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Whole-genome investigations of Salmonella enterica isolates from poultry meat uncovered multidrug-resistant and outbreak-associated serovars circulating in Accra, Ghana

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Research Article

Keywords: Salmonella enterica, Whole Genome Sequencing, Poultry meat, Antimicrobial resistance, Outbreak, Serovars, Africa

Posted Date: July 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10120722/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Microbiology on November 3rd, 2026. See the published version at https://doi.org/10.1186/s12866-026-05755-5.

Abstract

Background

Salmonella enterica (*S. enterica*) is a prominent foodborne pathogen associated with severe and potentially fatal infections in humans. This study investigated the prevalence, antimicrobial resistance (AMR) profiles, genetic diversity, and AMR mechanisms of *S. enterica* serovars isolated from poultry meat from various vendors in Ghana.

Methods

Poultry meat samples were collected from markets, retail shops, poultry farms, and live poultry retailers. *Salmonella* isolates were identified using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-ToF-MS). Antimicrobial susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method. Whole Genome Sequencing was performed on the Illumina NextSeq platform and data analyzed using bioinformatic tools.

Results

Of the 246 poultry meat samples investigated, *Salmonella spp* was detected in 71 (29%). AST revealed resistance to tetracycline (44%), trimethoprim/sulphamethoxazole (18%), and ciprofloxacin (14%), ampicillin (8%), gentamicin (8%), chloramphenicol (4%), ceftazidime (1%), and meropenem (1%). Multi-drug resistant (MDR) phenotypes were observed among 30% of the isolates. WGS identified resistance genes for tetracycline *(tetA*; 42%), sulfonamides, (*sul*; 38%), quinolones (*qnr*; 31%), trimethoprim (*dfrA1*; 20%), beta-lactams (bla_{TEM-1B}; 8%), and chloramphenicol (*cmlA1*; 4%). Among the twenty-four serovars were detected, *S*. Typhimurium, and *S.* Derby were predominant (n = 7, 29%), followed by *S*. Liverpool (n = 6), and *S*. Hato (n = 5). Three of the *S. Typhimurium* belonged to the outbreak-associated clone ST19. Other significant STs included ST11 (*S. Enteritidis)*, ST96 (*S*. Schwarzengrund), ST314 (*S*. Kentucky), ST1954 (*S*. Chester), and ST308 (*S*. Poona). *Col* and *Inc* plasmid replicons responsible for multiple resistance gene transfers were detected along with virulence genes associated with adhesins, transport, and secretion systems.

Conclusion

The detection of MDR and outbreak-associated *Salmonella* enterica serovars in poultry meat sold in Accra underscores a significant threat to public health and food safety. These findings highlight the urgent need for heightened public awareness and sustained surveillance efforts to mitigate and monitor the risk of foodborne infections.

Background

Salmonella enterica subsp. *enterica* is one of six subspecies of *S. enterica* responsible for infections in both humans and animals and ranks among the most common foodborne pathogens of animal origin (1). It accounts for over 90% of global foodborne associated with consumption of contaminated foods (2, 3). According to WHO estimates, *Salmonella enterica* causes approximately 93.8 million infections and 155,000 deaths globally each year (4). Over 2,500 serovars of *Salmonella* have been identified, including *S. enterica* serovars Typhimurium and Enteritidis, which are commonly found in seemingly healthy poultry and have been linked to infection outbreaks in humans (5–7).

A major concern is the multidrug-resistant (MDR) nature of *Salmonella* strains found in food-producing animals, largely driven by the prolonged use of antimicrobials for prophylaxis and growth promotion, which has serious implications for human health (8). Numerous studies have established an association between the misuse of these antimicrobials in animal production and the emergence of resistant foodborne pathogens responsible for human infections (3, 9).

Poultry continues to serve as a key source of dietary protein globally, including in Ghana (10). To meet rising demand, poultry farmers in Ghana frequently rely on antimicrobials such as tetracyclines and aminoglycosides for growth promotion, a practice that contributes to the proliferation of antimicrobial resistance genes (ARGs) within the country (10, 11). Additionally, Ghana relies heavily on imported chicken to meet its high domestic demand. The Ghana National Association of Poultry Farmers (GNAP) reports that approximately 95% of poultry consumed in the country is imported from Brazil, the Netherlands, and the United States (12). This heavy dependence on imports may facilitate the cross-border spread of antibiotic-resistant pathogens, including pandemic Salmonella serovars.

To better understand the epidemiology of Salmonella, especially from food origin, advanced research techniques such as whole-genome sequencing (WGS) are now being employed in routine investigations. WGS provides early detection of emerging and re-emerging pathogenic threats that can result in worse transmission outcomes for both animals and humans, making it a comprehensive tool for food safety management and public health (13).

In Ghana, while numerous studies have been conducted on Salmonella, most have relied on conventional characterization methods (3, 14, 15). The use of WGS in Salmonella surveillance and research, particularly from animal food sources, remains limited. The few existing WGS-based studies have primarily focused on isolates from human and non-poultry sources, revealing epidemic clones such as ST11 (*S.* Enteritidis), ST33 (*S.* Hadar), and ST951 (*S.* Ajiobo) (16, 17). These clones carry various ARGs, including *qnrB11* (quinolones), *aadA1* (aminoglycosides), *tetA* (tetracycline), and *dfrA* (trimethoprim). Identified serovars such as Enteritidis, Hadar, and Chester further highlight the genetic diversity of Salmonella strains circulating in the country (17)

The current study investigated Salmonella isolates from poultry meat, with the aim of identifying dominant clones and characterizing their resistance and virulence profiles. This work expands our

understanding of the epidemiology of Salmonella originating from poultry in Ghana and generates crucial data on the mechanisms driving resistance and pathogenicity. Ultimately, the study highlights the importance of WGS as a powerful tool for evidence-based food safety risk assessment to guide public health policies and local decision-making.

Methods

Study design

As part of the Ghana Food and Drugs Authority's regulatory efforts to mitigate human health risks posed by antimicrobial-resistant (AMR) pathogens in the food chain, locally produced poultry meat intended for human consumption was sampled from 31 sites across the Greater Accra Region between February and March 2022. Sampling locations included markets, retail shops, intensive poultry farms, and live poultry retailers.

