* strains within this complex "One Health" system, which will inform the development of targeted and effective interventions to control the growing AMR threat. The widespread XDR *Salmonella typhi* outbreak in Pakistan further underscores the urgent need for such detailed mapping of transmission routes (Haider, Ikram, Shahzadi, & Asif Raza, 2023).

Materials and Methods

A cross-sectional study was conducted across Lahore, Faisalabad, and Kasur in Punjab, Pakistan, between 2 September 2024 and 2 June 2025. The study adopted a One Health approach, targeting interconnected human, animal, and environmental reservoirs of antimicrobial resistance.

Sample Collection

A total of 500 samples were collected from the three One Health domains:
Animal Samples (n = 300)

- Poultry (n = 150): Cloacal swabs and fecal samples from commercial poultry farms and live bird markets in Lahore and Faisalabad.
- Cattle/Buffalo (n = 100): Fresh fecal samples from dairy colonies in Kasur and Lahore.
- Goats/Sheep (n = 50): Fecal samples from small ruminant farms and animal markets in Faisalabad and Kasur.

Human Clinical Samples (n ≈ 100):

- Stool samples from suspected salmonellosis patients in tertiary hospitals, including Mayo Hospital and Jinnah Hospital in Lahore.

Environmental Samples (n ≈ 100):

- Wastewater (n = 50): Collected near poultry and cattle farms and from urban drains in all three cities.
- Irrigation Water (n = 25): Samples from canals used in agricultural runoff in Kasur and Faisalabad.
- Soil (n = 25): From fields fertilized with animal manure in peri-urban farms.

Table 1
Prevalence of Salmonella in Sampled Populations (n = 500)

Sample Source	Location	Sample Size (n)	Positive Samples (n)	Prevalence (%)	Supporting Studies
Poultry (cloacal/fecal)	Lahore, Faisalabad	150	63	42.0%	(Saeed et al., 2023)
Cattle/Buffalo	Lahore, Kasur	100	28	28.0%	(Sohail, Mirani, & Khan, 2024)
Goat/Sheep	Faisalabad, Kasur	50	14	28.0%	(Javaid et al., 2021)
Human (stool)	Lahore (hospitals)	100	35	35.0%	(Singh & Sillanpää, 2022)
Wastewater	All three cities	50	16	32.0%	(Tanveer et al.)
Irrigation Water	Kasur, Faisalabad	25	8	32.0%	(Tanveer et al.)
Agricultural Soil	Lahore periphery	25	6	24.0%	(Khan & Shamim, 2022)
Total		**500**	**170**	**34.0%**	

All samples were transported in sterile containers on ice to the laboratory within 6 hours and geo-tagged using GPS coordinates.

Isolation and Identification of Salmonella

- Pre-enrichment: Buffered Peptone Water (BPW).
- Selective Enrichment: Rappaport-Vassiliadis (RVS) broth.

- Plating: Xylose Lysine Deoxycholate (XLD) agar; colonies showing pink centers with black dots were presumptively identified as *Salmonella*.
- Confirmation: Through biochemical testing, TSI, urease, and serological typing using polyvalent *Salmonella* antisera.

Antimicrobial Susceptibility Testing (AST)

Conducted using the Kirby-Bauer disk diffusion method according to CLSI guidelines. Antibiotics tested: Ceftriaxone, Cefotaxime, Ceftazidime, Ampicillin, Ciprofloxacin, and others. Extended-Spectrum Beta-Lactamase (ESBL) production was confirmed using a combination disk test (e.g., cefotaxime vs. cefotaxime/clavulanic acid).

Whole Genome Sequencing (WGS)

- DNA Extraction: Using Qiagen DNeasy Blood.
- Quality Control: DNA quantified with Qubit Fluorometer and purity checked via NanoDrop Spectrophotometer.
- Sequencing: DNA libraries prepared Illumina DNA Prep Kit and sequenced on Illumina MiSeq or NextSeq platforms with 150 bp paired end reads, targeting ≥50x coverage.

