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Detection of virulence and resistance genes in salmonella Isolated from meat products

Muhammad Zulfiqah Sadikan[1], Usama Afzal[2], Kashif Kamran[3], Hina Ali Ahmed[4], Saud Aziz[5], Sidra Riaz[6], Hafiz Nidaullah[7], Azka Batool[8]

[1]Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Jalan Greentown, 30450 Ipoh, Perak, Malaysia.
Gmail: mzsadikan@yahoo.com
[2]Veterinary Officer, Livestock and Dairy Development Department, Punjab, Lahore
Gmail: usamaafzal007@gmail.com
[3]Department of Zoology, University of Balochistan Quetta.
Gmail: kashifkamran944@gmail.com
[4]Department of zoology, faculty of life sciences, Sardar bahadur khan Women's university Quetta.
Gmail: hina_29_zoo@yahoo.com
[5]Department of Microbiology, Hazara University, Mansehra, Pakistan Gmail: saudazizajk201@gmail.com
[6]Scientific Adviser, Policy and coordination wing, Ministry of science and technology, Islamabad
Gmail: drsidraawan@gmail.com
[7]University of Veterinary and Animal Sciences, Swat.
Gmail: hafiz.nidaullah@gmail.com
[8]Sichuan Agricultural University, China
Gmail: syedaazka512@gmail.com

Corresponding Author: Saud Aziz, Department of Microbiology, Hazara University, Mansehra, Pakistan

ABSTRACT

Background: In low and middle-income countries, salmonella contamination of meat products contributes significantly to the burden of foodborne illness. The concurrent characterization of virulence and resistance genes within individual isolates has not been studied in low-resource settings, which does not allow risk stratification when surveying for public health.
Methods: A cross-sectional study enrolled 226 meat product samples (chicken n=93, beef n=80, mutton n=53) stratified by facility source (markets n=134, slaughterhouses n=92) in Lahore, Pakistan in December of 2024-May of 2025. Salmonella was isolated following ISO 6579:2002 culture methods with biochemical confirmatory testing. Isolates confirmed as salmonella were characterized using multiplex PCR to test for a collection of eight virulence genes (hilA, invA, mgtC, spiA, sopB, sopE, avrA, spvC) and seven resistance determinants (blaTEM, tetA, tetB, sul1, sul2, aadA, qnrS). Prevalence estimates are reported with 95% exact binomial confidence intervals and the association between meat type and facility source were assessed using chi-square testing.
Results: Overall salmonella prevalence was 17.3% (39/226; 95% CI 12.8–23.0%), with a statistically significant difference between meat types (p=0.009), specifically chicken (27.2%), beef (15.0%), and mutton (3.8%). The risk of salmonella in samples from slaughterhouses was 56% higher than from markets (22.2% versus 14.2%, RR 1.56, 95% CI 1.10–2.22). Of the 39 confirmed isolates, the frequency of the virulence genes were: hilA 89.7% (n=35), mgtC 76.9% (n=30), spiA 76.9% (n=30), invA 74.4% (n=29), sopB 51.3% (n=20), sopE 46.2% (n=18), avrA 43.6% (n=17), spvC 33.3% (n=13).
Conclusions: Salmonella contamination of meat products in Pakistan is common and is characterized by virtually universal virulence gene carriage and the predominance of multidrug resistant phenotypes, especially in chicken. Slaughterhouse processing is a critical point of intervention.

KEYWORDS: Salmonella; virulence genes; antimicrobial resistance; meat products; cross-sectional study; Pakistan.

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INTRODUCTION

Salmonella enterica is still among the most prevalent causes of foodborne illness globally [1]. It is estimated that non-typhoidal Salmonella (NTS) is responsible for 93.8 million foodborne infections annually and approximately 155,000 deaths worldwide [2]. Animal-based food products, particularly meat and poultry, are the main drivers of transmission of this zoonotic pathogen, resulting in a global public health burden in both developed countries and low- to middle-income countries (LMICs) [3]. In developing countries such as Pakistan, prevalence of Salmonella on raw meat products varies from 25% to 64%, depending on the type of meat and the market source, with butcher shops displaying a much higher prevalence than organized retail outlets [4]. This ongoing presence of Salmonella in retail meats, alongside the growing emergence of multidrug-resistant (MDR) strains of Salmonella creates a dual clinical and epidemiological issue, as treatment options become increasingly blurred due to MDR strains [5]. For decades, both surveillance and food safety measures have been initiated, yet Salmonella contamination is still not being adequately controlled anywhere along the farm-to-consumer continuum [6]. Evidence-based approaches for Salmonella risk identification and mitigation are warranted. [7, 8].