Sample Collection and Bacterial Isolation

Samples included fresh, chilled, or frozen poultry meat, which were either whole, cut-up, or internal organs of the poultry. Preliminary isolation and identification were carried out in the FDA microbiology laboratory. Except for the edible internal organs, test portions were taken from the skin of the poultry. All samples were pre-enriched in buffered peptone water (BPW) (Oxoid Limited, UK) and incubated overnight at 37˚C. For selective enrichment, samples were inoculated in Rappaport-Vassiliadis broth (Oxoid Limited, UK) as well as Muller Kauffman broth base (Oxoid Limited, UK) and incubated for 18–24 hours at 37˚C. Following incubation, the enriched cultures were streaked onto Xylose Lysine Deoxycholate agar (Oxoid Limited, UK) and Bismuth Sulfite Agar (Oxoid Limited, UK) and incubated at 37˚C for 18–24 hours. Two presumptive *Salmonella* colonies were transferred from both agar plates and streaked on nutrient agar for purification. Biochemical testing to confirm the Salmonella was conducted using urease and sugar fermentation tests. Preliminary methods of Salmonella detection were done in accordance with ISO 6579-1:2002 (Microbiology of the food chain–Horizontal method for the detection and serotyping of *Salmonella*). Presumptive Salmonella isolates were transported to the Bacteriology Department at the Noguchi Memorial Institute for Medical Research (NMIMR) for further analysis: Salmonella confirmation, antimicrobial susceptibility testing (AST), and WGS.

Salmonella confirmation and antimicrobial susceptibility testing

Upon arrival of isolates at NMIMR, the presumptive *Salmonella* isolates were re-cultured on Salmonella Shigella Agar (SSA) (Oxoid, USA) and indicative isolates were confirmed using the Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-ToF) mass spectrometry (Bruker Daltonics, Germany). Further testing was performed on only the MALDI-ToF positive *Salmonella* sp.

AST using 11 antibiotics was performed via the Kirby-Bauer disk diffusion method, and zone of inhibition values were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2022 edition). Antibiotics tested include tetracycline (30 µg), trimethoprim/sulphamethoxazole (25 µg), ciprofloxacin (5 µg), ampicillin (10 µg), gentamicin (10 µg), chloramphenicol (30 µg), meropenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), ceftriaxone (30 µg), and cefoxitin (30 µg). *Escherichia coli* ATCC 25922 was used as a standard control strain. Multi-drug resistance (MDR) was determined by phenotypic resistance to ≥ 1 agent in ≥ 3 categories of antimicrobials (18).

Data analysis

Information on *Salmonella enterica* prevalence and distribution, as well as data from laboratory investigations, was entered into a Microsoft Excel database for editing and analysis. Categorical variables were summarized with frequencies and percentages. Data visualization was performed in Python (version 3.10.19) using pandas (version 2.0.3) for data manipulation, matplotlib (version 3.10.8) and seaborn (version 0.13.2) for figure generation, and SciPy (version 1.15.3) for statistical testing.

DNA extraction, Library preparation, and Sequencing

The QIAGEN QIAamp DNA mini kit (Qiagen Inc. GmbH, Hilden, Germany) was used to extract the DNA of the Salmonella isolates from overnight culture. Concentrations of the DNA were determined using the Qubit 4.0 Fluorometer Assay Kit (Thermo Fisher Scientific, MA, USA). DNA libraries were prepared using the Illumina DNA library prep - (M) Tagmentation kit (Illumina Inc.,San Diego, CA). Library quantification was achieved with the 2100 Bioanalyzer System (Agilent) and Kapa Sybr Fast qPCR Kit. An Illumina NextSeq instrument (Illumina Inc., San Diego, CA, USA) was used for a 2x150 paired-end sequencing. Quality control checks were done on the raw sequencing reads to a Phred score ≥ 20, and trimming was performed using Trimmomatic (19). Quality of reads were ascertained with the FastQC tool (20)The resultant high-quality reads were assembled using the Unicycler assembler v0.5.0 (21).

Bioinformatics analysis

Kmerfinder v3.2 was used to confirm the species of the 71 Salmonella strains (22). AMR and virulence genes were detected using Abricate v4.0.23 (https://github.com/tseemann/abricate) with the ResFinder, Comprehensive Antibiotic Resistance Database (CARD) and Virulence Finder Database (VFDB), respectively (23, 24). PointFinder (integrated within ResFinder v4.7.2) was used to detect chromosomal mutations. PlasmidFinder v2.1 was used to detect plasmid replicons (https://cge.food.dtu.dk/services/PlasmidFinder/). Sequence types (STs) were assigned based on the seven housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA, and thrA*) using the MLST 1.0.1 tool (https://genepi.food.dtu.dk/mlstfinder). Serotypes of the Salmonella strains were determined with SeqSero v2.1(25) database. To detect integrons in the genomes, the IntegronFinder v2.0.6 was used (26). All the tools were used at default settings. (27)

Phylogenetic Analysis

Core Genome MultiLocus Sequence Typing

Enterobase was used to analyze the core genome Multi Locus Sequence Typing (cgMLST) of the Salmonella genomes. The core-genome database for Salmonella was employed, which contains 3002 loci. A minimum spanning tree based on the allelic profiles was generated. The tree was exported in newick format and annotated in iTOL (28).

Maximum Likelihood Single Nucleotide Polymorphism (SNP) Analysis

SNP analysis was performed on the predominant serovars – Typhimurium (n = 7), Derby (n = 7), and Kentucky (n = 5). Briefly, assembled genomes were uploaded to CSIPhylogeny v1.4 (https://cge.food.dtu.dk/services/CSIPhylogeny/) to generate a maximum likelihood phylogenetic tree and a SNP matrix. The generated tree was uploaded and annotated in iTOL (28)

Results

Identification and distribution of Salmonella sp.

The 71 *Salmonella* sp. (29%; 71/246) isolates were confirmed by WGS as *Salmonella enterica*. Majority were isolated from whole poultry (n = 53; 75%), followed by chilled cut portions (n = 11; 15%), fresh cut portions (n = 5; 7%), and frozen cut portions (n = 2; 3%). The highest number of isolates were obtained from Tema Community 1. Figure 1 shows the sample collection sites. Table 1 shows the distribution of Salmonella isolates and study sites.

Antimicrobial resistance phenotype

We observed that 49% (n = 35) of the *Salmonella enterica* were resistant to at least one of the antimicrobial agents tested. Highest resistance was recorded against tetracycline (44%, n = 31), followed by trimethoprim-sulphamethoxazole (cotrimoxazole) (18%, n = 13), ciprofloxacin (14%, n = 10), ampicillin (8%, n = 6), gentamicin (8%, n = 6), chloramphenicol (l4%, n = 3), ceftazidime (1%, n = 1) and meropenem (1%, n = 1). The isolates were susceptible to cefepime, cefoxitin, and ceftriaxone antibiotics. Thirty percent (n = 21) of the isolates were multidrug resistant. Five of the MDR isolates (17%) exhibited concurrent resistance to tetracycline, ampicillin, and trimethoprim-sulfamethoxazole, while three isolates were resistant to tetracycline, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin. AMR was predominantly seen among the fresh whole samples (n = 24), with isolates in this category showing resistance to tetracycline (n = 19). Both frozen samples had Salmonella isolates showing resistance, with one isolate from the frozen category being resistant to tetracycline, trimethoprim-sulphamethoxazole, and ampicillin (Table 1).

