FOODBORNE PATHOGENS AND DISEASE
Volume XX, Number XX, 2024

DOI: 10.1089/fpd.2023.0181

Megaplasmid Dissemination in Multidrug-Resistant *Salmonella* Serotypes from Backyard and Commercial Broiler Production Systems in the Southeastern United States

Jessica L. Parzygnat,[1] Rocio Crespo,[1] Mary Fosnaught,[2] Muhammed Muyyarrikkandy,[3] Dawn Hull,[4] Lyndy Harden,[1] and Siddhartha Thakur[1]

Abstract

Over the past decade, there has been a rise in U.S. backyard poultry ownership, raising concern for residential area antimicrobial-resistant (AMR) *Salmonella* contamination. This study aims to lay the groundwork to better understand the persistence of AMR *Salmonella* in residential broiler production systems and make comparisons with commercial systems. Ten backyard and 10 commercial farms were sampled at three time points across bird production. Both fecal ($n = 10$) and environmental (soil, $n = 5$, litter/compost, $n = 5$, feeder, and waterer swabs, $n = 6$) samples were collected at each visit on days 10, 31, and 52 of production for backyard farms and days 10, 24, and 38 of production for commercial farms. AMR *Salmonella* was characterized phenotypically by broth microdilution and genotypically by whole-genome sequencing. Overall, *Salmonella* was more prevalent in commercial farm samples (52.31%) over backyard farms (19.10%). Kentucky (sequence type (ST) 152) was the most common serotype found in both backyard and commercial farms. Multidrug-resistant (MDR, resistance to ≥3 or more antimicrobial classes) isolates were found in both production systems, while ciprofloxacin- and nalidixic acid-resistant and intermediate isolates were more prevalent in commercial (33%) than backyard samples (1%). Plasmids that have been associated with MDR were found in Kentucky and Infantis isolates, particularly IncFIB(K)_1_Kpn3 megaplasmid (Infantis). Our study emphasizes the need to understand the selection pressures in disseminating megaplasmids in MDR *Salmonella* in distinct broiler production systems.

Keywords: *Salmonella*, broilers, antimicrobial resistance, whole-genome sequencing, backyard poultry

Introduction

Salmonella is a top foodborne pathogen worldwide and is known to be strongly associated with the number one consumed meat in the United States, broiler chicken (CDC, 2023b). In the United States, backyard broiler farming is on the rise due to consumer push for local foods (Gwin et al., 2013). Outbreaks of *Salmonella* linked to backyard poultry have occurred consecutively in recent years (2019–2023), which highlights the growing concern for this issue (CDC, 2023c; CDC, 2022b; CDC, 2021; CDC, 2020; CDC, 2019b). Special consideration should be taken for backyard production, as it brings the risk of infection to residential environments surrounding homes. Children and

[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA.
[2]Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, USA.
[3]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brooking, South Dakota, USA.
[4]Bacterial Diseases Branch, Center for Infectious Diseases Research, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA.

immunocompromised individuals are especially at risk given their immune systems are not as robust (CDC, 2023d: Christenson, 2013).

Despite the increase in backyard ownership and recent outbreaks, little research is currently investigating *Salmonella* in backyard production systems in the United States. Severe salmonellosis cases warrant antibiotic administration, but emerging antimicrobial-resistant (AMR) *Salmonella* limits safe treatment options (Asperilla et al., 1990; CDC, 2023a). Multidrug-resistant (MDR) *Salmonella*, having resistance to three or more antimicrobial classes, has been reported by the U.S. National Antimicrobial Resistance Monitoring System (NARMS) in retail chicken (Punchihewage-Don et al., 2022). Although antimicrobial use is a known driver for AMR, resistant strains can exist even in the absence of any antimicrobial (Keelara et al., 2013; Quintana-Hayashi and Thakur, 2012). Given the threat of AMR *Salmonella* to consumers and farm owners, more research needs to be put into understanding AMR dynamics in different broiler production environments.

Our farm-to-genome study characterizes *Salmonella* in terms of on-farm prevalence, phenotypic AMR, and genotypic AMR profiling. This study aims to detail the prevalence and AMR of *Salmonella* in backyard broiler production systems and compare outcomes with more established commercial systems.

Materials and Methods

Farms

Ten backyard and ten commercial broiler farms in the Southeast United States were recruited for the study. The time frame of farm collection was April 2021 to April 2023. Backyard flocks were selected for sampling if they consisted of broiler breeds raised in a residential setting that were sold for meat consumption or used for home consumption. The flocks ranged in size from 22 to 1000 birds in a flock. Commercial farms were considered to be indoor raised birds as part of a large commercial company. Flock size ranged from 13,500 to 30,900 birds. For commercial farms, a ''farm'' was considered a single house at a particular location. In other words, there were 10 different commercial farm locations, and the same house was sampled at each visit. Commercial farm visits were split among three different companies.

For the backyard, farms 1 and 7 and farms 5 and 9 were in the same residential location. However, they were sampled in a different season than the first time. Each farm was visited three times across production on days 10, 31, and 52 for backyard farms and days 10, 24, and 38 for commercial farms. Commercial farms had a shortened sampling time line because commercial birds, on average, went to market quicker than backyard birds.

At each backyard and commercial farm visit, fecal samples ($n=10$), litter or compost ($n=5$), soil ($n=5$), and swabs of the feeders and waterers ($n=6$) were collected. Fecal, litter/compost, and soil samples were collected in sterilized closure bags, while swabs were collected in conical cap tubes containing buffered peptone water. For backyard farms, birds tend to be in a brooder with litter for the first visit and on grass for the remaining visits. Depending on availability, compost or leftover litter was collected for all five samples in the remaining visits. No compost samples were taken from commercial farms, as birds were housed on litter throughout the three visits. For commercial farms, soil samples were taken from just outside the houses.

