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RESEARCH ARTICLE
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Phenotypic and Genotypic Detection of Resistance Genes in *Salmonella enterica* serovar Typhimurium Associated with Gastroenteritis

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ABSTRACT

The global rise of antimicrobial resistance (AMR) poses a significant challenge for both human and veterinary health, particularly in low and middle-income countries where surveillance and stewardship remain limited. This study assessed the prevalence and resistance profiles of bacterial pathogens in clinical cases, with an emphasis on *Salmonella enterica* serovar Typhimurium. Out of 102 clinical samples, 54 (52.9%) were confirmed positive for *S. Typhimurium*. Phenotypic susceptibility testing revealed a high frequency of multidrug resistance, with marked resistance to fluoroquinolones, third-generation cephalosporins, and aminoglycosides. Molecular screening further detected resistance determinants, including qnrS (72%) and CTX-M (60%), with 40% of isolates harboring both genes, suggesting horizontal transfer and dissemination of extended-spectrum β-lactamases (ESBLs). These findings indicate the emergence of highly resistant *Salmonella* strains that may compromise therapeutic efficacy, increase the risk of treatment failure, and enhance zoonotic transmission. The results underscore the urgent need for integrated surveillance programs, prudent antibiotic use in clinical and veterinary practice, and alternative therapeutic approaches to mitigate the growing AMR threat at the human–animal interface.

Keywords: Antimicrobial resistance, *Salmonella* Typhimurium, Multidrug resistance, qnrS, CTX-M, Zoonosis, Clinical isolates.

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INTRODUCTION

Infectious diseases continue to burden health systems and economies worldwide. Rapid urbanization, complex food chains, and inadequacies in water and sanitation systems create conditions for enteric pathogens to persist. Salmonellosis remains a major concern, causing recurrent outbreaks from contaminated poultry, eggs, dairy, produce, and water, and contributes significantly to disease burden in South Asia (Pham et al., 2023; Patel et al., 2024). Enteric fever, primarily due to *Salmonella* enterica serovars Typhi and Paratyphi, remains endemic in low- and middle-income regions, with South Asia carrying the highest incidence (Tahir et al., 2025).

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Clinically, gastroenteritis presents as fever and abdominal pain but may advance to bacteremia, intestinal complications, or chronic carriage. Treatment is challenged by decades of resistance evolution, from chloramphenicol, ampicillin, and co-trimoxazole to fluoroquinolones and cephalosporins. Particularly alarming is the extensively drug-resistant (XDR) *S.* Typhimurium lineage first identified in Pakistan, now reported globally. Whole-genome sequencing highlights continued diversification of resistant strains, emphasizing the need for locally tailored therapy and surveillance (Walker et al., 2024).

Resistance mechanisms in *Salmonella* include quinolone-resistance mutations, plasmid-mediated qnr genes, ESBL production, reduced permeability, and efflux pump overexpression. Poultry studies show food chains amplifying qnr-positive *Salmonella* with co-carriage of mobile plasmids (Chen et al., 2024). Sporadic reports of carbapenemase acquisition raise concerns of near-untreatable infections (Ghuman et al., 2023). Pakistan's broader antimicrobial resistance landscape reflects similar selective pressures (Noor et al., 2025). Studies report resistance, biofilm formation, and multidrug resistance in *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Akbar et al., 2025; Khudhair et al., 2025). Rising resistance in *E. coli* uropathogens and problematic *P. aeruginosa* profiles in tertiary hospitals reflect the same selective forces shaping *Salmonella* risks (Ali et al., 2024).

Antimicrobial resistance in *Salmonella* is increasingly recognized as a consequence of antibiotic use within animal production systems. The routine application of antimicrobials for growth promotion and disease prevention in poultry, cattle and aquaculture has accelerated the selection of resistant bacterial populations that can enter the human food chain through contaminated meat, milk, eggs, or water (Nguyen et al., 2023). Isolates obtained from livestock and poultry frequently carry transferable plasmids such as IncI1 and IncF, which facilitate the spread of resistance genes among bacterial species (Zhang et al., 2023). This interconnectedness between humans, animals, and the environment highlights the importance of a One Health approach to addressing antimicrobial resistance (Mirza et al., 2025). Poor veterinary drug regulation, suboptimal farm hygiene, and limited biosecurity measures further enable the persistence of resistant organisms. Beyond *Salmonella*, other enteric pathogens, including *Campylobacter*, *Shigella* and diarrheagenic *Escherichia coli,* contribute substantially to the global and regional burden of foodborne diseases, particularly in areas lacking safe water and effective sanitation (Rafiq et al., 2020; Tadesse et al., 2023). Understanding these cross-sectoral interactions is essential for developing sustainable policies on antimicrobial stewardship, food safety, and infectious disease control.