Resistance gene profiles

ResFinder analysis of ARGs revealed a total of 16 ARGs. Majority of these genes were associated with tetracycline resistance. The *tet(A)* gene was detected in 42% (n = 30) followed by *tet(M)* (4%, n = 3). Among the aminoglycoside genes, *aph*(6)-*Id* was predominant (32%, n = 23), compared to *aph*(3)-*Ib* (30%, n = 21), and *aadA* (14%, n = 10). For sulfonamide resistance, the identified genes included *sul2* (18%, n = 13), *sul3* (13%, n = 9), and *sul1* (7%, n = 5). Quinolone resistance genes detected were *qnrB* (31%, n = 22) and *qnrS* (8%, n = 6). A single point mutation in the quinolone resistance-determining region (QRDR) of *gyrA* (p.D87N), conferring resistance to quinolones, was found in one *S. enterica* isolate.

Beta-lactams resistance genes [bla_{TEM-1B} (8%, n = 6)], chloramphenicol resistance gene [*cmlA1* (4%, n = 3)], and trimethoprim [*dfrA14* (14%, n = 10); *dfrA15* (6%, n = 4)] were also detected among the isolates. Chromosomal resistance genes such as *fosA*, conferring resistance to Fosfomycin, were present in three (4%) isolates (Table 1).

Overall, high concordance was observed between phenotypic and genotypic resistance across most of the antimicrobials tested. The highest concordance rates (> 90%) were observed for chloramphenicol, tetracycline, and ampicillin, primarily driven by the presence of *cmlA1*, *tet(A)*, and *blaTEM-1B* resistance genes, respectively. Ciprofloxacin and sulphamethoxazole/trimethoprim showed moderate concordance values of 87% and 82% respectively, while the lowest concordance was observed for gentamycin (75%). Table 2 presents the detailed concordance for all antimicrobials tested.

Plasmid replicons

A total of thirteen unique plasmid replicons were detected in the isolates, with 63% (n = 45) of the isolates carrying at least one plasmid (Table 1). Two major groups of plasmid replicons were identified, including the *Inc* and *Col* plasmid families. *Col (pHAD28)* was the most prevalent, detected in 38% (27/71), and showed the broadest association with multiple resistance genes, including *aph(3")-Ib*; 55.6% (15/27), *aph*(6)-*Id* ; 55.6% (15/27), *qnrB19*; 51.9% (14/27), and *sul2*; 37% (10/27). *IncQ1* plasmids were observed in 10 isolates and consistently cooccurred with *tet(A)* 100% (10/10). Association with *aph(3")-Ib* ; 70% (7/10), *sul2* ; 70% (7/10;), and *dfrA51* 40% ; (4/10;) was also observed. *IncX1* (5/72 isolates) exhibited complete co-occurrence with *blaTEM-1*, *qnrS1*, *dfrA14*, *sul3*, and *tet(A)*. Table 4 shows the detailed co-occurrence between resistance genes and plasmid replicons.

Integrons

Of the 71 Salmonella genomes, 16.9% (n = 12) harbored integrons comprising both complete (41.7%, n = 5) and CALIN (clusters of attC sites lacking integron-integrase; 58.3%, n = 7) types (Table 3). All complete integrons belonged to Class 1 and predominantly carried trimethoprim resistance genes (dfrA1 or dfrA1-like). In contrast, the CALIN type integrons were more genetically diverse and carried multiple resistance gene, including aminoglycoside-modifying enzymes such as *ANT(3")-I*, *aac*(3)-*I*, and *aadA1*, chloramphenicol efflux (*cmlA*), and quaternary ammonium compound resistance (*qacE*) genes.

Distribution of serovars and virulence gene profiles

All 71 *Salmonella enterica* belonged to 24 different serovars. Predominant serovars are *S*. Typhimurium (n = 7), *S*. Derby (n = 7), *S*. Liverpool (n = 6), and *S*. Kentucky (n = 5), mainly from the fresh whole samples (n = 21). *S*. Enteritidis (n = 1) was detected from a chilled cut sample. Other serovars such as *S.* Schwarzengrund (n = 4) were distributed among fresh whole (n = 2), fresh cut (n = 1) and chilled cut (n = 1) samples. *S*. Poona (n = 2) serovar was identified in a fresh cut and fresh whole sample whilst *S*. Agona (n = 1) serovars were identified in a fresh whole sample. *S. Hato* (n = 5) and S. *Typhimurium* were present across all sample types except in frozen samples.

Fresh whole samples accounted for the highest diversity of serovars, representing 75% (n = 53/71) of the isolates, followed by chilled cut samples at 14% (n = 10/71), fresh cut at 7% (n = 5/71), and frozen meat at 3% (n = 2/71). The distribution of the various serovars is outlined in Table 1.

A total of 123 virulence genes (Table S1) were identified among the isolates, with several associated with transport and secretion systems. These included multiple genes encoding type III secretion system effectors, such *as sspH1, sspH2, sseI/srfH, pipB*, and *gogB*. Additionally, *ssaI*, which encodes the type III secretion system inner rod protein, was also detected. The *shdA* gene, encoding an AIDA autotransporter-like protein, was found in 17 isolates. Toxin-related virulence factors were also present, including *cdtB*, which encodes the cytolethal distending toxin B. Other notable virulence genes included *ratB*, encoding a putative outer membrane protein, and sinH, responsible for production of an intimin-like protein. Furthermore, four isolates harbored the *rck* gene, which confers resistance to complement-mediated killing as part of the host immune response.

Clonal diversity of the Salmonella enterica isolates

Multi-locus sequence type results revealed that 66 (93%) out of the 71 *S. enterica* isolates belonged to 21 known STs, and 5 (7%) novel STs. *S.* Liverpool ST1959 (n = 6), *S*. Hato ST3899 (n = 5), *S.* Kentucky ST314 (n = 5), *S.* Typhimurium ST19 (n = 3), and *S.* Saintpaul ST27 (n = 3) were the predominant sequence types. The novel STs were ST13123 (n = 2), ST13124 (n = 1), ST13125 (n = 1), and ST13126 (n = 1). MDR serovars were associated with specific sequence types, notably ST314 with *S*. Kentucky (n = 5) and ST3899 with *S.* Hato (n = 5), both exhibiting phenotypic resistance to multiple antibiotics supported by corresponding resistance genes.

ST1954, associated with *S.* Chester (n = 3), carried resistance genes conferring resistance to multiple antibiotics and harbored diverse virulence factors. Similarly, a *S*. Schwarzengrund isolate belonging to ST96 was identified as MDR and carried several virulence-associated genes. Notable sequence types such as ST308 (S. Poona), ST11 (*S*. Enteritidis), and ST19 (*S*. Typhimurium) did not exhibit multidrug resistance, although they possessed various resistance and virulence markers.

