Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387 Print ISSN 3007-2379

Molecular Detection and Antimicrobial Resistance Profiling of Salmonella spp. in Commercial Poultry Farms

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Keywords: Salmonella spp., nontyphoidal Salmonella, poultry, molecular detection, PCR, CRISPR-SeroSeq, antimicrobial resistance, multidrug resistance, zoonotic foodborne illness, prevalence, biosecurity

Received on 5 Apr 2026

Accepted on 7 May 2026

Published on 17 May 2026

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Abstract

Salmonella spp., particularly nontyphoidal serovars, remain a leading cause of zoonotic foodborne illness globally, with commercial poultry serving as a primary reservoir and contributing to an estimated 153 million human cases and over 155,000 deaths annually. This review synthesizes recent advances in molecular detection and antimicrobial resistance (AMR) profiling of Salmonella in poultry production systems. Conventional culture-based methods are increasingly supplemented or replaced by rapid, sensitive techniques including PCR (targeting invA, ttr, hilA), real-time qPCR, loop-mediated isothermal amplification (LAMP), digital PCR, and CRISPR-based serotyping (CRISPR-SeroSeq), enabling high-throughput identification and serovar discrimination even in complex matrices (cloacal swabs, litter, embryonated eggs). Prevalence varies widely (pooled estimates 12–14% in meta-analyses from Ethiopia, Bangladesh, and Nepal), with S. Typhimurium, S. Enteritidis, and S. Kentucky frequently dominant. AMR profiling reveals alarming trends of multidrug resistance (MDR), including resistance to critically important classes (fluoroquinolones, third-generation cephalosporins, colistin), driven by extended-spectrum β-lactamases (ESBLs), plasmid-mediated quinolone resistance (PMQR), and integrons. Horizontal gene transfer and biofilm formation further complicate control. Integrated strategies—stringent biosecurity, vaccination,

competitive exclusion, and prudent antimicrobial stewardship—are emphasized to mitigate transmission and preserve treatment efficacy in the face of rising AMR threats.

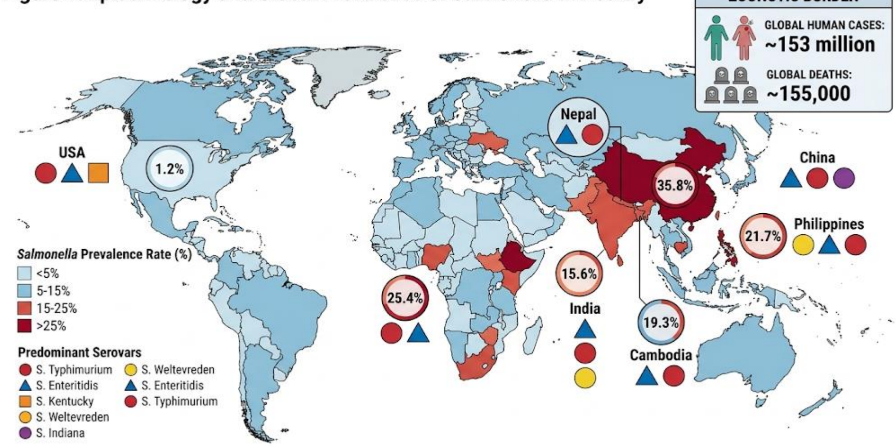
1. Introduction

The global poultry industry represents a cornerstone of agricultural economics and food security, yet it remains one of the most significant reservoirs for Salmonella species, a genus of Gram-negative, facultative anaerobic bacilli within the Enterobacteriaceae family (Basazinew et al., 2025). Nontyphoidal Salmonella (NTS) is a primary etiological agent of zoonotic gastroenteritis, responsible for an estimated 1.35 million illnesses and 420 deaths annually in the United States alone, with global estimates reaching 153 million cases and over 155,000 deaths (Frontiers in Microbiology, 2025). The persistence of Salmonella in commercial poultry complexes is facilitated by its ability to undergo both vertical transmission through the egg and horizontal transmission via environmental contamination, vectors, and direct contact (Siceloff, 2024). As international trade and intensive production systems expand, the challenges associated with managing Salmonella are compounded by the emergence of multidrug-resistant (MDR) strains, which significantly limit therapeutic options and increase the public health burden (Djemaioune et al., 2025).

