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Original Article

An Empirical Study on the Prevalence and Multidrug Resistance Profiles of Salmonella and Campylobacter Species: A One Health Context

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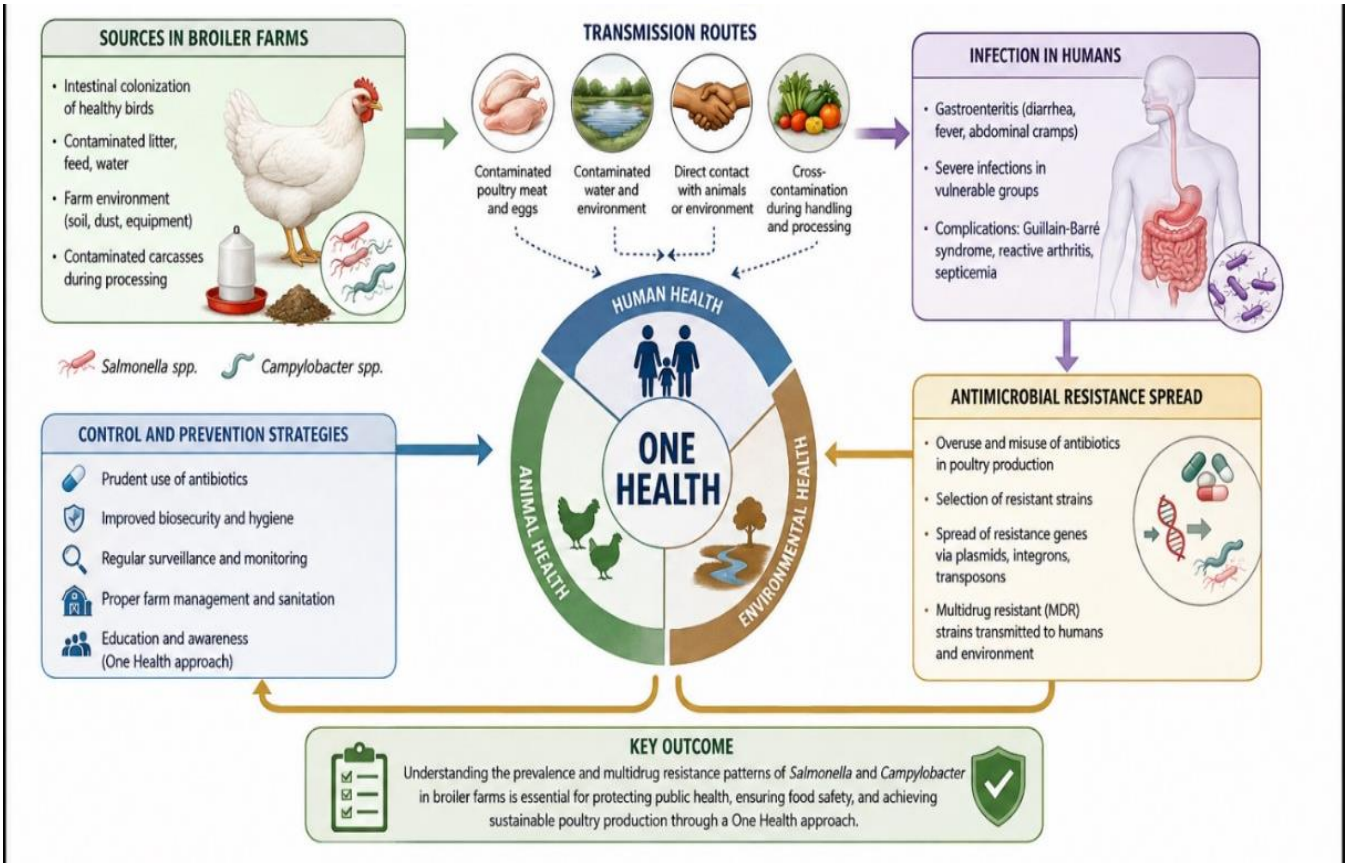
ABSTRACT

Salmonella and Campylobacter are foodborne pathogens that pose public health risks, particularly in commercial poultry production, where antimicrobial resistance may compromise the treatment of infected birds and undermine food safety. The present cross-sectional study aimed to determine the prevalence, antimicrobial resistance profile, and multidrug resistance (MDR) pattern of Salmonella and Campylobacter in commercial broiler farms of Punjab, Pakistan. Samples (ecal contents, cloacal swabs, bootsocks, and fecal grabs) were taken from 70 farms, with a total of 350 samples collected and processed by microbiological and molecular techniques. The overall Salmonella prevalence was 22.0% (77 samples were positive), and the overall Campylobacter prevalence was 34.0% (119 samples were positive). Campylobacter prevalence was also found to be highest in bootsocks (48.6%), whereas the prevalence of Salmonella was highest in bootsocks (31.4%). The highest prevalence of both pathogens was found on small farms. The highest resistance level was seen for Salmonella isolates against tetracycline (80.5%) and nalidixic acid (71.4%), and 61.0% were considered MDR. The antimicrobial resistance rate for Campylobacter was high for tetracycline (89.1%), ciprofloxacin (81.5%), and nalidixic acid (76.5%), and the MDR rate was 93.3%. Pathogen prevalence was significantly associated with farm size, antimicrobial use, and biosecurity. These findings indicate a threat from One Health and underscore the importance of better biosecurity, responsible use of antimicrobials, surveillance, and targeted interventions for commercial broiler production.

Keywords: Salmonella, Campylobacter, Antimicrobial Resistance, Multidrug Resistance (MDR), Commercial Broiler Farms.

