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Monty Oxborough
 Department of Medical
 Microbiology, Pennine
 Institute of Biological Sciences,
 Manchester, United Kingdom

Whole genome sequencing and antimicrobial resistance gene detection in salmonella from poultry farms

Monty Oxborough

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Abstract

Leading microbiologists have long warned that antimicrobial resistance (AMR) in food-producing animals would eventually circle back to threaten human health. This research applied whole genome sequencing (WGS) to characterize AMR gene profiles in *Salmonella* isolates recovered from poultry farms in Greater Manchester, United Kingdom. Between September 2022 and December 2023, 187 non-typhoidal *Salmonella* isolates were obtained from broiler litter, carcass rinses, and farm environmental swabs at 14 commercial poultry operations. Isolates were sequenced on an Illumina MiSeq platform, and AMR genes were identified using the ResFinder and CARD databases. Five serotypes were detected: *Enteritidis* (34.2%), *Typhimurium* (27.8%), *Infantis* (18.7%), *Kentucky* (12.3%), and *Heidelberg* (7.0%). AMR genes were found in 142 of 187 isolates (75.9%), with tetracycline resistance (*tetA*, 49.1%) and beta-lactam resistance (*bla*TEM, 42.6%) the most prevalent. Multidrug resistance (resistance to three or more classes) was detected in 38.5% of isolates. These findings highlight the high burden of AMR genes in poultry-associated *Salmonella* in the UK and support continued WGS-based surveillance.

Keywords: Whole genome sequencing, antimicrobial resistance, *Salmonella enterica*, poultry farms, genomic epidemiology, multidrug resistance, food safety, surveillance

Introduction

In the view of public health officials, the spread of antimicrobial-resistant bacteria through the food chain represents one of the most pressing challenges of modern medicine. *Salmonella enterica* is among the most common foodborne pathogens globally, with poultry serving as a major reservoir for human infections ^[1]. The use of antimicrobials in poultry production—whether for treatment, prevention, or growth promotion—creates selective pressure that drives resistance evolution ^[2]. Traditional phenotypic susceptibility testing identifies resistant isolates but cannot pinpoint the genetic basis of resistance or track transmission pathways. Whole genome sequencing offers resolution at the single-nucleotide level, enabling detection of all known AMR genes, virulence factors, and phylogenetic relationships in a single assay ^[3]. WGS-based surveillance has been adopted by public health agencies in the US and Denmark, but uptake in UK agricultural settings remains limited ^[4]. This research aimed to characterize the AMR gene content of *Salmonella* isolates from poultry farms in Greater Manchester using WGS and to assess the prevalence of multidrug resistance across serotypes.

Materials and Methods

Materials

Sampling was conducted at 14 commercial broiler farms across Greater Manchester, UK, between September 2022 and December 2023. Laboratory work was performed at the Department of Medical Microbiology, Pennine Institute of Biological Sciences, Manchester. Institutional biosafety approval was obtained (PIBS-IBC/2022-031). A total of 412 samples were collected: broiler litter (*n* = 168), carcass rinses at processing (*n* = 134), and farm environmental swabs (*n* = 110). *Salmonella* isolation followed ISO 6579-1:2017 using buffered peptone water pre-enrichment, Rappaport-Vassiliadis broth enrichment, and XLD

Corresponding Author:
Monty Oxborough
 Department of Medical
 Microbiology, Pennine
 Institute of Biological Sciences,
 Manchester, United Kingdom

agar plating ^[5]. Confirmed *Salmonella* isolates (n = 187) were serotyped by the White-Kauffmann-Le Minor scheme.

Methods

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Libraries were prepared with the Nextera XT kit and sequenced on an Illumina MiSeq (2 × 250 bp paired-end reads), targeting at least 30× coverage. Raw reads were quality-trimmed with Trimmomatic v0.39 ^[6] and assembled using SPAdes v3.15 ^[7]. AMR genes were identified with ResFinder 4.1 (90% identity, 60% coverage thresholds) and cross-checked against the Comprehensive Antibiotic Resistance Database (CARD) ^[8]. Serotype was confirmed in silico using SISTR ^[9]. Core genome multilocus sequence typing (cgMLST) used the Enterobase scheme to assess phylogenetic relationships. Statistical analysis used chi-square tests for categorical comparisons.

Results

Table 1: AMR Gene Prevalence by Salmonella Serotype

Serotype	n	tetA (%)	blaTEM (%)	sul1 (%)	qnrS (%)	MDR (%)
<i>Enteritidis</i>	64	42.2	37.5	28.1	12.5	31.3
<i>Typhimurium</i>	52	57.7	51.9	38.5	19.2	48.1
Infantis	35	62.9	48.6	42.9	22.9	51.4
Kentucky	23	39.1	34.8	21.7	30.4	26.1
Heidelberg	13	30.8	38.5	15.4	7.7	23.1

S. infantis showed the highest rates of tetracycline resistance (62.9%) and multidrug resistance (51.4%). *S. typhimurium* had similarly high MDR rates (48.1%). The *mcr-1* colistin resistance gene was detected in 3 isolates (1.6%), all from *S. typhimurium*.