Processing

Sample processing began the day samples were collected from the farm. Processing procedures were adopted from NARMS Retail Meat Surveillance Laboratory Protocol, but slightly adjusted to fit our project (McDermott, 2021). Day 1 of processing consisted of pouring 90 mL of buffered peptone water into all sterile closure bags containing samples. All sterile closure bags with samples and buffered peptone water were placed on an automatic shaker at 200 RPM for 15 min and then placed in a 35.0°C incubator for 24 h.

On day 2, 100 μL of the incubated buffered peptone water from each sample was placed in a tube of RV broth and then incubated at 35°C for 24 h. On day 3, a 10 μL inoculation loop was used to streak the incubated RV (BD 218581) broth onto XLT4 (BD 223420) and then incubated at 35.0°C for 24 h. After incubation, one black colony was selected from each plate with a sterile inoculation loop, streaked onto blood agar plates (BAP; BD 221261), and incubated at 35.0°C for another 24 h. The bacterial cultures were then placed into tubes with Brucella broth (BD 211088) and 15% glycerol and stored in a −80°C freezer.

For backyard farms, multiple isolates were taken from a single plate for the first five farms. For commercial isolates and remaining backyard farms, only one isolate was taken per plate/sample. Loop-mediated isothermal amplification (LAMP) assay was conducted for 65 random isolates to confirm our isolation protocol.

Antimicrobial susceptibility testing

The NARMS 2020 Manual of Laboratory Methods was followed for antimicrobial susceptibility testing (NARMS, 2020). Frozen glycerol tubes with *Salmonella* isolates were streaked onto XLT4 (BD 223420) and incubated overnight at 35.0°C for 24 h. One colony was streaked onto BAP (BD 221261) and incubated overnight again at 35.0°C for 24 h. Using a 1 μL inoculation loop, the culture was inoculated in 5 mL of demineralized water and vortexed until adjusted to a 0.5 McFarland Standard. Ten microliters of the adjusted McFarland Standard was then transferred to 11 mL of Mueller Hinton Broth (T3462; Thermo Scientific™) and vortexed. The inoculated Mueller Hinton Broth tube was placed into a Sensititre™ System, which dispensed 50 μL into each well of a 90-well plate.

The NARMS Gram-negative plate was used for broth microdilution testing (CMV3AGNF; Thermo Scientific, Sensititre). Bacterial susceptibility was tested for the following antimicrobials: cefoxitin (FOX; 0.5–32 μg/mL), azithromycin (AZI; 0.12–16 μg/mL), chloramphenicol (CHL; 2–32 μg/mL), tetracycline (TET; 4–32 μg/mL), ceftriaxone (AXO; 0.25–64 μg/mL), amoxicillin/clavulanic acid (AUG2; 1/0.5–32/16 μg/mL), ciprofloxacin (CIP; 0.015–4 μg/mL), gentamicin (GEN; 0.25–16 μg/mL), nalidixic acid (NAL; 0.5–32 ug/mL), ceftiofur (XNL; 0.12–8 μg/mL), sulfisoxazole (FIS; 16–256 μg/mL), trimethoprim/sulfamethoxazole (SXT; 0.12/2.38–4/76 μg/mL), ampicillin

(AMP; 1–32 μg/mL), and streptomycin (STR; 2–64 μg/mL). Plates were manually read with a Sensititre Manual Viewbox ~24 h later. We detected the minimum inhibitory concentration (MIC) for each isolate in the study. MIC is defined as the minimum concentration required to inhibit the growth of the bacterial isolate.

NARMS breakpoints were used to determine if isolates were susceptible (therapeutic success likely), intermediate (therapeutic success unknown), or resistant (therapeutic failure likely) (NARMS, 2019; Rodloff et al., 2008).

DNA isolation and whole-genome sequencing

For DNA isolation, *Salmonella* freezer stock was streaked onto XLT4 and then BAP (BD 221261). Bacterial genomic DNA was extracted using the Qiagen DNeasy PowerLyzer Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. Quality and concentration of extracted DNA were determined using a NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and a Qubit Flex Fluorometer (Thermo Fisher Scientific). DNA libraries were prepared using the Illumina DNA Prep Kit (Product No. 20060059; Illumina, San Diego, CA) following the manufacturer's instructions. The resulting DNA libraries were requantified using the Qubit Flex Fluorometer. Whole-genome sequencing (WGS) was performed on the Illumina MiSeq System using the MiSeq Reagent Kit v3 600 cycles (Illumina). All samples can be found on the National Center for Biotechnology Information (NCBI) under BioProject: PRJNA293224.

Bioinformatic pipeline

The bioinformatic pipeline is as described in Hull et al. (2022). Briefly, Amazon Web Services was used to send whole-genome shotgun sequence data through Shovill v1.1.0 and SPAdes version 3.15.2 for *de novo* assembly (Bankevich et al., 2012). Quality of genome assembly was conducted by QUAST v 5.0.2 (Gurevich et al., 2013). The resulting fasta files were blasted against databases to identify AMR and virulence genes using ABRICATE version 1.0.1 (Seemann, 2020). This produced output from databases CARD, MEGAres, VFDB, and Plasmidfinder (Alcock et al., 2020; Carattoli et al., 2014; Chen et al., 2016; Doster et al., 2020; Feldgarden et al., 2019). Sequences were also passed through MLST v 2.19.0 and AMRFinderPlus. Plasmid data were also assembled through Shovill and PlasmidSPAdes. Plasmid fasta files were screened for AMR and virulence genes using the same pipeline detailed above.

Results from the WGS data and plasmid data were compared to assess carriage of resistance genes on plasmid versus chromosomal DNA.

Serotyping and phylogenetic trees

Serotypes were determined through a combination of SeqSero2 v1.1.0, bettercallsal, and NCBI Pathogen Detection (Konganti et al., 2023; NCBI, 2023; Zhang et al., 2019). Phylogenetic trees were created using the online open-access tool REALPHY v1.13 (Bertels et al., 2014). The results were visualized using Interactive Tree of Life (Letunic and Bork, 2021).