GRAPHICAL ABSTRACT



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INTRODUCTION

Foodborne diseases are a major public health problem all over the world and a major cause of morbidity and mortality in both developed and underdeveloped countries. Salmonella and Campylobacter are the most important causal agents of bacterial gastroenteritis worldwide. Non-typhoidal Salmonella is estimated to infect an estimated 93.8 million people and result in 155,000-230,000 deaths annually worldwide. Likewise, Campylobacter is estimated to cause 21,000 deaths and 96 million cases of gastroenteritis annually (Aljasir *et al*., 2025). The sources of these pathogens in poultry and poultry products and the routes for transmission to humans have been well documented. Salmonella and Campylobacter are common inhabitant pathogens of chickens and can multiply to high levels in the chicken intestine without harming the bird, leading to widespread contamination of the farm environment and the carcasses during processing (Poudel *et al*., 2022). Poultry production is considered a key contributor to food security and economic development in developing countries, not only for supplying a low-cost, readily available source of high-quality animal protein, but also for generating income for farmers and traders. As a result, the issue of antibiotic resistance among pathogens within this industry is a significant concern for human health and industry sustainability (Chiu *et al*., 2010).

Significance of Pathogens

Of the thousands of Salmonella serovars, those most important public health-wise are the nontyphoidal variants that include S. Enteritidis and S. Typhimurium, which are widely distributed among many species of animals. These serovars are often associated with large foodborne outbreaks that occurred when people ate food contaminated with meat and eggs (Shakir *et al*., 2021). Campylobacter spp. is a genus of Gram-negative organisms; the most commonly found in humans are C. jejuni and C. coli, which are responsible for more than 90-95% of campylobacteriosis cases. In humans, clinical symptoms of these infections vary from mild gastroenteritis with fever, abdominal cramps, and diarrhea (often bloody) to more serious infections. Infants, the elderly, and those with compromised immune systems are especially susceptible to Salmonella causing life-threatening bacteremia and systemic infections. In addition, there are serious sequelae to Campylobacter infections, such as Guillain-Barré syndrome (a serious autoimmune disorder that causes paralysis), reactive arthritis, and septicemia (Kim *et al*., 2012).

Antimicrobial Resistance Crisis

Antimicrobial resistance (AMR) is a worldwide challenge that affects the effectiveness of treatments that save lives. Resistance arises through several mechanisms such as spontaneous chromosomal mutations (e.g., in the gyrA gene that is responsible for fluoroquinolone resistance) and through the acquisition of resistance determinants on plasmids, integrons and transposons (Popa *et al*., 2025). Furthermore, bacteria can actively pump a number of different classes of antibiotics out of the cell, creating multidrug resistance (MDR).

One Health Perspective

Human, animal, and environmental health are intertwined, and due to the AMR crisis, a "One Health" approach is needed to ensure proper surveillance and control. Pathogens and resistance genes are transmitted over complex pathways from farm to fork. When poultry litter or manure is used as fertilizer, resistant bacteria can be spread to the soil and water and may contaminate vegetables and natural waterways (Bort *et al*., 2022). These implications for public health and the economy are staggering. In addition to the medical care and hospitalization expenses, AMR results in reduced productivity, loss of income for farmers due to flock mortality, and may also cause trade restrictions for poultry products. Solutions to these challenges must come from a multi-sectoral approach, with a coordinated effort for veterinary, medical, and environmental sectors, in terms of stewardship and interventions (Ahmed *et al*., 2019).

Knowledge Gap

There is a lack of detailed and up-to-date information about the presence and resistance patterns of Salmonella and Campylobacter in many areas in commercial broiler farms. There are many countries that do not have a coordinated surveillance system, such as Nigeria, Bangladesh, and Kenya, and many studies are limited in geographical scope and/or number of isolates. In particular, data about intensively produced, large-scale, commercial broilers, which constitute a large

proportion of the food supply, are often much less available than that of retail meat or small-scale backyard production (Han *et al*., 2016).

Study Objectives

1. To establish the occurrence of Salmonella and Campylobacter species in commercial broiler farms.
2. To determine the antimicrobial resistance pattern of the recovered pathogens
3. To evaluate the level and pattern of multiple drug resistance
4. To determine the factors linked to the prevalence and resistance of pathogens

LITERATURE REVIEW

Epidemiology of Salmonella and Campylobacter in Broiler Production

Global Prevalence Data

The incidence of Salmonella and Campylobacter within poultry production systems also depends on the geographical location and the differences in management, biosecurity, and environment. In the Middle East, representative Salmonella serovars were found in the poultry environment, and the reported prevalence of 9.3% for Salmonella and 35.8% for Campylobacter in the poultry environment in Saudi Arabia were used as representative benchmarks for the region. These pathogens are found at high prevalence worldwide, and in Pakistan, it has been reported at high prevalence in retail meat in big cities, for example in Lahore (Agada *et al*., 2014). Epidemiological factors vary with farm size, with higher prevalence rates seen on small-scale and backyard farms, where birds may be more likely to be exposed to environmental contaminants due to scavenging and be less likely to adopt rigorous biosecurity measures. On the other hand, a high pathogen count can be present in intensively managed commercial broiler houses in spite of good infrastructure because of cyclic contamination; this can take place through shared resources such as transport crates and people (Caffrey *et al*., 2021).

Regional Studies and Context

The levels of contamination are found to be high in South Asian and Middle Eastern countries, in comparison to the other parts of the world, through regional studies. Salmonella prevalence has been reported up to 82 % in cloacal swabs from broiler farms in Bangladesh, and Campylobacter was isolated from 24 % of various poultry sources in Iraq. These are sometimes higher than the figures recorded in Europe and North America for the same time period on the same unit of measurement (Talukder *et al*., 2021). For instance, the European Union (EU) summary reports, the prevalence of Campylobacter in flocks has been reported at around 71.2%, and this is lower than the 82-98% prevalence reported in market-ready broilers in parts of Kenya in recent years. Likewise, the US experience with longitudinal data demonstrated a progressive decline in Campylobacter in raw poultry meat down to 34% in 2011, but the longitudinal data for developing poultry sectors remained high.