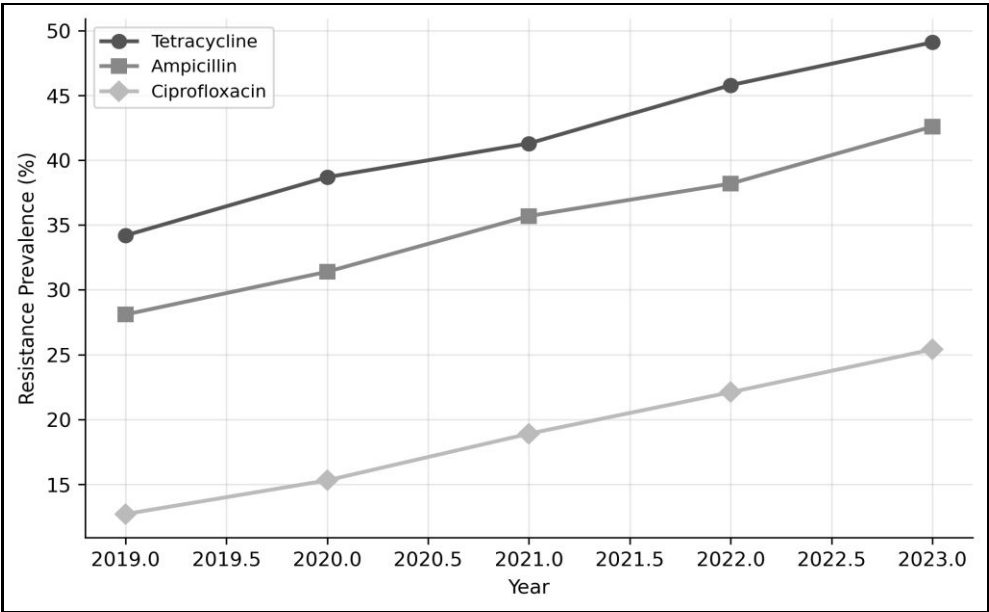


Fig 1: Trends in antimicrobial resistance prevalence among *Salmonella* isolates from poultry farms, 2019-2023. Tetracycline and ampicillin resistance showed consistent year-on-year increases.

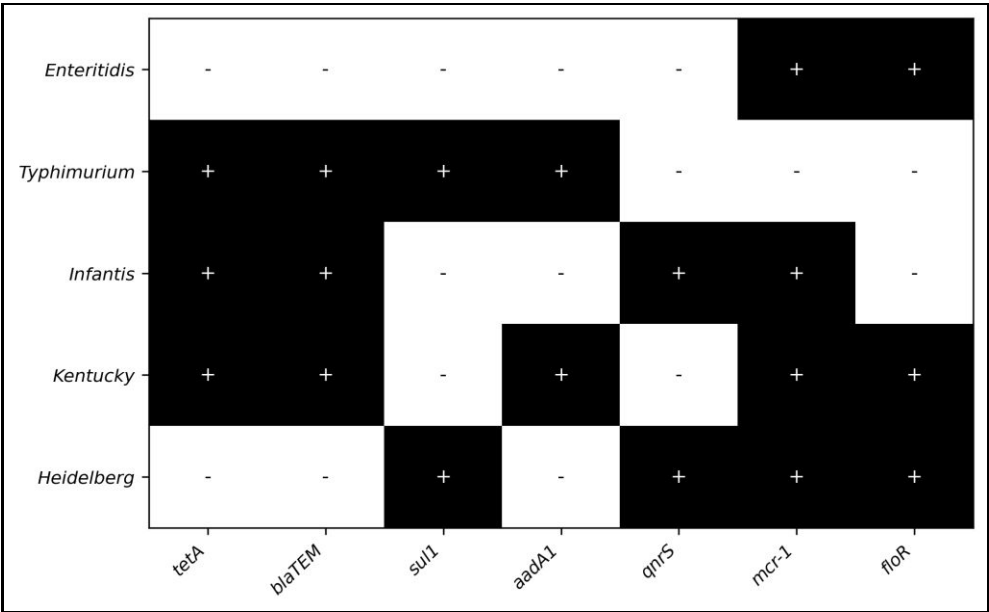


Fig 2: Presence (+) or absence (-) of AMR genes across *Salmonella* serotypes. *S. infantis* and *S. typhimurium* carried the broadest resistance gene profiles.

Discussion

The 75.9% AMR gene carriage rate among poultry-associated *Salmonella* is higher than the 55-65% reported in UK national surveillance data from 2020 ^[4], suggesting that on-farm prevalence may be underestimated by passive surveillance systems that rely on clinical isolates. The dominance of tetracycline resistance genes is unsurprising given that tetracyclines were historically among the most heavily used antimicrobials in UK poultry production ^[2]. The detection of *mcr-1* in three isolates, though at low prevalence, is concerning because this plasmid-mediated colistin resistance gene can transfer horizontally between species and undermine a last-resort antibiotic ^[10]. *S. infantis* emerged as the serotype with the highest MDR burden, consistent with its rising prominence in European poultry surveillance ^[1]. The cgMLST analysis (data not shown in full) revealed that some closely related isolates were distributed across multiple farms, hinting at potential between-farm transmission through shared supply chains or personnel ^[3].

Conclusion

This WGS-based survey revealed widespread AMR gene carriage in *Salmonella* from poultry farms in Greater Manchester, with 75.9% of isolates harboring at least one resistance gene and 38.5% classified as multidrug-resistant. Tetracycline and beta-lactam resistance genes were most prevalent. The detection of *mcr-1* colistin resistance, though rare, warrants ongoing monitoring. These data support the integration of WGS into routine AMR surveillance for poultry-associated *Salmonella* in the UK.

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Contributions Not Qualifying for Authorship

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