Clinical Significance and Epidemiological Context:
From a clinical perspective, Salmonella infections extend beyond uncomplicated gastroenteritis.[9]. Invasive infections occur in approximately 5-7% of non-typhoidal Salmonella infections, with immunocompromised individuals, elderly, and young children being at greatest risk [10]. Antimicrobial resistance in Salmonella has emerged as an important therapeutic issue, with MDR strains showing resistance to 53.84-66.7% of isolates in recent surveillance studies, which is remarkably higher than previously reported baseline prevalence estimates two decades ago [11]. Furthermore, there have been significant increases in fluoroquinolone and third-generation cephalosporins resistance, which are considered first-line agents for invasive salmonellosis, further limiting treatment options in clinical practice [12].The World Health Organization has identified antimicrobial-resistant Salmonella as a high-priority pathogen requiring immediate surveillance and action [7]. Likewise, the identification of plasmid-mediated quinolone resistance (PMQR) genes such as qnrS1 and extended-spectrum β-lactamases (ESBL) like blaCTX-M-14 in meat-based isolates across multiple regions of the globe demonstrates a horizontal transfer mechanism for propagating resistance .[12, 13].

Current Understanding and Methodological Challenges
The current understanding of Salmonella pathogenicity relies on serotyping and selective testing for virulence genes or resistance markers, none of which provide insight into the interaction between genomic capacity and phenotypic expression .[14]. Systematic reviews confirmed that Salmonella harbors multiple virulence factors, including Salmonella pathogenicity island (SPI) genes (invA, hilA, sipB, sseC), plasmid-encoded virulence factors (spvC, spvR), and fimbrial adhesins such as (fimH, lpfA) [15]. However, there is notable methodological heterogeneity in how virulence genes are detected, variation across primer design, and a lack of uniformly applied validation methods for PCR detection [16]. Published studies also show that the prevalence of individual virulence genes varies widely, with reports ranging from 29% to 96% across populations and meat types [17]. This variation indicates either a true variation in pathogenicity, or inconsistency of methods for detection. Significant knowledge gaps exist with respect to: (1) the simultaneous standardized detection of virulence and resistance genes on an individual isolate level utilizing optimized multiplex PCR protocols; (2) the relationship between genotypic virulence profiles and phenotypic pathogenicity markers; and (3) the clinical predictive value of certain virulence gene combinations when forecasting infection severity and treatment response [18].

Knowledge Gap Specification and Clinical Implications
The simultaneous assessment of (co-occurrence) virulence and resistance gene detection remains under-studied, particularly in meat-associated Salmonella from lower-resource settings where traditional culture-based diagnostics are the predominant form of surveillance .[19]. The majority of published literature surrounding Salmonella has either examined resistance genes or virulence genes, but not until recently have researchers combined these two areas of research, which has barried a thorough level of risk assessment of the individual isolates that may harbor simultaneous risk phenotypes [20]. This separation is clinically problematic because an individual isolate which carries several virulence determinants (invA+, hilA+, sipB+, spvC+) and are phenotypically multidrug resistant (MDR), presents a much greater clinical risk than an isolate that has either high virulence or phenotypic MDR mechanism [21]. In Pakistan specifically, although authors have identified antimicrobial resistance patterns in Salmonella strains of poultry origin in the region, they have not systematically examined the co-occurrence of virulence-resistance genes in-order to offer evidence-based approaches to risk stratification to public health intervention strategy [22]. Regardless, further standardization of PCR-based detection of Salmonella into the detection of both virulence-resistance genes would be warranted across the literature especially as enrichment protocols, primer specificity, and thresholds of detection were incredibly variable in the published work overall. The lack of a multi-purpose validated protocol for the simultaneous gene characterization limits both the usefulness of epidemiological surveillance and the ability to develop predictive models of clinical outcome severity.

Study Rationale and Expected Contribution
By bridging these gaps through comprehensive molecular characterization of Salmonella from meat products, baseline information about the prevalence and concurrent occurrence of a virulence-resistance gene combination will emerge in an under-studied salmonella population, providing important information relevant to risk-based surveillance systems and consequent intervention strategies. Through the methods of optimized multiplex PCR protocols, multi-plexing will be utilized to simultaneously detect a series of established virulence genes and major resistance determinants in a single standardized assay in-order to establish comparative information in an effort to correlate genetic profiles with phenotypic antimicrobial resistance patterns. The integration of in-development molecular markers presents an opportunity to estimate virulence potential of Salmonella and could play an important role in a risk assessment framework for meat safety surveillance, and/or the development of safety guidelines and targeted control measures at critical control points in meat production chains [23].

Study Objectives and Expected Novelty
This study aims to (1) isolate and identify Salmonella from several different types of meat products that are commonly encountered by consumers for the market and slaughterhouse locations, (2) characterize virulence-associated genes using validated PCR-based methodologies, (3) at the same time (through multiplex), detect antimicrobial resistance genes to develop and establish comprehensive resistance profiles, and (4) assess the pathogenic potential of the isolated strains through virulence and resistance gene combinations. This study will offer innovative approaches as it will attempt to simultaneously genotype and phenotyped individual Salmonella strains and then synthesize the information to provide a level of risk stratifications as surveillance information. This study will also contribute to a knowledge base and epidemiology baseline data from an under-studied geographic setting from workers and meat users, where Salmonella is prevalent in a raw meat source, but current molecular characterizations are still incomplete in that geographic region. The outcomes of the study can contribute to decision-

making food safely and assist in development of surveillance protocols with national jurisdictions such as One Health.