Antimicrobial Resistance Patterns

Salmonella Resistance Profiles

The distribution of resistance among Salmonella isolates from commercial poultry is often high for traditional first-line antimicrobials, with resistance to ampicillin, tetracycline, and nalidixic acid typically around 70–90%. In particular, all isolates from some Nigerian studies were found to be resistant to ampicillin and cephalosporins. Emerging resistance has also been observed to critical antibiotics such as third-generation cephalosporins (e.g., cefotaxime) and colistin, with resistance rates of almost 90% in some isolates from Bangladesh (Hasan *et al*., 2025). The resistance trend is also highlighted in Pakistan, where zoonotic serovars isolated from broilers showed resistance to ampicillin (78.4%) and ciprofloxacin (39.0%) (Munim *et al*., 2024).

Campylobacter Resistance Profiles

Campylobacter species are extensively resistant to fluoroquinolones (ciprofloxacin) and macrolides (erythromycin, azithromycin), which are the main antibiotics used to treat human infections. The high tetracycline resistances are world-wide characteristics and often ranges between 63.8% and 92% and even to 100% in intensive production systems in Kenya. More

worryingly, multidrug resistance (MDR) is becoming commonplace and is no longer uncommon (that is, the proportion of isolates showing MDR has increased; 97% to 100% was observed in studies in Ghana and India) (Zhang *et al*., 2020).

Multidrug Resistance

MDROs are typically those that are resistant to at least three categories of antimicrobials. There are other classifications: Extensively Drug-Resistant (XDR) is resistance to two or fewer classes, and Pan-Drug Resistant (PDR) is resistance to all tested classes. The MDR trends that were observed over the last 10 years indicate a disturbing trend, possibly brought about by the horizontal transfer of resistance determinants and by spontaneous mutations in the presence of high selection pressure in poultry farms (Khan *et al*., 2018).

Antimicrobial Use in Poultry Production

Typical antimicrobials used in broiler production include tetracyclines, penicillins, fluoroquinolones (e.g., enrofloxacin), macrolides (e.g., tylosin), and the sulfonamides. They are used for therapeutic treatment of clinical diseases, for prophylaxis (prevention), and as growth promoters as sub-therapeutic level supplements to the feed. In certain areas, the use of fluoroquinolones such as enrofloxacin has been among the highest of all groups, with resulting selection of resistant strains (Kariuki *et al*., 2020). Antimicrobial usage in commercial broiler production in South Asia is poorly controlled. In Bangladesh, up to 92% of farmers use antimicrobials, often for growth promotion and prophylaxis purposes without veterinary guidance. Based on estimates, Asia is estimated to use the highest volume of non-therapeutic antibiotics worldwide, and this is expected to continue rising until 2030. The enforcement of the regulatory framework is difficult, and drugs are given according to the recommendations of feed dealers or company representatives, not according to qualified veterinarians (Gahamanyi *et al*., 2021).

Genetic Basis of Resistance

Resistance to tetracyclines is mostly due to the presence of tetA, tetB and tetO genes that encode efflux pumps or ribosomal protection proteins. Genes such as blaTEM, blaSHV, blaCTX-M, and blaCMY-2 contribute to resistance to β-Lactams including the third-generation cephalosporins. Resistance to fluoroquinolones is most frequently due to point mutations in the gyrA and gyrB genes (e.g., C257T missense mutation) that result in a change in the DNA gyrase target. Campylobacter has multidrug efflux systems such as cmeB and cmeG, which are responsible for the efflux of multiple unrelated antibiotics and are responsible for complex multidrug resistance phenotypes (Vinueza-Burgos *et al*., 2017).

Molecular tests for resistance genes are PCR-based screening and DNA microarrays. The use of the applications of whole genome sequencing (WGS) in surveillance programs is growing and is increasingly used to provide high-resolution data on transmission of resistant clones and to detect new resistance markers that could be missed by other surveillance tools (Shang *et al*., 2023).

Public Health Implications

Human Health Burden

Infection with food-borne pathogens imposes a huge burden on human health. Non-typhoidal Salmonella causes an estimated 93.8 million cases of gastroenteritis and more than 150,000 deaths every year around the world. Campylobacter is also significant, as its sequelae can be serious, such as Guillain-Barré syndrome and reactive arthritis. MDR infections are also problematic, with more costly, less available, and probably more toxicity alternative treatments, resulting in increased morbidity and mortality (Kwon *et al*., 2021).

Economic Impact

Economically, the cost of health services for treating foodborne illnesses is high, and people's productivity levels are also greatly affected by human illness. In the poultry industry, losses due to MDR pathogens are twofold – direct losses include flock mortality, and indirect losses include potential import bans from countries with high levels of AMR by countries with stringent safety requirements.

METHODS AND MATERIALS

Study Design and Setting

A cross-sectional study was conducted to determine the prevalence and antimicrobial resistance profiles of Salmonella and Campylobacter species in commercial broiler farms. The study was conducted in Punjab, Pakistan, the country's largest poultry-producing province. Farms were selected from major poultry districts. The region represents approximately 60% of Pakistan's commercial broiler production, including:

- Central Punjab: Lahore, Sheikhupura, Kasur
- Northern Punjab: Rawalpindi, Gujranwala
- Southern Punjab: Multan, Bahawalpur

Study Duration and Sampling Strategy

The study was conducted over 12 months (January - December 2025) to account for seasonal variations in pathogen prevalence. The farm selection was done using:

Inclusion Criteria:

- Active commercial broiler farms in Punjab
- Birds aged 25-35 days (pre-slaughter)
- Documented history of antimicrobial usage
- Willingness to participate

Exclusion Criteria:

- Backyard or non-commercial farms
- Farms with disease outbreaks in the previous 2 weeks
- Birds undergoing antimicrobial treatment at sampling
- Unwilling farms

Sample Size Determination

Total Sample Size: 350 samples from 70 farms (5 samples per farm)
Sample Size Calculation (for each pathogen):
$n = (Z^2 \times p \times (1-p)) / d^2$
Where Z=1.96 (95% CI), p=expected prevalence (35% for Campylobacter), d=5% precision
Calculated:

- Campylobacter: 350 samples (using p=35%)
- Salmonella: 288 samples (using p=25%)
- Final: 350 samples (sufficient for both)

Table 1
Stratification by Farm Size: Farms were stratified into three categories

Category	Flock Size	Number of Farms	Samples per Farm
Small	1,000 - 5,000 birds	23 farms	5
Medium	5,001 - 20,000 birds	24 farms	5
Large	>20,000 birds	23 farms	5
Total		70 farms	350 samples

Farms were randomly selected from each district using stratified random sampling.