The core genome multilocus sequence typing (cgMLST) analysis revealed several distinct lineages reflecting the allelic diversity and sequence type distribution among the Salmonella genomes. Isolates of

the same serovar and sequence types clustered together.

The *S.* Liverpool isolates (n = 6) formed a monophyletic clade and belonged to the same sequence type (ST-1959). They were recovered from different sites, AMJB (n = 3) and DLB (n = 3), and none of the genomes harbored any resistance gene. The *S.* Typhimurium genomes formed two distinct clades, with the ST-9479 (n = 4) clustering close to *S.* Anesho (ST-3292) while ST-19 (n = 3) formed a monophyletic lineage closely related to the single *S.* enteritidis (ST-11) isolate. Two of the ST-19 genomes harbored aminoglycoside, tetracycline, and sulphonamide resistance genes, while the ST-9479 genomes harbored no resistance genes. The *S.* Derby genomes (n = 7) formed 2 clades that clustered together. One of the clades contained genomes of ST-3135 (n = 4). Three of the isolates originated from KLB, and the other from SAM. Genomes in the second clade originating from TC1 belonged to ST-8786 (n = 3). Other larger clusters that formed monophyletic clades include *S.* Kentucky (ST314), originating from 3 sources (MMPD, TC7, TC1), and *S.* Hato (ST3839) from KSC. Figure 2 illustrates the allelic variation and genetic relatedness among the Salmonella isolates, annotated with sequence types, location, and resistance genes.

The SNP analysis (Fig. 3) of the 3 serovars (Derby, Kentucky, and Typhimurium) showed very close genetic relatedness among some of the isolates. Among the Derby serotypes with ST-3135, isolates XLD_RV_KM_77 and KLS_RV_KM_78 showed close genetic relatedness, with only 7 SNP differences. Both isolates originated from KLB and harbored identical resistance gene profiles, including aminoglycosides (*aph(3″)*-Ib, *aph*(6)-*Id*), tetracycline (*tetA*), and sulphonamide (*sul*), along with the plasmid replicon *IncQ1*. XLD_RV_092, XLD_MK_093, and XLD_RV_091 were more distantly related with 16 SNP differences.

The *S.* Kentucky genomes were more closely related with < 10 SNP differences. Isolates XLD_RV_096 and XLD_RV_089, which originated from different sites most, TC7 and TC1), were more closely related, with 3 SNP differences. Both genomes harbored aminoglycoside (*aac*(3)-*Id*, *aph(3″)*-Ib, *aph*(6)-*Id, aadA7*), tetracycline (*tetA*), quinolones (*qnrB19*) and sulphonamide (sul1) resistance genes. They also carried the *col(pHAD28)* plasmid replicon.

Among the *S.* Typhimurium isolates, XLD_RV_TC_53 and XLD_RV_TC_55 showed high genetic relatedness, differing by only 1 SNP. Both genomes originated from TCFAL and carried the resistance genes for aminoglycosides (*aph(3″)*-Ib, *aph*(6)-*Id*), tetracycline (*tetA)*, and sulphonamide (*sul1*). They also harbored multiple Inc plasmids, including *IncFIB(S), IncFII(S)*, *InFII(S)*, as well as *the Col(pHAD28)* col plasmid.

Plasmid-mediated quinolone resistance (PMQR)

Out of the 71 Salmonella strains, 32%(n = 23) harboured plasmid-mediated quinolone resistant (PMQR) genes. Four distinct PMQR genes were detected with *qnrB19* being the predominant gene, 74% (n = 17), followed by *qnrS1* (26%, n = 6)), *qnrB5* (13%, n = 3), and *qnrB81* (13%, n = 3) (Fig. 4A). Three isolates also harbored more than 1 type of PMQR gene.

Of the 23 isolates that carried the quinolone resistance genes, only 32% (n = 9) exhibited concurrent phenotypic resistance (Fig. 4B). This occurred in only carriers of the *qnrB* genes. In contrast, all the *qnrS* carriers showed no phenotypic resistance (Fig. 4E). Isolates that showed both phenotypic and genotypic resistance belonged to ST314 (n = 3), ST1954 (n = 4), ST3135 (n = 1). ST473 (n = 1) and ST308 (n = 1). The ST3899 (n = 6) isolates carrying *qnrS* genes did not exhibit any phenotypic resistance (Fig. 4C).

The *qnrB19* gene co-occurred exclusively with Col(pHAD28) plasmid in all 17 carriers, with additional IncQ1 plasmid co-carriage in 4 of these isolates (Fig. 4D). In contrast, *qnrS1* co-occurred exclusively with IncX1 in all six carriers, with no association to Col plasmids. Among the 10 Col-only carriers, 7 (70.0%) were ciprofloxacin-resistant, compared to only 2 of 13 conjugative Inc plasmid carriers (15.4%). This difference was statistically significant (Fisher's exact test: OR = 0.08, p = 0.013), with Inc plasmid carriage being inversely associated with ciprofloxacin phenotypic resistance (Fig. 4F).

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Discussion

Salmonella enterica is a leading cause of foodborne and zoonotic infections globally. While most infections are self-limiting, they can become life-threatening if the pathogen invades beyond the gastrointestinal tract. According to the Centers for Disease Control and Prevention, consumption of contaminated poultry meat remains a major source of human Salmonella infections (29). This study employed WGS to identify resistance genes, virulence factors, and key sequence types (STs) of *S. enterica* serovars isolated from poultry meat sold across 31 sites in Accra. The findings fill a critical knowledge gap on the genomic characteristics of Salmonella in poultry meat in Ghana and complement existing data from human infections and other sources, further highlighting the diversity and public health significance of Salmonella strains circulating in the country.

Poultry meat serves as an important reservoir for antimicrobial-resistant *S. enterica* serovars (30, 31). The widespread use of antimicrobials in local poultry production contributes to selective pressure, promoting the emergence and persistence of resistant Salmonella strains in the environment. This presents a significant public health concern, particularly in relation to the handling and consumption of contaminated poultry meat (9). In this study, the prevalence of *S. enterica* was 29%, which is higher than previously reported figures from similar studies in Accra (25%) (14), the Ashanti Region (6%) (3), Tamale (3%) (32), and Nigeria (4%) (33). These differences were expected due to the variations in sample types, study objectives, sample sizes, and geographical contexts.