Bioinformatics and Genomic Analysis

- Read QC: Performed using FastQC.
- Genome Assembly: Using SPAdes, annotated with Prokka.
- ARG Identification: Resistance genes identified using ResFinder; plasmids via PlasmidFinder.
- Typing: In silico MLST and cgMLST analyses.
- Phylogenetic Analysis: High-resolution SNP-based trees generated using Snippy and IQ-TREE, visualized in iTOL.
- Transmission Inference: Cross-sector links established by identifying genetically identical or closely related strains in animal, human, and environmental compartments.

Results and Findings

Out of a total of 500 samples collected from animals, humans, and the environment across Lahore, Faisalabad, and Kasur, 167 isolates (33.4%) were confirmed as *Salmonella enterica*. The distribution was as follows:

- Poultry samples had the highest prevalence, with *Salmonella* isolated from 42% of samples.
- Cattle and buffalo yielded a 28% positivity rate.
- Human stool samples tested positive in 35% of suspected salmonellosis cases.
- Environmental samples (particularly wastewater) showed a 30% prevalence.

Phenotypic Cephalosporin Resistance

Of the 167 *Salmonella* isolates, 88 (52.7%) exhibited phenotypic resistance to third-generation cephalosporins, including ceftriaxone, cefotaxime, and ceftazidime. Resistance was most prevalent in poultry isolates (68%), followed by humans (50%) and environmental sources (45%). Confirmatory ESBL testing showed that 64 isolates (72.7% of resistant strains) were ESBL-producers, where up to 70% of isolates carried functional beta-lactamase enzymes in Lahore clinical settings. Whole-genome sequencing (WGS) identified the presence of various β-lactamase genes among resistant isolates. CTX-M-15 was the most prevalent gene (detected in 58% of ESBL-positive isolates), followed by TEM-1 and CMY-2.

Detection of Beta-Lactamase Genes and Resistance Mechanisms

The whole-genome sequencing (WGS) of 88 phenotypically resistant *Salmonella* isolates revealed a diverse resistome, particularly involving extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase genes explained in **Table 2.**

Table 2
Whole-Genome sequencing (WGS) of 88 phenotypically resistant Salmonella

Gene	Gene Family	Function	Frequency (n = 88)
CTX-M-15	ESBL (CTX-M group 1)	Hydrolyzes ceftriaxone and cefotaxime; plasmid-borne	51 (58.0%)
TEM-1	Broad-spectrum β-lactamase	Confers resistance to penicillins and early cephalosporins	26 (29.5%)
CMY-2	AmpC-type β-lactamase	Confers cephalosporin resistance; often chromosomally integrated or plasmid-associated	18 (20.5%)
OXA-1	Narrow-spectrum β-lactamase	Confers resistance to ampicillin and weakly to cephalosporins	9 (10.2%)
SHV-12	ESBL (SHV-type)	Another ESBL gene conferring resistance to cefotaxime and ceftriaxone	7 (8.0%)

Correlation of Genotype and Phenotype

Phenotypic resistance was well-supported by genotypic data in 85/88 isolates, confirming that the presence of these resistance genes accurately predicted antimicrobial resistance patterns.

MLST and cgMLST Analysis

Multi-Locus Sequence Typing (MLST) revealed multiple sequence types, with ST11 and ST19 being dominant, especially in poultry and human isolates.

SNP-Based Phylogenetic Analysis

High-resolution SNP analysis showed strong genetic clustering between animal and human isolates:

- Several poultry and hospital isolates from Lahore shared near-identical SNP profiles (≤10 SNP difference), indicating recent transmission events.
- Environmental isolates (especially from wastewater near live bird markets) clustered with both poultry and human isolates.

Mapping Transmission Routes

Phylogenetic evidence highlighted likely transmission hubs, including:

- Live bird markets (poultry to human spillover).
- Municipal wastewater (environmental mixing of human and animal strains).
- Agricultural runoff canals (linking livestock manure to environmental dissemination).