Statistical analysis

A combination of R-Studio and MedCalc was used for chi-squared testing and overall analysis (MedCalc, 2023; RStudioTeam, 2020). CDC EpiCalc Info StatCalc v5.5.11 software was used to conduct Fisher's exact two-tailed test (CDC, 2022a). Significance was determined with a p-value <0.05.

Results

Of the 780 samples collected from each farm type, 149 tested positive in backyard farms for *Salmonella* (19.10%) and 408 tested positive in commercial farms (52.31%; chi-squared, $p<0.05$). The distribution of positive samples among sample types is displayed in Figure 1, and the exact numbers within farm type and sample are in Table 1. The proportion of positive isolates between farm types was significant for only fecal samples (Fisher's exact, p-value <0.05). Of the total 557 *Salmonella* isolates collected, 345 of them were sequenced ($n=73$ backyard, $n=272$ commercial). Isolates were selected for sequencing to obtain representative

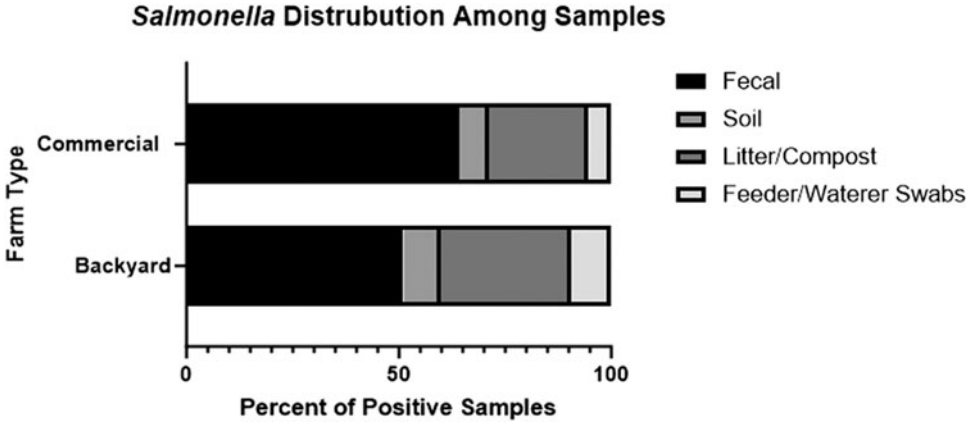

FIG. 1. Sample distribution. This stacked bar chart shows what proportion of samples came from each sample type (fecal, soil, litter/compost, feeder/waterer swabs). The proportion of all *Salmonella*-positive samples from commercial and backyard farms is included. The proportion of fecal samples was found to be significant between farm types (Fisher's exact, p-value <0.05).

Table 1. Positive Samples Across Farm and Sample Type

Farm	*Fecal* (n = *30*)	*Soil* (n = *15*)	*Litter/compost* (n = *15*)	*Swab* (n = *18*)	*Total*
Backyard 1	0	0	0	0	0
Backyard 2	0	0	8	0	8
Backyard 3	1	1	4	0	6
Backyard 4	12	2	6	3	23
Backyard 5	13	1	4	4	22
Backyard 6	3	2	8	2	15
Backyard 7	15	0	6	3	24
Backyard 8	8	1	1	1	11
Backyard 9	1	0	0	0	1
Backyard 10	23	6	9	1	39
Commercial 1	23	3	9	10	45
Commercial 2	29	6	10	1	46
Commercial 3	29	0	13	3	45
Commercial 4	28	0	14	6	48
Commercial 5	20	1	5	0	26
Commercial 6	24	10	9	0	43
Commercial 7	19	0	1	0	20
Commercial 8	30	2	9	0	41
Commercial 9	30	2	12	0	44
Commercial 10	30	4	14	2	50

This table shows the distribution of positive *Salmonella* samples among the individual backyard and commercial farm types as well as how they were spread among sample types. This includes data from all three visits. A total of $n = 30$ fecal, $n = 15$ soil, $n = 15$ litter/compost, and $n = 18$ feeder and waterer swabs were taken in total from each farm.

samples based on phenotypic resistance, sample type, and farm.

Serotyping revealed the top serotype from both farm systems to be Kentucky (Overall: $n = 103/345$, 29.86%; Commercial: $n = 72/103$; Backyard: $n = 31/103$). Along with Kentucky, Anatum ($n = 10/73$) and Infantis ($n = 7/73$) made up the top three serotypes for backyard farms while Infantis ($n = 53/272$) and Thompson ($n = 38/272$) were predominant in the commercial production system.

Antimicrobial susceptibility testing was conducted for all isolates (203, backyard; 408, commercial) and the results are displayed in Table 2. Overall, commercial farms did not have a statistically significant proportion of pan-susceptible isolates (48.53%; $n = 198/408$) compared with backyard farms (49.26%; $n = 100/203$; Fisher's exact; p-value >0.05). Proportions of phenotypic MDR isolates were not statistically significant between farm types, as 25% ($n = 102/408$) of commercial isolates and 17.73% ($n = 36/203$) of backyard isolates displayed MDR (Fisher's exact; p-value >0.05). The MDR isolates were concentrated in 2 of the 10 backyard farms and 4 of the 10 commercial farms. Of the phenotypic MDR isolates that were sequenced ($n = 64/137$), the most common serotype was Infantis ($n = 49/64$; 76.56%) and the second most was Kentucky ($n = 11/64$; 17.18%) (Fig. 2).

The most prevalent backyard MDR pattern was STR TET FIS GEN (Backyard 12.32%; Commercial 0.98%) and the most prevalent commercial pattern was STR TET FIS NAL (Backyard 0.99%; Commercial 14.95%).

Phenotypic resistance was detected against tetracycline, chloramphenicol, aminoglycoside, and sulfonamide in both farm systems (Table 2). To highlight specific antimicrobials, in commercial farms, 33% ($n = 136/408$) of isolates were phenotypically resistant to nalidixic acid and 33% ($n = 136/408$) were intermediate to ciprofloxacin. In backyard farms, two isolates ($\sim 1\%$) were resistant to nalidixic acid and two isolates ($\sim 1\%$) were intermediate to ciprofloxacin. The proportion of isolates that were resistant to these two antimicrobials was statistically significant between backyard and commercial farms (Fisher's exact; p-value <0.05). Of those carrying this phenotype, 71 were sequenced and the top serotype associated was Infantis ($n = 55/71$; 77.46%) followed by Enteritidis ($n = 12/71$; 16.90%).