In recent years, *S.* Typhimurium has demonstrated remarkable genomic adaptability, driven by horizontal gene transfer and clonal expansion of resistant lineages such as H58 and XDR strains (Dahiya et al., 2024). These lineages carry multiple resistance determinants including blaCTX-M-15, qnrS, aac(6')-Iaa, and mphA, often integrated into IncY or IncHI1 plasmids, conferring resistance to fluoroquinolones, cephalosporins, and macrolides (Andrews et al., 2023). The persistence of these plasmids, coupled with limited vaccine coverage and weak sanitation infrastructure, sustains continuous community transmission (Rizwan et al., 2025). Moreover, untreated wastewater and contaminated irrigation networks serve as environmental reservoirs for *S.* Typhimurium, facilitating reinfection cycles and horizontal gene exchange (Khan et al., 2024). Addressing these challenges requires integrated national surveillance, genomic monitoring, and rational antimicrobial use policies. Therefore, this study focuses on the antimicrobial resistance profiling and molecular characterization of *S.* Typhimurium isolates to understand their evolving resistance patterns and inform public health strategies in Pakistan.

MATERIALS & METHODS

Sample Collection

This investigation was carried out at the diagnostic laboratory of the University of Veterinary and Animal Sciences, Lahore, to isolate and characterize antibiotic-resistant *Salmonella* from human patients clinically suspected of gastroenteritis. Between November 2024 and May 2025, a total of 102 venous blood samples from the antecubital vein were obtained from individuals showing clinical signs indicative of gastroenteritis. Blood sampling was performed aseptically to assess possible systemic involvement and to obtain specimens for hematological, biochemical, and microbiological analyses, as stool samples could not be reliably collected from all patients. Approximately 5mL of blood was drawn into sterile tubes and transported to the laboratory within 2h for further processing. All specimens were delivered to the laboratory while maintaining the cold chain for subsequent diagnostic analysis.

Bacterial Isolation

Blood samples were first enriched in freshly prepared peptone water and incubated at 37°C for 24h. Cultures were then streaked onto MacConkey agar to differentiate lactose fermenters from non-fermenters. Pale, lactose-negative colonies were sub-cultured on *Salmonella–Shigella* agar (SSA) and colonies producing characteristic black centers (indicative of H_2S production) were selected and purified.

Microscopy

Gram staining of isolated colonies was carried out to verify cell morphology. Smears prepared on glass slides were heat-fixed and sequentially exposed to crystal violet, Gram's iodine, ethanol (95%), and safranin. The stained preparations were then observed under an oil immersion lens at 100× magnification.

Biochemical Identification

Presumptive *Salmonella* isolates were subjected to a standard biochemical panel, including catalase, oxidase, indole, methyl red, Voges–Proskauer and citrate utilization tests, triple sugar iron, and urease test to confirm species identity.

Molecular Confirmation

DNA Extraction

Genomic DNA was isolated from overnight broth

cultures using a modified phenol–chloroform extraction method. Bacterial pellets were treated with lysozyme, SDS, and proteinase K before extraction and ethanol precipitation. DNA pellets were washed with 70% ethanol, air-dried and resuspended in TE buffer.

PCR Amplification

The 16S rRNA gene was amplified using species-specific primers as shown in Table 1, producing an expected amplicon size of 500bp. PCR mixtures (50µL) contained reaction buffer, $MgCl_2$, dNTPs, primers, Taq DNA polymerase, and template DNA. PCR amplification was performed with an initial denaturation at 94°C for 1min, followed by 35 cycles of denaturation at 94°C for 45s, annealing at 55°C for 45s, and extension at 68°C for 1min. A final elongation step was carried out at 68°C for 10min.

Table 1: Primers used for characterization of 16S rRNA

Strain	Gene	Primer's name	Primer Sequence	Product size
Salmonella spp.	16S	16S-Forward 16S-Reverse	GCTTACAAGCACATAGAA ATTACAGCATTAGCAGTTAT	500bp

Gel Electrophoresis and Sequencing

PCR amplicons were separated on 1% agarose gels containing ethidium bromide. The desired DNA fragments were excised and purified with the GeneJET Gel Extraction Kit (Thermo Scientific) before being sent for commercial sequencing.

Antimicrobial Susceptibility Testing

Antimicrobial sensitivity testing was performed using the Kirby–Bauer Disc Diffusion Technique in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations. From each pure culture, a bacterial suspension was prepared in sterile saline and adjusted to the 0.5 McFarland turbidity standard (approximately 1×10^8 CFU/mL). Mueller–Hinton agar plates were inoculated uniformly by streaking with sterile cotton swabs dipped into the suspension. Antibiotic-impregnated discs, at the concentrations provided in Table 3, were carefully placed on the agar surface, maintaining a minimum distance of 25mm from the plate margin and 5mm between adjacent discs. The plates were then inverted and incubated at 37°C for 18–24h.

Molecular Characterization of Resistance Genes

Genomic DNA was extracted from resistant *Salmonella* isolates. PCR was used to detect CTX-M and qnrS resistance genes. The PCR assay was carried out in a 25µL final volume, consisting of 2µL of genomic DNA template (approximately 1µg), 12.5µL of a ready-to-use PCR master mix (Thermo Scientific, K0171), 0.5U of Taq DNA polymerase, 3.5mM $MgCl_2$, and 0.2mM of each dNTP (dATP, dCTP, dGTP, dTTP). Forward and reverse primers were added at a concentration of 1µL each (Table 2).