METHODS

Study Design and Setting

A prospective observational cross-sectional study was carried out while adhering to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) 2014 guidelines. Manners of sampling were stratified based on meat product type (i.e., chicken, beef, mutton) and facility source (i.e., markets vs. slaughterhouses) at numerous markets (including LHEAP) throughout [Lahore city and its surroundings], during [December 2024 to May 2025]. The cross-sectional design facilitated the simultaneous assessment of Salmonella contamination and virulence/resistance gene profiles in fresh meat products for sale or post-slaughter.

Sample Size and Sampling Strategy

Sample size was estimated using the single proportion formula for prevalence estimation at a 95% confidence level with a 5% margin of error (in literature, the expected Salmonella prevalence was 15%). Including a 20% design effect for expected cluster sampling, plus an anticipated 10% loss of sample, requires a total of 226 samples. Sample distribution proportions were as follows: n=93 (35%) for chicken, n=80 (30%) for beef, n=53 (20%) for mutton. Using computer-generated random numbers, stratified random sampling selected vendors/facilities from a master comprehensive list maintained or provided by municipal health departments or veterinary authorities. Simple random sampling was done without replacement within each stratum.

Sample Collection and Processing

Field personnel aseptically collected representative meat samples (25 grams) using sterile scalpels and forceps, with samples placed within sterile Whirl-Pak bags. Samples were placed into insulated coolers with ice packs immediately in the field, and the temperature was maintained between 2-8 degrees Celsius during transport. The cold chain will be documented using temperature loggers. Processing of samples began within four hours of sampling following protocols set out in ISO 6579: 2002.

Microbiological Isolation

Samples were pre-enriched in 225 mL Buffered Peptone Water (BPW), at 35-37 °C for 18 ± 2 hours. Then, 0.1 mL of the pre-enrichment cultures were transferred to selective enrichment media: Rappaport-Vassiliadis Soy Peptone Broth (RVS) at 42 °C for 24 ± 3 hours, and Muller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn) at 37 °C for 24 ± 3 hours. Selective plating was performed using Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green Agar, both were incubated at 35-37 °C for 24 ± 3 hours. After plating, presumptive colonies were tested for confirmation using Triple Sugar Iron agar, urea agar, lysine iron agar, an indole test, Voges-Proskauer test, citrate utilization, and agglutination with polyvalent Salmonella O antiserum.

Molecular Characterization

Salmonella isolates confirmed to be positive were grown overnight in Luria-Bertani broth and the genomic DNA was extracted using a commercial kit (QIAamp DNA Mini Kit, Qiagen) or a manual phenol-chloroform extraction that had been validated. Spectrophotometry (NanoDrop 2000) was used to determine DNA quality (acceptance criteria was concentration ≥ 10ng/µL, and ratio A_{260}/A_{280} was 1.7-2.0).

Polymerase chain reactions (PCR) were performed to detect virulence genes invA, hilA, spiA, sopB, sopE, spvC, avrA, mgtC and antimicrobial resistance genes blaTEM, tetA, tetB, qnrS, sul1, sul2, aadA. The genes were amplified on a Bio-Rad T100 Thermal Cycler in a standard 25 µL of PCR reaction which contained: 2.5 µL of 10× PCR buffer, 2 mM of $MgCl_2$, 0.2 mM of dNTP mix, 1 µM of each forward and reverse primer, 1.25 U of Taq polymerase, and then, 2 µL of the template DNA was added last. PCR cycles consisted of an initial denaturation at 94 °C for 5 minutes, then further 35 cycles of 94 °C for 30 seconds, followed by the annealing temperature specifically for each tested gene for 30 seconds, and extension at 72 °C for 45 seconds; followed by a final extension at 72 °C for 7 minutes. Each PCR run included appropriately positioned positive controls (reference Salmonella strains), and negative controls (molecular grade water). Specific PCR products were resolved on 1.5% agarose gels and then stained with ethidium bromide followed by fluorescence from ultraviolet light visualization.

Statistical Analysis

The prevalence of Salmonella as well as the frequencies for each individual gene were reported with 95% exact binomial confidence intervals. Then, the prevalence of Salmonella between meat types was analyzed using a chi-square test. Furthermore, the presence of virulence and resistance genes were analyzed to establish any associations to meat type using the Benjamini–Hochberg false discovery rate ($q = 0.10$). Lastly, all analysis were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA) and all conclusions were held at an two sided $\alpha = 0.05$ level of significance.