Genotypic data revealed resistance genes and point mutation that all together cover multiple antimicrobial classes: aminoglycosides [*aac(3)-IV*, *aac(3)-VIa*, *aac(6′)-Iaa*, *aac(6′)-Iy*, *ant(3″)-IIa*, *aph(3″)-Ib*, *aph(4)-Ia*, *aph(6)-Id*; $n = 343/345$; 99.42%], sulfonamides (*sulI*, *sulII*; $n = 86/345$; 24.93%), beta-lactam (*ampH*, *CTX*; $n = 280/345$; 81.16%), TET (*tetracycline*, *tetB*, *tetD*; $n = 119/345$; 34.49%), phenicol (*floR*; $n = 9/345$; 2.61%), trimethoprim (*dfrA*; $n = 9/345$; 2.61%), fosfomycin (*fosA*; $n = 2/345$; 0.58%), and quinolone (*gyrA-D87y*; $n = 69/345$; 20%). In addition, genes responsible for efflux pumps were identified (*acrA*, *acrB*, *emrA*, *emrB*, *gesA*, *gesB*, *gesC*). Of sequenced isolates, 89% ($n = 307/345$) carried a plasmid. The most common plasmid detected among all sequenced isolates in both farm types was IncX1_3 ($n = 132/307$; 42.86%). The most common plasmid detected among MDR isolates was IncFIB(K)_1_Kpn3 ($n = 49/64$). Phenotypic, genotypic, and plasmid data are displayed in Figure 2 for sequenced MDR isolates.

Discussion

Backyard broiler production is increasing in the United States, yet studies investigating the prevalence and AMR *Salmonella* in these environments are scarce. In our study, 90% of flocks tested positive for *Salmonella*, which is higher than previous studies conducted in Massachusetts (2% of flocks tested positive), Vermont (19% of flocks tested positive), and Washington State (3% of flocks tested positive) (Larsen et al., 2022; McDonagh et al., 2018; Shah et al., 2020). Our commercial farm prevalence was higher than

Table 2. Squashtogram of *Salmonella* Minimum Inhibitory Concentrations

			Distribution of MIC (ug/mL) (# of isolates)															
*	*Source*	*% resistant*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	8	*16*	32	*64*	*128*	*256*	*512*
AUG2	Commercial	0%							385	23	0	0	0	0				
	Backyard	0%							191	8	4	0	0	0				
AMP	Commercial	0%							382	26	0	0	0	0				
	Backyard	0%							190	9	3	1	0	0				
AZI	Commercial	0%				0	0	0	4	121	217	59	7					
	Backyard	0%				0	0	0	0	50	141	12	0					
FOX	Commercial	0.25%						2	3	242	155	4	1	**1**				
	Backyard	0%						0	3	111	82	6	1	0				
XNL	Commercial	0%				0	0	97	304	7	0	0						
	Backyard	0%				0	0	78	119	6	0	0						
AXO	Commercial	0%					408	0	0	0	0	0	0	0	0			
	Backyard	0%					203	0	0	0	0	0	0	0	0			
GEN[a]	Commercial	1.47%					140	190	12	1	3	56	**1**	**5**				
	Backyard	15.27%					43	123	6	0	0	0	**7**	**24**				
STR[a]	Commercial	33.82%								1	36	147	86	**95**	**17**	**26**		
	Backyard	46.80%								1	9	43	55	**5**	**14**	**76**		
NAL[a]	Commercial	33.33%						1	0	21	246	3	1	0	**136**			
	Backyard	0.99%						2	3	86	110	0	0	0	**2**			
CIP	Commercial	0%	238	33	0	16	121	0	0	0	0							
	Backyard	0%	162	39	0	1	1	0	0	0	0							
SXT[a]	Commercial	8.82%				357	14	0	1	0	0	**36**						
	Backyard	0%				203	0	0	0	0	0							
FIS[a]	Commercial	33.33%											90	179	3	0	0	**136**
	Backyard	20.69%											45	40	63	6	7	**42**
CHL[a]	Commercial	9.07%								6	240	123	2	0	**37**			
	Backyard	0%								1	99	99	4	0				
TET[a]	Commercial	34.56%									267	0	0	0	**141**			
	Backyard	43.35%									115	0	0	0	**88**			

Displays the range of antimicrobial concentration for each antibiotic used for testing. It shows the number of isolates that had an MIC at each concentration for both backyard and commercial farms. Backyard farm samples tested for include all isolates collected as opposed to all positive samples. The bold numbers represent isolates to be considered resistant according to NARMS breakpoints.

[a]Indicates the proportion of resistant isolates was statistically significant according to farm type (Fisher's exact, p-value <0.05).

AMP, ampicillin; AUG2, amoxicillin/clavulanic acid; AXO, ceftriaxone; AZI, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; FIS, sulfisoxazole; FOX, cefoxitin; GEN, gentamicin; MIC, minimum inhibitory concentration; NAL, nalidixic acid; NARMS, National Antimicrobial Resistance Monitoring System; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; XNL, ceftiofur.

