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Whole-genome sequencing and antimicrobial resistance profiling of salmonella enterica serovars from poultry farms

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Abstract

How effectively can Whole-Genome Sequencing (WGS) unravel the antimicrobial resistance (AMR) landscape of *Salmonella enterica* circulating in commercial poultry operations? This research addressed that question by examining 240 cloacal swab samples collected from eight broiler farms across the Gyeonggi Province of South Korea between March 2023 and February 2024. *Salmonella* was isolated using standard enrichment and selective plating, yielding 87 confirmed isolates belonging to five serovars: *S. Enteritidis* (36.8%), *S. Typhimurium* (27.6%), *S. Infantis* (19.5%), *S. Gallinarum* (10.3%), and *S. Albany* (5.8%). All 87 isolates underwent WGS on an Illumina MiSeq platform with 2 x 250 bp paired-end chemistry, and the assembled genomes were screened against the ResFinder and CARD databases. A total of 23 distinct AMR genes were detected, with *bla*TEM-1 (78.2%), *tetA* (71.3%), and *sul1* (64.4%) being the most prevalent. Multi-drug resistance, defined as resistance to three or more antimicrobial classes, was observed in 62.1% of all isolates and reached 83.3% among *S. Typhimurium*. Phylogenetic reconstruction based on core-genome single nucleotide polymorphisms revealed two dominant clonal clusters of *S. Enteritidis* shared across geographically separate farms, pointing to a possible common contamination source. These findings highlight the value of integrating WGS-based surveillance into poultry health programmes to track resistance gene flow and support targeted intervention.

Keywords: Whole-genome sequencing, antimicrobial resistance, *Salmonella enterica*, poultry farms, AMR genes, serovar diversity, multi-drug resistance, genomic epidemiology, ResFinder, cloacal swabs

Introduction

What drives the persistence and spread of antimicrobial-resistant *Salmonella* within intensively managed poultry flocks? That question has gained urgency as global consumption of poultry meat continues to rise and resistance rates climb in tandem ^[1]. *Salmonella enterica* remains one of the leading foodborne zoonotic pathogens, responsible for an estimated 93.8 million cases of gastroenteritis worldwide each year ^[2]. Poultry are recognised as a major reservoir, and contamination of carcasses during processing creates a direct route of human exposure ^[3].

Antimicrobial resistance in *Salmonella* is not a new concern, but the pace at which resistance determinants accumulate has accelerated. Widespread prophylactic and therapeutic use of antibiotics in poultry production, especially tetracyclines, beta-lactams, and sulfonamides, exerts strong selection pressure on commensal and pathogenic bacteria alike ^[4]. Once resistance genes become established on mobile genetic elements such as plasmids and integrons, they can transfer horizontally between strains and even between species ^[5]. Traditional phenotypic susceptibility testing provides a snapshot of resistance, yet it cannot identify the specific genetic mechanisms involved or trace how those mechanisms move through bacterial populations.

Whole-genome sequencing has transformed the capacity to investigate AMR at molecular resolution. By interrogating the complete chromosomal and plasmid content of an isolate, WGS simultaneously identifies resistance genes, virulence factors, serotype determinants, and

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phylogenetic relationships [6]. Large-scale WGS-based surveillance programmes in Europe and North America have already shown their worth in outbreak detection and source attribution [7, 8]. In the Republic of Korea, however, poultry-associated *Salmonella* has been monitored largely through phenotypic methods and limited PCR-based gene screening, leaving a gap in genomic-level understanding [9].

The Gyeonggi Province surrounding Seoul supports a high density of commercial broiler farms and acts as a key node in the national poultry supply chain [10]. Despite this, no comprehensive WGS survey of *Salmonella* serovars from the region had been reported at the time this research began. The present work therefore aimed to (i) determine the serovar distribution and AMR gene repertoire of *Salmonella enterica* isolated from eight broiler farms over a 12-month period, (ii) characterise the genomic context of key resistance determinants, and (iii) reconstruct the phylogenetic relationships among isolates to assess clonal transmission patterns. The findings are intended to support evidence-based decisions on antimicrobial stewardship in Korean poultry production.