2. Epidemiological Trends and Global Prevalence Dynamics

The prevalence of Salmonella in commercial poultry is highly variable, reflecting differences in geographic climate, biosecurity rigor, and the maturity of national surveillance programs (Siceloff, 2025). Systematic reviews and meta-analyses covering the period through 2025 highlight an overall pooled prevalence in some regions of approximately 12.46%, although specific localized studies report rates ranging from 2.20% in broad cross-matrix surveys to nearly 100% in certain retail environments (Tsogtbayar et al., 2025). Salmonella prevalence in poultry varies widely across regions, influenced by production systems, biosecurity, and sampling matrices. A global overview of prevalence and dominant serovars is summarized in Figure 1.

Figure 1: Epidemiology and Global Prevalence of *Salmonella* in Poultry



2.1 Regional Variation and Metadata Analysis

In Ethiopia, meta-regression models indicate that the highest pooled prevalence is found in the Western region (23.18%), with broilers exhibiting significantly higher infection rates (28.23%) compared to other production types (Chu et al., 2024). Age is a critical determinant, as poultry under 6 months of age show a higher susceptibility (14.45%) due to incomplete immune development. Research in Bangladesh has identified a 14.4% prevalence at the farm level, with layers showing higher rates than broilers, often attributed to longer production cycles that increase exposure time to environmental reservoirs (Siddiky et al., 2025). Recent surveillance of dead-in-shell embryonated eggs in Nepal further revealed a prevalence of 11.42%, identifying S. Kentucky and S. Typhimurium as significant isolates (Khanal et al., 2025).

Table 1. Regional Variation and Pooled Prevalence of Salmonella in

Commercial Poultry

Geographic Region	Pooled Prevalence (%)	Predominant Serovars	Primary Sample Type
Ethiopia (National)	12.46%	S. Typhimurium, S. Enteritidis	Feces, Litter, Cloacal (Abdi et al., 2025)
Bangladesh	14.4%	S. Typhimurium, S. Enteritidis	Feces, Cloacal (Siddiky et al., 2025)
India (Patna/Haryana)	23.7%	S. Gallinarum, S. Typhimurium	Meat, Broiler Flocks (Microbiology Journal, 2025)
Cambodia	6.30%	S. Weltevreden, S. Typhimurium	Farm Swabs (Cambodian Journal, 2025)
Philippines	37.5%	S. Infantis, S. Brancaster	Retail Cut Samples (Frontiers in Microbiology, 2025)
China (Shandong)	18.2%	S. Infantis, S. Derby	Retail Chicken (Chu et al., 2024)
USA (Organic)	37.08%	S. Kentucky, S. Infantis	Carcass Rinse (Marchello et al., 2020)
USA (non-organic)	61.67%	S. Kentucky, S. Infantis	Carcass Rinse (Marchello et al., 2020)

3. Molecular Detection Methodologies: Evolution and Optimization

The necessity for rapid and accurate detection has driven a shift from traditional culture-based "gold standard" methods to molecular diagnostics. Traditional protocols, such as ISO 6579-1:2017, involve sequential enrichment and can take 3 to 5 days for a definitive result (Awang et al., 2021).

3.1 Polymerase Chain Reaction and Genetic Markers

The most frequently targeted genetic marker for Salmonella identification is the invA gene, which encodes a component of the Type III Secretion System essential for host cell invasion (Mohammed, 2024). Multiplex PCR allows for the simultaneous detection of multiple serovars or resistance genes, such as the spy gene for S. Typhimurium and the sdfI gene for S. Enteritidis (Worley, 2025).

3.2 AI-Powered Diagnostics and Vision Transformers

Novel diagnostic platforms are increasingly integrating Artificial Intelligence (AI) to enhance detection speed. A Modified Hierarchical Vision Transformer (HViT) model has been developed to detect Salmonella and other poultry diseases from fecal images, achieving a validation accuracy of 90.90% (IET Image Processing, 2025).

Similarly, MobileNetV2-based systems have demonstrated 98.02% accuracy in distinguishing Salmonella infections in resource-constrained settings, providing a scalable, farmer-friendly tool for early diagnosis (Poultry Science, 2025).

3.3 MALDI-TOF MS and Proteomic Profiling

Matrix-Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a transformative tool for the rapid detection of AMR. By analyzing metabolic or proteomic changes induced by antibiotic exposure, this phenotypic method enables the direct inference of resistance in just 2 to 4 hours (Beyer, 2025). Recent workflows using MALDI-TOF MS have achieved high accuracy in detecting resistance across multiple classes, including the direct identification of hydrolyzed beta-lactam products (Chung et al., 2023).

3.4 Isothermal Amplification and Point-of-Care Tools

Loop-mediated Isothermal Amplification (LAMP) has emerged as a powerful alternative to PCR for field applications, operating at a constant temperature of 60 to 65 degrees C (Zhang et al., 2014). Recent advancements have optimized HNB-based LAMP assays to detect the aadA1 gene responsible for streptomycin resistance in as little as 35 minutes with high sensitivity (Veterinary Sciences, 2025).