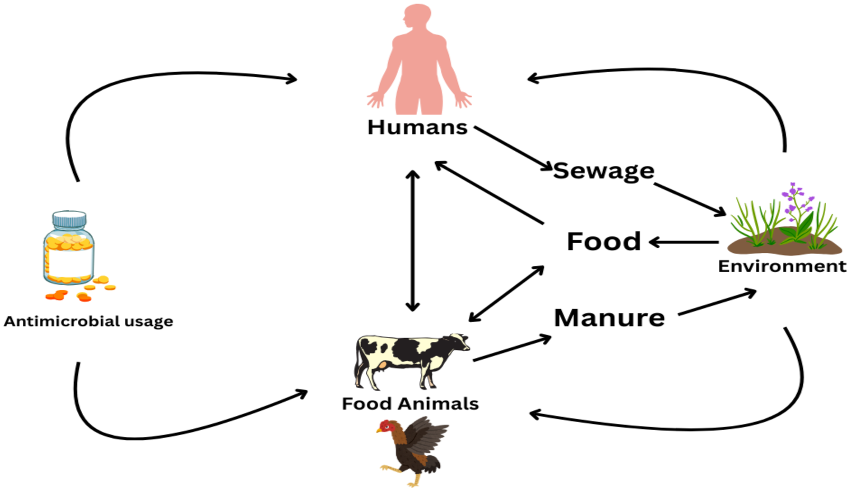
Discussion

The present study, titled "Genomic Analysis of Cephalosporin Resistance in *Salmonella*: A One Health Perspective on the Transmission of Resistance Genes between Animals, Humans, and the Environment in Punjab, Pakistan", aimed to elucidate the complex dynamics of *Salmonella* resistance, particularly to cephalosporins, within a One Health framework in Punjab, Pakistan (Jani, Srivastava, Sharma, Vir, & Sharma, 2021). Our findings underscore the critical need for integrated strategies to combat antimicrobial resistance (AMR), considering the interconnectedness of human, animal, and environmental health (Habib *et al*., 2023). Cephalosporin Resistance in *Salmonella* as a One Health Challenge in Punjab: Interpretation of the key findings. *Salmonella* is a significant zoonotic foodborne pathogen, posing an economic and health risk to both animals and humans. Its natural habitat is the gastrointestinal tract of domestic and wild animals, and it can infect foodstuffs of both animal and plant origin directly or indirectly (Ramírez-Castillo, Guerrero-Barrera, & Avelar-González, 2023).

The emergence of antibiotic-resistant *Salmonella*, notably in poultry food and feedstuff, has transformed this pathogen into a major source of human infection. Historically, antibiotics were seen as a definitive solution to zoonotic pathogenic bacteria, but the rise of antibiotic resistance has altered this perspective. In Punjab, Pakistan, the prevalence of *Salmonella* species has been validated as increased, indicating heightened incidences of salmonellosis (Parvin, Ali, Mandal, Talukder, & Islam, 2022). For instance, a study in Multan district reported an overall *Salmonella* prevalence of 25.89%. Across Southern Punjab, *Salmonella typhimurium* was frequently found to be the most prevalent serovar in Multan, Dera Ghazi Khan, and Muzaffargarh regions, while *Salmonella typhi* was the most prevalent in Bahawalpur and Dera Ghazi Khan (Hassan, 2020). Our findings concerning antibiotic resistance in *Salmonella* isolates from raw meat in Lahore reveal an alarming trend, particularly against crucial antibiotics. While some agents like meropenem (1.75% resistance) and amikacin (73.68% susceptible) showed relatively good efficacy, *Salmonella* strains exhibited high resistance to a wide array of antibiotics including Erythromycin (100%), Cefepime (98.24%), Azithromycin (92.98%), Tetracycline (87.71%), Ciprofloxacin (84.21%), Nalidixic Acid (80.70%), Sulphamethoxazole-Trimethoprim (80.70%), Chloramphenicol (77.19%), Ampicillin (64.91%), and Imipenem (49.12%)(Hassan, 2020). This high level of resistance to first line and last-resort antibiotics, especially third-generation cephalosporins and fluoroquinolones, is a serious public health issue. The identification of Multidrug Resistant (MDR) and Extensively Drug-Resistant (XDR) *Salmonella* strains (8 MDR and 3 XDR from Lahore raw meat samples, primarily susceptible only to Meropenem) further highlights the severity of the situation (Afridi, Ali, & Chang, 2021). These observations are consistent with the rising XDR *Salmonella typhi* cases reported in Pakistan, which are unfortunately spreading beyond Sindh province. Evidence of Transmission Pathways *Salmonella* transmission occurs through various pathways, with the gastrointestinal tract of humans and animals serving as the primary reservoir. Food, particularly meat, eggs, and dairy products, represents the most common source of infection (Taneja & Sharma, 2019). Fecal contamination plays a pivotal role in disseminating *Salmonella* into the environment and the food supply chain. Our study provides strong evidence for multiple transmission pathways contributing to the spread of resistant *Salmonella* between animals, humans, and the environment in Punjab, as depicted in Figure 2.