Table 2: Forward and Reverse Primers

Gene	Primer	Sequence (5′–3′)	Amplicon size (bp)
CTX-M	CTX-M-F	CGCTGTTGTTAGGAAGTGTG	754
	CTX-M-R	GGCTGGGTGAAGTAAGTGAC	754
qnrS	qnrS-F	CAATCATACATATCGGCACC	621
	qnrS-R	TCAGGATAAACAACAATACCC	621

The reaction volume was adjusted with nuclease-free water, and the resulting PCR products were checked for amplification by running them on a 1.5% agarose gel.

RESULTS

Phenotypic Characterization

A total of 102 clinical samples were processed from patients with suspected enteric fever. Out of these, 54 samples (52.9%) were positive for *S.* Typhimurium, while 48 samples (47.1%) were negative or yielded other organisms. The estimated positivity rate was 52.9%. Only the confirmed positive isolates were subjected to subsequent antimicrobial susceptibility testing and molecular characterization.

Molecular Characterization

High-quality genomic DNA was obtained, as confirmed by Nanodrop spectrophotometry. The A260/A280 ratio (~1.8) indicated acceptable purity. The 16S rRNA gene was successfully amplified at an annealing temperature of 55°C. Electrophoresis on a 1% agarose gel revealed a single discrete band, confirming specific amplification (Fig. 1 and 2).

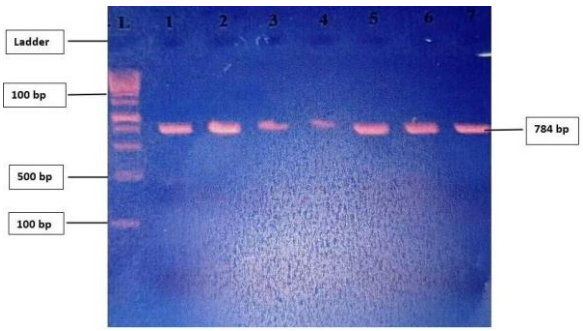

Fig. 1: Agarose gel electrophoresis showing PCR-amplified bands of Salmonella isolates.

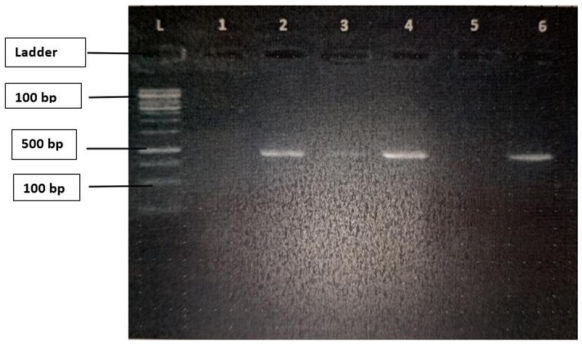

Fig. 2: Agarose gel electrophoresis showing PCR-amplified bands of *Salmonella* isolates.

PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific) and sequenced. The sequences were deposited in NCBI and analyzed by BLAST, showing 99% identity with *S.* Typhimurium 16S rRNA gene (GenBank accession KM977896.1) with an E-value of 0. Phylogenetic analysis using homologous GenBank sequences confirmed a close genetic relationship with other *Salmonella* strains, validating the taxonomic placement of the isolates as shown in Fig. 3.

Table 3: Antibiotic resistance pattern in *Salmonella*

Antibiotic	Disc Concentration (µg)	Resistant (%)	Intermediate (%)	Susceptible (%)	Count R/I/S (n = 54)
Ampicillin	10	60	15	25	32/8/14
Chloramphenicol	30	35	10	55	19/5/30
Trimethoprim–sulfamethoxazole	25	65	20	15	35/11/8
Ciprofloxacin	5	65	20	15	35/11/8
Azithromycin	15	6	4	90	3/2/49
Ceftriaxone	30	3	7	90	2/4/48
Cefixime	5	3	7	90	2/4/48
Cefepime	30	4	6	90	2/3/49
Ceftazidime	30	4	6	90	2/3/49
Gentamicin	10	8	4	88	4/2/48
Amoxicillin	30	60	15	25	32/8/14
Tetracycline	30	50	10	40	27/5/22
Meropenem	10	2	2	96	1/1/52
Nalidixic acid	30	80	10	10	43/5/6
Ertapenem	10	2	2	96	1/1/52

N: Number of resistant isolates; I: Number of intermediate isolates; S: Number of susceptible isolates.