In silico analysis identified a genetically diverse Salmonella population across the study sites, comprising 24 distinct serovars and 21 unique sequence types. While *S.* Typhimurium and *S.* Enteritidis are recognized globally as predominant serovars (34, 35), our findings revealed that *S.* Typhimurium and *S. Derby* were the most prevalent among the isolates. This finding is not surprising, as Andoh et al. reported similar results in their 2016 study (14)

The global public health significance of *S.* Typhimurium, particularly the ST19 lineage, has been documented in several studies due to its frequent involvement in foodborne outbreaks across diverse settings (36, 37). Transmission mostly occurs through the handling or consumption of contaminated products such as poultry meat, dairy, nuts, and cheese (38). In other African countries and Ghana, ST19-S.Typhimurium has been associated with human invasive diseases and multidrug resistance to aminoglycosides, sulphonamides, and tetracyclines (35, 39, 40). Despite this, its detection in poultry has only recently been documented, with Thye et al. reporting the first poultry-associated ST19 isolate in 2025, which was a pan-susceptible isolate (41). In contrast, 2 of the ST19-S.Typhimurium identified in this study carried resistance gene patterns similar to those described in clinical isolates causing human infections (35, 39, 40). Given the established zoonotic potential of this clone, the identification of resistant strains in poultry is concerning, as it highlights poultry as a potential reservoir for transmission to humans through contaminated poultry products, direct animal products, or environmental contamination.

S. Kentucky isolates in this study carried multiple antimicrobial resistance genes, including those linked to reduced susceptibility to ciprofloxacin, a trend also reported in Ghana by Ofori et al. (3). *S.* Kentucky, especially ST314, is endemic in Africa, Southeast Asia, and the Middle East. It is commonly isolated from both human and poultry sources and is frequently associated with MDR. Notably, it has also been implicated in disease outbreaks across North America and Europe (42). Considering the lack of basic hygienic practices at local slaughterhouses and retail areas, the presence of this serovar and a possible cross-transmission may result in a serious foodborne public health crisis if not monitored (35). Our study also identified other serovars: *S.* Hadar, and *S.* Chester, which have also been implicated in outbreaks in the USA(43) and China(44) respectively.

S. Chester was isolated from samples sourced from two different farms and one supermarket, while *S.* Hadar was detected in samples from three separate supermarkets in this study. This indicates a widespread distribution of these serovars within the city. However, due to the nature of the sampling methods, it was not possible to determine whether a common external distributor was involved, particularly in the supermarket samples.

We also detected serovar Hato, consistent with previous findings from Ghana (32) and Burkina Faso (45). Additionally, four isolates were identified as *S.* Schwarzengrund, all of which harbored multiple antimicrobial resistance and virulence genes. *S.* Schwarzengrund is primarily associated with poultry but has also been implicated in human infections in countries such as the United States, Denmark, and China and Thailand (46–48). The presence of this serovar in Ghana may be linked to international trade of poultry products from these regions (16).

Interestingly, although *S.* Enteritidis is one of the most common serovars globally (34), this study identified only one strain showing susceptibility to all antibiotics. *S.* Enteritidis is a well-known pandemic foodborne pathogen linked with outbreaks in USA, Europe, and sub-Saharan Africa (35, 38, 49). An earlier

report in Ghana revealed ST11 *S*. Enteritidis in human infections (16). Variability in the prevalence of Salmonella serovars among poultry has been noted across countries and time periods (34).

The SNP-based phylogeny provided higher resolution into the genetic relationships among isolates within the same serovar. We observed that some isolates among the *S.* Derby ST3135, *S.* Kentucky ST314, and *S*. Typhimurium ST19 showed less than 10 SNP differences, suggesting recent clonal dissemination events within the poultry supply chain (50). These genetically related isolates from different sites highlight the possibility of inter-market distribution network in Accra. The closely related strains in different markets may reflect shared poultry sources, slaughterhouses, or transport routes. Cross-contamination during slaughtering, processing, or retail handling may also pose as major drivers of transmission (51). All these factors indicate that transmission likely occurs throughout the poultry supply chain and the movement of these poultry products between markets may contribute to the spread of multidrug resistance stains. These findings underscore the value of WGS and the importance of integrating genomic surveillance into routine food safety monitoring to enable rapid detection of contamination events, guide targeted interventions, and support timely responses to prevent widespread outbreaks (52).

The phenotypic profile of the Salmonella isolates revealed resistance to tetracycline (44%), trimethoprim-sulfamethoxazole (18%), ciprofloxacin (14%), ampicillin (8%), and gentamicin (8%), which aligns with findings from other studies (53, 54). Of particular concern is resistance to ciprofloxacin, as the WHO has classified fluoroquinolone-resistant Salmonella as a high-priority public health threat (55). Encouragingly, most isolates remained susceptible (99%) to third- and fourth-generation cephalosporins as well as meropenem (99%), in line with reports from Ghana (14, 56). The low resistance to these higher-tier antibiotics may reflect their limited use in poultry production due to cost constraints. Nonetheless, the presence of resistant Salmonella strains underscores the potential risk of therapeutic failure in future outbreaks.

The study showed a strong concordance between phenotypic resistance and the presence of corresponding resistance genes, particularly for chloramphenicol, tetracycline, and ampicillin. The most prevalent resistance determinant was the tetracycline resistance gene, detected in 42% of isolates, with a 99% phenotypic resistance concordance. Tetracyclines are among the most commonly used antibiotics in poultry production, often administered for prophylactic purposes (57). This likely explains the high prevalence observed in this study, consistent with previous reports(58). However, the increasing detection of the tetracycline gene with corresponding phenotypic expression is concerning as it significantly limits therapeutic options for managing Salmonella infections (59).

Additionally, resistance genes such as bla_{TEM-1B}, *sul, tet(A), qnr*, and *cmlA*, which confer resistance to commonly used antibiotics like ampicillin, sulfonamides, tetracyclines, quinolones, and chloramphenicol, were identified in several isolates. These findings align with studies from other regions (56, 60) and raise significant public health concerns, particularly regarding the diminishing efficacy of empirical therapies in managing Salmonella infections.

Quinolone resistance due to a chromosomal point mutation in the *gyrA* gene [*gyrA* (p.D87N)] was identified in the single *S.* Enteritidis isolate. However, this mutation did not result in reduced susceptibility to ciprofloxacin. Additionally, the fosfomycin resistance gene *fosA7*, previously reported as chromosomally encoded in serovars such as *S.* Derby, *S.* Heidelberg, and *S.* Reading (61), was detected in *S.* Agona and *S.* Stanleyville isolates in this study. Although phenotypic testing for fosfomycin resistance was not performed, the presence of *fosA7* in these serovars suggests a broader distribution of this gene than previously recognized.

Resistance to quaternary ammonium compounds-disinfectants (*qacE*) were detected in 5 isolates. Detergents are crucial in maintaining hygienic conditions in the poultry industry. Data of *qacE* gene in poultry meat is still scarce with a few studies reporting its presence (62).