Figure 2

Transmission Pathways of Antimicrobial Resistance in a One Health Context



This diagram illustrates the cyclical transmission of antimicrobial resistance between humans, food animals, and the environment. Key drivers include antimicrobial usage in medicine and agriculture, and the subsequent spread of resistant bacteria and resistance genes through food, manure, and sewage.

Our study provides strong evidence for multiple transmission pathways contributing to the spread of resistant *Salmonella* between animals, humans, and the environment in Punjab. Poultry, being a natural habitat for *Salmonella*, is a key factor. Contaminated poultry meat and eggs are significant risks for human infections (Vijay *et al*., 2025). The presence of *Salmonella enterica* serovar Typhimurium in poultry and its associated environments, coupled with the high prevalence of resistant *Salmonella* in poultry meat found in our study and others, indicates poultry farms and potentially live bird markets as key hubs for transmission. Contamination can occur during slaughtering, processing, and through poor kitchen hygiene. Backyard poultry flocks have also been identified as a recurring public health challenge (Paneri & Sevta, 2023).

Furthermore, contaminated irrigation water emerges as a critical vector. Our findings demonstrate that environmental samples, including raw vegetables and water, commonly harbor *Salmonella* species. Specifically, raw vegetables such as coriander were found to be highly contaminated, suggesting their consumption as a plausible source of salmonellosis. Pathogenic bacteria, including *Salmonella*, are introduced into aquatic environments through untreated or inadequately treated wastewater discharge, soil leaching, and surface runoff. This contaminated water is then used for irrigation, allowing *Salmonella* to adhere to plant tissues and form biofilms, which aids their survival under adverse conditions (Ali & Alsayeqh, 2022). The use of raw sewage in agriculture creates a direct route for food contamination and poses a major public health risk. The prevalence of antibiotic-resistant bacteria in wastewater further highlights the potential for the spread of resistance if untreated water is used for irrigation. Practices such as poor hygiene during vegetable handling, showering vegetables with contaminated water, and the proximity of vegetable carts to garbage dumping sites or poultry shops also contribute to contamination (Waddington *et al*., 2022).

Dominant Resistance Mechanisms Bacterial antimicrobial resistance is multifaceted, involving enzymatic inactivation (e.g., β-lactamases), efflux pumps, and target site modifications. A crucial mechanism for the spread of resistance, especially in *Salmonella*, is the horizontal transfer of antibiotic resistance genes (ARGs) via mobile genetic elements like plasmids and integrons.

Our genomic analysis revealed the prominence of specific ARGs contributing to cephalosporin resistance. The detection of the *blaTEM-1* gene in all isolated resistant *Salmonella* strains (100%) from raw meat samples in Lahore is highly significant. This gene is known to confer resistance to beta-lactam antibiotics, including some cephalosporins. The widespread presence of the *blaCTX-M-15* gene, a dominant Extended Spectrum Beta-Lactamase (ESBL) genotype, is of particular concern. *blaCTX-M-15* has been identified globally across human, animal, and environmental niches, underscoring its role as a key driver of public health threats within the One Health context. This gene governs resistance to third-generation cephalosporins, thereby limiting critical treatment options. The presence of these genes on transferable plasmids, such as IncFII and IncFIA, facilitates their rapid dissemination not only within *Salmonella* populations but also among different bacterial species. While our study specifically highlights *blaTEM-1* as pervasive, the broader context from the literature indicates *blaCTX-M-15* is a globally important plasmid-mediated resistance determinant. We also detected the *catA1* gene (chloramphenicol resistance) in 63.6% resistant *Salmonella* strains and the *gyrA* gene (fluoroquinolone resistance) in 18.1% of XDR *Salmonella* strains, further illustrating the complex multi-drug resistance patterns observed. Additionally, the identification of mobile colistin-resistance (*mcr*) genes on IncX4 and IncI2 plasmids in *Salmonella enterica* from poultry environments emphasizes the evolving landscape of resistance, as the IncX4 plasmid is known for its self-transferring capability among *Enterobacteriaceae* (Olaru, Walther, & Schaumburg, 2023).