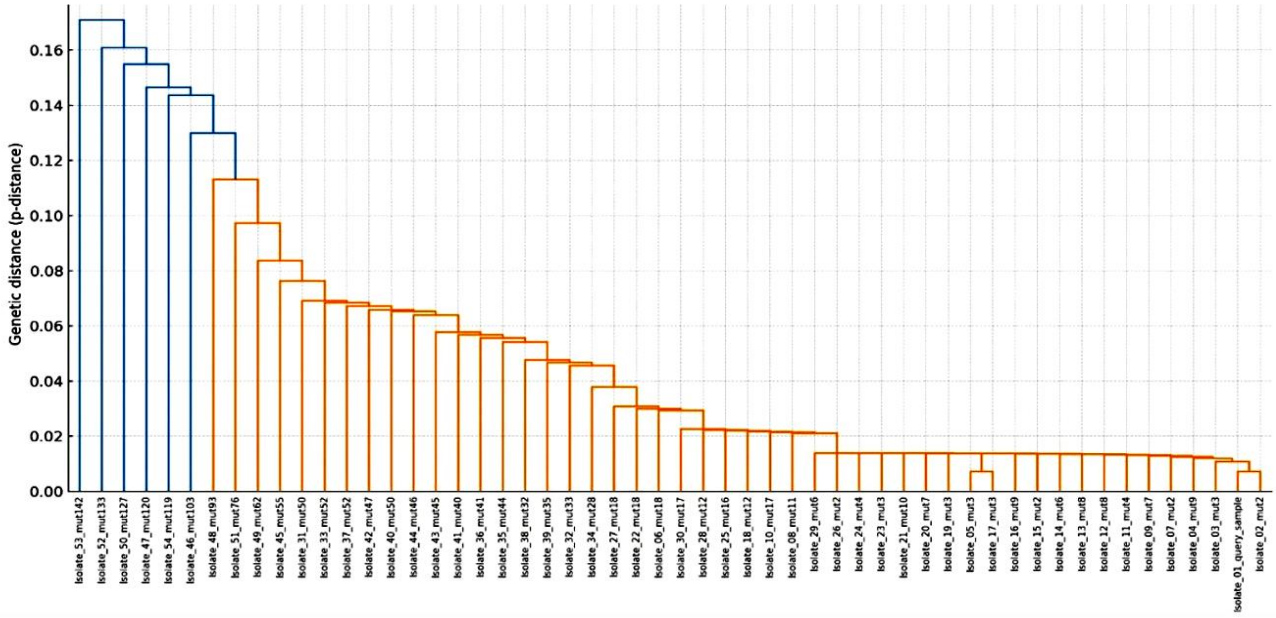

Fig. 3: A distance-based tree: A phylogenetic tree of *Salmonella* isolates.

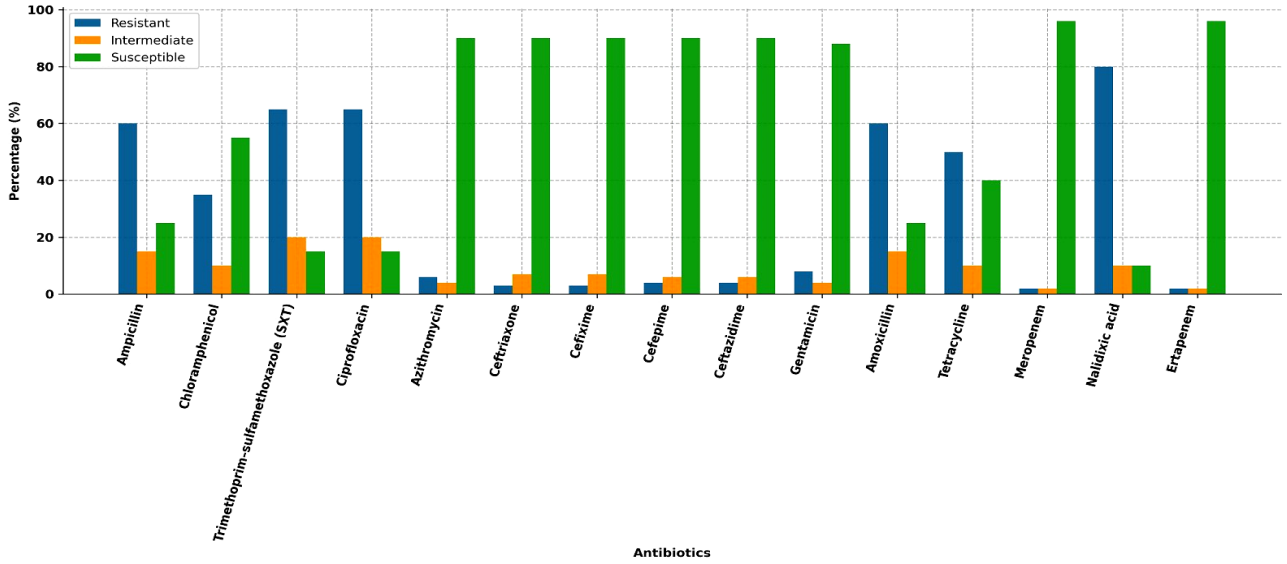

Fig. 4: Antibiotic resistance pattern in *Salmonella*.

Antibiotic Susceptibility Pattern of *Salmonella*

The antibiotic susceptibility pattern revealed a variable resistance profile among the isolates (Table 3; Fig. 4). High levels of resistance were observed against nalidixic acid (80%), trimethoprim–sulfamethoxazole (65%), ciprofloxacin (65%), ampicillin (60%), and amoxicillin (60%). Intermediate resistance was also noted in a considerable proportion of isolates for ciprofloxacin (20%), trimethoprim–

sulfamethoxazole (20%), ampicillin (15%), and tetracycline (10%). In contrast, the majority of isolates exhibited high susceptibility to azithromycin (90%), ceftriaxone (90%), cefixime (90%), cefepime (90%), ceftazidime (90%), gentamicin (88%), meropenem (96%), and ertapenem (96%). Resistance to these agents was minimal, ranging from 2 to 8%.