Sample Collection Methods

Table 2

Sample Types (5 per farm)

Sample Type	Number	Collection Method	Purpose
Cecal contents	5 birds (pooled)	Collected during slaughter; entire caecum with contents placed in a sterile bag	Detection in intestinal tract
Cloacal swabs	5 birds (pooled)	Sterile swab inserted 2-3 cm into cloaca; placed in Amies transport medium	Detection of shedding
Bootsocks	1 per house	Walked through entire poultry house; placed in sterile Whirl-Pak bag	Environmental contamination
Fecal grabs	10 deposits (pooled)	Fresh deposits collected with sterile spoon; pooled into one container	Detection in environment

Note: For each farm, samples were pooled from 5 birds (cecal + cloacal), one bootsock, and one pooled fecal sample, totaling 5 sample types per farm × 70 farms = 350 samples.

Sample Transport

All samples were transported to the laboratory in insulated coolers with ice packs (2-8°C) and processed within 4 hours of collection.

Laboratory Methods

Isolation of Salmonella spp

Table 3

Procedure following ISO 6579

Step	Method	Medium/Conditions	Duration
Pre-enrichment	25g sample + 225mL Buffered Peptone Water (BPW)	35°C ± 1°C	18-24 hours
Selective Enrichment	0.1mL BPW culture → 10mL RV broth	41.5°C ± 1°C	24 hours
	1mL BPW culture → 10mL TT broth	35°C ± 1°C	24 hours
Plating	Streak onto XLD, BGS, HE agar	35°C ± 1°C	24-48 hours
Biochemical Confirmation	TSI, LIA, urease, citrate, motility, oxidase	Standard methods	-
Serotyping	O and H antisera (Kauffmann-White scheme)	Commercial antisera	-
Molecular Confirmation	PCR targeting invA gene (284 bp amplicon)	35 cycles at 58°C	-

Presumptive Salmonella Colonies

- XLD: Red with black centers (H_2S positive)
- BGS: Pink-red colonies on green background
- HE: Blue-green with black centers

Isolation of Campylobacter spp

Table 4

Procedure following ISO 10272-1

Step	Method	Medium/Conditions	Duration
Enrichment	25g sample + 225mL Bolton broth + supplements	Microaerophilic (5% O_2, 10% CO_2, 85% N_2); 37°C for 4h, then 41.5°C	44 ± 4 hours
Plating	Streak onto mCCD agar and Preston agar	Microaerophilic; 41.5°C	44 ± 4 hours
Biochemical Confirmation	Oxidase (positive), Catalase (positive), Hippurate hydrolysis	C. jejuni: hippurate +; C. coli: hippurate -	-
Molecular Confirmation	Multiplex PCR: mapA (C. jejuni, 589 bp), ceuE (C. coli, 462 bp)	35 cycles at 56°C	-

Presumptive Campylobacter Colonies

- mCCD agar: Gray, flat, moist, spreading with metallic sheen
- Preston agar: Gray, flat, moist with irregular edges

Antimicrobial Susceptibility Testing

Testing Method

Kirby-Bauer disk diffusion method on Mueller-Hinton agar following CLSI guidelines.
Quality Control Strains:

- E. coli ATCC 25922
- S. aureus ATCC 25923
- P. aeruginosa ATCC 27853
- C. jejuni ATCC 33560

Antimicrobial Panels

Table 5

Salmonella (10 antimicrobials)

Antimicrobial Class	Agent	Concentration (µg)	CLSI Breakpoints (Zone Diameter, mm)
Aminoglycosides	Gentamicin	10	S≥15, I=13-14, R≤12
β-Lactam/Inhibitor	Amoxicillin-Clavulanic Acid	20/10	S≥18, I=14-17, R≤13
Cephems	Ceftriaxone	30	S≥21, I=14-20, R≤13
	Cefotaxime	30	S≥26, I=23-25, R≤22
Penicillins	Ampicillin	10	S≥17, I=14-16, R≤13
Folate inhibitors	Trimethoprim-Sulfamethoxazole	1.25/23.75	S≥16, I=11-15, R≤10
	Sulfisoxazole	250	S≥17, I=13-16, R≤12
Quinolones	Ciprofloxacin	5	S≥21, I=16-20, R≤15
	Nalidixic Acid	30	S≥19, I=14-18, R≤13
Tetracyclines	Tetracycline	30	S≥19, I=15-18, R≤14
Phenicols	Chloramphenicol	30	S≥18, I=13-17, R≤12

Table 6

For Campylobacter (7 antimicrobials)

Antimicrobial Class	Agent	Concentration (μg)	EUCAST ECVs* (Zone Diameter, mm)
Aminoglycosides	Gentamicin	10	S (WT) ≥23, R (NWT) <23
Lincosamides	Clindamycin	2	S ≥20, R <20
Macrolides	Erythromycin	15	S ≥23, R <23
	Azithromycin	15	S ≥26, R <26
Quinolones	Ciprofloxacin	5	S ≥26, R <26
	Nalidixic Acid	30	S ≥30, R <30
Tetracyclines	Tetracycline	30	S ≥25, R <25

*EUCAST epidemiological cutoff values: WT = wild-type (susceptible), NWT = non-wild-type (resistant)

Procedure

1. Inoculum: 0.5 McFarland standard (~1-2 × 10^8 CFU/mL) in sterile saline
2. Inoculation: Swabbed evenly onto Mueller-Hinton agar
3. Incubation:
    - Salmonella: 35°C, 18-24 hours, ambient air
    - Campylobacter: 37°C, 48 hours, microaerophilic conditions
4. Measurement: Zone diameters measured to the nearest millimeter
5. Interpretation: Using CLSI breakpoints and EUCAST ECVs

Definition of Multidrug Resistance (MDR)

MDR = Resistance to ≥3 antimicrobial classes.