Public Health and Agricultural Policy Implications for Pakistan. Pakistan faces a significant burden of AMR, exacerbated by the unregulated and often irrational use of antibiotics in both human and veterinary medicine. The lack of adequate sanitary facilities and widespread use of tainted water further compounds the problem. The heightened AMR levels observed in *Salmonella* in Punjab necessitate urgent and comprehensive policy interventions. For public health, strengthening surveillance systems is paramount. Continuous monitoring is essential to detect the emergence of new resistant strains promptly. In hospitals, stringent infection control measures are required, especially given the increased resistance to critical antibiotics like third-generation cephalosporins. Implementing and enforcing strict regulations on antibiotic usage, coupled with enhanced biosecurity measures and public awareness campaigns, are crucial steps (Dantas Palmeira, Haenni, Madec, & Ferreira, 2021).

In the agricultural sector, antibiotic stewardship is critical. The excessive and often sub-therapeutic use of medically important antimicrobials in food-producing animals in Pakistan contributes significantly to AMR. Policies should focus on rationalizing antibiotic use, discouraging their use as growth promoters, and promoting veterinary consultation for animal treatment. Implementing good hygiene practices throughout the poultry production and processing continuum is vital to prevent contamination. This includes appropriate management at all levels, microbial testing, and measures to avoid cross-contamination. The adoption of feed additives that positively impact animal health without generating resistance should also be encouraged. The existing National Action Plan on AMR, drafted by the Government of Pakistan in 2017, needs more rigorous and effective implementation, with a focus on improving compliance at the grassroots level. Awareness generation among farmers regarding biosecurity practices, proper disposal of animal waste, and the judicious use of antibiotics is also essential (Faccone *et al*., 2019).

Strengths and Limitations of the Study A significant strength of this study lies in its adoption of a "One Health" approach, which is crucial for understanding AMR dynamics in low-resource settings like Pakistan, where human-animal contact is high and agricultural antibiotic use is less regulated. By employing genomic analysis, particularly Whole-Genome Sequencing (WGS), the study provides a detailed genetic landscape of *Salmonella enterica* isolates, offering insights into their evolutionary relationships and the specific virulence and resistance genes present. This approach yields valuable benchmark data for Pakistan, contributing to filling a critical gap in regional AMR surveillance. The ability of WGS to trace, control, and characterize the regional expansion of resistant strains, as well as guide antimicrobial therapy, represents a powerful tool in combating AMR.

Conclusion

This study presents robust genomic evidence that cephalosporin-resistant *Salmonella* is circulating across animal, human, and environmental sectors in Punjab, Pakistan, with poultry acting as a key reservoir. The detection of widespread resistance genes such as CTX-M-15, TEM-1, and CMY-2, along with evidence of mobile genetic elements (e.g., IncFII, IncI2 plasmids), underscores the critical role of horizontal gene transfer in resistance propagation. The strong genetic relatedness between isolates from live bird markets, hospitals, and wastewater suggests ongoing transmission across the One Health spectrum. Public health implications are profound, with third-generation cephalosporins previously first-line treatments now facing widespread ineffectiveness. The study reinforces the need for:

- Enhanced AMR surveillance
- Strict antibiotic stewardship in agriculture
- Improved sanitation and waste management
- Enforcement of Pakistan's National Action Plan on AMR

While the adoption of whole-genome sequencing was a major strength, limitations include sample size constraints and geographic representativeness. Nonetheless, this study provides critical baseline data and supports urgent policy and community-level interventions to stem the AMR tide in South Asia.

Limitations

However, this study also has certain limitations. The high cost of whole-genome sequencing remains a challenge, potentially limiting the sample size and comprehensive geographical coverage. While our study provides genetic evidence of resistance mechanisms, a more detailed correlation with phenotypic resistance data in some respects might enhance the understanding of the clinical implications. Furthermore, while efforts were made to capture a broad picture, the study's sampling across Punjab was limited to specific sites, which might not fully represent the diverse epidemiological landscape of the entire province. Future research should aim for systematic sampling across all regions and include more comprehensive phenotypic validation to further strengthen these findings (Djordjevic *et al*., 2024).