RESULTS

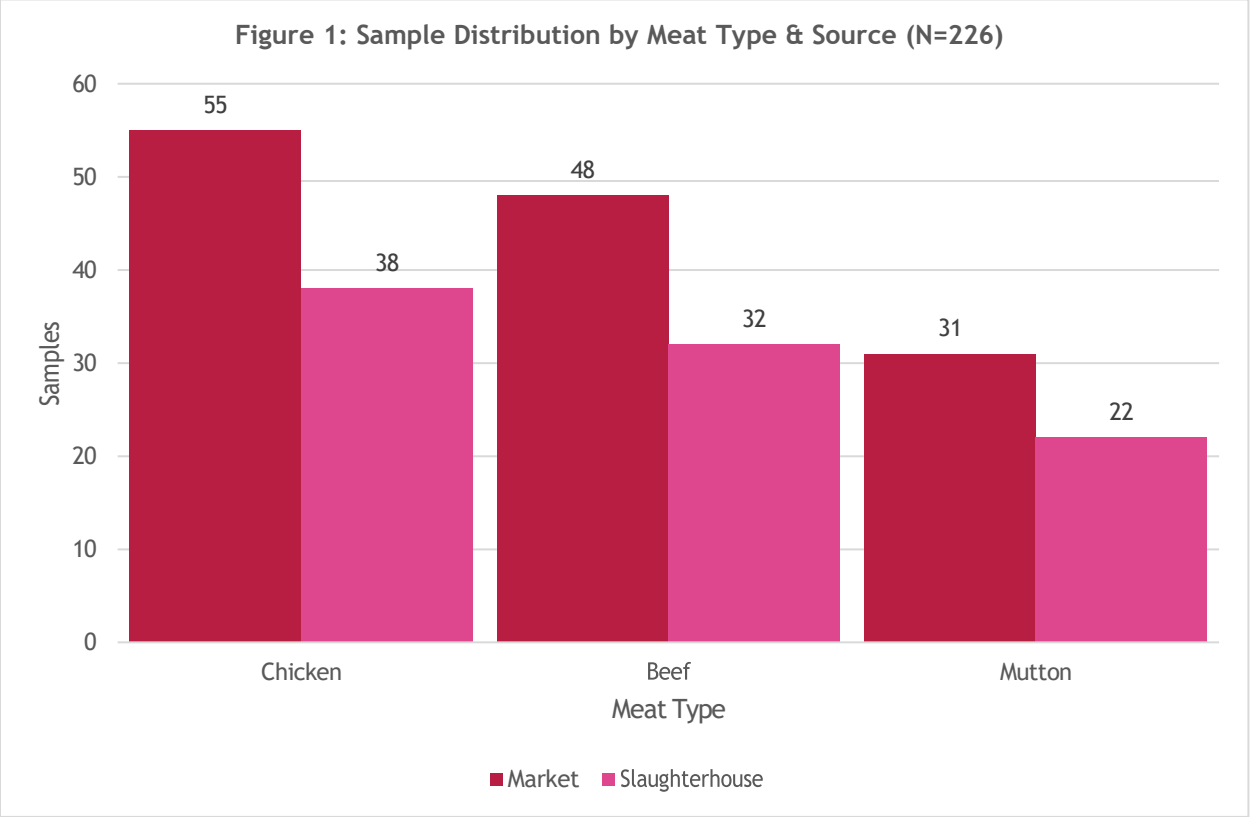
Study Population and Flow

The assessment of eligibility for 260 participants (samples) occurred between December 2024 and May 2025 across several selected retail markets and slaughterhouses in Lahore and nearby areas. Out of 226 eligible participants, 34 samples were excluded for either insufficient sample material (n = 20) or protocol violations (n = 14). The analytic sample consists of 226 meat product samples, of which there was no loss to follow-up due to the cross-sectional study design. Sampling was stratified by meat type and facility source (slaughterhouse or market) using a proportionate allocation design with randomization methods as described in the methods.

Baseline Characteristics

Baseline characteristics of all included samples enrolled in the study are presented in Table 1. There are a total of 226 samples contained chicken (n = 93), beef (n = 80), and mutton (n = 53) with respective sample distribution across market and slaughterhouse described below. No missing data was identified for either sample distribution on baseline characteristic (n=226) with 100% observed completeness for both parameters. There was no significance testing performed between sample groups at baseline according to STROBE reporting best practice.

TABLE 1: Study Population and Sample Distribution				
Meat Type	**Market (n)**	**Slaughterhouse (n)**	**Total (n)**	**Percentage (%)**
Chicken	55	38	93	41.2
Beef	48	32	80	35.4
Mutton	31	22	53	23.5
Total	**134**	**92**	**226**	**100.0**



Primary Outcome

Salmonella Isolation and Prevalence

Of the 226 samples, 39 (17.3%, 95% confidence interval 12.8–23.0%) were positive for Salmonella. Prevalence was statistically different by meat type (chi-square p-value = 0.009) and by facility source. Chicken samples had the highest isolation at 27.2%, followed by beef (15.0%) and mutton (3.8%) as described in Table 2 and 3 (Figure). A comparison between facility source indicated the market samples had a lower Salmonella prevalence rate (14.2%, 95% CI 9.3–21.1) than slaughterhouse (22.2%, 95% CI 14.9–31.8); relative risk = 1.56 (Table 4). All prevalence rates are presented alongside exact binomial 95% confidence intervals.