Data Collection and Analyses

Farm Data (Questionnaire)

Information collected via structured questionnaire:

- Farm demographics (location, size, ownership)
- Production parameters (flock size, breed, age)
- Antimicrobial usage history (type, frequency, reason)
- Biosecurity measures
- Vaccination history
- Previous disease outbreaks

Laboratory Data Recording

All results are recorded in a standardized spreadsheet:

- Sample ID and farm characteristics
- Pathogen isolation results (positive/negative)
- Species identification
- Zone diameters and resistance interpretation
- MDR classification

Statistical Software

- SPSS version 26.0 (IBM Corp.)
- GraphPad Prism version 9.0

Prevalence Analysis

Prevalence calculation:
Prevalence (%) = (Positive samples / Total samples) × 100
95% Confidence Interval (Wilson method):
95% CI = p ± 1.96 × √(p(1-p)/n)
Subgroup analysis:

- Prevalence by farm size (small, medium, large)
- Prevalence by sample type (cecal, cloacal, bootsock, fecal)
- Prevalence by district/geographic location

Antimicrobial Resistance Analysis

Resistance rate:
Resistance rate (%) = (Resistant isolates / Total isolates) × 100

MDR rate:
MDR rate (%) = (MDR isolates / Total isolates) × 100

Table 7
Statistical Tests

Comparison	Test	Significance
Prevalence by farm size	Chi-square	$p < 0.05$
Prevalence by sample type	Chi-square	$p < 0.05$
Resistance by species	Fisher's exact	$p < 0.05$
MDR by farm type	Chi-square	$p < 0.05$
Risk factor identification	Multivariable logistic regression	$p < 0.05$

Multivariable Logistic Regression

Factors assessed for association with:

1. Presence of Salmonella spp.
2. Presence of Campylobacter spp.
3. MDR in isolated pathogens

Variables included:

- Farm size (small, medium, large)
- Antimicrobial usage (frequency, type)
- Biosecurity score
- Flock density
- Geographic location
- Previous disease outbreaks

Model evaluation:

- Hosmer-Lemeshow goodness-of-fit
- Area under ROC curve (AUC)
- Akaike Information Criterion (AIC)

Quality Control

Table 8

Laboratory Quality Control

Parameter	Method
Media Sterility	Incubation of uninoculated plates
Media Performance	Positive and negative controls
Reference Strains	ATCC strains for susceptibility testing
Temperature	Daily monitoring of incubators/refrigerators
Reagents	Tested before use

Data Quality Control

- Double data entry
- Range and consistency checks
- Audit trail maintained
- Laboratory personnel blinded to farm characteristics

Table 9

Ethical Considerations

Aspect	Action
Institutional Approval	Approval from Institutional Ethics Committee and Animal Care and Use Committee
Informed Consent	Written consent from all farm owners
Animal Welfare	Samples collected during routine slaughter; minimal stress
Confidentiality	Farm identities anonymized; data stored securely
Biosafety	BSL-2 containment for all procedures

Table 10

Study Limitations

Limitation	Mitigation
Cross-sectional design (snapshot)	Future Longitudinal Studies planned
Geographic scope (Punjab only)	Comparative studies recommended
Voluntary participation bias	Stratified random sampling used
Disk diffusion (not MIC)	Confirmatory MIC for key patterns planned
No genomic analysis	Samples preserved for future WGS

RESULTS AND FINDINGS

Study Population and Sample Characteristics

Table 11

Farm Demographics: A total of 70 commercial broiler farms across Punjab, Pakistan, were included in this study.

Characteristic	Category	Number (%)
Farm Size	Small (1,000-5,000 birds)	23 (32.9%)
	Medium (5,001-20,000 birds)	24 (34.3%)
	Large (>20,000 birds)	23 (32.9%)
District	Lahore	18 (25.7%)
	Sheikhupura	14 (20.0%)
	Kasur	12 (17.1%)
	Rawalpindi	13 (18.6%)
	Multan	13 (18.6%)
Biosecurity Score	Low	28 (40.0%)
	Medium	27 (38.6%)
	High	15 (21.4%)