3.5 Digital PCR and Absolute Quantification

Digital PCR (dPCR) represents the third generation of PCR, enabling absolute quantification of target DNA without external standard curves (Cleveland et al., 2024). This technology is extremely tolerant to PCR inhibitors common in poultry rinses, such as peracetic acid and chlorine, and can provide a standardized estimate of Salmonella load in 8 to 9 hours (Mao et al., 2019).

4. Genomic Characterization and High-Resolution Surveillance

Whole-Genome Sequencing (WGS) has transformed Salmonella surveillance by consolidating serotyping, virulence profiling, and AMR genotyping into a single workflow (Clark, 2021).

4.1 CRISPR-SeroSeq and Deep Serotyping

CRISPR-SeroSeq analyzes the spacers within CRISPR arrays to detect multiple serovars within a single sample, overcoming the bias of culture-based methods that often isolate only the dominant strain (Thompson et al., 2018). This method has identified multiserovar populations in 18% of environmental samples from broiler breeder farms, revealing complex dynamics where certain serovars, like S. Kentucky, may exclude others (Quinn et al., 2023).

5. Antimicrobial Resistance (AMR): Mechanisms and Profiling

Widespread antimicrobial use in poultry has led to high rates of multidrug resistance. In Algeria, 100% of S. Gallinarum isolates from laying hens exhibited resistance to quinolones, aminoglycosides, and sulfonamides (Djemaioune et al., 2025).

Table 2. Key Resistance Genes and Molecular Mechanisms Associated with Salmonella AMR

Antimicrobial Class	Key Resistance Genes	Molecular Mechanism	Notable Prevalence/Findings
Beta-lactams	blaTEM, blaSHV, blaCTX-M-65	Enzymatic Hydrolysis	92.68% carry blaCTX-M (Nigeria Study, 2021)
Tetracyclines	tetA, tetB	Efflux Pump	89.2% of TET-R isolates harbor tetB (Abdi et al., 2025)

Sulfonamides	sul1, sul2	Target Bypass (DHPS)	94.4% resistance in Bangladesh (Siddiky et al., 2025)
Fluoroquinolones	qnrS, qnrB, gyrA mutations	Target Protection	21.13% qnr prevalence in China (Chu et al., 2024)
Aminoglycosides	aadA1, aadA2, strA/B	Enzymatic Inactivation	Associated with S. Infantis pESI (Nhung et al., 2024)

5.1 The Role of the pESI Megaplastic in S. Infantis

The emergence of MDR S. Infantis is inextricably linked to the acquisition of the pESI megaplasmid (approximately 300 kb), which carries ESBL, tetracycline, and sulfonamide resistance genes. pESI-positive strains demonstrate increased biofilm formation and environmental fitness, facilitating their spread through the broiler production chain (Kim et al., 2024).

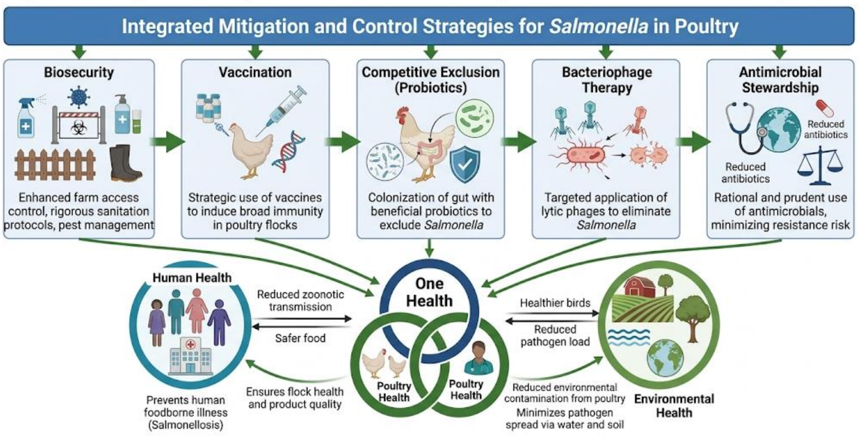
6. Contributing Factors to Salmonella Persistence and AMR Spread

The presence of Salmonella and the dissemination of resistance are driven by a multifactorial system encompassing management practices and environmental reservoirs. Large flock sizes (more than 8,000 to 10,000 birds) are statistically associated with increased Salmonella prevalence (Lamichhane et al., 2024). Poor biosecurity protocols, such as sourcing chicks from multiple multiplication centers, facilitate the entry of various serovars (Berry & Wells, 2018).