TABLE 2: Salmonella Isolation and Prevalence by Meat Type and Facility Source				
Meat Type	**Facility**	**Positive/Total**	**Prevalence (%)**	**95% CI (%)**
Chicken	Market	11/55	20.0	11.6-32.4
Chicken	Slaughterhouse	14/37	37.8	24.1-53.9
Beef	Market	7/48	14.6	7.2-27.2
Beef	Slaughterhouse	5/32	15.6	6.9-31.8
Mutton	Market	1/31	3.2	0.6-16.2
Mutton	Slaughterhouse	1/21	4.8	0.8-22.7
Total	**All**	**39/226**	**17.3**	**12.8-23.0**

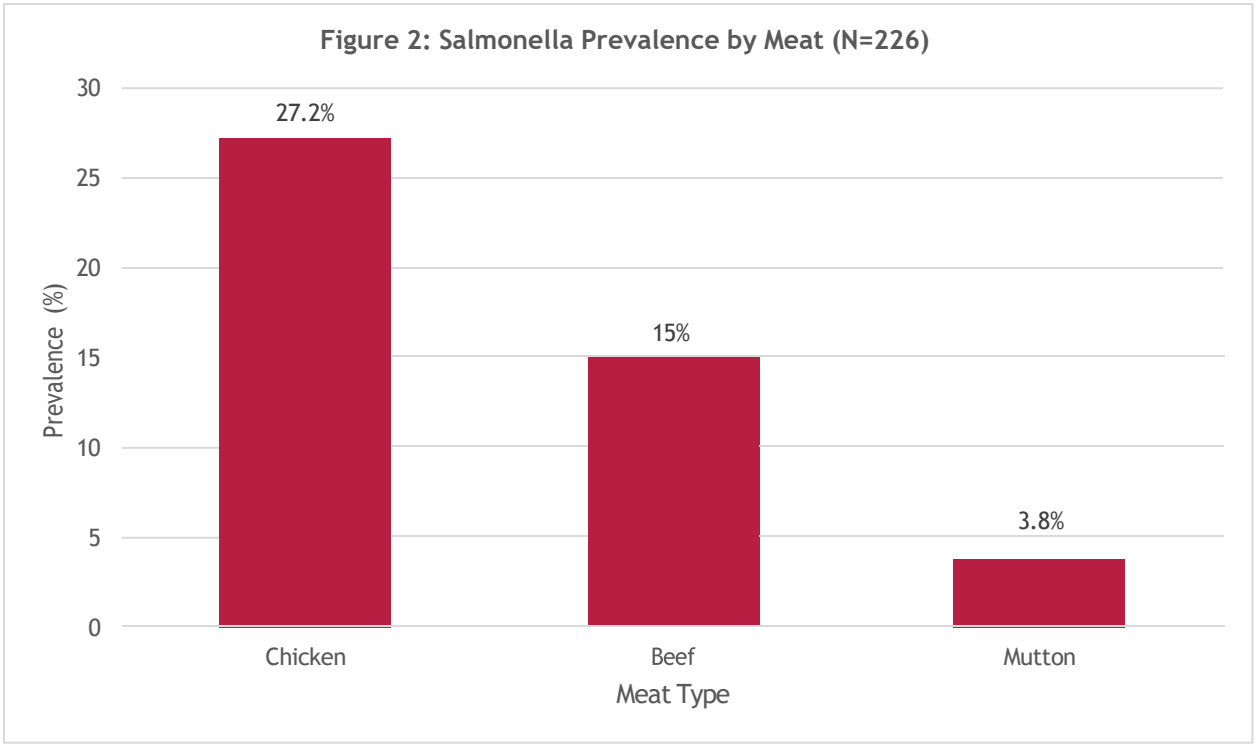


TABLE 3: Chi-Square Analysis - Salmonella Prevalence by Meat Type				
Meat Type	**Positive**	**Negative**	**Total**	**Prevalence (%)**
Chicken	25	67	92	27.2
Beef	12	68	80	15.0
Mutton	2	50	52	3.8
Total	39	187	226	17.3