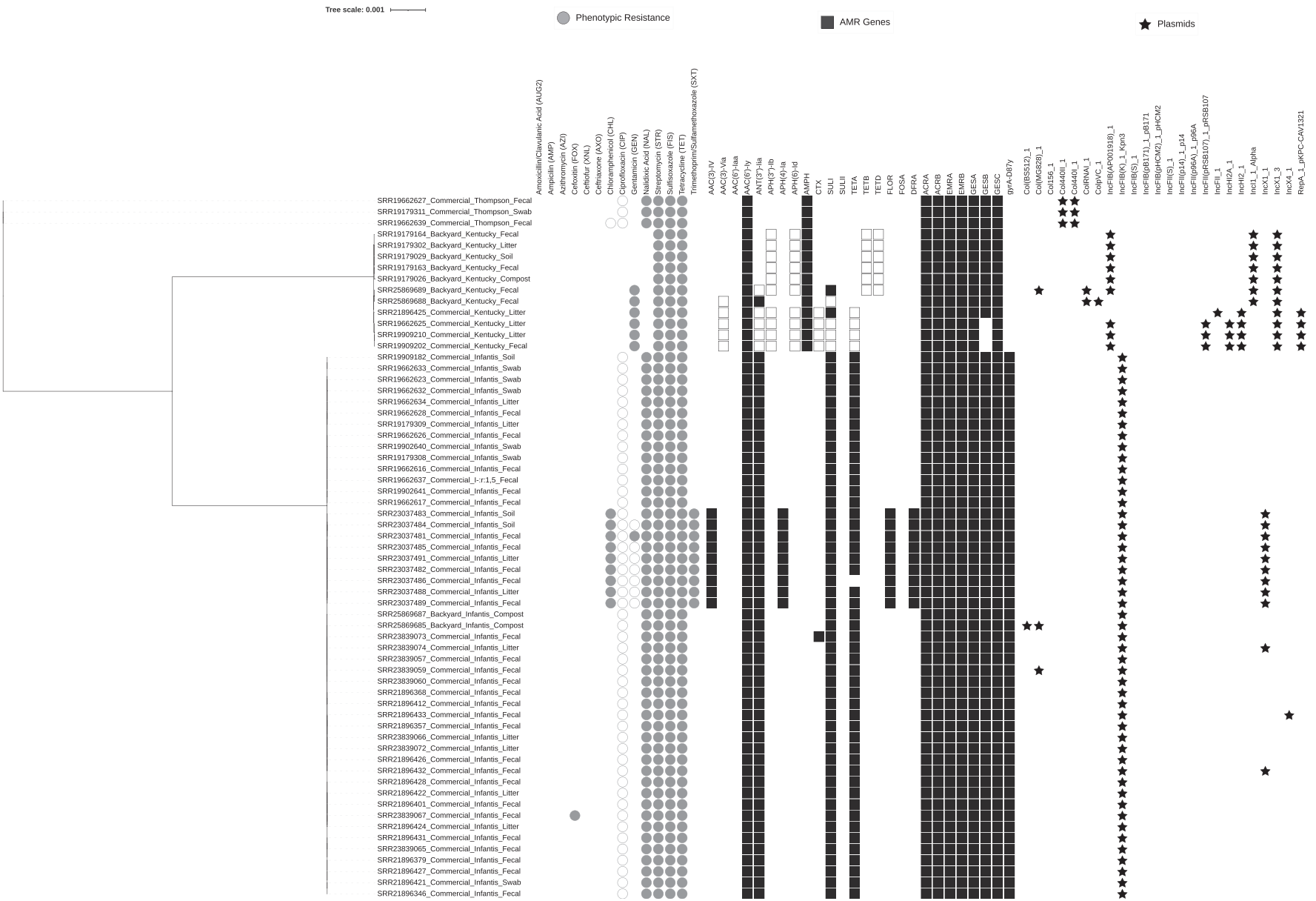

FIG. 2. Resistance characterization of MDR isolates. Phenotypically MDR isolates that have been sequenced ($n = 63$ sequenced/137 Phenotypically MDR) are displayed in the dendrogram. Isolates are listed as SRR#_Farm Type_Serotype_Sample source. Circles represent phenotypic resistance to the listed antimicrobials. Outlined circles mean the isolate was determined to be intermediate. Squares show the presence of antimicrobial resistance genes. Squares that are outlined and not filled in represent genes that were found on plasmids. Genes detected in the entire data set beyond MDR isolates are listed. Stars indicated the presence of the plasmids listed. All plasmids found in the entire data set ($n = 307$) aside from just what is found in MDR isolates are listed. MDR, multidrug resistant.

reported in literature. Our study found 100% of farms testing positive with a 52.31% sample prevalence. A study based on a meta-analysis from literature review estimated a sample prevalence of 22.9% from conventional chicken farms (Golden and Mishra, 2020). Another study investigating conventional and organic commercial broiler farms found a sample prevalence of 38.8% for conventional and a lower prevalence for organic (5.6%) (Alali et al., 2010).

For our study, we sampled both conventional and organic commercial farms. The two farms with lowest percentages of positive samples (25.64% and 33.33%) for *Salmonella* happen to be from organic farms, but other organic farms had comparable numbers with conventional farms in this study (55.13–64.10%).

The dominant serotype for both farm types in our study was Kentucky. All the Kentucky sequence types (STs) in this study were identified as 152 according to multilocus sequence typing (MLST), which is commonly associated with U.S. poultry but not necessarily human illness (Soltys et al., 2021; Tate et al., 2022). Another ST that is commonly associated with Kentucky as well as human illness is ST 198; however, this ST does not appear to be common in U.S. domestic poultry and we did not find it in this study (Soltys et al., 2021; Tate et al., 2022; Vosik et al., 2018; Xiong et al., 2020).

Our study establishes a widespread prevalence of ST 152 in both backyard and commercial production systems in the Southeastern United States, as well as emphasizes its MDR importance. ST 152 is not currently known for *Salmonella* outbreaks; however, it was the second-most common ST associated with MDR in our study. It has been speculated that ST 152 potentially causes mild symptoms that recover quickly and therefore illness is not reported (Tate et al., 2022). This has concerning implications for backyard farms as they potentially expose immunocompromised individuals or children, who may require antimicrobial assistance in recovery.