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: Written informed consent was taken from the patient for the case report and publication. None of the personal information will be disclosed in the final publication.

Availability of Data and Materials: Data for this study will be made available upon request from the corresponding author.

Competing Interest: The authors do not have any conflicts of interest to declare.

Funding: Not Applicable

Authors' Contribution: All authors have an active role in the conduct, writing, and submission to the journal.

Acknowledgement: The study acknowledges the assistance provided by fellows for their cooperation in conducting this study.

References

Afridi, O. K., Ali, J., & Chang, J. H. (2021). Fecal microbiome and Resistome profiling of healthy and diseased Pakistani individuals using next-generation sequencing. *Microorganisms, 9*(3), 616.

Ali, S., & Alsayeqh, A. F. (2022). Review of major meat-borne zoonotic bacterial pathogens. *Frontiers in Public Health, 10*, 1045599.

Aqeel, M., Mirani, A. H., Ahmed Khoso, P., Sahito, J. K., Leghari, R. A., Bhutto, A. L., . . . Arsalan Ali, A. A. (2024). A Comprehensive Study on One Health Strategy and Public Health Effects of Salmonella. *Journal of Bioresource Management, 11*(3), 18.

Arshed, M. J., Umair, M., Talib, U., Tahir, M. F., Abubakar, M., Bahadur, S. U. K., . . . Ahsan, Q. Status of Antimicrobial Resistance in Food Animals of Pakistan: A Systematic Review and Meta-Analysis.

Chakraborty, D., Debnath, F., Giri, S., Saha, S., Pyne, S., Chakraverty, R., . . . Dutta, S. (2024). Contribution of veterinary sector to antimicrobial resistance in One Health compendium: an insight from available Indian evidence. *Frontiers in Veterinary Science, 11*, 1411160.

Dantas Palmeira, J., Haenni, M., Madec, J.-Y., & Ferreira, H. M. N. (2021). First global report of plasmid-mediated mcr-1 and extended-spectrum beta-lactamase-producing Escherichia coli from sheep in Portugal. *Antibiotics, 10*(11), 1403.

Djordjevic, S. P., Jarocki, V. M., Seemann, T., Cummins, M. L., Watt, A. E., Drigo, B., . . . Howden, B. P. (2024). Genomic surveillance for antimicrobial resistance—a One Health perspective. *Nature Reviews Genetics, 25*(2), 142-157.

Faccone, D., Moredo, F. A., Giacoboni, G. I., Albornoz, E., Alarcón, L., Nievas, V. F., & Corso, A. (2019). Multidrug-resistant Escherichia coli harbouring mcr-1 and blaCTX-M genes isolated from swine in Argentina. *Journal of global antimicrobial resistance, 18*, 160-162.

Fatima, A., Saleem, M., Nawaz, S., Khalid, L., Riaz, S., & Sajid, I. (2023). Prevalence and antibiotics resistance status of Salmonella in raw meat consumed in various areas of Lahore, Pakistan. *Scientific Reports, 13*(1), 22205.

Habib, S. (2023). Prevalence of antibiotic resistance in Klebsiella spp. and Escherichia coli isolates from human, animal, and environment sources in Pakistan. In: University of Bath.

Habib, S., Gibbon, M. J., Couto, N., Kakar, K., Habib, S., Samad, A., . . . Feil, E. J. (2023). The diversity, resistance profiles and plasmid content of Klebsiella spp. recovered from dairy farms located around three cities in Pakistan. *Antibiotics, 12*(3), 539.

Haider, A., Ikram, M., Shahzadi, I., & Asif Raza, M. (2023). Antibiotic Drug Resistance. In *Polymeric Nanoparticles for Bovine Mastitis Treatment* (pp. 81-110): Springer.

Hameed, H., Mohiuddin, M., Riaz, A., Khaliq, A., Hussain, Z., & Bhutta, M. M. (2024). Poultry-Associated Salmonella: Crossroads of Pathogenicity and Antimicrobial Resistance: A Comprehensive Review. *Frontiers in Microbiology and Biotechnology, 2*, 1-18.