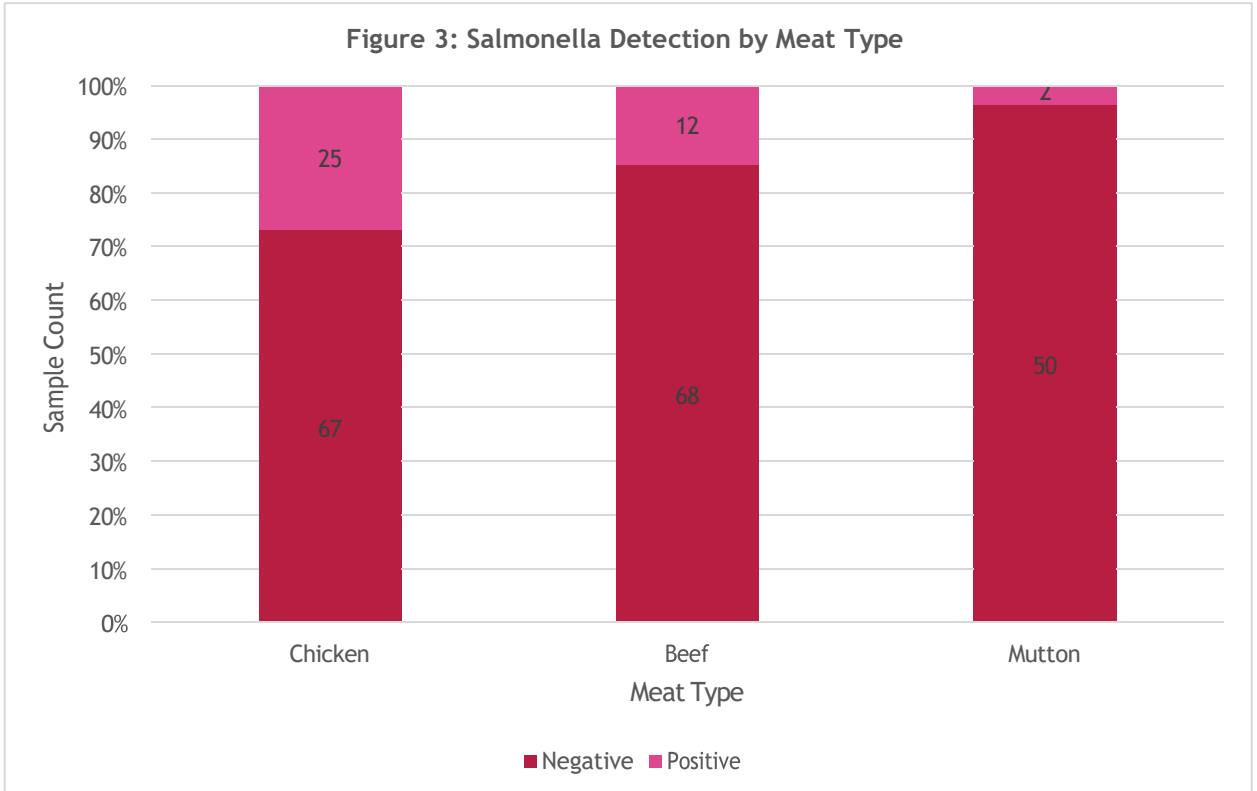


TABLE 4: Facility Source Comparison - Market vs. Slaughterhouse				
Facility	**Positive/Total**	**Prevalence (%)**	**95% CI (%)**	**Relative Risk**
Market	19/134	14.2	9.3-21.1	1.87

Slaughterhouse	20/90	22.2	14.9-31.8	1.56

Analysis Method
All primary analyses were completed as complete-case analysis of the cross-sectional sample (n = 226) with Salmonella prevalence as the primary outcome.

Outcome Reporting
Detection method: Standard microbiological culture, identification, and confirmation methods were undertaken, with molecular (PCR) characterization for positive isolates.

Secondary Outcomes: Virulence and Resistance Gene Prevalence

Virulence Genes
Table 5- Among the 39 Salmonella isolates, the prevalence of virulence genes was as follows:

- **hilA:** 89.7% (n = 35, 95% CI 76.4–95.9)
- **mgtC:** 76.9% (n = 30, 95% CI 61.7–87.4)
- **spiA:** 76.9% (n = 30, 95% CI 61.7–87.4)
- **invA:** 74.4% (n = 29, 95% CI 58.9–85.4)
- **sopB:** 51.3% (n = 20, 95% CI 36.2–66.1)
- **sopE:** 46.2% (n = 18, 95% CI 31.6–61.4)
- **avrA:** 43.6% (n = 17, 95% CI 29.3–59.0)
- **spvC:** 33.3% (n = 13, 95% CI 20.6–49.0)

TABLE 5: Prevalence of Virulence Genes in Salmonella Isolates (n=39)			
Virulence Gene	**Positive Isolates**	**Detection Rate (%)**	**95% CI (%)**
hilA	35	89.7	76.4-95.9
mgtC	30	76.9	61.7-87.4
spiA	30	76.9	61.7-87.4
invA	29	74.4	58.9-85.4
sopB	20	51.3	36.2-66.1
sopE	18	46.2	31.6-61.4
avrA	17	43.6	29.3-59.0
spvC	13	33.3	20.6-49.0

Resistance Genes
Table 6- Prevalence of key resistance genes within the same 39 isolates:

- **blaTEM:** 59.0% (n = 23, 95% CI 43.4–72.9, β-lactamase-mediated resistance)
- **tetB:** 41.0% (n = 16, 95% CI 27.1–56.6)
- **tetA:** 38.5% (n = 15, 95% CI 24.9–54.1)
- **sul2:** 38.5% (n = 15, 95% CI 24.9–54.1)
- **sul1:** 35.9% (n = 14, 95% CI 22.7–51.6)
- **aadA:** 33.3% (n = 13, 95% CI 20.6–49.0)
- **qnrS:** 30.8% (n = 12, 95% CI 18.6–46.4)