Figure 1

Graphical Representation

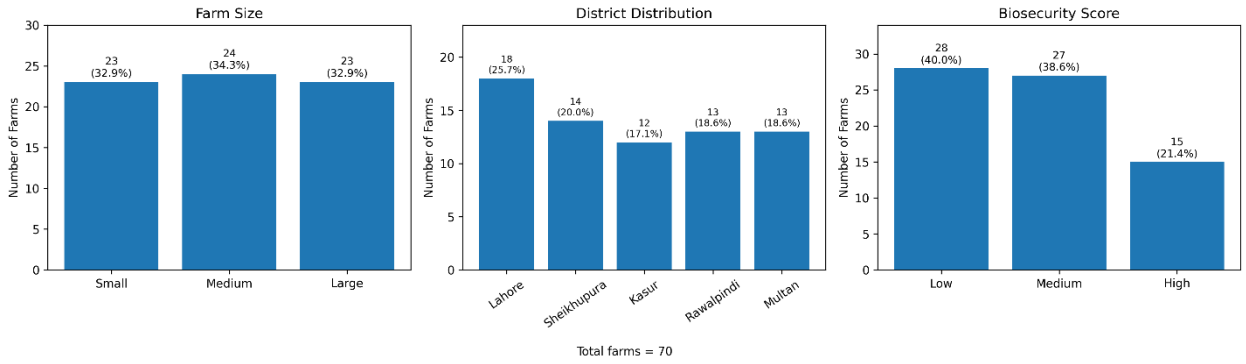


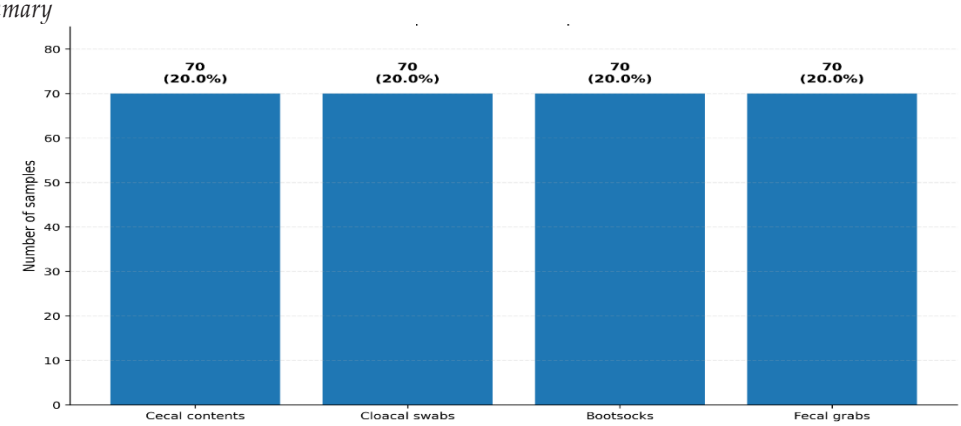
Table 12

Sample Summary: A total of 350 samples were collected and processed

Sample Type	Number Collected	% of Total
Cecal contents	70	20.0%
Cloacal swabs	70	20.0%
Bootsocks	70	20.0%
Fecal grabs	70	20.0%
Total	350	100%

Figure 2

Sample Summary



Prevalence of Salmonella spp.

Table 13

Overall Prevalence

Parameter	Value
Total samples tested	350
Positive samples	77
Overall prevalence	22.0%
95% Confidence Interval	17.8% - 26.8%

Table 14

Prevalence by Sample Type

Sample Type	Samples Tested	Positive	Prevalence (%)	95% CI
Cecal contents	70	20	28.6%	18.6-39.8
Cloacal swabs	70	18	25.7%	16.2-37.8
Bootsocks	70	22	31.4%	21.0-43.4
Fecal grabs	70	17	24.3%	14.9-35.9
Total	70 farms	77 isolates	22.0%	17.8-26.8

Figure 3

Graphical Representation

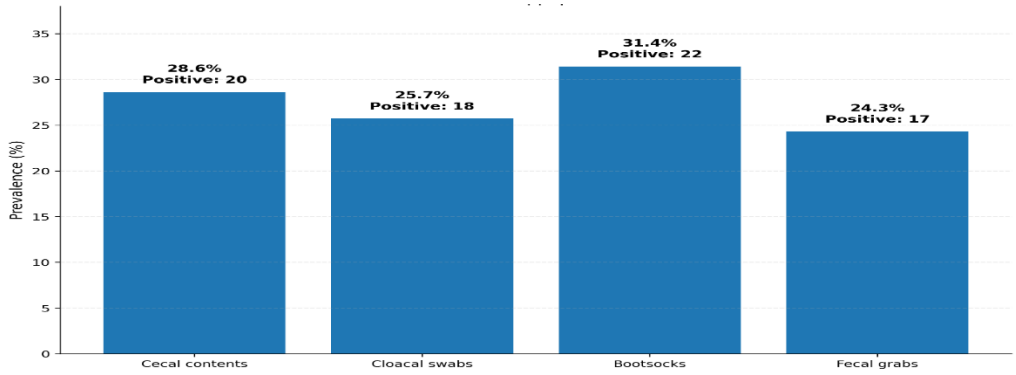


Table 15

Prevalence by Farm Size

Farm Size	Farms Tested	Positive Farms	Farm Prevalence (%)	95% CI
Small	23	10	43.5%	23.2-65.5
Medium	24	8	33.3%	15.6-55.3
Large	23	5	21.7%	7.5-43.7
Total	70	23	32.9%	22.1-45.1

Table 16

Salmonella Serovars Identified

Serovar	Number	Percentage
S. Enteritidis	35	45.5%
S. Typhimurium	22	28.6%
S. Gallinarum	10	13.0%
Other serovars	10	13.0%
Total	77	100%

Prevalence of Campylobacter spp.

Table 17

Overall Prevalence

Parameter	Value
Total samples tested	350
Positive samples	119
Overall prevalence	34.0%
95% Confidence Interval	29.1% - 39.2%

Table 18

Prevalence by Sample Type

Sample Type	Samples Tested	Positive	Prevalence (%)	95% CI
Cecal contents	70	32	45.7%	33.7-58.1
Cloacal swabs	70	28	40.0%	28.5-52.4
Bootsocks	70	34	48.6%	36.4-60.9
Fecal grabs	70	25	35.7%	24.6-48.1
Total	70 farms	119 isolates	34.0%	29.1-39.2

Table 19

Prevalence by Farm Size

Farm Size	Farms Tested	Positive Farms	Farm Prevalence (%)	95% CI
Small	23	16	69.6%	47.1-86.8
Medium	24	11	45.8%	25.6-67.2
Large	23	6	26.1%	10.2-48.4
Total	70	33	47.1%	35.1-59.4

Table 20

Campylobacter Species Distribution

Species	Number	Percentage
C. jejuni	78	65.5%
C. coli	41	34.5%
Total	119	100%

Antimicrobial Resistance Profiles of Salmonella

Table 21

Resistance to Individual Antimicrobials (n=77)