MDR *Salmonella* is an increasing public health threat worldwide (Ferreira et al., 2020; Gambino et al., 2022; Zhang et al., 2018). Both backyard and commercial farms in our study contained MDR isolates, and the proportions of MDR isolates were not statistically significantly different between farm types. The most recent NARMS (2019) report marks increases in MDR *Salmonella* between 2018 and 2019 with MDR prevalence rising from 20% to 32% (NARMS, 2022). Infantis was identified in both farm types and was the top MDR serotype, supporting NARMS and other literature highlighting this serotype as a culprit for worldwide MDR *Salmonella* emergence and human illness (Alvarez et al., 2023; Hull et al., 2022; NARMS, 2022). We discovered that the prevalence of MDR serotypes was concentrated to specific farms within each system, which highlights the need to identify what environmental pressures or genomic factors could be contributing.

We found many resistance genes not associated with plasmids, potentially creating a stable passing from one generation to the next within serotypes in these farm systems (Storey et al., 2022).

Another important genomic consideration is plasmids, as they are notorious for their spread of AMR genes in *Salmonella* (McMillan et al., 2020). The most common plasmid found overall was IncX1_3 and was contained in serotypes Kentucky or -8:zi6, a closely associated strain variant (NCBI, 2023). This plasmid has been noted as MDR previously in association with Kentucky from chicken retail samples (Hull et al., 2022). Our pipeline also identified the IncFIB(K)_1_Kpn3 megaplasmid (coverage: 99.64%; identity: 82.80%) in 55 of the total 60 Infantis isolates and in all 3 I -:r:1,5 isolates, which appears to be associated with Infantis (NCBI, 2023). This plasmid has also previously been identified as an MDR plasmid associated with chicken samples (Hull et al., 2022).

MDR *Salmonella* Infantis appears to be a major emerging public health threat, given the latest NARMS report and other literature detailing its prevalence in the United States (Hull et al., 2022; NARMS, 2022; Tyson et al., 2021). Two of our backyard farms contained Infantis isolates, and the IncFIB(K)_1_Kpn3 megaplasmid was prevalent in the isolates from the farm with MDR isolates. Our findings support concerns about MDR *Salmonella* Infantis spread as well as the role of megaplasmids in AMR dissemination.

Antibiogram results revealed resistance to first-line antimicrobials used for treating *Salmonella* infections, nalidixic acid and ciprofloxacin (CDC, 2019a; Mølbak et al., 2002; Walker et al., 2021). Although this was more prevalent in commercial farms, presence in backyard farms is still concerning given the importance of these antimicrobials. Our findings support data from NARMS noting a decrease in ciprofloxacin-susceptible *Salmonella* isolated from poultry samples (NARMS, 2022). The *gyrA_D87Y* point mutation is known to confer resistance to these antimicrobials and was present in almost all isolates displaying resistance ($n = 49/52$) (CDC, 2019a; Mølbak et al., 2002; Walker et al., 2021). Various efflux pumps, such as AcrAB-TolC and EmrAB, could be the reason for resistance in the remaining isolates, which all happened to be serotype Thompson (Alcock et al., 2020; Alenazy, 2022).

It is important to note that fluoroquinolones have been banned in poultry since 2005 in the United States, emphasizing the need to understand the environmental pressures causing the persistence of resistant bacteria (FDA, 2017). In this study, the majority of isolates containing these resistance markers were Infantis and Enteritidis. These serotypes (Infantis and Enteritidis) have also been linked to recent outbreaks linked to backyard poultry (CDC, 2023c; CDC, 2022c). Serotype Infantis is of particular concern given our findings of MDR in this study, as resistance to first-line antimicrobials as well as other antimicrobial classes threatens viable treatment options.

Conclusion

Overall, it is evident that risk of AMR *Salmonella* is present without the use of antimicrobials in both backyard and commercial farm settings. We found MDR Kentucky ST 152 to be established in both commercial and backyard broiler farms in the Southeastern United States. Our research also supports the role of megaplasmids in MDR *Salmonella* Infantis, which is a pathogen emerging as a concern worldwide. The prevalence of MDR *Salmonella* serotypes, particularly Infantis, is concerning given its implications for health care burden. Our research highlights the need to assess the effect of unique environmental conditions to better understand AMR *Salmonella* persistence.

Acknowledgments

We would like to thank Erin Harrell, Luke Raymond, and our undergraduate team for field sampling and laboratory processing support.

Authors' Contributions

S.T. presented the idea and obtained funding. R.C. found commercial farms to sample. M.F. found backyard farms to sample and traveled to farms to assist on sampling day. J.L.P., L.H., and M.M. conducted field sampling, planning, and sample processing. L.H. prepared samples for and conducted WGS. J.L.P. performed bioinformatic analysis with insight and assistance from D.H. and L.H. J.L.P. wrote the article with feedback from all the coauthors.

Disclosure Statement

The authors declare that they have no competing interests.

Funding Information

This study was funded by the USDA NIFA SAS Grant 410553 and WGS was funded by the FDA GenomeTrakr program-funded grant 5U19FD007113.

References

Alali WQ, Thakur S, Berghaus RD, et al. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. Foodborne Pathog Dis 2010;7(11):1363–1371; doi: 10.1089/fpd.2010.0566

Alcock BP, Raphenya AR, Lau TTY, et al. CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 2020; 48(D1):D517–D525; doi: 10.1093/nar/gkz935

Alenazy R. Antibiotic resistance in *Salmonella*: Targeting multidrug resistance by understanding efflux pumps, regulators and inhibitors. J King Saud Univ Sci 2022;34(7):1–8; doi: 10.1016/j.jksus.2022.102275

Alvarez DM, Barron-Montenegro R, Conejeros J, et al. A review of the global emergence of multidrug-resistant *Salmonella enterica* subsp. enterica serovar Infantis. Int J Food Microbiol 2023;403:1–13; doi: 10.1016/j.ijfoodmicro.2023.110297

Asperilla MO, Smego RA, Scott LK. Quinolone antibiotics in the treatment of *Salmonella* infections. Rev Infect Dis 1990; 12(5):873–889; doi: 10.1093/clinids/12.5.873

Bankevich A, Nurk S, Antipov D, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19(5):455–477; doi: 10.1089/cmb.2012.0021