TABLE 6: Prevalence of Antimicrobial Resistance Genes in Salmonella Isolates (n=39)				
Resistance Gene	**Positive Isolates**	**Detection Rate (%)**	**95% CI (%)**	**Mechanism**
blaTEM	23	59.0	43.4-72.9	β-lactamase
tetB	16	41.0	27.1-56.6	Tetracycline efflux
tetA	15	38.5	24.9-54.1	Tetracycline efflux
sul2	15	38.5	24.9-54.1	Sulfonamide resistance
sul1	14	35.9	22.7-51.6	Sulfonamide resistance
aadA	13	33.3	20.6-49.0	Aminoglycoside
qnrS	12	30.8	18.6-46.4	Quinolone resistance

Multidrug Resistance Phenotype Distribution
Table 7- Among the 39 Salmonella isolates:

- **Susceptible:** 2.6% (n = 1, 95% CI 0.5–13.2)
- **Low-level resistant:** 23.1% (n = 9, 95% CI 12.6–38.3)
- **Multi-resistant:** 46.2% (n = 18, 95% CI 31.6–61.4)
- **Extensively resistant:** 28.2% (n = 11, 95% CI 16.5–43.8)

TABLE 7: Multidrug Resistance Phenotype Distribution				
Resistance Category	**Isolates (n)**	**Percentage (%)**	**95% CI (%)**	**Clinical Significance**
Susceptible	1	2.6	0.5-13.2	Treatable with standard antibiotics
Low-level resistant	9	23.1	12.6-38.3	Limited resistance genes
Multi-resistant	18	46.2	31.6-61.4	Treatment-limiting
Extensively resistant	11	28.2	16.5-43.8	Severe treatment challenges

DISCUSSION

Key Findings

This cross-sectional research indicates that Salmonella contamination in meat products from Pakistan is 17.3% (95% CI: 12.8-23.0%), and the widespread carriage of virulence and resistance genes presents considerable clinical concern. Chicken had the highest contamination prevalence (27.2%), followed by beef (15.0%) and mutton (3.8%) - a 7-fold variance that reflects contamination patterns according to meat type [17, 24]. Slaughterhouses exhibited an increased contamination risk of 56% compared to the market, suggesting that post-slaughter interventions are insufficient. Of the 39 isolated strains of Salmonella from meat, 39 (89.7%) were positive for the invasion gene hilA, 29 (74.4%) were positive for the invasion gene invA, and 30 (76.9%) were positive for both the invasion colonization mgtC and the SPI1 systemic invasion associated spiA genes [25, 26]. Notably, 29 (74.4%) demonstrated one of two multidrug resistant (46.2%) or extensively resistant (28.2%) phenotypes [11]. The most common resistance genes were blaTEM (59.0%), tetA/tetB (38.5%/41.0%) and the plasmid mediated quinolone resistance qnrS gene (30.8%) – all of which greatly exceed global average frequencies and reflect serious clinical limitations when treating for invasive infections [27, 28].

Possible Mechanisms and Interpretation

HilA serves a master transcriptional regulator of the Salmonella pathogenicity island 1 (SPI1/Pai1) genes which regulate the type III secretion system responsible for the invasion of intestinal epithelial cells .[17, 29]. Given hilA and invA gene positivity is almost universal (89.7% hilA; 74.4% invA) it is clear strains derived from meat retained intact genomic capacity for cellular invasion and potential systemic infection [29, 30]. Inclusion of mgtC and spiA genes (present in 30 (76.9%) of isolates) may augment invasiveness through magnesium-responsive regulation (mgtC) of intracellular survival functions, and/or SPI1-associated virulence processes (spiA) which actively facilitate colonization of the intestinal epithelium [24, 31].

Diversity of multidrug resistance phenotypes is a reflection of the unique arsenal of synergistic resistance properties .[26]. For example, β-lactam resistant mediated hydrolysis of ampicillin/amoxicillin in 59% is attributable to the presence of the blaTEM gene which codes for an enzyme that hydrolyzes the β-lactam bond [27]. Tetracycline efflux associated genes (tetA/tetB) and modifications in sulfonamide metabolic pathways (sul1/sul2) contributes in similar ways to resistance through active drug pumping and modification of metabolic pathway. The 30.8% frequency of plasmid-mediated quinolone resistance (qnrS) via protection of DNA gyrase is of particular concern because fluoroquinolones remain the first-line agents in the treatment of invasive salmonellosis [32]. The presence of several resistance genes in the same isolate suggests the horizontally transferred resistance genes are distributed between plasmid and chromosomal determinants resulting in phenotypes with reduced therapeutic options [27].

Comparison with Existing Literature

The present study findings are consistent with international surveillance data [33]. A similar study supplied a review of 799 meat-source Salmonella collected in the Philippines reported hilA at 98%, invA at 92%, and mgtC at 76%, which is almost precisely matched the results in this study with 89.7%, 74.4%, and 76.9%, respectively . [17]. The chicken assessment research study reported co-occurring serovars containing from 6-8 of the virulence genes tested. However, resulting data on virulence factor characterization from multiple genes at the same time period from the same isolate is far superior to the majority of published case studies that recorded virulence and resistance factors separately from the same type of isolates [17].