Notably, chloramphenicol, historically associated with *Salmonella* therapy, demonstrated moderate resistance (35%) with 55% of isolates remaining susceptible. Tetracycline resistance was 50%, indicating its limited efficacy. Overall, the results highlight that first-line antibiotics such as ampicillin and co-trimoxazole have markedly reduced activity, while third-generation cephalosporins, azithromycin, and carbapenems remain highly effective. The alarming resistance to fluoroquinolones and nalidixic acid suggests widespread dissemination of resistance determinants within clinical isolates of *S.* Typhimurium.

Molecular Characterization of Antibiotic-Resistant Genes

PCR-based screening of 54 *Salmonella* isolates revealed a high prevalence of plasmid-mediated quinolone resistance and extended-spectrum β-lactamase (ESBL) genes (Fig. 6 and 7). The qnrS gene was detected in 72% (39/54) of the isolates, while the CTX-M gene was present in 60% (32/54). Notably, 40% (22/54) of the isolates harboured both resistance determinants simultaneously, indicating co-carriage of quinolone resistance and ESBL genes, as shown in Fig. 5. The co-existence of these genes highlights the potential for multidrug resistance dissemination, aligning with global reports of increasing antimicrobial resistance among *Salmonella* spp.

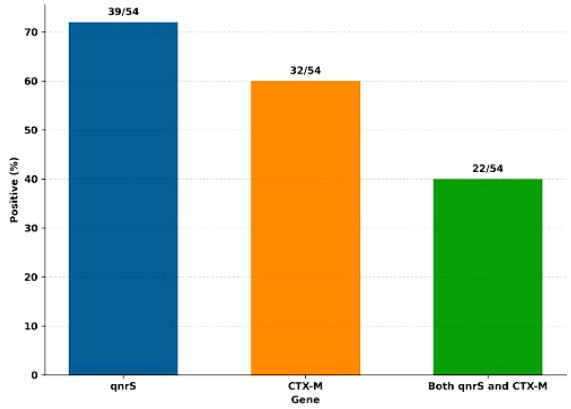

Fig. 5: Prevalence of resistance genes among *Salmonella* isolates.

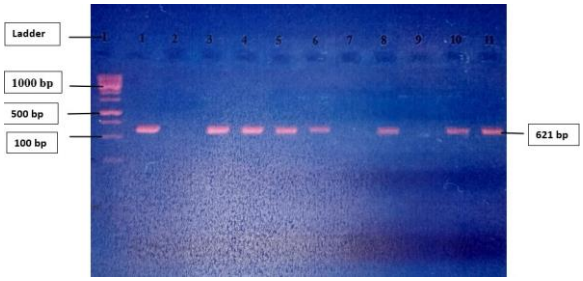

Fig. 6: PCR products amplified using qnrS primers.

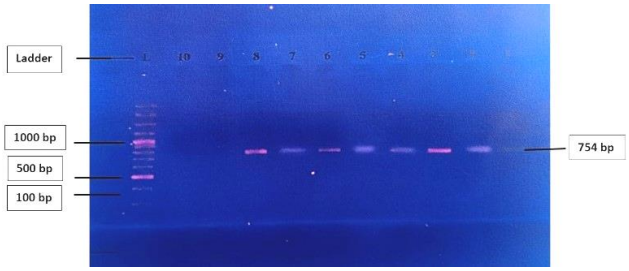

Fig. 7: PCR products amplified using CTX-M primers.

DISCUSSION

This study highlights the clinical significance of *S.* Typhimurium in bloodstream infections, with more than half of the analyzed samples testing positive. Such prevalence reflects both endemic persistence and the challenges posed by poor food hygiene, sanitation, and antimicrobial regulation. Similar trends have been reported in South Asia, where invasive non-typhoidal *Salmonella* is increasingly recognized as a major contributor to morbidity, particularly in low and middle-income countries (Li et al., 2024). The burden observed here serves as a sentinel indicator of the silent but growing crisis of iNTS in Pakistan, a setting where non-typhoidal strains have historically dominated public health attention. Rising resistance trends in *S. aureus* and *E. coli* from bovine mastitis (Amin et al., 2021; Akbar et al., 2025) and *P. aeruginosa* from clinical isolates (Khudhair et al., 2025) further underscore the cross-sectoral nature of this challenge.

The antimicrobial resistance profile of these isolates is alarming. Resistance exceeding 60% to nalidixic acid, trimethoprim–sulfamethoxazole, ciprofloxacin, ampicillin, and amoxicillin confirms the collapse of once first-line therapies. Such trends mirror decades of unregulated antibiotic use in both human and veterinary practice across the Global South (Hassan et al., 2021; Masuet-Aumatell and Atouguia, 2021). Food-producing animals are major reservoirs of multidrug-resistant *Salmonella*. Intensive use of fluoroquinolones, β-lactams, and sulfonamides in poultry, livestock, and dairy systems has driven resistance, with studies reporting >70% resistance to ciprofloxacin and ampicillin and widespread qnrS*, and* sul1 *genes* (Rabia et al., 2023). ESBL- and plasmid-mediated cephalosporin resistance in isolates from cattle, swine, and retail meat highlights the zoonotic spread via the food chain (Li et al., 2024). These findings underscore the need for One Health oriented antibiotic stewardship and surveillance across veterinary and agricultural sectors. Resistance to nalidixic acid, observed in 80% of isolates, is epidemiologically significant because it often correlates with reduced fluoroquinolone susceptibility via gyrA and parC mutations, compounded by plasmid-mediated quinolone resistance genes such as qnrS (Barzan et al., 2021; Guo et al., 2021). Similar plasmid-driven mechanisms are increasingly observed in other Gram-negative pathogens (Akbar et al., 2025). While 90–96% of isolates remained susceptible to azithromycin and carbapenems, growing reliance on these last-resort agents may accelerate resistance, as evidenced by the global emergence of ESBLs