Materials and Methods

Materials

Cloacal swab samples ($n = 240$) were collected from apparently healthy broiler chickens across eight commercial farms located in four districts of Gyeonggi Province, South Korea. The research was conducted from March 2023 to February 2024, with each farm sampled once per quarter, yielding four collection rounds. Thirty swabs per farm were obtained during each visit, targeting birds at market age (35–42 days). Swabs were transported in Cary-Blair medium at 4 degrees Celsius and processed within 6 hours of collection. Ethical clearance for the sampling protocol was granted by the Institutional Animal Care and Use Committee of the Seoul Institute of Veterinary Biosciences (Protocol No. SIVB-IACUC-2023-017, approved 12 January 2023). Farm selection was stratified by production capacity: four high-capacity farms (more than 50,000 birds per cycle) and four medium-capacity farms (20,000–50,000 birds per cycle).

Reagents and Chemicals

All microbiological media were sourced from BD Difco (Sparks, MD, USA). Selenite cystine broth served as the primary enrichment medium, while Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green agar were used for selective isolation. Biochemical confirmation relied on the API 20E identification system (bioMérieux, Marcy-l'Etoile, France). Serotyping antisera were obtained from the Korea National Institute of Health reference laboratory. Genomic DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), and DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing libraries were prepared with the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Antimicrobial susceptibility testing used Mueller-Hinton agar and Sensititre plates (Trek Diagnostic Systems, East Grinstead, UK) following CLSI guidelines. A Qubit 3.0 fluorometer (Thermo Fisher Scientific) provided secondary quantification of library DNA before loading onto the MiSeq cartridge.

Methods

Each swab was inoculated into 10 mL of selenite cystine broth and incubated at 37 degrees Celsius for 18 hours. A loopful of enriched broth was then streaked onto XLD and Brilliant Green agar plates and incubated overnight. Presumptive *Salmonella* colonies showing characteristic morphology were picked and confirmed biochemically using the API 20E system. Confirmed isolates were serotyped following the Kauffmann-White scheme with slide agglutination [11]. Genomic DNA was extracted, and quality was verified by a 260/280 absorbance ratio above 1.8 and a minimum concentration of 20 ng per microlitre. Sequencing libraries were generated with the Nextera XT kit and sequenced on an Illumina MiSeq platform using 2 x 250 bp paired-end chemistry, targeting a minimum 40-fold coverage depth.

Raw reads were quality-trimmed with Trimmomatic v0.39, retaining reads longer than 50 bp with a Phred score above 20 [12]. De novo assembly was performed using SPAdes v3.15.4 and assembly metrics were evaluated with QUAST [13]. Acquired resistance genes were identified by screening assemblies against the ResFinder 4.1 database with a minimum identity threshold of 90% and coverage of 60%, and additionally against the Comprehensive Antibiotic Resistance Database (CARD) using RGI v5.2 [14]. Phylogenetic relationships were inferred through core-genome SNP analysis using CSI Phylogeny v1.4 on the CGE server, with *S. Enteritidis* strain P125109 as the reference. A maximum-likelihood tree was constructed with 1,000 bootstrap replicates. Statistical comparisons of resistance prevalence among serovars were carried out in R v4.3.1 using Fisher's exact test with Bonferroni correction, and significance was set at $p < 0.05$ [15].

Quality control

Stringent quality control measures were applied at each stage of the workflow. For bacteriology, American Type Culture Collection (ATCC) reference strain *S. Typhimurium* ATCC 14028 was included in every enrichment batch as a positive control, while uninoculated media served as negative controls. Serotyping was repeated on 10% of isolates selected at random, achieving 100% concordance. DNA extracts failing the 260/280 ratio criterion (below 1.8 or above 2.1) were re-extracted. Sequencing run quality was monitored through the MiSeq Reporter software dashboard; lanes producing fewer than 100,000 quality-filtered read pairs per sample were repeated. Post-assembly, contigs shorter than 500 bp were discarded, and only assemblies with an N50 above 30,000 bp and fewer than 300 contigs were retained for downstream analysis. ResFinder hits were manually curated to exclude partial gene fragments shorter than 80% of the reference length.