Bertels F, Silander O, Pachkov M, et al. Automated reconstruction of whole-genome phylogenies from short-sequence reads. Mol Biol Evol 2014;31(5):1077–1088; doi: 10.1093/molbev/msu088

Carattoli A, Zankari E, Garcia-Fernandez A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58(7):3895–3903; doi: 10.1128/aac.02412-14

Centers for Disease Control and Prevention (CDC). Advice to Clinicians. 2019a. Available from: https://www.cdc.gov/salmonella/infantis-10-18/advice.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). Outbreaks of *Salmonella* Infections Linked to Backyard Poultry. 2019b. Available from: https://www.cdc.gov/salmonella/backyardpoultry-05-19/index.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). Outbreaks of *Salmonella* Linked to Backyard Poultry. 2020. Available from: https://www.cdc.gov/salmonella/backyardpoultry-05-20/index.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). *Salmonella* Outbreaks Linked to Backyard Poultry. 2021. Available from: https://www.cdc.gov/salmonella/backyardpoultry-05-21/index.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). Epi Info™ StatCalc. 2022a. Available from: https://www.cdc.gov/epiinfo/user-guide/statcalc/statcalcintro.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). *Salmonella* Outbreaks Linked to Backyard Poultry. 2022b. Available from: https://www.cdc.gov/salmonella/backyardpoultry-06-22/index.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). Snapshots of Serotypes. 2022c. Available from: https://www.cdc.gov/salmonella/reportspubs/salmonella-atlas/serotype-snapshots.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). Information for Healthcare Professionals and Laboratories. 2023a. Available from: https://www.cdc.gov/salmonella/general/technical.html#:~:text=Recommended%20antibiotics%20for%20severe%20infections,azithromycin%2C%20and%20third%20generation%20cephalosporins [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). *Salmonella* and Food. 2023b. Available from: https://www.cdc.gov/foodsafety/communication/salmonella-food.html#:~:text=CDC%20estimates%20that%20Salmonella%20causes,if%20it%27s%20not%20cooked%20thoroughly [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). *Salmonella* Outbreaks Linked to Backyard Poultry. 2023c. Available from: https://www.cdc.gov/salmonella/backyardpoultry-05-23/index.html [Last accessed: October 26, 2023].

Centers for Disease Control and Prevention (CDC). *Salmonella*: Questions & Answers. 2023d. Available from: https://www.cdc.gov/salmonella/general/index.html#:~:text=Children%20under%205%20years%20old,likely%20to%20have%20severe%20infections [Last accessed: October 26, 2023].

Chen L, Zheng D, Liu B, et al. VFDB 2016: Hierarchical and refined dataset for big data analysis—10 Years on. Nucleic Acids Res 2016;44(D1):D694–D697; doi: 10.1093/nar/gkv1239

Christenson JC. *Salmonella* infections. Pediatr Rev 2013;34(9): 375–383; doi: 10.1542/pir.34-9-375

Doster E, Lakin S, Dean C, et al. MEGARes 2.0: A database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Res 2020;48:561–569; doi: 10.1093/nar/gkz1010

Feldgarden M, Brover V, Haft D, et al. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother 2019; 63(11):1–19; doi: 10.1128/aac.00483-19

Ferreira V, Cardoso MJ, Magalhaes R, et al. Occurrence of *Salmonella* spp. in eggs from backyard chicken flocks

in Portugal and Romania—Results of a preliminary study. Food Control 2020;113:1–6; doi: 10.1016/j.foodcont.2020.107180

Gambino D, Gargano V, Butera G, et al. Food is reservoir of MDR *Salmonella*: Prevalence of ESBLs profiles and resistance genes in strains isolated from food. Microorganisms 2022;10(4):1–8; doi: 10.3390/microorganisms10040780

Golden CE, Mishra A. Prevalence of *Salmonella* and *Campylobacter* spp. in alternative and conventionally produced chicken in the United States: A systematic review and meta-analysis. J Food Prot 2020;83(7):1181–1197; doi: 10.4315/JFP-19-538

Gurevich A, Saveliev V, Vyahhi N, et al. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 2013;29(8):1072–1075; doi: 10.1093/bioinformatics/btt086

Gwin L, Thiboumery A, Stillman R. Local Meat and Poultry Processing. Economic Research Report Number 150; 2013. Available from: https://www.ers.usda.gov/webdocs/publications/45094/37949_err-150.pdf?v=4821.3 [Last accessed: October 21, 2023].

Hull D, Harrell E, Harden L, et al. Multidrug resistance and virulence genes carried by mobile genomic elements in *Salmonella enterica* isolated from live food animals, processed, and retail meat in North Carolina, 2018–2019. Int J Food Microbiol 2022;378:1–18; doi: 10.1016/j.ijfoodmicro.2022.109821

Keelara S, Scott HM, Morrow WM, et al. Longitudinal study of distributions of similar antimicrobial-resistant *Salmonella* serovars in pigs and their environment in two distinct swine production systems. Appl Environ Microbiol 2013;79(17):5167–5178; doi: 10.1128/AEM.01419-13

Konganti K, Reed E, Mammel M, et al. bettercallsal: Better calling of *Salmonella* serotypes from enrichment cultures using shotgun metagenomic profiling and its application in an outbreak setting. bioRxiv 2023;1–27; doi: 10.1101/2023.04.06.535929

Larsen KM, DeCicco M, Hood K, et al. *Salmonella enterica* frequency in backyard chickens in Vermont and biosecurity knowledge and pracices of owners. Front Vet Med 2022;9:1–14; doi: 10.3389/fvets.2022.979548

Letunic I, Bork P. Interactive Tree of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. Nucleic Acids Res 2021;49(W1):W293–W296; doi: 10.1093/nar/gkab301

McDermott S. National Antimicrobial Resistance Monitoring System (NARMS) Retail Meat Surveillance Laboratory Protocol. 2021. Available from: https://www.fda.gov/media/93332/download [Last accessed: October 23, 2023].