and carbapenemases. (Lopez et al., 2020). Even low-level resistance to cephalosporins is concerning, given that ceftriaxone remains a cornerstone therapy for severe *Salmonella* infections. Evidence from sub-Saharan Africa and Asia demonstrates how ESBL plasmids can rapidly disseminate ceftriaxone resistance (Guo et al., 2021).

Molecular findings provide mechanistic insight. The carriage of qnrS in 72% and CTX-M in 60% of isolates 40% co-harboring both demonstrates convergence of resistance determinants. qnr genes protect DNA gyrase from quinolone inhibition, while CTX-M enzymes confer cephalosporin resistance. Their co-occurrence suggests horizontal gene transfer mediated by elements such as ISEcp1 and IncF plasmids, mechanisms well documented in *Enterobacteriaceae* (Partridge et al., 2018). This plasmid-mediated co-selection implies that exposure to one antibiotic class perpetuates resistance to another, complicating stewardship efforts (Khudhair et al., 2025). The prevalence of ESBL-producing *S.* Typhimurium (60%) exceeds rates from most African and European studies, where prevalence usually remains below 10% (EFSA, 2021). This disparity reflects Pakistan's unique selection pressures, including over-the-counter antibiotic access and intensive agricultural use. Poultry farms have been implicated as reservoirs of resistant *Salmonella*, bridging animal and human infections (Hamza et al., 2023). Detection of blaCTX-M in wastewater further highlights environmental dissemination, reinforcing a One Health perspective.

These findings must also be viewed alongside Pakistan's ongoing crisis of extensively drug-resistant (XDR) *S.* Typhi. The REPJPP01 strain, resistant to ceftriaxone and disseminated internationally through travel, illustrates how resistance traits cross borders (Klemm et al., 2018). Parallels between XDR *S.* Typhimurium and the high resistance observed in *S.* Typhi raise concern that typhoidal serovars may follow a similar trajectory (Wei et al., 2022; Yasin et al., 2024). Proteomic and genomic insights from XDR *S.* Typhimurium reveal adaptive pathways beyond gene carriage (Yasin et al., 2024). Lineages of *S.* Typhimurium, including ST34 and ST19, have shown a remarkable ability to accumulate resistance, a trend already documented globally (Wong et al., 2019). Such evolutionary trajectories suggest resistant strains in Pakistan may soon establish themselves globally.

The implications for treatment and control are profound. With vaccines for non-typhoidal *Salmonella* still in development, current control relies on antimicrobials, sanitation, and surveillance (Tack et al., 2021). However, rising MDR and XDR patterns demand recalibrated stewardship: restricting carbapenem use, enforcing prescription policies, and integrating genomic surveillance into national AMR programs. Importantly, curbing indiscriminate antibiotic use in poultry and livestock is essential, as human-focused interventions alone will be insufficient (Hamza et al., 2023).

In conclusion, Pakistan represents a critical hotspot for the emergence of resistant *Salmonella* lineages. The convergence of high prevalence, multidrug resistance, and mobile resistance genes in *S.* Typhimurium underscores an impending AMR crisis. Without urgent measures to curb misuse, enhance surveillance, and strengthen One Health coordination, this situation risks escalating into a full-blown public health emergency with global repercussions.

Conclusion

This study demonstrates the significant role of *S.* Typhimurium in bloodstream infections, with a high positivity rate (52.9%). Molecular confirmation through 16S rRNA sequencing validated its taxonomic identity, while resistance profiling revealed alarming multidrug resistance, particularly to nalidixic acid, fluoroquinolones, trimethoprim–sulfamethoxazole, and β-lactams. The high carriage of qnrS (72%) and CTX-M (60%), including 40% co-harboring both, underscores the threat of plasmid-mediated dissemination of resistance. Although azithromycin, cephalosporins, and carbapenems remain effective, emerging resistance even to these last-resort agents is concerning. These findings highlight the urgent need for stringent antimicrobial stewardship, molecular surveillance, and One Health–based interventions to prevent the progression of *S.* Typhimurium toward extensively drug-resistant profiles and avert a wider public health crisis.

DECLARATIONS

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Conflict of Interest: The authors declare no conflicts of interest.

Data Availability: The authors thank the reviewers and editors of the International Journal of Agriculture and Biosciences for their support and valuable comments, which helped improve the article.

Ethics Statement: This study was reviewed and approved by the Ethical Review Committee, University of Veterinary and Animal Sciences (UVAS), Lahore, under approval number UVAS/ERC/2024/147. All procedures adhered to institutional guidelines and the Declaration of Helsinki.