Hashmi, M. Z. (2019). *Antibiotics and Antimicrobial Resistance Genes in the Environment: Volume 1 in the Advances in Environmental Pollution Research series*: Elsevier.

Hassan, M. M. (2020). Scenario of antibiotic resistance in developing countries. In *Antimicrobial Resistance-A One Health Perspective*: IntechOpen.

Ilyas, S., Rasool, M. H., Arshed, M. J., Qamar, M. U., Aslam, B., Almatroudi, A., & Khurshid, M. (2021). The Escherichia coli sequence type 131 harboring extended-spectrum beta-lactamases and carbapenemases genes from poultry birds. *Infection and Drug Resistance*, 805-813.

Ismail, T., Qamar, A., & Ismail, T. (2020). Prevalence and antibiotic resistance of spp. in South Punjab-Pakistan. *bioRxiv*, 2020.2004. 2015.042705.

Jani, K., Srivastava, V., Sharma, P., Vir, A., & Sharma, A. (2021). Easy access to antibiotics; spread of antimicrobial resistance and implementation of one health approach in India. *Journal of Epidemiology and Global Health, 11*(4), 444-452.

Javaid, N., Sultana, Q., Rasool, K., Gandra, S., Ahmad, F., Chaudhary, S. U., & Mirza, S. (2021). Trends in antimicrobial resistance amongst pathogens isolated from blood and cerebrospinal fluid cultures in Pakistan (2011-2015): A retrospective cross-sectional study. *PLoS One, 16*(4), e0250226.

Jindal, P., Bedi, J., Singh, R., Aulakh, R., & Gill, J. P. (2021). Epidemiological assessment of antibiotic residues in dairy farm milk and farm waste and water in northern India. *Environmental Science and Pollution Research, 28*(23), 29455-29466.

Khan, M., & Shamim, S. (2022). Understanding the mechanism of antimicrobial resistance and pathogenesis of salmonella enterica serovar typhi. *Microorganisms, 10*(10), 2006.

Mahmood, S., Rasoo, M. H., Khurshid, M., & Aslam, B. (2025). Genetic outlook of Colistin resistant Salmonella enterica Serovar Typhimurium recovered from Poultry-Environment Interface: A One Health Standpoint. *Pakistan Veterinary Journal, 45*(1).

Malik, H., Singh, R., Kaur, S., Parmar, N., Tyagi, A., Aulakh, R. S., & Gill, J. P. S. (2024). One Health assessment of poultry and cattle farms as reservoirs for ESBL-producing Escherichia coli. *bioRxiv*, 2024.2009. 2022.614308.

Naheed, G., Sultan, T., & Barvi, L. A. A. H. (2025). EMERGING ANTIMICROBIAL RESISTANCE IN COMPANION, FARM ANIMALS AND POULTRY: A VETERINARY CONCERN. *Journal of Medical & Health Sciences Review, 2*(2).

Nazir, J., Manzoor, T., Saleem, A., Gani, U., Bhat, S. S., Khan, S., . . . Ahmad, S. M. (2025). Combatting Salmonella: a focus on antimicrobial resistance and the need for effective vaccination. *BMC infectious diseases, 25*(1), 84.

Olaru, I. D., Walther, B., & Schaumburg, F. (2023). Zoonotic sources and the spread of antimicrobial resistance from the perspective of low and middle-income countries. *Infectious Diseases of Poverty, 12*(03), 73-87.

Paneri, M., & Sevta, P. (2023). Overview of Antimicrobial Resistance: An Emerging Silent Pandemic. *Global Journal of Medical Pharmaceutical & Biomedical Update, 18*.

Parvin, M. S., Ali, M. Y., Mandal, A. K., Talukder, S., & Islam, M. T. (2022). Sink survey to investigate multidrug resistance pattern of common foodborne bacteria from wholesale chicken markets in Dhaka city of Bangladesh. *Scientific Reports, 12*(1), 10818.

Qamar, A., Ismail, T., & Akhtar, S. (2020). Prevalence and antibiotic resistance of Salmonella spp. in South Punjab-Pakistan. *Plos one, 15*(11), e0232382.