Forty-five of the 71 Salmonella isolates harbored plasmids from the *Inc* and *Col* families. *IncF* plasmids are particularly notable for carrying a wide range of antibiotic resistance and virulence genes, facilitating their spread among Salmonella serovars and other members of the Enterobacterales family (63). In this study, serovars possessing *IncF* plasmids were MDR, each carrying genes conferring resistance to more than four classes of antibiotics. Two *S.* Typhimurium isolates carried both *Col* and *IncF* plasmids *[Col (pHAD28), IncFIB(S), IncFII(S)]*, which encoded resistance to aminoglycosides, tetracyclines, and sulphonamides. Additionally, the *IncFIB(S)/IncFII(S)* plasmid combination was identified in one *S.* Typhimurium and one *S.* Enteritidis isolate, both associated with aminoglycoside resistance genes. This contrasts with the findings of Akinyemi et al. (33), where four *S.* Typhimurium isolates carrying *IncFIB(B)/IncFII(S)* plasmids were associated with multiple resistance genes.

In this study, the *IncX1* plasmid was identified in *S.* Hato and *S.* Anatum serovars, both of which carried multiple resistance genes conferring resistance to β-lactams, aminoglycosides, quinolones, and sulfonamides. Similarly, strains carrying *IncQ1* plasmids in this study also possessed genes mediating resistance to aminoglycosides, tetracyclines and sulfonamides, consistent with known associations between *IncQ1* plasmids and these multidrug resistance traits (63). The dissemination may also be promoted by integrons, which facilitate the acquisition and expression of multiple antimicrobial resistance gene cassettes.

Conclusion

To conclude, WGS revealed the presence of MDR and outbreak-associated *Salmonella* enterica clones in poultry meat sold in Accra, Ghana. Given poultry's importance as a major protein source, the detection of these strains presents a serious public health and food safety concern. These findings underscore the need for strengthened but practical, risk-based regulatory approaches. These include integrating genomic surveillance into routine monitoring systems and improving hygiene practices at slaughter and retail levels. A coordinated One-Health approach linking human, animal and food systems is essential for long-term control.

Abbreviations

AMR	Antimicrobial Resistance
MALDI-ToF-MS	Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
AST	Antimicrobial Susceptibility Testing
ARGs	Antimicrobial Resistance Genes
GNAP	Ghana National Association of Poultry Farmers
WGS	Whole Genome Sequencing
BPW	Buffered Peptone Water
NMIMR	Noguchi Memorial Institute for Medical Research
SSA	Salmonella Shigella Agar
CLSI	Clinical and Laboratory Standards Institute guidelines
MDR	Multi-Drug Resistance
CARD	Comprehensive Antibiotic Resistance Database
VFDB	Virulence Finder Database
STs	Sequence Types
cgMLST	core genome Multi Locus Sequence Typing
SNP	Single Nucleotide Polymorphism
CALIN	Clusters of attC sites lacking integron-integrase

Declarations

Ethics approval

Sample collection and isolation of Salmonella were performed by the Food and Drugs Authority (FDA) of Ghana in accordance with its statutory regulatory mandate under the Public Health Act 851 of 2012 (Parts 6–8). Section 127(2) of the Act empowers FDA laboratories to monitor the quality of regulated products, including food analysis (Section 127(2)(b)) and the conduct of related research (Section 127(2)(c)). Part 7, Section 135(1a) of the Act further permits authorized FDA officers to obtain samples from any business premises without requiring owner approval in the execution of their regulatory duties. Nonetheless, verbal informed consent to participate was obtained from all business owners prior to metadata collection and sample collection from their premises.

Samples were obtained from poultry carcasses slaughtered on-site by owners through standard commercial practices (ventral neck cutting, bleeding, scalding, and defeathering). No live animals were

subjected to experimental procedures, and no anesthesia or euthanasia was employed.

Ethical approval for all procedures from isolate recovery through whole-genome sequencing and analysis was obtained from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, University of Ghana (approval number FW00001824). All procedures were conducted in accordance with relevant local legislation and institutional requirements.

Consent for publication

Not applicable

Data availability

The whole genome sequenced data generated during this study have been deposited in the National center for Biotechnology Information (NCBI) repository with project accession number PRJNA1293397 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1293397/). All other data supporting the findings of the study have been included in the main manuscript.

Competing interests

The authors declare no competing interests.

Funding

Whole-genome sequencing was supported by SeqAfrica project (FF RGR2 FF25) funded by the Department of Health and Social Care's Fleming Fund using UK aid. The views expressed in this publication are those of the authors and not necessarily those of the UK Department of Health and Social Care or its Management Agent, Mott MacDonald. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

Author Contributions

BE was responsible for funding acquisition, project administration, resources, validation, visualization, writing original draft, writing – review & editing, conceptualization, data curation, investigation, and methodology. HAO was responsible for investigation, methodology, writing - review & editing, and conceptualization. FAO, and CON were responsible for data curation, formal analysis, investigation, methodology, software, writing original draft, and review & editing. HA, JKD, AO, WB, AB, GRA, QM, JBS, MOA, DAF, MNG, NS, AOA, JB, EKB and BA were responsible for methodology and writing – review & editing. CTG and PN were responsible for funding acquisition and writing – review & editing. RH was responsible for writing – review & editing, funding acquisition, project administration, resources, and validation.

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Tables

Tables 1 to 3 are available in the supplementary files section.