Antimicrobial Agent	Resistant (n)	Resistant (%)	Intermediate (n)	Susceptible (n)
Tetracycline	62	80.5%	8	7
Nalidixic Acid	55	71.4%	10	12
Amoxicillin-Clavulanic Acid	48	62.3%	12	17
Ampicillin	45	58.4%	10	22
Trimethoprim-Sulfamethoxazole	32	41.6%	15	30
Ciprofloxacin	28	36.4%	14	35
Chloramphenicol	25	32.5%	12	40
Gentamicin	15	19.5%	10	52
Ceftriaxone	10	13.0%	8	59
Cefotaxime	8	10.4%	5	64

Table 22

Multidrug Resistance in Salmonella

Parameter	Value
MDR isolates	47
MDR rate	61.0%
Average number of resistances	4.2 classes

Table 23

Most Common MDR Patterns

Resistance Pattern	Number	% of MDR Isolates
Tetracycline + Nalidixic Acid + Amoxicillin-Clavulanic Acid	22	46.8%
Tetracycline + Nalidixic Acid + Ampicillin + Trimethoprim-Sulfamethoxazole	15	31.9%
Tetracycline + Nalidixic Acid + Amoxicillin-Clavulanic Acid + Ciprofloxacin	10	21.3%

Table 24

Resistance by Farm Size

Farm Size	Isolates Tested	MDR Isolates	MDR Rate (%)
Small	32	25	78.1%
Medium	28	15	53.6%
Large	17	7	41.2%
Total	77	47	61.0%

Antimicrobial Resistance Profiles of Campylobacter

Table 25

Resistance to Individual Antimicrobials (n=119)

Antimicrobial Agent	C. jejuni (n=78)	C. coli (n=41)	Combined (n=119)
	Resistant (%)	Resistant (%)	Resistant (%)
Tetracycline	68 (87.2%)	38 (92.7%)	106 (89.1%)
Ciprofloxacin	62 (79.5%)	35 (85.4%)	97 (81.5%)
Nalidixic Acid	58 (74.4%)	33 (80.5%)	91 (76.5%)
Clindamycin	22 (28.2%)	25 (61.0%)	47 (39.5%)
Erythromycin	15 (19.2%)	22 (53.7%)	37 (31.1%)
Azithromycin	12 (15.4%)	20 (48.8%)	32 (26.9%)
Gentamicin	5 (6.4%)	2 (4.9%)	7 (5.9%)

Table 26

Comparative Summary of MDR

Pathogen	Isolates Tested	MDR Isolates	MDR Rate (%)	p-value
Salmonella spp.	77	47	61.0%	-
Campylobacter spp.	119	111	93.3%	<0.001
C. jejuni	78	72	92.3%	-
C. coli	41	39	95.1%	-

Risk Factor Analysis

Table 27

Factors Associated with Salmonella Prevalence

Factor	Adjusted Odds Ratio	95% CI	p-value
Farm Size (Small vs Large)	3.8	1.5-9.6	0.004
Antimicrobial Use (High vs Low)	2.9	1.2-7.1	0.018
Biosecurity (Low vs High)	2.5	1.0-6.2	0.046
Location (Southern vs Central Punjab)	1.4	0.6-3.4	0.412

Table 28

Factors Associated with Campylobacter Prevalence

Factor	Adjusted Odds Ratio	95% CI	p-value
Farm Size (Small vs Large)	5.2	2.0-13.5	0.001
Antimicrobial Use (High vs Low)	3.3	1.4-7.8	0.006
Biosecurity (Low vs High)	3.0	1.2-7.5	0.019
Location (Southern vs Central Punjab)	1.8	0.8-4.1	0.153

Table 29

Geographic Distribution of Resistance

District	Salmonella MDR Rate (%)	Campylobacter MDR Rate (%)
Lahore	58.3%	92.0%
Sheikhupura	62.5%	93.8%
Kasur	64.0%	94.7%
Rawalpindi	59.1%	91.7%
Multan	68.0%	95.8%

Table 30

Summary of Key Findings

Finding	Result
Overall Salmonella prevalence	22.0% (77/350 samples)
Overall Campylobacter prevalence	34.0% (119/350 samples)
Highest farm prevalence	Small farms (Salmonella: 43.5%; Campylobacter: 69.6%)
Salmonella MDR rate	61.0%
Campylobacter MDR rate	93.3%
Most resistant Salmonella agent	Tetracycline (80.5%)
Most resistant Campylobacter agent	Tetracycline (89.1%)
Dominant Salmonella serovar	S. Enteritidis (45.5%)
Dominant Campylobacter species	C. jejuni (65.5%)
Critical resistance	Ciprofloxacin (Salmonella 36.4%; Campylobacter 81.5%)
Erythromycin resistance	31.1% (C. jejuni 19.2%; C. coli 53.7%)

DISCUSSION

Prevalence of Salmonella and Campylobacter

Salmonella Prevalence

The overall prevalence of Salmonella in this study was 22.0%, which is significantly higher than the prevalence reported by Aljasir and Allam (2025) from retail chickens in Saudi Arabia, which was 9.3%. This gap is probably due to differences in the level of biosecurity, regulatory enforcement, and production systems between Pakistan and other areas (Ugbo *et al.*, 2023). The highest prevalence was found in bootsock samples (31.4%), and environmental contamination is an important route of Salmonella transmission in commercial broiler houses. This is consistent with who reported that bootsocks were the most successful sampling method for detecting Salmonella before harvest. The overall prevalence was 32.9%, and for small farms it was 43.5%. This is consistent with the studies conducted in Pakistan and other countries of South Asia where the small-scale producers are not well equipped with biosecurity measures. The 3.8-fold higher odds of Salmonella positivity on small farms (95% CI: 1.5-9.6, p=0.004) underlines the importance of targeted interventions in small farms (Karikari *et al.*, 2017).