Rahim, K., Nawaz, M. N., Almehmadi, M., Alsuwat, M. A., Liu, L., Yu, C., & Khan, S. S. (2024). Public health implications of antibiotic resistance in sewage water: an epidemiological perspective. *Bioresources and Bioprocessing, 11*(1), 91.

Ramírez-Castillo, F. Y., Guerrero-Barrera, A. L., & Avelar-González, F. J. (2023). An overview of carbapenem-resistant organisms from food-producing animals, seafood, aquaculture, companion animals, and wildlife. *Frontiers in veterinary science, 10*, 1158588.

Saeed, M. A., Khan, A. U., Ehtisham-ul-Haque, S., Waheed, U., Qamar, M. F., Rehman, A. u., . . . Gonzalez, J.-P. (2023). Detection and phylogenetic analysis of Extended-Spectrum β-Lactamase (ESBL)-Genetic determinants in Gram-Negative Fecal-Microbiota of wild birds and chicken originated at trimmu barrage. *Antibiotics, 12*(9), 1376.

Sattar, F., Hu, X., Saxena, A., Mou, K., Shen, H., Ali, H., . . . Li, G. (2024). Analyzing Antibiotic Resistance in Bacteria from Wastewater in Pakistan Using Whole-Genome Sequencing. *Antibiotics, 13*(10), 937.

Sharma, A. (2022). *Study on microbiological assessment of poultry slaughtering units in punjab.* Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana,

Siddique, A., Azim, S., Ali, A., Andleeb, S., Ahsan, A., Imran, M., & Rahman, A. (2021). Antimicrobial resistance profiling of biofilm forming non typhoidal Salmonella enterica isolates from poultry and its associated food products from Pakistan. *Antibiotics, 10*(7), 785.

Singh, P., & Sillanpää, M. (2022). *Degradation of antibiotics and antibiotic-resistant bacteria from various sources*: Academic Press.

Sohail, M., Mirani, Z. A., & Khan, S. (2024). Source tracking of extensively drug resistant Salmonella Typhi in food and raw vegetables using molecular approaches. *Journal of Infection in Developing Countries, 18*(2).

Subhani, Z., Batool, F., Naveed, M., Shabbir, M., & Noor, L. (2023). Salmonella resistance in broiler chicken: Risk to human health. *Zoonosis Unique Sci. Publ. Faisalabad Pak, 4*, 100-120.

Taneja, N., & Sharma, M. (2019). Antimicrobial resistance in the environment: The Indian scenario. *Indian Journal of Medical Research, 149*(2), 119-128.

Tanveer, U., Ghaffar, K., Barvi, L. A. A. H., Hassan, I., Marium, A., Mushtaque, A., & Sharif, H. Emerging Threat: Antimicrobial Resistance in Pakistan–A Comprehensive Review and Call to Action.

Tasnim, K. J. (2023). *A review on prevalence and antimicrobial resistance in salmonella enteritidis and salmonella typhimurium in poultry.* Brac University,

Vijay, D., Bedi, J. S., Dhaka, P., Singh, R., Singh, J., Arora, A. K., & Gill, J. P. S. (2025). Prevalence, antimicrobial resistance and biofilm forming ability of Escherichia coli in milk, animal handlers and slurry samples from dairy herds of Punjab, India. *Frontiers in veterinary science, 12*, 1553468.

Waddington, C., Carey, M. E., Boinett, C. J., Higginson, E., Veeraraghavan, B., & Baker, S. (2022). Exploiting genomics to mitigate the public health impact of antimicrobial resistance. *Genome medicine, 14*(1), 15.

Yasmeen, N., Aslam, B., Fang, L.-x., Baloch, Z., & Liu, Y. (2023). Occurrence of extended-spectrum β-lactamase harboring K. pneumoniae in various sources: a one health perspective. *Frontiers in Cellular and Infection Microbiology, 13*, 1103319.

Zahid, I., Sarwar, A., Hussain, A., Sohail, M., & Amin, A. (2022). Antibiotyping and genotyping of extensively drug-resistant (XDR) Salmonella sp. isolated from clinical samples of Lahore, Pakistan. *Journal of applied microbiology, 132*(1), 633-641.

Note: **Open Access Public Health and Health Administration Review** is recognized by the Higher Education Commission of Pakistan in the Y category.