Results

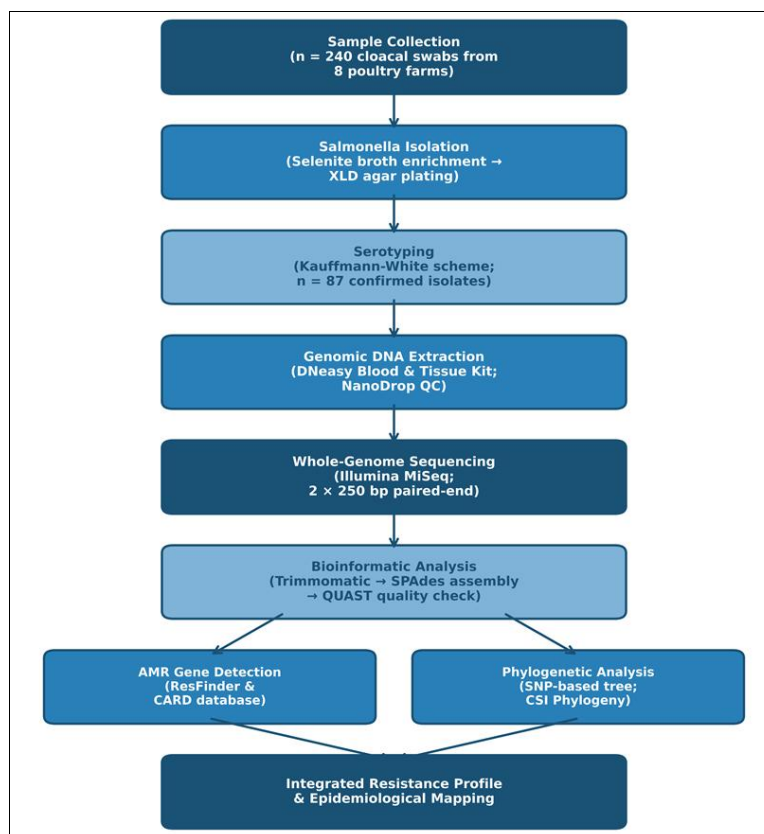
Of the 240 cloacal swabs examined, 87 (36.3%) yielded confirmed *Salmonella* isolates. Recovery rates differed between farm capacity groups: high-capacity farms contributed 52 of the 87 isolates (59.8%), while medium-capacity farms accounted for the remaining 35 (40.2%). Seasonal recovery peaked during summer (June–August), when 31 of the 87 isolates (35.6%) were obtained, compared with 14 (16.1%) during winter months.

Table 1: Distribution of *Salmonella enterica* serovars isolated from broiler farms (n = 87)

Serovar	No. of Isolates	Percentage (%)	Farm sources
<i>S. Enteritidis</i>	32	36.8	7 of 8
<i>S. Typhimurium</i>	24	27.6	6 of 8
<i>S. Infantis</i>	17	19.5	5 of 8
<i>S. Gallinarum</i>	9	10.3	3 of 8
<i>S. Albany</i>	5	5.8	2 of 8
Total	87	100.0	8 of 8

The serovar distribution revealed *S. Enteritidis* as the dominant serovar at 36.8%, followed by *S. Typhimurium* at 27.6%. Together these two serovars represented nearly two-

thirds of all isolates. *S. Infantis*, increasingly recognised as an emerging multidrug-resistant lineage in poultry globally, accounted for 19.5%.

**Fig 1:** Workflow for *Salmonella* isolation, whole-genome sequencing, and antimicrobial resistance gene profiling from broiler farm cloacal swabs

Genome assemblies met quality thresholds for all 87 isolates, with a median N50 of 127,400 bp and median total assembly length of 4.78 Mb. Screening against ResFinder and CARD

identified 23 unique AMR genes spanning eight antimicrobial classes.