7. Integrated Mitigation and Control Strategies

Combating Salmonella requires balancing biosecurity with innovative alternatives to antibiotics. Recent research has focused on encapsulated leads for AMR mitigation, which have proven safe for commensal gut lactobacilli while effectively reducing pathogen load (Sindhushree et al., 2025). Effective control of Salmonella requires a multifaceted One Health strategy integrating biosecurity, vaccination, probiotics, bacteriophage therapy, and prudent antimicrobial use. A summary of these mitigation measures is shown in Figure 2.

Figure 2: Integrated Mitigation and Control Strategies for *Salmonella* in Poultry



7.1 Probiotics and Bacteriophage Therapy

Probiotics like Lactobacillus and Bifidobacterium promote gut health through competitive exclusion, adhering to the mucosa and producing short-chain fatty acids. Therapeutic bacteriophages offer a cost-effective tool for selectively lysing Salmonella without disrupting the beneficial microbiota (Mills, 2025).

8. Conclusion

The persistence of Salmonella in commercial poultry systems, coupled with the rapid emergence and dissemination of multidrug-resistant strains, poses a persistent and escalating threat to public health and food safety worldwide. Molecular diagnostics particularly real-time PCR, LAMP, digital PCR, and CRISPR-based serotyping have revolutionized surveillance by providing rapid, sensitive, and serovar-specific detection that outperforms traditional culture methods, enabling earlier intervention and traceability across the production chain. Concurrently, AMR profiling underscores the urgent need for global harmonized monitoring, as resistance to last-resort antimicrobials (e.g., colistin, carbapenems) compromises both human and veterinary therapeutics. Effective mitigation demands a One Health approach integrating enhanced biosecurity, Salmonella-free breeder programs, targeted vaccination, competitive exclusion probiotics, and strict antimicrobial stewardship policies. Continued investment in molecular epidemiology, whole-genome sequencing for resistance gene tracking, and international collaboration will be essential to curb transmission, reduce zoonotic burden, and safeguard poultry-derived food supplies in an era of intensifying production and AMR pressure.

REFERENCES

Basazinew, E., Dejene, H., & Tegegne, D. (2025). A systematic review and meta-analysis of salmonellosis in poultry farms in Ethiopia: prevalence, risk factors, and antimicrobial resistance. Frontiers in Veterinary Science, 12, Article 1538963. https://doi.org/10.3389/fvets.2025.1538963

Beyer, A. (2025). MALDI–TOF MS as a leading candidate for next-generation diagnostic workflows in antimicrobial resistance profiling. *Antibiotics*, 14(3), 221. https://doi.org/10.3390/antibiotics14030221

Chu, X., Li, Y., Wang, Y., Zhang, Y., Li, J., Wang, X., ... & Wu, C. (2024). Prevalence and molecular characteristics of qnr-positive Salmonella strains from chicken farms and markets in China. Microorganisms, 12(4), Article 767. https://doi.org/10.3390/microorganisms12040767

Clark, T. (2021). Using genomics to trace Salmonella transmission and antimicrobial resistance. *London School of Hygiene and Tropical Medicine*.

Djemaioune, A., Todorović, D., Novović, K., Dramlić, S. M., Cherak, Z., Miković, S., Ayachi, A., & Rašeta, M. (2025). High level fluoroquinolone resistance and multidrug resistance in Salmonella spp. isolated from poultry, turkey flocks and slaughterhouses in Algeria. Acta Veterinaria, 75(3), 309–325. https://doi.org/10.2478/acve-2025-0024

Frontiers in Microbiology. (2025). Multidrug-resistant Salmonella serovars in chicken meat from wet markets in Metro Manila, Philippines. *Frontiers in Microbiology*, 16, 1496685. https://doi.org/10.3389/fmicb.2025.1496685

IET Image Processing. (2025). A modified hierarchical vision transformer model for poultry disease detection. *IET Image Processing*, 19(1). https://doi.org/10.1049/ipr2.70115

Mills, J. (2025). Innovative NGS workflow accelerates Salmonella detection and typing, aiding control. *Modern Poultry Media*.

Kim, M. B., Lee, Y. J., & others. (2024). Genomic analysis of pESI-like megaplasmid in Salmonella Infantis from the poultry industry in Korea. Veterinary Microbiology, 299, Article 110283. https://doi.org/10.1016/j.vetmic.2024.110283

Poultry Science. (2025). Accessible AI-powered poultry disease diagnostics: Development, validation, and web deployment of a farmer-friendly MobileNet-based system. *Poultry Science*, 105(3), 106338. https://doi.org/10.1016/j.psj.2025.106338

Siceloff, A. (2024). *Deep serotyping by CRISPR-SeroSeq to investigate multiserovar Salmonella population dynamics in domestic poultry production* (Doctoral dissertation). University of Georgia.