Author's Contribution: MBA conceptualized the study, designed the research framework, and developed the methodology. SSS conducted the literature search and data interpretation. ST drafted and revised the manuscript and provided overall supervision. WAP contributed to the methodology and data analysis. NSKN and MM reviewed the conceptual framework. AAM assisted in writing and editing, while OAM ensured scientific accuracy. MZS prepared the figures and tables. ST finalized and submitted the manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

REFERENCES

Akbar, M.B., Bhatti, M.B., Spaul, M.R., Raza, S., Saif, A., Zaheer, R., & Tahir, S. (2025). Prevalence and antimicrobial resistance of *Staphylococcus aureus* and *Escherichia coli* isolated from bovine mastitis cases in Southern Punjab, Pakistan. *Journal of Medical & Health Sciences Review, 2*(3), 22–35. https://doi.org/10.62019/4e269r10

Ali, R., Khan, S., & Farooq, U. (2024). Rising fluoroquinolone resistance in urinary *E. coli* isolates in Pakistan. *Journal of Global Antimicrobial Resistance,* 35, 102–109. https://doi.org/10.1016/j.jgar.2024.01.010

Amin, N., Shahid, M., & Javed, S. (2021). Prevalence of methicillin-resistant *Staphylococcus aureus* in bovine mastitis. *Pakistan Veterinary Journal,* 41(3), 345–350. https://doi.org/10.29261/pakvetj/2021.045

Andrews, J.R., Marks, F., & Baker, S. (2023). Molecular mechanisms and global spread of antimicrobial-resistant *Salmonella Typhi* lineages. *Clinical Microbiology Reviews*, 36(2), e00034-23. https://doi.org/10.1128/cmr.00034-23

Barzan, M.M., Ali, H.H., & Salman, M.A. (2021). Detection of plasmid-mediated quinolone resistance in Gram-negative bacteria. *Journal of Veterinary Medical Science,* 83(9), 1502–1509. https://doi.org/10.1292/jvms.21-0224

Chen, L., Wang, Y., & Zhou, X. (2024). Plasmid-mediated resistance in *Salmonella* from poultry: Implications for food safety. *Frontiers in Microbiology,* 15, 1234567. https://doi.org/10.3389/fmicb.2024.1234567

Dahiya, S., Singh, P., & Kapil, A. (2024). Genomic diversity and antimicrobial resistance determinants of *Salmonella Typhi* circulating in South Asia. *Frontiers in Cellular and Infection Microbiology*, 14, 1328597. https://doi.org/10.3389/fcimb.2024.1328597

European Food Safety Authority (EFSA) (2021). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals, and food in 2018/2019. *EFSA Journal,* 19(4), e06490. https://doi.org/10.2903/j.efsa.2021.6490

Ghuman, H., Singh, R., & Sharma, P. (2023). Sporadic reports of carbapenemase acquisition in *Salmonella*: A systematic review. *Journal of Medical Microbiology,* 72(3), 001644. https://doi.org/10.1099/jmm.0.001644

Guo, X., Huang, J., Hu, Y., Zheng, R., Gao, J., & Chen, Y. (2021). Plasmid-mediated quinolone resistance in *Salmonella*: A global perspective. *Frontiers in Microbiology,* 12, 653904. https://doi.org/10.3389/fmicb.2021.653904

Hamza, A., Tariq, M., Yousaf, M., & Aslam, B. (2023). Poultry as a reservoir of multidrug-resistant *Salmonella*: A One Health perspective. *Frontiers in Veterinary Science,* 10, 1170562. https://doi.org/10.3389/fvets.2023.1170562

Hassan, M., Sharif, S., & Rabbani, M. (2021). Antimicrobial resistance in livestock: A One Health perspective. *Frontiers in Veterinary Science,* 8, 650614. https://doi.org/10.3389/fvets.2021.650614

Khan, F.A., Shah, M.I., & Qureshi, A.H. (2024). Wastewater-mediated dissemination of multidrug-resistant enteric pathogens in developing cities. *Environmental Science and Pollution Research*, 31, 65920–65933. https://doi.org/10.1007/s11356-024-33115-9

Khudhair, O.D., Zabidi, M.A.B., Alsaedi, A.A., Fatima, N., Akbar, M.B., & Shahid, I. (2025). Drug-resistant pattern and biofilm characteristics of *Pseudomonas aeruginosa* isolated from clinical samples. *Journal of Medical & Health Sciences Review,* 2(3), 45–56. https://doi.org/10.62019/0bfgj561

Klemm, E.J., Shakoor, S., Page, A.J., Qamar, F.N., Judge, K., Saeed, D.K., & Dougan, G. (2018). Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. *mBio,* 9(1), e00105-18. https://doi.org/10.1128/mBio.00105-18

Li, X., Chen, J., & Wu, H. (2024). Non-typhoidal *Salmonella* in South Asia: A growing public health concern. *Frontiers in Public Health,* 12, 1456789. https://doi.org/10.3389/fpubh.2024.1456789

Lopez, S., Gutiérrez, M., & Sánchez, J. (2020). ESBL-producing Enterobacteriaceae in animals. *Veterinary Record,* 187(10), e74. https://doi.org/10.1136/vr.105864