McDonagh A, Leibler JH, Mukherjee J, et al. Frequent human-poultry interactions and low prevalence of *Salmonella* in backyard chicken flocks in Massachusetts. Zoonoses Public Health 2018;66(1):92–100; doi: 10.1111/zph.12538

McMillan EA, Jackson CR, Frye JG. Transferable plasmids of *Salmonella enterica* associated with antibiotic resistance genes. Front Microbiol 2020;11(562181):1–9; doi: 10.3389/fmicb.2020.562181

MedCalc. MedCalc® Statistical Software Version 22.009. Ostend; 2023. Available from: https://www.medcalc.org [Last accessed: October 23, 2023].

Mølbak K, Gerner-Smidt P, Wegener HC. Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerg Infect Dis 2002;8(5):514–515; doi: 10.3201/eid0805.010288

National Antimicrobial Resistance Monitoring System (NARMS). Antibiotics Tested by NARMS. 2019. Available from: https://www.cdc.gov/narms/antibiotics-tested.html [Last accessed: October 22, 2023].

National Antimicrobial Resistance Monitoring System (NARMS). The National Antimicrobial Resistance Monitoring System Manual of Laboratory Methods. 2020. Available from: https://www.fda.gov/media/101423/download [Last accessed: October 22, 2023].

National Antimicrobial Resistance Monitoring System (NARMS). 2019 NARMS Update: Integrated Report Summary. 2022. Available from: https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/2019-narms-update-integrated-report-summary [Last accessed: October 22, 2023].

National Center for Biotechnology Information (NCBI). Pathogen Detection. 2023. Available from: https://www.ncbi.nlm.nih.gov/pathogens [Last accessed: October 23, 2023].

Punchihewage-Don AJ, Hawkins J, Adnan AM, et al. The outbreaks and prevalence of antimicrobial resistant *Salmonella* in poultry in the United States: An overview. Heliyon 2022;8(11):e11571; doi: 10.1016/j.heliyon.2022.e11571

Quintana-Hayashi MP, Thakur S. Longitudinal study of the persistence of antimicrobial-resistant *Campylobacter* strains in distinct swine production systems on farms, at slaughter, and in the environment. Appl Environ Microbiol 2012;78(8):2698–2705; doi: 10.1128/AEM.07723-11

Rodloff A, Bauer T, Ewig S, et al. Susceptible, intermediate, and resistant—The intensity of antibiotic action. Dtsch Arztebl Int 2008;105(39):657–662; doi: 10.3238/arztebl.2008.0657

RStudioTeam. Integrated Development for R. R Studio, PBC: Boston, MA; 2020.

Seemann T. ABRicate. 2020. Available from: https://github.com/tseemann/abricate [Last accessed: October 23, 2023].

Shah DH, Board MM, Crespo R, et al. The occurrence of *Salmonella*, extended-spectrum β-lactamase producing *Escherichia coli* and carbapenem resistant non-fermenting Gram-negative bacteria in a backyard poultry flock environment. Zoonoses public Health 2020;67(6):742–753; doi: 10.1111/zph.12756

Soltys R, Sakomoto C, Oltean H, et al. High-Resolution Comparative Genomics of *Salmonella* Kentucky Aids Source Tracing and Detection of ST198 and ST152 Lineage-Specific Mutations. CDC; 2021. Available from: https://www.ars.usda.gov/research/publications/publication/?seqNo115=383870 [Last accessed: October 24, 2023].

Storey N, Cawthraw S, Turner O, et al. Use of genomics to explore AMR persistence in an outdoor pig farm with low antimicrobial usage. Microb Genom 2022;8(3):000782; doi: 10.1099/mgen.0.000782

Tate H, Hsu C-H, Chen JC, et al. Genomic diversity, antimicrobial resistance, and virulence gene profiles of *Salmonella* serovar Kentucky isolated from humans, food, and animal ceca. Foodborne Pathog Dis 2022;19(8):509–521; doi: 10.1089/fpd.2022.0005

Tyson GH, Li C, Harrison LB, et al. A multidrug-resistant *Salmonella* Infantis clone is spreading and recombining in the United States. Microb Drug Resist 2021;27(6):1–8; doi: 10.1089/mdr.2020.0389

U.S. Food & Drug Administration (FDA). Withdrawal of Enrofloxacin for Poultry. 2017. Available from: https://www.fda.gov/animal-veterinary/recalls-withdrawals/withdrawal-enrofloxacin-poultry [Last accessed: November 24, 2023].

Vosik D, Tewari D, Dettinger L, et al. CRISPR typing and antibiotic resistance correlates with polyphyletic distribution

in human isolates of *Salmonella* Kentucky. Foodborne Pathog Dis 2018;15(2):101–108; doi: 10.1089/fpd.2017.2298

Walker GK, Suyemoto MM, Hull DM, et al. Genomic characterization of a nalidixic acid-resistant *Salmonella* Enteritidis strain causing persistent infections in broiler chickens. Front Vet Sci 2021;8:725737; doi: 10.3389/fvets.2021.725737

Xiong Z, Wang S, Huang Y, et al. Ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky ST198 in broiler chicken supply chain and patients, China, 2010–2016. Microorganisms 2020;8(1):1–9; doi: 10.3390/microorganisms8010140

Zhang L, Fu Y, Xiong Z, et al. Highly prevalent multidrug-resistant *Salmonella* from chicken and pork meat at retail markets in Guangdong, China. Front Microbiol 2018; 9(2104):1–9; doi: 10.3389/fmicb.2018.02104

Zhang S, den Bakker H, Li S, et al. SeqSero2: Rapid and improved *Salmonella* serotype determination using whole-genome sequencing data. Appl Environ Microbiol 2019; 85(23):1–13; doi: 10.1128/AEM.01746-19

Address correspondence to:
Siddhartha Thakur, BVSc, MVSC, PhD
Department of Population Health and Pathobiology
College of Veterinary Medicine
North Carolina State University
CVM Research Building RM 472
1051 William Moore Drive
Raleigh, NC 27607
USA

E-mail: sthakur@ncsu.edyu