Siddiky, A., Uddin, M. M., Akter, M. R., Islam, M. S., & Hossain, M. A. (2024). Prevalence, serovars, virulence genes, and antimicrobial resistance of Salmonella in poultry farms in Bangladesh. Frontiers in Microbiology, 15, Article 1365873. https://doi.org/10.3389/fmicb.2024.1365873

Sindhushree, S. R., Pradeep, S., Sai Chakith, M. R., Reddy, P., & Achar, R. R. (2025). Encapsulated leads for mitigating antimicrobial resistance in poultry: Safety and efficacy analysis. *ICAR Annual Report 2022-23*.

Veterinary Sciences. (2025). Simple, rapid, and low-cost molecular method known as loop-mediated isothermal amplification (LAMP) to detect the aadA1 gene. *Veterinary Sciences*, 12(11), 1094. https://doi.org/10.3390/vetsci12111094

Zhang, X., Li, Y., Khan, A., Ali, M., Chen, J., & Wang, Z. (2014). Loop-mediated isothermal amplification for the rapid detection of Salmonella. *Journal of Food Safety*.

Siceloff, A. T. (2025). High-Resolution Salmonella Surveillance in Commercial Broiler Breeder Production (Doctoral dissertation, University of Georgia).

Tsogtbayar, O., Munkhbayarlakh, B., Dorjsurenkhor, N., & Gray, G. C. (2025). Major Livestock-associated Zoonoses in Mongolia: An Overview. Zoonoses (Shannon, Ireland), 5(1), 23.

Khanal, S., Luitel, H., Adhikari, S., Marasini, A., & Bhattarai, R. K. (2025). Molecular characterization, antimicrobial resistance profiling, and biofilm analysis of Salmonella isolates from dead-in-shell embryonated eggs. Poultry Science, 105773.

Awang, M. S., Bustami, Y., Hamzah, H. H., Zambry, N. S., Najib, M. A., Khalid, M. F., ... & Abd Manaf, A. (2021). Advancement in Salmonella detection methods: from conventional to electrochemical-based sensing detection. Biosensors, 11(9), 346.

Mohammed, B. T. (2024). Identification and bioinformatic analysis of invA gene of Salmonella in free range chicken. Brazilian Journal of Biology, 84, e263363.

Worley, M. J. (2025). Salmonella type III secretion system effectors. International Journal of Molecular Sciences, 26(6), 2611.

Chung, C. R., Wang, H. Y., Chou, P. H., Wu, L. C., Lu, J. J., Horng, J. T., & Lee, T. Y. (2023). Towards accurate identification of antibiotic-resistant pathogens through the ensemble of multiple preprocessing methods based on MALDI-TOF spectra. International journal of molecular sciences, 24(2), 998.

Cleveland, M. H., He, H. J., Milavec, M., Bae, Y. K., Vallone, P. M., & Huggett, J. F. (2024). Digital PCR for the characterization of reference materials. Molecular aspects of medicine, 96, 101256.

Mao, X., Liu, C., Tong, H., Chen, Y., & Liu, K. (2019). Principles of digital PCR and its applications in current obstetrical and gynecological diseases. American journal of translational research, 11(12), 7209.

Thompson, C. P., Doak, A. N., Amirani, N., Schroeder, E. A., Wright, J., Kariyawasam, S., ... & Shariat, N. W. (2018). High-resolution identification of multiple Salmonella serovars in a single sample by using CRISPR-SeroSeq. Applied and Environmental Microbiology, 84(21), e01859-18.

Quinn, M. W., Linton, N. F., Leon-Velarde, C. G., & Chen, S. (2023). Application of a CRISPR sequence-based method for a large-scale assessment of Salmonella serovars in Ontario poultry production environments. Applied and Environmental Microbiology, 89(3), e01923-22.

Lamichhane, B., Mawad, A. M., Saleh, M., Kelley, W. G., Harrington, P. J., Lovestad, C. W., ... & Helmy, Y. A. (2024). Salmonellosis: an overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistant infections. Antibiotics, 13(1), 76.

Berry, E. D., & Wells, J. E. (2018). Reducing foodborne pathogen persistence and transmission in animal production environments: challenges and opportunities. Preharvest Food Safety, 177-203.