Figures

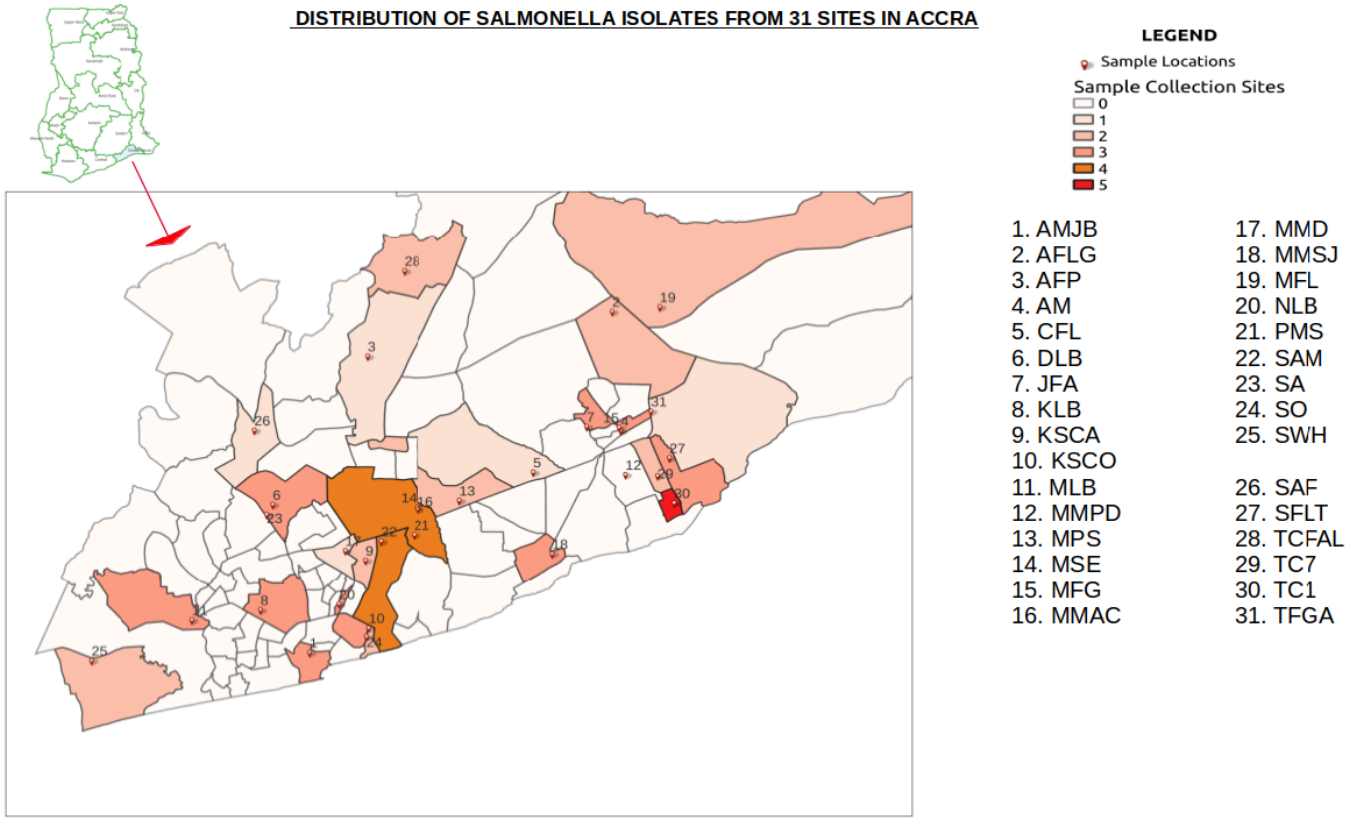


Figure 1

Distribution of *Salmonella* sp. isolates across study sites.

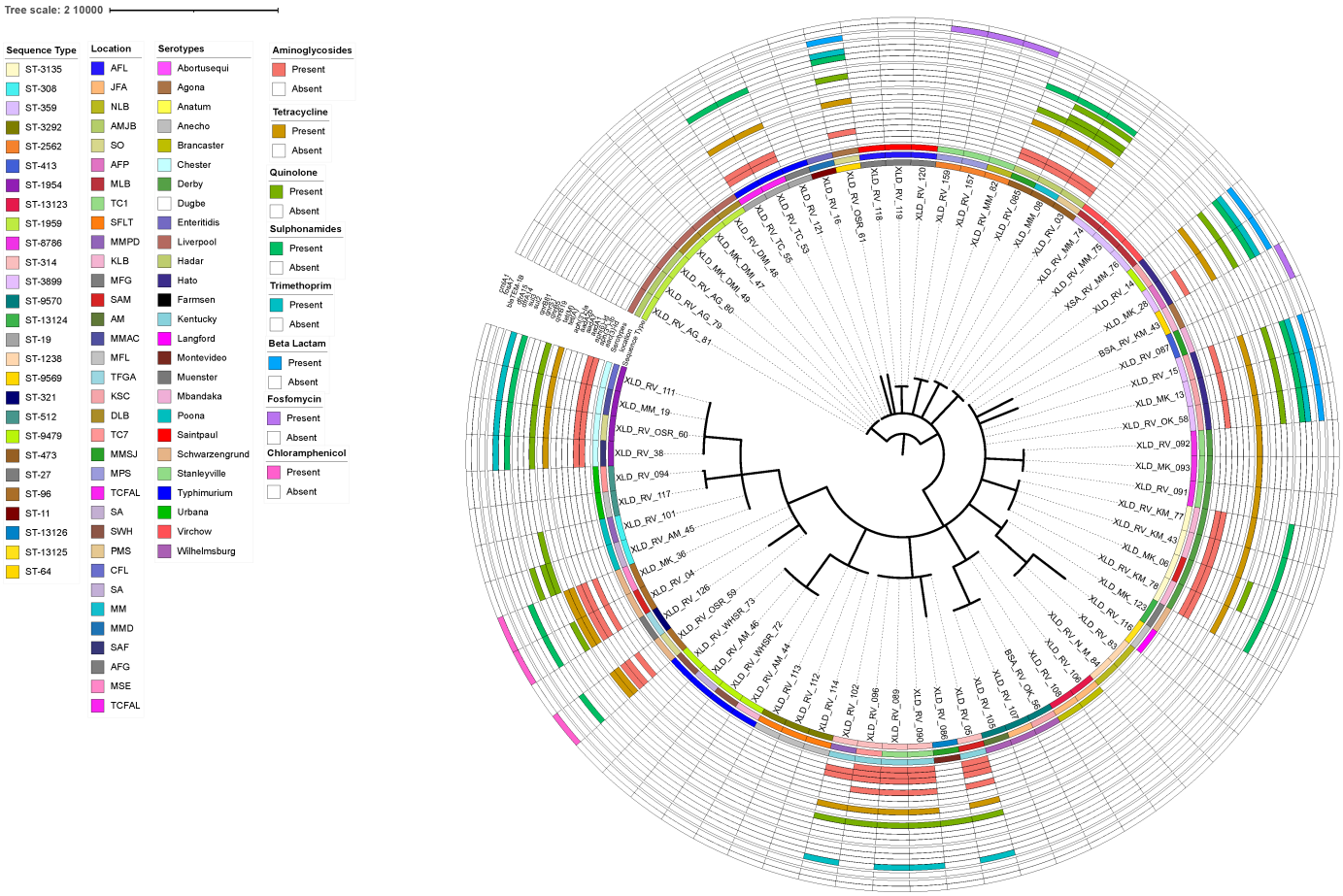


Figure 2: Minimum spanning tree based on cgMLST profiles of 71 Salmonella enterica isolates constructed using the enterobase and visualized in iToL. Each node represents a single Salmonella isolate, labeled with isolate ID The tree is annotated with Sequence type, location, serotype, and resistance genes