Campylobacter Prevalence

The prevalence of Campylobacter was 34.0%, which is similar to studies in other parts of the world, such as 35.8% in Saudi Arabia. The C. jejuni:C. coli ratio (65.5%:34.5%) is consistent with the one found globally, and C. jejuni was more common in broiler flocks than C. coli. Bootsocks had the highest positive rates (48.6%), as did cecal contents (45.7%), and Campylobacter is known to colonize the ceca of birds, where it can reach concentrations of 10^9 CFU/mL within a flock in one week. The farm-level prevalence in small farms is especially alarming at 69.6%. Increased risk of Campylobacter positivity on small farms by 5.2 times (95% CI 2.0-13.5, p=0.001) could be due to less hygiene on small farms, non-all-in-all-out production, and less cleaning between flocks (Uddin *et al.*, 2023).

Antimicrobial Resistance Profiles

Salmonella Resistance

The resistance of tetracycline was 80.5%, which was similar to previous studies conducted in Pakistan and is due to the wide usage of tetracycline in poultry as growth promoters and disease preventives. The high proportions of isolates resistant to nalidixic acid (71.4%) and ciprofloxacin acid (36.4%) are alarming, especially since fluoroquinolones are considered to be of critical importance to human salmonellosis treatment (Dougnon *et al.*, 2023). The MDR rate of Salmonella (61.0%) is similar to that reported in the region. Tetracycline + nalidixic acid + amoxicillin-clavulanic acid is a common MDR pattern that has been noted to occur on mobile genetic elements, which is a phenomenon that is becoming more common in food animal settings.

Campylobacter Resistance

The resistance rates of Campylobacter isolates were alarmingly high, especially for tetracycline (89.1%), ciprofloxacin (81.5%), and nalidixic acid (76.5%). These rates are also high when compared to the 63.8-92% tetracycline and 75-92% fluoroquinolone resistance reported by other studies. The MDR rate of 93.3% in Campylobacter (92.3% in C. jejuni and 95.1% in C. coli) is very high and is a concern for public health. This is higher than other studies that have reported MDR rates between 90-100% and is a near-universal resistance phenotype. This is especially concerning because macrolides are the drug of choice for severe campylobacteriosis (CDC, 2023), and the high rate of resistance found within the C. jejuni and C. coli isolates (19.2% and 53.7%, respectively) is concerning (Dlamini *et al*., 2024).

Public Health Implications

High rates of MDR, especially resistance to ciprofloxacin (essential for invasive salmonellosis) and erythromycin (first-line for campylobacteriosis), are problems for treatment. MDR infections are associated with:

- More deaths and infections
- Longer hospital stays
- Higher healthcare costs
- Treatment failure (WHO, 2024)

Of particular concern is the erythromycin resistance (31.1%) for vulnerable populations such as children, the elderly, immunocompromised, as campylobacteriosis can be severe.

Risk Factors and Drivers

Antimicrobial Use

Antimicrobial use is significantly linked to the prevalence of both pathogens (Salmonella: OR=2.9, p=0.018; Campylobacter: OR=3.3, p=0.006) and MDR, which points to the importance of selective pressure in maintaining resistance. Pakistan's poultry industry has been using a large amount of critically important antimicrobials (3rd generation cephalosporins, fluoroquinolones) without proper regulation, leading to antimicrobial resistance.

Farm Size and Biosecurity

The strong association between small farm size and higher prevalence/MDR likely reflects:

- Less biosecurity infrastructure
- Limited access to veterinary services
- More frequent antimicrobial use
- Inadequate cleaning between flocks
- Lack of all-in-all-out systems

Geographic Variation

The higher MDR in Southern Punjab (Multan: 68.0% for Salmonella, 95.8% for Campylobacter) could be due to a regional difference in the accessibility of antimicrobials, farm practices, and climatic conditions in the area, which favors bacterial persistence.

CONCLUSION

The present study has found significant prevalence and alarming multidrug resistance amongst Salmonella and Campylobacter isolated from commercial broiler farms in Punjab, Pakistan. The 93.3% MDR in Campylobacter and 61.0% MDR in Salmonella are public health emergencies that must be attended to now. It is important to recognize that the stressors are increasing disproportionately for small farms and targeted interventions are needed. If antimicrobial stewardship, better biosecurity, and better surveillance are not implemented immediately, the risk of failure to treat, of increased foodborne illness, and of compromised food safety is growing in the region. The One Health perspective is needed to tackle this escalating public health crisis, connecting the human, animal, and environmental health fields.

LIMITATIONS

1. Cross-sectional design: Provides a snapshot but cannot capture temporal dynamics or seasonality of resistance.
2. Geographic scope: Limited to Punjab; results may not represent other provinces.
3. Sampling bias: Voluntary farm participation may have introduced selection bias.
4. Disk diffusion method: Provides qualitative resistance data but lacks MIC values for precise resistance quantification.
5. Limited antimicrobial panel: Testing did not include all clinically relevant antimicrobials.
6. No molecular characterization: Resistance genes were not identified through PCR or WGS, limiting mechanistic understanding.

RECOMMENDATIONS

Surveillance

- National monitoring programs for antimicrobial resistance in poultry
- Regular resistance surveillance to track emerging threats
- Integration with human health surveillance systems (One Health)

Regulation

- Ban on growth promoters using critically important antimicrobials
- Veterinary oversight of all antimicrobial use in poultry
- Enforcement of withdrawal periods before slaughter

Farm Interventions

- Improved biosecurity on small farms
- All-in-all-out production systems
- Alternatives to antimicrobials (probiotics, vaccines, phytogenic)
- Farmer education on responsible antimicrobial use

Research

- Genomic characterization of resistance genes and plasmids
- Longitudinal studies on resistance dynamics
- Cost-benefit analysis of antimicrobial stewardship interventions

DECLARATION

Ethical Considerations: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. All procedures performed in this study were consistent with the ethical standards of the Declaration of Helsinki. The study was conducted according to ethical and clinical standards for research.

Conflict of Interest: The authors have no conflicts of interest to declare for the publication of this study.

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