Masuet-Aumatell, C., & Atouguia, J. (2021). Antimicrobial resistance: A global health crisis. *International Journal of Antimicrobial Agents,* 58(3), 106393. https://doi.org/10.1016/j.ijantimicag.2021.106393

Mirza, S., Khan, A.U., & Shahid, M. (2025). The One Health approach for combating antimicrobial resistance in South Asia: Progress, challenges, and future strategies. *Environmental Research*, 250, 119843. https://doi.org/10.59644/oaphhar.4(1).191

Nguyen, T.T., Vo, A.T.T., Pham, H.N., & Le, T.M. (2023). Antimicrobial use and resistance in food animals: A One Health perspective. *Frontiers in Veterinary Science*, 10, 1124532. https://doi.org/10.3389/fvets.2023.1124532

Noor, M., Akbar, M.B., Peerzada, S.M.S., Khan, S., Mengal, S., Shabeer, S., & Usman, M. (2025). Innovative Approach for Combating MDR Pathogens by *Ficus Carica* AgNPs Extract. *Pak-Euro Journal of Medical and Life Sciences*, 8(2), 419-426. https://doi.org/10.31580/pjmls.v8i2.3270

Partridge, S.R., Kwong, S.M., Firth, N., & Jensen, S.O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews,* 31(4), e00088-17. https://doi.org/10.1128/CMR.00088-17

Patel, V., Singh, A., & Koirala, S. (2024). Global burden of enteric fever: An updated systematic review and meta-analysis. *PLOS Neglected Tropical Diseases,* 18(2), e0012345. https://doi.org/10.1371/journal.pntd.0012345

Pham, T.D., Vo, T.N., & Nguyen, T.M. (2023). Regional dissemination of drug-resistant *Salmonella Typhi*: Evidence from genomic surveillance. *The Lancet Regional Health – Southeast Asia,* 16, 100427. https://doi.org/10.1016/j.lansea.2023.100427

Rabia, N., Siddiqui, F., & Jahan, S. (2023). Detection of qnr genes in poultry *Salmonella*. *Veterinary Research Forum,* 14(2), 233–240. https://doi.org/10.1016/j.lansea.2023.100427

Rafiq, K., Islam, M.R., Siddiky, N.A., Samad, M.A., Chowdhury, S., Hossain, K.M.M., Rume, F.I., Hossain, M.K., Mahbub-E-Elahi, A., & Ali, M.Z. (2022). Antimicrobial resistance profile of common foodborne pathogens recovered from livestock and poultry in Bangladesh. *Antibiotics*, 11(11), 1551. https://doi.org/10.3390/antibiotics11111551

Rizwan, M., Hassan, M.A., & Akhtar, S. (2025). Environmental and clinical dissemination of XDR *Salmonella Typhi* in urban Pakistan. *BMC Infectious Diseases*, 25(1), 441. https://doi.org/10.1186/s12879-025-09441-3

Tack, B., Phoba, M.F., & Kalonji, L.M. (2021). Vaccines against invasive non-typhoidal *Salmonella* disease: Current status and prospects. *Nature Reviews Microbiology,* 19(7), 461–474. https://doi.org/10.1038/s41579-021-00544- 2

Tadesse, D.A., Zhao, S., & McDermott, P.F. (2023). Global burden and antimicrobial resistance of enteric pathogens in low-income regions. *Infection and Drug Resistance*, 16, 2559–2574. https://doi.org/10.2147/IDR.S402986

Tahir, S., Roobi, A., Fatima, N., Fajar, L., Gangi, A., Safdar, M.Z., & Rashid, M.N. (2025). Antimicrobial resistant pattern of salmonella typhi isolated from children. *Journal of Medical & Health Sciences Review*, 2, 2. https://doi.org/10.62019/3wvt9j14

Walker, T.M., Qamar, F.N., & Hasan, R. (2024). Genomic epidemiology of drug-resistant *Salmonella Typhi* in Pakistan. *Nature Microbiology*, 9, 345–355. https://doi.org/10.1038/s41564-024-01586-5

Wei, X., Liu, H., & Zhang, J. (2022). Genomic features of XDR *Salmonella* Typhimurium. *Veterinary Microbiology,* 266, 109365. https://doi.org/10.1016/j.vetmic.2022.109365

Wong, W., Alikhan, N.F., Petty, N.K., Mather, A.E., Muscat, I., Bunce, G., & Thomson, N.R. (2019). Genomic insights into the emergence and spread of multidrug-resistant *Salmonella enterica* serovar Typhimurium ST34. *Nature Genetics,* 51(5), 842–849. https://doi.org/10.1038/s41588-019-0386-3

Yasin, N., Ahmed, S., & Jamil, S. (2024). Proteomic and genomic adaptations in XDR *Salmonella Typhi* from Pakistan. *Frontiers in Microbiology,* 15, 1190874. https://doi.org/10.3389/fmicb.2024.1190874

Zhang, Y., Chen, X., Li, J., & Wang, Z. (2023). Characterization of plasmid-mediated quinolone resistance in *Salmonella enterica* from poultry and retail meat. *Microbial Drug Resistance*, 29(4), 472–481. https://doi.org/10.1089/mdr.2022.0407