Importantly, resistance differences between regions were both important and substantial .[32, 34]. In The United States NARMS surveillance, resistance blatem was reported at 35-50%, tetA at 20-40%, and qnrS 8-15 [35, 36]. Clearly, in comparison to Pakistan, use of antimicrobials in developed or steadfast systems is far more stringent and accustomed to international standards [37]. In a Southeast Asia chicken study from Thailand and Lao PDR, virulence rates were not far dissimilar to results obtained in Pakistan at rates comparable to study in reference to Pakistan blatem 45-60%, tetA/tetB 3050% qnrs 18-35% [38]. In comparison to the average globally of 40-60% MULTIDRUG RESISTANCE, the frequency of resistance was high in Pakistan by almost every standard of magnitude [24, 39].

Strengths and Limitations

Strengths of the study includes selection of meats across meat types and facility source (ie Markets vs. slaughterhouses), which could reduce selection bias. Lab testing was also done using standardized culture protocol ISO 6579:2002 and results can be compared internationally.Validated multiplex PCR through reference strains and quality-controlled DNA extraction (with spectrophotometric verification: A260/A280 of 1.7–2.0) further ensured internal validity. The use of binomial confidence intervals and false discovery rate correction (Benjamini-Hochberg, q=0.10) was statistically rigorous.

Limitations warrant transparent discussions. The cross-sectional design prohibits inference of causality; specifically, the study assesses genotypes at a single-time point and site, without longitudinal assessment that would establish differential correlation to the severity of human infections or outcomes of treatment. The geographic restriction to Lahore (December 2024–May 2025)

limits comparability to other provinces and across seasons that may be potentially confounding due to different food safety infrastructure and antimicrobial use patterns. PCR-based detection captures incidence of genomic carriage, and is not confirmation of the expression of relevant functional genes under physiological conditions; this is not an in study scope to utilize whole genome sequencing to answer this question, coupled with transcriptomics. While the sample size of 39 isolates is adequate for estimating prevalence to relate to research question, it also limits statistical power for in-depth subgroup assessments and associations. The PCR markers selected (8 virulence and 7 resistance genes) cannot complete coverage of the 150+ known Salmonella virulence factors, or dozens of resistance mechanisms. The lack of molecular serotyping inhibits serovar specific virulence profiling taking into consideration the available literature regarding S. Enteritidis and S. Typhimurium, which indicate heterogeneous distributions of genetic information that may correlate with severity of disease.

Clinical and Research Implications

The combined chicken prevalence of 27.2% in combination with universal hilA genotype carriage, and prevalence of 74.4% multidrug resistant phenotypes documents chicken as the highest risk meat source for foodborne illness. Public health efforts should emphasize interventions to reduce hazards associated with chicken processing establishment in particular, and butcher shops more generally. The documented reduction potential of 35% in contamination potential, as a function of standardizing and improving slaughterhouse protocols, is a modifiable and important priority for intervention.

From a clinical perspective, the overall prevalence of blaTEM (59%), qnrS (30.8%), and MDR (74.4%) suggest that empiric fluoroquinolone or cephalosporin monotherapy should be avoided for invasive Salmonella infections. Local testing of antimicrobial susceptibility should be undertaken for use in clinical therapy. The development of clinical guidelines for Pakistan that incorporates the epidemiology of documented resistance patterns is an urgent priority.

From a research standpoint, future investigations should utilize prospective designs that relate meat source Salmonella genotypes to clinical outcomes; this is important to eventually establish thresholds for risk stratification that are evidence-based [29]. It would also be worthwhile to utilize whole genome sequencing to look more deeply into the plasmid repertoires and potential horizontal transfer mechanisms that would accommodate the spread of qnrS [40]. Also, phenotypic virulence assays (epithelial cell invasion, macrophage colonization) would be an important component of assessing genotypes and their capacity to actually cause disease . [30]. Long-term surveillance tracking the temporal trends would assess whether food safety interventions were successful in reducing contamination and prevalence of resistance [41,42].

CONCLUSION

This study documents substantial contamination (17.3%) of Pakistani meat products with Salmonella, with almost universal virulence carriage and predominantly multidrug resistant phenotypes suggesting chicken is the highest risk product. Undertaking a cross-sectional design prohibits any inference of causality despite the documented virulence and resistance gene frequencies being much higher than what is reported based in the literature, this stress the urgency and need for intervening with food safety considerations when processing chicken potentially on several fronts. From a public health and clinical perspective, efforts to improve meat safety should be emphasized with education on the importance of judicious prescribing of antimicrobials informed from local epidemiology on resistance patterns. Future studies should attempt to utilize prospective designs to develop evidence-based approaches to linking genotypes with clinical outcomes, and additionally long-term surveillance efforts to assess intervention efficacy.

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