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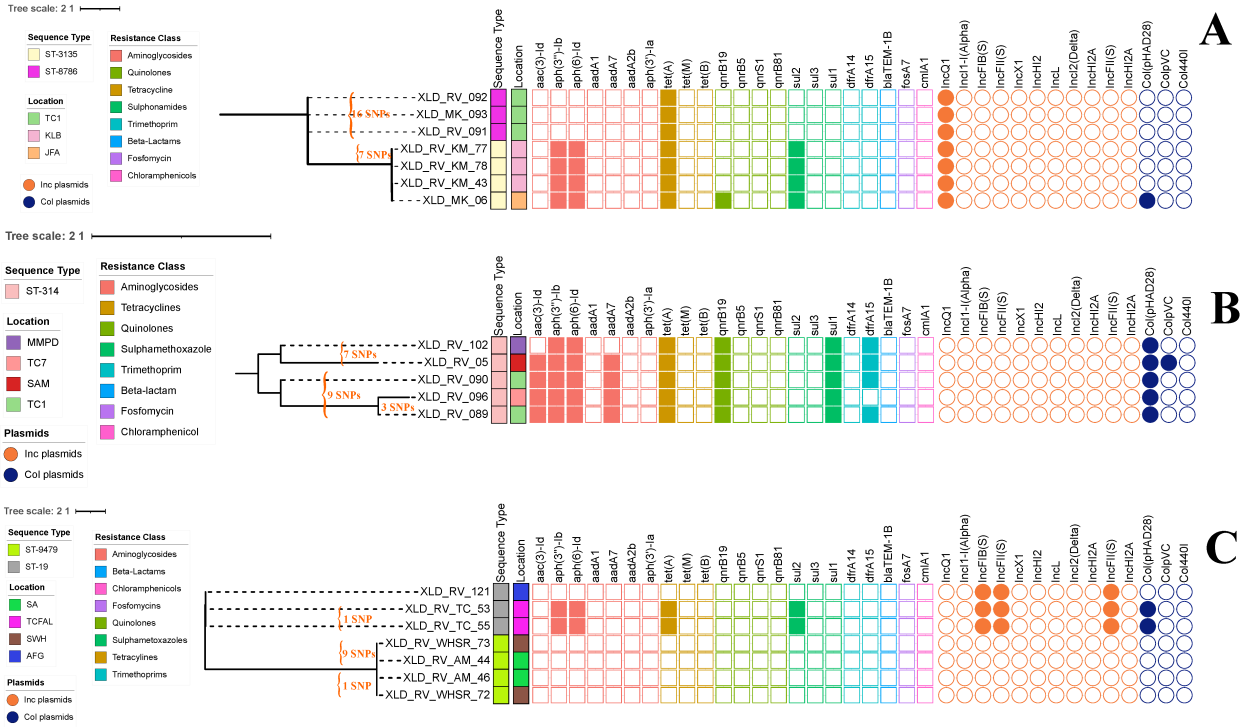
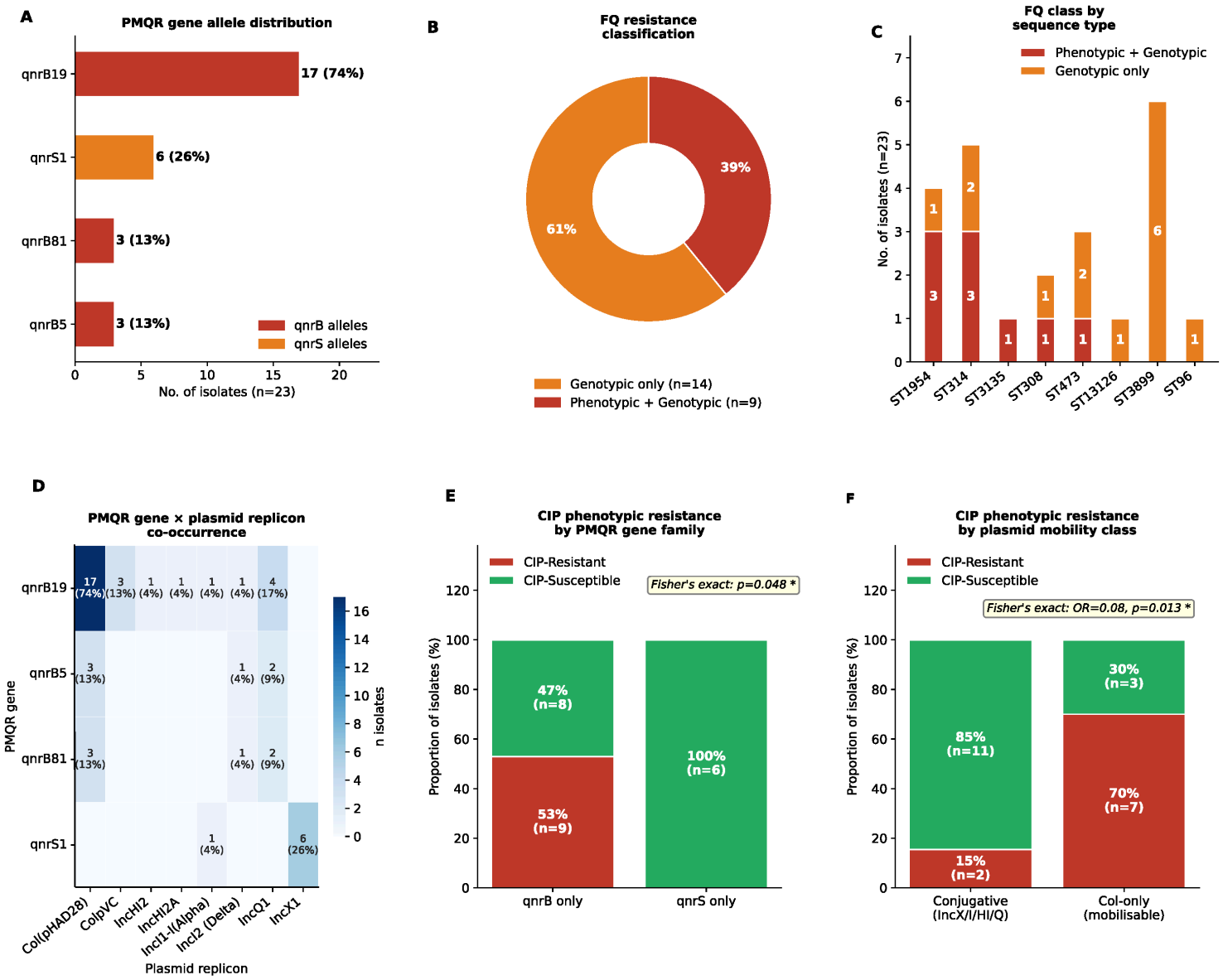


Figure 3: Single Nucleotide Polymorphism (SNP) based phylogeny of the Salmonella enterica serotypes Derby (A), Kentucky (B), and Typhimurium (C). The tree was constructed using CSIphylogeny. The tree is annotated with sequence types, location, resistance genes, and plasmids.

Figure 3

Single Nucleotide Polymorphism (SNP) based phylogeny of the *Salmonella enterica*serotypes Derby (A), Kentucky (B), and Typhimurium (C). The tree was constructed using CSIphylogeny. The tree is annotated with sequence types, location, resistance genes, and plasmids.



PMQR - Plasmid-Mediated Quinolone Resistance, FQ - Fluoroquinolone, CIP - Ciprofloxacin

Figure 4

Plasmid-mediated quinolone resistance gene distribution, plasmid association and determinants of quinolone phenotypic resistance among the Salmonella strains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xls
- Table2.xlsx

- Table3.xlsx
- TableS1.xlsx