Table 2: Antimicrobial resistance genes identified across *Salmonella* isolates by drug class

Antimicrobial class	Gene(s) detected	No. positive	Prevalence (%)	p-value*
Beta-lactams	blaTEM-1, blaCTX-M-15	68	78.2	<0.001
Tetracyclines	tetA, tetB	62	71.3	<0.001
Sulfonamides	sul1, sul2	56	64.4	<0.001
Aminoglycosides	aadA1, strA, strB	50	57.5	0.003
Amphenicols	floR, cmlA	38	43.7	0.012
Quinolones	qnrS, qnrB	32	36.8	0.024
Polymyxins	mcr-1	11	12.6	0.041
Macrolides	ermB	7	8.1	0.108

*Fisher's exact test comparing prevalence between serovars

Multi-drug resistance (MDR), defined here as genotypic resistance to three or more antimicrobial classes, was detected in 54 of 87 isolates (62.1%). *S. Typhimurium* exhibited the highest MDR proportion at 83.3%, significantly exceeding *S.*

Enteritidis at 53.1% ($p = 0.017$). Notably, the plasmid-mediated colistin resistance gene *mcr-1* was found in 11 isolates (12.6%), all of which belonged to either *S. Typhimurium* or *S. Infantis*.

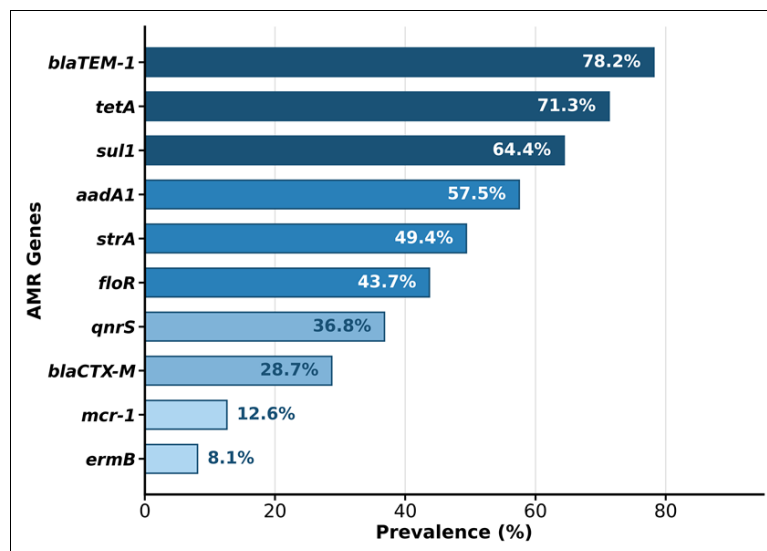


Fig 2: Prevalence of the ten most frequently detected antimicrobial resistance genes among 87 *Salmonella enterica* isolates from broiler farms

Phylogenetic analysis grouped the 32 *S. Enteritidis* isolates into two main clusters. Cluster I contained 19 isolates from five different farms and differed by fewer than 15 core-genome SNPs, suggesting recent clonal expansion from a

shared source. Cluster II comprised 13 isolates from three farms with wider SNP distances (42-78 SNPs), indicating a distinct but related lineage.

Table 3: Multi-drug resistance classification by serovar and resistance class count

Serovar	n	3 Classes	4 Classes	5+ Classes	Total MDR (%)
<i>S. Enteritidis</i>	32	8	6	3	17 (53.1)
<i>S. Typhimurium</i>	24	5	7	8	20 (83.3)
<i>S. Infantis</i>	17	4	5	3	12 (70.6)
<i>S. Gallinarum</i>	9	2	1	0	3 (33.3)
<i>S. Albany</i>	5	1	1	0	2 (40.0)
Total	87	20	20	14	54 (62.1)

Comprehensive interpretation

Taken as a whole, the genomic data reveal a poultry *Salmonella* population under heavy antimicrobial selection, with beta-lactam, tetracycline, and sulfonamide resistance genes nearly ubiquitous among isolates. The co-occurrence of *mcr-1* with extended-spectrum beta-lactamase genes in a subset of isolates is of particular concern, as it narrows the therapeutic options available for human salmonellosis originating from poultry sources. The clonal relatedness of *S. Enteritidis* across multiple farms raises questions about the role of shared inputs such as day-old chick suppliers or feed mills in disseminating resistant strains.

Discussion

The 36.3% *Salmonella* recovery rate documented here is consistent with rates reported from broiler farms in Southeast Asia and southern Europe, which typically range from 25% to 45% [3, 7]. By contrast, farms in northern European countries with mature antimicrobial stewardship programmes tend to report lower prevalences below 10% [8], underscoring the influence of on-farm management practices. The dominance of *S. Enteritidis* aligns with Korean national monitoring data from 2019 to 2022, which ranked this serovar first among poultry-origin isolates [9]. And the rising share of *S. Infantis*, which didn't feature prominently in earlier Korean surveys, mirrors a trend already well-documented in Europe and Japan [6].

The genetic basis of resistance followed predictable patterns for several drug classes. *blaTEM-1* remains the most widespread beta-lactamase in livestock-associated *Salmonella* worldwide, and its 78.2% prevalence here sits near the upper

end of published figures [4, 14]. *tetA*-mediated tetracycline resistance and *sul1*-associated sulfonamide resistance reflect decades of heavy use of these agents in Korean poultry production. But the 12.6% prevalence of *mcr-1* warrants particular attention. Colistin has been classified as a last-resort antimicrobial by the World Health Organization, and plasmid-borne *mcr* genes can spread rapidly through Enterobacteriaceae populations [5]. Korea banned the agricultural use of colistin in 2018, yet the gene clearly persists, possibly maintained through co-selection with other resistance determinants on the same plasmid.

The phylogenetic clustering of *S. Enteritidis* across geographically separate farms implies lateral movement of strains within the supply chain rather than independent emergence. Similar supply-chain-mediated dissemination has been reported in Denmark and the Netherlands, where WGS tracing linked farm clusters to common hatcheries [7, 8]. Identifying the specific transmission vehicle in the present dataset was beyond scope, but follow-up sampling of hatcheries and feed sources is warranted.

Conclusion

This research set out to map the genomic resistance landscape of *Salmonella enterica* in commercial broiler farms of Gyeonggi Province, South Korea, using whole-genome sequencing. Across 87 isolates representing five serovars, 23 distinct AMR genes were identified, and multi-drug resistance affected 62.1% of the collection, reaching 83.3% in *S. Typhimurium*. The detection of *mcr-1* at 12.6% prevalence, despite a national colistin ban, signals that colistin resistance genes can persist through co-selection mechanisms long after

direct selection pressure is removed. Phylogenomic analysis exposed clonal *S. Enteritidis* clusters spanning multiple farms, pointing to supply-chain-level dissemination rather than independent on-farm emergence.

For poultry producers and regulators, these results carry a clear practical message: phenotypic susceptibility testing alone is insufficient to capture the full complexity of AMR in *Salmonella* populations. WGS-based surveillance provides the resolution needed to track resistance gene flow, detect emerging threats like *mcr*-mediated colistin resistance, and identify transmission pathways that connect apparently unrelated farms. Integrating routine WGS into the national *Salmonella* monitoring programme would strengthen early-warning capabilities and help target antimicrobial stewardship efforts where they are most needed.

Future directions should include extending WGS surveillance to hatcheries and feed mills to pinpoint the source of clonal strain dissemination, as well as longitudinal tracking of *mcr*-1-carrying plasmids to determine whether their prevalence is stable, rising, or declining following the colistin ban. Comparative analysis with human clinical *Salmonella* isolates from the same region would further clarify the public health implications of the resistance patterns observed here.

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Contributions not qualifying for authorship

Dr. Choi Hyun-Suk provided technical guidance on bioinformatic pipeline configuration. Ms. Yoon Da-Eun assisted with DNA extraction and library preparation.

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